

Poster Presentations

Poster Session 1 Disease Modifying Therapy – Part 1

P1

Treatment by glatiramer acetate improves cell therapy in experimental autoimmune encephalomyelitis

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Background: Stem cell transplantation is a promising therapeutic strategy. In multiple sclerosis (MS) in particular, stem cells differentiating into oligodendrocytes and neurons may lead to myelin repair and neuron replacement. However, application of this strategy in MS resulted in limited clinical success. Glatiramer acetate (GA, COPAXONE®) an approved therapy for MS, is an immunomodulator that reduces the chronic inflammation implicated in the destruction of the transplanted cells. GA was also shown to suppress immune rejection in several transplantation models and thus may augment cell engraftment into the central nervous system (CNS). Moreover, GA elevates neurotrophic factor expression in situ, inducing a growth-promoting environment and neurogenesis. **Objective:** The present study aimed at exploring the capacity of GA to improve cell therapy in experimental autoimmune encephalomyelitis (EAE) - the animal model of MS. **Methods:** Muscle progenitor cells were obtained from adult mouse skeletal muscles and propagated as myospheres. Genetically marked (B-gal expressing) myosphere cells were administered either locally by intra-ventricle inclusion, or into the peritoneal cavity (i.p.) of MOG-induced EAE mice injected daily (sub-cutaneously) with GA. Tracing of the transplanted cells was studied immunohistochemically. **Results:** Transplanted myosphere cells were spread in various brain regions when injected into brains of adult EAE affected mice, but not in brains of naive mice. A fraction of the migrating cells gained neuronal morphology and expressed the neuronal marker B-Tubulin-III. GA treatment enhanced the migration and the differentiation of the transplanted cells as manifested by an increased number of B-gal positive cells recruited to injury sites and their B-Tubulin-III expression. Furthermore, even when the myosphere cells were transplanted i.p., their number in brains of GA-treated mice was three times higher than in EAE transplanted mice that were not treated by GA. **Conclusions:** These results warrant further assessment of GA for the improvement of stem cell therapy in CNS pathologies, particularly in MS.

Supported by: This study was supported in part by a research grant from Teva Pharmaceutical Industries.

P2

Plasma exchange after immunosuppressive treatment failure in neuromyelitis optica

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Background: Plasma exchange (PLEX) has shown therapeutic efficacy in steroid-refractory demyelinating disorders, especially in patients with NMO. Besides anti-NMO antibody, chemokines inducing helper T cell type 1 and 2 are also elevated in NMO patients. Removal of these circulating factors by PLEX may reduce the inflammatory response. Early use of PLEX is believed to be associated with better prognosis. **Objective:** To evaluate the PLEX therapeutic response in immunosuppression refractory NMO patients. **Methods:** Inclusion criteria were diagnosis of NMO (Wingershuck et al., 2006), therapeutic failure to intravenous methylprednisolone and cyclophosphamide

or mitoxantrone. Exclusion criteria were active bacterial infectious or hemodynamic instability. Outcome was measured using the Expanded Disability Status Score (EDSS). **Results:** Our sample was of six females, all Afro-Brazilian, with ages ranging from 12 to 46 years. Disease duration varied from 2 to 10 years and relapses were characterized by ON in three individuals and LETM in three individuals. Interval between relapse and onset of PLEX varied from 10 to 90 days. From four to seven sections of PLEX were performed, with every other day exchanges. Anti-NMO was positive in four, and negative in two patients. Clinical improvement occurred after two or three exchanges in five patients and one patient remained stable. Treatment was discontinued in one patient because of concurrent infection. EDSS score had a marked improvement in one patient (decrease of 3 points), moderate in three (reduction of 2 points) and mild in one (decrease of 1 point). **Conclusions:** PLEX was effective in reducing disability in five of six patients with relapsing NMO refractory to immunosuppression. It was a well tolerated procedure which might be considered even after several weeks of symptoms relapsing. In our series, better results were obtained in younger patients.

P3

Short-term immunosuppression with mitoxantrone followed by long-term glatiramer acetate vs. glatiramer acetate alone: Results at 36 months in patients with relapsing multiple sclerosis

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Background: Brief induction immunosuppression with mitoxantrone followed by glatiramer acetate (Mito-GA) was evaluated in a 15-month randomized trial in relapsing-remitting multiple sclerosis (RRMS) patients. Mito-GA resulted in significantly greater reductions in new Gd-enhancing lesions (GEL), T2w and T1w lesion volume accumulation, and the evolution of GEL to chronic black holes compared with GA only. **Objective:** To determine whether benefits of Mito-GA over GA-only persist at 36 months for patients in this ongoing study. **Methods:** Patients with 1–15 GELs and EDSS score 0–6.5 were randomized to either initial monthly mitoxantrone infusions (12mg/m²) for 3 months followed by daily GA (20mg SC), or to daily GA-only (20mg SC), for a total of 36 months. Neurological examinations were performed every 6 months. MRIs were acquired at months 6, 9, 12, 15, 24, and 36. **Results:** At 36 months, 15/21 (71%) Mito-GA and 14/19 (74%) GA-only patients continued in the study. EDSS scores (mean±SE) were unchanged from baseline (-0.07±0.29 vs. 0.04±0.26 with Mito-GA and GA-only, respectively). In the Mito-GA vs. GA groups, the cumulative number of GELs from months 6 to 36 was 1.33±0.37 vs. 11.47±3.51, p=0.02, and the cumulative volume (mL) was 0.06±0.02 vs. 1.20±0.51, p=0.02. In the Mito-GA vs. GA groups, the per cent change from baseline to 36 months in the volume of T2w lesions was -9.17±4.04% vs. 10.16±5.44%, p=0.01, and the volume of T1w lesions was 28.47±9.09% vs. 17.94±62.25%, p=0.21. For the Mito-GA and GA groups at 36 months, the proportion of GEL at baseline that evolved into chronic black holes was 45±10% vs. 31±10%, p=0.37; the proportion evolving on treatment was 27±8% vs. 9±6%, p=0.06. Atrophy did not differ between the groups. Differences between the Mito-GA and GA groups at 36 months were not found in clinical or other MRI endpoints. **Conclusions:** Brief immunosuppression with mitoxantrone prior to GA therapy resulted in sustained differences at 36 months in the cumulative number and volume of GELs, the accumulation of T2w lesion volume, and the proportion of GELs that evolved into chronic black holes. **Supported by:** Teva Neuroscience, Kansas City MO. Grant PMO25.

P4

Use of monthly cyclophosphamide and methylprednisolone in rapidly progressive multiple sclerosis: pilot study

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Background: Multiple sclerosis (MS) is a chronic demyelinating disease among young persons. In rapidly progressive multiple sclerosis (RPMS) the use of mitoxantrone or cyclophosphamide (CPM) is recommended. The latter is used in patients with mitoxantrone contraindications. **Objective:** To demonstrate that monthly CPM plus methylprednisolone (MPN) in patients with RPMS or interferon-B (IFN-B) treatment failure diminish the progression index (PI) to less than 0.6 (by natural history a PI of 0.4/year is expected) after 1 year of treatment. **Methods:** Pilot study. Patients with MS were divided into three groups: 1. IFN-B treatment failure: three or more relapses in 1 year or two or more relapses in 6 months. 2. RPMS: at least one point increase over the previous expanded disability status scale (EDSS) in 1 year, or two point increase, relapse related. 3. Secondly progressive MS (SPMS): at least one relapse within the past 6 months and at least one point increase over the previous EDSS. Evaluation includes: brain magnetic resonance image (MRI), general laboratory studies, EDSS, multiple sclerosis severity score (MSSS), and PI defined as EDSS divided by disease duration in years. **Results:** Seventeen patients were included (eight RPMS, two IFN-B failure, and seven SPMS); 10 completed the first 6 months, and seven completed 1 year. At six months there was an EDSS increase (5.6 vs. 5.9) without changes on MRI. At 1 year, the PI (mean 1.18) has slowed down in the RPMS group (1.34 obtained vs. 1.58 expected) with a p value of 0.809, whereas the PI in the SPMS group remained stable. **Conclusions:** Even though the results are not statistically significant, there is a tendency of the PI to slow down in relation to the expected PI. To determine whether these observations are real, it is justified to include more patients in this study.

P5

Therapeutical approaches in childhood multiple sclerosis

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Background: Multiple sclerosis (MS) is an inflammatory disabling disease of the central nervous system of young adults. In 50% the first symptoms occur between the age of 20 and 40 years. Since 1995 4260 MS-patients have been documented in our Berlin database (MUSIS); 144 (3%) of them were age <16 years at manifestation. One hundred and eight of them were female (77%). Only 15% of those were 10 years of age or younger at the time of manifestation. **Objective:** The mean annual progression rate in childhood MS was lower (EDSS 0.2 per year versus 0.25 per year) The new immunotherapies are supposed to alter the course of the disease and to ameliorate the long-term outcome. There are no licensed drugs for the treatment of childhood MS. **Methods:** Since 1995, in 60 MS patients the immunological therapy has been started below the age of 18 years. We started the initial therapies with immunoglobulines (n=23) or glatirameracetate (n=15) due to minor side effects. Meanwhile experiences with INF beta in children aged >=12 years were reported. We used INFb1a im (n=12), INFb 1b sc (n=3), INFb 1a sc (n=5). **Results:** Initial therapies needed to be changed: 27 of 60 patients had high activity of the disease (74%), 19% side effects. The discontinuation rates for IVIG were 50%, INFbeta 40% and Copaxone 33%. Seventeen patients needed a third change of therapy and seven patients even a fourth - Natalizumab (n=4), Mitoxantron (n=3) and Rituximab (n=1) plasmapheresis (n=3). In two cases we decided to perform bone marrow transplantation because of the aggressive, life-threatening course of the disease. **Conclusions:** An early immunoprophylactic therapy is recommended for adults and children respectively. At the age of 20 years 60% of our patients with childhood MS were not disabled (EDSS<=2). The adherence to initially established therapies corresponds to the experiences in adults. A highly aggressive course of the disease, which requires an escalating therapy, seems, however, to be more common during childhood.

P6

Comparison of two sources of Interferon B 1a to induce increases in vascular cell adhesion molecule

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Background: Adhesion molecules are described as responsible of immune activation and particularly in relapsing-remitting multiple sclerosis (RRMS) to correlate with disease progression by initiating lesion formation. The mode of action of IFN β therapy in MS may involve the induction of sVCAM, and by binding with T cells intercept their adhesion to the blood-brain barrier. This mechanism has previously been discussed. **Objective:** To evaluate a comparison of two different sources of Interferon Beta 1a (IFN β 1a) on induction of vascular cell adhesion molecules (sVCAM-1) in patients with RRMS and their early correlation with quality of life. **Methods:** Twenty patients, of age range 19 to 58 years, 55% female, were randomized to receive either IFN β 1a 44mcg subcutaneous from a standard International source, or IFN β 1a 44mcg subcutaneous from a Mexican source, three times weekly for 12 weeks. Serum sVCAM-1 levels were measured at baseline and monthly during treatment. Quality of life was assessed before and three months after treatment. **Results:** sVCAM-1 levels were increased after treatment more than 80% in comparison with baseline in both groups. Adverse events as well as the effect on serum sVCAM-1 levels were similar for both groups; no differences were found regarding gender, age, baseline nor final disease status. **Conclusions:** A significant positive correlation was found between sVCAM-1 increment and quality of life improvement, disregarding the IFN β 1a source.

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P7

Intravital 2-photon imaging of encephalitogenic effector cells during fingolimod (FTY720) treatment of experimental autoimmune encephalomyelitis

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Background: Oral fingolimod (FTY720) is a sphingosine-1-phosphate receptor modulator, which exerts a beneficial effect in multiple sclerosis (MS) by retaining auto-aggressive lymphocytes in the lymph nodes and away from the CNS. **Objective:** To study the effects of fingolimod on adoptively transferred brain-auto-reactive CD4+ effector T cell-induced Experimental Autoimmune Encephalomyelitis (tEAE) in the Lewis rat - a model for MS. **Methods:** We studied the migratory pattern and the functional state of the auto-aggressive T cell population in blood, spleen, lymph nodes and the central nervous system (CNS) at various stages of tEAE in order to detail the mechanisms underlying the therapeutic effects of fingolimod. Fingolimod, administered once-daily per os at 1mg/kg was given prophylactically (from day of cell transfer; day 0) or therapeutically (at onset of symptoms), and T cell count and expression profiles were assessed on days 2, 4 and 6. Movement and tissue distribution of T cells were also evaluated at these timepoints using intravital 2-photon imaging. **Results:** Oral fingolimod clearly ameliorated tEAE disease severity. As expected from the MoA of fingolimod, treatment strongly reduced the number of effector T cells within the blood circulation by approximately 60%. However, this effect was transient and auto-aggressive effector T cell count was restored within 5-6 days. In contrast, in the CNS of fingolimod-treated rats, effector T cell counts did not recover over time but remained low throughout the entire tEAE episode. However, locomotion and tissue distribution of the residual, invading T cells remained unaltered. **Conclusions:** This study indicates that, in addition to retaining

CD4+ effector T cells within the lymph nodes, oral fingolimod inhibits their transition into their target organs.

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P8

Effect of natalizumab on relapses in patients with relapsing multiple sclerosis who experience MRI activity during treatment

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Background: Natalizumab (TYSABRI®) significantly reduced the number of gadolinium-enhancing (Gd+) lesions in the AFFIRM and SENTINEL phase 3 studies of patients with relapsing multiple sclerosis. Furthermore, natalizumab has been shown to reduce the number of new T1-hypointense lesions compared with placebo. Despite the demonstrated efficacy of natalizumab in reducing lesion formation, some patients will have Gd+ lesions during treatment. The extent to which these patients benefit from natalizumab treatment in terms of clinical outcomes is not clear. **Objective:** To assess the proportion of patients with Gd+ lesions who were relapse free after 2 years of natalizumab therapy. **Methods:** A post hoc analysis was performed in subgroups of patients from AFFIRM and SENTINEL with no Gd+ lesions or with ≥ 1 Gd+ lesion at year 1 and/or year 2. The proportions of patients who were relapse free after 2 years were evaluated. In AFFIRM, patients received natalizumab 300mg or placebo intravenously once every 4 weeks. In SENTINEL, patients in the natalizumab and placebo groups also received interferon beta-1a. **Results:** In AFFIRM, the proportion of relapse-free patients over 2 years was not different between those with no Gd+ lesions and those with ≥ 1 Gd+ lesion during the study. Among the subgroup of patients with ≥ 1 Gd+ lesion at 1–2 years in AFFIRM (natalizumab, n=30; placebo, n=126), 60% of natalizumab-treated patients were relapse free over 2 years compared with 37% of placebo patients (P=0.019). After excluding patients who were persistently positive for anti-natalizumab antibodies from the ≥ 1 Gd+ lesion subgroup, the proportion of relapse-free patients in the natalizumab treatment group was higher and significantly greater than that in the placebo group (78% vs. 37%; P<0.001). Similar results were seen in patients treated with natalizumab plus interferon beta-1a in the SENTINEL study. **Conclusions:** Patients treated with natalizumab experience clinical efficacy despite the occurrence of Gd+ lesions.

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P9

Glatiramer acetate in treatment-naïve and immunomodulatory pre-treated relapsing-remitting multiple sclerosis patients

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Background: Currently immunomodulatory therapies represent the first line therapeutic options for treatment of relapsing-remitting multiple sclerosis (RRMS). **Objective:** This study evaluated the efficacy, safety and tolerability of glatiramer acetate (GA) in treatment-naïve RRMS patients and in patients who switched to GA from another immunomodulatory therapy (beta-interferons or IVIGs). Aspects of cognitive performance were evaluated using the Digit Symbol Modalities Test (DSMT). **Methods:** Prospective open-label non-interventional study over 18 months. Assessments: baseline and every 3 months. Objectives: relapse rate, Expanded Disability Status Scale (EDSS), DSMT score, global judgements. Inclusion/exclusion criteria according to SmPC of GA. Treatment cohorts were defined based on pre-study therapy. Descriptive statistical methods were used for data analysis. **Results:** Overall 350 patients were enrolled in specialized

Austrian MS-centers: treatment-naïve patients (n=211) and pre-treated patients (n=139). Major reasons for switching were: INF-beta: side-effects (64.2%) and lack of efficacy (34.0%); IVIGs: lack of efficacy (67.1%) and side-effects (15.3%). During GA therapy annual relapse rate declined by -73% in the total cohort from 1.9 (± 1.0) to 0.5 (± 0.7). This effect was equivalent in both cohorts. However, in the pre-treated cohort, the effect of GA on relapse rate was more pronounced in patients who switched due to insufficient efficacy compared with patients switching due to intolerability: 2.3 (± 1.2) to 0.7 (± 0.8) vs. 1.6 (± 1.0) to 0.5 (± 0.8). Mean changes in EDSS were less than 0.5 points in both cohorts. DSMT scores remained unchanged indicating stabilization. Twenty-one (6%) of 350 patients discontinued prematurely, therefrom 12 patients due to side-effects or insufficient stabilization. **Conclusions:** In the treatment-naïve patient cohort the well-known efficacy, safety and tolerability of GA was documented under conditions of daily practice in a large study population. The switch from prior INF-beta or IVIG treatment to GA resulted in beneficial effects for most of the patients regardless of the switch reason. The reported adverse reactions resembled the well-known safety profile of GA.

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P10

Escalating treatment of optic neuritis by repeated high-dose corticosteroid-therapies and plasma exchange in pediatric multiple sclerosis

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Background: Severe relapses in adults with multiple sclerosis (MS), refractory to a single high-dose corticosteroid-treatment, are currently treated with an escalation design consisting of repetitive high-dose corticosteroid-therapies and, if relevant improvement is not achieved, with plasma exchange (PE). In relapsing-remitting (RR) pediatric MS, defined by onset before the age of 16 years, there is only one published case treated with PE for a steroid-refractory relapse before the age of 16 years. **Objective:** We report on two female pediatric RRMS patients with severe optic neuritis and insufficient or lacking response to a single high-dose corticosteroid-treatment. **Methods:** Both patients with severe optic neuritis were treated with an escalation regimen with PE after repetitive corticosteroid treatments. **Results:** Patient 1: A 14-year-old girl with RRMS developed a relapse with bilateral optic neuritis, visual acuity (VA) finger counting right eye (oculus dexter, OD), left eye (oculus sinister, OS) 0.02 (decimal equivalent). After the first high-dose corticosteroid-pulse (1g methylprednisolone i.v. daily over 5 days) VA improved to 0.05 on OD and 0.2 on OS, after the second (2g methylprednisolone i.v. daily over 5 days) to 0.2 OD and 0.8 OS. After a subsequent five PEs VA improved on OD to 0.5 and to 1.0 on OS. After 3 months VA was 1.0 on both eyes. Patient 2: An 11-year-old girl with RRMS developed optic neuritis on OS with a VA of 0.03. After two high-dose corticosteroid pulses (850mg prednisolone daily i.v. over 5 days, 1.7g prednisolone daily over 5 days) VA improved only slightly to 0.05. After a subsequent 5 PEs there was no further improvement. Treatments were well tolerated in both patients. **Conclusions:** These cases illustrate that the escalation design for relapse treatment used in adult MS patients is also well tolerated in pediatric MS. At least single pediatric MS patients may benefit from that regimen.

P11

There is no rebound worsening of multiple sclerosis after stopping disease-modifying therapies

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Background: Many auto-immune diseases are suppressed by immune-inhibiting drugs, but they exhibit an abrupt flare if treatment is suddenly stopped. This rebound worsening has been reported in

some (but not all) studies of multiple sclerosis (MS) patients whose natalizumab (Tysabri) therapy was abruptly discontinued. **Objective:** To determine if rebound worsening occurs after cessation of interferon or glatiramer therapy. **Methods:** We analyzed 573 relapsing-remitting MS patients treated with glatiramer and various interferon therapies, followed prospectively in a longitudinal database. **Results:** A total of 103 patients (18%) discontinued or interrupted treatment, usually because of side effects or perceived lack of benefit. Only 19 of these patients (18%) had a relapse in the first year off of drugs. This relapse rate of 0.18 attacks per year was less than the on-treatment average of 0.31 attacks per year. There was no difference between glatiramer and any of the interferon preparations in the attack rates. (Genetic analysis of these patients will also be presented.) **Conclusions:** We conclude that prophylactic MS therapy with glatiramer or the interferons can be interrupted with little fear of precipitating any rebound worsening of the underlying MS.

P12

Disease modifying therapies prevent secondary progression in multiple sclerosis patients

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Background: The large use of disease modifying therapies (DMTs) in multiple sclerosis (MS) is mostly based on their demonstrated efficacy in reducing inflammation, and consequently relapse-rate. In addition, a recent post-marketing study suggests a beneficial effect on long-term disability progression (Trojano et al., *Ann Neurol* 2007). Recently, we assessed the effectiveness of a risk score (Bayesian risk estimate for MS, BREMS) able to early predict the unfavorable long-term evolution of MS, namely to reach the secondary-progressive (SP) phase (Bergamaschi et al., *JNS* 2007). **Objective:** In the present study, we aimed to evaluate the efficacy of DMTs in preventing SP by applying the BREMS score. **Methods:** We studied 1192 patients (median onset age 25.1 years; mean disease duration 17.3 years) with a relapsing form at onset and at least 10 years of disease duration; 835 of them (70%) have been treated with at least one DMT. We analyzed clinical and demographical data, so calculating BREMS for each single patient within the first year of disease. We performed a regression analysis with Cox proportional hazard model, with BREMS as predictive variable and the time to reach SP as main end-point. **Results:** Three hundred and eighty seven patients (32.5%) became SP by the end of the entire observation. BREMS scores were significantly higher in the SP patients than in the progression-free patients in the whole group ($p < 0.00001$). A significantly higher probability to reach SP was found for non-treated patients compared with patients treated with DMTs ($p = 0.004$). When we analysed the DMTs separately, glatiramer acetate seemed to be a little more 'protective' than interferons towards SP ($p = 0.05$). **Conclusions:** Our results confirm the ability of BREMS to predict the risk of reaching SP and reinforce the role of DMTs in modifying the natural disease course, suggesting a favorable effect not only on the inflammatory but also on the neurodegenerative processes. **Supported by:** The study was partially supported by a grant from FISM (Federazione Italiana Sclerosi Multipla) - Project 2005/R/3.

P13

Clinical response to glatiramer acetate in interferon beta-resistant relapsing-remitting multiple sclerosis patients

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Background: Interferon beta (IFN-beta) therapy is widely used to reduce disease activity in multiple sclerosis (MS). However, there is a number of patients who do not benefit from these drugs. A switch to

glatiramer acetate (GA) is used in multiple sclerosis (MS) patients who cannot tolerate or fail to respond to IFN-beta. **Objective:** To assess the efficacy and tolerability of GA in IFN-beta-resistant and IFN-beta-intolerant MS patients. **Methods:** Thirty nine relapsing-remitting MS patients (28 women and 11 men, average age 37.4 years) who discontinued IFN-beta therapy and switched to GA were enrolled in this retrospective study and classified in two groups depending on the reason for discontinuation: side effects or lack of efficacy. Duration of treatment with IFN-beta and GA, number of relapses, relapses per year and causes of discontinuation of both treatments were collected and analyzed as well as demographic data. **Results:** Twenty five patients discontinued IFN-beta because of adverse effects (11 with lymphopenia, four with hypertransaminasemia, four with depression, four with flu-like syndrome, one with thyroid dysregulation and one with local effects, whereas only 11 did it because of lack of effectiveness. Patients who discontinued because of adverse effects changed from 0.33 relapses per year with IFN-beta to 0.31 with GA and three patients (12%) interrupted GA. Patients who discontinued for inefficacy changed from 1.55 relapses per year with IFN-beta to 0.62 with GA and five patients (36%) interrupted this treatment, four of them because of lack of efficacy. **Conclusions:** A switch from IFN-beta treatment to GA in IFN beta-intolerant and IFN beta-resistant MS patients was beneficial. Patients who had discontinued IFN-beta treatment because of side-effects seemed to tolerate GA well. A switch to GA in this kind of patient must be considered.

P14

Avonex® 15-year long-term follow-up study of patients with relapsing multiple sclerosis

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Background: Treatments that offer long-term benefits to patients with multiple sclerosis are crucial, but long-term placebo-controlled studies are not viable. In pivotal studies, intramuscular interferon beta-1a (IM IFNβ-1a; Avonex) reduced relapse rates and delayed progression of disability during a 2-year period in patients with relapsing-remitting multiple sclerosis (RRMS). **Objective:** To assess factors that may predict which patients may do well using IM IFNβ-1a. **Methods:** ASSURANCE is an open-label, multicenter, 15-year follow-up study that includes patients who received ≥2 years of treatment in the pivotal phase 3 trial (n=172). Patients completed a self-reported questionnaire. As of March 5, 2008, 116 of a possible 172 questionnaires have been returned, and enrollment is continuing. Patients were categorized as IM IFNβ-1a long-term users (patients currently on IM IFNβ-1a) and non-long-term IM IFNβ-1a users. **Results:** Of 116 patients enrolled, 11% (13) are deceased. The 103 patients still living have a mean age of 52.0 years. Overall, 51% (53/103) of non-deceased patients are IM IFNβ-1a long-term users and have received IM IFNβ-1a for up to 17.3 years. Median duration of IM IFNβ-1a use was 13.3 years in long-term users and 6.0 years in non-long-term users. Eighty-nine percent of long-term users stated their health was good to excellent. In the long-term-user groups, significantly fewer patients reached an Expanded Disability Status Scale (EDSS) score of 6.0 compared with the non-long-term users (30% vs. 60%; $P = 0.002$). After analysis of multiple demographic and disease characteristics, baseline annualized relapse rate was the only parameter that was different between long-term and non-long-term users. Analysis of factors predicting long-term use of IM IFNβ-1a is ongoing. **Conclusions:** We report on a group of patients who continue to use IM IFNβ-1a for up to 17 years. Identifying factors that predict long-term continuing use of IM IFNβ-1a without significant clinical disease progression could assist in treatment decisions early in the course of RRMS. **Supported by:** Biogen Idec, Inc.

P15**Retrospective study to assess the efficacy and safety of mitoxantrone in multiple sclerosis patients with different therapy courses**I. Bonaventura¹, M. Marco², S. Romero¹, S. Quintana¹, T. Delgado²¹Hospital Mutua de Terrassa, Terrassa, Barcelona, Spain; ²Hospital de Sabadell, Sabadell, Barcelona, Spain

Background: Mitoxantrone has been approved for worsening relapsing-remitting (RR) and secondary progressive (SP) multiple sclerosis (MS), unresponsive to standard therapy. The standard protocol is 12mg/m² every 3 months up to 120mg/m², but in patients with more aggressive disease we can use 8mg/m² every month to try to be more effective. The purpose of this study is to evaluate the efficacy and safety of mitoxantrone in these different courses. **Objective:** To compare the efficacy and safety in a cohort of active MS patients with different courses of mitoxantrone. **Methods:** To analyze retrospectively the response in relapse rate and disability (Expanded Disability Status Scale - EDSS) between 1 and 2 years before and after treatment. Statistics: variance analysis with repeated measures. **Results:** N=20, 11 female. Clinical forms: 9 RRMS, 11 SPMS. The duration average of the disease is 13.1 years. Mean age at the beginning of treatment: 40.85 years. Mean EDSS at onset: 6.15. Mean relapse rate 1 year before mitoxantrone: 1.75. Treatment protocol: 11 patients monthly and 9 every 3 months. Mean relapse rate 1 year after starting mitoxantrone decreased to 0.4 (p<0.001), similar in the two courses of treatment, but higher in RRMS than in SPMS. The mean EDSS 1 year after treatment was 5.87, improving in monthly treatment and stable in patients every 3 months. Also, the mean EDSS difference also improved in RRMS and was stable in SPMS. These results are maintained in the second year. Minor side effects in 45% of patients were observed, without differences between both treatments' courses. **Conclusions:** Mitoxantrone is effective in reducing relapse rate and in delaying disability in patients with active MS. This treatment is very effective in patients with RRMS, but stabilizes disease activity in patients with SPMS. Probably the therapeutic benefits are higher when it is used monthly, without more secondary effects.

P16**Characterization of a novel sphingosine-1-phosphate-1 receptor agonist with activity in experimental autoimmune encephalomyelitis**

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Background: Multiple sclerosis (MS) is a central nervous system (CNS) demyelinating disease that often presents with a relapsing-remitting course leading to progressive disability. Autoreactive CD4+ T cells contribute to the pathophysiology of the disease with additional immune cells contributing to the demyelination and ultimate destruction of axons. Sphingosine-1-phosphate (S1P) is a key regulator of lymphocyte recirculation mediated through S1P1 receptor signaling. Activation of the S1P1 receptor in vivo results in a marked lymphopenia and a dramatic therapeutic effect in models of cell-mediated autoimmunity. Recently, fingolimod, an S1P receptor agonist, has been shown to be effective in a Phase II clinical trial in MS. Identification of a novel S1P1 agonist, Compound 1, with antagonist activity at S1P3 has recently been reported. **Objective:** The objective of this study was to determine the effect of Compound 1 on peripheral blood cell count and experimental autoimmune encephalomyelitis (EAE). **Methods:** Lymphopenia was determined 24 hours after a single administration of Compound 1. A relapsing-remitting rat model of EAE induced by MOG was used to assess efficacy of compound 1. **Results:** Twenty-four hours after a single administration, Compound 1 induced a dose dependent lymphopenia with an ED90 of 1mg/kg. When administered daily in a rat EAE model, Compound 1 induced a dose dependent reduction in clinical signs with an ED90 of 0.3–1mg/kg. Drug levels that induced 90% lym-

phopenia were required to achieve a complete therapeutic response in EAE, consistent with the hypothesis that lymphocyte retention in peripheral lymph nodes is the primary mechanism of action. In the clinic, fingolimod has been associated with significant bradycardia and impaired pulmonary function, purportedly due to agonist activity on S1P3. Compound 1, which is an antagonist at S1P3, did not induce bradycardia or reduce pulmonary function. **Conclusions:** We propose that S1P1 agonism has been demonstrated as a viable approach for the treatment of MS and that agents such as Compound 1, which lack S1P3 agonism, may offer an advantage by avoiding potential cardiac and pulmonary side effects.

P17**Alemtuzumab Phase 2 Extension Study Design (CAMMS223): Assessing long-term outcomes and potential benefits of additional alemtuzumab treatment in patients with relapsing-remitting multiple sclerosis**

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Background: CAMMS223 is a randomized, rater-blinded, phase 2 study comparing the safety and efficacy of up to three annual alemtuzumab (alem) cycles with three times/week SC interferon beta-1a (IFNβ-1a) in treatment-naïve patients with relapsing-remitting multiple sclerosis (RRMS). In the 3-year analysis, alem reduced the risk for 6-month sustained accumulation of disability (i.e., elevated Expanded Disability Status Scale (EDSS) lasting ≥6 months) by 71% and the risk for relapse by 74% compared with IFNβ-1a (both p<0.0001). Alem-related adverse events included infusion reactions, immune thrombocytopenic purpura, thyroid dysfunction, and predominantly mild to moderate infections. **Objective:** Present the design and rationale for the CAMMS223 Extension Study **Methods:** Patients who received alem during the initial 3-year study and consent to retreatment will be randomized at a 1:2 ratio to 'fixed' or 'as-needed' retreatment. In the fixed arm, patients receive two annual cycles of alem (12mg/day for 3 consecutive days/cycle). In the as-needed arm, retreatment is deferred until a patient relapses or develops ≥2 new/active lesions on magnetic resonance imaging (MRI). All patients, including alem-treated patients who decline retreatment and those randomized to SC IFNβ-1a at study onset, will continue with scheduled assessments through at least 3 additional years of follow-up. **Results:** The CAMMS223 extension study will examine safety and efficacy outcomes beyond 3 years, and compare two distinct retreatment strategies. **Conclusions:** Results should help elucidate the long-term effects of prior alem treatment as well as the safety and efficacy of additional alem retreatment delivered in fixed annual cycles or as needed for resumed MS disease activity.

Supported by: Genzyme Corporation.**P18****Estimates of disease-modifying-drug effect size in relapsing-onset multiple sclerosis tend to decline as disability severity, years since onset and treatment years increase**Murray G. Brown¹, Sarah Kirby², Chris Skedgel³, Ingrid Sketris⁴, Jock Murray², John D. Fisk⁵, Virender Bhan², for Dalhousie MS Research Group.

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Background: Canada approved IFN-a, IFN-b and glatiramer acetate disease-modifying drugs (DMDs) for relapsing-onset definite MS (R-onset MS) between 1994 and 1997. In 1998 the province of Nova Scotia provided 100% insurance coverage for these DMDs. Estimates of DMD effect size under 'real world' conditions were made using Nova Scotia 1979–2004 clinical data. **Objective:** To describe how effect size estimates for DMDs, as a class, in R-onset MS tend to decline as disability severity (Expanded Disability Status Scale - EDSS),

years since onset (YSO) and treatment years (DMDy) increase. **Methods:** Treatment effect size was estimated in absolute terms and relative to EDSS natural history by a fixed effects model of annual EDSS change in pre-treatment-years, treatment-years on first DMD, treatment-years on second or third DMD, and post-treatment years. The model was populated, sequentially, with data from various DMD-treated and never-treated groups. Estimates were reported for treated relapsing-remitting (RRMS, EDSS<=3.5), secondary progressive (SPMS, EDSS<=6.5) and combined R-onset MS final classification reference groups. Sensitivity analysis focused on effect size estimates for the first DMD; ranges were EDSS <=2 to <=6.5, YSO <=5 to <=30 and DMDy <=1 to <=10. **Results:** Reference group estimates of absolute EDSS increase avoided per year on first DMD were significant for RRMS (-0.103, 0.000), SPMS (-0.065, 0.011) and R-onset MS (-0.162, 0.000); effect size relative to natural history was 112%, 21% and 105%. Sensitivity analysis estimates varied greatly (RRMS: 0.06 to 0.27; SPMS: 0.03 to 0.34) and tended to decline for treated groups with greater disability, longer YSO and more treatment years. **Conclusions:** Sensitivity analysis found that the RRMS reference group estimate was centered within estimates for other RRMS groups. The SPMS reference group estimate was not similarly centered, being much smaller than estimates for SPMS groups with lower EDSS scores and YSO. **Supported by:** Health Canada. MS Society of Canada. Nova Scotia Health Research Foundation. Capital Health Nova Scotia.

P19

Autologous non-myeloablative hematopoietic stem cell transplantation for relapsing-remitting multiple sclerosis

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Background: Hematopoietic stem cell transplantation (HSCT) in patients with late secondary progressive multiple sclerosis (MS) has demonstrated little evidence for improvement in neurologic performance. We, therefore, evaluated the outcome of HSCT earlier in the course of disease during the immune-mediated relapsing-remitting phase. **Objective:** To determine the safety and neurologic effects of non-myeloablative HSCT in RRMS with frequent relapses despite interferon. **Methods:** Candidates had an Extended Disability Status Scale (EDSS) between 2.0 and 5.5 and despite treatment with interferon beta had at least two acute attacks treated with corticosteroids or at least one such attack and MRI gadolinium enhancing lesion(s) on an occasion distinct from the clinical relapse. Hematopoietic stem cells (HSC) were mobilized with cyclophosphamide (2.0g/m²) and granulocyte colony stimulating factor (10ug/kg/day and infused intravenously after immune suppression with cyclophosphamide (200mg/kg) and either alemtuzumab (20mg) or rabbit anti-thymocyte globulin (6mg/kg). **Results:** Engraftment of white blood cells and platelets was on mean day 9 and hospital discharge day 11. The only infections were one episode of Clostridium difficile diarrhea and two cases of dermatomal zoster. Two patients receiving alemtuzumab developed late immune thrombocytopenic purpura (ITP) that remitted with standard therapy. All 21 patients are surviving at a mean follow-up of 38 months. Five patients (24%) relapsed following initial improvement but achieved another remission after further immunosuppression. Statistically significant improvement occurred in the EDSS, NRS, PASAT, 9-hole peg test, 25 foot walk, and quality of life SF-36. The EDSS improved by at least 1.0 point in 66%. Progression-free survival is 100%, relapse-free survival is 76%, disease-activity free survival is 62%. **Conclusions:** Non-myeloablative autologous HSCT performed in MS patients having frequent relapses results in improvement in neurologic function with low risk of infection. Rabbit antithymocyte globulin should be used instead of alemtuzumab to avoid late ITP.

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P20

A Phase I/II dose-escalation trial of oral vitamin D3 with calcium supplementation in patients with multiple sclerosis

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Background: Increasing distance from the equator, low UV radiation and low serum 25-hydroxyvitamin D [25(OH)D] are associated with increased multiple sclerosis (MS) prevalence and risk. While this relationship provides insight into prevention, it begs the question, 'Is vitamin D3 (VD3), known to have immunoregulatory properties, beneficial in established MS?' To answer this, a safe, effective dose must be determined. **Objective:** To characterize the safety profile of high-dose oral VD3 in MS. **Methods:** A prospective controlled 52-week trial matched MS patients for demographic and disease characteristics, randomizing them to treatment or control groups. Treatment patients started VD3 at 4000 IU/day and escalated over 28 weeks to 40 000 IU/day. This was followed by maintenance with 10 000 IU/day for 12 weeks, 4000 IU/day for 8 weeks and a 4-week wash-out, translating into roughly 14 000 IU/day over 52 weeks. Calcium (1200mg/day) was given throughout the trial. The primary endpoint was mean change in serum calcium in treatment patients at each VD3 dose, and a comparison of calcium between treatment and control groups. Secondary endpoints included 25(OH)D, urine calcium/creatinine (Ca/Cr) and PTH. Cytokines, lymphocyte response and matrix metalloproteinase-9 were also measured, as were Expanded Disability Status Scale (EDSS) and relapses. **Results:** Forty-nine patients were enrolled (25 treatment, 24 control) with mean age 40.5 years (21–54 years), EDSS 1.34 (0–6.0) and 25(OH)D 78nmol/l (38–154). No abnormalities or differences in serum calcium, urine Ca/Cr or PTH occurred, nor were there differences in calcium between groups. Despite a maximum mean 25(OH)D of 413nmol/l (66–729), no significant clinical or biochemical adverse events occurred. A greater proportion of treatment patients had stable/improved EDSS vs. control patients (p=0.018). Treatment patients also had fewer relapses and a greater reduction in relapse rate vs. controls. Immunological data will be presented. **Conclusions:** High-dose VD3 (~10 000 IU/day, possibly higher) in MS is safe and tolerable, with evidence of clinical improvement.

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P21

Immunomodulatory therapy reduces the development of inflammatory cortical lesions in relapsing-remitting multiple sclerosis

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Background: The great majority of multiple sclerosis (MS) patients develop a relevant inflammatory pathology in the cortex, i.e., inflammatory cortical lesions (CLs), that likely play a relevant role in determining clinical and cognitive disability. **Objective:** To assess the efficacy of 2-year immunomodulatory agent (IMA)-based treatments in preventing the development of CLs in relapsing-remitting MS (RRMS). **Methods:** One hundred and fourteen patients with a diagnosis of RRMS (MacDonald's criteria) were included in the study; 58 patients (mean Expanded Disability Status Scale - EDSS - 2.0±1.0; mean age 36.4±10.0 years) were treated with an immunomodulatory agent (IMA), 39 with interferon beta 1a (IFNβ1a), 19 with glatiramer acetate (GA). Fifty-six patients (mean EDSS 1.9±1.8; mean age

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39.4±11.0) remained therapy free. Each patient underwent clinical (EDSS) and magnetic resonance (MR) examination (including double inversion recovery for the demonstration of CLs) at baseline and after 24 months. The following MR parameters were analyzed: CLs number and volume, gray matter fraction (GMf) and white matter fraction (WMf), T2 weighted white matter lesion load (T2-WMLV), number of contrast enhancing lesions. **Results:** In IMA-treated patients the number of CLs did not change from baseline to month 24 (3.6±4.9 versus 3.9±4.1, $p=0.5$), while a significant increase in CLs number was observed in therapy-free patients (2.8±3.6 versus 4.8±5.0, $p=0.007$). The appearance of new CLs was observed in 13/58 (22.4%) IMA-treated patients and 28/56 (50.0%) untreated patients, and the difference was significant ($\chi^2=9.415$, $p=0.002$). No difference was observed between IFN β 1a- and GA-treated patients. **Conclusions:** IMA therapy is effective in reducing the accumulation of CLs in RRMS patients. While further confirming the inflammatory origin of CLs, our findings strongly support an early IMA-based treatment of RRMS.

P22

An open-label study of high dose cyclophosphamide for moderate to severe refractory multiple sclerosis

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Background: Treatment-refractory clinically active multiple sclerosis (MS) can quickly lead to irreversible neurological disability. High dose cyclophosphamide (CTX) could benefit the most severely affected patients, resistant to traditional treatment. This effect appears to be durable. In Brazil, the monoclonal antibodies were not approved and treating patients with devastating MS can be a frustrating challenge to the clinician. **Objective:** To evaluate the therapeutic response to High Doses Immunosuppression (HDI): CTX and Methylprednisolone (MP) for moderate severe refractory MS patients. **Methods:** Assessment prospectively, consecutive patients fulfilled the inclusion criteria: diagnosis of relapsing-remitting MS (RRMS) (McDonald criteria), Expanded Disability Status (EDSS) >3.5, new severe or >2 relapses within the previous year, despite use of standard therapies, active lesions measured by gadolinium enhancement (Gd+) on Magnetic Resonance Imaging (MRI). Patients received HDI: MP 1g/day for 8 days plus CTX 50–200mg/Kd/day for 4 days. The exclusion criteria were: active infection, contraindication for use of MP or CF. The response was measured by EDSS and free time of relapse (FTR). **Results:** Eleven patients: 10 female, aged 18–51 years, 6 months to 7 years after MS diagnosis. The median EDSS pre-treatment was 6.5 (4.0–8.0). MRI discloses active Gd+ lesions and three showed pseudo tumor hemispheric lesions (Marburg Variant). All patients received complete schedule treatment. Moderate clinical improvement occurred in seven, discrete in three and no improvement in one. The median EDSS final was 3.5 (1.5–6.5). The most common complications: leukopenia (10), infection (4), alopecia (6). Nine patients reach a clear stabilization in 6–36 months follow-up, and median FTR reach was 12. **Conclusions:** HDI (MP and CTX) was beneficial and safe for all patients in this series. HDI showed a clear stabilization in refractory patients, who had a more aggressive course from the onset. High dose CTX has not been described in South America.

P23

Cutaneous necrosis in multiple sclerosis patients: misuse of interferon beta or individual susceptibility?

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Background: Cutaneous necrosis (CN) is known to be a possible complication of interferon beta (Ib) treatment in multiple sclerosis (MS). Few data describe the potential conditions for its outcome or susceptible conditions to undergo such a complication. **Objective:** To describe the individual and clinical context for the occurrence of CN in MS patients treated with interferon. **Methods:** We identified five MS patients with CN. Their clinical profile and the outcome of this

adverse event are described together with potential conditions susceptible to increasing the risk of CN. **Results:** There were three women and two men with relapsing-remitting MS (RRMS). Ages of onset of MS were 22 (n=2), 27, 49 and 53 years. Three patients had no relapse for more than 2 years, one had had 1 relapse and the fourth had a relapse rate of 2/year in the last 2 years. Three patients were treated by Ibf 1a SC. In one patient two episodes were noted. The first was typical CN but the second was severe panniculitis lasting more than 3 months. Both episodes were on the abdomen. For the two remaining patients, the CN episode was unique, at the shoulder, and qualified as severe because it was extremely painful. In all three cases we noted errors in the use of Ibf with frequently absent local asepsia and injections repeated in the same site. Two patients were treated with Ibf 1b SC. All the patients experienced three or more episodes. Site of CN was variable in the same patient: the thighs, shoulders or abdomen. All episodes resumed spontaneously. No errors of treatment administration could be noted. **Conclusions:** CN is not a rare complication of Ibf treatment. We noted differences between our five cases. With Ibf 1a SC, episodes were unique and severe. In all these cases errors of asepsia and injections done on the same site seem to be a hallmark for CN occurrence. With Ibf 1b, multiple and mild episodes were described. We then hypothesized that CN with Ibf 1b may be due to individual susceptibility or to the treatment itself. Conversely, with Ibf 1a SC, CN may be linked to a treatment misuse.

P24

Evaluating patient satisfaction on transition from subcutaneous interferon beta-1a to a new human serum albumin-free formulation: the TRANSFER study

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Background: Rebif® New Formulation (RNF; sc IFN beta-1a) has been developed without fetal bovine serum and human serum albumin, with the aim of improving tolerability and immunogenicity while maintaining efficacy. **Objective:** To evaluate satisfaction among patients with relapsing MS transitioning from Rebif® to RNF (44mcg sc three times weekly) and receiving prophylactic (PRO) or as-needed (PRN) ibuprofen. **Methods:** Patients (n=117, aged 18–60 years with relapsing MS [McDonald criteria], Expanded Disability Status Scale (EDSS) score ≤ 5.5 , on stable Rebif® for ≥ 6 months) were randomized to this multicenter, two-arm, open-label, Phase IIIb study and transitioned to RNF plus PRO ibuprofen (400mg 30–60min pre-injection) or PRN for 4 weeks, followed by a 4-week safety extension. The primary endpoint was the flu-like symptoms (FLS) domain score (0 [good]–20 [poor]) of the validated Multiple Sclerosis Treatment Concern Questionnaire (MSTCQ; total score 100). **Results:** An equal proportion of patients in each group completed the study (93%: PRO, 56/60; PRN, 53/57). Mean [SD] MSTCQ FLS scores in both groups showed no meaningful change from baseline (PRO, 9.4 [3.7]; PRN, 8.4 [4.0]) to week 4 (PRO, 8.5 [4.0], PRN, 9.1 [3.7]). MSTCQ injection-system satisfaction and global side-effect scores were unchanged, while total and injection-site reactions scores improved moderately in both groups between baseline and week 4. A median (range) of two (0–24) ibuprofen doses were taken in the PRN group during the 8-week study, and one (0–31) dose was taken in addition to the mandated doses in the PRO group. The most commonly reported adverse events ($\geq 10\%$ patients) in weeks 1–4, reported in the PRO and PRN groups, were FLS (66% and 67%), headache (39% and 32%), and fatigue (25% and 12%). **Conclusions:** These data indicate that patients transitioning from the current to the new formulation remained similarly, if not increasingly, satisfied with their treatment in both PRO and PRN groups.

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P25

Compliance and complications after 5 years of continual therapy with interferon-beta or glatiramer acetate in relapsing-remitting multiple sclerosis

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Background: Currently, interferon beta (IFNB) or glatiramer acetate (GA) are recommended to be taken indefinitely, which can be challenging to many patients. Long-term compliance to GA or IFNB may be affected by associated toxicity. **Objective:** We conducted a study to examine the local and systemic toxicity after 5 years of continual IFNB or GA therapy and if it affected the degree of compliance in relapsing-remitting multiple sclerosis (RRMS). **Methods:** A detailed ascertainment of clinical features, chart review and patient interview was carried out. **Results:** Two hundred and seventy three RRMS patients were studied. Of these, 101 received GA, 93 SC IFNB, and 79 weekly low dose IFNB for 5 years continually. There were no significant differences in clinical demographics. Toxicity data showed flu-like symptoms persisted in 37% high dose and 21% low dose IFNB but none in GA. Local injection site reactions were seen in 90% of GA but 67% of the high dose and 21% of the low dose IFNB patients. Injection site necrosis was seen in 10% of high dose but only 1.7% of low dose IFNB or GA. Severe post-injection reactions (high fever with generalized weakness) after IFNB injection or severe systemic reaction post GA injection were seen in 7% of high dose IFNB, 24% of low dose IFNB, and 10% of GA patients. Lipoatrophy was seen in 78% of GA, 40% of high dose and 1% of low dose IFNB. Headaches were also reported. Compliance was arbitrarily divided into three levels by the percentage of injections taken monthly (I=>90%, II=70-90%, and III=<70%). The best compliance was seen in low dose IFNB, followed by high dose IFNB and GA ($p<0.05$). Further analyses and comparison to adverse events reported in pivotal trials will be presented. **Conclusions:** Despite 5 years of continual therapy, adverse effects associated with IFNB or GA therapy persisted in a large number of patients that affected compliance. Of note, post-injection fever and weakness was seen in 25% of low dose IFNB patients and headaches attributed to high dose IFNB in 25% of patients. Lipoatrophy (78%) led to suboptimal compliance in GA. This raises the need to re-evaluate our approach to encourage long-term compliance, which in turn is likely to improve clinical outcomes.

P26

The impact of long-term treatment with disease modifying therapy on disability progression in relapsing-remitting multiple sclerosis patients

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Background: Natural history studies in multiple sclerosis (MS) define disease progression as the progression to certain landmark disability scores (Expanded Disability Status Scale - EDSS 4-6-8). This approach does not distinguish between the clinical effects of focal demyelinating lesions and progressive axonal degeneration, since disability may be caused by residual symptoms following relapses as well as progressive axonal degeneration. Both interferon (IFNs) and glatiramer acetate (GA) have demonstrated efficacy on relapses and on the rate of disability progression. The monitoring of MS patients can provide important information on the long-term safety and effectiveness of current disease-modifying drugs (IMDs). **Objective:** To assess the impact on clinical outcomes of treatment with a single IMD over time. Primary endpoints: 1) time to EDSS 4, 6 and 8; 2) the risk of disability progression according to the length of exposure to the first IMD applied. Secondary endpoints: annualized relapses rate and changes in EDSS scores. **Methods:** Data analysis was performed with: Epi Info 2007 software; χ^2 test; non-parametric tests (Kruskal Wallis for multiple groups); Wilcoxon signed rank test. The time to EDSS score was estimated using Kaplan Meier survival analysis. Markov modeling of disability progression was used to deal with missing data.

Results: Out of 914 RRMS patients, $n=451$ (49.3%) naïve patients had been exposed to IMDs; INFB $n=267$ (59.2%); GA: $n=184$ (40.8%). 73.4% of patients on GA remain ambulatory vs. 67% on IFNB, despite having had MS for over 20 years. The risk of reaching EDSS ≥ 6 was 1.5 times higher in patients treated with INFB vs. those treated with GA. Comparisons with an untreated control group were performed and will be presented. **Conclusions:** Our results demonstrate that continuous long term IMD treatment delayed progression of disability and reduced relapses. Patients exposed to GA showed higher risk reduction to EDSS ≥ 6 than those exposed to IFNB over the 14 years follow-up.

P27

The risk of optic neuritis during immunomodulatory treatment in relapsing-remitting multiple sclerosis patients

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Background: Optic neuritis (ON) results in the death of retinal ganglion cells (RGC) and is responsible for the usually temporary loss of visual function during multiple sclerosis (MS). Axonal destruction and neuronal loss also occurs, manifested as optic atrophy and loss of the nerve fiber layer. Neuroprotection is a therapeutic paradigm for delay or prevention of death of neurons from injury. Results of the clinical trials suggest that patients treated with immunomodulatory agents (IMD) experience fewer relapses. Nevertheless data is not available about the clinical effect of IMD on RGC (corresponding neuron) in patients experiencing ON; neither the risk of repeated ON attacks during treatment. **Objective:** To assess the risk of subsequent development of ON after treatment initiation with glatiramer acetate (GA) or interferon beta (IFN), compared with the pre-treatment period. **Methods:** Two hundred and thirty three patients with clinically definitive RRMS, with or without ON prior to treatment, were followed for a period of 12 years. Statistical analysis: Epi info 2007 software. **Results:** Of 140 patients (60%) who did not have ON before treatment 11 patients (7.9%) went on to develop ON during treatment. Of these, distribution of therapy was: GA: $n=4$ (36.4%); INFB: $n=7$ (63.7%); RR:2.14. Ninety three patients (40%) had a history or findings suggestive of ON prior to treatment. Sixteen patients (17.2%) had a recurrence of ON: five of them (31.2%) were on GA while 11 of them (68.8%) were on IFNB; RR:2.18. **Conclusions:** The concept of neuroprotection involves preventing damage to the cell body and its axon. Although GA and IFNB treatment showed benefit in patients with or without ON prior to treatment only GA showed two times lower RR than INFB to develop ON during treatment after 12 years of follow-up. These results suggest GA may have a better protective effect of the optic nerve from ON in the context of MS.

P28

The MS-STAT trial: a Phase II trial of high dose simvastatin in secondary progressive multiple sclerosis

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Background: Options are limited in trying to modify the disease course of secondary progressive multiple sclerosis (SPMS). An attractive candidate is the HMGCoA reductase inhibitor simvastatin, currently being evaluated in several trials in relapsing-remitting MS. The rationale for these studies is the recognition that these drugs possess broad spectrum immunomodulatory and anti-inflammatory effects including inhibiting MHC class II-restricted antigen presentation, attenuating antigen-presenting cell maturation, inducing a shift from a pro-inflammatory Th1 to a regulatory Th2 phenotype, and blocking adhesion molecule expression and leukocyte migration. Germane to SPMS, recent evidence also attributes neuroprotective properties to statins such as up-regulation of the major cell survival protein bcl-2,

protection against glutamate-mediated excitotoxicity and free radical damage and promotion of myelin repair. **Objective:** To investigate whether simvastatin can reduce the rate of brain atrophy, as measured using volumetric magnetic resonance imaging (MRI), in SPMS. **Methods:** A Phase II randomised placebo-controlled trial of 80mg of simvastatin in SPMS is proposed. The major inclusion criteria are: age 18–65 years, Expanded Disability Status Scale (EDSS) 4.0–6.5, progression within the previous 2 years, no immunosuppressants or disease-modifying treatments within the previous 6 months. One hundred and forty patients will be randomized (1:1) and followed up at 1 and 2 years. Brain atrophy rate will be utilized as the primary outcome measure, and will be determined from T1-weighted volumetric MRI using the brain boundary shift integral. Secondary outcome measures include changes in EDSS, MSIS-29, MSFC, SF-36, T1/T2 lesion load and cognitive function as determined by serial neuropsychological assessment. Full statin immunological profiling will be carried out. **Results:** This trial has now commenced. **Conclusions:** The results of this trial will be reported in 2011. ClinicalTrials.gov, number NCT00647348

Supported by: Multiple sclerosis trials collaboration (registered UK charity).

P29

S1P₁ receptor signaling on cells of astrocytic lineages in experimental autoimmune encephalomyelitis: a role in disease progression and the efficacy of fingolimod (FTY720)

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Background: Sphingosine-1-phosphate (S1P) is a lysophospholipid that can function as an extracellular signaling molecule. To date, at least five cognate G-protein-coupled receptors have been identified (S1P₁₋₅). This receptor system has therapeutic relevance in multiple sclerosis (MS) given the activities of oral fingolimod (FTY720), which is being assessed in ongoing Phase III trials for MS. The phosphorylated metabolite of FTY720, FTY720-phosphate, is an S1P receptor modulator that regulates four of the five known S1P receptors. Retention of lymphocytes in the lymph nodes is believed to be the primary mechanism of action of FTY720, which antagonizes some of the normal functions of S1P in this system. **Objective:** This study examined the potential neuronal and non-neuronal CNS effects of FTY720 in the mouse experimental autoimmune encephalomyelitis (EAE) model of MS. **Methods:** Genetically altered mice that were conditionally null for S1P₁ receptors were assayed behaviorally following induction of EAE. Synapsin-Cre lines were used to produce conditional deletion in neurons and pan-neuronal lineages were produced by use of nestin-Cre. Histological samples were assessed by immunohistochemistry, particularly the use of anti-gial fibrillary acidic protein (GFAP), which selectively labels astrocytes; controls were compared with experimental conditions and genotypes. **Results:** As we reported previously, the efficacy of FTY720 in EAE does not depend entirely on lymphocyte retention in the lymph nodes. Nestin-Cre conditional deletion of floxed-S1P₁ from cells of neural lineages both reduced the severity of EAE and rendered EAE clinical scores refractory to FTY720 treatment, despite maintained reductions in lymphocyte counts. The synapsin-Cre mutant for S1P₁ in neural tissues was indistinguishable from wild-type, suggesting the involvement of a non-neuronal lineage. Astroglia is a histopathologic feature of EAE, but was attenuated in both the nestin-Cre conditional mutants and in FTY720-treated controls. **Conclusions:** These data support a role for S1P₁ receptors on astrocytes in EAE and in the efficacy of FTY720 in MS.

P30

Oral fingolimod (FTY720) versus interferon beta-1a in relapsing-remitting multiple sclerosis: baseline patient demographics and disease characteristics from a Phase III trial (TRANSFORMS)

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Background: Oral fingolimod (FTY720), a sphingosine-1-phosphate receptor modulator, reduced annualized relapse rate by >50% and number of gadolinium-enhancing lesions by up to 80% at doses of 1.25 or 5.0mg in a Phase II, 6-month placebo-controlled trial of 281 patients with relapsing multiple sclerosis (MS). A large Phase III trial is currently underway to compare the efficacy and safety of oral fingolimod with that of interferon beta-1a (IFNβ-1a) injected intramuscularly in patients with relapsing-remitting MS (RRMS). **Objective:** To report baseline patient demographic and MS disease characteristics from the TRANSFORMS trial. **Methods:** Patients diagnosed with RRMS according to the 2005 revised McDonald criteria were randomized to receive once-daily oral fingolimod 0.5 or 1.25mg, or once-weekly intramuscular IFNβ-1a 30mcg for 12 months in this global, double-blind, double-dummy, parallel-group trial. Eligible patients were aged 18–55 years with Expanded Disability Status Scale (EDSS) score 0–5.5 and were required to have at least one relapse in the previous year or two relapses in the previous 2 years. **Results:** Study enrollment took place over 16 months between May 2006 and September 2007, and 1292 patients with a mean age of 36 years were randomized; 67% were female. At baseline, mean MS duration was 7.5 years, with a mean of 1.5 relapses in the past year and 2.2 in the past 2 years. Mean baseline EDSS score was 2.2. Approximately 56% of patients previously received a disease-modifying treatment (27% received intramuscular IFNβ-1a, 18% subcutaneous IFNβ-1a, 15% glatiramer acetate, 14% IFNβ-1b, and 1% natalizumab); some patients received more than one treatment. **Conclusions:** Baseline results for patient demographic and MS disease characteristics from the ongoing TRANSFORMS phase III trial represent an RRMS population with active disease, which is consistent with those of previous Phase III studies of parenteral MS disease-modifying treatments (IFNβ-1b [1993], glatiramer acetate [1995], intramuscular IFNβ-1a [1996], subcutaneous IFNβ-1a [1998], natalizumab [2006]).

P31

Oral laquinimod in patients with relapsing-remitting multiple sclerosis: 9-month double-blind active extension of the multicenter, randomized, double-blind, parallel-group placebo-controlled study

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Background: Patients who received 0.6mg/day oral laquinimod for the initial placebo-controlled (PC) 36 weeks had a 40% and 55% reduction in the mean and median number of Gd-enhancing lesions (GdE) on T1-weighted images taken on the last trimester compared with placebo. **Objective:** To determine whether the effects observed during

the first PC phase with daily 0.3 and 0.6mg laquinimod are sustained and those seen in the group originally randomized to placebo are reproducible when patients switched to either low or high dose laquinimod. **Methods:** Subjects originally assigned to placebo were equally randomized to receive either 0.3 or 0.6mg/day laquinimod, while others continued their original treatment for another double blind extension for 36 weeks. Magnetic resonance imaging (MRI) was performed at the beginning and at the end of the active extension phase. **Results:** Two hundred and fifty seven patients (91%) entered the extension phase to receive laquinimod 0.3mg/day (119 patients) or 0.6mg/day (138 patients). Two hundred and thirty five patients had an MRI measurement at the end of the active extension. In patients who switched from placebo to laquinimod, the mean number of GdE was significantly reduced, by 52% (from 4.46±6.55 to 2.12±3.73, $p<0.0007$). The reduction was significant for both patients switching to a high or a low dose ($p<0.009$ and $p<0.03$). A significant reduction of the mean number of GdE was also observed in patients initially treated with 0.6mg/day ($p=0.0062$) and 0.3mg/d ($p=0.0013$), with no further increase in the proportion of GdE-free patients. The proportion of GdE-free patients increased from 31% at the onset to 47% at the end of the extension phase ($p<0.012$). Neither new safety signals nor increase in incidence rate of AEs and laboratory abnormalities have emerged following new or prolonged exposure to laquinimod. **Conclusions:** Patients on continuous 18 months laquinimod 0.6mg treatment continued to show low MRI disease activity, a high proportion still remaining free of Gd-enhancing lesions with good tolerability. The treatment effects on MRI activity seen during the PC phase were reproduced when placebo patients switched to laquinimod. **Supported by:** TEVA Pharmaceutical Industries Ltd.

P32

Treatment with glatiramer acetate delays conversion to clinically definite multiple sclerosis in patients with clinically isolated syndrome: subgroup analyses

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Background: The PreCISe study examined glatiramer acetate (GA) treatment in patients with clinically isolated syndrome (CIS) and at least two T2-weighted brain lesions of >6mm diameter. Results from interim analysis showed that GA reduced the risk of developing clinically definite multiple sclerosis (CDMS) by 45% compared with placebo (Hazard ratio 0.55, 95% CI [0.40; 0.77], $p=0.0005$). **Objective:** To evaluate the efficacy of early treatment with glatiramer acetate (GA, COPAXONE®) in delaying conversion to CDMS in subgroups of patients presenting with CIS suggestive of MS. **Methods:** Subgroups of 481 patients (GA (n=243) or placebo (n=238)) were analyzed in terms of the risk to progression to CDMS using Cox proportional hazards regression, with respect to demographics, clinical and magnetic resonance imaging characteristics at study baseline. **Results:** A significant risk reduction of 48% by GA treatment compared with placebo was demonstrated for females (Hazard ratio 0.52, 95% CI [0.34, 0.81], $p=0.0037$) and 53% for young patients (<30 years, Hazard ratio 0.47 95% CI [0.27, 0.80], $p=0.0060$). A significant risk reduction of 39% and 54% was obtained for patients with or without corticosteroid treatment for the initial attack, respectively, and 66% risk

reduction was demonstrated for patients presenting with optic neuritis (Hazard ratio 0.34 95% CI [0.17, 0.68], $p=0.0022$). The results of the logistic regression comparing GA treatment vs. placebo in reference to MRI disease activity at baseline demonstrated significant and pronounced effects for patients with MRI active disease. A risk reduction of 71% (Hazard ratio 0.29 95% CI [0.16, 0.54], p value <0.0001) for patients with more than one T1 gadolinium (Gd)-enhancing lesion at baseline and 58% (Hazard ratio 0.42 95% CI [0.27, 0.64], $p<0.0001$) for patients with nine or more T2 lesions were obtained. **Conclusions:** GA treatment effect was robust across the study population including subgroups of patients with and without MRI disease activity and less clinical or MRI disease dissemination at onset and patients not receiving steroids for the CIS.

Supported by: TEVA Pharmaceutical Industries Ltd.

P33

Rationale for the use of multipotent mesenchymal stromal cells in multiple sclerosis

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Background: Multipotent mesenchymal stromal cells (MSCs) are a population of stem cells that can be derived from the bone marrow of humans throughout life. They have been proposed as a potential neuroprotective/repair therapy in several neurological diseases, with trials in MS patients now underway. **Objective:** To summarize the proposed rationale for MSC therapy in MS. **Methods:** A systematic review of MSC translational research literature. **Results:** The rationale of using MSCs as an exogenous source of cells for CNS tissue repair is controversial. Despite several reports of in vitro neuronal/glia transdifferentiation, there is debate that non-standard growth conditions may result in artefactual neuronal morphology and marker expression. In addition, there is currently no evidence of transdifferentiation occurring in vivo. An alternative rationale of using MSCs to facilitate endogenous repair processes, either directly (such as via the promotion of oligodendrogenesis) or indirectly, is also recognized. Existing disease models demonstrate axonal neuroprotection following MSC therapy, with some evidence that this is potentially mediated through the production of neurotrophic/growth factors, and/or immunomodulatory effects of MSCs. A growing body of in vitro literature confirms these intrinsic MSC biological properties, and they provide a plausible mechanism of action to guide clinical trial design. **Conclusions:** MSCs have effects on MS disease models through neuroprotective mechanisms. These plausibly include known biological properties of MSCs such as the production of neurotrophic/growth factors and/or immunomodulatory effects.

P34

DMT adherence in the VHA MS Surveillance Registry

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Background: The effectiveness of disease modifying therapy (DMT) for multiple sclerosis (MS) is thought to be limited, in part, by adherence. Few systematic studies of adherence have been reported. **Objective:** Determine the impact of DMT side-effects on adherence and determine whether there are characteristics that predict which patients will be non-adherent to therapy. **Methods:** We mailed surveys to a nationally representative, stratified, random sample of 3905 veterans with MS. The survey included a chronological accounting of DMT utilization and side-effects. Results were validated against outpatient pharmacy records. Analysis was carried out on 1001 patients with relapsing disease. DMT adherence was defined as continuous DMT utilization (regardless of type) from initial prescription to the present. **Results:** The number of patients returning completed questionnaires

was 1226 (31%) and they comprise the VHA MS Surveillance Registry (MSSR). Respondents were nearly identical to the veteran MS population with only minor differences compared with non-respondents (married: respondents - 61%; non-respondents - 53%). A total of 1461 DMTs were utilized sequentially by 882 patients with relapsing MS: 484 (55%) used only one DMT; 255 (29%) used two DMTs; 108 (12%) used three DMTs; 32 (4%) used four DMTs; and 3 (0.4%) used five DMTs. Of the 882 patients prescribed DMT 556 (63%) were adherent with therapy. Of these, 62% achieved adherence with one DMT, 26% used two DMTs, 12% or less used three or more DMTs. The side-effects related to non-adherence were: flu symptoms (24%), not effective (26%), injection site reactions (16%), and 10% developed progressive MS. There were no differences in age, gender, race, subtype or region between patients who were adherent and those who were non-adherent. **Conclusions:** These data show that treatment with multiple DMTs sequentially is common and suggest that non-adherence to DMT therapy is related to side effects and perceived effectiveness. No predictive demographic factors were identified. **Supported by:** Berlex Laboratories (currently Bayer Pharmaceuticals, Inc.).

P35

Serum ferritin levels and β -interferon treatment in multiple sclerosis: a clue for interferon liver toxicity

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Background: Transient transaminases (ALT and AST) elevation is frequently observed within the first months of interferon therapy. Liver toxicity is rarely severe but few cases of severe hepatic injury are reported. Recently, increased iron turnover with elevated serum ferritin has been described. **Objective:** To evaluate the correlation between serum ferritin and liver enzymes within a group of β -interferon treated patients. **Methods:** We retrospectively analyzed serum liver enzymes (ALT, AST and GGT) modifications within a cohort of 161 multiple sclerosis (MS) patients treated with β -interferon. Serum ferritin was monitored to see if there is a significant correlation between iron metabolism and liver toxicity. When ferritin levels reached more than 5 times the upper limit of normal (ULN) and were associated with ALT or AST significant elevation (grade 3 toxicity), patients underwent bleeding in order to obtain ferritin normalization. **Results:** Out of 161 interferon-treated patients, 70 (43.5%) had serum ferritin elevation with a median of 1.6 times ULN (maximum 12.0 times). Seven patients (4.3 %) had more than 5 times ULN. ALT perturbation was more frequently observed than AST or GGT elevation (28.6%, 21.7% and 27.9% of patients, respectively). ALT, AST and GGT modifications were highly significantly correlated ($p < 0.001$). Four patients (2.5%) presented grade 3 toxicity. Median ALT increase was 1.6 times ULN (maximum 12.6 times) for the whole cohort of patients. Ferritin and liver enzyme serum levels were linearly correlated ($r = 0.74$, $p < 0.001$ for ALT, $r = 0.73$, $p < 0.001$ for AST and $r = 0.55$, $p < 0.001$ for GGT). Four patients (2.5%) were bled until the ferritin level normalized. For each of them, liver enzyme abnormalities improved below grade 3 and interferon therapy was maintained. Serum ferritin and transaminase modifications were strictly correlated during the follow-up. **Conclusions:** Serum ferritin elevation is observed more frequently than liver enzyme abnormalities during interferon therapy. These parameters are strictly correlated in time and amplitude. Blood punctures are associated with liver function improvement and allow the maintaining of interferon therapy.

P36

JC virus in multiple sclerosis patients treated with natalizumab

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Background: JC virus (JCV) is a human polyomavirus that establishes life-long latency in kidney and in other organs. JCV is

known for its capability of inducing progressive multifocal leukoencephalopathy (PML). In a recent clinical trial with beta-1a interferon and natalizumab, two multiple sclerosis (MS) patients developed PML as a consequence of an opportunistic JCV infection of the CNS. **Objective:** We analyzed the effect of natalizumab treatment on the active replication of JCV in relapsing-remitting MS (RRMS) patients. **Methods:** We have analyzed 45 peripheral blood mononuclear cells (PBMCs), serum and urine samples obtained from the 21 RRMS patients who are being currently treated with natalizumab (between 2 and 7 months) in the Hospital Clinico San Carlos of Madrid. The patients (mean age 33.9 years; range 26–46; and 71.4% women) were treated previously with beta-interferon. The samples were extracted monthly, and the DNA was extracted and finally analyzed by a quantitative real time polymerase chain reaction assay to detect JCV genomes. **Results:** We found that 7.7% of the MS patients had viral DNA in the serum (mean viral load: 1510 JCV copies/ml serum), and the 71.4% of MS patients had JCV in their urine samples (mean viral load: 64.2x10⁵ JCV copies/ml urine; range 8.6–8560x 10⁵ copies/ml). We did not find any difference between the prevalence or the viral load of JCV in the urine samples extracted before and after the treatment; there was no relation between the viral load and the duration of the treatment. Regarding the serum samples, the positive was found in one patient after the beginning of the treatment. **Conclusions:** We have found presence of JCV in the serum of MS patients treated with natalizumab; therefore, we should be aware of a possible spread to the brain and the risk of the development of PML.

P37

Mitoxantrone treatment of multiple sclerosis patients enhances in vitro TH2 type cytokine production

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Background: Mitoxantrone is an immunosuppressive drug that is used in the treatment of progressive forms of multiple sclerosis (MS). In addition to the cytotoxic effects immunomodulatory properties of mitoxantrone have been described. The migration of mononuclear cells and B-cell function are inhibited by mitoxantrone. However, the effects of mitoxantrone on the cytokine pattern of peripheral blood monocytes and T-cells subsets have not been studied in great detail. **Objective:** To investigate the effect of mitoxantrone treatment in MS patients on the cytokine patterns produced by peripheral blood monocytes and CD4+ and CD8+ T-cell subsets ex vivo. **Methods:** Eighteen patients were recruited into the study. Blood was obtained before and 6, 12 and 18 days following mitoxantrone infusion. CD4+ and CD8+ lymphocytes were isolated using bead selection. Proliferation and TH1 and TH2 type cytokines were determined following in vitro stimulation with PHA or antiCD3/CD28 stimulation. In addition, cytokine patterns derived from de novo patients were compared with patients with >1 year of mitoxantrone treatment. **Results:** The proliferative responses were not inhibited in this experimental setting. There was an increased production of IL-4 ($p = 0.0218$), IL-5 ($p < 0.0001$) and IL-10 ($p = 0.0099$) in CD4+ T-cells within 18 days of treatment. The cross sectional study comparing treatment naïve patients with those receiving mitoxantrone for at least 12 months revealed enhancement of in vitro IL-4 ($p = 0.0166$) and IL-5 ($p = 0.0199$) production in PBMC. **Conclusions:** This study indicates that mitoxantrone induces TH2 type cytokines, which may contribute to its beneficial effects in the treatment of MS.

P38

Therapy-related acute leukemia with mitoxantrone: what is the risk and can we minimize it?

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Background: Therapy-related acute leukemia (TRAL) is a concern for neurologists and patients when considering treatment with mitoxantrone for multiple sclerosis. **Objective:** To improve understanding of

this risk we undertook a literature review, combined with local experience, to determine the risk of TRAL and its relationship to exposure. **Methods:** We undertook a literature search for relevant publications and hand searched abstracts to identify case series reporting follow-up and complications of treatment with mitoxantrone. We combined this with our local database of 250 cases treated since 1997. We also identified all reported individual cases of TRAL and extracted data from cases reporting exposure (dose or mg/m²), timing and outcome of TRAL. **Results:** Case series including 5472 patients were identified; mean dose of mitoxantrone was 74.2mg/m² (range 12–120mg/m²). TRAL was diagnosed in 0.30% (1 in 333). In 29 TRAL cases sufficient data was available to inform analysis of exposure. Onset was a median of 18 months following treatment (range 4–60). Acute myelocytic leukemia (AML) was the most common leukaemia subtype (52%) and acute promyelocytic leukemia (APL) the second most frequent (39%). Five of 20 TRAL patients, where outcome was reported, had died (25%). Eighty percent of cases occurred in patients exposed to >60mg/m², with a relative risk of 1.44 (CI 95% 1.43–1.46) when comparing total dose <60mg/m² against >60mg/m², strongly suggesting a relationship between risk of TRAL and total dose. **Conclusions:** The risk of TRAL following mitoxantrone is around 1 in 333 with a trend suggesting a relationship to dose. Treatment regimes which limit total dose of mitoxantrone to <60mg/m² should minimize this risk in addition to that of cardiotoxicity and menstrual irregularities.

P39

A multicenter, open label, non-comparative trial investigating the recovering of IFN-beta efficacy in breakthrough relapsing-remitting multiple sclerosis patients with neutralizing IFN-beta antibodies (RECOVER)

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Background: Interferon-beta (IFN-β) treated multiple sclerosis (MS) patients have a risk of developing neutralizing antibodies (NABs) against the medication. The incidence of such NABs varies substantially between the different commercial preparations, and is highest among those using subcutaneous injection. **Objective:** To determine the proportion of patients with a NAB titer ≤20 at 15 months (responders), in patients who were NAB positive on s.c. IFN-β therapy and who switched to a low immunogenic i.m. IFN-β-1a therapy after a wash-out period. **Methods:** The study was an open-label non-comparative study in Caucasian relapsing-remitting MS patients. Fourteen NAB-positive patients treated with s.c. IFN-β were included. After a wash-out period of 3 months where the patients were treated with monthly oral methylprednisolone, the patients self-administered treatment with IFN-β-1a 30 μg i.m. once weekly for 12 months. The following variables were determined: the proportion of patients with a NAB titer ≤20 at 15 months (responders), change in MxA (biomarker of the biological effect of IFN-β) induction, annualized relapse rate, and score on the Expanded Disability Status Scale (EDSS), and the overall incidence of adverse events. **Results:** Only four out of 14 patients were responders (NAB titer ≤20; non-significant); however, the MxA values were inconclusive as to whether any of the patients had a biological response to IFN-β treatment. The annualized relapse rate decreased from 1.63 at screening to 0.70 at end of study. The mean EDSS score increased from 3.21 at screening to 3.42 at end of study. No safety issue was observed. **Conclusions:** This study showed no clear evidence supporting a decrease of NAB titers over time in patients who are switched from an s.c. IFN-β treatment to a low immunogenic i.m. IFN-beta-1a therapy. The study was limited by the small patient number and relatively short follow-up period, which possibly could be the reason for non-significance of the results. **Supported by:** Biogeldec, Denmark.

P40

Evaluation of activity, aggregation and NAB testing with generic interferon beta products: implications for treating multiple sclerosis

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Background: Currently, several IFNβ products are being marketed in developing countries for therapy of relapsing-remitting multiple sclerosis (RRMS), including both innovator (approved in US and Europe) and subsequent entry IFNβ products. To a large extent the latter are copies of the original products. **Objective:** To obtain and test several new IFNβ-1a products (A, B, C, D) for biological potency and molecular characteristics. **Methods:** Products were evaluated using both antiviral and reporter gene assays, immunoblots were used to evaluate product aggregation. **Results:** In antiviral and reporter gene assays for estimation of biological potency, using the WHO international standard of IFNβ-1a (NIBSC 00/572) as calibrant, we have found that IFNβ-1a products A and B had a similar specific activity (262 and 295MIU/mg, respectively) to an approved IFNβ-1a product (average 271MIU/mg), while that of the other products, C and D, was significantly lower (148 and 160MIU/mg, respectively). In immunoblots, all IFNβ-1a products formulated with human serum albumin (HSA) variably displayed slower migrating protein bands, these being most prominent with products C and D, in comparison with the main IFNβ band at 22kDa. Evaluating performance in the Luciferase Reporter Gene assay at 20IU/ml - as described on the product labeling, was equal to 7.22LU/ml (A), 7.07LU/ml (B), 2.08LU/ml (C) and 6.33LU/ml (D) of each biosimilar, equivalent to 10LU/ml for an approved IFNβ-1a product. IFNβ products with the lowest specific activity produced higher NAB titers and higher rate of positive NAB results on screening. **Conclusions:** These findings suggest a tendency of IFNβ-1a to form aggregates and/or adducts with HSA, which may affect biological activity and immunogenicity and results in an antigen-dependent variation in NAB titers. They indicate that the biological and physical properties of entry IFN-β-1a products are variable and differ significantly from those of approved IFNβ-1a products. This influences the outcome of immunogenicity assays and will have efficacy implications for patients treated with some of these products.

P41

Risk-benefit assessment of concurrent slow dose of mitoxantrone to control active multiple sclerosis under interferon beta-1a: an exploratory study

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Background: Mitoxantrone monotherapy is registered for worsening multiple sclerosis, but concurrent combined therapy of mitoxantrone with other immunomodulators is an interesting option to be investigated. **Objective:** To investigate the risk-benefit ratio of a slow-dose therapeutic protocol of mitoxantrone concurrent with beta-interferon 1a in highly active multiple sclerosis. Interim results of the first quarter of treatment are reported. **Methods:** Multiple sclerosis (MS) subjects with >2 exacerbations or >1 point increase Expanded Disability Status Scale (EDSS) in the last year despite interferon-beta-1a treatment were supplemented with a loading dose of 10mg/month for 3 consecutive months. Then mitoxantrone was administered quarterly at a single dose of 10mg for up to 30 months of treatment. Blood analysis and echocardiography were performed. Incidence and severity of adverse reactions will assess the risks, whereas both relapse rate

and EDSS changes will assess the benefit. **Results:** Up to now, 20 subjects have completed the first 3 months of loading dose of mitoxantrone. At study entry, eight subjects had progressed >1 EDSS point in the last 12 months and 13 subjects had two or more exacerbations in the previous year. Mean time from IFN- β beginning to mitoxantrone start had been 2.8 \pm 1.5 years. At informed consent signature, mean annual exacerbation rate was 2.1 \pm 1.2 and mean EDSS score 4.1 \pm 1.4. After 3rd month of treatment, four subjects had experienced 1 relapse, one subject 2 relapses, and no subjects 3 or more while in 12 months pre-baseline fifteen subjects had 1–2 relapses, and 5 subjects 3 or more. Regarding risks, no subject presented leukopenia worse than grade I, and basal left ventricular ejection fraction remained unchanged. Eight subjects reported adverse events, none of them was severe. **Conclusions:** This interim evaluation suggests that concurrent supplementation of IFN- β 1a with a slow dosage scheme of mitoxantrone can be applied without major risks and the benefit seems promising. The planned long-term follow-up of the study will eventually substantiate the true risk-benefit of this new therapeutic approach.

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P42

Therapeutic plasma exchange for steroid unresponsive multiple sclerosis relapses: a long term follow-up observation

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Background: Therapeutic plasma exchange (TPE) is proposed in the escalation therapy of steroid unresponsive multiple sclerosis relapses, although there are only few data on predictors of the response rate, long term data and specific follow-up therapies after TPE. **Objective:** We conducted a long term follow-up observation to gain data on these issues. **Methods:** Clinical, electrophysiological and, where applicable, radiological data on the disease course of 22 multiple sclerosis (MS) patients (mean age 36 years, 18 female, 4 male) treated with TPE (50ml/kg BW, replacement fluid albumin) within 4 weeks after steroid unresponsive MS relapses (RRMS or PRMS) were analyzed, including follow-up investigations after 6 to 12 months. **Results:** Overall response rate 4 weeks after TPE (defined as a clinically relevant improvement) was 91% with 41% of the patients responding after the third TPE. At follow-up examination we found a sustained amelioration of the index symptom in 76%. Four patients responding to TPE exhibited further steroid-refractory relapses, again highly responsive to TPE, in one patient in an interval as short as 4 weeks. One patient with severe side-effects during earlier TPE was effectively and safely treated with immune adsorption (tryptophane column, exchange volume 1500ml). The immune therapy initiated after first TPE (Interferon beta (n=7), glatiramer acetate (n=1), natalizumab (n=6), mitoxantrone (n=4), rituximab (n=3), mycophenolate mofetil (n=1), azathioprine (n=1), partially in sequential combination) was tolerated without severe side-effects. **Conclusions:** In this ongoing long term observation we could affirm a lasting success of TPE on steroid refractory MS relapses. Non-responsiveness to steroids and efficacy of TPE may be predictive for the individual patients' future treatment response. Immune therapy can be safely initiated after TPE.

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P43

Persistency of neutralizing antibodies against interferon beta after discontinued treatment in patients with multiple sclerosis

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Background: Interferon beta (IFN β) is an antiviral and immunomodulatory cytokine and a first-line treatment in multiple sclerosis (MS). However, 32% of treated patients develop neutralizing antibodies (NAbs) against recombinant IFN β , which at high titers block the biological effect of IFN β and thus reduce drug efficiency. Natalizumab is an alternative treatment for patients with high NAbs against IFN β , which inhibits activated leukocyte migration into brain tissue. Immuno-

modulating treatment with natalizumab might potentially increase the risk of virus reactivation in the brain, especially if the patients have hampered ability to fight viral infections due to high NAbs. **Objective:** In this study we investigated NAb titer levels after withdrawal of IFN β treatment in patients converting to natalizumab. **Methods:** Serum from 50 natalizumab-treated MS patients (n=50) that were previously treated with IFN β was analyzed; one sample taken during IFN β treatment and one during natalizumab treatment, respectively, at an average 16.5 months after cessation of IFN β (range 1.1–42). NAbs were measured using an in vitro bioassay, where MxA mRNA expression induced by IFN β was detected with real-time PCR. A critical titer level of 150 10-fold reduction units per ml (TRU/ml) was set, since we previously identified NAb titers above this level to correlate with loss of IFN β bioactivity in vivo. **Results:** The overall appearance in our study was a decrease in NAb titers. Of patients with titers >150 TRU/ml during IFN β treatment, 25% shifted to levels with regained bioactivity. However, three patients with NAb titers <150 TRU/ml shifted to become >150 TRU/ml when on natalizumab. The changes in titer levels did not correlate with time since IFN β withdrawal. **Conclusions:** The majority of patients maintain their NAb status even over 1 year after switching from IFN β to an alternative treatment. However, compared with patients continuing on IFN β treatment, slightly more patients do lose their NAbs after discontinuation.

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P44

Continuous long-term immunomodulatory therapy in relapsing multiple sclerosis: results from the 15-year analysis of the US prospective open-label study of glatiramer acetate

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Background: The ongoing US glatiramer acetate (GA) Trial, begun in 1991, is the longest prospective evaluation of continuous immunomodulatory therapy in relapsing-remitting multiple sclerosis (RRMS). **Objective:** To assess benefits of continuous long-term GA treatment, as sole disease-modifying therapy. **Methods:** The modified intention-to-treat (mITT, n=232) cohort included all study patients receiving \geq 1 GA dose. Ongoing patients (n=100; 43%) have continued on-study to February, 2008. Patients were evaluated every 6 months (Expanded Disability Status Scale - EDSS). Confirmed disability progression was defined as \geq 1.0 EDSS point increase sustained for 6 months. Patients were classified as 'stable/improved' if EDSS score changes were less than or equal to 0.5 points. Proportions of patients who reached confirmed thresholds of EDSS 4, 6, or 8 while on GA, and Kaplan-Meier (KM) estimates of median times to these thresholds were obtained. **Results:** Mean GA exposure was 8.6 \pm 5.2 and 13.6 \pm 1.3 years for mITT and ongoing cohorts. While on GA, mITT cohort relapse rates declined from 1.18 \pm 0.82 to 0.43 \pm 0.58/year; 32% experienced disease progression; 54% had stable/improved EDSS scores; and 39%, 23%, and 5% reached EDSS 4, 6, and 8, respectively. All ongoing patients completed the 15 year clinical visit. For ongoing patients, relapse rate declined from 1.12 \pm 0.82 to 0.25 \pm 0.34/year, 38% experienced disease progression; 57% were stable/improved; and 38%, 18%, and 3% reached EDSS 4, 6, and 8, respectively. Estimated time for one quartile of patients to reach EDSS 4 was 3.98 years in the mITT cohort and 6.8 years in the ongoing cohort. Less than 25% of either cohort reached EDSS 6 and 8. An excellent safety profile was maintained. **Conclusions:** This prospective, ongoing, open-label study of continuous use of GA demonstrates the benefits of long-term

GA use. With a mean disease duration of ~22 years, approximately one-third of patients experienced disease progression and >80% of patients remained ambulatory without aids. Patients will continue to be followed for 20 years.

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P45

Cognition, fatigue, depression and health-related quality of life in early multiple sclerosis: baseline data from CogniMS, a multinational longitudinal study

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Background: Cognition in early MS patients is rarely documented longitudinally, and there are few data on measures appropriate for worldwide studies. **Objective:** To measure cognition, fatigue, depression and health-related quality of life (HRQoL) and the interrelation of these parameters in early multiple sclerosis (MS) patients within a global study. **Methods:** CogniMS is an observational study involving 1509 patients with early MS (diagnosed within 2 years) treated with Betaferon (IFNB-1b) and assessed every 6 months over 2 years. Cognitive function was assessed by the Faces Symbol Test (FST) and the Paced Auditory Serial Addition Task (PASAT). A subset of 388 patients completed the Brief Repeatable Battery of Neuropsychological Tests (BRB-N). Fatigue was assessed by the Fatigue Severity Scale (FSS), depression by the Center of Epidemiologic Studies Depression Scale (CES-D), HRQoL by the European Quality of Life 5-dimensional questionnaire (EQ-5D) and the Functional Assessment of MS (FAMS). **Results:** We report baseline data on 1509 patients from 32 countries (Europe 1140; Middle East 135; Asia 111; New World 123). Median age was 34 years, 67.4% of patients were female. Median time since disease onset was overall 17.4 months. Median EDSS was 2.0 (Europe 2.0, Middle East 1.0, Asia 2.0, New World 1.5). Median PASAT score was 49.0 (Europe 48.0, Middle East 56.0, Asia 48.0, New World 47.0); overall 11.4% were likely impaired. The median FST score was 2.67. In patients assessed using the BRB-N, likely impaired results in =>2 tests occurred in 9.2%. Of FSS scores 45.1% were suggestive of fatigue; 36.1% of CES-D scores were indicative of depression. The EQ-5D visual analogue median was 80 (Europe 80, Middle East 80, Asia 70, New World 80). Correlations of cognitive tests, fatigue, depression and HRQoL will be presented. **Conclusions:** This study suggests similar worldwide characteristics of cognitive impairment in patients with early MS and explores regional differences. Further refinements and data collection are necessary to validate current scales.

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P46

High intensity immunoblation and autologous stem cell treatment for aggressive multiple sclerosis can improve quality of life and fatigue

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Background: Immunoblation and autologous stem cell treatment (ASCT) is a potential treatment for aggressive MS but can be associated with significant morbidity. Quality of life (QoL) data provide information about patient perception of themselves and their illness during and after treatment. Fatigue can also be brought on by or worsened by the treatment. **Objective:** To apply QoL measures and the fatigue impact scale (FIS) to patients in the early post-transplant period (PoT), to assess the immediate impact of treatment, and the late post-transplant period to examine the possible benefit of treatment. **Methods:** Validated MS-QoL-54 and FIS were given to consented patients to complete in the 3 month pre-transplant (PrT),

and every 6 months thereafter for 3 years. **Results:** Sixteen out of 17 treated patients and two controls completed evaluations in the immediate (1 year) PoT period. Ten treated patients and one control completed questionnaires out to 36 months. One patient died in the first 3 months PoT. All treated patients remained free of MS relapses or any new MRI activity. FIS: all but two treated patients improved (9) or remained unchanged (5) compared with the PrT period in the first year. Two patients reported worsening and neither had significant PoT complications. In contrast, at 36 months all but one patient reported an improved (6) or stable (3) level of fatigue compared with the PrT period. One patient had significant worsening along with a noted progression of his illness. One control was worse and one improved. Overall physical (pQoL) and mental (mQoL) QoL scores: all but three treated patients reported improvement (9) or stabilization (4) in pQoL and all but two improved (12) or stabilized (2) in mQoL in the immediate PoT period. The patients who worsened did not experience any particular complication of the treatment. At 36 months all but two had scores that improved (5) or stabilized (3) in pQoL and all but one patient improved (6) or stabilized (3) in mQoL. **Conclusions:** These data show that the procedure of immunoblation and ASCT does not compromise patient-related outcomes of wellbeing (FIS and MSQoL54) in either the immediate or 3 year post-treatment periods.

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P47

Oral mycophenolate mofetil versus weekly im interferon beta 1a: an MRI and clinical safety investigation

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Background: Mycophenolate mofetil has been utilized as an anti-rejection agent in transplant recipients and in patients with a myriad of autoimmune disorders. The advent of an oral immunomodulator for multiple sclerosis (MS) would represent an important advance in biological therapeutics and with respect to quality of life. This agent is often used as an adjunctive agent in MS, hence the systematic collection of safety information in this population is crucial for the future utilization of this agent. **Objective:** To present the final radiographic, clinical, and safety results of a head-to-head randomized blind investigation involving daily oral mycophenolate mofetil versus weekly intramuscular interferon beta 1a in relapsing MS. **Methods:** We organized a randomized, open-label, parallel-group, multicenter study to determine the safety and efficacy of mycophenolate mofetil (Cellcept®) in mono- and combination-therapy with interferon beta-1a (Avonex®), in patients with relapsing-remitting MS (RRMS) and evidence of gadolinium enhancement on a screening magnetic resonance imaging (MRI) of the brain. We sought to assess the 6 months safety and efficacy of Cellcept versus Avonex by comparing month-to-month changes of Gd enhancing and T2 lesions in patients with RRMS. Further, we evaluated the safety and efficacy of Cellcept in combination therapy with Avonex for RRMS patients using inflammatory MRI outcome measures over months 7–12. We also explored the possible effect of Cellcept, as mono- or combination-therapy, on the progression of standard clinical and two non-conventional MRI measures; magnetization transfer imaging (MTI) and development of persistent T1-hypointensities (black holes), in a head-to-head comparison and in combination phases of the study. **Results:** We will present the final radiographic, clinical, and safety outcomes of the head-to-head 6-month monotherapy investigation. **Conclusions:** Mycophenolate mofetil is a unique oral immunomodulator of potential benefit for patients with relapsing forms of MS.

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P48

Clinical effect of the neuroprotectant MN-166 in relapsing multiple sclerosis: year 2 data

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Background: MN-166 (ibudilast) is an orally administered small molecule with neuroprotectant and anti-inflammatory properties. In year 1 of this 2-year study (Drulovic et al., ECTRIMS 2008), MN-166 at 60mg/day significantly reduced percent brain volume (PBV) loss and prolonged time-to-first relapse by 157 days ($p=0.04$), but did not significantly reduce cumulative new lesion count, the primary study endpoint, in relapsing-remitting multiple sclerosis (RRMS) patients. MN-166 produced a dose-related reduction in change of new inflammatory lesions to PBH ($p=0.01$; Gammans et al., ENS 2008). **Objective:** To evaluate the clinical effects and safety of MN-166 in RMS patients over 2 years. **Methods:** RRMS (93%) or SPMS with relapses (7%) patients and ≥ 1 T1 Gd-lesion were randomized to placebo (PBO, $n=100$) or MN-166 at 30 ($n=94$) or 60mg/day ($n=98$); for year 2 PBO patients were pre-assigned to MN-166 at 30 ($n=49$) or 60mg/day ($n=51$). Clinical and magnetic resonance imaging (MRI) evaluations were every 2 months for 2 years. **Results:** Two hundred and sixty four (96% of PBO - 30mg/day, 97% of PBO - 60mg/day, 82% of 30mg/day and 87% of 60mg/day) patients entered year 2. Significantly fewer patients treated with MN-166 for 2 years at 30 or 60mg/day than patients treated with PBO followed by MN-166 at either dose for 1 year had a sustained disability progression ($+1$ point on Extended Disability Status Scale - EDSS - for ≥ 4 months) (2 year MN-166 10.4%, PBO to MN-166 21%, $p=0.03$). MN-166 at 60mg/day for 2 years significantly ($p=0.04$) attenuated loss in PBV compared with the remaining groups. MN-166 was well tolerated at either dose. Three of 85 MN-166 60mg/day patients discontinued in year 2 for adverse effects compared with none in the other groups. Only GI events were more frequent in year 2 on MN-166 at 60mg/day (11%) and 30mg/day (4.2%) PBO-to-60mg/day (2%) PBO-to-30mg/day (0%). Depression was reported in 5% of the 60mg/day and 3% of the PBO-to-60mg/day patients towards the end of the second year of the study. **Conclusions:** The findings in this 2-year study on three independent measures - reduced disability progression (50%), reduced conversion of inflammatory lesions to PBH (37%) and reduced PBV loss - suggest that MN-166's clinical benefit results predominantly from protecting neurons from damage. These data suggest that MN-166 reduces progression of MS.

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P49

Three pleiotropic acting recombinant interferon beta agents display pharmacologically varying RNA expression over time

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Background: Three beta interferon products are available for multiple sclerosis (MS) treatment. They differ according to formulation, route of administration and dosage regimens. Large-scale analysis of peripheral blood gene transcripts should be stratified for the respective drug. **Objective:** To compare and integrate longitudinal gene expression profiles derived from PBMCs of relapsing-remitting MS (RRMS) patients that received distinct IFN β therapies. **Methods:** We monitored (microarray technology) 59 RRMS patients receiving IFN β 1a sc ($n=12$), im ($n=22$) or 1b sc ($n=25$) over a period of 2 years. Ficoll-treated blood samples were processed before first treatment (t_0) and correspondingly before upcoming injections after 2 days (t_1) - 1 week, respectively - 4 weeks (t_2), 1 year (t_3) and 2 years (t_4). Transcriptome analyses were realized including data curation by MASS.0 (Absent/No change calls), probeset quality by GeneAnnot (specificity and sensitivity) and a self-made algorithm (intensity-

dependent fold change criterion) as well as available R software packages (paired t-test, PCA). Gene regulatory network inference and transcription factor binding site identification (TRANSFAC, JASPAR) analyses are underway. **Results:** At t_1 , of 383 filtered genes, Fc fragment of IgE (FCER1A) was uniformly down-regulated; increased were monocyte related CD163, acyl-CoA synthetase (ACSL1), and free fatty acid receptor (FFAR2). Otherwise, 30 differentially expressed genes were shared by 1b sc and 1a sc treated individuals, mainly IFN-inducible genes. Five hundred and forty five filtered genes at t_2 contained 120 genes associated both with 1b sc and with 1a sc; significant reduction of cell cycle gene CDKN1C was detected in 1a im treated patients exclusively. At t_3 , of 308 interesting genes intersection between all three products revealed XAF1, IFI44, IFI44L, LY6E, OAS1, and PLSCR1. Interestingly, after 2 years, of 454 significantly regulated genes more were shared between 1b sc and 1a im ($n=24$) than 1b sc and 1a sc ($n=13$). For all three formulations, upregulated IFI44 and CD20 antigen-like 1 (MS4A4A) were assigned. **Conclusions:** Evident discrepancies between IFN β formulations should be considered when pooling data for meta analyses.

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P50

Safety profile of BG00012, an oral formulation of dimethyl fumarate for patients with relapsing MS

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Background: BG00012 is an oral formulation of dimethyl fumarate that is currently in phase 3 studies for the treatment of relapsing multiple sclerosis (MS). **Objective:** To present the current safety profile of BG00012. **Methods:** In a phase 1 QTC study, healthy volunteers were randomized to receive single oral doses each of BG00012 240mg, BG00012 360mg, placebo, or moxifloxacin 400mg in one of four treatment sequences. The effect of BG00012 on the QTc interval was assessed. In a phase 2b study, patients with relapsing MS were randomized to receive BG00012 120mg QD, 120mg TID, 240mg TID, or placebo during a 24-week treatment period. During a 24-week, dose-blinded safety extension, placebo patients were transitioned to BG00012 240mg TID and all other patients maintained their original BG00012 dosing regimen. AEs and laboratory values were assessed. **Results:** In the QTC study, time-matched analysis results presented means of ~ 10 msec for the assay sensitivity arm (moxifloxacin) whereas no timepoints crossed the 10msec upper boundary for any BG00012 dosage group, defining a negative study with respect to QTc prolongation. During the placebo-controlled phase 2b study period, AEs significantly more common in patients receiving BG00012 240mg TID vs. placebo were abdominal pain ($P<0.029$), hot flush ($P<0.013$), and flushing ($P<0.000$). MS relapse was the only serious AE reported in >1 patient. Infections were reported in 34% of both BG00012-treated and placebo patients. Hematology shifts were minor and clinically significant anemia or neutropenia did not occur. The safety profile during the phase 2b safety extension was similar. Overall, the frequency of gastrointestinal events and flushing in BG00012-treated patients decreased from month 1 to month 6 of treatment (52.4% to 4.0% and 66.5% to 4.7%, respectively). **Conclusions:** BG00012 has demonstrated a favorable safety profile in clinical studies to date. The overall tolerability of BG00012 improves with continued dosing.

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P51

Association of interferon beta and inosine in relapsing-remitting multiple sclerosis (ASIMS): a multi-centre, randomized, double-blind, placebo-controlled phase II (proof of concept) trial in 157 patients

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Background: Neuronal and axonal loss occurs early in relapsing-remitting multiple sclerosis (RRMS) and is mediated by inflammatory processes as well as by oxidative stress and excitotoxicity. Effective anti-inflammatory drugs are available but the development of neuroprotective agents remains a challenge. Experimental and clinical observations suggest that uric acid (UA), a natural antioxidant, has a neuroprotective effect in EAE and possibly in MS. Oral administration of inosine, a precursor of UA, increases serum and CSF UA levels in MS patients and is well tolerated. **Objective:** Combination therapy with an anti-inflammatory drug (IFN β) and a potentially neuroprotective agent (inosine) might delay disability progression. **Methods:** Patients with RRMS who had been on IFN β for at least 6 months at baseline were randomized to IFN β + inosine (n=79) or IFN β + placebo (n=78) for 2 years. The two groups were not significantly different in terms of age (~37 years), disease duration (~6.2 years), mean EDSS (~2.3) and annual relapse rate (~0.8). The dose of inosine was adjusted to maintain serum UA levels in the range of asymptomatic hyperuricemia (females \leq 8mg/dL; males \leq 9mg/dL). Expanded Disability Status Scale (EDSS) and MSFC components were assessed every 3 months by a blinded evaluating physician. The primary end points were: percentage of patients with progression of disability (EDSS+1 confirmed at 3 months), time to sustained progression (Kaplan-Meier analysis) and mean EDSS. **Results:** The combination of IFN β and inosine was safe and well tolerated. Urinary lithiasis occurred in 3.8% of treated patients and 1.3% of controls. No change in blood pressure was noted in patients with induced asymptomatic hyperuricemia. At 24 months no significant differences were observed between the two groups in any of the primary end points. **Conclusions:** Combination therapy with IFN β and inosine is not superior to IFN β monotherapy. Potential reasons will be discussed. Inosine effects might be masked by those of IFN β and the negative results of this combination therapy do not rule out a possible benefit of UA in naive patients.

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P52

Early treatment with interferon beta-1b associated with improved long-term outcome in multiple sclerosis

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Background: There seems to be a relationship between how early disease-modifying treatment (DMT) is started in multiple sclerosis (MS) patients and the effectiveness of therapy. **Objective:** This study used data from the 16-year Long-Term Follow-up study of interferon beta-1b (IFN β -1b; Betaseron®) to investigate the relationship between timing of drug-exposure and long-term outcomes (Expanded Disability Status Scale [EDSS] score=6, secondary progressive MS, wheelchair requirement, or death). **Methods:** For this analysis, drug exposure was

measured as the medication-possession-ratio (MPR), defined as the actual time that a patient received therapy divided by the total time possible before an undesirable outcome was reached (or at data-censor). The MPR was adjusted up or down according to 161 different weighting schemes based on the EDSS score and the disease-duration at treatment-onset. A recursive partitioning algorithm was then used to optimally divide patients into groups with 'more' or 'less' exposure and to determine whether exposure group was associated with long-term outcome. Propensity-score adjustment was used to reduce bias for group-comparisons and multiple sensitivity-analyses were run using different definitions of long-term outcome and alternative statistical adjustment methods. **Results:** In each of these analyses, the same weighting scheme was consistently selected by the recursive partitioning algorithm. This scheme was one that reduced (down-weighted) the effective MPR either for increasing disease duration or for increasing EDSS at treatment-onset. For example, if treatment was started 2 years after onset and at EDSS score=1.5, the MPR would be reduced (by the selected weighting scheme) to only 60% of its measured value. Applying this scheme and propensity scoring, the group with 'more' exposure had a lower risk of reaching an undesirable clinical outcome compared with the group with 'less' exposure (Cox hazard ratio=0.420, 95% confidence limits=0.277-0.636, P<0.001). **Conclusions:** These results suggest that early initiation of DMT therapy with IFN β -1b has a greater impact on long-term outcome than delayed therapy.

Supported by: Bayer Schering Pharma AG.

P53

Identification and characterization of mefloquine efficacy against JC virus in vitro

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Background: Progressive multifocal leukoencephalitis (PML) is a rare but frequently fatal disease caused by uncontrolled replication of JC polyomavirus (JCV) in the brain of some immunocompromised individuals. Currently, no effective anti-viral treatment for this disease has been identified. Therefore, further search for therapeutics that target JCV directly is necessary. **Objective:** To screen SPECTRUM collection of 2000 approved drugs and biologically active molecules for anti-JCV activity. **Methods:** We used an in vitro infection model of JC virus susceptible human cells with JC virus to look for drugs that effectively inhibited active virus production and to characterize mefloquine activity. **Results:** We identified a number of different drugs and compounds with significant anti-JCV activity at micromolar concentrations and no toxicity to the cells. Of the compounds with anti-JCV activity only mefloquine has been reported to show sufficiently high CNS penetration such that it would be predicted to achieve efficacious concentrations in the brain; hence, we studied it in more detail. Our in vitro experiments have demonstrated mefloquine inhibition of the viral infection rate by two different JCV strains, Mad4 and Mad1/SVE Δ , in two different cell types, human glial cell line (SVG-A) and primary human astrocytes, with an IC₅₀ of ~4 μ M. Mefloquine is similarly effective in the inhibition of JCV infection rate even when added to the culture system 24 hours after infection of cells with the virus, suggesting that it inhibits the virus in previously infected cells. Using qPCR to quantify the number of viral copies in the culture, we have also shown that mefloquine inhibits viral DNA replication. Both (+) and (-) enantiomers of mefloquine racemate were similarly potent in inhibiting JCV infection in the JCV inhibition assay. Mefloquine is approved for use as an antimalarial agent for prophylaxis and treatment of mild to moderate acute malaria. **Conclusions:** Although no animal model of PML or JCV infection is available to test mefloquine in vivo, our in vitro results combined with the published biodistribution literature suggest that mefloquine could be an effective PML therapy.

P54

Assessment of the utility of the Canadian Treatment Optimization Recommendations (CanTOR) in the routine care of multiple sclerosis

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Background: Clinicians lack evidence-based criteria for defining sub-optimal responses to disease modifying drugs (DMDs). The Canadian Multiple Sclerosis Working Group developed the Treatment Optimization Recommendations (TOR) so neurologists could assess the status of patients on DMDs and decide when it may be necessary to modify treatment. The relevance and utility of these recommendations in clinical practice have yet to be studied prospectively. **Objective:** To evaluate the utility of the TOR to the DMD treatment optimization process in the clinical setting and to determine the proportion of subjects on DMD treatment meeting a medium or high level of concern according to the TOR, who might therefore warrant a change in treatment. **Methods:** This is a Canadian observational, multi-center, phase IV study of patients with relapsing-remitting multiple sclerosis or clinically isolated syndrome. The focus was on non-academic office practices. All subjects were assessed according to the TOR by their neurologist at baseline (representing the response to treatment over the previous year) and will be reassessed over a prospective 12-month observation period. **Results:** At this time baseline results are available for 110 of 193 subjects. Baseline data for all 193 subjects will be presented in the poster. Twenty-three (20.9%) patients presented medium or high levels of concerns with respect to relapse outcomes, five (4.6%) with respect to progression outcomes and two (1.8%) with respect to MRI outcomes. Optimization was recommended based on the TOR in eight (7.3%) patients and was consistent with management by the physician in three patients. The TOR was felt by the investigators to be useful in the majority of patients with a medium or high level of concern. **Conclusions:** Neurologists found the Canadian TOR useful in facilitating treatment decision-making, especially in patients with medium or high levels of concern. **Supported by:** EMD Serono.

P55

Preferential effects of cladribine on lymphocyte subpopulations

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Background: Cladribine (2-chloro-2'-deoxyadenosine), a synthetic chlorinated analog of deoxyadenosine used effectively to treat hairy cell leukemia and lymphoma, has been assessed in its parenteral form as treatment for multiple sclerosis (MS) in three randomized, double-blind trials. Based on promising results in these trials, an oral formulation has been developed. **Objective:** A re-analysis of data from the MS-001 study on the effect of cladribine on lymphocyte subpopulations is presented to improve understanding of the pharmacodynamic effects of cladribine in MS. **Methods:** The study enrolled 159 patients with primary or secondary progressive MS. Patients received six monthly, 5-day courses of cladribine, administered subcutaneously (sc) at total doses of 0.7mg/kg (n=53) or 2.1mg/kg (n=52), or placebo (n=54). CD3+ (T cell), CD4+ (helper T cell), CD8+ (cytotoxic T cell), CD19+ (B cell), and CD16+/CD56+ double-positive (natural killer) cell counts were measured at baseline and at monthly intervals throughout the 12-month, double-blind phase. **Results:** The most pronounced and sustained effect of cladribine (0.7 or 2.1mg/kg) was a dose-dependent reduction in the mean CD4+ T-cell count from baseline to final evaluation. Thirty-one patients in the cladribine 2.1mg/kg group had CD4+ cell counts of $\leq 0.20 \times 10^9/L$ (n=31), compared with five receiving cladribine 0.7mg/kg and one receiving

placebo. Cladribine treatment was associated with less marked dose-dependent reductions in mean CD8+ T-cell counts than in CD4+ T-cell counts. A dose-dependent reduction of the CD4:CD8 ratio was observed in cladribine-treated patients, compared with a slight increase in placebo-treated patients. During the first 2–7 months of cladribine treatment, mean CD19+ and CD16+/CD56+ lymphocyte counts decreased, recovering to baseline after 10 or 7 months, respectively. **Conclusions:** Cladribine caused a preferential and sustained depletion of CD4+ T cells, less pronounced dose-dependent reductions in CD8+ T cells, and smaller dose-dependent reductions in CD19+ and CD16+/CD56+ lymphocytes.

Supported by: Analyses supported by Merck Serono International S.A., Geneva, Switzerland (an affiliate of Merck KGaA, Darmstadt, Germany).

P56

Investigation of the effect of early initiation of therapy with interferon beta-1a (i.m.) on disease course, quality of life and cognition in patients with relapsing-remitting multiple sclerosis

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Background: Results of the data collection 'Frühe Initiale Therapie bei MS' ('Early Initial Therapy in MS'). **Objective:** This prospective data collection was intended to observe the course of disease of patients with a first or maximally second demyelinating event who underwent early therapy with interferon beta-1a i.m. for at least 12 months. **Methods:** A total of 231 patients who had experienced a first demyelinating event or patients who had experienced at most a second relapse received interferon beta-1a i.m. once weekly. Five documentations were performed over a period of 12 months (at baseline, after 3, 6, 9 and 12 months). Besides the evaluation of the number of relapses, assessment included Expanded Disability Status Scale (EDSS) and quality of life by using the EuroQol-questionnaire (EQ-5D). In addition, cognitive abilities and extent of fatigue, the latter by MUSIC-Test (Multiple Sclerosis inventory Cognition), were evaluated at baseline and after 6 and 12 months. **Results:** Data of 231 patients (28.7% men, 71.3% women, mean age 35.6 years) were collected and evaluated. Mean EDSS score at treatment initiation was 1.76. The first demyelinating event was characterized by paraesthesias in 56% and by vision disturbances in 36.4% of the patients. After 12 months of interferon beta-1a i.m. therapy EDSS score remained constant, as was expected, and nearly 70% of the patients had no relapse since initiation of therapy. A positive effect of interferon beta-1a i.m. on the cognitive abilities could be shown by the MUSIC-Test: the percentage of patients with normal cognitive functions relatively increased by approximately 20% after 12 months of treatment. **Conclusions:** This prospective data collection has demonstrated that two-thirds of patients who received early therapy with interferon beta-1a i.m. had no relapse within 1 year after treatment initiation. In addition, therapy had a positive influence on cognitive abilities. These results underline the relevance of an early immunomodulatory therapy after the first relapse in daily practice.

Supported by: Biogen Idec GmbH.

P57

Long-term follow-up of immunomodulatory therapies in early relapsing-remitting multiple sclerosis

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Background: Immunomodulatory therapies (IMTs) represent the first line therapeutic options for the treatment of relapsing-remitting multiple sclerosis (RRMS). Comparative data on efficacy, tolerability and patient adherence are needed for recommending initial treatment. **Objective:** The objective of this study was to evaluate the long-term efficacy and patient adherence of four IMTs in daily clinical practice over 8 years. **Methods:** This follow-up analysis was based on a prospectively organized clinical database, including 285 patients treated with beta-interferons (INFBeta-1a i.m. (Avonex), INFBeta-1b

s.c. (Betaferon), INFBeta-1a 22µg s.c. (Rebif), and glatiramer acetate (Copaxone) for 8 years. Adherence to initial treatment was analyzed by Kaplan-Meier Survival Analysis. Additional efficacy parameters were annual relapse rates, and progression of the disease. **Results:** After 8 years, 20% of the 285 patients were still on the initial treatment (glatiramer acetate 29.1%, INFBeta-1a i.m. 13.8%, INFBeta-1b s.c. 18.2% and INFBeta-1a 22µg s.c. 18.4%). It was shown by Kaplan-Meier Analysis that adherence to IMT treatment was significantly higher in the glatiramer acetate group, compared with all other groups. The mean survival time under glatiramer acetate was 53 months, compared with INFBeta-1a i.m. (42 months), INFBeta-1b s.c. (44 months) and INFBeta-1a 22µg s.c. (43 months). Annual relapse rates after 8 years were significantly lower compared with the annual relapse rates of the 2 years prior to the study in all treatment groups. The number of patients with progression was lowest, although not significantly in the glatiramer acetate group (16%) compared with the other IMTs. **Conclusions:** Kaplan-Meier Analyses confirmed that significantly more patients adhered to glatiramer acetate than to any other treatment regimen and that the time of adherence was longest under glatiramer acetate treatment. Relapse rates and freedom from progression remained on favorable levels in all treatment groups.

P58

Long-term maintenance treatment with glatiramer acetate after mitoxantrone in rapidly progressive multiple sclerosis

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Background: Several clinical trials proved the efficacy of mitoxantrone (MTX), an immunosuppressive agent, to reduce the relapse rate and delay the progression of disease in patients with secondary progressive multiple sclerosis (SPMS), progressive relapsing MS (PRSM) or worsening relapsing-remitting MS (WRRMS). **Objective:** Since MTX has been used to treat patient 'non responders' to previous interferon beta (INFB) therapy, we employed glatiramer acetate (GA) as an alternative to maintain the disease stability gained by MTX. **Methods:** Twelve patients (six WRRMS and six PRMS, two males and ten females, mean age 47.4 years), were treated with MTX every 3 months up to a maximum cumulative dose of 140mg/m² (mean treatment period of 17.4 months). After MTX, 6 patients received maintenance therapy with GA (20mg/day subcutaneously), while the others received no treatment. At 24 months follow-up since the last administration of MTX, we compared pre- and post-treatment annualized relapse rate (ARR), Expanded Disability Status Scale (EDSS) and brain magnetic resonance imaging (MRI) in two groups. **Results:** After 2 years follow-up, in patients treated with maintenance GA therapy, the mean ARR was 2.8 (SD: 1.32) during the 2 years before MTX, and 0.2 (SD: 0.48) during GA (p=0.009). The EDSS pre-treatment (mean: 5.4; SD: 1.7) also decreased by 1 point (mean: 4.4; SD 1.57; p=0.34). Moreover in these patients no increased lesion load was identified on brain MRI. In the non-treated group, the ARR-pre-treatment (mean:1.1; S.D: 0.9) was not significantly reduced (mean:0.25; S.D. 0.4; p=0.1), while the mean EDSS was stable (p=0.8). Gadolinium enhancing lesions were identified on brain MRI in two patients. No unexpected side-effects were encountered. **Conclusions:** GA following immunosuppression with MTX could provide effective and well-tolerated treatment for rapidly progressive MS patients and may induce disease stability.

P59

Feasibility of using autologous neural progenitors as a regenerative strategy in multiple sclerosis

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Background: In multiple sclerosis (MS) there is a need to develop therapies that induce neural repair and regeneration to reverse established disability. Use of autologous stem cells to induce nerve and

myelin growth is one option under investigation. **Objective:** We characterized mesenchymal stem cells (MSC)-derived neural progenitors (NPs) to establish their normalcy in MS patients and their differentiation potential; a prerequisite for consideration of using these cells as a repair strategy in MS. **Methods:** We obtained MSCs from bone marrow aspirates from 20 MS subjects by informed IRB-approved consent. MSCs were purified from hematopoietic cells by culture and adherent properties and further characterized by morphology, and surface antigen expression. The differentiation potential of MSCs into adipocytes, osteoblasts, and chondroblasts was verified. Following expansion of MSCs, cells were induced into NPs by growth in neural progenitor maintenance media. NP induction was verified by protein (immunofluorescence) and gene expression (real-time quantitative PCR using gene-specific markers) of nestin, neurofilament M, GFAP, CXCR4 (NP markers) and vimentin and smooth muscle actin (MSC markers). NPs and MSCs are also compared by FACS for homogeneity. Long-term viability and safety of the MSC is verified by maintenance in culture with several passages and karyotype analysis. All experiments with MS patient aspirates are compared from samples obtained from healthy donors. MSCs and NPs are compared for their ability to differentiate into specific neural lineages. **Results:** NPs from MS patients and controls exhibit characteristic neurosphere morphology and express increased levels of neural progenitor genes Nestin, Neurofilament-M, GFAP, and CXCR4, and decreased levels of MSC-associated genes. FACS analysis confirmed that NPs represent a more homogenous cell population than MSCs. NPs, but not MSCs, are capable of in vitro differentiation into oligodendroglial (O4+) or neuronal (B3-tubulin+) cell types. Long-term passage of these cells is not associated with mutations. **Conclusions:** NPs derived from autologous bone marrow are a viable option for consideration in regenerative therapies in MS.

P60

Risk tolerance in multiple sclerosis patients: a re-survey to assess stability and change

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Background: Progress in multiple sclerosis (MS) therapeutics is leading to effective, but risky, therapies. To appropriately use risky therapies, clinicians need to understand how patients tolerate risk and how this tolerance shifts over time. **Objective:** To assess stability of risk tolerance (RT) over time and identify factors which influence RT in MS patients. **Methods:** A follow-up telephone survey of 128 MS patients who took part in a previous survey of RT was administered. Patients were given three scenarios to assess RT: a miracle drug which would cure MS, the same drug after a year with MS disease progression, and natalizumab. Demographic, disability, MS therapy and disease activity data were collected. **Results:** One hundred and five of the original 128 patients (82%) were re-surveyed after 18 months. Median RT for each scenario remained unchanged except for miracle drug after progression, where median RT changed from 1:12 to 1:50. In each scenario, RT was stable in approximately 25% of patients, with the remainder evenly split between increased or decreased RT. Defining actual natalizumab risk according to the estimated 1:1000 risk of progressive multifocal leukoencephalopathy, RT scenarios defined 13 patients (12%) as tolerant to this risk who were initially intolerant. Conversely, 18 patients (17%) previously tolerant were now intolerant. Median RT in the 22 patients who received natalizumab since its 2006 re-release was higher than those who did not receive natalizumab. Five natalizumab-treated patients (22.7%) had been intolerant of natalizumab risk (1:1000) at baseline, but had shifted to tolerant at follow-up. Two patients treated with natalizumab reported intolerance of 1:1000 natalizumab risk; both patients also reported adverse reactions and were no longer on the treatment. **Conclusions:** Patient-reported RT changes over time, with some patients becoming tolerant of medications to which they had previously reported intolerance. Patient experience with a medication (both positive and negative) may also impact reported RT to medication. A better understanding of the factors that alter MS patient RT over time will be useful to clinicians using therapies with potentially serious complications.

P61

Phase 2 follow-up results of the BHT-3009 DNA vaccine for multiple sclerosis

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Background: BHT-3009 is a tolerizing DNA vaccine for multiple sclerosis (MS), encoding full-length human myelin basic protein. In a 48-week phase 2 trial of relapsing-remitting MS patients (RRMS), BHT-3009 was shown to cause antigen-specific immune tolerance and to reduce the number of gadolinium (Gd) enhancing lesions on brain magnetic resonance imaging (MRI) in patients with high CSF IgG levels at baseline. **Objective:** The objective of the current follow-up study is to evaluate BHT-3009's durability of effect. **Methods:** Two hundred and eighty-nine RRMS patients were initially randomized to placebo, 0.5mg BHT-3009, or 1.5mg BHT-3009, given approximately every 4 weeks until week 44. Patients who received BHT-3009, but not patients who received placebo, were enrolled into a follow-up observational study. Eight months after the last dose of study drug, brain MRI with Gd and evaluation for clinical relapses were performed. **Results:** Ninety-five of the 104 patients (91.3%) randomized to 0.5mg BHT-3009 and 83 of 87 patients (95.4%) randomized to 1.5mg BHT-3009 participated in the follow-up study. The expected number of Gd-enhancing lesions and relapse rates were calculated based on the rate of lesions and relapses observed during the phase 2 trial. The expected number of new Gd-enhancing lesions per MRI was 1.003, but the observed number was 0.731 for all patients (27% less than expected). The expected annualized relapse rates were 0.46 and 0.61 on 0.5mg and 1.5mg of BHT-3009, respectively. The observed annualized relapse rates during the follow-up period were 0.17 (63% less than expected) and 0.38 (38% less than expected) on 0.5mg and 1.5mg of BHT-3009, respectively. Further, there was no evidence of delayed adverse events, and BHT-3009 continues to demonstrate an excellent safety profile. **Conclusions:** Follow-up data at 8 months after the last dose of BHT-3009 suggest a durable effect of the DNA vaccine with fewer Gd-enhancing lesions and relapses than expected. **Supported by:** Bayhill Therapeutics.

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Natalizumab increases the proportion of disease-free patients in relapsing multiple sclerosis

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Background: As more effective therapies become available for the treatment of relapsing multiple sclerosis (MS), there may be the potential for these therapies to induce a disease-free state. **Objective:** Analyses of data from the pivotal phase 3 AFFIRM study of natalizumab monotherapy were performed to evaluate the proportions of disease-free patients over 2 years based on clinical and magnetic resonance imaging (MRI) criteria. **Methods:** Patients were considered clinically disease free if they had no relapses and no disability progression sustained for 12 weeks, and free of MRI disease

activity if they had no gadolinium-enhancing (Gd+) lesions and no new or enlarging T2-hyperintense lesions. A more rigorous definition of disease free combined the clinical and MRI criteria. A subgroup analysis compared outcomes in patients with highly active (≥ 2 relapses and ≥ 1 Gd+ lesions) and non-highly active disease at baseline. **Results:** Over 2 years, more patients were without relapses (70.6% vs. 43.3%, $P < 0.0001$) and did not experience disability progression sustained for 12 weeks (83.6% vs. 71.7%, $P < 0.0001$) in the natalizumab group compared with placebo. Moreover, the proportions of patients with no Gd+ lesions (94.9% vs. 56.6%, $P < 0.0001$) or no new or enlarging T2-hyperintense lesions (58.3% vs. 14.9%, $P < 0.0001$) were greater in the natalizumab group. In addition, natalizumab significantly increased the proportions of disease-free patients according to clinical (64.3% vs. 38.9%, $P < 0.0001$), MRI (57.7% vs. 14.2%, $P < 0.0001$), and combined clinical and MRI criteria (36.7% vs. 7.2%, $P < 0.0001$). Natalizumab also increased the proportion of disease-free patients according to the combined clinical and MRI criteria in subgroups of patients with highly active (27.4% vs. 1.7%, $P < 0.0001$) or non-highly active (39.6% vs. 8.6%, $P < 0.0001$) disease at baseline. **Conclusions:** Natalizumab increased the proportion of disease-free patients defined according to clinical and MRI criteria and the combination of these criteria, irrespective of baseline disease activity.

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P63

Acute myelogenous leukemia in MS patients with sequential treatment by mitoxantrone and azathioprine: is there a synergistic or additive effect?

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Background: Mitoxantrone (MTX) is used to treat cancer and acute leukemia. MTX is indicated for worsening relapsing and secondary progressive multiple sclerosis (MS). Therapy-related acute leukemia (TRAL) has been reported in MS patients with an incidence of 0.07%. TRAL following MTX has a short latency period (2 years), acute onset and is associated with cytogenetic abnormalities such as translocations of chromosome bands 11q23 and 21q22. An increased risk of cancer has been reported with azathioprine (AZA). Treatment duration and cumulative dose increase the risk. TRAL induced by AZA is preceded by a long lasting period of preleukemic state associated with cytogenetic abnormalities including deletions or losses of chromosomes 5 and 7. They are resistant to anti-leukaemic therapy. **Objective:** To report a 48-year-old MS patient who developed acute myelogenous leukemia after mitoxantrone and azathioprine therapy. **Methods:** Case report. **Results:** In 1991, this woman developed relapsing-remitting MS (RRMS). She had 10 relapses in the first 4 years of the disease and Expanded Disability Status Scale (EDSS) score was 3.5. INFB-1b was introduced in November 1995 but she had three severe relapses and EDSS score was 7.0 in June 1996 and IFNB-1b was stopped. From July 1996 to April 1997 she received MTX (120mg). EDSS score improved (6.0) and AZA 150mg/day was introduced. From 1997 to 2007 she had only one relapse but the disease became progressive in 2001. In November 2007 blood tests disclosed pancytopenia. A bone marrow biopsy showed an increased cellularity with blasts. Karyotype was 46, XX (7)/45, XX, -7. A diagnosis of therapy-related acute myeloblastic leukemia type II according to the French American British classification was made. AZA was interrupted. She received induction chemotherapy followed by allogenic transplant. At day 86 she developed a GVHD involving the gut. At day 105 a relapse was observed on a new bone marrow aspirate. The patient declined any new treatment and died in November 2007. **Conclusions:** Leukemia in this case fulfilled criteria of TRAL induced by alkylating agents. The fact that the patient first received MTX is suggestive of a synergistic or additive effect.

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Long-term follow-up study of very active multiple sclerosis (MS) patients under interferon beta-1b therapy

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Background: Interferon beta-1b (IFNB-1b; Betaferon®) has demonstrated efficacy and safety in relapsing-remitting MS (RRMS). It was the first approved interferon in France for RRMS with ≥ 2 relapses in the last 2 years. Impact of IFNB-1b on very active MS (VAMS) has not been studied. The 12-year long-term follow-up (LTF) French cohort ATU of IFNB-1b will evaluate the long-term effect of IFNB-1b on clinical parameters in a cohort of patients with VAMS. **Objective:** To assess the clinical status MS patients included in the French cohort ATU of IFNB-1b after 12-year follow-up. **Methods:** This study is a multi-center, open-label, observational study that evaluates outcomes in patients included in the French cohort of ATU between July 1995 and June 1996. ATU is an administrative procedure aimed to propose treatment to patients before definite agreement by the French health regulatory agency. During this period, inclusion criteria for IFNB-1b were: 1) RR clinically definite MS; 2) ≥ 3 relapses with objective sequelae confirmed by a neurologist in the last 12 months; 3) Expanded Disability Status Scale (EDSS) score: $3.0 \leq \text{EDSS} \leq 6.0$; 4) Age: 18–50 years; 5) MS onset <10 years; 6) Abnormal magnetic resonance imaging (MRI). When a neurologist wanted to treat a patient he sent the medical record to a selection committee of 12 members, who checked the inclusion criteria and allowed the delivery of IFNB-1b. All the patients were followed by a neurologist for safety end efficacy every month for the first 3 months and then every 3 months. The procedure of the ATU ended when IFNB-1b was marketed in August 1996. All the patients included in the cohort ATU were identified. Their medical history was reviewed to collect the following information: duration of treatment, reason for treatment interruption, survival, course of the disease (relapsing-remitting or secondary progressive), relapse rate, EDSS score. **Results:** Two hundred and thirty seven MS patients were included in the cohort. At inclusion, mean age was 34 years, mean MS duration was 5 years, mean relapse rate was 3.5; median EDSS was 4.0. Follow-up data will be presented at the meeting. **Conclusions:** The French cohort ATU is the first LTF of VAMS patients under IFNB-1b.

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Immunomodulatory effects on postpartum relapse rate in patients with multiple sclerosis: a comparison between three groups

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Background: Multiple sclerosis (MS) often affects young women during a period of life when they desire children. Whereas the annual relapse rate (ARR) decreases continuously during pregnancy, after birth the ARR increases significantly. Little is known about satisfactory postpartum relapse prevention and the effects of early reinitiation of immunomodulatory treatment (DMT) with IFN or GLAT. Recently the GAMPP Study postulated that intravenous immunoglobulins (IVIG) could have a beneficial effect on postpartum ARR, but they did not provide any placebo arm. **Objective:** We aimed to investigate the effects of IVIG, DMT or non-treatment on the postpartum RR. **Methods:** We identified out of our pregnancy database MS patients, who were treated with IVIG postpartum, or where an immunomodulatory treatment was initiated shortly after birth. The control group was a postpartum DMT naïve collective out of the database, matched for disease activity before and during pregnancy with the IVIG group to allow a better comparison. **Results:** We followed 124 pregnancies in total. The ARR was continuously and significantly decreasing during pregnancy over all groups, most distinctly in the third trimester ($F(5,600)=9.35$; $p<.001$), to increase significantly after birth. In the

first trimester postpartum 8 (15.7%) of the IVIG group, 17 (30%) of the control group and 4 (18%) of the DMT group had an exacerbation. The differences between the postpartum relapse rates was lowest in the IVIG treated patients but did not reach statistical significance ($F(10,590)=0.55$ $p=0.853$). **Conclusions:** Our results confirm the known reduced relapse rate during pregnancy, which is followed by increased relapse rate after birth. Unfortunately they indicate that the common clinical practice of IVIG postpartum or early initiation of DMT in patients who do not breastfeed only results in a trend to reduce the RR. Faster acting therapeutic alternatives or combination therapies should be investigated further.

Supported by: The study was partly supported by Biogen Idec Germany, Biotest, Merck Serono and Teva/Sanofi Aventis.

P66

Antibodies to native myelin oligodendrocyte glycoprotein do not predict progression from clinically isolated syndrome to multiple sclerosis: complete analysis of the Betaferon® in Newly Emerging multiple sclerosis For Initial Treatment (BENEFIT) study

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Background: Previously, anti-MOG antibody testing was done by Western blot (WB) using a recombinant, extracellular MOG domain as target antigen and did not show any correlation. Here, we employed a high-sensitivity cell-based assay that allows detection of autoantibodies binding to the extracellular part of the native human MOG (nMOG) protein. **Objective:** To determine whether the presence of antibodies against native myelin oligodendrocyte glycoprotein (MOG) is related to earlier progression to clinically definite multiple sclerosis (CDMS) or advanced disability in patients with a first event suggestive of multiple sclerosis (MS) in the BENEFIT study. **Methods:** The BENEFIT study was designed to evaluate the impact of early versus delayed interferon beta-1b (IFNB-1b; Betaferon®) treatment in patients with a first event suggestive of MS. Patients were randomized to IFNB-1b 250µg or placebo, s.c. e.o.d., for 2 years or until developing CDMS. Patients then were eligible for a follow-up with IFNB-1b 250µg being offered for up to 5 years. Primary outcomes included time to CDMS and time to confirmed disability progression. Samples for anti-MOG antibody testing were taken at baseline and analyzed to identify any association between nMOG antibodies and the occurrence of CDMS and confirmed disability progression over 3 years. **Results:** Four hundred and ten patients' baseline samples were analyzed. Antibody titers did not correlate with time to CDMS or time to confirmed disability progression after 3 years. Even with this high-sensitivity nMOG assay, which appears to detect a different repertoire of antibodies to MOG than the conventional solid phase assay, we were unable to demonstrate any significant correlation. **Conclusions:** Quantitative analysis of antibodies to MOG or nMOG are of no prognostic value in terms of predicting progression to CDMS or time to confirmed disability progression. Further studies are underway to determine whether changes in nMOG antibody status are associated with clinical parameters during the course of MS. **Supported by:** Bayer Schering Pharma AG.

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Antibodies to aquaporin-4 in patients with a clinically isolated syndrome: analyses from the BENEFIT study (BETAferon® in Newly Emerging multiple sclerosis For Initial Treatment)

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Background: It is possible that some patients with a first event suggestive of multiple sclerosis (MS) (clinically isolated syndrome - CIS) turn out to have other demyelinating diseases such as neuromyelitis optica (NMO). **Objective:** To measure antibodies directed against aquaporin-4 (AQ4) in a large group of prospectively followed CIS patients. **Methods:** The BENEFIT study was designed to evaluate the impact of early versus delayed interferon beta-1b treatment in CIS patients with cerebral magnetic resonance imaging (MRI) findings suggestive of MS. Samples were evaluated for anti-AQ4 at baseline and at study year 1. AQ4 antibodies were determined by a commercial test for NMO IgG employing recombinant AQ4 in a radioimmunoprecipitation assay (RIPA). Selected samples were retested by a flow cytometry based bio-assay, utilizing a human astrocytoma cell line stably expressing AQ4 under native conditions [nativeAQ4]. **Results:** By RIPA, 383 baseline samples and 384 year 1 samples were analyzed. Four (1%) baseline and six (1.6%) year 1 samples met criteria for a 'positive test result' (AQ4 ratio ≥ 15 , threshold level for positivity as provided for this commercial assay); one patient had positive findings at baseline and year 1. Positive samples had anti-AQ4 ratios in the lower range (no anti-AQ4 ratio ≥ 22.0). None of the RIPA positive samples (and none of 18 randomly selected RIPA negative samples) were found positive for native AQ4 antibodies. Eight of the nine patients with positive RIPA findings had either optic or spinal symptoms at disease onset. Whether or not these patients meet further clinical/MRI criteria for NMO is currently under evaluation. **Conclusions:** Lower titers of antibodies to AQ4 can infrequently be detected in CIS by commercially available RIPA. These findings may represent a non-specific phenomenon, since they could not be confirmed by a cell-based assay detecting antibodies against nativeAQ4. Whether positive findings corresponded to the clinical/MRI phenotype of NMO will be reported.

Supported by: Bayer Schering Pharma AG.

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Electronic tool to optimize multiple sclerosis treatment

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Background: An electronic algorithm (e-Tool) has been developed to allow to automatize the recommendations of the CMSWG for optimizing MS treatment. **Objective:** To determine and quantify the potential clinical advantage of e-Tool versus clinical criteria (CC) to take decisions on treatment optimization in multiple sclerosis (MS). **Methods:** A cohort of 410 MS subjects from 22 centers is being surveyed. The survey consists in the application of the e-Tool we designed and the comparison of the resulting therapeutic recommendation versus the CC on the appropriateness to optimize ongoing treatment. The eTool is loaded with retrospective exacerbation number, magnetic resonance imaging (MRI) data, and current Expanded Disability Status Scale (EDSS) score. The relevant decision on treatment maintenance or change/optimization is up to the treating neurologist. No matter what the decision taken, subjects will be followed for 2 years to compare the disease evolution among the resulting groups. Specificity, sensitivity, and positive predictive value of the e-Tool will be determined. **Results:** Currently, the e-Tool has been

applied to a total of 410 subjects. EDSS score at e-Tool application was 0-3.5 points in 326 patients (80%) and above 3.5 in 50 (12%), data missed in 34 patients (8%). Mean duration from MS diagnosis to e-Tool application was 6.1+/-6.9 years. Distribution of patients in the main subsets according to clinical criteria and e-Tool results was: CC+ and eTool + 44/410 (10,7%), e-Tool + 116/410 (28,3%), CC-/eTool+ 72/410 (17,6%) being e-Tool - in 166/410 (40,5%). Out of the 116 patients where e-Tool was + and a treatment optimization was suggested, only 36 did change treatment (31%) whereas the percentage of changes when CC and e-Tool were coincident and positive increased to 88% (36/44). CC+ and eT- occurred in six cases and two of them optimized treatment. **Conclusions:** MS activity profile to trigger clinical criteria for treatment optimization is different from the one required by the e-Tool. Given the observed discrepancy, the disease evolution during the 2 years of follow-up will determine the predictive value and accuracy of the tool to detect suboptimal response in MS patients.

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P69

Breakthrough relapses after rituximab in NMO patients: incidence and other treatment options

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Background: Neuromyelitis optica (NMO) is a CNS inflammatory disease characterized by optic neuritis and longitudinally extensive myelitis (LEM). Two-thirds of the cases are relapsing-remitting. NMO is hypothesized to be caused in part by abnormal B cell response. Consistent with this concept, plasmapheresis and IVIG, treatments that decrease humoral responses, partially abrogate disease. Rituximab has significant efficacy in preventing relapses in NMO. This monoclonal antibody effectively depletes most B cells, including memory B cells. However, we find some patients continue to have relapses, sometimes soon after rituximab treatment. **Objective:** Describe the incidence of relapses within 3 months of rituximab treatment. **Methods:** Retrospective analysis of all patients in our NMO database. **Results:** Fifteen patients who had received rituximab treatment were identified. Four (27%) had a clinical relapse plus active MRI lesions within 2 months of rituximab infusion. CD19 counts were available for two patients, and were 0. Serum immunoglobulin levels (IgG, IgM, IgA) were available from one patient, which were within the normal range even though the CD19 count was 0, as expected. All four failures were treated with IV methylprednisolone and plasmapheresis. In two cases where there was significant cord edema after steroid and plasmapheresis treatment, one dose of cyclophosphamide (750mg/m2) resulted in further improvement. **Conclusions:** Rituximab may not be effective in all NMO patients. A significant number of patients may relapse soon after rituximab. This may be due to the differential effects of rituximab on B cells versus plasma cells, which continue to produce potentially pathogenic antibodies and are unaffected by rituximab. It may be judicious to prophylactically treat patients with plasmapheresis prior to and for a few months post rituximab treatment to potentially prevent early relapse.

P70

Benefit/risk evaluation of mitoxantrone and natalizumab in multiple sclerosis

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Background: Since the mid-1990s, four disease-modifying drugs (DMDs) have been approved for first-line treatment for relapsing-remitting multiple sclerosis (RRMS). After 2000, two rescue therapies, mitoxantrone and natalizumab, became available as second-line treatments. Mitoxantrone is approved for worsening forms of MS,

whereas, natalizumab is recommended in patients who have inadequate response to or cannot tolerate DMDs. The efficacy and risks of these therapies have not been adequately compared in MS. **Objective:** To evaluate the therapeutic benefit of two rescue therapies in MS using number needed to treat (NNT). **Methods:** Efficacy and safety data from the MIMS (mitoxantrone) and AFFIRM (natalizumab) studies were obtained from published literature and prescribing information. Therapeutic benefit for each agent was derived by calculating absolute risk reduction and NNT (1/absolute difference) for relapse, disability, and MRI. Safety data were analyzed descriptively. **Results:** Annualized relapse rate (ARR) was significantly reduced with mitoxantrone (0.35) versus placebo (1.02); $P=0.001$; NNT, 1.5, and with natalizumab (0.22) versus placebo (0.67); $P<0.001$; NNT, 2.2. Therapeutic benefit was also observed for progression-free, relapse-free, and Gd+ lesion-free measures. Serious adverse events (SAEs) were associated with both mitoxantrone and natalizumab including acute myeloid leukemia and PML, respectively. Mitoxantrone has an established long-term safety profile including the risk of cardiotoxicity. The long-term safety profile of natalizumab is not fully known. Both products carry black box warnings; appropriate monitoring for potential SAEs is strongly recommended. **Conclusions:** Both mitoxantrone and natalizumab were found to be effective based on therapeutic benefit in the MS populations studied. For mitoxantrone, the population studied was consistent with the approved indication. Natalizumab was studied in treatment-naïve RRMS population, but after reintroduction to the market following safety concerns, is recommended in patients who have not adequately responded to DMDs. The benefit and risks of these therapies, including the known and unknown long-term safety profiles, are important considerations for MS patients and practitioners.

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P71

Recurrent autoimmune thrombocytopenia after exposure to interferon beta-1a and natalizumab

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Background: Registry data revealed the possibility of interferon-induced thrombocytopenia, but no other case reports on natalizumab associated thrombocytopenia have been published. **Objective:** To describe a case of probable immune thrombocytopenia associated with treatment with natalizumab, a new disease-modifying therapy for relapsing-remitting multiple sclerosis (RRMS). Natalizumab is a humanized monoclonal antibody which binds to alpha(4)beta(1)-integrin. The patient had a previous history of low platelet count with interferon beta-1a therapy. **Methods:** Case presentation. **Results:** A 58-year-old woman presented with a severe infusion reaction (thrombocytopenic purpura) with her third treatment with natalizumab. She had a prior history of low platelet count ($99 \times 10^3/\text{mm}^3$), which improved ($119 \times 10^3/\text{mm}^3$) after discontinuation of weekly intramuscular injection of interferon beta-1a. After natalizumab, her platelet count dropped from $119 \times 10^3/\text{mm}^3$ to $66 \times 10^3/\text{mm}^3$ on post-treatment day 56. Laboratory testing revealed stable red blood cell count, hemoglobin, and hematocrit values; and an increased white blood cell count ($22 \times 10^3/\text{mm}^3$) that normalized after 48 hours. Antibodies against natalizumab were persistently positive after 6 months and were measured using an enzyme-linked immunosorbent assay (ELISA) method. Despite the immediate withdrawal of natalizumab, her platelet count further decreased to $62 \times 10^3/\text{mm}^3$. Bone marrow aspirate was normal. She was treated with intravenous and oral administration of steroids and two weeks later she received rituximab. Her platelet count returned to the normal level 60 days later. **Conclusions:** Natalizumab-associated immune thrombocytopenia case is reported here. Clinicians should be aware of this adverse event.

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Oral fingolimod (FTY720) in patients with relapsing multiple sclerosis: 3-year results from a phase II study extension

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Background: Oral fingolimod (FTY720) 1.25 or 5.0mg, once-daily, reduced annualized relapse rate (ARR) by >50% and cumulative number of gadolinium-enhancing (Gd+) lesions by up to 80% versus placebo during a 6-month, placebo-controlled trial of 281 patients with relapsing MS. All patients who subsequently opted to enter a long-term extension of the study received this sphingosine-1-phosphate receptor modulator once-daily for up to 36 months. **Objective:** To report the long-term safety, tolerability and efficacy results from the study extension. **Methods:** Patients entering the extension from the placebo group were re-randomized to fingolimod 1.25 or 5.0mg; all other patients continued fingolimod treatment at their original dose (1.25 or 5.0mg). During months 15–24, patients receiving fingolimod 5.0mg were switched to 1.25mg. **Results:** Of the 250 patients who entered the extension, 173 completed month 36. The discontinuation rate during months 24–36 (8.0%) was notably lower than during months 12–24 (17.2%). After 36 months of continuous fingolimod treatment, ARR remained low (0.20–0.21) and 68–73% of patients remained relapse-free. At month 36, the majority of patients in each group were free from Gd+ lesions (88–89%) or new T2 lesions (70–78%). The majority (76–80%) of patients continuing in the extension were also free from 6-month sustained disability progression. The most frequently reported AEs (>15% of patients) were nasopharyngitis, headache, fatigue, and influenza. Frequently reported AEs associated with fingolimod during months 0–6 (dyspnea, diarrhea, and nausea) were rarely reported during months 24–36 (1.1%, 2.7%, and 2.1%, respectively). **Conclusions:** After 3 years of follow-up, there was persistent inhibition of clinical and MRI activity in patients who received continuous oral fingolimod treatment at the 1.25mg dose and those who initially received the 5.0mg dose and were switched to 1.25mg in the second year of the study. The 1.25mg dose had a good safety profile and ongoing phase III studies will further evaluate the benefit-risk of oral fingolimod 1.25mg and 0.5mg in relapsing MS.

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Oral fingolimod (FTY720) in relapsing-remitting multiple sclerosis: baseline patient demographics and disease characteristics from a 2-year phase III trial (FREEDOMS)

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Background: A 6-month, phase II, placebo-controlled trial in 281 patients with relapsing multiple sclerosis (MS) demonstrated that the sphingosine-1-phosphate receptor modulator oral fingolimod (FTY720), 1.25 or 5.0mg once-daily, reduced annualized relapse rate by >50% and number of gadolinium-enhancing lesions by up to 80%; the higher dose did not confer additional treatment benefits. A large, 24-month, phase III trial is currently underway to evaluate the efficacy and safety of oral fingolimod versus placebo in patients with relapsing-remitting MS (RRMS). **Objective:** To report baseline patient demographic and MS disease characteristics from the ongoing FREEDOMS trial. **Methods:** Patients diagnosed with RRMS according to the 2005 revised McDonald criteria were randomized to once-daily oral fingolimod 0.5 or 1.25mg, or placebo for 24 months in this global, double-blind, placebo-controlled, parallel-group trial. Eligible patients were aged 18–55 years with an Expanded Disability Status Scale (EDSS)

score of 0–5.5 and were required to have had at least one relapse in the previous year or two relapses in the previous 2 years. **Results:** Study enrolment took place over 18 months between January 2006 and August 2007, and a total of 1272 patients have been randomized in the FREEDOMS study (mean age 37 years; 70% female). At baseline, mean duration of MS was 8.3 years, with a mean of 1.5 relapses in the year prior to baseline and 2.1 in the 2 years prior to baseline. Mean baseline EDSS score was 2.4. Approximately 33% of patients previously received a disease-modifying treatment (14% received intramuscular interferon beta [IFN β]-1a, 12% subcutaneous IFN β -1a, 10% IFN β -1b, 11% glatiramer acetate, and 1% natalizumab); patients could have received more than one of these treatments. **Conclusions:** These baseline results indicate that the RRMS patient population randomized to the presently ongoing FREEDOMS trial appears to be similar to that of previous phase III studies of parenteral MS disease-modifying treatments (IFN β -1b [1993], glatiramer acetate [1995], intramuscular IFN β -1a [1996], subcutaneous IFN β -1a [1998], natalizumab [2006]).

P74

Efficacy and safety of natalizumab in children with highly active relapsing-remitting multiple sclerosis

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Background: There are few studies for multiple sclerosis (MS) in children. Onset before age 11 accounts for 0.2–0.6% of total MS cases and the use of disease modifying treatments (DMTs), although less common than in adults, has been presented recently (IFN β , GA, IVIG). There are no studies for the management of highly active MS cases unresponsive to DMTs in children. **Objective:** To evaluate the efficacy and safety of natalizumab in relapsing-remitting multiple sclerosis (RRMS) in children unresponsive to DMTs. To our knowledge, this is the first reported open observational study in children with active RRMS refractory to DMTs. **Methods:** After obtaining informed consent from parents, five children (two females and three males; 13.2 \pm 0.3 years old) were started on natalizumab monotherapy, one week after previous DMTs were stopped. Previous treatments were IFN β (two patients), GA (one patient) and IVIG (two patients). Mean MS duration was 3.2 years with an annual relapse rate between 5 and 8, with >20 T2 lesions in magnetic resonance imaging (MRI). All patients had Definitive RRMS according to the McDonald criteria and Expanded Disability Status Scale (EDSS) 3–6. All patients were evaluated monthly, both clinically and with blood tests. MRIs were performed before and at 6 and 12 months after natalizumab initiation. Adverse events (AEs) were monitored for all patients. A comparison of the results was done between last and previous treatments. **Results:** After 6 months of treatment there were no drop-outs, no AEs, no relapses, no new T2 lesions, no new Gd+ lesions, no signs of PML. The patients returned to school. Comparing the quality of life under DMTs and under natalizumab there is a significant improvement. **Conclusions:** Natalizumab may be an alternative treatment for highly active RRMS refractory to DMTs in children. These preliminary data are promising but more studies are warranted.

P75

Treatment of multiple sclerosis with glatiramer acetate and albuterol: results of a clinical trial

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Background: The mechanism of action of glatiramer acetate (GA) is thought to be by induction of anergy of GA reactive lines and enhanced production of Th2 cytokines. We hypothesized that albuterol may enhance the effects of GA in vivo or accelerate the induction of anergy and Th2 cytokine production. **Objective:** In a randomized, double-masked, two-arm pilot study we investigated the effect of adding oral albuterol versus placebo to GA in relapsing-remitting multiple sclerosis (RRMS). **Methods:** Eligibility criteria were

clinically definite RRMS with positive brain magnetic resonance imaging (MRI), and an Expanded Disability Status Scale (EDSS) score between 0 and 3.5. No prior treatment with GA or oral myelin. No treatment with immunomodulating therapy within the past 3 months. No prior treatment with immunosuppressants. No steroid treatment 1 month prior to study entry. Subjects were randomized to two treatment arms: 20mg SQ of GA daily + 4mg PO of placebo daily for 2 years or 20mg SQ of GA daily + 4mg PO of albuterol daily, for 2 years. The primary outcome measures were the change in the MS Functional Scale (MSFC), and the change in IL-13 and IFN- γ cytokine secretion by GA reactive T cell lines. Secondary outcome variables included changes in percentage of IL-12-producing monocytes by intra-cytoplasmic staining, time of first exacerbation, number and severity of exacerbations, and MRI evidence of progression. **Results:** Forty four subjects were randomized (21 in the GA+placebo arm and 23 in the GA+albuterol arm). There was a treatment effect of albuterol on MSFC at 6 months that diminished over time ($p=0.026$) and a trend for improved MSFC in the albuterol arm at 12 months. Analysis of the immunologic endpoints is ongoing. **Conclusions:** Albuterol added to GA treatment in RRMS enhanced treatment response in the early time points and may be beneficial in accelerating the beneficial effects of therapy.

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Magnetic resonance imaging activity predicts multiple sclerosis patients' response to treatment with interferon beta-1a

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Background: Disease course and treatment effects of interferon beta (IFN β) and glatiramer acetate in multiple sclerosis (MS) are variable; therefore, it may take years before response to therapy is determined on clinical grounds. Hence, finding biologic markers that more rapidly predict treatment response may provide guidance for neurologists and ultimately improve patient outcomes. **Objective:** To determine whether magnetic resonance imaging (MRI) characteristics shortly after starting therapy can predict response to treatment with intramuscular (IM) IFN β -1a in patients in the Controlled High-Risk Subjects Avonex Multiple Sclerosis Prevention Study (CHAMPS). **Methods:** In CHAMPS, 383 patients with a first demyelinating event and cranial MRI evidence of prior subclinical demyelination received IFN β -1a 30 mcg (n=193) or placebo (n=190) IM once weekly for \leq 3 years. The primary endpoint was the cumulative probability of developing clinically definitive MS (CDMS). MRI scans were obtained every 6 months for \leq 18 months. IFN β -1a- and placebo-treated patients were categorized into 1 of 2 subgroups based on MRI activity at study month 6: (1) 0 gadolinium-enhancing (Gd+) and <2 new T2-hyperintense lesions, and (2) \geq 1 Gd+ or \geq 2 new T2-hyperintense lesions. The cumulative probability of CDMS over 30 months of treatment was determined in subgroups in both IFN β -1a and placebo groups. **Results:** In the 59% of IFN β -1a-treated patients with 0 Gd+ and <2 T2-hyperintense lesions at 6 months, the cumulative probability of CDMS was significantly lower than in the 41% of patients with \geq 1 Gd+ or \geq 2 T2 hyperintense lesions ($P=0.0002$, hazard ratio: 3.94). In patients on placebo, there was no difference in the cumulative probability of CDMS between patients with 0 Gd+ lesions and <2 T2-hyperintense lesions compared to patients with \geq 1 Gd+ lesion or \geq 2 T2 hyperintense lesions ($P=0.1022$, hazard ratio: 1.62). **Conclusions:** MRI activity on a single contrast-enhanced scan obtained 6 months after starting IFN β -1a predicts response to therapy over the subsequent 2 years.

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P77

Worsening of lupus symptoms after IFN- β 1a injections in a patient with multiple sclerosis and systemic lupus erythematosus

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Background: Multiple sclerosis (MS) and systemic lupus erythematosus (SLE) can co-exist in an individual, but the effects of treatment with IFN β on lupus symptoms are unknown. Observations of effects of immunomodulators on inducing or exacerbating autoimmune disease may offer insight into how upsetting the delicate balance among the components of the immune system may lead to pathogenic autoimmunity. **Objective:** To present a patient with MS and SLE in whom lupus symptoms were triggered by IFN β 1a therapy. To discuss the possible mechanisms whereby IFN β can aggravate SLE and review the published literature on IFN β -induced lupus. **Methods:** Case report. **Results:** A 30-year-old woman experienced episodes of diplopia, vertigo, motor and sensory disturbances since age 8 and of malar rash, ankle and digit swelling, oral ulcers, discoid skin lesions, photosensitivity and alopecia since adolescence. She was diagnosed with MS and SLE at age 30 during hospitalization for right-sided weakness and numbness. At the time she had malar rash, ankle swelling and anti-nuclear antibody titers of 1:640 (nucleolar). Brain MRI demonstrated numerous periventricular lesions, some enhancing, typical for MS. She was initiated on oral hydroxychloroquine and weekly intramuscular IFN β 1a, but within hours of each injection developed malar rash, swelling of digit joints, diffuse body pain and high fevers (102-3 F), which initially lasted for 2-3 days, but eventually became more persistent. After over a year of therapy, she opted to discontinue the injections and lupus symptoms abated. **Conclusions:** IFN β therapy increases levels of interleukins-6, -10 and TNF α , which are elevated during lupus flares. We hypothesize that IFN β therapy may lead to SLE exacerbation by altering cytokine balance. Cytokine imbalance may also explain the phenomenon of IFN β -induced lupus, of which more than 10 instances have been reported to date.

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Immunoablative therapy with autologous hematopoietic stem cell transplantation in the treatment of poor-risk multiple sclerosisTomas Kozak¹, Eva Havrdova², Marek Trnecny², Jiri Pitha¹, Eva Krasulova², Petra Lhotakova¹, Petr Kobylka³, Zuzana Trnkova¹, Jiri Fiedler⁴, Vladimir Koza⁴*¹Dept. of Clinical Hematology, University Hospital Kralovske Vinohrady, Prague, Czech Republic; ²General University Hospital, Prague, Czech Republic; ³Institute of Hematology and Blood Transfusion, Prague, Czech Republic; ⁴University Hospital Pilsen, Pilsen, Czech Republic*

Background: The prognosis of the intractable form of multiple sclerosis (MS) is poor; however, high-dose immunotherapy with autologous stem cell transplantation (ASCT) may lead to stabilisation or improvement in disability. **Objective:** 37 patients with secondary progressive MS not responding to all other modalities were included in the phase II clinical trial of ASCT between 1998 and 2006. **Methods:** 33 patients underwent high-dose chemotherapy (immunoablation) BEAM (carmustin, etoposide, cytosinabinoside, melphalan). T cell depletion in vitro was performed in 20 grafts depending on number of harvested progenitors and available resources. Four patients initially included in the study were not transplanted. **Results:** Median follow-up is 60 months (12-108). Median Expanded Disability Status Scale (EDSS) score of grafted patients at inclusion was 6.5 (5.0-8.5), median EDSS of grafted patients at the last follow up was 7.0 (6.0-10.0). Two patients died 31 and 58 months after transplantation, one because of progression of MS and the other of a cause not related to transplantation, respectively. Two patients were lost for follow-up. There was no treatment-related mortality. At last follow-up, the significant improvement (by at least 1.0 point on EDSS) remains in one patient, stabilisation of the disease occurred in 23 patients (70%), nine patients gained disability significantly (by 1.0 point and more on EDSS). Of the four patients initially included and not transplanted, one patient improved by 1.5 points and remains stable after successful stem cell mobilisation with cyclophosphamide. Two other patients

slightly worsened and one patient worsened significantly in disability; all these patients are undergoing various types of modifying therapies. There was no significant difference in neurological outcome between patients transplanted with T cell-depleted stem cell graft and those without T cell depletion. **Conclusions:** A significant majority of patients at least stabilized in their disability after transplantation. We consider the results as promising and wait for confirmation in the randomized trial.

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Efficacy of interferon beta treatment and the role of magnetic resonance imaging in Korean multiple sclerosis patientsJae Gyu Kwak¹, Jae Young An², Joo Hong Min³, Kyong Jin Shin⁴, Man Seok Park⁵, Byoung Joon Kim¹, Kwang Ho Lee¹*¹Samsung Medical Center, Seoul, South Korea; ²Kangnam St Mary's Hospital, Seoul, South Korea; ³Chung-Ang University Hospital, Seoul, South Korea; ⁴Hanmaeum General Hospital, Jeju-si, Jeju-do, South Korea; ⁵Chonnam National University Hospital, Gwangju-si, South Korea*

Background: The clinical features of neuromyelitis optica (NMO) and multiple sclerosis (MS) are often overlapped and the efficacies of interferon-beta (IFN β) treatment in either disease are not well known in Korea. **Objective:** To evaluate the effect of IFN β on MS patients and the role of magnetic resonance imaging (MRI) findings. **Methods:** Fifty Korean patients with clinically defined MS by Poser or McDonald criteria who had received IFN β therapy between 2001 and 2007 were reviewed. We compared the IFN β responses in groups 1) with short cord lesion (SCL) and with long cord lesion (LCL) extending over 3 vertebral segments; 2) with typical (compatible with MS) and atypical/normal (compatible with NMO) brain MRI lesions. We retrospectively analyzed clinical features, brain/spinal MRI findings, and annual relapse rate (RR)/change in Expanded Disability Status Score (EDSS) after IFN β therapy in MS with different MRI findings. **Results:** Fifty patients (20 men and 30 women) were included. IFN β treatment was associated with a significant reduction in the number of relapses in Korean MS patients (before IFN β : 1.7 \pm 1.4, after 1 yr: 0.8 \pm 1.0). The patients with LCL tended to have more annual RR after IFN β treatment (baseline: 2.0 \pm 1.8, after 1 yr: 1.2 \pm 1.1) than those with SCL (baseline: 1.6 \pm 1.1, after 1 yr: 0.5 \pm 0.8). The patients with atypical/normal brain MRI lesions tended to have more annual RR after IFN β treatment (baseline: 1.9 \pm 1.5, after 1 yr: 0.8 \pm 1.1) compared with those with typical brain lesions (baseline: 1.6 \pm 1.2, after 1 yr: 0.7 \pm 0.8). Mean baseline EDSS score and EDSS score after IFN β were higher in patients with LCL (baseline: 3.9 \pm 2.0, after 1 yr: 4.1 \pm 2.8) than those with SCL (baseline: 2.3 \pm 1.6, after 1 yr: 2.0 \pm 1.7). **Conclusions:** This study demonstrated that IFN β treatment led to a significant reduction in the number of relapses in Korean MS patients, and that IFN β treatment would be less effective in patients with spine and brain MRI findings suggestive of NMO.

P80

Cladribine exerts a modulatory effect on T-cell activation

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Background: Cladribine (2-chlorodeoxyadenosine, 2-CdA) is a synthetic purine nucleoside analog structurally very similar to adenosine, a natural endogenous compound with anti-inflammatory effects on many immune cell types. Cladribine exerts an anti-inflammatory effect by preferentially depleting T cells. This effect suggests a potential role for cladribine in the treatment of certain autoimmune diseases, including multiple sclerosis (MS). Additionally, direct adenosine-like anti-inflammatory activities might contribute to cladribine's therapeutic mechanism of action. **Objective:** To investigate the activity of cladribine on the effector functions of human T cells. **Methods:** T cells isolated from the peripheral blood of healthy donors were exposed to high concentrations of cladribine and stimulated either with microtiter plate-bound anti-CD3 antibodies or with mixed allogeneic peripheral blood mononuclear cells. Cell survival and surface phenotype were assessed by flow cytometry and levels of T-cell

cytokine were measured by cytokine bead arrays or Luminex technology. **Results:** Cladribine markedly down-regulated the in vitro secretion of pro- and anti-inflammatory cytokines by T cells in short-term activation assays. This modulatory effect on T-cell function is independent of its lymphocyte-depleting effects. Upon extended exposure to cladribine most T cells underwent apoptosis. A discrete T-cell population that divided extensively, however, persisted in culture up to 2 weeks after the initial stimulation. These cells displayed a reduced CD4+:CD8+ ratio as well as distinct cell surface phenotype and functional properties, and were skewed towards a Th2 profile (up-regulation of interleukin [IL]-5, IL-9 and IL-13). **Conclusions:** These data show that cladribine markedly inhibits in vitro T-cell effector functions and promotes the survival of a discrete T-cell population. Therefore, in addition to its preferential lymphocyte-depleting properties, cladribine seems to directly influence and shape the T-cell response through mechanisms that are distinct from those of adenosine. The dual effects of cladribine described here may contribute to its therapeutic effects in autoimmune diseases such as MS.

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P81

Could long-term therapy with interferon beta-1b induce pulmonary arterial hypertension? a case report

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Background: Interferon beta 1-b (IFN β -1b) is widely used in relapsing-remitting (RRMS) and secondary progressive multiple sclerosis (SPMS). **Objective:** IFN β -1b-induced pulmonary arterial hypertension (PAH) is not described as side effect. In the literature we found five cases of interferon alpha-induced PAH. **Methods:** Case presentation: An 18-year-old female patient with RRMS was treated for 24 months with IFN β -1b. During IFN β -1b therapy the patient was stable, with no relapses and with no serious side effects. **Results:** After 24 months of IFN β -1b treatment the patient reported increasing dyspnea on exertion accompanied by sudden malaise and edema of the lower leg. Electrocardiography showed sinus tachycardia and right axis deviation. The patient was diagnosed with decompensated right heart failure and was therefore hospitalized. There were no signs of vasculitis, hypercoagulability or rheumatologic disorders. Transthoracic echocardiography showed right ventricular hypertrophy and dilatation. Diagnostic right heart catheter revealed a PAH. The IFN β -1b therapy was stopped with no improvement of PAH after two months. Treatment with sildenafil was initiated. **Conclusions:** Interferons could induce PAH by activation of the thromboxane cascade and by induction of expression of various chemokines. According to data from literature, if IFN β -1b treated patients develop respiratory symptoms PAH should be considered in the differential diagnosis.

P82

Inappropriate use of immunomodulatory therapy in severely disabled patients with progressive multiple sclerosis

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Background: Immunomodulatory therapy (IMT) is not effective in slowing progression in disabled patients (Expanded Disability Status Scale (EDSS) score >6.5) with secondary progressive multiple sclerosis (SPMS) without superimposed relapses and is ineffective in primary progressive multiple sclerosis (PPMS). Many patients with SPMS, who initially had relapsing-remitting MS 10–12 years ago, continue to use IMT. They often experience adverse side-effects; the enormous costs of inappropriate IMT further burden health service budgets. Regular neurological assessment is recommended to monitor progression and treatment responses, and to guide discontinuation of IMT if no longer effective. However, this is unavailable to many patients, particularly in rural areas. **Objective:** To observe in an epidemiological study of MS the use of IMT in patients with progressive MS and EDSS > 6.5. **Methods:** During an epidemiological study of MS in two regions in

Ireland (South Dublin city, an urban area and Donegal County, a rural area), we recorded the IMT details of patients with SPMS or PPMS and EDSS >6.5. **Results:** A total of 194 patients were seen; 72 from south Dublin city and 122 from Donegal County. Ninety-five patients had RRMS (49%), 49 had SPMS (25%), 44 had PPMS (23%) and six (3%) had benign MS. In the SPMS group, 13/47 had EDSS > 6.5. Of these, five were on IMT (31%): four were from Donegal and one was from Dublin. In the PPMS group, 4/16 had EDSS >6.5 and were receiving IMT; all four patients were from Donegal. **Conclusions:** A significant proportion of disabled patients with SPMS or PPMS (31%, 9/29) were receiving inappropriate therapy. Eight of the nine were from Donegal, reflecting poorer access to neurology services in this county. The cost of IMT in this group (approximately 8,000/patient/annum) could be re-directed towards development of neurology and rehabilitation services, to optimise management of these patients. Patients in south Dublin are close to hospital MS clinics and only one patient was receiving inappropriate therapy.

P83

Reactivation of BK virus during natalizumab therapy in relapsing-remitting multiple sclerosis

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Background: Natalizumab therapy in multiple sclerosis (MS) has been associated with JC virus-induced progressive multifocal leukoencephalopathy (PML). Reactivation of BK virus, another polyomavirus, has resulted in significant morbidity, typically renal (BK Virus-Associated Nephropathy), during other immunosuppressive therapies. BK virus reactivation during natalizumab therapy has not been reported. We hypothesised that if JC virus can reactivate during natalizumab therapy, then so too could BK virus (BKV). **Objective:** To prospectively monitor for reactivation of BK virus in relapsing-remitting MS (RRMS) patients receiving natalizumab for 1 year. **Methods:** In 21 RRMS patients treated for 1 year with natalizumab, a polyomavirus screen including JC and BK polymerase chain reaction was analysed on blood and urine samples at enrollment, monthly for the first 3 months, and 3-monthly thereafter. CD4:CD8 ratios were analysed in peripheral blood (as surrogate markers of immunocompetence). Baseline renal profile was recorded, and monitored monthly in patients with BKV reactivation. BKV subtyping and NCCR sequencing was carried out on samples demonstrating reactivation. **Results:** BK reactivation occurred in seven patients (at mean dose 8; range 1–13). BK viruria was transient in five patients and persistent in two. Persistent viruria was associated with transient viremia. Concomitantly JC viral loads were undetectable. Reactivating BKV subtypes were heterogeneous: subtype 1 was seen in four patients, subtype 2 was seen in one patient, and subtype 4 was seen in one patient. Although CD4:CD8 ratios fluctuated, absolute CD4 counts remained within normal limits. In 5 of the 7 patients with BKV reactivation, transient reductions in CD4:CD8 ratios were observed prior to onset of BK viruria. Renal function remains normal in all 7 patients. **Conclusions:** BK virus reactivation can occur during natalizumab therapy, but the significance of viruria or transient viremia, in the absence of renal dysfunction, is unclear. We propose regular monitoring for BK reactivation and for renal dysfunction, in addition to screening for JC virus reactivation, in patients receiving natalizumab.

P84

Cerebrospinal fluid cell phenotypic analysis from a phase 2 trial of the BHT-3009 DNA vaccine for multiple sclerosis

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Background: BHT-3009 is a tolerizing DNA vaccine for multiple sclerosis (MS), encoding full-length human myelin basic protein. In a 48-week phase 2 trial of relapsing-remitting MS patients, BHT-3009 was shown to cause antigen-specific immune tolerance and to reduce brain lesions in patients with high cerebrospinal fluid (CSF) IgG levels at baseline. **Objective:** The objective of the current study is to examine the CSF of patients treated with BHT-3009 for any changes in immunological activity. **Methods:** Two hundred eighty-nine relapsing-remitting MS patients were randomized to placebo, 0.5 mg BHT-3009, or 1.5 mg BHT-3009, given approximately every 4 weeks until week 44. CSF from 80 of these patients at baseline and week 44 were analyzed by fluorescence-activated cell sorting (FACS) stains for various cell-surface markers of immunological activity. **Results:** Several significant changes in immune cell populations were noted in the placebo group from baseline to week 44. For example, there was a decrease in CD25+/CD8+ cells from 13.4% at baseline to 5.2% at week 44 with placebo ($p=0.03$), which could represent a relative reduction of Treg cells. With BHT-3009 there was no statistically significant change (from 7% to 9.7% on 0.5 mg, from 10.8% to 4.2% on 1.5 mg). There was also an increase in CD26+/CD3+ cells from 18.6% at baseline to 49.8% at week 44 ($p=0.004$) and in CD80+/CD8+ cells from 2.7% at baseline to 16.6% at week 44 ($p=0.018$) with placebo, which could represent an increase in the activation of T cells. With BHT-3009 there was no statistically significant change in either of these cell types (CD26+/CD3+ cells: from 16.1% to 31.3% on 0.5 mg, from 20% to 33.6% on 1.5 mg; CD80+/CD8+ cells: from 15.6% to 14.2% on 0.5 mg, from 9.9% to 12% on 1.5 mg). **Conclusions:** These data, as well as others which will be presented, could suggest a relative activation of immune cell populations within the placebo group compared with the BHT-3009 groups. **Supported by:** Bayhill Therapeutics.

P85

Effects of subcutaneous interferon beta-1a on disease parameters and cognition in patients with early relapsing-remitting multiple sclerosis: longitudinal clinical and cognitive results from the COGIMUS (COGNITION IMPAIRMENT IN MULTIPLE SCLEROSIS) study

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Background: Cognitive impairment (CI) develops in 40–65% of patients with multiple sclerosis (MS) and can be detected at early disease stages before the onset of physical disability. **Objective:** To evaluate the impact of two doses of interferon (IFN) beta-1a on CI and disease in patients with early relapsing-remitting MS (RRMS). **Methods:** Patients aged 18–50 years with RRMS (diagnosed by the McDonald criteria) and an Expanded Disability Status Scale (EDSS) score ≤ 4.0 were recruited to the prospective, multi-center, observational, dose-controlled COGIMUS study and received IFN beta-1a, 22mcg or 44mcg subcutaneously three times weekly. At baseline and regular intervals thereafter for 3 years, neurological examination and Rao's battery of neuropsychological tests were performed by psychologists or evaluating neurologists blinded to patients' treatment group. **Results:** 459 patients were recruited for treatment with IFN beta-1a

(22mcg, n=223; 44mcg, n=236). Three-year follow-up data were available for 331 (72.1%) patients (22mcg, n=162; 44mcg, n=169). Three-year cognitive function data were available for 318 patients. At 3 years, the proportion of patients developing CI in ≥ 3 tests was significantly lower with IFN beta-1a 44mcg than with 22mcg (15.2% versus 24.8%; $p=0.030$). A similar result was found for ≥ 2 impaired tests ($p=0.035$). Risk factors for CI at year 3 included lower-dose IFN beta-1a treatment and ≥ 3 impaired tests at baseline. Three-year relapse data were available for 376 (81.9%) patients. Of these, 62.0% remained relapse-free throughout; mean annualized relapse rate: 0.21 in year 3 and 0.23 overall. Sustained disease progression (≥ 1 point EDSS increase, confirmed 6 months later) occurred in 55/331 (16.7%) patients. Data collection and analysis are ongoing: 3-year cognition, clinical efficacy and safety data for the entire study population will be presented. **Conclusions:** This is the first study to demonstrate a dose-dependent effect of IFN beta-1a on cognition in RRMS and supports the clinical benefit of starting therapy early in the MS disease course. **Supported by:** European Biomedical Foundation.

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Betaferon®/Betaseron® in the early relapsing-remitting multiple sclerosis surveillance trial (BEST): data from all patients completing 2 years of treatment

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Background: Interferon beta-1b (IFNB-1b; Betaferon®/Betaseron®) was approved as treatment for relapsing-remitting (RRMS) and secondary progressive MS and for patients with a single demyelinating event, based on the results of randomized controlled trials. **Objective:** To investigate the long-term outcomes of early IFNB-1b treatment in a clinical setting representative of everyday practice. **Methods:** The BEST study is a large-scale, prospective, 5-year, observational trial of patients with early RRMS treated with IFNB-1b 250 mcg subcutaneously every other day. Parameters collected every 6 months include Expanded Disability Status Scale (EDSS) scores, relapse assessments and health-related quality of life (HRQoL) information. This is the first report on all the patients who have completed two full years of treatment. **Results:** By December 2005, 3566 patients had been recruited. Of these patients, 65.5% (2335/3566) have continued treatment over two years, whereas 23.8% (849/3566) were confirmed study dropouts. In the group of patients who continued treatment, 83.7% (1935/2312) had no disease progression (improvement or no increase by ≥ 1 in EDSS score) and there was a 55.7% reduction in relapse rate compared with pre-baseline (0.43 versus 0.97). The proportion of 'responders to treatment', defined as progression free and relapse free or experiencing relapse rate reduction compared with pre-baseline, was 66%. Functional Assessment of Multiple Sclerosis total score and EQ-5D Health State Scale remained stable over time and correlated with the clinical assessments. No new or unexpected adverse events were observed. Comprehensive results for all patients completing two years of treatment will be presented. **Conclusions:** The safety and efficacy data derived from 'real life' clinical settings are consistent with the results of published randomized controlled trials. The number of dropouts from the study at two years was higher than in controlled studies conducted at specialized MS centers. **Supported by:** Bayer Schering Pharma AG.

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Oral fingolimod (FTY720) reduces the severity of experimental autoimmune encephalomyelitis and protects against disease progression

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Background: Oral fingolimod (FTY720) is a sphingosine-1-phosphate receptor modulator under development for the treatment of relapsing multiple sclerosis (MS). In a 6-month, placebo-controlled trial of 281 patients with relapsing MS, oral fingolimod, 1.25 or 5.0mg once-daily, significantly reduced annualized relapse rate by >50% and

cumulative number of Gd-enhanced lesions by up to 80% versus placebo. **Objective:** To assess whether late treatment with oral fingolimod initiated after onset of demyelination can protect from experimental autoimmune encephalomyelitis (EAE). **Methods:** Female dark agouti rats were immunized (day 0) with 75mcg myelin oligodendrocyte glycoprotein. Daily treatment for 9 days with fingolimod 0.3mg/kg *p.o.* or vehicle was initiated after a second relapse once demyelination and axonal damage were pronounced. Demyelination in the white matter of the spinal cord was detected by magnetization transfer contrast imaging and quantified by magnetization transfer ratio (MTR) values prior to treatment and at study end. Inflammation, demyelination and axonal density were measured at study end by histochemistry. Severity of disease was assessed by EAE clinical score. **Results:** Delayed treatment with oral fingolimod starting at the second relapse (approximately 29 days post immunization) significantly reduced mean EAE clinical score compared with vehicle after 4 days of treatment. White matter loss (assessed by MTR) was evident in both groups before treatment was initiated. There was a further significant MTR decrease (indicating demyelination) in vehicle-treated rats approximately 8 days after treatment initiation, whereas no significant change was seen in the fingolimod group. Histology revealed pronounced reductions in inflammation, macrophage infiltration/microglial activation and demyelination, and an increase in axonal density in fingolimod-treated animals. **Conclusions:** Oral fingolimod reduced disease severity even when administered long after onset of clinical or magnetic resonance imaging evidence of EAE. This study supports the possibility that fingolimod may have protective effects during the progressive stages of MS.

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In vitro immunomodulation by quercetin and interferon- β in multiple sclerosis patients

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Background: Quercetin (3,3',4',5,7-pentahydroxy flavone), the key representative of the family of flavonoids, is known to exert significant immunomodulatory effects. Treatment with quercetin reduces the clinical symptoms of experimental autoimmune encephalomyelitis. Quercetin has the potential to positively impact multiple sclerosis (MS) therapeutics when used alone or in combination with other disease-modifying therapies. **Objective:** To determine the role of quercetin, alone or in combination with human interferon-beta (HuIFN- β), in modulating the immune response(s) of peripheral blood mononuclear cells (PBMC) isolated from MS patients. **Methods:** We determined the effect of quercetin alone or in combination with HuIFN- β on PBMCs' proliferative responses, production of pro-inflammatory cytokines (IL-1 β , TNF- α), and the ratio of cell migration mediator MMP-9 and its inhibitor TIMP-1. PBMCs were obtained from 23 MS patients, naive to disease-modifying therapies for the previous 6 months, and 18 age- and gender-matched healthy subjects. Phytohaemagglutinin-stimulated cells were cultured in the absence or presence of quercetin (0.2–200 μ M) and steady-state concentration of 2 IU of HuIFN- β , or a combination of these two drugs. Cell proliferation was assessed and the secretion of IL-1 β , TNF- α , total MMP-9 and TIMP-1 in the culture supernatants was measured by commercially available enzyme-linked immunosorbent assay kits. **Results:** Quercetin reduced the level of IL-1 β and TNF- α in the supernatant, and reduced proliferation of PBMCs in dose-dependent manner. Significant effects of quercetin were shown at concentrations of $\geq 5\mu$ M ($p < 0.001$). Quercetin had no effect on TIMP-1, but it reduced the MMP-9/TIMP-1 ratio via reducing MMP-9 production. Quercetin had additive effects when combined with HuIFN- β . The responses to quercetin were similar between MS patients and normal subjects. **Conclusions:** Our data suggest that quercetin's immunomodulatory effects are significant in the treatment of MS patients, when used alone or in combination with other disease-modifying therapies. **Supported by:** National Multiple Sclerosis Society (USA) #PP1481.

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Breakthrough disease in pediatric multiple sclerosis patients: a pediatric network experience

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Background: Pediatric multiple sclerosis (MS) comprises 2–5% of all cases of MS, and most patients are treated with first-line disease-modifying therapies (DMT). However, while in adult MS it is recognized that first-line DMT is only partially effective, little is known in pediatric MS about the frequency and management of treatment failure (breakthrough disease). **Objective:** To assess the frequency and management of breakthrough disease in a group of children and adolescents with MS evaluated within a network of pediatric MS centers in the United States. **Methods:** Retrospective chart reviews at three National Multiple Sclerosis Society designated Pediatric MS Centers of Excellence in the United States. MS diagnosis was established using the International Pediatric MS Study Group consensus criteria. Breakthrough disease was defined as two clinical relapses and/or accumulation of new magnetic resonance imaging (MRI) lesions on two consecutive MRI scans performed within a year while on therapy. **Results:** Records of 165 children (F/M 1.9:1; age at disease onset: 6–17 yrs) with MS and who were treated with first-line therapy were reviewed. First-line therapies consisted of: IM interferon (IFN) beta 1a 60/165 (36%), SQ IFN beta 1a 48/165 (29%), IFN beta-1b 24/165 (15%), and glatiramer acetate (GA) 33/165 (20%). Thirty-eight children (23%) experienced breakthrough disease requiring second-line agents. One third (14/38) of this group were switched from one IFN product to another and 12 patients switched from or to GA. Twelve patients received chemotherapy (8), IVIG (2) or natalizumab (2) after first-line treatment failure. Despite these treatment changes, 10 of 24 patients on second-line DMTs required initiation of natalizumab or chemotherapy as third-line therapy due to continued breakthrough disease. **Conclusions:** While first-line DMT is usually effective in pediatric MS, almost one quarter of patients have breakthrough disease and are switched to other therapeutic agents, of which a small group includes natalizumab or chemotherapy.

Epidemiology/Genetics – Part 1

P90

Epidemiological evidence of multiple sclerosis in Ecuador

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Background: Prevalence of multiple sclerosis (MS) is higher with increasing distance from the Equator. This is the first report of epidemiological evidence of MS in Ecuador (Latitude N0-S0, South America). **Objective:** To determine the prevalence of MS in Ecuador and its epidemiological characteristics. **Methods:** From May to June 2006, we conducted an observational study to calculate MS prevalence. Data of patients who achieved inclusion criteria (Poser's) were entered to a database. We chose a validated questionnaire used in a similar study in Uruguay. Patients were identified by neurologists from public centers, private hospital and FUNDEM (National Foundation for MS) in Quito, Guayaquil and Cuenca cities. **Results:** We identified 159 patients with MS, with a total prevalence of 1.2/100,000. Total prevalence in women: 1.71/100,000 in contrast with men: 0.67/100,000 (females/male ratio 2.51). Patients living at sea level: 0.67/100,000 patients living at high altitude: 1.7/100,000. Prevalence in Quito: 5.22/100,000; in Cuenca: 3.75/100,000; in Guayaquil (sea level): 1.59/100,000. Crude prevalence in the three cities: 3.3/100,000. Some age groups showed higher prevalence: 30–34 years with 2.15/100,000; 35–39 years with 3.11/100,000; 40–44 years (200) with 3.86/100,000; 45–49 years with 2.49/100,000; 50–54 years with 2.91/100,000. 74.8% were classified as clinically

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definite MS and 25.2% as probable MS. 71.3% of cases were relapsing-remitting MS; Disability Scale score was < 3 in almost 50% of patients. **Conclusions:** Ecuador is a low-risk area for MS. We found a positive correlation with altitude suggesting an altitudinal gradient. Prevalence was higher in women especially in their thirties and forties. Although this first report is not a community study it showed that the number of MS cases had been underestimated. We also believe in the necessity of prevalence studies of each area, even those where disease was reported as seldom or non-existing. We found a lack of Public Health responsibility in the treatment of MS patients. We recommend the creation of a surveillance system that would allow more complete data and better epidemiological analysis.

P91**NARCOMS Latino cohort**

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Background: Previous studies have investigated characteristics of multiple sclerosis (MS) between races, but data on Latinos are limited. **Objective:** To compare socio-demographic and disease characteristics of NARCOMS Latino enrollees with those of White and African American (AA). **Methods:** We analyzed US enrollees in the North American Research Committee on MS (NARCOMS) Registry who reported race/ethnicity as Latino (n=646), White (n=28949) or AA (n=1422). We compared these groups on demographic (sex, age at enrollment, education, annual income, region of residence) and clinical (age at symptom onset, age at diagnosis, disability level) characteristics. **Results:** The male:female ratio was similar across groups. The majority (68%) of Latinos resided in the South and West regions, while most Whites (53%) and AA (68%) resided in the Midwest and South regions. Education was similar in all cohorts, with 54% of Latinos, 57% of Whites and 51% of AA completing some college education. Fewer Latinos (37%) and Whites (31%) than AA (52%) had an income ≤ \$30,000. On average, Latinos were younger at enrollment (mean 41.9, SD 10.7 years) than Whites (47.4, 10.8) and AA (44.4, 10.4). On average, Latinos were diagnosed at an earlier age (34.3, 9.7) than Whites (37.2, 9.8 p<0.0001) and AA (35.8, 9.7 p=0.001). After the 1980s Latinos had a shorter delay from symptom onset to diagnosis (7.29, 8.92) compared with Whites (8.80, 9.48 p=0.0001) and AA (8.23, 10.43 p=0.06). Disability increased in all three groups with disease duration. On average, Latinos had the lowest Patient Determined Disease Step (PDDS) level. At disease duration ≤ 5 years, Latinos had lower levels than AA (p<0.0001) and were similar to Whites (p=0.18). **Conclusions:** Socio-demographic characteristics and diagnostic delay may differ between races. English-speaking Latinos report lower levels of MS disability and shorter diagnostic delay than White and AA. Language barrier may have affected the results.

P92**Clinical presentation of relapses in multiple sclerosis during pregnancy and postpartum period: an epidemiological overview in Mexican women**

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Background: Multiple sclerosis (MS) affects young persons, predominantly females. Therefore, studies have emerged to determine disease relation to pregnancy. Some have concluded that the relapse risk diminishes during the third trimester, increasing over the next three months postpartum; others concluded that there is no influence of MS over women's fertility, pregnancy complications, fetal malformations, nor spontaneous abortions. **Objective:** To determine the most frequent clinical presentation of relapses during pregnancy and postnatal period (PpNP) in women with MS in the Mexican population. **Methods:** Retrospective study of medical records of the National

Institute of Neurology in Mexico City from 1993–2006, assessing pregnancies before and after the diagnosis of MS. The Expanded Disability Status Scale (EDSS) scores both at the beginning of the disease and in the last medical visit were registered. Clinical relapse presentation and number of relapses during PpNP were noted. **Results:** 100 patients were included, divided in two groups. Group A: 40 patients with MS diagnosed before their first pregnancy. Group B: 60 patients diagnosed with MS after their first pregnancy. Spinal cord symptoms were the most common clinical presentation in relapses during pregnancy. The relapse rate percentage in the twelve-month period including pregnancy and three postnatal months was 17.1%. There were no differences in disease progression between patients who presented with a relapse during pregnancy and those who did not present relapses. **Conclusions:** The relapse rate percentage result differs from previous studies. This finding could be attributed to different genetic and environmental factors. It has been demonstrated that aquaporin 4 increases during pregnancy, particularly in the optic nerves and spinal cord. As the neuromyelitis optica (NMO) IgG antibody selectively binds to this molecule, the predominance of spinal cord symptoms during PpNP could be relevant whilst trying to demonstrate the presence of NMO-IgG as a predictive relapse factor during this period.

P93**Acute partial transverse myelitis as index events in relapsing neuromyelitis optica: analysis of 60 patients**

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Background: Relapsing neuromyelitis optica (RNMO) is defined by two index events: acute transverse myelitis (ATM) and optic neuritis (ON) followed by at least one subsequent episode of ATM or ON. Idiopathic ATM was recently classified according to clinical and laboratory findings in complete (ACTM) and partial (APTM) and related to different outcome and immunopathogenesis. **Objective:** To compare clinical and laboratory findings of APTM and ACTM in RNMO. **Methods:** Sixty patients (6 males and 54 females; 25 white and 35 of African descent) were followed-up between 1990 and 2004 at a referral center for multiple sclerosis (MS) in Rio de Janeiro, Brazil. All patients fulfilled the 1999 NMO diagnostic criteria. The spinal cord index event was classified as APTM or ACTM. **Results:** APTM was identified in 22 patients (4 males and 18 females; 14 white and 8 of African descent) and was clinically characterized by Lhermitte's sign, paresthesia of the limbs with a clearly defined upper sensory level or thoracic pain associated with mild (50%) or moderate (50%) sensory loss; mild motor and/or sphincter dysfunction was associated in few cases. Patients with APTM developed further spinal cord episodes characterized by partial or complete transverse myelitis. At the last follow-up, we found large spinal cord magnetic resonance imaging lesions in 76.2% and intrathecal synthesis of IgG in cerebrospinal fluid in 56.25%. Mortality actualized in 2007 showed a death rate of 13.6%. Demographic, clinical and laboratory findings were significantly different when compared with ACTM. **Conclusions:** APTM has traditionally been linked to MS; however, in the present study, 36% of the RNMO patients presented partial myelitis in the spinal cord index event. The outcomes of patients with APTM syndrome was quite different from those with ACTM, with less morbidity and mortality.

P94**Association of IL23R functional polymorphisms with multiple sclerosis**

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Background: Recent large-scale genetic association scans have shown a convincing association of several polymorphisms located in the gene IL23R with different inflammatory conditions, such as Crohn's

disease, psoriasis or ankylosing spondylitis. The IL-23 receptor is composed of two chains, one shared with the IL-12 receptor (IL-12R β 1) and another specific chain, encoded by the IL23R gene, located on human chromosome 1. IL-23 is involved in the development of Th17 cells, which have been postulated to be important in autoimmune processes, including experimental autoimmune encephalomyelitis (the murine model of multiple sclerosis (MS)). **Objective:** We aimed to analyze the role of IL23R polymorphisms in multiple sclerosis, another autoimmune disease. **Methods:** We performed a case-control study including 414 patients with MS and 546 healthy controls, all of them white Spaniards. All samples were genotyped, by TaqMan technology (Applied Biosystems, Foster City, CA, USA), for two single nucleotide polymorphisms, rs7517847 and rs11209026 (Arg381Gln). Statistical analyses were performed using chi-square tests or the Fisher's exact test (when necessary). **Results:** The minor allele (Gln) frequency of the coding variant Arg381Gln (rs11209026) was significantly increased in MS patients when compared with controls (9% in MS vs. 6% in controls, $p=0.006$ OR (95%CI)=1.64(1.14–2.37)). A stronger effect was observed in patients showing primary progressive disease (16%, $p=0.004$ OR (95%CI)=3.19 (1.45–6.89)). Moreover, heterozygotes for rs7517847 were significantly more frequent in this group of MS patients (81% in MS vs. 48% in controls, $p=0.0002$ OR (95%CI)=4.77 (1.88–14.36)). No differences were observed when patients were stratified by DRB1*1501 status. **Conclusions:** Contrary to what has been described previously for Crohn's disease, the less frequent allele of the functional polymorphism Arg381Gln (rs11209026) seems to increase susceptibility to MS, and a stronger effect is observed in patients with the primary progressive form of the disease.

P95

Profile of asymptomatic contrast sensitivity loss in multiple sclerosis

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Background: It is well established that optic nerve involvement in patients with multiple sclerosis (MS) is very common and not exclusively associated with optic neuritis (ON). Contrast sensitivity (CS) is affected early in optic neuropathies and may be a sensitive marker of optic nerve disease. **Objective:** To evaluate the occurrence and profile of decreased CS in visually asymptomatic MS patients. **Methods:** Monocular Low Contrast Sloan Letter acuity (LCSLA) was performed in MS patients with and without history of ON, and in a control group matched for age and gender. The total number of letters read correctly at four contrast levels (100%, 5%, 1.25% and 0.6%) was correlated with age, gender, disease phenotype, disease duration, number of relapses and the Expanded Disability Status Scale (EDSS) score. **Results:** There were 69 MS patients (29 with and 40 without ON) and 30 healthy controls. The mean LCSLA score was higher in healthy controls than in MS patients at any of the four contrast levels ($p<0.01$). This difference was greatest at the 1.25% level. MS patients with no history of ON had higher LCSLA score at all levels of contrast compared with MS patients with history of ON, but the differences were significant only on the 0.6% contrast chart. ($p<0.05$). The LCSLA scores were lower in progressive than in relapsing-remitting MS at 5% and 1.25% contrast levels ($p<0.05$), whereas patients with higher EDSS scores had decreased sensitivity at 5%, 1.25% and 0.6% contrast levels ($p<0.01$). **Conclusions:** CS tests disclose optic nerve involvement in visually asymptomatic MS patients without previous history of ON. The loss of CS has a good correlation with the course of disease and higher scores of the EDSS.

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P96

Cigarette smoking and sex ratio of multiple sclerosis

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Background: The female to male ratio in multiple sclerosis (MS) incidence has markedly increased over the past several decades. Parallel changes in smoking behavior, a risk factor for MS, could contribute to explain this trend. **Objective:** To examine if temporal trends in smoking behavior contribute to explain the increasing female to male ratio of MS in Denmark and Canada. **Methods:** Using data from Canada (birth cohorts 1931 to 1976) and Denmark (birth cohorts 1931 to 1956), we correlated the female to male ratio in smoking prevalence with the corresponding ratio in MS incidence. **Results:** The female to male ratios of MS incidence and smoking are strongly correlated. Assuming that the relative risk of MS comparing ever smokers to never smokers is 2.5 in men and 1.6 in women, as suggested by a previous study, the diverging trends in smoking behavior in men and women could explain up to 80.0% of the increase in the female to male MS ratio in Canada, and 62.4% in Denmark. **Conclusions:** The increasing female to male ratio of MS incidence could be largely, although not entirely, explained by the adverse effect of smoking on MS incidence and the relative increase in smoking prevalence among women.

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Low-contrast letter acuity scores reflect vision-specific, health-related quality of life in multiple sclerosis

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Background: Low-contrast letter acuity scores correlate with magnetic resonance imaging, visual evoked potentials, and retinal nerve fiber layer thickness; this test also demonstrated treatment effects in recent phase 3 trials. How these scores reflect functioning and quality of life is another element that is essential to the evaluation of new measures. **Objective:** To examine the relation between low-contrast letter acuity and vision-targeted health-related quality of life (HRQOL) in multiple sclerosis (MS) cohort. **Methods:** Patients in this cross-sectional study were part of an ongoing investigation of visual function in MS at the University of Pennsylvania. Testing was performed binocularly using low-contrast letter acuity (2.5% and 1.25% contrast) and high-contrast visual acuity (VA). The 25-Item National Eye Institute Visual Functioning Questionnaire (NEI-VFQ-25) and 10-Item Neuro-Ophthalmic Supplement, Impact of Visual Impairment Scale (IVIS), and Short Form-36 Health Survey (SF-36) were used to assess HRQOL. **Results:** Patients ($n=256$) were aged 46 ± 10 years, with Expanded Disability Status Scale score 2.5 (0–7.5), and binocular Snellen acuity 20/16 (20/12.5–20/80). HRQOL scores for all scales were reduced in the MS cohort compared with published reference and control group averages. Reductions in overall scores for vision-specific HRQOL scales were associated with lower (worse) scores for low-contrast letter acuity and high-contrast VA ($p<0.001$ for all scales, linear regression, accounting for age). Two-line differences in low-contrast acuity were associated, on average, with >4 -point worsening in NEI-VFQ-25 composite scores, a reduction that is considered clinically meaningful for this scale. Scores for a new 10-Item Neuro-Ophthalmic Supplement to the NEI-VFQ-25 also correlated well with visual function. **Conclusions:** Low-contrast letter acuity scores correlate well with HRQOL in MS. Two-line differences in scores for low-contrast acuity and VA reflect clinically meaningful differences in composite scores for the NEI-VFQ-25, the most widely used vision-targeted HRQOL scale. Low-contrast acuity testing provides information on patient-reported function, supporting use of these measures in MS clinical trials.

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P98

Clinical features of acquired demyelination of the central nervous system in Canadian children

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Background: The clinical presentation of acquired demyelination, herein termed clinically isolated syndromes (CIS) includes optic neuritis (ON), transverse myelitis (TM), symptoms referable to a single (monolesional) or multiple (polylesional) areas of the central nervous system, or acute disseminated encephalomyelitis (ADEM). **Objective:** To define the relative representation of different CIS presentations and multiple sclerosis (MS) outcome in children. **Methods:** Analysis of clinical features at presentation and current diagnosis of all children enrolled to date in a 23-site prospective national study of CIS in Canadian children. Descriptive statistics and logistic regression analyses were performed. **Results:** Of 219 patients enrolled, nine were withdrawn due to alternative diagnoses and 160 have been followed for greater than 6 months. The average age at first presentation was 10.0 years (range: 0.6–16.8), and the female to male ratio was 1.16:1. ON (n=44; 27.5%), ADEM (n=36, 22.5%) and TM (n=24; 15%) were the most common presentations. Average age and female to male ratio at presentation were lowest in children with ADEM (5.75 years; s.d. 3.7; range 1.1–15.4; F:M 0.83:1) and greatest in ON (11.5 years; s.d.2.8; range 5.4–16.8; F:M 1.32:1). 133 children (83.13%) were hospitalized for an average of 11.14 days (2–65). Cerebrospinal fluid (CSF) oligoclonal bands were detected in 25 (15.6%) of 94 children. Six children (4%) reported positive family history of MS. To date, 23 (14.4%) have been diagnosed with MS based on clinically confirmed relapse. An early diagnosis of MS was more likely in female children (OR=4.1, 95%CI:1.4–11.9) and in those who were older at CIS (OR=1.2, 95%CI:1.1–1.4), but was not related to clinical presentation (OR=0.7, 95%CI:0.4–1.3) **Conclusions:** Children with CIS most commonly manifest with ON, ADEM, and TM, which differ in terms of age at presentation and sex ratios. The vast majority of children require hospitalization. Both age at CIS and sex appear to be important predictors of an early MS diagnosis. Ongoing longitudinal observation will determine the rate of MS diagnosis following CIS in children, and integration of clinical, epidemiological, pathobiological and magnetic resonance imaging analyses will define predictors of this outcome.

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P99

Impact of maternity in the progression of disability in multiple sclerosis

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Background: Multiple sclerosis (MS) typically affects young women of childbearing age. The PRIMS study showed no negative effects of pregnancy on MS course. **Objective:** To analyze whether the medium to long-term evolution of disability in patients with MS is affected by maternity. **Methods:** We included all patients seen by us in a prospective and protocolised manner. We analyzed the evolution of disability (Expanded Disability Status Scale (EDSS) scores 4,5,6 and 7) by means of the Turnbull estimator of Kaplan-Meier curves (univariate) and AFT (accelerated failure time) parametric models. **Results:** N:451. Initial disease evolution: primary progressive 37. Relapsing 414 (currently secondary progressive: 88). Years since the first symptom: 11.4±9.5 (median:9.1; range:59.6). Age of onset: 30.3±9.6. Age of first maternity: 26±4.83. Number of outbreaks in the first 2 years/n women: 1/216;≥2/192. Without children: 200; with children only before clinical onset: 151; with children only after clinical onset: 69; with children before and after clinical onset: 31. Time to disability is

delayed by over 10 years in those who only had children after clinical onset of disease. This difference disappears when adjusted by age and number of outbreaks. The relative risk of reaching disability increases 0.05 for every year onset of MS is delayed and is multiplied by 3 if they have ≥ 2 outbreaks in the first two years. **Conclusions:** In our experience the evolution of disability in women with MS is independent of maternity. The age of onset of the disease and the number of outbreaks in the first two years are the variables most closely related to the medium to long-term prognosis of MS.

P100

TLR4 haplotypes in multiple sclerosis: a case-control study in the Spanish population

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Background: Epidemiological data suggest that some environmental factors, such as infectious agents, might be implicated in the development of multiple sclerosis (MS). TLR4 is a cellular receptor of lipopolysaccharide (a component of Gram-negative bacteria), and upon binding of its ligand, triggers a signaling cascade that induces the expression of inflammatory genes. Several polymorphisms have been described in this gene, some of them with proven functional effect. Case-control studies performed previously in MS have only analyzed a few selected polymorphisms, but no detailed analysis of the complete gene with a dense set of tagging single nucleotide polymorphisms (SNPs) is available. **Objective:** Our aim is therefore to comprehensively analyze the role played by the TLR4 gene in the susceptibility to multiple sclerosis. **Methods:** We included 362 MS patients and 467 healthy controls, all white Spanish individuals. DNA was extracted from peripheral blood leukocytes by standard methods. Nine polymorphisms were analyzed in all individuals: rs2737191, rs10818073, rs11536869, rs1927911, rs12377632, rs1927907, rs2770146, rs5030717 and rs1554973. Genotyping of the samples was performed by TaqMan assays under the conditions recommended by the manufacturer (Applied Biosystems, Foster City, CA, USA). Haplotype frequencies were estimated using the EM algorithm. Genotype, allele and haplotype frequencies in patients and controls were compared with a Chi-square test; corrected p values under 0.05 were considered significant. **Results:** No significant differences after Bonferroni correction were observed in allelic, genotype or haplotype frequencies. We did not observe differences when patients were stratified by gender, clinical form (primary progressive vs relapsing-remitting/secondary progressive) or DRB1*1501 positivity. **Conclusions:** This is the first complete study of the role of the TLR4 gene in MS. Our results suggest that TLR4 is at best a minor susceptibility locus for MS. Further studies are necessary to evaluate its relationship with specific infectious agents present in MS patients.

P101

KIAA0350 polymorphisms: association with multiple sclerosis in the Spanish population

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Background: Different genome-wide association studies have shown associations between several polymorphisms within a linkage disequilibrium block on chromosome 16p13 and type 1 diabetes (T1D) and multiple sclerosis (MS). This block contains a gene potentially involved in immunity regulation: KIAA0350, which presumably encodes a C-type lectin. **Objective:** We aimed to test the role of the associated polymorphisms within this gene in MS susceptibility in the Spanish population. **Methods:** Two KIAA0350 polymorphisms previously associated with either T1D (rs2903692) or MS (rs6498169) were analyzed using TaqMan chemistry (Applied Biosystems, Foster City, CA, USA), in 424 patients and 547 ethnically matched healthy controls. Statistical analyses were performed using chi-square tests or the

Fisher's exact test. **Results:** In agreement with the previously described association with T1D, a protective effect of the minor allele of rs2903692 was found in our MS cohort [$p=0.0006$; OR (95%CI)=0.72 (0.6–0.87)]. We also replicated the originally described association with MS for the other SNP (rs6498169) [G (minor allele) vs. A: $p=0.002$; OR (95%CI)=1.34 (1.11–1.63)]. No differences were observed when patients were subdivided by gender, clinical form (RR/SP vs. PP) or DRB1*1501 status. **Conclusions:** The already described association of KIAA0350 polymorphisms (rs6498169, and, in the present study, also rs2903692) with MS was successfully replicated in our population. We therefore confirm the relevance of the KIAA0350 gene as a risk factor in MS. The description of a possible genetic element shared with T1D will hopefully open new avenues for research and therapy.

P102

Matrix metalloproteinase-9 and matrix metalloproteinase-2 gene polymorphisms in multiple sclerosis

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Background: The role of matrix metalloproteinases (MMPs) as notable contributors to neuroinflammation and blood-brain barrier disruption in multiple sclerosis (MS) was studied. **Objective:** We investigated the association of matrix metalloproteinase-9 (MMP-9) (-1562C/T, +279R/Q) and matrix metalloproteinase-2 (MMP-2) (-1575G/A, -1306C/T) gene polymorphisms with MS susceptibility. **Methods:** The study group consisted of 244 patients, fulfilling McDonald's criteria of MS, with a mean age of 38 years. The control group consisted of 132 healthy controls, with a mean age of 36 years. Genomic DNA was isolated from peripheral blood leukocytes by standard technique using proteinase K. Genotyping in MMP genes was performed with polymerase chain reaction (PCR) methods and restriction analysis. Detection of known polymorphisms was performed by PCR with sequence-specific primers (PCR-SSP), proposed by the original authors after Lympny et al. (1998). **Results:** A marginally significant difference in genotype distribution ($P_g=0.03$, $P_g\text{-corr}=0.12$) between the MS group and the control group in -1562C/T MMP-9 polymorphism was found. The homozygotes CC were more frequent in patients with MS, (191/53 in MS patients vs 87/45 in control subjects, OR 1.86, 95% confidence interval 1.16–2.99, $P_{\text{corr}} = 0.02$). A significantly lower frequency of the -1562 T allele carrier was found in MS patients compared with healthy controls ($P_a = 0.01$, $P_{\text{acorr}} = 0.04$). Similarly, we demonstrated a marginally significant difference in genotype distribution ($P_g=0.04$, $P_{\text{gcorr}}=0.12$) between the MS group and healthy controls in -1575G/A MMP-2 polymorphism. The homozygotes GG and the heterozygotes GA were more frequent in MS patients (227/13 in MS patients vs 115/17 in control subjects, OR 2.58, 95% confidence interval 1.21–5.50, $P_{\text{corr}} = 0.03$). **Conclusions:** In conclusion, we confirmed that the genotypes and alleles of MMP-9 -1562C/T and MMP-2 -1575G/A gene polymorphisms were associated with higher MS susceptibility.

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P103

Neuromyelitis optica in Brazil: a retrospective study of 41 patients

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Background: Neuromyelitis optica (NMO) is a demyelinating autoimmune central nervous system disease that preferentially affects the optic nerve and spinal cord, although patients with NMO have been shown to have brain magnetic resonance imaging (MRI) lesions not typical of multiple sclerosis (MS). There are few studies on NMO in South America, and it is not known whether patients with

normal brain MRI (nMRI) evolve similarly to patients with abnormal brain MRI (aMRI). **Objective:** To describe the clinical characteristics of 41 patients with NMO from a tertiary care center in Brazil and compare the groups with nMRI and aMRI. **Methods:** Retrospective review of 41 patients followed with NMO at the Neuroimmunology Clinic of the Federal University of Sao Paulo, Brazil, from 1989 to 2007. **Results:** All patients had predominant optic-spinal disease, long extending spinal cord lesions and brain MRI not meeting Barkhof criteria for MS, thus fulfilling the 1999 and 2006 Wingerchuck criteria for NMO. All patients had a relapsing-remitting clinical course with a mean follow-up time of 52 months. Mean age of onset was 32.6 years and mean Expanded Disability Status Scale score on first evaluation was 3.9; mean relapse rate and progression index were 1.0 and 0.9 respectively. Patients were treated with one or a combination of immunosuppressive drugs. Twenty-four patients (59%) had brain lesions not typical of MS on MRI and there was no statistical difference on progression index, relapse rate and demographic data between patients who had brain lesions and patients who did not. Twelve patients were tested for NMO-Ig with positivity of 58%. **Conclusions:** This is the largest series to report clinical and epidemiological data of patients with NMO from South America. In this series, patients with and without brain lesions seemed to have no statistical difference on clinical and demographic data, suggesting that the presence of brain abnormalities does not affect disease progression.

P104

An extended Flemish pedigree affected by multiple sclerosis: genome-wide linkage analysis and study of clinical features

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Background: Multiple sclerosis (MS) is generally considered to be a multifactorial polygenetic disorder. A marked familial clustering is observed, with 20% of MS patients having another relative with MS. Families with seven or more affected individuals are extremely rare, but it is hoped that such pedigrees may facilitate the identification of genetic susceptibility genes. **Objective:** We have identified and clinically characterized an extended Flemish pedigree affected by MS and have performed a genome-wide linkage study. **Methods:** Affected individuals were clinically characterized. Blood samples were obtained from six persons with MS, and 12 connecting asymptomatic family members. HLA-DRB1 typing was done with single specific primer-based methods. Samples were genotyped with an Affymetrix 10K microarray. Linkage analysis was performed with the Merlin package. **Results:** The pedigree now counts 11 individuals affected by MS, amongst them four individuals out of a sibship of nine. The ancestors of the affected individuals could be traced back for at least five generations to two founding couples originating from a rural village in Flanders. The prevalence in this family is at least a magnitude higher than that of the Flemish population in general. Two affected family members did not give informed consent in order to obtain their clinical data or a DNA sample. The other individuals were all diagnosed with MS according to the revised McDonald criteria, except one person who was classified as possible MS. Average age at onset was 36. Five individuals suffered from relapsing-remitting MS, three persons from primary progressive MS. Three of the available affected individuals (50%) and three of the asymptomatic individuals (25%) carried the HLA-DRB1*15 allele. Two regions reached non-parametric lod scores (Kong and Cox exponential model) of ≥ 2 , an observation that remained unchanged after accounting for linkage disequilibrium. **Conclusions:** We identified and characterized an extended pedigree with multiple affected individuals and aimed to identify genomic regions possibly harbouring MS susceptibility loci acting within this family.

P105**Infection with specific strains of Epstein-Barr virus are a risk factor for multiple sclerosis**

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Background: A large body of epidemiological evidence indicates that infection with Epstein-Barr virus (EBV) has a role in the pathogenesis of several human autoimmune diseases including multiple sclerosis (MS). Recent studies have shown abnormal immunity to EBV and large numbers of EBV-infected B cells in the brains of MS patients, and have suggested that this contributes to the pathogenesis of the disease. Previous sequence analysis of various EBV antigens has shown significant polymorphism between isolates from healthy donors of different ethnic groups. **Objective:** We examined the hypothesis that EBV strains infecting patients with MS may differ from those infecting healthy subjects in the amino acid sequence of the EBNA-1 gene of EBV. This antigen has been chosen because previous studies have shown that elevated IgG reactivity to EBNA-1 increases the risk of MS. **Methods:** Spontaneous lymphoblastoid cell lines were established (without exogenous EBV addition) from 40 MS patients and 43 controls in order to study the resident EBV strains, and DNA sequence analysis of the EBNA-1 gene was carried out. **Results:** This analysis has revealed six EBNA-1 amino acid polymorphisms occurring at a significantly ($p=0.05$) different frequency in EBV strains infecting MS patients versus controls. **Conclusions:** These results indicate that a comprehensive investigation into the functional and immunological impact of this EBNA-1 sequence polymorphism is required, along with sequence analysis of other EBV genes from strains infecting MS patients. The identification of specific EBV strain variation associated with MS may open new avenues for fighting this debilitating disease. **Supported by:** Multiple Sclerosis Research Australia Grant NHMRC Program Grant.

P106**Global expression profiling in multiple sclerosis: a disease of the central nervous system, but with relapses triggered in the periphery?**

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Background: Expression profiling using a microarray technique enables the total transcriptome to be surveyed in the search for genes whose transcript levels are affected by disease status. **Objective:** Our objectives were to utilize both peripheral blood lymphocytes (PBL) and cerebrospinal fluid (CSF) cells and perform a global gene expression study on multiple sclerosis (MS) patients in the search for biomarkers and pathways involved in the disease. **Methods:** We performed gene expression profiling on 44 MS patients and controls with other non-inflammatory neurological disorders. The expression of approximately 39 000 genes was detected in each sample using 88 Human Genome U133 plus 2 arrays (Affymetrix). Relevant transcripts were further investigated by real-time polymerase chain reaction (PCR). All analyses were conducted in R using packages from Bioconductor. **Results:** We observed an abundance (>4000) of differentially expressed (DE) transcripts in CSF, comparing MS and controls, but none in PBL. However, when comparing MS patients in relapse with those in remission numerous (>900) DE transcripts were detected in PBL, but none in CSF. KEGG pathways were analyzed for coordinated changes in expression, where several pathways showed significance. **Conclusions:** It is tempting to speculate that our results imply the importance of peripheral events in driving disease bouts in

MS. The nature of DE transcripts and significant KEGG pathways will be discussed further.

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P107**Genetic analysis of caspase 8 polymorphisms in multiple sclerosis**

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Background: The etiology of multiple sclerosis (MS) remains unclear, although it is considered to be a result of the interaction of environmental factors in genetically susceptible individuals. The combination of genes included in susceptibility regions for MS identified in previous studies by our group, and differentially expressed genes reported from gene expression profiling studies in MS using microarrays pointed to caspase 8 (CASP8) as a candidate gene for MS susceptibility. CASP8 encodes a member of the caspase family, which plays a central role in the execution-phase of cell apoptosis. **Objective:** To perform a case-control study of the CASP8 by means of single nucleotide polymorphisms (SNP) and evaluate whether CASP8 polymorphisms are associated with susceptibility to MS. **Methods:** We genotyped 3 SNPs [SNP1 (C/T) and SNP2 (A/G) located in intron 2, and SNP3 (A/T) located in exon 10] in 548 healthy controls (HC) and 539 MS patients [430 patients with relapse-onset MS and 109 patients with primary progressive MS (PPMS)], by using the 5' nuclease assay (Taqman®) on an ABI PRISM® 7000. **Results:** Comparisons of genotype frequencies between PPMS patients and HC revealed associations of CT heterozygosity at SNP1 (OR=1.8; $p=0.005$), GG homozygosity at SNP2 (OR=1.8; $p=0.009$), and AA homozygosity plus AT heterozygosity at SNP3 (OR=1.8; $p=0.014$) with the disease. In addition, CT heterozygosity at SNP1 (OR=1.8; $p=0.008$), GG homozygosity at SNP2 (OR=1.7; $p=0.022$), and AA homozygosity plus AT heterozygosity at SNP3 (OR=1.7; $p=0.029$) were associated with PPMS when compared with relapse-onset MS. Finally, GG homozygosity for SNP2 was associated with overexpression of CASP8 compared with AA and AG genotypes. **Conclusions:** These findings support the hypothesis that individual polymorphisms within the CASP8 gene may have an influence on genetic predisposition for MS, especially in PPMS patients. Functional studies are currently in progress to investigate differences in apoptosis induction related to the risk genotypes found in PPMS patients.

P108**Epigenetic marks in the brain of patients with secondary progressive multiple sclerosis**

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Background: The gender prevalence of multiple sclerosis (MS), the low-level concordance in homozygous twins and the linkage to several genetic loci, suggest an epigenetic component to the definition of this demyelinating disorder. Epigenetics refers to the changes in gene expression that are consequent to secondary modifications of nucleosomal histones, the basic unit of chromatin, and/or DNA methylation. **Objective:** Since modification of lysine residues in the tail of nucleosomal histones associated with repression are essential for developmental myelination in rodents, we asked whether specific patterns of post-translational modifications of the histones could be identified in human MS brains compared with non-MS controls. **Methods:** Protein extracts were generated from the normal appearing white matter in brain samples obtained from the UCLA MS Brain Bank. Only samples from patients with secondary progressive MS, matched by age, sex, brain region and equivalent autolysis time were analyzed and compared with brain samples from non-neurological patients. Proteins were quantified and processed for Western-blot analysis using antibodies specific for lysine residues in the histone tails. **Results:** We detected decreased levels of epigenetic marks for

repression of gene expression (that is, repressive methylation of lysine 9) and decreased expression of the enzymes responsible for deacetylation of nucleosomal histones (that is, histone deacetylases). **Conclusions:** The epigenetic marks defining the oligodendrocyte lineage progression during development are decreased in the normal appearing white matter of patients with secondary progressive MS. **Supported by:** National Multiple Sclerosis Society (USA) RG-3957.

P109

Age modifies multiple sclerosis phenotype at onset

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Background: Pediatric onset (PO) multiple sclerosis (MS) has been reported to have a more benign disease course than adult onset (AO) MS. **Objective:** To determine the effect of age at presentation on disease phenotype in pre- and post-pubertal children and adults with MS. **Methods:** We queried the UCSF MS database for POMS (age at onset of 18 years old or less), and AOMS (age at onset more than 18 years old) with relapsing-remitting disease. We studied the predictors of disease phenotype at onset (severity, recovery, symptoms at onset) in both groups using uni- and multivariate analyses. **Results:** We identified 131 POMS (20 patients less than 11 years old, 111 patients between 11 and 18 years of age) and 305 AOMS patients. The proportion of monoregional onsets was similar in both groups (82 and 84% of the patients). The initial demyelinating episode was more often severe in POMS compared with AOMS (28% versus 16%, $p=0.004$). This was especially true in children under the age of 11 years compared with children between 11 and 18 (50% versus 24%, $p=0.03$). Onset involved the brainstem/cerebellum more frequently in POMS than AOMS (36% versus 21%, $p=0.004$). Among POMS patients, those under 11 years had more frequent encephalopathic changes at onset compared with those between 11 and 18 (24% versus 4%, $p=0.04$). **Conclusions:** POMS onset has a distinct phenotype compared with AOMS. While it should be confirmed in a larger population, age is a distinct disease modifier of MS presentation in our cohorts. Whether these findings relates to environmental, biological or genetic factors is unknown.

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P110

Comparison of the prevalence of sensorineural hearing loss and the impaired speech reception threshold in multiple sclerosis patients and controls

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Background: Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS). Hearing loss occurs in 6–10% of MS patients and the impaired speech reception threshold (SRT) is 40–50% in MS patients. **Objective:** The aim of this study is to compare the prevalence of sensorineural hearing loss and SRT in MS patients and a control group. **Methods:** Demographic findings, pure tone audiometry (PTA) findings and SRT findings were considered in a case-control cross-sectional study of 112 people in Isfahan. In 112 MS patients, factors such as presenting symptoms and the number of lesions visible on magnetic resonance imaging (MRI) were also obtained. **Results:** The 112 MS patients that were investigated in this study were 75% female and 25% male, with the same split in the 112 members of the control group (75% female/25% male). The mean of age in of the MS patients and members of the control group was 28.02 ± 3.8 and 25.22 ± 3.8 years, respectively (P -value < 0.011). Sensorineural hearing loss in MS patients was 40.2%, compared with 6.3% in the control group (P -value < 0.001). Sensorineural hearing loss in female patients was 40.5% and 3.3% (P -value = 0.69). In patients with sensorineural hearing loss, the mean age was 29.64 ± 6.62 years compared with 28.42 ± 4.75 years in the control group (P -value < 0.05). Mild and profound SRT was found in 33.9% and 1.8% of patients, while there was moderate SRT in 0.9% of our control group. Mild and profound SRT was

found in 38.1% and 2.4% of female patients while mild and profound SRT was found in 33.9 and 1.8% of male patients (P -value = 0.16). Normal SRT was reported in 100% of the women in the control group while there was moderate in SRT of 3.5% of men in the control group (P -value = 0.33). **Conclusions:** In patients with MS, we found a high prevalence of sensorineural hearing loss of 40.2% compared with the control group value of 6.3%, similarly to recent studies (85%, 11.7%). Impaired SRT was found in 35.7% of MS patients, similarly to other recent studies (40–50%). Higher age may be a potential source of sensorineural hearing loss in MS patients and the control group. The female sex may also be a potential source of impaired SRT.

P111

Early treatment to prevent conversion of clinically isolated syndrome to clinically defined multiple sclerosis: a Cochrane meta-analysis

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Background: A Cochrane metaanalysis showed the efficacy of Interferon (IFN) beta therapy in relapsing-remitting multiple sclerosis (RRMS) patients, years after diagnosis, as limited to the first year of follow-up. **Objective:** To assess the efficacy of very early IFN beta therapy, given at the time of the first episode suggestive of MS. **Methods:** We applied the Cochrane criteria for considering studies: types of studies (including only randomized controlled trial (RCT) studies), types of participants, interventions, outcome measures. Primary outcomes: number of patients converting from clinically isolated syndrome (CIS) to clinically defined multiple sclerosis (CDMS), defined by the occurrence of a second clinical episode; number of those with side effects. **Results:** We identified 454 papers; 430 were not eligible; 7 referred to three studies: CHAMPS and ETOMS (6 papers) both using once-weekly low-dose (22/30 mcg) IFN beta 1a; BENEFIT (1 papers) using multiple weekly 250 mcg IFN beta 1b. The three studies had different outcomes (occurrence of a second clinical episode or of disease progression in CHAMPS; occurrence of a second clinical episode in ETOMS; time to CDMS in BENEFIT). IFN beta treatment significantly prevented conversion to CDMS in: per protocol analyses in all studies; in both metaanalysis at 1 year (all scenarios but the worst one) as well as at 2 years (all scenarios). Metaanalysis on the number of patients with serious side effects: no significant differences comparing IFN- and placebo-treated patients. **Conclusions:** IFN beta given very early during disease course showed a persistent efficacy over both the first and the second year of follow-up. An open question remains: do the different types of CIS presentation have a different sensitivity to immunomodulatory treatment? The future development of this review will be focused on this issue, in order to give neurologists practical indications on early treatment.

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Factors influencing MS outcome in Sardinia: a clinical and genetic study

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Background: A wide variety of therapies aimed at preventing permanent disability are currently available for the treatment of multiple sclerosis (MS). Nevertheless MS outcome is extremely heterogeneous and the early identification of prognostic factors is becoming more

stringent. **Objective:** To identify factors able to predict MS severity and outcome and to test the most reliable definition of benign MS. **Methods:** We categorized 919 Sardinian MS patients with at least 10 years of disease duration on the basis of disease severity: malign (M) if they reached an Expanded Disability Status Scale (EDSS) score of 6 in 5 years or less; benign (B), defined using two different EDSS cut-off scores: ≤ 2 (B2) and ≤ 3 (B3) after 10 years of disease. The remaining patients were classified as classical (C). Considering the different cut-off, differences in clinical variables were analyzed using a chi-squared test and probabilities of B, M or C were calculated using logistic regression analysis. Differences in EDSS progression at 20 years were calculated in B versus C using the Mann-Whitney test. The percentages of stable B2 and B3 at 15 and 20 years of disease were calculated. In a subgroup of 651 unrelated patients the HLA DRB1-DQB1, the P/C ratio (frequencies in MS/controls) was calculated and compared between groups. **Results:** We identified 83 (10%) patients as M, while 464 (55%) and 581 (69%) were B2 and B3 and 300 (35%) and 183 (22%) were C2 and C3, respectively. The probability of being M was higher with primary progressive disease and increased with the age of onset while, with both criteria, B are mostly relapsing-remitting with a younger age at onset with respect to M and C, but have less cerebellar onset with respect C. No differences were observed in HLA DRB1-DQB1 between groups, apart from a trend for less DRB1* 01 in M. Slower progression in B than C (median 1 versus 2) was evidenced. The percentage of B2 and B3 stable patients was 71% and 57% at 15 years and 50% and 42% at 20 respectively. **Conclusions:** Our data suggest that MS outcome is influenced by clinical variables and are in line with previous studies indicating a modifying role of DRB1. It is noteworthy that less than half of B are unchanged after 20 years. In conclusion the available criteria are not sensitive enough for the early identification of B and a better definition of B MS is advocated.

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Optic neuritis and contrast letter acuity at baseline in CombiRx
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Background: Optic neuritis (ON) affects many relapsing-remitting (RR) multiple sclerosis (MS) patients presenting as a first symptom of disease in 25% of patients. Contrast letter acuity (CLA) change is associated with MS and ON in particular. In the CombiRx trial, patients have been recruited who are in the earlier phase of the RRMS disease process, affording an opportunity to assess the loss of CLA early in the disease in relation to a history of ON. **Objective:** To study ON and CLA in RRMS patients in CombiRx, a multi-center randomized controlled trial (RCT) of combination IFN b-1a and GA in 50% and single agent/corresponding placebo in 25% (1000 subjects). **Methods:** In August 2007, subjects were asked to complete an additional trial referral questionnaire, including history of ON. Baseline MSFC and bilateral Sloan Letter Chart testing and a CLA Eye Exam were administered to all subjects. **Results:** By April 2008, 49% completed both the ON history and CLA Eye Exam (401/820), with 30.4% indicating a definitive history of ON (3.9 \pm 4.5 years): 24.6% were bilateral. No differences were seen in disease (4.3 \pm 5.4 years) or symptom duration (1.2 \pm 3.1 years) or in the percentage correct on CLA Eye Exam at 100% contrast (94% correct); there was marginal evidence that those with a history of ON score lower at both the 2.5% (59% versus 62%, $p=0.0565$) and 1.25% (42% versus 46%, $p=0.0851$) contrast levels. Those with bilateral ON scored lower at the 2.5% ($p=0.0383$; B:52% versus L:62%, R:61%) contrast and marginally lower on 1.25% ($p=0.0918$; B:36% versus L:46%, R:44%) contrast. As the number of years after ON increases, percentage correct decreases for 2.5% ($p=0.0513$) and 1.25% ($p=0.0376$). **Conclusions:** Subjects with ON history, especially bilateral, have lower CLA than non-ON subjects. Those with longer ON history also have lower scores at baseline. The 3 year trial is ongoing to determine whether deficits persist. **Supported by:** CombiRx-Phase III-NINDS 1U01NS45719-01A1, National Multiple Sclerosis Society (USA).

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Race, ethnicity, country of origin and infections in CombiRx
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Background: Environmental exposure, including infection, has been investigated for association with multiple sclerosis (MS). Historically, the prevalence in high Northern latitudes and, more recently, infections such as Chlamydia pneumoniae (CP) and human herpes virus 6 (HHV-6) have been investigated. The CombiRx trial will afford the opportunity to further explore these potential associations. **Objective:** To classify race, ethnicity, country of origin and history of infections patients in CombiRx, a multi-center randomized controlled trial (RCT) of combination IFN b-1a and GA in 50% and single agent/corresponding placebo in 25% (1000 relapsing-remitting MS (RRMS) subjects). **Methods:** In August 2007, subjects were asked to complete an additional trial referral questionnaire, including country of birth and history of childhood infections. Race and ethnicity are self-reported as separate questions at baseline for all subjects. **Results:** By April 2008, 61% completed both initial and additional history questions (599/820). The majority of subjects were white (88%) and female (71%); 7% of subjects classified their ethnicity as African-American and 6% as Hispanic/Latino. Ninety-two percent of subjects were born in the US, 3% in Canada, 1% Mexico, with the other 4% of subjects born in 19 other countries. By continent 97% were born in North America, 1% each Europe/Asia, and 0.5% South America. Over half of subjects (54%) indicated a history of infection(s): 42% mononucleosis (16.1 \pm 4.9 years old at infection), 36% measles (6.7 \pm 3.7 years), 11% chicken pox (6.5 \pm 2.0 years); 23 subjects reporting a history of CP (20.7 \pm 11.9 years) and 9 of HHV-6 (18.1 \pm 8.6 years). Hispanic/Latino subjects indicated a significantly lower proportion of infections compared with other subjects (21% versus 56%, $p<0.0001$); Africa-American and White subjects reported higher rates of infections compared with other races (56% versus 26%, $p=0.0041$). **Conclusions:** While predominately a North American cohort, there is wide variability in reported history of infections. Hispanics tended to have fewer infections reported than others. The 3 year trial is ongoing to determine whether relationships persist. **Supported by:** CombiRx-Phase III-NINDS 1U01NS45719-01A1.

P115

Genome-wide association study using high-density single nucleotide polymorphism arrays on pooled genomic DNA from multiple sclerosis patients responders and non-responders to treatment with interferon-beta

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Background: Interferon-beta (IFN β) is a partially effective treatment for patients with relapsing-remitting multiple sclerosis (RRMS). IFN β has been shown to decrease clinical relapses, reduce brain magnetic resonance imaging activity, and possibly slow progression of disability. Nevertheless, the cost of IFN β is significant, the drug is associated with a number of adverse reactions, and there is a relatively large proportion of patients that do not respond to therapy. **Objective:** To identify genes that are associated with the responder and non-responder status in RRMS patients using high-density single nucleotide polymorphism (SNP) arrays. **Methods:** RRMS patients treated with IFN β were classified into responders or non-responders based on the increase in the Expanded Disability Status Scale (EDSS) score and the presence of relapses after 24 months of follow-up. DNA from 53 responders and 53 non-responders to IFN β was pooled and nine pooling replicates from each group were hybridized to high-density SNP arrays (Affymetrix GeneChip Mapping 500K). SNPs were

ranked based on statistical tests that incorporated both relative allele signals (RAS)-1 and RAS-2, SNP variability between pools, and allelic frequencies. Top scoring SNPs were selected for validation by means of individual genotyping in a confirmatory and independent cohort of 51 responders and 45 non-responders to IFN β . **Results:** A genome-wide association study using SNP arrays in RRMS patients responders and non-responders to IFN β lead to a number of candidate genes that are associated with the responder status. **Conclusions:** These results may help to elucidate the molecular basis of the heterogeneity found in the clinical response to IFN β .

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Change of demographic data over time in multiple sclerosis. The Lyon Multiple Sclerosis Cohort experience: Part I: sex ratio

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Background: Studies from several countries have shown that the female-to-male sex ratio in multiple sclerosis (MS) has been increasing over the recent decades. This was seemingly resulting from a disproportional increase in MS incidence in women. **Objective:** To confirm these results in the Lyon MS Cohort which has been enrolling patients with MS for five decades. **Methods:** Patients were identified through the Lyon MS Cohort, which was set up in the Lyon's Service de Neurologie in 1957. Since then, the cohort has included all of the patients with a diagnosis of MS examined at least once in the department. The data have been entered in the EDMUS (European Database for Multiple Sclerosis) system. Only cases with definite or probable MS according to Poser's classification have been included in the cohort. Sex-ratio analyses were assessed stratified by year of MS onset, then by year of birth. The change over time according to initial disease course (exacerbating-relapsing MS (ERMS) versus primary progressive MS (PPMS)) was also studied. Linear regression and the Wald test were used to assess the statistical significance of the trend. **Results:** Of the 4495 cases included in the analysis (born 1930–1980), 3030 were women (67.4%). According to year of MS onset, the female-to-male sex ratio increased from 1.68 in 1960 to 2.45 in 2005 ($p=0.017$) with modification of trend mostly in the 1990s. According to year of birth, the sex ratio increased from 1.79 in 1930 to 2.49 in 1979 ($p=0.026$). Major differences appeared according to initial disease course: the female-to-male sex ratio increased for ERMS ($p=0.009$) whereas it did not change for PPMS ($p=0.53$). **Conclusions:** These data seem to confirm the change in female-to-male sex ratio over time but this appears to be recent and moderate (less important than in other countries). In addition, this change does not concern patients with PPMS.

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Change of demographic data over time in multiple sclerosis. The Lyon Multiple Sclerosis Cohort experience: Part II: age of onset

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Background: Female to male sex ratio of multiple sclerosis (MS) seems to increase over time. Differences in age of onset could potentially affect the sex ratio because women are slightly younger at MS onset than men. **Objective:** To assess the age at MS onset over time in the Lyon MS Cohort which has been enrolling patients with MS for five decades. **Methods:** Patients were identified through the Lyon MS Cohort, which was set up in the Lyon's Service de Neurologie in 1957. Since then, the cohort has included all patients with a diagnosis of MS examined at least once in the department. The data have been entered

in the EDMUS (European Database for Multiple Sclerosis) system. Only cases with definite or probable MS according to Poser's classification have been included in the cohort. The change over time according to initial disease course (exacerbating-relapsing MS (ERMS) versus primary progressive (PPMS)) and sex were studied. Linear regression and the Wald test were used to assess the statistical significance of the trend. **Results:** Of the 4495 cases included in the analysis (born 1930–1980), 3030 were women (67.4%). The proportion of PPMS was 16.6%. Females onset of MS occurred earlier than male (mean=0.41 year, median=1 year; $p=0.06$). Age at onset of the disease increased from 28.4 years in 1960 to 34.0 years in 2005 ($p<0.0001$). According to initial disease course, age at onset increased from 27.0 years in 1960 to 31.4 years in 2005 ($p<0.0001$) for ERMS and from 36.0 years in 1960 to 41.8 years in 2005 ($p=0.0014$) for PPMS. According to sex the change over time was no different ($p=0.14$). **Conclusions:** Age of onset of MS has been increasing over the last decades regardless of initial disease course and sex. Due to the increasing female-to-male sex ratio, a mild decrease of the age at MS onset was expected. In fact, the reverse is observed.

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Multiple sclerosis: increase over time in the ratio of women to men in patients with an early onset

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Background: It has been reported that, over the last five decades, the ratio of women to men with multiple sclerosis (MS) has increased from 2.0 to 3.7. The cause of this stunning rise is unknown. It seems unrelated to the fact that women tend to consult more frequently than men do. **Objective:** We wanted to verify whether this pattern is consistent for the various courses of MS. We also examined the pattern of ages at onset over time. **Methods:** Data was extracted from our living database of 2070 MS patients followed regularly. Selected patients had a definite diagnosis of MS, or a clinically isolated syndrome (CIS), according to Poser or McDonald criteria. We calculated women-to-men ratios for patients born before 1950 and those born after 1950, according to their age of onset. We also calculated this ratio according to year of birth for patients born before 1940 and in the following five decades. Patients were also stratified according to course: CIS, relapsing-relapsing MS, secondary progressive MS and primary progressive MS. **Results:** In patients born before 1950, 25% have an age of onset lower than 30 years; their gender ratio is 2.4. For those born after 1950, 46% have an age of onset lower than 30 years; their gender ratio is 3.1. When patients are split into consecutive decades (according to their year of birth), the ratio gradually increases from 2.2 to 4.8. When disease course is considered, we observe that the increase in ratio is distributed among patients with a relapsing-relapsing course (3.3 to 3.9) and with a CIS (1.4 to 4.3). There is no change in ratio for patients with a primary or with a secondary progressive course. **Conclusions:** We confirm the increase in the women-to-men ratio over the last five decades. This is exclusive to patients with relapsing-relapsing MS and with CIS. We also observed that a larger proportion of patients have an earlier age of onset over time. Our data suggests that the increasing gender ratio could be related to the fact that MS is now detected at an earlier age than in previous decades, and that women are more prone than men to a relapsing-relapsing course with an early onset. Improved disease awareness and the availability of magnetic resonance imaging have allowed earlier detection.

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Familial multiple sclerosis in Isfahan, Iran

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Background: The aetiology of MS is not well understood; it is probably heterogeneous, and both genetic and environmental factors might be important in susceptibility and in the outcome of the disease. **Objective:** In this study we aimed to study the prevalence and

clinical and demographic characteristics of familial MS in a relatively large cohort of Iranian patients in Isfahan. **Methods:** The clinical records of MS patients who reported at least one other family member suffering from MS, who were prospectively followed by the Isfahan MS Society (IMSS), were gathered. The IMSS database consists of almost all MS patients in the province. **Results:** Among 2247 MS patients (1751 women, 496 men) registered with the IMSS from April 2003 to January 2008, 227 patients (161 female, 66 male) who had a positive family history of MS were enrolled in the study. Familial MS occurred in 10.1% of patients. The mean age for the included patients was 34.52 years and the age of onset was 27.68 years. Interestingly, our results showed that the closer the familial relation between the two affected members, the higher the chance of finding another affected family member (P -value < 0.001). **Conclusions:** Our calculated crude familial rate among a relatively large sample of Iranian patients was similar to those reported in other regions. These results are consistent with the hypothesis that genes play a role in the susceptibility to MS.

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The risk of multiple sclerosis for the offspring of consanguineous mating

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Background: Consanguineous matings are rare among Western populations. A small study showed that the multiple sclerosis (MS) recurrence risk for sibs of index cases whose unaffected parents represented a consanguineous mating approaches a fourfold increase compared with the risk when unaffected parents do not represent a consanguineous mating. **Objective:** This study was designed to study the impact of consanguineous mating on the susceptibility to MS, in children of such mating, in Isfahan province of Iran, which like other parts of Iran has a relatively high rate of consanguineous mating. **Methods:** The records of clinically definite patients who were prospectively followed by the Isfahan MS Society (IMSS) were gathered. Patients whose parents had familial kinship were included in the study. The IMSS database consists of almost all MS patients in the province. **Results:** Among 2247 MS patients (1751 women, 496 men) registered with the IMSS from April 2003 to January 2008, 557 (24.8%) reported a consanguineous kinship between their parents, and of these 437 (19.4%) were first cousins. According to a study which evaluated the frequency of consanguineous mating in every province of Iran among the normal population, the frequency of consanguineous marriage in Isfahan province was 34% of which 26.1% were first cousins. **Conclusions:** If consanguineous mating would increase the risk of MS for the children of such unions then, compared with the normal population, MS patients should be more commonly the offspring of such unions. However, our results, which are based on relatively large numbers, may suggest that children of consanguineous mating are not at a higher risk of MS.

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A study of 718 consecutive Greek patients with multiple sclerosis: demographic characteristics, clinical variables and early predictors of disease severity

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Background: The demographic and clinical characteristics of Greek multiple sclerosis (MS) patients have not been extensively studied in large consecutive patient cohorts. **Objective:** To assess demographic characteristics, clinical variables and early predictors of disease severity in a consecutive cohort of Greek MS patients and compare findings with data from other populations. **Methods:** We studied 718 patients with MS assessed consecutively during the period 2004

to 2008 at the Department of Neurology, University of Athens. We used the Multiple Sclerosis Severity Score (MSSS) to assess the influence of early clinical variables on disease severity. **Results:** The studied cohort included 473 women and 245 men (1.9:1) with mean age at onset 30.8 ± 9.9 years. At the time of first clinical assessment 215 patients had clinically isolated syndrome (CIS), 346 relapsing-remitting MS, 82 secondary progressive MS and 69 primary progressive MS; mean age was 37.1 ± 10.9 years and mean disease duration 6.3 ± 7.1 years. Symptoms at onset were optic neuritis in 23.8%, brainstem/cerebellar dysfunction in 21.0%, isolated sensory long tract dysfunction in 24.8%, motor long tract dysfunction in 22.3%, polysymptomatic in 4.8% and other in 3.2%. Mean Expanded Disability Status Scale (EDSS) was 2.5 ± 2.2 and mean MSSS 3.40 ± 2.80 . Mean MSSS was higher in men ($p < 0.001$) and in patients with a progressive course from onset ($p < 0.001$). Regarding symptoms at onset, mean MSSS was highest in patients with motor long tract dysfunction (4.70 ± 2.82) and polysymptomatic onset (4.39 ± 3.04), lower in patients with brainstem/cerebellar dysfunction (3.23 ± 2.81) and lowest in patients with optic neuritis (2.72 ± 2.67) and isolated sensory long tract dysfunction (2.63 ± 2.22) (overall $p < 0.001$). Higher age at onset correlated with higher MSSS ($p < 0.001$). **Conclusions:** Demographic characteristics, clinical variables and their prognostic significance in Greek patients with MS are similar to findings from large patient cohorts published in the literature.

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MS register in Germany 2008: symptoms of multiple sclerosis

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Background: In 2001, a national multiple sclerosis (MS) register was initiated under the auspices of the German MS Society, National Association (DMSG Bundesverband e.V.). **Objective:** The project aimed at gathering data about clinical characteristics, socio-demographic aspects, and information on the health care situation of people with MS in Germany. **Methods:** Until March 2008 standardized datasets of 12 288 patients in 96 MS centers across the country, covering different areas of medical care, have been recorded. After a two-step quality check, 10 465 data sets remained for further analysis. **Results:** 71.4% of patients were female, mean (\pm SD) age was 44 ± 11.7 years, mean (\pm SD) disease duration was 12.3 ± 9.3 years. 53.3% suffered from relapsing-remitting MS, median Expanded Disability Status Scale (EDSS) was 3.5, and 63.3% had an EDSS ≤ 4 . The most frequent symptoms were fatigue (64.7%), spasticity (63.2%), bladder dysfunction (60.7%), and ataxia (48.4%). Likewise, cognitive dysfunction (38.6%) and depression (38.0%) also occurred rather frequently. Patients with disease duration less than two years suffered mainly from fatigue (47.8%), whereas spasticity (20.4%), pain (27.6%), bladder dysfunction (25.8%), and ataxia (22.1%) appeared less often. The rate of fatigue increased in patients with MS duration of more than 15 years (67.5%); however, these patients suffered eminently from spasticity (78.0%), bladder dysfunction (75.2%), ataxia (55.5%), and pain (44.1%). The most frequent initial symptoms were sensory problems (42.4%), paresis (37.1%), and visual dysfunction (30.0%). Compared with people with disease onset after the age of 50 years, patients who developed MS before the age of 20 suffered more often from visual dysfunction and sensory problems and less frequently from paresis and motor incoordination. **Conclusions:** With more than 10 000 datasets from the German MS Register is amongst the largest databases worldwide. The high impact of the disease on quality of life is underscored by the high number of persons who suffered from fatigue and other 'invisible' symptoms, such as cognitive dysfunction and depression during early and late stages of the disease.

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Quality of life in patients with multiple sclerosis: design and first results of a large cross-sectional study in Germany

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Background: The improvement of quality of life (QoL) is a primary objective in the care of patients with multiple sclerosis (MS). Previous studies showed different results with regard to its influencing factors. **Objective:** The aim of this cross-sectional study was to evaluate the effects of MS, its impairments and the immunomodulatory treatment on QoL in a large cohort of MS patients. **Methods:** Between April and August 2007, 1106 patients with MS were investigated in 71 German centers all over the country. Patients treated with glatiramer acetate (GA), interferon beta (IFN B) or those who were untreated for at least one year were documented. The physician-based case record form (CRF) enclosed demographical and clinical data such as disease course, relapses, Expanded Disability Status Scale (EDSS), current treatment and data concerning working ability. The patient-based CRF contained validated questionnaires addressing QoL (EQ-5D, FAMS), fatigue (WEIMuS), depression (BDI), social support (F-SozU) and control belief (KKG). **Results:** From the cohort of 1106 patients, 39 had to be excluded from the analysis, mainly due to prior treatment with corticosteroids. Physician- and patient-based CRFs were available from 939 patients. Mean age was 42.2 years (\pm 10.6), 73% were female, mean disease duration was 9.6 years (\pm 7.3), and median EDSS 2.0 (0–8.5, IQR 1.0–4.0). The majority of patients (79%) suffered from relapsing-remitting MS, 17% from secondary progressive MS, and 2% from primary progressive MS. 21% were treated with GA, 16% with IFN B1-a IM, 24% with IFN B1-b SC, 20% with IFN B1-a SC, and 19% were untreated. Depression was present in 16% of the patients, and 30% suffered from fatigue. Quality of life was rather high (FAMS 127 \pm 30, EQ-5D 0.864 \pm 0.173), as well as the perceived social support (4.3 \pm 0.7). **Conclusions:** This most extensive cross-sectional survey concerning physical and social aspects of MS shows a high level of QoL in a cohort of patients with rather low disability. The relevant factors pertaining to the primary outcomes are analysed and presented.

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Multiple sclerosis relapses and meteorological factors in a Portuguese population

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Background: Environmental factors are thought to be important in the pathophysiology of multiple sclerosis (MS). Some factors, such as temperature, have been related to worsening of neurological symptoms. However, the information available in the literature regarding the relationship between MS relapses and meteorological conditions is scarce and refers to small groups of patients. **Objective:** The aim of this study was to determine whether there was a correlation between the number of MS relapses and some meteorological factors (maximum and minimum temperatures, atmospheric pressure, and humidity) in a Portuguese population. **Methods:** A retrospective study, from January 2004 to December 2007, analysing 414 MS relapses in 250 (179 females) patients was performed at the Santa Maria Neurology Department in Lisbon. Data was collected from the Neurology ward discharge notes and from the Outpatient steroid administration registries. The mean number of relapses per month, mean maximum and minimum atmospheric temperatures, mean humidity and atmospheric pressure per month were determined. A multivariate analysis of variance (MANOVA) test was used to analyze the monthly distribution of MS relapses. A multivariate analysis was performed to investigate whether there was a relationship between MS relapses and the different meteorological variables. **Results:** There was no significant

difference between months regarding the number of relapses (maximum of 48 relapses occurring in January and May and a minimum of 27 in June). No correlation was found between any of the meteorological variables and the number of relapses. **Conclusions:** A higher frequency of MS relapses in the warmest months of the year has been reported in several series of patients including those from Spain. To the best of the authors' knowledge our series is the largest addressing the influence of temperature, humidity and atmospheric pressure on MS relapses. We have analyzed for the first time this issue in a Portuguese population. The number of MS relapses seems to be unrelated to meteorological factors.

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The French prevalence gradient of multiple sclerosis according to gender

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Background: The distribution of multiple sclerosis (MS) is heterogeneous between the countries. However, these data are not comparable because they are obtained with different methodologies. The main health insurance system (Caisse Nationale Assurance Maladie, CNAM), which insures 84% of the French population, supplies us with data on MS patients declared to have a chronic disease in France in October 2004. **Objective:** To compare MS prevalence standardized by age and gender in different geographic areas (departments) using the same methodology. **Methods:** We have calculated national MS prevalence (NMSP) standardized by age and gender, prevalence in each department and the standardized incidence ratios (SIRs). The SIR is obtained by applying the NMSP to each department to calculate expected cases). Thus, we can compare them with observed cases throughout France. The SIR obtained is compared with the chi-squared test. **Results:** NMSP was 95.66 per 105 [CI 95.22–96.09] patients insured by CNAM, 139.90 [134.19–135.62] for women and 54.12 [53.66–54.58] for men [sex ratio (SR) 2.58]. SIR was statistically significantly superior to 1 (that is, observed cases are superior to expected cases) in northeast departments and inferior to 1 in southwest departments in France. Concerning the distribution of SIRs by gender, the gradient was identical except in some departments (Aisne, Allier, Creuse, Doubs, Eure et Loire, Haute Marne, Haute Saône, Indre, Loiret, Meuse, Sarthe, Territoire de Belfort, Yonne) with a SIR superior to 1 only for women. **Conclusions:** This study confirms the existence of a gradient of MS prevalence in France in a representative French population. Moreover, it shows differences in SIRs by gender in certain departments of high prevalence of MS where women are more often affected than men. These differences are not yet explained, but it could be due to increase of incidence of MS among women.

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Antiviral activity of interferon beta treatment in multiple sclerosis patients

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Background: Viral infections have been appointed as the main component of environmental susceptibility to multiple sclerosis (MS). Interferon beta has antiviral, immunomodulatory and antiproliferative properties and it is able to modify the natural course of the disease. **Objective:** To analyze the antiviral activity of interferon beta and to correlate it with its clinical efficiency in MS patients treated with interferon beta 1b for two years. **Methods:** Pairs of blood and serum samples were collected from 54 MS patients along seven programmed visits in two years: one before the start of the treatment (basal visit) and six during interferon beta treatment. In the case of relapse, another pair of samples was collected. Human herpes virus 6 (HHV6) and Epstein-Barr virus (EBV) genomes were detected by quantitative polymerase chain reaction (qPCR). **Results:** HHV-6 prevalence in blood samples decreased from 66.6% at basal visit to 40% at

24 months ($p=0.0001$). In serum samples, it decreased from 18% to 10.3% along the study period ($p=0.1$). Moreover, in healthy blood donors, the HHV-6 prevalence in blood was 22.1% ($p=0.005$), whereas in serum no positive sample was found ($p=0.001$). EBV prevalence in blood only presented slight differences between visits. Any positive sample was found in the serum. Moreover, we also found that in samples obtained at non-programmed visits from patients in relapse the prevalence of HHV-6 was significantly higher than in programmed visits: 81.8% in blood ($p=0.009$), 63.3% in serum ($p<0.001$). Regarding EBV, its prevalence in relapses did not differ from samples in remission ($p<0.001$). Furthermore, 55.5% of patients with reactivations of HHV-6 experienced at least one relapse during the follow up versus 13.9% of patients without viral reactivations. **Conclusions:** Clinical efficiency of interferon beta treatment seems to be linked to its antiviral activity against HHV-6, suggesting a possible role of this virus in the pathogenesis of MS.

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Pregnancy reverts the imbalanced expression of inflammation-related genes: the GEXPRIMS study

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Background: In order to gain better insight into the biological mechanisms underlying the pregnancy-related decrease in disease activity, we have previously analyzed the gene expression profiles in peripheral blood mononuclear cells (PBMCs) from nine multiple sclerosis (MS) patients and five healthy controls using microarray technology. Expression of 347 genes was found to be altered in PBMCs of non-pregnant MS patients with respect to healthy controls; complementary changes in expression occurring during pregnancy reverted this imbalance particularly for eight transcripts, namely SOCS2, TNFAIP3, NR4A2, CXCR4, ZFP36L1, RASA4, FAM49B, and DKFZP434A0131. All of these genes are involved in inflammation. **Objective:** To corroborate and extend by real-time polymerase chain reaction (RT-PCR) the findings in a larger cohort of women. **Methods:** Quantitative PCR measurements were performed in 24 women with MS and 10 healthy controls. Women were followed during their pregnancy and samples were obtained before pregnancy and at the third, sixth, and ninth month of gestation. **Results:** Five gene expression profiles from RT-PCR agreed with the previous microarray profiles; significant changes in expression were for TNFAIP3, NR4A2, SOCS2, CXCR4, and FAM49B (analysis of variance (ANOVA): all $p\leq 0.0136$). Longitudinal analysis showed that pregnancy reverted the imbalance within the third month of gestation (Mann-Whitney: all $p\geq 0.345$). In contrast, the remaining three PCR results did not agree with those indicated by microarray analysis, as genes did not show a pregnancy-related regulation. Four (16.7%) of the 24 MS patients had a relapse during pregnancy, mostly in the first trimester. The latter showed different expression profiles when compared to relapse-free patients: in these patients gene expression imbalance was reverted later in the pregnancy, that is, at the sixth or even ninth month. **Conclusions:** Data indicate for the first time that pregnancy regulates inflammation-related genes, mostly by inducing anti-inflammatory molecules. This specific pattern of expression seems to be associated to the pregnancy-related decrease in disease activity.

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Relapses are more frequent in pediatric-onset than adult-onset multiple sclerosis

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Background: Disease progression is slower in patients with pediatric-onset (PO) multiple sclerosis (MS) compared with adult-onset (AO) MS. It is unclear whether this disparity is due to differences in relapses

between these two groups. Prior studies examining the effect of age of MS onset on relapse rates provide discrepant results. None have controlled for use of disease-modifying treatment (DMT). **Objective:** To compare relapse rates in patients with POMS (<18 years old at first symptom onset) and AOMS. **Methods:** Patients seen at the Adult and Pediatric MS Centers at Brigham and Women's and Massachusetts General Hospitals, respectively, up to 12 months from first symptom onset in July 2001 or later and followed for at least 12 months were included. Data was abstracted from a clinical database and validated by one of the authors. Annualized relapse rates (ARRs) were compared between POMS and AOMS patients using the proportional means model. **Results:** Data from 110 AOMS and 21 POMS patients were included. There were significantly more non-Caucasian patients in the POMS group (66.7% versus 94.5%; $p=0.001$). The overall ARR in the POMS group was significantly higher than the ARR in the AOMS group (1.13 versus 0.40; $p<0.0001$) with an adjusted rate ratio of 2.81 (95% CI: 2.07, 3.81). When time on DMT was controlled for in the analysis, the difference remained highly significant (adjusted rate ratio of 2.82; 95% CI: 2.08, 3.83; $p<0.0001$). When age at MS onset was treated as a continuous variable, a highly significant association between age and ARR was observed ($p<0.0001$). There were no significant differences in relapse location between the two groups. **Conclusions:** Relapses are more frequent in patients with POMS compared with AOMS in the DMT era. Differences in relapse rates do not appear to explain slower disease progression in POMS patients.

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Time to regression from trial-defined disease progression in multiple sclerosis is dependent upon baseline Expanded Disability Status Scale

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Background: Trials in multiple sclerosis (MS) utilize a one-point progression of the Kurtzke Expanded Disability Status Scale (EDSS) sustained at 3 months as a measure of permanent disability progression. Utilizing data from a prospective multi-center cohort study, the MSBase Investigators have identified that 3-month confirmed disability progression in relapsing-remitting MS (RRMS) patients reverts to baseline in 25% of cases at 2 years, and >40% at 5 years, with no significant difference seen with 6 month confirmed progression. It is unknown whether baseline EDSS influences the probability of regression after 3-month confirmed progression. **Objective:** To determine whether long-term sustained progression after 3-month confirmation in MS is dependent on baseline EDSS. **Methods:** At the time of analysis, the MSBase Registry database consisted of 4801 patients. Baseline EDSS was defined as the first recorded EDSS. Sustained EDSS progression was defined as an increase of ≥ 1 (or ≥ 1.5 if baseline EDSS = 0), with confirmation at 3 months. Time to regression back to baseline EDSS was compared for different baseline EDSS values using Cox regression analysis. **Results:** At least one confirmed progression during the observational period was seen in 1137 cases (24%). The rate of regression after a 3-month confirmed progression for baseline EDSS scores ≤ 1.0 was assigned a hazard ratio (HR) of 1.0. Regression was significantly slower for baseline EDSS ≥ 4.0 (HR 0.542, 95% CI 0.374–0.785, $p=0.001$) or 2.5–3.5 (HR 0.688, 95% CI 0.496–0.955, $p=0.025$). A baseline EDSS of 2.0 showed no statistical difference (HR 1.1, $p=0.6$). Patients with a baseline EDSS of 1.5 had a significantly higher probability of regression, with a HR of 1.73 (95% CI 1.2–2.6, $p=0.006$). **Conclusions:** Recovery back to baseline (regression) after a 3-month confirmed progression is dependent on baseline EDSS. The probability of long-term sustained disability progression after 3-month progression is greatest if the baseline EDSS score is ≥ 2.5 (indicative of a moderate level of disability in more than one functional system), intermediate for EDSS scores of 0, 1 or 2 and, interestingly, lowest for cases with a baseline EDSS score of 1.5.

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WITHDRAWN

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Vestibular evoked myogenic potential in multiple sclerosis patients

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Background: Patients with multiple sclerosis (MS) frequently report symptoms which could be related to vestibular disorders in the course of their disease. So it could be considered that vestibular evoked myogenic potential (VEMP), which assesses the vestibulospinal pathway, could be abnormal in numerous of the MS patients. **Objective:** The aim of our study was to determine the sensitivity of the VEMP abnormality in MS patients as well as its relation with clinical signs, course of disease and other evoked potentials. **Methods:** The VEMP test was performed in 20 definite relapsing-remitting MS patients. Unilateral clicks (0.1 ms, 2 Hz, 95 dB NHL), delivered during a steady sternocleidomastoid (SCM) contraction evoked myogenic responses which consist of two components, an initial positive (P1 or P13) followed by a negative (N1 or N23). Individuals were requested to be seated on the chair and rotated their heads to the opposite side of the stimulated ear to activate the SCM. We applied 200 stimuli in each ear by click and repeated for two consecutive cycles to show its reproducibility. VEMPs were recorded from symmetrical sites over the upper half of each SCM belly by means of a surface electrode with a reference over the medial clavicle and ground electrode over the upper sternum. The test was performed in 20 healthy controls whose demographic data had no significant difference to that of the MS group to find the normal values. Mean $+2.5SD$ (standard deviations) for latencies and mean $-2.5SD$ for amplitudes of VEMPs were obtained. **Results:** An abnormal VEMP was observed in 14 out of 20 MS patients (70%). This could be compared with visual and auditory brain stem evoked potential which was abnormal in 75% and 65% of participants, respectively, in our study. On the other hand VEMP abnormalities were not statistically related to the course of disease or clinical signs or symptoms of the patients, but there was a significant relation between its abnormality with duration of disease ($P < 0.01$). **Conclusions:** It seems that VEMP has a high sensitivity for diagnosis of MS disease.

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Modeling of staying times for multiple sclerosis patients

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Background: Knowledge regarding the time multiple sclerosis (MS) patients are in each different disease level as well as regarding the demographic and clinical covariates influencing this time is important for evidence-based decision support. **Objective:** The aim was to develop a methodology to analyze times MS patients remain at different disease levels subject to covariates taking into account that disability accumulates faster in the progressive phase of MS than in the relapsing-remitting phase. The ability to handle interval censored data resulting from the fact that the disease course is only observed at a certain number of examinations was essential. **Methods:** A model was developed which includes a random change point for each patient at which the change to the progressive phase occurs. The disease course is split according to this and the data from the different phases is assigned to two differing Markov processes, each describing the staying times depending on covariates. This model was applied to data of 1043 MS patients seen in London, ON, Canada, between 1972

and 1984. To investigate whether any of the characteristics gender, age at onset, number of attacks in the first two years after disease onset and certain initial symptoms have an effect on the staying times, a heuristic forward selection procedure was applied. **Results:** Age at onset and number of attacks were selected by the procedure. For example, for a patient with age at onset between 20 and 29 years and one attack in the first two years, an expected staying time at the level covering Kurtzke disability status scale (DSS) score of 0–2 of 34 years resulted for the relapsing-remitting phase. When facing two attacks, the expected staying time shortens to 26 years. **Conclusions:** The methodological concept and the first results are promising. However, the statistical assumption underlying the selection procedure is not assured and the results have not been validated so far. Therefore, simulation studies will be conducted in order to examine the properties of the selection procedure. Moreover, the model shall be applied to more comprehensive data to investigate the validity of the results and to also enable a reliable assessment of the transition from the level comprising DSS 6–7 to that spanning DSS 8–10.

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Classical twin studies in multiple sclerosis: time to move on?

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Background: The classical twin study quantifies genetic and environmental components, and may guide further research, provided that the methodology is correct. Despite several multiple sclerosis (MS) twin studies, a clear message does not emerge. **Objective:** To evaluate the larger population-based twin studies in MS. **Methods:** We evaluated all MS twin studies but excluded (i) volunteer-based collections because of their tendency to systematic bias and (ii) population-based studies with less than 50 pairs. This left six population surveys totalling more than 2000 twins from France, the UK, Canada, Denmark, Italy and North America. Where possible the raw data were re-analyzed using Structural Equation Modelling to enable comparison of heritability (h^2) estimates. **Results:** If confidence intervals are ignored, the data from France and North America indicate weak genetic factors (h^2 : 0.25–0.31); from Italy they are modest (h^2 : 0.45); and from the UK, Canada and Denmark they are substantial (h^2 : 0.53–0.76). Confidence intervals are large because of small sample sizes. **Conclusions:** Although all six studies indicate some degree of genetic influence, it is not possible to quantify this, principally because of small sample sizes which in turn relate to the relative rarity of MS or the size of individual populations. Common biases in collection methods tend to elevate genetic estimates. We suggest that MS twin prevalence studies should now be replaced by the co-twin control method, where the healthy co-twin provides a near-perfect matched control.

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Steps towards validation of a severity-based multiple sclerosis classification system using the Ian McDonald multiple sclerosis database

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Background: The global Multiple Sclerosis Severity Score (MSSS) is a severity scale which relates clinical disability to disease duration [1]. Recently, an MSSS-based classification system was proposed to define subpopulations of varying disease severity [2]. **Objective:** To independently validate the MSSS and classification system within a meta-analysis using the Ian McDonald MS Database of Sylvia Lawry Centre, with focus on supposed MSSS stability over time. **Methods:** About 12 000 Expanded Disability Status Scale (EDSS) assessments obtained from 1134 clinical trial placebo arm patients followed for up to 5 years (median: 2 years) were available for this study. We re-calculated 'local' MSSS tables at study entry and exit (MSSS-McD/entry and

MSSSMcD/exit) which were then compared with the published table (MSSS-Rox). Variations between tables were assessed using the concordance correlation coefficient (CCC, with CCC=1 corresponding to perfect agreement), which combines measures of precision and accuracy, and Bland-Altman plots for association examinations. Changes over time within severity subgroups were assessed by weighted scatterplot smoothing. **Results:** Mean disease duration at baseline was 9 years (range: 0–40) with corresponding EDSS scores varying from 0 to 8 (mean: 3.7). Published and recalculated MSSS tables (MSSS-McD/entry vs. MSSS-Rox) were similar, with CCC=0.92 at baseline indicating almost perfect agreement. Considering overall MSSS variation over time (MSSS-McD/entry versus MSSS-McD/exit), moderate agreement was observed (CCC=0.7). Considering changes within MSSS severity subgroups, slopes of mean trajectories of longitudinal MSSS approximated zero, demonstrating stability over time. **Conclusions:** We reproduced the published MSSS table on an independent dataset of untreated patients. In exploratory analyses stability over time within severity groups was confirmed, although individual MSSS fluctuations exist. In further investigations we will assess predictive validity of MSSS and severity subgroups in order to provide evidence-based decision support for patient care, from prognostic estimates to treatment individualization. References [1] Roxburgh RHSR et al. *Neurology* 2005; 64: 1144–1151. [2] Herbert. *Mult Scler* 2006; 12: S21. **Supported by:** Hertie Foundation, National Multiple Sclerosis Society (USA) Pilot Award to JH, Porticus Foundation.

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Two polymorphisms of angiotensinogen gene and their association with multiple sclerosis

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Background: Multiple sclerosis (MS) is a chronic inflammatory disease which is characterized by the imbalance of pro- and anti-inflammatory factors. Angiotensin II (AT II) and its precursor angiotensinogen (ATG) whose are known as key factors for blood pressure control, are also widely involved in the inflammatory process. AT II directly activates infiltrating immunocompetent cells, recruits inflammatory cells into the tissues, increases vascular permeability, and induces the expression of pro-inflammatory and pro-oxidant genes. **Objective:** The aim of the study was to investigate the association between A(-6)G, M235T polymorphisms in ATG gene and MS. The presence of alleles A and T increases the final plasma levels of ATG. **Methods:** A total of 194 unrelated patients (50 men, 144 women) with definitive MS according to McDonald criteria and 126 healthy controls matched for age and sex were genotyped for two polymorphisms of ATG gene. The cohort included patients with mean age 37 years. Genotyping was performed using polymerase chain reaction (PCR) with restriction analysis. Genotype frequencies were compared using chi-squared and Fisher's exact tests. **Results:** We observed remarkable differences in double genotype coincidence in the case-control comparison. Statistically significant differences were found in three of the four possible heterozygous combinations: GAMM [P=0.003, odds ratio (OR)=6.34, 95% confidence interval (CI) 1.45–27.82], GATT [P=0.0009, OR=7.53, 95% CI 1.73–32.69], AAMT [P=0.0012, OR=8.98, 95% CI 1.15–69.51] whose were more frequent in MS patients versus controls and in two homozygous combinations: GGMM [P=0.00006] and AATT [P=0.0038] whose were more frequent in controls. **Conclusions:** Many processes are involved in the regulation of the final AT II concentration. The first step in this path is the regulation through functional polymorphisms of ATG gene.

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Risk alleles for multiple sclerosis in a Dutch genetic isolate

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Background: The study of multiple sclerosis (MS) in genetic isolates may facilitate the identification of novel genes that determine MS risk in the general population. **Objective:** To identify novel MS risk genes. **Methods:** We ascertained 46 MS patients, with regular clinical phenotypes, in a Dutch genetic isolate according to standard diagnostic criteria. Residents in this isolate are generally related via multiple lines of descent. We performed a screening phase of a genomewide screen (GWA, 250K SNP Affymetrix) in 46 MS patients and 194 controls. We compared our results with the results from the International MS Consortium. The single nucleotide polymorphisms (SNPs) that were not available from the Affymetrix GeneChipMapping 250K Array were imputed, using HAPMAP CEU haplotypes as a reference. A novel risk gene was confirmed which we further validated in an independent set of 1318 MS patients from the Canadian Collaborative Project on the Genetic Susceptibility to MS (CCPGSMS), performing transmission disequilibrium test (TDTs). We also replicated 21 SNPs that gave highest odds ratios (ORs) in the screening phase of our GWA in an independent set of 479 Dutch MS patients and 600 controls. **Results:** We checked our data from the screening phase of our GWA study with the 17 MS SNPs that gave highest statistical association in the recent International GWA study. Apart from the human leukocyte antigen (HLA) locus, two SNPs, both in the EVI5 gene on chromosome 1, gave significant p-values. The risk effect of EVI5 was confirmed in an independent set of Canadian MS patients. The replication phase of our genomewide scan yielded seven genetic variants with significant results, not overlapping with the results of the International MS Consortium. **Conclusions:** This study confirms EVI5 as a fourth risk locus for MS. In addition, seven new genetic variants were identified as heritable risk factors for MS.

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ApoE ε4 positive multiple sclerosis patients develop more gray matter and whole brain atrophy: a 4-year longitudinal study using the 15-year disease onset model

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Background: Multiple sclerosis (MS) is a disease with considerable individual variation, and genetic background plays a key role in disease susceptibility and severity. **Objective:** To evaluate the relationship between ApoE genotype and the evolution of different MRI parameters in relapsing-remitting MS over 4 years. **Methods:** We investigated a group of 150 patients from the original ASA (Avonex-Steroid-Azathioprine) study that completed 4-year follow-up. The mean age was 30 years, disease duration 44 months (minimum 0.7; maximum 179 months), Expanded Disability Status Scale (EDSS) was 2.0 and an annualized relapse rate before study was 2. Measures of brain parenchymal volume (BPV), gray matter volume (GMV), white matter volume (WMV) and peripheral gray volume (PGV) were obtained by SIENAX. In addition, T2-lesion and lateral ventricle volumes were assessed with the semiautomated methods. Using a mixed effect model, a 15-year evolution of MRI parameters with respect to the disease onset was built. Different demographic parameters (age, gender, disease duration) were used as covariates to assess

inter-individual variation. **Results:** We identified 36 ApoE ϵ 4 positive and 114 ApoE ϵ 4 negative patients. Over the 4-year interval, a borderline trend for higher decrease of GMV was found in ApoE ϵ 4 pos patients ($p=0.074$). In the 15-year disease onset model, the higher decline in GMV evolution became highly significant in ApoE ϵ 4 positive patients ($p=0.009$). Moreover, there was significant difference in the higher decrease of PGV ($p=0.003$) and BPV ($p=0.029$) in ApoE ϵ 4 pos patients. No differences were found for the lesion measures. **Conclusions:** Our results showed higher development of brain (in particular, GM) atrophy in ApoE ϵ 4 pos MS patients over 4 years and in the 15-year disease onset model. **Supported by:** Biogen Idec, Gedeon Richter, 'Neuropsychiatric aspects of neurodegenerative diseases' (MSM 0021620849).

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Sleep-related disorders in multiple sclerosis

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Background: A few, mostly uncontrolled, studies have investigated the prevalence of sleep-related disorders in multiple sclerosis (MS), with conflicting results. **Objective:** To determine whether most common sleep disorders are more prevalent in MS patients than in control subjects and to establish their disease-related determinants. **Methods:** We performed a single center, case-control study. A blinded neurologist asked MS patients and age- and gender-matched controls (acquaintances not relatives of patients) for the main sleep disorders, using a semistructured interview. Student's T test, chi-squared test, and logistic regression were used for data analysis. **Results:** Fifty MS patients (72% female, female:male ratio 2.6:1; mean age 40.7 years, range 18–67) and 50 age- and sex-matched controls (mean age 40.7, range 23–63) were included. Forty-five patients (90%) had relapsing-remitting MS and five patients (10%) had secondary progressive MS. Mean age at MS onset was 31.4 (range 14–52) years, mean MS duration was 9.2 (range 0–25) years, mean Expanded Disability Status Scale (EDSS) score was 2.2 ± 1.36 . Sleep latency, total sleep time, presence of naps, duration of naps, excessive daytime sleepiness, insomnia, bruxism, snoring/sleep apnoea were not significantly different in MS patients and control. Restless legs syndrome (RLS) was significantly more frequent in MS patients (32.0% versus 14.0%, odds ratio (OR) = 2.3, 95% confidence interval (CI) = 1.0–5.1). In the multivariate analysis, RLS correlated also with increasing EDSS ($p=0.01$) and female gender ($p=0.03$). **Conclusions:** In our case series of mild to moderate MS we found no increase in sleep disorders, except for RLS. In particular insomnia was not increased, probably due to sleep preservation in less advanced stages of the disease.

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The correlation of multiple sclerosis prevalence and distance from a river

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Background: Multiple sclerosis (MS) is a disease with a poorly understood genetic and environmental contribution. Some reports suggest a high prevalence of MS along the course of rivers. Isfahan is situated along the banks of the River Zayandehrood with medium MS prevalence. We aimed to explore the correlation between MS prevalence and distance from this river. **Objective:** To determine the correlation between MS prevalence and distance from the River Zayandehrood. **Methods:** Demographic information was collected from the Isfahan MS Society registry between April 2003 and December 2007. All patients were verified according to the McDonald criteria. For each town we calculated the MS prevalence rate and measured its distance from the river bank. Towns were classified into three groups: (a) beside the river bank; (b) Less than 50 km from the river; (c) more than 50 km from the river. **Results:** Prevalence rates (per 100,000 inhabitants) were as follows. Group A: Isfahan (66.3); Lenjan (36.6).

Group B: Mobarake (43.6); Najafabad (28.1); Felavarjan (22.4); Khomeini shahr (20.0) Shahin shahr (19.8); Group C: Naein (3.68); Kashan (2.35); Ardestan (1.27); Semirrom (1.37); Golpayegan (1.22). There was a significant negative correlation between MS prevalence and distance from the river bank ($p<0.000$; $r = -0.89$). **Conclusions:** A significant association between proximity to the River Zayandehrood and prevalence of MS was shown. This may relate to a variety of neurotoxic heavy metals or agrochemicals contained in industrial effluent, agricultural waste or untreated human sewage. The association may not be casual and could relate to other variables such as socio-economic group, quality and density of housing. These factors need further exploration.

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Sex ratios in multiple sclerosis records are robust epidemiological markers

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Background: Sex ratios are skewed toward female preponderance in many autoimmune diseases. In multiple sclerosis (MS) female to male sex ratios are accepted to be approximately 2:1, but are highly variable even within populations with similar prevalence rates. Possibly, variations in sex ratios carry informational value on epidemiological patterns which can be utilized in MS research. **Objective:** This study evaluates whether variations in sex ratios in comparison to prevalence rates measures may be an appropriate analytical variable for studies in MS epidemiology. **Methods:** The analysis was based on published records from the Danish Multiple Sclerosis Registry as of 1996 and records of admissions to Danish hospitals with the diagnosis MS in the period January 1st 1978 to December 31st 2000 from The National Patient Registry, Denmark. A statistical analysis of the relationship between proposed prevalence rates, age at admission to hospital and sex ratios was performed for 15 counties. **Results:** This study shows that sex ratios in comparison with prevalence rates measures are robust markers in Danish epidemiological records. Choice of measures of prevalence rates, such as crude and age standardized prevalence rates, will have a marked effect on the relative level of prevalence among Danish counties. As a consequence prevalence data, adjusted in accordance with given specific hypotheses on MS causality and/or development, leads to an uncountable number of uncorrelated prevalence rate outcomes. Each outcome may through statistical analyses point to several causative factors. Sex ratios, in several widely different prevalence rate measures are highly correlated. Hence, these markers are likely to point to far fewer possible factors. Finally, it was observed that a second-order relationship exists between sex ratios and MS incidence and prevalence rates in the Danish population suggesting a systematic relationship. **Conclusions:** Sex ratios seem to be a robust analytical parameter in Danish MS epidemiology.

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Environmental factors determine multiple sclerosis risk in migrants to Israel

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Background: Multiple sclerosis (MS) is more common in developed than in underdeveloped regions. Several studies have shown that MS frequency may depend on age at immigration from low to high MS frequency zones. Israel's population includes immigrants from high and low MS frequency regions. MS is high in native-born Jewish Israelis, Israeli immigrants from Europe and in Israeli-born Jewish offspring of Afro-Asians (AA). **Objective:** To investigate whether MS risk depends on age at migration or length of exposure to lifestyle/geoclimatic factor(s). **Methods:** The Israeli National MS Registry (INMSR) listed 3935 MS patients with onset after 1950; 476 were AAs. We

analyzed MS frequency by age at immigration and length of residence in Israel. We also compared the incidence rate of MS in 300 Israeli-born individuals whose parents were AAs and immigrated to Israel less than or more than five years before the birth of the Israeli born offspring with MS (assuming adaptation takes about 5 years). The Central Bureau of Statistics provided the total population at risk for MS. **Results:** Among AA immigrants, the incidence of MS decreased linearly with increasing age at immigration and increased with increased length of exposure to Israel's environment; there was no sharp change at any particular age. MS incidence in Israeli-born offspring was highest when both parents immigrated to Israel from AA more than 5 years before the child's birth in Israel and was lowest when both parents immigrated to Israel less than 5 years before birth of the child in Israel (annual IR of 2.3 versus 0.9/1 000 000). Adopting the Israeli lifestyle appeared to increase risk of MS in these offspring. **Conclusions:** Cumulative exposure to Israeli lifestyle/geo-climatic factors appeared to determine MS with no critical exposure age. **Supported by:** National Multiple Sclerosis Society (USA) (grant RG3647-A-8).

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IL7RA polymorphisms and disease severity in multiple sclerosis
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Background: We recently confirmed an association between a functional non-synonymous single nucleotide polymorphism (SNP) (rs6897932) of IL7RA and susceptibility to multiple sclerosis (MS). **Objective:** To uncover associations between IL7RA SNPs and severity of MS. **Methods:** We genotyped nine SNPs in IL7RA in a population-based cohort of 208 patients from USA, and 292 patients from Northern Ireland. In the population-based cohort, disability information (measured using the Expanded Disability Status Scale (EDSS)) collected at two time points 10 years apart was transformed into a ranked severity scale (RSS) based on duration. Outcome measures were: time to secondary progressive MS; time to EDSS \geq 3 and EDSS \geq 6; RSS; extremes of lower and upper third of RSS distribution (restricted to information available for having EDSS \leq 3 or EDSS \geq 6 after 10 years of disease in the Belfast cohort). All typing was performed at the Belfast site using Sequenom technology. **Results:** Rs6897932, previously associated with disease susceptibility, was not associated with the outcome measures in either population. Rs7711202 G allele was associated with worse RSS values; as a continuous variable ($p=0.017$) and as upper and lower third extremes (odds ratio (OR):3.2; 95% confidence interval (CI): 1.4–7.3, $p=0.007$); with shorter time to secondary progression ($p=0.035$) but not with time to EDSS=3 ($p=0.066$) or time to EDSS=6 ($p=0.088$). Rs11567762 G allele was independently associated with time to secondary progression ($p=0.031$), with time to EDSS=3 ($p=0.014$) and time to EDSS=6 ($p=0.031$), worse RSS values as a continuous variable ($p=0.025$) but not as upper and lower third extremes ($p=0.117$). Neither of these SNPs was associated with outcome measures defined in the Northern Irish population. None of the other SNPs were associated with disease outcome. **Conclusions:** A modest association may exist between IL7Ra polymorphisms and disease severity in MS.

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Sequencing of IL2RA in multiple sclerosis patients

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Background: A whole genome association study in MS has identified two intronic single nucleotide polymorphisms (SNPs; rs12722489 and rs2104286) in the interleukin-2 receptor-alpha-chain gene (IL-2RA) that are associated with susceptibility to multiple sclerosis (MS), albeit with small effect size (odds ratio (OR) 1.2). We hypothesized that the association of the intronic SNPs is due to linkage disequilibrium with genetic variants that affect protein expression or structure of IL2RA.

Objective: To uncover mutations in genomic DNA encoding IL2RA that may account for the recent association of two intronic SNPs with susceptibility to MS. **Methods:** Thirty six Caucasian patients were randomly selected from a population-based cohort of 221 MS patients from Olmsted County, Minnesota, set to detect minor allele frequency of 0.03. The promoter (1000 bp); exons 1–8; exon-intron splice sites and three untranslated region were sequenced in forward and reverse directions by the dye-terminator technique. Results were analyzed using the Mutation Surveyor v.3.12 (Softgenetics, State College, PA) with settings sensitive to heterozygous mutations. The frequency of the risk allele for both SNPs were tested in the selected patients by restriction fragment length polymorphism assays. **Results:** We obtained reliable sequences in 27 patients (powered to detect an allele frequency 0.04). The frequency of the risk allele among the selected patients was 59.2% for rs12722489 and 84.6% for rs2104286. We detected 12 previously reported SNPs in IL2RA with frequencies consistent with those reported in public databases (<http://www.ncbi.nlm.nih.gov/SNP>). Two patients (7.5%) had an exon 2 SNP (rs4308625) and two patients had an exon 4 SNP (rs2228149), both synonymous. The only known IL2RA missense SNP (exon 8; rs 12722712; minor allele frequency of 0.007) was not polymorphic in our patients. We did not identify any novel exonic SNPs. **Conclusions:** We did not identify SNPs that would affect protein structure or expression of IL2RA that would account for the recent association of two intronic SNPs with susceptibility to MS. **Supported by:** National Multiple Sclerosis Society (USA).

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Disease course rather than age at onset as the main disability factor in late-onset multiple sclerosis

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Background: It is generally assumed that late onset is an adverse prognostic factor in MS. However, being frequently associated with a progressive course, it is difficult to assess its individual contribution to prognosis. **Objective:** To balance the contribution of late onset versus primary progressive disease course on disability progression in MS. **Methods:** Using the longitudinal, population-based LORSEP cohort of definite or probable MS patients (Poser's criteria), we assessed time from disease onset to irreversible EDSS 4 and 6 according to age at onset (<50 vs \geq 50 years, respectively young-onset MS (YOMS) and late-onset MS (LOMS)) and initial disease course (relapsing-remitting (RR) versus primary progressive (PP)). Statistical analyses were carried out using Kaplan-Meier estimates (95% confidence interval (CI)) and multivariate Cox regression model. **Results:** Among 3602 patients, 3356 (93%) had YOMS and 246 (7%) had LOMS. The percentage of RR cases in each group was 90.9% and 52%, respectively. In the RR group, time to EDSS 4/6 was 15.7 \pm 0.3/23.3 \pm 0.5 years in YOMS but only 7.0 \pm 0.6/10.8 \pm 0.9 years in LOMS and time from onset to secondary progression was 23.3 \pm 0.6 years in YOMS and 9.6 \pm 0.6 years in LOMS; for PP patients, time to EDSS 4/6 was 4.8 \pm 0.3/10.8 \pm 0.6 years in YOMS and 3.4 \pm 0.3/7.8 \pm 0.5 years in LOMS ($p<0.0001$ for all comparisons). Nevertheless, multivariate analysis showed that a PP disease course (versus RR) is a much stronger prognostic factor of disease progression (hazard ratios for time to EDSS 4=8.6 (6.0–12.4) and 17.8 (7.2–43.9) for time to EDSS 6) than LOMS (versus YOMS) (hazard ratios for time to EDSS 4=1.9 (1.7–2.1) and 2.2 (1.8–2.7) for time to EDSS 6); ($p<0.0001$ for all comparisons). **Conclusions:** These data support PP disease course rather than older age at onset as the main prognostic factor for disease progression in LOMS.

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Four familial cases with anti-aquaporin-4 antibodyMin Su Park¹, Ho Jin Kim¹, Byoung-Jo Kim², Jae-Young An³¹Department of Neurology, Research Institute and Hospital of National Cancer Center, Goyang, Gyeonggi-do, South Korea; ²Department of Neurology, Korea University College of Medicine, Seoul, South Korea; ³Department of Neurology, Catholic University College of Medicine, Seoul, South Korea

Background: Neuromyelitis optica (NMO) is an idiopathic inflammatory demyelinating disease of the central nervous system and the disease-specific biomarker, NMO-IgG, binds to aquaporin-4 (AQP4) water channel. Most cases of NMO are sporadic and familial cases have been rarely reported. **Objective:** We describe two familial cases of identical twin sisters and a brother-sister pair, which suggest a genetic contribution to this disorder. **Methods:** We carried out a review of the case reports of the two families. **Results:** Family 1 (twin sisters): a 33-year-old woman experienced recurrent attacks including longitudinally extensive transverse myelitis (LETM), optic neuritis (ON), and multiple brain symptoms. She had six severe attacks over 13 months in spite of various immunotherapies, and was unable to walk without assistance due to poor recovery from each attack. She fulfilled the diagnostic criteria for Sjögren syndrome (SS) and anti-AQP4 antibody was positive. She was treated with rituximab and had no further relapse over 26 months. Her disability was dramatically improved as demonstrated by a reduction in EDSS from 8.0 to 3.5. Her identical twin sister developed LETM at the age of 34. She had two more LETM over the next 8 months. She was also seropositive for anti-AQP4 antibody, and fulfilled the diagnostic criteria for SS. Rituximab was initiated, and has been relapse-free for over 12 months, resulting in a reduction in EDSS from 4.0 to 1.5. Family 2 (brother and sister): a 34-year-old woman had one attack of ON and four attacks of LETM over a period of 42 months. After treatment with rituximab, she has been relapse-free for 8 months, resulting in a reduction in EDSS from 3.5 to 2.0. Four months after she was treated with rituximab, her brother, a 36-year-old man presented with sensory symptoms. Spinal magnetic resonance imaging (MRI) revealed an abnormal cord lesion extending less than two vertebral segments. He experienced another period of mild myelitis 4 months later. Learning from his sister, he was treated with rituximab and has had no further relapse. Both of them were seropositive for anti-AQP4 antibody. **Conclusions:** Our four familial cases suggest the genetic influence on NMO spectrum disorder with anti-AQP4 antibody. In addition, the beneficial effect of B cell depletion by rituximab emphasizes an important role of B cell in this disorder.

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Changes in diagnostic and referral delays in multiple sclerosis could impact incidence and prevalence studies: findings from Western and Eastern CanadaElaine Kingwell¹, Elaine Roger², Pierre Duquette², Peter Rieckmann¹, Helen Tremlett¹¹Medicine (Neurology), University of British Columbia, Vancouver, British Columbia, Canada; ²Hôpital Notre-Dame du CHUM, University of Montreal, Montreal, Quebec, Canada

Background: Diagnostic or referral delay can have an impact on: (i) incidence and prevalence studies in multiple sclerosis (MS); (ii) treatment options and opportunity for early intervention. **Objective:** To investigate changes in diagnostic or referral delays within two geographically distinct MS Canadian cohorts over a 20-year period. **Methods:** Patients with: definite or probable MS (Poser or McDonald criteria); onset age ≥ 18 years and first MS clinic visit between 1985–2004 were selected from: 1. the population-based British Columbian (BC) MS database (estimated to include 80% of MS patients in BC) and 2. the clinic-based Hôpital Notre-Dame (CHUM) database, Quebec. The primary outcome was referral delay - time from symptom onset to first clinic visit (available in both datasets); the secondary was diagnostic delay (available in CHUM only). Both delays were examined by year of first clinic visit, sex and age at onset of MS. Cohorts were analysed separately using analysis of variance (ANOVA) and non-parametric tests. **Results:** This study included 5446 patients

(3973, 73% women) from the BCMS cohort and 1326 patients (974, 73% women) from the CHUM cohort. Median referral delay did not differ significantly by sex (BCMS: 5 years; CHUM: 4 years), but varied by onset age, with younger patients having the longest referral delays ($p < 0.001$). Referral delay was significantly reduced over time in both cohorts ($p < 0.01$), by an average of 3 weeks per year (BCMS). However, women aged 45+ ($p = 0.02$) and men aged < 25 ($p = 0.04$) at onset of MS experienced the greatest reduction in referral delay (BCMS cohort). A similar trend in women with older onset age was observed in the CHUM cohort. Diagnostic delay was significantly reduced over time ($p < 0.001$), decreasing by an average 5 weeks per year (CHUM cohort). **Conclusions:** (1) If early intervention in MS is warranted, then referral delays for younger adult patients must be addressed. Whether delays represent under-recognition of MS symptoms in the community remains to be determined. (2) The potential for disproportionate changes in referral rates for older women and younger men (at onset) must be considered when conducting incidence and prevalence studies.

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Prevalence of multiple sclerosis in Lithuania and the influence of immunomodulating treatment on annual hospitalizationRasa Kizlaitiene¹, Valmantas Budrys¹, Gintaras Kaubrys¹, Ieva Runkauskaitė¹, Dalia Petrauskienė²¹Vilnius University Hospital Santariskiu Klinikos, Department of Neurology, Vilnius, Lithuania; ²Vilnius University Hospital Santariskiu Klinikos, Department of Informatics, Vilnius, Lithuania

Background: Multiple sclerosis (MS) is relapsing and often eventually progressive disorder of the white matter of the central nervous system. According to earliest published data of an epidemiologic MS study in Lithuania, the prevalence of MS was about 56 cases per 100 000 inhabitants. **Objective:** Our aim was to specify the number of patients with MS diagnosis during period 2004–2007 in Lithuania, to calculate the number of outpatient visits, hospitalizations due to MS and to reveal MS hospitalization tendencies, when the treatment with immunomodulating drugs was reimbursed since the year 2006. **Methods:** Using data from the Lithuanian Department of Statistics we calculated the prevalence of MS, the number of hospitalizations and hospitalized patients per year in Lithuania. **Results:** At the beginning of year 2008 the population of Lithuania was 3 361 100 inhabitants according to data from the Department of Statistics. The number of patients diagnosed with MS Lithuania, registered during the period 2004–2007 was 2621 (male 875, female 1746). We have found that the prevalence of MS in Lithuania is 78 cases per 100 000 inhabitants. So, Lithuania could be called an area of high MS prevalence. During year 2007, almost 500 patients with relapsing-remitting MS were treated with immunomodulating drugs: interferons beta (INF β) 378 patients, glatiramerum acetatis (GA) 72 patients and in MS clinical trials 50 patients. The number of MS outpatient visits per year was: 2004, 14 268; 2005, 15 054; 2006, 17 631; 2007, 17 764; the number of hospitalizations was 982, 967, 986, 933 cases and 774, 756, 756, 726 patients during this period, respectively. The number of hospitalizations in 2007 decreased by almost 6% compared with the previous period. We have collected data in Vilnius University Hospital and have found similar tendencies. **Conclusions:** We concluded that Lithuania belongs to an area of high MS prevalence. Since immunomodulating treatment with INF β and GA has started to be used widely, decreases in the hospitalization rate and number of hospitalized patients were observed indicating that immunomodulating treatment is effective in preventing relapses and expenses in relapsing MS treatment.

P148**A prospective study of the time to conversion to clinically definite multiple sclerosis in 172 Greek patients with clinically isolated syndrome**

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Background: Prospective studies of patients with clinically isolated syndrome (CIS) have not been systematically undertaken in Greece. As a result it is unclear whether Greek patients with CIS convert to clinically definite multiple sclerosis (CDMS) at rates similar to those observed in other better-studied populations. **Objective:** To assess the time to conversion to CDMS in a prospective cohort of disease-modifying treatment-naïve Greek patients with CIS and to compare our results with findings from better-studied populations. **Methods:** We used Kaplan-Meier analysis to study 172 Greek patients presenting with CIS and at least 2 T2 lesions on brain magnetic resonance imaging (MRI). Patients were prospectively followed-up during a period of 4 years (2004 to 2007) at the Department of Neurology, University of Athens. **Results:** Our patient cohort consisted of 119 women and 53 men (ratio 2.2:1). Mean patient age was 31.8 ± 8.4 years, mean age at onset 31.2 ± 8.4 years and mean disease duration 0.9 ± 0.8 years. Presenting symptoms were optic neuritis in 26.7%, brainstem/cerebellar dysfunction in 21.5%, isolated long tract sensory dysfunction in 28.5%, long tract motor dysfunction in 15.1%, multisymptomatic in 5.8% and others in 2.4%. The probability of conversion to CDMS was 53% at 24 months and 73% at 48 months. Mean time to conversion was 26.5 months. Time to conversion to CDMS was independent of sex ($p = 0.98$), symptoms at presentation ($p = 0.56$) and presence of cerebrospinal fluid (CSF) oligoclonal bands ($p = 0.22$). A trend was observed in patients with gadolinium enhancing lesions in their initial scan to convert sooner to CDMS ($p = 0.057$). **Conclusions:** The rate at which Greek patients with CIS suggestive of multiple sclerosis convert to CDMS is comparable to previous findings from other CIS patient cohorts published in the literature.

P149**Viral triggers of multiple sclerosis attacks**

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Background: Several observational studies have linked the common cold with multiple sclerosis (MS) attacks. However, no specific viruses have been conclusively linked to MS attacks. Polymerase chain reaction (PCR) technology now allows the specific amplification of viral pathogens responsible for individual colds in MS patients. **Objective:** This proposal seeks to identify possible viral triggers of MS attacks in adults with relapsing disease. This could lead to new prevention strategies or treatments for MS attacks. **Methods:** This is an ongoing observational and prospective study. Nasal secretions from MS patients with a new cold were analyzed by multiplex and nested PCR for rhinoviruses A/B and for influenza A/B, parainfluenza, respiratory syncytial virus, adenovirus, metapneumovirus, and coronaviruses. Each subject received an initial evaluation with 5 weeks of neurologic followup to detect attacks. **Results:** Thirty-six colds occurred among 32 subjects at the two centers. Thirteen MS attacks occurred among 13 subjects, 5 of which were confirmed by definite changes in the EDSS. Viral nucleic acids were amplified from 19 of the 28 tested specimens (10 rhinovirus, 5 coronavirus, 2 adenovirus, 2 respiratory syncytial virus). Among the 10 attacks with virologic data, 4 were associated with rhinovirus, 2 with coronavirus, and 2 with adenovirus amplifications (2 with no virus). Nine of 11 (82%) MS attacks were associated with an amplified virus; 10 of 18 (59%) colds without an

attack were associated with viral amplification ($p = \text{NS}$). Three of the 4 definite attacks with virologic data were associated with rhinovirus amplification. **Conclusions:** Preliminary analysis of data from 28 colds in MS patients does not suggest a definite association between the amplification of specific viruses and MS attacks. The number of subjects included in this preliminary analysis is small and the study is ongoing.

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P150**Gender ratio in the National Swedish MS Register**

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Background: Recently evidence has been presented indicating an increase in the female-to-male ratio in multiple sclerosis (MS) patients in Canada and Denmark. Environmental factors have been focused to explain this tendency. **Objective:** To analyze the gender ratio in Swedish MS patients according to year of onset. **Methods:** Data was extracted from the Swedish MS Register ($n=9392$). Only patients with a defined time of onset were included. Patients with possible MS, clinically isolated syndrome (CIS) or registration artifacts were excluded. **Results:** We selected 6467 patients for calculation of gender ratio covering onset periods from 1950 to 2007. The female-to-male ratio showed a rather great stability, with a mean value of 2.43. However, among patients with onset during the time period 1956–1970 the ratio was increased to a mean of 3.35. The female-to-male ratio during onset time 1970–2000 was 2.35. There was an increase of incidence in both sexes, especially after onset year 1990. **Conclusions:** The early figures must be interpreted with caution since the number of patients with onset 1950–1960 is small, only 183. The Swedish data does not reveal an ongoing change in gender ratio. The increase of incidence may be due to diagnostic procedures.

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2008 update on the French EDMUS databases

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Background: Multiple sclerosis (MS) affects about 60 000 patients in France. Since 1990, the EDMUS software has been implemented in more than 50 French MS centers, which have also agreed to work together since 2003. **Objective:** The objective is to provide a dynamic picture of MS in France, by selecting few but pertinent information only. It is not intended to collect exhaustive data on all MS patients. This would not have been very realistic regarding the high variability in data collection between centers. **Methods:** In January 2008, French EDMUS users were asked to provide an automatic and anonymized extraction of selected data within their EDMUS databases. Data have been pooled and analyzed by the EDMUS Coordinating Center. Before analysis, a search for doublons was performed in order to identify patients that could have moved and be registered in more than one database, using the Unique International Number. **Results:** By March 8th, 2008, we could pool data from 24 754 computerized files coming from 20 regional centers. There were 430 patients found registered in at least two different databases, sometimes three. There were (71%) females and 7184 (29% males). Mean age at onset was 32.1 (± 10.4) years and 66.3% started between 20 and 40 years. Onset was exacerbating-remitting in 18 692 patients (87%) aged 30.8 years at onset, and progressive in 2817 (13%) aged 41.4 years. A secondary progressive course of the disease was experienced by 23%. Further analyses will be provided at the ECTRIMS meeting. **Conclusions:** Sharing the same language and software to describe MS patients has demonstrated to be a useful tool for individual clinical practice, but also gives power to implement further epidemiological studies in very large and well-described cohorts of MS patients.

P152

Clinically confirmed varicella and the risk of childhood-onset multiple sclerosis in offspring

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Background: The possibility of a link between viral infections and incident multiple sclerosis (MS) has been raised. The role of varicella zoster virus is debated. Most studies show no association between the frequency of varicella and MS. However, methodological limitations were pointed out in some of them, including seroepidemiological studies. **Objective:** To investigate whether clinically confirmed varicella increases the risk of incident MS in childhood. **Methods:** We conducted a population-based, case-control study. The cases were patients with incident MS occurring between 1994 and 2003, before the age of 16 years, in France. Each case was matched for age, sex, and geographic origin with 12 controls, randomly selected from the French general population. Information about occurrence of varicella in the cases and controls was collected with a standardized questionnaire and controlled in the health certificate. Conditional logistic regression was used to estimate the odds ratio (OR) of MS associated with clinically confirmed varicella, with adjustment on additional potential confounding factors (familial MS history, family history of another autoimmune disease, socioprofessional status of the head of the family, parental smoking at home). **Results:** The 137 cases of MS were matched with 1061 controls. Information about varicella was obtained for all of these cases and controls. Clinically confirmed varicella was noted in 76.6% of cases and 84.9% of their matched controls. The adjusted OR of a first episode of MS associated with varicella occurrence was 0.58 (95% confidence interval (CI): 0.36–0.92). Moreover, stratification for age showed that the decrease in risk concerned mostly the cases with an older age at onset of disease (over 10 years of age at the time of the index episode), OR 0.52 (0.29–0.92), than younger cases. **Conclusions:** Clinically confirmed varicella has a protective effect against occurrence of childhood-onset MS in offspring, mostly in patients with older age at onset.

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Multiple sclerosis and subsequent hospitalization with myocardial infarction and stroke: a population-based cohort study

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Background: Increased risk of cardiovascular mortality has been reported in patients with multiple sclerosis (MS). However, few data are available on the association between MS and hospitalization with myocardial infarction (MI) or stroke. **Objective:** To investigate the risk of hospitalization for MI and stroke among patients with MS compared with matched population controls. **Methods:** The Danish National Registry of Patients (DNRP), which covers all Danish hospitals since 1977, and the Danish Civil Registration System, were used to identify two cohorts: patients with MS (N=14 733), and a matched (sex, age, municipality) population control cohort (N=75 428). All study patients with a diagnosis of MI or stroke before their first MS diagnosis (or index date for control patients) were excluded. The DNRP was used to identify hospitalizations for acute MI or stroke. We computed the risk of MI and stroke for MS and control patients within the first year after MS diagnosis/index date and for the following 2–30 years and used Cox regression analysis to calculate hazard ratios as a measure of relative risk (RR) for endpoint diagnoses.

Results: During the first year of follow up 34 (0.2%) patients with MS and 118 (0.16%) control patients were hospitalized with MI; RR=1.78, (95% confidence interval (CI): 1.26–2.49). Forty-four (0.3%) patients with MS and 89 (0.1%) controls were hospitalized with stroke; RR=2.38, (95% CI: 1.76–3.22). During the following 2–30 years 293 (2.0%) patients with MS and 2196 (2.9%) control patients were hospitalized with MI, corresponding to RR=1.05 (95% CI: 0.93–1.18). A total 313 (2.1%) patients with MS were hospitalized with stroke compared with 2254 (3.0%) controls; RR=1.17 (95% CI: 1.04–1.32).

Conclusions: Patients with MS were at increased risk of MI and stroke during the first year after MS diagnosis. The increased risk of stroke but not of MI persisted during long-term follow-up.

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Physician preferences for the specialty care of multiple sclerosis patients

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Background: Multiple sclerosis (MS) requires lifelong care by an MS-specialist, a general neurologist, or a general practitioner (GP). A few publications have addressed patient's preferences for specialty care for MS patients, but none have focused on physician's preferences. This study is part of a larger 'pre-design' study of MS prevalence, funded by the Canadian Institutes of Health Research. **Objective:** To determine the preferences of neurologists, GPs, and ophthalmologists for specialty care of MS patients. **Methods:** A self-administered questionnaire was developed and mailed to all Quebec neurologists (n=236) and ophthalmologists (n=313) and a random sample (n=900) of the province's 8837 GPs. It included four typical clinical scenarios: clinically-isolated syndrome (CIS), stable relapsing-remitting MS, aggressive MS, and stable secondary progressive MS. For each scenario physicians were asked whether they would manage the patient or refer to a specialist, and whether they expected the specialist to assume care. **Results:** The percentage of neurologists who reported that they would continue to manage the patient themselves was 64%, 65%, 51%, and 64% for each clinical scenario. If they referred the patient they wanted the specialist to assume care (82%, 96%, 79%, 69%). GPs preferred to refer CIS cases or aggressive MS cases (76%, 73%) to a specialist. If they referred the patient they did not necessarily expect the specialist to take over care (43%, 46%, 54%, 31%). Ophthalmologists would refer patients (76%, 78%, 85%, 66%) and expect the specialist to take over care (77%, 86%, 91%, 96%). **Conclusions:** Although neurologists feel they are the most appropriate specialty group to follow MS patients, GPs do prefer to follow MS patients themselves in at least two common disease scenarios reflecting more chronic but stable stages of MS. This is an important finding given recent evidence of increased MS prevalence and the likelihood that specialty MS clinics will become busier.

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P155

Divorce and separation in multiple sclerosis

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Background: The current literature does not include population-based prospective studies with long-term follow-up of duration of marriage or partnership. **Objective:** To evaluate the probability of multiple sclerosis (MS) patients to remain in marriage or a relation-

ship with the same partner after onset of MS, compared with matched controls from the Danish background population. **Methods:** We included all MS patients in Denmark with disease onset between 1980 and 1989, identified through the Danish MS Registry. Twenty matched control persons per patient were randomly drawn from the civil registration system. So were the partners of both. Information on family status was retrieved from Statistik Denmark. A total of 2538 patients with definite or probable MS were included in the study. We used survival statistics for the analyses. Time was defined as time from onset to endpoint (cessation of relationship, that is, divorce, separation, or end of cohabitation), or time to death or end of observation (January 1st, 2004), whichever came first. **Results:** In the patient group, 71.1% had a partner at the start of the study compared with 70.9% of the control population. There was no difference in gender. After 10 years, the cumulative probability of remaining in the same relationship differed little between patients and controls (81% versus 85%). After 15 years, however, there was a pronounced separation of the curves, and at 20 years, the cumulative probability was only 37% in patients as opposed to 75% in the control persons (p<0.001). There was no difference in gender. **Conclusions:** MS seriously affects the probability of remaining in the same relationship, compared with the background population, but it does not take effect until several years after onset. This is probably a consequence of late occurring handicaps or cognitive symptoms rather than caused by early symptoms or the diagnosis itself.

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P156

Early retirement in multiple sclerosis

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Background: Time to retirement pension has not been evaluated in comparative prospective population-based studies. **Objective:** To evaluate the probability of remaining in work after onset of multiple sclerosis (MS) compared with matched controls from the Danish background population in a prospective population-based study. **Methods:** We included all MS patients in Denmark with disease onset between 1980 and 1989, identified through the Danish MS-Registry. Twenty matched control persons per patient were randomly drawn from the civil registration system. Information on employment status was retrieved from Statistik Denmark. A total of 2538 patients with definite or probable MS were included in the study. We used survival statistics for the analyses. Time was defined as time from onset to endpoint (disability pension), if it occurred, else time to death, to age 65, to retirement unrelated to health, or to end of observation January 1st, 2004, whichever came first. **Results:** At time of onset 2.5% of the patients received disability pension compared with 3.2% of the matched controls. For those not receiving disability pension at entry, the probability of remaining without disability pension at 5 years was 70% for patients and 97% for controls; at 10 years it was 45% for patients and 93% for controls; at 15 years it was 31% for patients and 89% for controls; and at 20 years it was 23% for patients and 85% for controls. The figures were almost equal for male and female patients, but female controls had a slightly higher probability of receiving disability pension than males 15 years after entry. **Conclusions:** Not unexpectedly the risk of disability pension is higher in MS patients than in controls. However, the difference is distinct even 5 years after onset, and the gap continues to widen, so after 20 years the probability of staying in employment is less than 1/4 in patients and more than 4/5 in controls.

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P157

A multicenter retrospective study of Thai multiple sclerosis patients

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Background: Manifestations of multiple sclerosis (MS) seem to be different among Eastern and Western countries in many aspects including prevalence, MS types, presentations, cerebrospinal fluid (CSF) analyses and imaging findings. **Objective:** To determine the clinical data in Thai MS patients regarding demographic data, first clinical manifestation, progression of the disease, CSF and imaging findings. **Methods:** A multicenter retrospective study from 130 patients attending eight Neurological Centers in Thailand between June 2004 and December 2004 was performed. Each patient fulfilled MS criteria according to Poser's criteria. Baseline characteristics were collected. The ethics committee of each center approved the study. **Results:** From 130 patients, four were excluded being classified as Devic's syndrome. Of the remaining 126, there were 96 female (76.2%) and 30 male patients (23.8%) with the female-to-male sex ratio of 3.2:1. The age at onset was 32.9 ± 11.3 years with the mean duration of illness of 3.8 ± 5.1 years. The mean number of relapses was 4.6 ± 4.4 and the mean relapse rate was 1.5 ± 1.3 attacks per annum. No patient reported a family history of MS. Recurrent opticospinal form of MS was found in 34 patients (27.0%) among all MS types followed by spinal form in 21 patients (16.7%) then classic (Western form of MS) in 18 patients (14.3%). The most common presenting symptom was visual impairment (50%). The presence of CSF oligoclonal bands was positive in only 24.2%. The median score of the Kurtzke's Expanded Disability Status Scale (EDSS) at their latest visits was 3.0 with mean score of 3.8 ± 3.0 . **Conclusions:** MS in Thailand is different from Western countries. In this study there were no occurrence of MS in families, higher incidence of visual impairment at the onset of illness, more common recurrent opticospinal form in first clinical manifestations and lower incidence of oligoclonal bands in the CSF.

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Association of HLA DR2 haplotype DQB1*0602 and DRB1*1501 alleles in Brazilian primary progressive multiple sclerosis

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Background: Although the participation of the major histocompatibility complex (MHC) genes in the pathogenesis of multiple sclerosis (MS) has not yet been proven, in Caucasian populations of the Northern Hemisphere there are genetic differences between the two initial clinical phenotypes of the illness, relapsing-remitting MS (RRMS) and primary progressive (PPMS). **Objective:** Based on the knowledge of the association of alleles HLA DRB1, DQA1 and DQB1 in the Brazilian RRMS patients, we investigate the occurrence of the same alleles in the PPMS patients, in order to prove susceptibility or resistance to disease. **Methods:** The studied population consisted of 180 healthy individuals (88 African-Brazilians and 92 White Brazilians) and 33 PPMS patients (10 African-Brazilians and 23 White Brazilians) enrolled in MS genetic susceptibility study performed in Hospital Regional Universitario Carlos Haya, Malaga, Spain. The HLA class II sub-regions DRB1, DQA1 and DQB1 were analyzed by polymerase chain reaction (PCR) and sequence-specific oligonucleotide probe hybridization (PCR/SSO) for DRB1 and DQB1 and with sequence-specific primers (PCR/SSP) for DRB1 subtypes and DQA1. **Results:** Associations were detected between PPMS and HLA class II alleles DRB1*1501 (36% versus 6.6%, $p=0.0013$) and DQB1*0602 (42% versus 15%, $p=0.008$). The DR2 haplotype (DRB1*1501, DQA1*0102, DQB1*0602) was associated with PPMS (36% versus 6.6%, $p=0.0013$). **Conclusions:** Our results confirm the positive association of DR2 haplotype with PPMS Brazilian patients, mainly with the alleles DRB1*1501 and DQB1*0602. Similar associations were observed in previous genetic Brazilian studies of RRMS, confirming the similarity between the two clinical phenotypes in a low prevalence area.

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Change of sex-ratio over time in multiple sclerosis: national and regional data from the French 'Observatoire de la Sclérose en Plaques'

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Background: Several recent studies have shown some evolutions in sex-ratio over time in multiple sclerosis (MS). In France, MS centers using the EDMUS software have agreed to share their data for the purpose of epidemiological studies since 2003. **Objective:** Our primary objective was to evaluate the female-to-male ratio over time in the whole French cohort of MS patients. A secondary objective was to also provide some regional data, as a North-East to South-West gradient in the prevalence of MS has been shown previously in France. **Methods:** In January 2008, French EDMUS users were asked to provide an automatic and anonymized extraction of selected data within their EDMUS databases. Data have been pooled and analyzed by the EDMUS Coordinating Center. Before analysis, a search for doublons was performed in order to identify patients that could have moved and be registered in more than one database, using the Unique International Number. Only cases with definite or probable MS according to Poser's classification were included. Sex-ratio analyses were assessed stratified by year of onset then by year of birth. The change over time according to initial disease course (relapsing-remitting versus primary progressive) was also studied. Linear regression and the Wald test were used to assess the trend statistical significance. **Results:** By March 8th, 2008, we could pool data from 24 754 computerized files coming from 20 regional centers. There were 430 patients found to be registered in at least two different databases, sometimes three. There were (71%) females and 7184 (29%) males). Detailed results on the sex-ratio evolution over time will be provided at theECTRIMS meeting. **Conclusions:** Our study could give further insights into the knowledge of MS epidemiology and evolution over time.

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HLA B7/A2, Epstein-Barr Virus antibodies and magnetic resonance imaging in multiple sclerosis: a case for gene-environment interactions

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Background: The underlying gene-gene interactions and gene-environment interactions can potentially cause the heterogeneity in brain inflammation, demyelination and axonal loss in patients with multiple sclerosis (MS). **Objective:** To determine the role of gene-environmental interactions between the Class I and Class II HLA alleles and the humoral anti-Epstein Barr Virus (EBV) responses in the development of brain injury and clinical disability in MS patients. **Methods:** A total of 93 MS patients (62 females; 31 males) and 122 healthy controls underwent HLA typing and testing for antibodies against EBV. The MS patients underwent brain magnetic resonance imaging (MRI) and quantitative measurements of T1- and T2-weighted lesion volumes (LVs) and brain parenchymal fraction (BPF) were obtained. There were 54 MS cases who underwent MRI and EBV-antibody assessments at the 3-year follow-up. The anti-EBV panel included measurements of the levels of anti-EBV early antigen (EA) IgG, anti-EBV nuclear antigen (EBNA) IgG and anti-EBV viral capsid antigen (VCA) IgM and anti-EBV VCA IgG. The relationships between HLA alleles, anti-EBV antibody levels, MRI and clinical parameters were assessed in regression analysis. **Results:** The presence of HLA B7 was associated with increased anti-EBV VCA IgG levels, higher disability (Expanded Disability Status Scale (EDSS) score) and more destructive MRI parameters (higher T2-LV, T1-LV, and decreased BPF). The presence of HLA A2 had protective role and was associated with lower anti-EBV VCA IgG levels, EDSS and MRI lesion metrics (T2-LV and T1-LV). **Conclusions:** Our data suggest that gene-environment interactions between specific HLA Class I loci and EBV exposure are associated with MRI markers of lesion injury and brain atrophy in MS patients.

Experimental Disease Models

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A potential new therapy to alleviate experimental autoimmune encephalomyelitis symptoms: an animal model of multiple sclerosis
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Background: multiple sclerosis (MS) disabilities appear across a broad spectrum including motor spasticity, weakness, cognitive impairment, and balance disorder. Our recent experimental autoimmune encephalomyelitis (EAE) rat model revealed robust changes in motor/cognitive/balance functions that are significant hallmark MS disabilities. **Objective:** To expand the evidence base for new rehabilitative interventions. **Methods:** We used this animal model to study the neurobiology of MS disabilities and to comprehensively evaluate outcome measures relative to two experimental treatment approaches, cycle locomotor training and transcranial magnetic stimulation (TMS). **Results:** By week 5 post-inoculation (pi), MBP in CFA), the EAE rats revealed significant tonic and dynamic ankle extensor spasticity, which progressively increased up to pi week 17. Moreover, in acute stage of the disease, motor-evoked potential (tcMMEPs) amplitudes were increased in soleus (SOL) and tibialis

anterior (TA) and forelimb flexor (FF) (at pi week 3). From pi week 5 through 17, these were drastically decreased or absent (~50%) in SOL and TA and remained increased in FF. In addition, these animals showed significant motor weakness in the forelimbs, a cognitive deficit for serial learning in the Morris water maze, and a significant reduction in balance tested on a rotarod. Our data to date indicate that EAE animals treated using cycle training (two 20-minute sessions of locomotor exercise for 3 weeks) or TMS (25 single magnetic pulses with graded stimulus intensities from 30% to 70% of maximum) revealed significantly decreased spasticity, increased forelimb grip strength, improved scores for serial learning, and increased balance performance. **Conclusions:** We propose that a significant portion of these disabilities are companion disorders correlated with decreased noradrenergic (NE) function in neural regions that are critical to these functions. We hypothesize that the therapeutic treatments using locomotor training or TMS significantly improved MS symptoms through upregulation of NE function in selected spinal, brainstem, and cortical regions.

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Estrogen and progesterone prevent demyelination and affect oligodendrocyte function in the cuprizone model

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Background: We have previously shown that combined estrogen and progesterone treatment prevents cuprizone-induced acute demyelination. Sex hormones are thought to affect and delay progression of multiple sclerosis (MS) during pregnancy. Both steroid hormones are regarded as neuroprotective factors in the brain. Only the influence of estrogen supplementation on disease progression has so far been tested in clinical trials. **Objective:** In this study, we focused on the underlying mechanisms of protective hormonal effects. **Methods:** Adult male mice were fed with cuprizone for a defined time interval to induce demyelination of the corpus callosum. Animals were exposed to estrogen and progesterone by subcutaneous injection. The status of myelination was analyzed by histological stainings and magnetic resonance imaging (MRI). Functional markers of oligodendrocytes, microglia, and astrocytes were additionally analyzed. Direct hormonal effects on oligodendrocytes were analyzed by cell culture experiments. **Results:** A combined treatment with both hormones nearly completely counteracted the process of demyelination. Furthermore, premature and mature oligodendrocyte markers were significantly increased. Stronger astrogliosis was observed in hormone-treated compared with only cuprizone-fed animals. Microglia invasion was detected in the midline of the demyelinated CC in hormone-treated animals, whereas in only cuprizone-fed animals, microglia invasion was detected in the lateral part of the CC. Molecular analysis of IGF-1 gene expression showed higher levels of IGF-1 mRNA in hormone-treated animals. Direct hormonal effects on oligodendrocyte proliferation and differentiation were marginal. **Conclusions:** These data support the concept that sex steroids can protect the brain from demyelination during MS. Our findings suggest that beneficial steroid effects require complex interactions between astrocytes, microglia and oligodendrocytes by either preventing oligodendrocyte cell death and/or recruitment of premature oligodendrocytes for new myelin formation. Since IGF-1 expression is under hormonal control and known to be implicated in the regulation of myelination, we assume that IGF-1 regulation is a key event in neuroprotection by steroids.

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Oligodendroglial expression of DCC is required for the organization of paranodal junctions *in vivo*Sarah-Jane Bull¹, Mohammadali Almasieh², Adriana Di Polo², Timothy E. Kennedy¹¹Montreal Neurological Institute, Department of Neurology and Neurosurgery, McGill University, Montreal, Quebec, Canada; ²Department of Pathology and Cell Biology, Université de Montréal, Montreal, Quebec, Canada

Background: Expression of paranodal proteins is altered in demyelinating lesions in multiple sclerosis (MS) patients and paranodal architecture may remain disrupted following remyelination. We have previously reported that netrin-1 and DCC proteins are enriched at paranodal axo-oligodendroglial junctions both *in vivo* and *in vitro*. Studies carried out *in vitro* provided evidence that netrin-1 and DCC are required for the maintenance of normal paranodal junctions. **Objective:** To determine whether DCC is required for paranode maintenance *in vivo*, we assessed the capacity of oligodendrocyte precursor cells (OPCs) derived from *dcc*^{-/-} mice to myelinate retinal ganglion cells. Retinal ganglion cell axons are myelinated in the optic nerve; however OPCs do not invade the retina during development. The unmyelinated proximal segment of ganglion cell axon within the retina provides a unique opportunity to assess the capacity of OPCs transplanted into the retina to myelinate. **Methods:** Isolated OPCs derived from *dcc*^{-/-} mice or heterozygote and wild-type littermates were transplanted into the eyes of wild type adult mice and myelination was analyzed 2 months later. **Results:** Abundant myelinated internodal segments were present in eyes that received OPCs. Abnormal paranodal specializations were detected in retinas myelinated by *dcc*^{-/-} OPCs compared with retinas myelinated by wild-type or heterozygotes OPCs. **Conclusions:** These findings indicate that DCC expressed by oligodendrocytes is essential for maintenance of paranodal integrity *in vivo*.

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A cyclooxygenase 2 inhibitor limits demyelination in a murine model of multiple sclerosis and protects oligodendrocytes from excitotoxic death in cultureNoel G. Carlson¹, Monica Rojas², Jonathan Redd³, Blair Wood³, Kenneth E. Hill², John W. Rose³¹GRECC, VA SLC HCS, Salt Lake City, Utah, USA; ²University of Utah, Salt Lake City, Utah, USA; ³VA SLCHCS Neurovirology, Salt Lake City, Utah, USA

Background: We previously found that cyclooxygenase 2 (COX-2) was expressed in dying oligodendrocytes at the onset of demyelination in the Theiler's Murine Encephalomyelitis Virus (TMEV) model of multiple sclerosis (MS). **Objective:** In order to determine whether COX-2 expression in oligodendrocytes might render these cells more susceptible to glutamate-mediated excitotoxicity in much the same manner that COX-2 expression in neurons increases neuronal cell death, we examined whether COX-2 inhibitors can decrease demyelination in TMEV induced demyelination (TMEV-IDD) and limit excitotoxic death of oligodendrocytes *in vitro*. **Methods:** Mice were infected intracerebrally with the DA strain of TMEV and either given a control diet or chow containing the COX-2 inhibitor CAY10452 (a derivative of celecoxib). Spinal cords of control and CAY10452-treated mice were harvested 35 days after infection and scored for inflammation and demyelination following Luxol fast blue staining. For the *in vitro* experiments, organotypic cultures were prepared from post-natal day 8 spinal cords and exposed to the excitotoxin kainic acid (KA) (300 μ M) in the presence or absence of the COX-2 inhibitor CAY10452 (30 μ M). Cell death in white matter and gray matter was assessed 24 hours later by immunofluorescence confocal microscopy using the cell death indicator activated caspase 3 and the oligodendrocyte marker cyclic nucleotide phosphodiesterase (CNase). **Results:** In the TMEV-IDD model of MS, COX-2 inhibitors had no effect on the score for inflammation but significantly decreased the amount of demyelination ($p < 0.05$ by the Mann-Whitney nonparametric t-test). *In vitro*, COX-2 inhibitors decreased

the amount of KA-induced cell death in white matter and gray matter ($p < 0.05$ by analysis of variance (ANOVA) Tukey-Kramer). **Conclusions:** The COX-2 inhibitor CAY10452 can reduce demyelination in the TMEV-IDD model. Investigations with *in vitro* spinal cord cultures demonstrated that this COX-2 inhibitor has a protective effect which reduces glutamate-mediated excitotoxicity and oligodendrocyte death.

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Longitudinal monitoring of an animal model of opticospinal demyelination by magnetic resonance imagingNicolas Collongues¹, Frederic Blanc¹, Jerome Steibel², Blandine Guignard², Dao Lam², Said Ghandour², Jerome de Seze¹¹neurologie, Hospices Civils, Strasbourg, France; ²CNRSUMR7191, Strasbourg, France

Background: MOG-induced experimental autoimmune encephalomyelitis (EAE) in the Brown Norway rat is a reproducible model of opticospinal demyelination. Despite the topography of this demyelination, inflammatory cells as activated microglia may be found in the brain, indicating a diffuse inflammatory process. At this point, no longitudinal monitoring of this MOG-induced model by magnetic resonance imaging (MRI) has been performed. **Objective:** We investigate for the first time the accurate topography of the lesions by MRI at different stages of the immunological process in the brain, optic nerve and spinal cord. **Methods:** Multiparametric MRI (Diffusion, T1- and T2-weighted) was performed at 4.7 Tesla. We studied 13 Brown Norway rats immunised with 100 μ g of soluble MOG associated with incomplete adjuvant. We longitudinally followed-up the brains, optic nerves and spinal cord by MRI at 10, 20 and 30 days post-immunization. **Results:** Most of the rats developed clinical signs after 15 days post-immunization. MRI signals changes occurred early in the optic nerves and the spinal cord, but also in the periventricular areas in the brain. **Conclusions:** MRI monitoring confirms that opticospinal inflammation is induced by MOG in the Brown Norway rat. MRI follow-up suggests a more diffuse process including the area around the cerebrospinal fluid (CSF)-like periventricular areas. These results provide an argument for a major role of the CSF in the inflammatory process in this model of EAE.

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Didox - a pluripotent polyphenol for treating demyelinating diseaseChristian Campbell¹, R. Fletcher Schwartz¹, Doug Feinstein², Becker Catania⁴, Jeff Dupree³, Hannah Sims⁵, Howard Elford¹, George H. DeVries³¹Hines VA Hospital, Hines, Illinois, USA; ²University of Illinois Chicago, Department of Anesthesiology, Chicago, Illinois, USA; ³University of Illinois Chicago, Department of Anatomy & Cell Biology, Chicago, Illinois, USA; ⁴Health Science Division Virginia Commonwealth University Dept Anatomy and Neurobiology, Richmond, Virginia, USA; ⁵Molecules for Health, Richmond, Virginia, USA

Background: Didox (N-3,4 trihydroxy benzamide) is a free radical scavenger, an iron chelator and an antiproliferative agent. Since these biological activities are known to be involved in demyelinating disease, we investigated the ability of didox to influence chronic experimental autoimmune encephalomyelitis (EAE) induced by myelin oligodendrocyte glycoprotein (MOG). **Objective:** To evaluate the ability of didox *in vivo* to ameliorate the symptoms of EAE and *in vitro* to influence T-cell proliferation, secretion of inflammatory cytokines and microglial nitrite production. **Methods:** C57BL/6 mice were treated with didox (250 mg/kg i.p.) two days prior or 18 days after the last MOG booster and scored for clinical symptoms for at least three weeks. T cells from C57BL/6 mice were activated via CD3/CD28 antibodies in the presence of didox. T cell proliferation was evaluated by BrdU incorporation, and inflammatory cytokines (IL-17, IFN-gamma) were determined by enzyme-linked immunosorbent assay. Microglia were isolated from rat central nervous system, stimulated with lipopolysaccharide in the presence of didox

and evaluated for nitrite production via the Greiss assay. **Results:** Treatment of mice two days prior to the final MOG booster delayed the onset and decreased the clinical severity of the disease. Remarkably, mice treated with didox at the peak of disease severity showed significant improvement in clinical symptoms within five days. Increasing dose of didox in the presence of stimulated T cells reduced proliferation to background levels. Didox was a potent inhibitor of T-cell secretion of IL-17 and IFN-gamma. Didox also attenuated the production of nitrite by microglia to background levels in a dose-dependent manner. **Conclusions:** We conclude that didox is a potent therapy for this animal model of demyelinating disease by virtue of its ability to greatly decrease the production of nitrites and inflammatory cytokines and to suppress T-cell proliferation, cumulatively reversing clinical symptoms even when given after disease onset.

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Targeted experimental autoimmune encephalomyelitis with persistent demyelination and delayed functional loss

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Background: Although chronic and relapsing-remitting models for demyelinating disease exist, the demyelination is modest and spatially and temporally disseminated. Current focal models of experimental autoimmune encephalomyelitis (EAE) yield transitory demyelination which does not allow correlation of demyelination events with functional losses. **Objective:** To develop a targeted EAE model in which the focal demyelination is permanent and can be related to functional loss. **Methods:** Female Lewis rats were pre-trained in a skilled forelimb reaching task (pellet retrieval), followed by immunization with myelin oligodendrocyte glycoprotein (MOG) in complete Freund's adjuvant (FA) followed two weeks later with a booster MOG in incomplete FA. One week later a cytokine cocktail injection (IFN-gamma, TNF-alpha, IL-1, IL-17) was given in the sub-cortical white matter area beneath the forelimb reaching cortex. The animals were subsequently evaluated for loss of function and morphological analysis of immune infiltration (CD11b, H&E) myelin (Luxol fast blue) and axonal integrity (EM). **Results:** No EAE symptoms were evident throughout the experiments. Immune infiltration occurred within a week, functional and axonal loss 3 to 4 weeks after the cytokine injection with initial functional loss beginning 2 to 3 weeks after cytokine injection. Functional loss persisted for at least a month after the first significant functional loss was observed. **Conclusions:** Although significant, functional loss is not great enough to allow evaluation of therapeutic potential of proposed treatments (stem cell therapy, anti-Nogo A therapy). Increased sensitization by injection of guinea pig myelin basic protein in incomplete Freund's will allow for greater sensitization and subsequent demyelination and a more profound behavioral loss. Multiple sclerosis (MS) is initially an exacerbating relapsing inflammatory disease followed by demyelination, axonal loss and concomitant functional deficits. The persistent t-EAE model resembles MS since there is initial inflammation with no functional loss, followed by demyelination, axonal loss and persistent functional loss.

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Atacept inhibits disease in multiple forms of experimental autoimmune encephalomyelitis

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Background: Pathogenic autoreactive T cells have traditionally been considered to be the main contributors in the pathogenesis of multiple sclerosis (MS). However, there is now increasing clinical evidence that B cells also contribute to MS pathology. Atacept is a fully human B-cell modulating recombinant fusion protein comprising the extracellular portion of the TACI receptor linked to an Fc domain of immunoglobulin (Ig)G. Atacept modulates B cells by blocking BlyS (B-Lymphocyte Stimulator) and APRIL (A Proliferation-Inducing Ligand), important regulators of B-cell function and survival. Inhibition of BlyS and APRIL in mice using murine TACI-Ig inhibits mature B-cell and plasma cell survival and lowers Ig levels without affecting immature or memory B-cells. This may translate into anti-inflammatory and neuroprotective effects of atacept in MS. Here we present the results of pre-clinical studies of atacept in experimental autoimmune encephalomyelitis (EAE) models of MS. **Objective:** To evaluate the efficacy of B-cell modulation therapy using atacept in two EAE models of MS. **Methods:** We evaluated atacept prophylactic and/or therapeutic treatment in two mouse models of MS (B-cell-dependent recombinant (r) MOG-EAE, and primarily T cell-dependent MOG peptide (p)-EAE) and monitored the effects of treatment on disease severity and incidence. **Results:** In both the rMOG- and MOGp-EAE models, prophylactic atacept treatment markedly reduced circulating mature B-cell numbers and serum IgM and IgG levels and significantly delayed disease onset compared with vehicle controls. Atacept treatment significantly lowered disease severity and incidence in the rMOG-EAE model, which was associated with reduced B-cell infiltration into the central nervous system. **Conclusions:** Our results demonstrate that atacept acts on both the cellular and humoral effector arms of the immune response in EAE, a novel feature that represents a new mechanism of action for the treatment of MS. This potential therapeutic value is currently being further investigated in Phase 2 clinical studies of atacept in MS and optic neuritis.

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Adaptive angiogenesis in response to normobaric hypoxia is protective in experimental autoimmune encephalomyelitis

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Background: While the pathologic events associated with multiple sclerosis (MS), diffuse axonal injury, cognitive damage, and white matter plaques have been known for some time, the etiology is unknown and therapeutic efforts are somewhat disappointing. This may be due to a lack of fundamental knowledge on how to maintain homeostasis and buffer the brain from secondary injury. Maintenance of homeostasis is the result of regulatory adjustments by cellular constituents of the neurovascular unit (pericytes, endothelial cells, astrocytes, neurons) that include induction of adaptive/physiological angiogenesis. Results from our lab and others suggest that although aspects of adaptive angiogenesis are induced in MS and the murine model experimental autoimmune encephalomyelitis (EAE), vascular remodeling is ineffective and the balance between metabolic need and oxygen and glucose availability is disrupted. **Objective:** We hypothesize that restoration of angiodynamics will augment neuroprotection, mitigating the extent of secondary injury and sparing cognitive deficit in patients with MS. **Methods:** To test this hypothesis we have characterized angiodynamics in myelin oligodendrocyte glycoprotein (MOG) peptide (35-55)-induced EAE in C57BL/6 mice and then examined the effect of exposure to mild-graded normobaric hypoxia. **Results:** In murine white matter, capillary density is half

that observed in gray matter. Hypoxia inducible factor alpha (HIF-1 α) and capillary vascular endothelial growth factor (VEGF) are induced by 3–5 days following immunization. HIF-responsive genes are induced at later time points in all cells of the neurovascular unit. It is unclear whether the pro-angiogenic response is sufficient to promote repair during ongoing disease as vascular density is diminished. Exposure of animals to normobaric hypoxia (10%) increased vascular density and significantly delayed the onset of clinical EAE. Normobaric hypoxia also diminished the clinical score and enhanced the rate of recovery. **Conclusions:** While the mechanisms of neuroprotection are unclear, results suggest that normobaric hypoxia stabilizes the stress response, promotes physiological angiogenesis and is neuroprotective.

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Identification of the RNA targets bound by the QUAKING RNA binding proteins

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Background: The pathology of multiple sclerosis (MS) disease is triggered by an autoimmune response resulting in central nervous system (CNS) demyelination. The naturally occurring Quaking viable mouse (*qk*^v) has been widely used as a model for studying myelination. In the CNS the *qk*^v mouse displays high levels of uncompacted myelin as well as a lack of oligodendrocyte maturation. The *qk*^v mouse model exhibits a genetic deletion within the *qki* gene, preventing the proper expression of QUAKING (QKI) 6 and QKI-7 isoforms in oligodendrocytes. QKI isoforms belong to the heteronuclear ribonucleoprotein particle K (hnRNP K) homology (KH) domain family of RNA-binding proteins. The QKI proteins dimerize and bind RNA with high affinity and in a sequence-dependent manner. Previously, we performed SELEX to identify RNA ligands from a random library of RNA aptamers and we defined the RNA binding site as ACUAAAY (N1-20) UAAAY direct repeats. Bioinformatic studies identified 1430 putative mRNA targets with this QKI response element (QRE). **Objective:** We propose that in rat oligodendrocytes the QKI isoforms interact with RNA targets in order to promote myelination, thus explaining why the lack of QKI isoforms in *qk*^v mice leads to dysmyelination. **Methods:** To identify the physiological RNA targets of QKI, we are utilizing the ultraviolet cross-linking and immunoprecipitation (CLIP) method. CLIP permits the capturing of the physiological RNA targets bound directly to the QKI proteins; in addition, this method will identify the specific RNA sequences of the targets. **Results:** We identified a cross-linked and RNase protected RNA-QKI ribonucleoprotein complex. The RNAs will be identified and the data presented. **Conclusions:** Our findings will further shed light on the role of the QKI RNA-binding proteins during myelination.

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Experimental autoimmune encephalomyelitis induction in mice with cuprizone-induced demyelination leads to cerebral MS-like lesions

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Background: Multiple sclerosis (MS) is an autoimmune demyelinating disease affecting the brain and spinal cord. This is in contrast to the most widely used animal model, experimental autoimmune encephalomyelitis (EAE), where inflammation is located predominantly in the spinal cord. **Objective:** Our aim was to develop an experimental model where mechanisms and consequences of inflam-

matory demyelination in the mouse brain could be studied. **Methods:** We combined toxin-induced demyelination by the copper chelator cuprizone with MOG35-55 peptide immunization. **Results:** This approach led to demyelinated brain lesions with infiltration of T cells and macrophages through an open blood-brain barrier, thus closely resembling MS pathology. Inflammation led to abundant axonal damage in contrast to cuprizone induced demyelination alone where only few APP-positive axons were detected. In immunized animals, macrophages expressed proinflammatory proteins such as iNOS and S100A9/MRP14 as well the anti-inflammatory protein HO-1. Withdrawing cuprizone from the chow led to rapid remyelination to a similar extent in immunized and non-immunized animals. **Conclusions:** In summary, we present a new model which is particularly suitable to study mechanisms of damage and repair of MS-like lesions in the mouse brain.

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Transgene expression levels in bone marrow grafts determine its immunogenicity in sublethally myeloablated mice

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Background: One of the major challenges that gene therapy has to face is the immunogenicity of the transgene products. Hematopoietic stem cell (HSC) gene therapy after myeloablative conditioning constitutes an exceptional situation, since the gene-modified HSCs reconstitute a new lymphohematopoietic system in which the transgene product is presented as an autoantigen, resulting in immune tolerance. In spite of this, immune responses to the transgene products have been described. **Objective:** To determine whether transgene expression level is critical in the outcome of transduced graft in partially myeloablated mice. **Methods:** An LTR-EGFP vector (pSF1-EGFP) and an IRES-EGFP vector (pSF91-IRES-EGFP), driving respectively high or low levels of EGFP expression, were used. Female mice C57Bl/6J (CD45.2, recipients) and B6/SJL-PtprcaPep3b/BoyJ (CD45.1, donors) were used. Cells transduced with these retroviral vectors were transplanted into sublethally conditioned recipients. At the time of transplantation, cells were analyzed for EGFP expression and fluorescence intensity by flow cytometry. Donor engraftment and EGFP expression (molecular chimerism) were also analyzed in the peripheral blood and in bone marrow, and the presence of anti-EGFP antibodies was determined. **Results:** All recipients had evidence of donor engraftment in their hematopoietic tissues four weeks after transplantation. However, the presence of molecular chimerism was detected only in a fraction of the recipient mice; it was observed in recipients receiving grafts transduced with the IRES-EGFP vector but not in those transplanted with grafts expressing LTR-EGFP. The presence of anti-EGFP antibodies did not correlate with a specific outcome (tolerance vs. rejection) suggesting that rejection was mediated by cellular immune mechanisms. **Conclusions:** The level of transgene expression is a critical factor to determine whether tolerance or an immune response is achieved. This should be considered when designing vectors for tolerance induction in autoimmune diseases such as multiple sclerosis.

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P2x7 deficiency attenuates experimental autoimmune encephalomyelitis

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Background: The purinergic receptor P2x7 is expressed throughout the body on cells of myeloid lineage, including macrophages and lymphocytes, and on central nervous system (CNS) cells including glia and neurons. Previous studies showed that activation of P2x7 increases activation of glial cells and influences T-cell functions,

suggesting a role in the progression of autoimmune diseases including multiple sclerosis (MS). However, the exact role of P2x7 during disease progression in experimental autoimmune encephalomyelitis (EAE) is unclear. **Objective:** To determine whether EAE disease is reduced in P2x7-deficient mice and, if so, whether T-cell or glial activation is modified. **Methods:** EAE was induced in P2x7 null and wildtype C57BL/6 mice using myelin oligodendrocyte glycoprotein peptide 35–55. Clinical symptoms were monitored daily, T-cell activation assessed by enzyme-linked immunosorbent assay for cytokine (IFN γ , IL-17) production, and brain pathology assessed by immunocytochemical staining and histology. **Results:** The incidence of disease in P2x7 null mice was reduced 4-fold compared with wildtype mice (from 65% to 16%); however, the mice that developed illness showed a similar day of onset and similar maximal clinical scores. Splenic T cells from P2x7 null mice produced greater quantities of IFN γ and IL-17 than did wildtype cells, and histological examination revealed infiltrating cells in brains from both wildtype and P2x7 null mice. In contrast, whereas GFAP staining was increased throughout the brain of wildtype mice, increased GFAP staining was less and regionally restricted in P2x7-deficient mice. Axonal damage was significantly reduced in P2x7 null mice compared with the controls. **Conclusions:** Despite the presence of activated T cells, EAE disease is reduced in P2x7 null mice, suggesting that activation of glial P2x7 is critical to development of EAE disease. These results suggest that methods to reduce P2x7 activation in the CNS may be of therapeutic value in the treatment of MS.

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Armed CNS CD8⁺ T cells promote demyelination through a perforin-independent mechanism

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Background: Accumulating evidence indicates that CD8⁺ T cells may contribute significantly to multiple sclerosis pathogenesis. To gain insights into the potential mechanisms by which this T cell subset may induce nervous tissue injury, we study a novel animal model (herein referred to as L31 mice) that spontaneously develops a CD8⁺ T cell-mediated demyelinating disease associated with axonal damage primarily due to the transgenic expression of the B7.2 (CD86) T cell costimulatory ligand on microglia. **Objective:** To further understand the pathological process that occurs in this animal model, we characterized the phenotypic and functional status of the CD8⁺ T cells that accumulate in the central nervous system (CNS) of symptomatic L31 mice and determined whether neurons and/or oligodendrocytes have the potential to become targets of a MHC-I-restricted perforin-dependent CD8⁺ T cell-mediated cytotoxic attack. **Methods:** CD8⁺ T cells were isolated from the CNS of symptomatic animals and characterized by flow cytometry. MHC class I expression in the CNS was studied by immunohistochemistry. The role of perforin was assessed by generating L31 mice deficient in perforin expression. **Results:** CNS CD8⁺ T cells have phenotypic and functional characteristics of effector-memory cells in an acute activated state, and are well equipped to kill target cells through various cytotoxic pathways. MHC-I expression was detected on oligodendrocytes but not on neurons in pre-clinical L31 animals. L31 mice deficient in perforin expression exhibited the same clinical and pathological features as perforin-competent animals but with an accelerated onset and hastened accumulation of CD8⁺ T cells in the CNS. **Conclusions:** Our data show that the CD8⁺ T cells in the CNS of L31 mice are potent effector cells and that the oligodendrocytes have the potential to interact with armed CNS CD8⁺ T cells in a MHC-I-dependent fashion before clinical manifestations of disease. However, demyelination does not occur through a perforin-dependent mechanism. Perforin appears rather to regulate the expansion of CD8⁺ T cells within the CNS.

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The mechanism of experimental autoimmune encephalomyelitis tolerance induced by stress-related chaperone Hsp70 complexed with endogenous peptides depends on H60 and NKG2D interaction

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Background: Inflammatory processes induce a site stress response which might be important in the recovery phase. **Objective:** Hsp70 complexed with endogenous peptides (Hsp70-pc) induces tolerance to experimental autoimmune encephalomyelitis (EAE); however, the mechanisms of this effect are not well understood. **Methods:** To assess Hsp70-pc-induced tolerance the effect of preinjection with anti-H60 and anti-NKG2D antibody on EAE in SJL/J was examined. We estimated function of dendritic and NK cells in co-culture with proteolipid protein (PLP)-reactive T cells. Assessment of PLP-reactive T-cell death and number of Treg cells was done by flow cytometry. **Results:** Peptides derived from brain tissue of mice with EAE complexed with stress-induced chaperone, hsp70 (Hsp70-pc) induced an NK-cell-dependent tolerance for subsequent EAE sensitization. We have shown that the MHC class I-related glycoprotein H60 determines Hsp70-pc-induced EAE inhibition. Hsp70-pc led to significant and selective upregulation of H60 expression in SJL/J mice, and antibody blocking of H60 expression led to loss of EAE tolerance. Similarly, blocking of the NK cell receptor for H60, NKG2D, also reversed the Hsp70-pc-induced EAE inhibition. In contrast, in C57BL/6 mice in which H60 was naturally not expressed, Hsp70-pc-induced tolerance was not detected. Direct killing of H60+ PLP-reactive cells seems not to be involved in the Hsp70-pc-induced tolerance mechanism as no enhanced loss of CD3+H60+ over CD3+H60- cells in Hsp70-pc-induced EAE tolerance was found. The NK cell-mediated Hsp70-pc-induced tolerance to EAE was dependent on modulation of dendritic cell (DC) function. Similarly, we have not seen increased number of Tregs, CD4+ CD25+ FoxP3+ T cells. In co-culture system, DC from mice treated with Hsp70-pc with PLP-reactive cells reduced proliferative response to PLP. The mechanism of reduced PLP reactivity involved enhanced autoreactive cell death as shown by Annexin V and 7AAD staining. **Conclusions:** These findings might be of significance in tailoring immune regulatory strategies for autoimmune diseases such as multiple sclerosis.

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Fingolimod reduces axonal loss in relapsing secondary progressive experimental autoimmune encephalomyelitis

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Background: Whilst relapsing-remitting multiple sclerosis (MS) responds to immunomodulatory agents, immunosuppression alone does not control progressive MS, which is related to nerve loss. Fingolimod is an immunomodulatory agent in phase III trials in MS, that could have some influence not only on the immune elements that drive relapsing MS, but may also promote repair process that could influence the neurodegenerative process associated with disease progression. The immunomodulatory capacity has been demonstrated in experimental autoimmune encephalomyelitis (EAE) models in rodents and clinical trials in MS. However, rodent models can also demonstrate secondary progressive disease which, like the human counterpart, may not respond to immunosuppressive agents, and thus provide a model system to examine the neuroprotective potential of fingolimod in progressive disease. **Objective:** The aim of the study was to determine whether fingolimod is neuroprotective in an experimental model of progressive MS. **Methods:** Relapsing progressive EAE was induced in ABH mice using active sensitization to spinal cord homogenate in Freund's adjuvant. Fingolimod was administered orally at different times following disease induction and the effect on

clinical progression was assessed clinically using subjective standard disability status scales and objective outcome measures, such as mobility on RotaRod activity for up to 120 days. Axonal counting in the spinal cord was performed, on toluidine blue stained sections, using a fractionator technique. **Results:** Fingolimod slowed the development of immobility that arises as a consequence of relapsing-progressive disease and reduced axonal loss. **Conclusions:** Fingolimod significantly reduced signs of disease when delivered at the relapsing stage of disease. Modulation of disease by fingolimod was reflected in functional analysis by RotaRod, which was able to detect an improvement over time in fingolimod-treated animals which was not reflected in the clinical score. Fingolimod significantly reduced axonal loss. **Supported by:** Novartis.

P177**The experimental autoimmune encephalomyelitis model in Lewis rats: can we ignore the concomitant arthritis?**

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Background: Experimental autoimmune encephalomyelitis (EAE) is a model of multiple sclerosis (MS) that can be induced in Lewis rats by injection of myelin basic protein (MBP) emulsified in complete Freund's adjuvant (CFA). Animals with EAE show signs of arthritis. This is not surprising because CFA is used to generate experimental arthritis in the same strain. Since this model is used for target validation of novel therapies, it is important to know if the simultaneous arthritis interferes with the results on EAE. **Objective:** We have addressed the following questions: do rats with EAE develop a true arthritic process? Is EAE sensitive to treatments that work in MS? Does the improvement of the underlying arthritis impact the course of the EAE? **Methods:** Lewis rats were treated with CFA + MBP or CFA alone to induce EAE or adjuvant arthritis, respectively. We evaluated the arthritic process in both groups by determining the degree of paw inflammation, joint damage (X-rays and histology), and hematological changes (blood cell counts, inflammatory markers). EAE in rats treated with MBP+CFA was evaluated by a classical clinical score of neurological symptoms. Animals with EAE or arthritis were treated with: 1) teriflunomide or fingolimod, drugs which improve multiple sclerosis; 2) enbrel or rofecoxib, drugs which improve rheumatoid arthritis, or 3) dexamethasone, which improves both human diseases. All treatments were oral and once a day, except for enbrel, which was i.p. every third day. **Results:** Arthritis associated with EAE is milder than that observed in rats treated with CFA alone in terms of paw inflammation, histology, hematological changes and radiological score. Furthermore, the drugs tested behave in EAE as expected: all but rofecoxib and enbrel reduced the clinical score. **Conclusions:** Our results suggest that the underlying arthritis does not have a strong impact on EAE, confirming the validity of the rat EAE model for validation and screening of new treatments to improve MS.

P178**Mesenchymal stromal cells derived from bone marrow and Wharton's Jelly as potential sources for cell-based therapies in multiple sclerosis**Evi Theunissen¹, Marjan Moreels¹, Peter Ponsaerts², Ivo Lambrichts¹, Piet Stinissen¹, Niels Hellings¹¹Hasselt University, Biomedical Research Institute, Diepenbeek, Belgium; ²Antwerp University, Laboratory of Experimental Hematology, Wilrijk, Belgium

Background: During the late stages of multiple sclerosis (MS), neurodegeneration and axonal loss is prominent without the occurrence of overt pro-inflammatory reactions. Therefore, new therapeutic strategies that act on remyelination and neuroregeneration are needed. Stem cell (SC) transplantation improves clinical outcome in experimental autoimmune encephalomyelitis (EAE), the animal model of MS. While mesenchymal stem cells (MSC) are described to be immunomodulatory, the mechanisms involved are only partially understood. Moreover, intravenously injected MSC may migrate to inflammatory brain lesions and promote survival of brain-resident

cells. Besides the well-characterized bone marrow-derived MSC (BMSC), recent evidence suggests that Wharton's jelly contains a substantial amount of progenitor cells, termed umbilical cord matrix stem cells (UCMS). **Objective:** To compare UCMS and BMSC according to 1) mesenchymal phenotype 2) multi-lineage differentiation 3) immunosuppressive potential 4) induction of allo-responses **Methods:** Primary cultures of UCMS were set up from human post-partum umbilical cords. Immunophenotype was determined by flow cytometry and immunocytochemistry. T cell suppression and allo-reactivity was analyzed in co-cultures of (anti-CD3 activated) peripheral blood mononuclear cells (of 4 healthy donors) and increasing amounts of SC. **Results:** We demonstrated that UCMS show immunophenotypical and ultrastructural similarities with MSC. UCMS are able to differentiate to multiple mesenchymal lineages (adipocytes, osteoblasts, and chondrocytes). Our initial results indicate that BMSC, in contrast to UCMS, dose-dependently suppressed anti-CD3 stimulated T cells. BMSC-derived supernatant strongly suppressed T-cell activation, suggesting a role for soluble factors. While BMSC induce only low proliferative allo-responses, UCMS induced significant alloreactivity in 3 out of 4 donors tested. Further experiments are ongoing to confirm these findings. **Conclusions:** Our preliminary data show that MSC populations derived from bone marrow versus Wharton's Jelly differ in their immunosuppressive capacity and immunogenicity. Currently, experiments are ongoing to test the effect of both SC populations in EAE.

P179**Poly(ADP)ribose polymerase inhibitor modifies the activities of various kinase cascades in the cuprizone-induced demyelination model**Sara Veto¹, Peter Acs², Antal Tapodi¹, Zoltan Lendvai³, Samuel Komoly², Zsolt Illes², Ferenc Gallyas¹¹Department of Biochemistry and Medical Chemistry, University of Pecs, Pecs, Hungary; ²Department of Neurology, University of Pecs, Pecs, Hungary; ³Department of Radiology, University of Pecs, Pecs, Hungary

Background: Cuprizone administration to young adult mice induces demyelination. It is assumed that cuprizone inhibits mitochondrial oxidative phosphorylation, causing energy depletion and also excessive production of reactive oxygen species in oligodendrocytes, resulting in the observed pathology. The nuclear enzyme poly(ADP)ribose polymerase (PARP) activated by DNA strand breaks plays an important role in oxidative stress-induced cell death. Inhibition of PARP showed protection in several pathological conditions involving oxidative stress, also affecting the modulation of several kinase cascades. **Objective:** Our aim was to investigate the involvement of PARP enzyme and the effect of the PARP inhibitor 4-hydroxyquinazoline (4HQ) in this model. **Methods:** Eight-week-old C57BL/6 male mice were fed cuprizone-containing diet for 5 weeks to induce demyelination. 4HQ was administered i.p. every day, started on the same day as the cuprizone treatment. For Western blot analysis tissue samples were taken from the corpus callosum. **Results:** Compared with controls, an increased PARP activity was found on cuprizone treatment, which was attenuated by 4HQ. PARP inhibition reversed the decreased expression of myelin basic protein induced by cuprizone, inhibiting the severe myelin loss. Cuprizone activated the phosphatidylinositol3 (PI3)-kinase/Akt pathway and the mitogen-activated protein kinase (MAPK) system, namely the extracellular signal-regulated kinase (ERK)1/2, p38 MAPK and c-Jun N-terminal kinase (JNK). 4HQ enhanced the activity of Akt, and dramatically attenuated the phosphorylation of p38 MAPK and JNK. **Conclusions:** Our results provide evidence for the involvement of PARP and MAPK cascades in the pathogenesis of demyelination and also for the beneficial effects of PARP inhibitors mediated, at least partially, through the regulation of the PI3-kinase/Akt and MAPK signaling pathways in the cuprizone model. These mechanisms may also play a role in the pathomechanism of multiple sclerosis and could be potential targets of treatment in the future.

P180**Connexin expression in acute and chronic mouse models of experimental autoimmune encephalomyelitis**Stephen Karlik¹, David E. Carter², Wendi Roscoe¹¹Diagnostic Radiology, University of Western Ontario, London, Ontario, Canada; ²Robarts Research Institute, London, Ontario, Canada

Background: Variations in severity and duration of experimental autoimmune encephalomyelitis (EAE) depend on the species and the method of induction. Our laboratory is characterizing chronic demyelinated and remyelinating mouse EAE models to understand possible therapeutic targets. Previous studies have shown that astrocytic connexin43 (Cx43) expression was decreased in demyelinating lesions and increased in remyelinating lesions in the chronic guinea pig model, indicating that astrocytic intercellular cell communication molecules were required for recovery. **Objective:** To use RNA microarray studies to investigate the modulation of gap junction proteins in acute and remyelinating EAE mouse models. **Methods:** In this study, we compared two methods of EAE induction, one with myelin oligodendrocyte glycoprotein (MOG 35–55) peptide, and the other with human recombinant MOG protein (rMOG), in female C57BL/6J mice. Sixteen mice were used in this study: 4 acute (MOG peptide-induced) and 4 non-immunized controls (sacrificed on day 21 post-immunization) and 4 with spontaneous remyelination (rMOG-induced) and 4 non-immunized controls (sacrificed on 55 days post-immunization). RNA was isolated from all spinal cords and gene expression was analyzed using the Affymetrix GeneChip system (London Regional Genomics Centre, London, ON). **Results:** MOG peptide caused an acute mouse model with extensive demyelination, immune cell infiltration and axonal loss, whereas rMOG immunization produced a less severe disease course with many animals showing partial clinical recovery and spinal cord remyelination. In the microarray studies, acute mice showed a decrease in Cx43, Cx47, Cx59, Cx32, and Cx30, and the remyelinating mice showed an increase in Cx43, Cx47, and Cx32, with no change observed in the other central nervous system (CNS) connexins. **Conclusions:** These are the first studies to compare an acute and remyelinating mouse model of EAE, and the first to show RNA expression, specifically connexin expression. The changes in several connexin transcripts indicate that connexin intercellular communication may be important for remyelination in the CNS.

P181**Experimental autoimmune encephalomyelitis in interleukin-15 knock out mice**

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Background: A complex interplay between cytokines exists during the induction and effector phases of experimental autoimmune encephalomyelitis (EAE). Interleukin-15 (IL-15) shares many activities exerted by proinflammatory interleukin-2 (IL-2) including the stimulation and expansion of T cells, thymocytes, B cells and natural killer (NK) cells. IL-15 and IL-2 may participate in negative regulation by binding to common hematopoietin receptor beta and gamma subunits. IL-15 also stimulates the expression of IL-17 by CD4+ T-cells. Mice deficient in IL-15 are unable to initiate an immune response to viral infection and show a deficiency in NK cells, T cells, and CD8+T cells in lymph nodes. **Objective:** To investigate the role of IL-15 in neuroinflammation. **Methods:** Acute EAE was induced in IL-15 knock out (KO) and wildtype mice. Clinical signs were monitored daily. Pathological changes in isolated cords were blindly assessed and terminal plasma samples were investigated using the RodentMAP 2.0 (Rules Based Medicine). **Results:** IL-15 (KO) mice (C57BL6Tac) immunized with the neuroantigen myelin oligodendrocyte glycoprotein peptide 35–55 and adjuvant showed enhanced inflammatory cell influx ($p=0.024$) into the central nervous system and increased clinical severity of EAE after day 18 post immunization ($p<0.05$) compared with immunized controls. Extensive demyelination was observed in the spinal cords of the IL-15 KO cohort ($p<0.014$). Consistent with these observations were altered levels of two molecules. A two-fold increase in CD40ligand (CD40L) ($p=0.021$) was detected in plasma samples from the IL-15 KO mice. CD40L facilitates leukocyte recruit-

ment and is necessary for microglial activation during acute EAE. There was also a 1.5-fold decrease in macrophage-derived chemokine (CCL22/MDC), which induces chemotaxis of Th2 cells and promotes recovery. **Conclusions:** We expected the IL-15 KO mice to show decreased EAE due to the loss of a proinflammatory input; however, altered cytokine levels appeared to compensate and resulted in enhanced disease.

P182**Cuprizone treatment induces demyelination in the hippocampus formation**

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Background: Cognitive impairment has been documented in multiple sclerosis (MS) patients. Memory impairment is especially prominent within the spectrum of cognitive deficits. One of the brain structures crucial for memory is the hippocampus formation. It is well known that hippocampal demyelination is seen in MS patients. The number of hippocampal lesions displays a strong correlation with cognitive decline as well as progression of cognitive dysfunction. **Objective:** So far no animal model for MS is known enabling investigation of mechanisms of hippocampal demyelination and development of appropriate therapy strategies against cognitive decline. **Methods:** Young adult and aged mice were fed with cuprizone for a defined time interval. The status of myelination in the hippocampus was analyzed by conventional histological stainings as well as expression analysis of mature oligodendrocytes. Functional markers microglia and astrocytes were additionally analyzed. **Results:** Feeding of cuprizone induced an almost complete demyelination of the perforant path. Demyelination was pronounced in both young adult and aged male and female mice. Demyelination of the perforant path was prominent in young adult mice after 5 weeks of cuprizone treatment (0.2%). Aged mice, however, required a higher concentration (0.4%) and longer exposure period (7 weeks). Other hippocampal fiber tracts such as the fimbria or alveus were not affected. Astrogliaosis was prominent throughout the entire hippocampus. **Conclusions:** These data strongly implicate that cuprizone-induced demyelination is an appropriate model to investigate mechanisms of hippocampal demyelination. Among the hippocampal pathways, evaluation of the perforant path seems to be most reliable and reproducible. This is of particular interest, since the perforant path provides the major input in the hippocampal formation. We conclude that cuprizone-induced demyelination might provide a new model to investigate appropriate therapy strategies for the prevention of cognitive decline in MS patients.

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P183**Remyelination is delayed in the gray matter compared with white matter tracts after cuprizone-induced demyelination**

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Background: Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system that leads to focal plaques of the white matter and axonal loss. In recent years however, studies have shown that lesions also arise within gray matter structures, particularly in the cortex. Cortical demyelination is mainly prominent in primary and secondary progressive MS, but is rare in the acute or relapsing form. It is not known to what extent underlying mechanisms of demyelination and remyelination differ in gray matter compared with white matter areas. **Objective:** The purpose of this study was to compare cortical lesions with white matter lesions in cuprizone-induced demyelination and remyelination. **Methods:** Demyelination was induced by feeding young male mice a diet containing 0.2% cuprizone for 5 weeks. To induce remyelination, cuprizone feeding was maintained for 5 weeks, and thereafter mice were put on a normal chow for 1–4 weeks. After the defined time interval,

cerebral hemispheres and the corpus callosum (CC) were dissected and analyzed for oligodendrocyte, astrocyte, and microglia markers as well as growth factor expression using array technology and rtPCR. **Results:** During demyelination, the decline of mature oligodendrocyte markers was similar in the gray and white matter. During remyelination, the appearance of myelin basic protein and proteolipid protein was delayed in the cortex compared with the CC. Beyond, both regions differed in their growth factor expression profile (i.e. higher IGF-1 and brain-derived neurotrophic factor levels in the CC) during early and late remyelination. **Conclusions:** Here, we show that cuprizone-induced demyelination is similar in white and gray matter areas while remyelination is delayed in the cortex. Differences in the growth factor expression profile implicate different underlying mechanisms. We conclude that these findings might be relevant for further therapeutic strategies with regard to white and gray matter pathology. **Supported by:** START grant from the Medical Faculty of the RWTH Aachen (M.K.).

P184

The TGF-beta inhibitor Smad7 controls t cell differentiation and susceptibility to autoimmune encephalomyelitis

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Background: T cells play an important role in the pathogenesis of multiple sclerosis (MS) and its model disease experimental autoimmune encephalomyelitis (EAE). The transforming growth factor (TGF)-beta is a crucial cytokine in T cell differentiation, blocking T helper (Th)1 and 2 differentiation under certain conditions and driving T regulatory (Treg) and Th17 polarization in others. **Objective:** To investigate the role of Smad7, the intracellular inhibitor of TGF-beta signaling, in T-cell differentiation and in autoimmune inflammation of the central nervous system (CNS). **Methods:** We used conditional gene targeting to generate mice with a specific deletion of Smad7 in T cells. We analyzed T-cell differentiation in vitro and in vivo after induction of EAE with MOG(35-55) peptide. Wild type mice and mice with T-cell specific overexpression of Smad7 served as controls. **Results:** When stimulated in vitro under differentiation conditions, Smad7-deficient T cells showed an increase in Treg cells, whereas Th17 differentiation was slightly and Th1 differentiation strongly inhibited. Mice lacking Smad7 in T cells were partially resistant to the induction of EAE and exhibited less inflammation in the CNS. In contrast, mice with a transgenic overexpression of Smad7 in T cells showed a significantly enhanced EAE disease course and Th1 response, despite the lack of Th17 differentiation and infiltration in the CNS. Importantly, we have shown that systemic inhibition of Smad7 with specific antisense oligonucleotides equally suppressed EAE in wild-type mice. **Conclusions:** Smad7 is a major regulator of T-cell differentiation and autoimmune CNS inflammation. Increased Smad7 expression in T cells aggravates EAE and Th1 responses despite reduced Th17 differentiation. Lack of Smad7 in T cells ameliorates EAE and reduces Th1 and Th17 effector mechanisms. Targeting of Smad7 might therefore be a therapeutic approach for T cell-mediated autoimmune diseases of the CNS.

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P185

p57kip2 is dynamically regulated in experimental autoimmune encephalomyelitis and functions as a negative regulator of post-mitotic oligodendroglial differentiation

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Background: In contrast to the peripheral nervous system the adult central nervous system has only a limited capacity for spontaneous regeneration. That is why traumatic lesions and neurodegenerative inflammatory diseases such as multiple sclerosis (MS) lead to chronic impairment featuring destroyed glial-neuronal architecture and demyelinated brain regions. **Objective:** In order to detect potential inhibitory components of cellular redifferentiation, we have investigated gene regulatory events in the spinal cord during myelin oligodendrocyte glycoprotein-induced experimental autoimmune encephalomyelitis (EAE). **Methods:** We performed array hybridisation and quantitative RT-PCR analysis on healthy and diseased rat spinal cords as well as long-term gene suppression by means of vector based RNA interference in cultured primary oligodendroglial cells. **Results:** This demonstrated that p57kip2 is expressed by oligodendroglial cells and that this gene is dynamically regulated during the disease course. Interestingly, the gene regulation pattern correlates with remyelination/repair activities. We then knocked down p57kip2 expression by means of long-term RNA interference. This revealed that decreased levels of p57kip2 lead to an accelerated morphological maturation of cultured primary oligodendrocyte precursor cells as well as an earlier onset of myelin marker expression. **Conclusions:** Our data suggest that p57kip2 exerts a negative effect on oligodendroglial differentiation, similar to what we have recently shown for Schwann cells (Heinen et al., Proc. Natl. Acad. Sci. U.S.A., 2008; in press). We therefore conclude that we have identified a novel negative regulator of oligodendrocyte differentiation which is probably involved in the control of remyelination and thus represents a potential target for new MS therapies.

P186

 1 integrin promotes survival of oligodendrocyte progenitor cells

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Background: Myelination results from oligodendrocyte precursor cells proliferating, migrating to appropriate locations, and differentiating into myelin producing cells within the central nervous system (CNS). Recent studies have demonstrated the importance of  1 integrin in oligodendrocyte maturation in vitro. **Objective:** To demonstrate the importance of  1 integrin in myelination in vivo. **Methods:** We have generated a transgenic mouse line that expresses a dominant-negative  1 integrin  C transgene under the control of the proteolipid protein (PLP) promoter that drives expression of the transgene specifically to CNS tissues. **Results:** The dominant-negative  1 integrin  C transgenic mice have hypomyelinated and increased numbers of unmyelinated axons in the spinal cord and the optic nerve; however, the corpus callosum remains unaffected. The dominant-negative  1 integrin  C transgenic mice, under a demyelination and remyelination model, demonstrate a significantly reduced number of remyelinated axons within the corpus callosum when compared with wild-type mice. The defects in myelination and remyelination in the dominant-negative  1 integrin  C mice were attributed to the disruption of the MAPK signaling pathway. Further histological and immunohistochemical analysis on tissue sections from the CNS of the dominant-negative  1 integrin  C transgenic mice under the demyelination and remyelination model demonstrates that there is a decrease in the number of proliferating oligodendrocytes, followed by a decrease in the number of mature oligodendrocytes. The reduction in the number of proliferating oligodendrocyte progenitors and mature oligodendrocytes may be attributed to an increase in the occurrence of apoptosis in the dominant-negative mice. We are currently investigating downstream signaling pathways that may be affected during remyelination. **Conclusions:** Our results highlight the importance of  1 integrin for oligodendrocyte survival in vivo.

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P187**The dominance of encephalitogenic peptide correlates to its ability to induce potent regulatory T cells**

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Background: Like their human counterpart multiple sclerosis (MS), animal MS models can show a great diversity in clinical courses. The most famous model is experimental autoimmune encephalomyelitis (EAE) and its susceptibility is believed to be determined by genetic factors. SJL/J mice are well known to develop relapsing-remitting EAE (RR-EAE) by immunization of PLP139–151. However, we found that priming with PLP136–150, overlapping peptide, induces monophasic EAE (M-EAE) resistant to relapse and also to re-induction of EAE with any peptides, unlike RR-EAE. We have already discovered that such resistance originated from a continuously high and selective induction of Foxp3+CD4+CD25+ regulatory T cells (Foxp3+Treg) co-expressing CD103 and CD69 in the LN cells during remission phase of M-EAE. **Objective:** To explore why the tiny difference in peptide sequence causes a greatly different ability in inducing potent Treg. **Methods:** We checked the dominance of proteolipid protein (PLP) peptides in SJL/J mice. We compared T-cell response in EAE primed with several peptides concerning with PLP136-150 and PLP139-151. **Results:** Contrary to conventional understanding, truncation of N-terminal residues in PLP136-150 or elongation of C-terminal residue in PLP139-151 exacerbated relapse and re-induction of EAE. As such, a dangerous self-peptide can differ from a less dangerous one only at flanking residues through prohibiting induction of potent Treg. Surprisingly, PLP136-150 was more dominant than PLP139-151 among immuno-dominant epitopes in SJL/J mice, and PLP136-150-primed LN cells could react more effectively than PLP139-151-primed ones. In the EAE and T cell response by low-dose PLP peptide experiments, we discovered that the more dominant the encephalitogenic peptide is, the more resistance to re-induction of EAE the induced peptide showed, indicating that TCR-peptide affinity determined the ability to induce potent Treg. **Conclusions:** These findings suggest that the variety of clinical prognoses in MS might depend on the different kinetics and potency of Treg induced by the different 'regulogenicity' in the individuals, controlled by the dominance of self-peptide. Through exploring this, a new therapy into full recovery and extended resistance to relapse may be developed.

P188**IL-6 trans-signaling modulates the early effector phase of experimental autoimmune encephalomyelitis and targets the blood-brain barrier**Ralf Linker¹, Fred Lühder², Karl-Joseph Kallen³, De-Hyung Lee¹, Britta Engelhardt⁴, Stefan Rose-John³, Ralf Gold¹*¹St. Josef-Hospital, Ruhr-University Bochum, Bochum, Germany; ²Institute for MS Research, University of Göttingen and Gemeinnützige Hertie-Stiftung, Göttingen, Germany; ³Department of Biochemistry, University of Kiel, Kiel, Germany; ⁴Theodor Kocher Institute, University of Bern, Bern, Switzerland*

Background: Interleukin-6 (IL-6) may play a crucial role in the pathogenesis of central nervous system (CNS) autoimmunity. It exerts its cellular effects by a membrane-bound IL-6 receptor (IL-6R), or alternatively by forming a complex with the soluble IL-6R (sIL-6R), a process named IL-6 trans-signaling. **Objective:** We investigated the role of IL-6 trans-signaling in myelin basic protein (MBP)-induced experimental autoimmune encephalomyelitis (EAE) in the Lewis rat, a model disease for autoimmune inflammation in the CNS. **Methods:** IL-6 trans-signaling was investigated in active and adoptive transfer MBP-EAE as well as in endothelial and T cell culture after application of specifically designed fusion proteins, including blockade of IL-6 trans-signaling with gp130-Fc or stimulation with hyper-IL-6. **Results:** Application of gp130-Fc delayed the onset of adoptively transferred EAE in comparison with control rats injected with PBS or isotype IgG. Histological evaluation on day 3 after immunization revealed reduced numbers of T cells and macrophages in the lumbar spinal cord of gp130-Fc treated rats. At the same time, blockade of

IL-6 trans-signaling resulted in a reduced expression of vascular cell adhesion molecule-1 on spinal cord microvessels whilst experiments in cell culture with hyper-IL-6 failed to show a direct effect of IL-6 trans-signaling on the up-regulation of endothelial adhesion molecules. In experiments including active EAE and T cell culture, inhibition of IL-6 trans-signaling mildly increased T cell proliferation, but did not change severity of active MBP-EAE or regulate Th1/Th17 responses. **Conclusions:** We conclude that IL-6 trans-signaling may play a role in autoimmune inflammation of the CNS mainly by regulating early expression of adhesion molecules, possibly via cellular networks at the blood-brain barrier.

P189**Hydroxytyrosol improves clinical and neuropathological signs of both relapsing-remitting and chronic non-relapsing experimental autoimmune encephalomyelitis**María José Mansilla¹, Carme Costa², Mireia Castillo², A. Geerlings³, Jordi Xaus³, J. Boza³, Manuel Comabella², Xavier Montalban², Carmen Espejo²*¹Neuroimmunology Unit, IR-Vall d'Hebron University Hospital, Barcelona, Spain; ²Vall d'Hebron University Hospital. Neuroimmunology Unit, Barcelona, Spain; ³Puleva Biotech, Granada, Spain*

Background: Hydroxytyrosol (HT), a phenolic compound present in virgin olive oil, has been shown to inhibit free radical generation, scavenger reactive oxygen, and nitrogen, and to increase plasma antioxidant capacity. It has anti-inflammatory activity and cytoprotective effects as well. **Objective:** Due to its antioxidant, anti-inflammatory, and cytoprotective properties, we aimed to study the effect of HT treatment in experimental autoimmune encephalomyelitis (EAE). **Methods:** For EAE induction, 8–10 week old female SJL/J mice were immunized with the PLP139–151 and C57BL6/J mice with the MOG40-55 peptides. As of one week before immunization until the day of sacrifice, mice were treated with 1–10 mg/mouse/daily of HT in drinking water. Mice had access to water ad libitum and were daily monitored for clinical signs. Animals were sacrificed at day 28 post-immunization and brain and spinal cord (for neuropathological studies) as well as spleen and lymphoid nodes (for immunological studies) were resected. **Results:** In four independent experiments (three in relapsing-remitting EAE and one in chronic non-relapsing EAE), the treatment with HT significantly decreased the incidence and/or severity of EAE clinical signs in a dose-dependent manner. In addition, animals treated with HT showed less inflammation and demyelization in the brain and spinal cord. Studies on the role of HT in the immune system are currently in progress in our lab in order to characterize HT function in T-cell proliferation, cytokine production and oxidative stress regulation. **Conclusions:** These results may set the rationale for the use of HT as an adjuvant treatment in multiple sclerosis therapy. **Supported by:** Puleva Biotech (Granada, Spain).

P190**Low-dose naltrexone (LDN) prevents development or delays onset and reduces severity of experimental autoimmune encephalomyelitis in mice**Kristen A. Rahn¹, Patricia J. McLaughlin¹, Robert H. Bonneau³, Anthony P. Turel², Gary A. Thomas², Ian S. Zagon¹*¹Neural & Behavioral Sciences, Penn State University, Hershey, Pennsylvania, USA; ²Penn State Univ - Neurology, Hershey, Pennsylvania, USA; ³Penn State Univ - Microbiology/Immunology, Hershey, Pennsylvania, USA*

Background: Naltrexone, a potent opioid antagonist, has been used at low concentrations (low-dose naltrexone, LDN) to block opioid receptors for a short time each day, and enhance the interaction of the compensatory elevation in endogenous opioids and their receptors in the remaining period. LDN depresses cell proliferation. **Objective:** To evaluate the efficacy of LDN treatment at the time of induction of experimental autoimmune encephalomyelitis (EAE). **Methods:** C57Bl/6 mice were inoculated with myelin oligodendrocyte glycoprotein (MOG)35–55 peptide and pertussis toxin. Immediately following MOG injection, mice were treated intraperitoneally once daily

with 0.1 mg/kg naltrexone (i.e., MOG-LDN) or saline (MOG-vehicle) and observed for changes in behavior and morphology. **Results:** 100% of the MOG-vehicle mice developed disease by day 30, and only 45% of the mice in the MOG-LDN group expressed the disease ($p < 0.0001$). The incidence of EAE (score of ≥ 1) was significantly reduced in the MOG-LDN group compared with the MOG-vehicle group. Of animals in the MOG-LDN group with disease, 28% of these had maximal behavioral scores of 1 (limp tail) in contrast to 100% MOG-vehicle mice displaying behavioral scores of >2 (wobbly gait). MOG-LDN animals (0.8 ± 0.2) had lower mean disease scores than MOG-vehicle mice (1.9 ± 0.1). Matched spinal cord sections showed that MOG-LDN mice had significantly ($p < 0.001$) fewer ($9.9 \pm 1.6/\text{grid}$) activated astrocytes relative to the MOG-vehicle group ($19.8 \pm 1.4/\text{grid}$). **Conclusions:** These data demonstrate that intermittent opioid receptor blockade by LDN prevents development of EAE, or delays the onset and severity of EAE, supporting further exploration of therapeutic use of LDN for multiple sclerosis patients.

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A role for integrin-linked kinase in oligodendrocyte mediated myelination of the central nervous system

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Background: Recent studies have shown integrin-linked kinase (ILK) to be a major structural binding partner of $\beta 1$ integrin, an essential component of oligodendrocyte myelin sheet formation in the central nervous system (CNS). To study the role of ILK in CNS myelination *in vivo* and avoid embryonic lethality associated with ILK deletion, we have created a conditional knockout mouse model employing the Cre-loxP system. **Objective:** To generate a line of ILK conditional knockout mice to study the morphological and ultrastructural changes in the CNS of ILK-depleted mice. **Methods:** Using the Cre recombinase model, recombination of floxed *Ilk* can be controlled spatially and temporally: the *Cre* gene is under the control of the proteolipid protein (*Plp*) promoter, allowing for oligodendrocyte-specific expression. The Cre recombinase used was a fusion with a mutant estrogen receptor (CreER⁺) which can only enter the nucleus upon activation by tamoxifen and not estradiol. Injection of tamoxifen would thus be required for translocation of Cre into the nucleus and for the excision of the floxed *Ilk* gene. Mice homozygous for floxed *Ilk* were crossed with heterozygous PLP-CreER⁺ mice to produce PLP-CreER⁺/ILK^{fllox/fllox} mice. Following tamoxifen injection, mice were sacrificed at different time points for analysis. **Results:** Initially, we have assessed for expression of Cre in CNS tissues, specifically in the oligodendrocyte lineages, and have determined the efficiency of *Ilk* excision. The effect of ILK deletion on formation and maintenance of CNS myelin, as well as remyelination following cuprizone-induced demyelination will be studied. **Conclusions:** Through our work we will demonstrate the importance of ILK in signaling CNS myelination. Understanding key steps in the modulation of remyelination is critical as we move towards developing therapeutic strategies for the treatment of multiple sclerosis.

Supported by: Multiple Sclerosis Society of Canada.

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Depletion of commensal microflora of the gut alters global immune homeostasis and influences the development of experimental autoimmune encephalomyelitis in mice

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Background: The role of commensal microorganisms in the immune balance and development of autoimmune diseases such as multiple sclerosis (MS) remains unknown. Recent studies demonstrated that peripheral tolerance induced in the gut might be a powerful tool to control experimental autoimmune encephalomyelitis (EAE), animal model of MS. Previous works have reported that germ-free animals present altered immune responses, with a default Th2-type bias and a

significant reduction of IL-17-producing CD4⁺ T cells. **Objective:** To analyze the role of gut commensal bacteria in the maintenance of immune homeostasis and development of EAE. **Methods:** C57BL/6 and SJL mice were treated orally or i.p. with a broad spectrum of antibiotics to deplete gut microflora and analyze the role of commensals in the development of EAE, induced by immunization with myelin oligodendrocyte glycoprotein (MOG)₃₅₋₅₅ or proteolipid protein (PLP)₁₃₉₋₁₅₁. Treg cell and dendritic cell populations in gut-associated lymphoid tissue and other peripheral lymph nodes, cytokine production and central nervous system (CNS) infiltration were analyzed. **Results:** Results show that depletion of commensal microflora impairs the development of EAE in SJL mice. We confirmed that this impairment is due to the reduction of commensal populations, since i.p. treatment with the same antibiotics did not diminish aerobic/anaerobic organism presence and animals developed comparable EAE clinical scores with naïve SJL mice. Depletion of aerobic and anaerobic commensals by oral administration caused a significant reduction of body and splenic weights. Splenic IFN- γ , TNF- α , MIP-1 α , MIP-1 β and IL-6 levels were significantly diminished after oral treatment with antibiotics, which also caused a significant increase in IL-13 when compared to naïve levels. Moreover, expression of IL-17 and ROR γ t was reduced in head and neck lymph nodes (HNLN) of orally treated mice. Significant accumulations of FoxP3⁺Treg cells were observed in PP, MLN and HNLN of animals with microflora depleted guts. **Conclusions:** We conclude that immune responses induced by commensal organisms of the gut modulate the global immune responses, affecting CNS autoimmune processes.

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Oral teriflunomide in patients with relapsing multiple sclerosis: baseline clinical features of patients in the TEMSO phase III trial

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Background: Teriflunomide is an oral immunomodulator with anti-inflammatory activity that has previously been investigated in a 36-week, Phase II randomized double blind placebo-controlled trial. In this study of 179 patients the mean number of unique lesions per magnetic resonance imaging (MRI) scan (primary end point) was more than 60% lower in teriflunomide 7 mg or 14 mg daily groups compared with the placebo group ($p = 0.023$ and $p = 0.005$ respectively). **Objective:** To present baseline clinical features of patients in the ongoing multicenter trial of oral teriflunomide for relapsing forms of multiple sclerosis (RMS). **Methods:** The TEMSO trial (Teriflunomide Multiple Sclerosis Oral trial) is an ongoing randomized, double-blind, placebo-controlled, parallel group design study that will evaluate the efficacy and safety of teriflunomide in reducing the frequency of relapses and accumulation of physical disability in subjects with RMS. 1088 patients aged 18–55, with Expanded Disability Status Scale (EDSS) score of 0–5.5, were included. Patients were randomized to receive teriflunomide 7 or 14 mg or placebo daily and will be followed for 108 weeks with clinical, MRI and safety evaluations. The primary endpoint is the annual relapse rate (ARR) defined as the number of relapses per subject-year, with several clinical and MRI secondary endpoints. The data are overseen by an independent data-monitoring-safety committee (IDMC). **Results:** Enrolment was completed in early 2008 with 1088 patients randomized in 21 countries. The mean age is 37.9 years (median 39 years), 73.0% of the patients are female, mean time (SD) from MS diagnosis is 5.3 (5.4) years and mean time from symptom onset is 8.5 (6.8) years. The median baseline EDSS was 2.5 (range 0.0 : 6.0). Further baseline demographic and clinical variables will be provided. **Conclusions:** The patient demographics and clinical features of this study population are comparable with those of other recent large trials in RMS. The TEMSO trial will provide additional information on the safety and efficacy of teriflunomide, one of the first putative oral disease-modifying treatments for RMS.

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The linked role Of TLR-4 And HO-1 in the experimental autoimmune encephalomyelitis model

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Background: Toll-like receptors (TLR) are sentinel sensors of the innate immunity which recognize microbial specific pathogen-associated molecular patterns. Although controversial, a role for TLR in experimental autoimmune encephalomyelitis (EAE) has been reported. Heme oxygenase-1 (HO-1) is an antioxidant protein induced by heat shock and oxidative stress stimuli, inflammation, statins and hemin treatment. It has protective effect in EAE. Data have suggested that TLR-4 signaling may have a repressor function on HO-1 expression in a model of liver ischemia/reperfusion injury. **Objective:** We hypothesized that similar interaction may contribute to EAE clinical outcome. **Methods:** EAE was induced with MOG35-55 peptide. C57Bl/6 WT and TLR-4 knockout (KO) mice were used. **Results:** Comparing with controls, TLR-4 KO mice had better disease clinical outcome during 42 days (P=0.0049). Our previous results have shown that simvastatin treatment enhanced HO-1 mRNA expression in EAE. In this case, simvastatin-treated mice had lower disease clinical scores. Therefore, we asked if simvastatin treatment would have an opposite effect in TLR-4 expression. Surprisingly, there was a tendency towards higher TLR-4 expression in simvastatin-treated mice (control: 5.81, simvastatin 1 mg/kg: 7.46, simvastatin 5 mg/kg: 10.75). **Conclusions:** TLR-4 KO mice have better EAE clinical outcome than wild-type control mice. However, the higher levels of TLR-4 expression in simvastatin treated mice do not enhance disease severity.

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P195

Identification of novel integrin-linked kinase interacting proteins in CG4 oligodendrocytes during proliferation and differentiation

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Background: Oligodendrocytes are responsible for the generation of myelin in the central nervous system. The formation of myelin relies on interactions between the extracellular matrix (ECM) and the β 1-integrin signaling pathway. Integrin-linked kinase (ILK) is a protein associated with the C-terminus of β 1-integrin. Recent work has suggested that ILK is involved in the stabilization of focal adhesions and transduction of signals received from the ECM. **Objective:** The current study aimed to determine novel binding partners of ILK in a CG4 oligodendrocyte cell line. **Methods:** An N-TAP-ILK Tap-Tag construct was used to determine the differential binding partners of ILK during proliferation versus differentiation. **Results:** Mass spectrometry results confirm the presence of known ILK binding partners such as parvin and myosin. Results also show that the binding partners of ILK change between proliferation and differentiation of CG4 cells. Two proteins were detected that are associated with ILK during both proliferation and differentiation. These are the non-heterogeneous nuclear ribonucleoprotein U and non-POU domain-containing octamer binding protein. Identification of ILK binding partners in proliferating CG4 cells include Cdc42 effector, contrasted by those identified in differentiated cells such as Ras suppressor protein1, spindlin, and GSK. **Conclusions:** Future work will involve validation of these interactions by co-immunoprecipitation of the newly identified binding partners of ILK. The results of this study will allow a better understanding of the key players involved in the proliferation and differentiation of oligodendrocytes. This knowledge may facilitate the development of regenerative treatment for those suffering from demyelinating diseases, such as multiple sclerosis.

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Proportional changes of T-independent B cell subsets during acute-recovery phases of experimental autoimmune encephalomyelitis

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Background: B cells appear to play an important role in the pathogenesis of multiple sclerosis and they are also involved in regulatory mechanisms in a number of ways, such as cytokines secretion, production of natural autoantibodies and their ability to function as antigen-presenting cells to suppress encephalitogenic T cells. **Objective:** To study the implication of different B cells with potential regulatory behavior we analyzed T-independent B cell subsets distribution in spleen and cervical nodes. **Methods:** Transitional 2 (T2), marginal zone (MZ) and B1a B cells distribution were analyzed in murine experimental autoimmune encephalomyelitis (EAE) models developed in sensitive SJL/J and resistant C57BL/10.S mice for 60 days of protocol. The phenotypic analysis was carried out using a classic flow cytometry technique. **Results:** Our preliminary data revealed particular T2 and MZ B cell distributions during acute-recovery clinical course of disease in SJL/J-EAE mice. In the spleen, at disease onset, both T2 and MZ B cells show decreased proportions than those found at time of induction. T2 B cell levels rose when the recovery process started, while MZ B cells continued to decrease. Once total recovery was reached both T2 and MZ B cells recovered similar levels than in non-EAE mice. In cervical nodes, only MZ B cells show modification of distribution. The highest levels were observed at disease onset. No differences were found regarding B1a B cells in spleen and cervical nodes. In C57BL/B10.S-EAE mice MZ B cell proportions were sustained higher than those found in SJL/J-EAE mice, while T2 and B1a B cell were similar. **Conclusions:** These results indicate a possible role of T2 and MZ B cells in the regulation of initial resolution process in EAE. On the other hand, B1a B cells may also be involved in a regulatory process, probably acting in different sites than T2/MZ B cells. **Supported by:** C. Lee Chang obtained scholarship from Centre Hospitalier Régional et Universitaire de Lille and Région Nord pas de Calais Schering Pharma supported this work.

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High resolution 1H nuclear magnetic resonance spectroscopic-based metabolomic urine analysis of experimental autoimmune encephalitis, a model disease of multiple sclerosis

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Background: Diagnosis of multiple sclerosis (MS) is made on the basis of clinical symptoms and signs, and assisted by magnetic resonance imaging (MRI), cerebrospinal fluid analysis (CSF) and evoked potentials (EPs). However, MRI is expensive and in high demand; CSF requires a spinal tap, and EPs are often non-specific. Therefore, alternative methods to assist in diagnosis and monitoring of MS and effects of treatments are highly desirable. Metabolomic analysis of easily available biofluids, such as urine, using 1H nuclear magnetic resonance (NMR) spectroscopy can be used to characterize low molecular weight metabolite profiles, and may have potential for developing novel biomarkers of MS. **Objective:** To use NMR-based metabolomics to characterize the urine samples obtained from a mice with experimental autoimmune encephalitis (EAE). **Methods:** Urines were collected from EAE mice at different disease stages (acute, relapse, remission, chronic) and from control animals. 1H NMR spectra were acquired at 500MHz. Specific metabolite concentrations were obtained by integration of selected spectral peaks and comparing intensities with a standard. Significant differences between levels of metabolites in different groups were assessed using ANOVA and post-Dunnetts testing. For metabolomic analysis principal components

analysis (PCA) was carried out. **Results:** A range of metabolites were detected and quantified by analysis of NMR signals. Using metabolomic analysis, all four EAE subgroups and controls mapped to separate metabolite spaces demonstrated by a 3D-PCA plot. Metabolite quantification revealed that hippurate was significantly reduced in the urines of all EAE animals. **Conclusions:** ¹H NMR spectroscopy has potential to generate new biomarkers for diagnosis of different stages of EAE. If confirmed, metabolomic analysis of biofluids should be performed in subjects with MS to translate this methodological approach into a clinical setting. **Supported by:** Wellcome Trust.

P198

Human endogenous retrovirus W envelope induces a preclinical multiple sclerosis model in C57/Bl6 myelin oligodendrocyte glycoprotein mouse: therapeutic effect of monoclonal antibody
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Background: The human endogenous retroviral family W (HERV-W) encodes a powerful immunopathogenic envelope protein (ENV), which activates a pro-inflammatory and autoimmune cascade through interaction with Toll-Like receptor 4 (TLR4) on antigen-presenting cells and triggers superantigen-like dysregulation of T-lymphocytes. The specific association of HERV-W RNA in circulating virion particles (multiple sclerosis-associated retroviral element, MSRV) with multiple sclerosis (MS), its evolution and prognosis has now been repeatedly reported. Its ENV protein was evidenced by several independent PCR and/or immunohistological studies in MS brain lesions post-mortem. ELISA immunodosage for HERV-W ENV protein revealed positive antigenemia in 73% of MS sera and a low percentage (~5%) of asymptomatic or healthy carriers in the normal population, suggesting that HERV-W ENV antigenemia is not simply a consequence of MS (manuscript submitted). **Objective:** To study the effects of HERV-W ENV protein compared with classical EAE in the C57/Bl6 mouse model. To evaluate the pre-clinical therapeutic effects of a neutralising monoclonal antibody against HERV-W ENV. **Methods:** Magnetic resonance imaging (MRI) follow-up, immunohistochemistry, immunoassays. **Results:** HERV-W ENV protein is here shown to reproduce the experimental autoimmune encephalomyelitis animal model in C57/Bl6 mice with important inflammatory demyelination and gliosis evidenced by MRI and histology, as well as anti-myelin autoimmunity. A significant inhibition and prevention of clinical symptoms, compared with untreated controls, were evidenced in this pre-clinical model by clinical and MRI follow-up, when injecting selected anti-HERV/ENV antibody in mice with ongoing central nervous system inflammation and clinical deficits. Untreated ENV EAE ill-control mice died or reached major neurological deficit. **Conclusions:** This model is thus useful for pre-clinical evaluations and these new results now pave the way for the assessment of a therapeutic version of this anti-ENV antibody.

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Human endogenous retrovirus W envelope promotes demyelination in C57/Bl6 MOG mouse model: effect of specific monoclonal antibody

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Background: The human endogenous retroviral family W (HERV-W) encodes a powerful immunopathogenic envelope protein (ENV), which activates a pro-inflammatory and autoimmune cascade through interaction with Toll-Like receptor 4 (TLR4) on antigen-presenting cells and triggers superantigen-like dysregulation of T-lymphocytes. The specific association of HERV-W RNA in circulating virion particles (multiple sclerosis-associated retroviral element, MSRV) with multiple sclerosis (MS), its evolution and prognosis has now been repeatedly reported. Its ENV protein was evidenced by several independent PCR and/or immunohistological studies, in MS brain lesions post-mortem. ELISA immunodosage for HERV-W ENV protein revealed positive antigenemia in 73% of MS sera and a low percentage (~5%) of asymptomatic or healthy carriers in the normal population, suggesting that HERV-W ENV antigenemia is not simply a consequence of MS (manuscript submitted). **Objective:** To evaluate a new MS pre-clinical model based on C57/bl6 mouse myelin oligodendrocyte glycoprotein (MOG) experimental autoimmune encephalomyelitis (EAE). **Methods:** HERV-W ENV protein was used to induce MOG EAE in C57/Bl6 mouse and specific anti-ENV monoclonal antibodies were evaluated in the model for therapeutic effects. **Results:** HERV-W ENV protein is here shown to reproduce the EAE animal model in C57/Bl6 mice with important inflammatory demyelination and gliosis evidenced by magnetic resonance imaging (MRI) and histology, as well as anti-MOG autoimmunity. Anti-HERV-W/ENV antibody injection after EAE induction caused dramatic clinical and imaging improvements in this pre-clinical model. **Conclusions:** This model is thus useful for pre-clinical evaluations and these new results now pave the way for the assessment of a therapeutic version of anti-ENV antibody. **Supported by:** Fondation CERAL, France.

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Progressive central atrophy precedes the onset of disability in a murine model of multiple sclerosis

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Background: The pathogenesis of brain atrophy remains unclear in multiple sclerosis (MS). The extensive central atrophy that often accompanies MS has not yet been reported in animal models. Theiler's murine encephalitis virus (TMEV) infection of mice is a known MS model. In SJL mice, the resulting disease is characterized by progressive demyelination and motor deficits. **Objective:** Our objective was to determine whether progressive central nervous system atrophy is detectable in the TMEV-induced MS model, and to assess its temporal relationship to disability and ¹H MRS markers. **Methods:** Eight TMEV-infected SJL mice and 4 controls were scanned at 1, 2, 3, 4, 6 and 12 months after disease induction, using volume acquisition T1 and T2 weighted sequences in a 7 Tesla small animal magnetic resonance imaging system. Total brain, ventricular volume and cord cross section measurements were performed using semiautomated tools in Analyze 8.0. Short TE (20 ms) magnetic resonance spectroscopy of single 8mm³ brainstem voxels were acquired and analyzed using LCModel. We monitored disability by monthly rotarod assay. Statistical analysis was performed in JMP (SAS Institute, Cary, NC). **Results:** Significant brain atrophy was detected as early as 3 months

($p=0.005$) and reached its peak at 6 months post infection. Cord atrophy progressed until 9 months. Disability became significant at 4 months ($p=0.0005$) but not at 3 months ($p=0.45$), and continued to progress until 9 months. A decrease in NAA ($p=0.025$) was seen at 3 months, progressing until 12 months ($p=0.00014$) consistent with axonal/neuronal damage. Its peak elevation was seen at 3 months ($p=0.034$) further increasing at 12 months ($p=0.014$) suggesting ongoing gliosis. Cho, a membrane turnover/cell infiltration marker was significantly increased at 3 months ($p=0.033$) but later normalized ($p=0.18$), suggesting less prominent inflammatory infiltrates in the chronic stage. **Conclusions:** Our results in the first known model of central atrophy demonstrate that brain atrophy precedes the development of motor deficits. Axonal/neuronal processes likely contribute as evidenced by significant progressive NAA decrease. The model will allow us to characterize the pathomechanism of atrophy and may lead to novel therapeutic strategies in MS.

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Magnetic resonance imaging quantification of lumbar spinal cord inflammation in mouse experimental autoimmune encephalomyelitis *in vivo*

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Background: Mouse experimental autoimmune encephalomyelitis (EAE), an autoimmune disease principally targeting myelin and axons, is used extensively to evaluate the actions of potential new multiple sclerosis (MS) therapies. In such therapeutic studies, the ability to monitor the severity of spinal cord (SC) inflammation *in vivo* would be desirable. Previous magnetic resonance imaging (MRI) investigations with the contrast agent ultrasmall superparamagnetic iron oxide (USPIO), an agent that accumulates in phagocytic cells after systemic injection, revealed uptake of the agent in areas of macrophage accumulation in the brain. However, cerebral inflammation in mouse EAE is scant in comparison with SC. **Objective:** Our objective was to investigate whether inflammatory infiltration in murine EAE lumbar SC could be detected using T2*-weighted MRI scanning. We used the most commonly described murine EAE model, namely MOG35-55 antigen induced EAE in C57Bl6 mice. **Methods:** EAE mice ($n=10/1$ timepoint) were used for developing a protocol for SC MRI. EAE mice ($n=5/2$ timepoints) and healthy controls (HC $n=5/2$ timepoints) underwent a baseline MRI scan (Bruker 4.7T, T2*-weighted), followed by intravenous injection of 0.1ml of 0.9mmol USPIO (SHU555C). Animals were rescanned 24 hours post-injection. The spinal cords were later processed for immunohistochemistry. **Results:** In mice with severe EAE, MRI analysis showed a 17% (range 14–18%) difference in the white matter to gray matter intensity ratio between pre- and post-injection scans, whereas the difference was only 5% (range 2–7%), in the HC group ($p<0.0001$). This indicates increased uptake of USPIO in the white matter of the SC. In EAE mice there was a moderate correlation between USPIO uptake and extent of immune infiltration in the dorsal and ventral columns of the lumbar SC ($r=0.58, P<0.001$). **Conclusions:** Utilising intravenously administered USPIO, MRI can detect inflammatory lesions in mouse EAE SC. This technique could be utilised for monitoring SC inflammation in animal models of auto-immune disease, in the context of pre-clinical therapeutic evaluations.

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P202

Exploring the neuro-vascular niche as potential target for treatment in MS

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Background: Proliferating neural stem cells have been detected in close contact with cerebral microvessels in the adult subventricular zone (SVZ). Blood-brain barrier damage as detected by gadolinium-enhancing T1-lesions is the hallmark of early lesion development but has also recently been associated with repair of multiple sclerosis (MS) lesions. **Objective:** To investigate inflammatory induced gene expression in cerebral endothelial cells and their functional impact on proliferation and differentiation of neural precursors in an *in vitro* co-culture model. **Methods:** Human (D3) and murine (bEnd-5) cerebral endothelial cells were grown to confluence and stimulated with tumor necrosis factor-alpha (TNF- α) +/- interferon beta (IFN- β). The gene expression pattern was determined by the R&D cytokine cDNA array and supernatants analyzed using ELISAs for brain-derived neurotrophic factor (BDNF), IL-6, SCF-1. For co-culture experiments neurospheres were obtained from the forebrain of E14 green fluorescent protein transgenic mouse embryos. Proliferation was determined by BRDU incorporation, whereas differentiating stem cells were detected by immunostaining for MAP-2, pTau (neuronal lineage) or NG-2 (glial lineage) and quantified by number and extension of processes. **Results:** In both human and murine cerebral endothelial cells TNF- α induced several inflammatory related genes among cytokines, chemokines and adhesion molecules, which were reduced by co-administration of IFN- β . Interestingly, trophic factors such as VEGF, SCF-1, PDGF- α and BDNF were also induced by TNF- α , but not inhibited by IFN- β . Using the co-culture system of cerebral endothelial cells and neural precursors we could induce differentiation with TNF- α treatment only in the presence of endothelial cells. This effect was largely inhibited by neutralizing antibodies against BDNF. **Conclusions:** Inflammatory induced transformation of cerebral endothelial cells leads to production of trophic factors and is associated with neural stem cell differentiation *in vitro*. The neuro-vascular niche may therefore be an interesting new target for regenerative treatment strategies in MS.

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P203

Axon stress and disruption of the axo-glial complex prior to the onset of disease in experimental allergic encephalomyelitis

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Background: Altered conduction and axonal damage are important features in multiple sclerosis (MS). We have previously shown that disruption of axo-glial contact at the node of Ranvier is concurrent with axonal stress in MS normal-appearing white matter (NAWM). However, the mechanisms of axon stress and damage in the early disease environment prior to demyelination are unknown. **Objective:** To investigate presymptomatic changes at the node of Ranvier in experimental allergic encephalomyelitis (EAE). **Methods:** Female C57BL/6 mice were immunized with 200 μ g of MOG35-55 peptide in Complete Freund's adjuvant at days 0 and 7 (100 μ l, s.c.). Tissue was sampled at determined time points for histological analysis. **Results:** Oligodendrocyte-specific neurofascin (Nf155) binds to proteins of the axonal membrane to paranodal axo-glial junctions that stabilize Nav+ channels at the node and K+ channels at the juxtaparanode, an arrangement vital for saltatory conduction. We assessed presymptomatic changes in these structures in MOG35-55 peptide-induced chronic EAE. Tissue sampled at 3 and 10 days post immunisation showed that paranodal axo-glial Nf155 expression was altered significantly in comparison with naive animals ($p<0.01$). Paranodal disruption resulted in an overlap of Nf155 with altered expression of Kv1.2 channels at the juxtaparanode, and the proportion of nodes expressing the adult Nav+ 1.6 isoform was reduced.

Increased TLR4 expression on IBA+ cells in the vicinity of paranodal disruption indicated microglial activation. Eleven days of prophylactic treatment with BIO5192, a small molecule VLA-4 antagonist did not reduce the extent of paranodal disruption. **Conclusions:** Our data suggests that distinct presymptomatic changes occur in the axo-glia complex that may be linked to neurofilament dephosphorylation in axons. Treatment with BIO5192 conferred no protection, implying that early changes in saltatory conduction may occur without the presence of peripheral immune cells, and that the resident central nervous system innate response initiates changes prior to focal antigen-mediated damage.

Supported by: aADI collaboration between GlaxoSmithkline and Imperial College, London.

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Effects of the anti VLA-4 small molecule inhibitor BIO5192 on clinical course and pathology in chronic experimental allergic encephalomyelitis

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Background: Therapeutic success with anti VLA-4 treatment in multiple sclerosis (MS) is well documented in reducing relapse frequency and the formation of new lesions as shown by magnetic resonance imaging. Evidence for the success of anti VLA-4 treatment in experimental allergic encephalomyelitis (EAE) ultimately led to the development of this therapy. However, contradictory evidence in numerous EAE models highlights the wider reaching effects of anti VLA-4 therapy. **Objective:** Analysis of the efficacy of prophylactic and therapeutic administration of BIO5192 in chronic EAE. **Methods:** Female C57BL/6 mice were immunized with 200µg of MOG35-55 peptide in Complete Freund's adjuvant at days 0 and 7 (100µl, s.c.). Prior to immunization, animals were assigned to treatment groups or vehicle controls. **Results:** Results in chronic EAE indicate that prophylactic treatment with BIO5192 (30mg/kg and 100mg/kg, uid, s.c.) leads to a reproducible delay in disease onset (17.8 +/- 0.75 Vehicle vs. 24.2 +/- 1.23 BIO5192 (30mg/kg) and 29.6 +/- 1.4 (100mg/kg)). Immunohistochemistry for T lymphocyte (CD4+ and CD8+) and macrophage/microglia (CD11b) markers highlighted that this temporal effect on disease delay correlated with a transitory reduction in inflammatory cell number in the spinal cord. No reduction in disease score was seen in the 100mg/kg treatment group compared with the vehicle controls as animals entered the chronic phase, indicating a loss of drug efficacy. Animals treated therapeutically showed no amelioration in disease compared with vehicle controls. In addition to changes in disease score and pathology, prophylactic treatment did not significantly change levels of IL-17 in the spinal cord of treated animals. **Conclusions:** This report confirms data previously published that the timing and concentration of anti VLA-4 treatment is vital for the reduction of clinical symptoms seen. Early data also hints that prolonged administration of BIO5192 does not alter the IL-17 axis of autoimmune damage. These studies emphasize the importance of evaluating preclinical data generated in more than one disease model in developing new therapies for MS.

Supported by: aADI collaboration between GlaxoSmithkline and Imperial College London.

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Monitoring of experimental autoimmune encephalomyelitis pathways during pharmacological modulation

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Background: Multiple sclerosis (MS) is a complex disease with a multifaceted etiology and heterogeneous pathology. Demyelinated cen-

tral nervous system (CNS) lesions are the pathologic hallmark of MS and are accompanied by inflammation, reactive gliosis, oligodendrocyte death and axonal loss. Experimental autoimmune encephalomyelitis (EAE) is widely used as an animal model of MS, serving as a valuable tool to study the pathogenesis and test new therapeutic approaches. **Objective:** The aim is to characterize the gene expression profile in different tissues of MOG-induced EAE in C57B/6 mice covering different states of the disease. This genomics paradigm enables an extensive concurrent representation of genes and pathways relevant to the pathological and drug treatment processes. **Methods:** The gene expression profile, characterizing the progression of EAE was studied by microarray analysis following temporal progression (7, 10, 14, 21 and 28 days) after disease induction. RNA from several tissues, CNS areas (spinal cord and cerebellum) lymph nodes, spleen and blood was studied in four individual mice with homogeneous clinical score per time point. The involvement of specific biological pathways and the over and under-representation of biological functions have been investigated by different analysis approaches including hierarchical clustering and pathway analysis. **Results:** We performed a stepwise analysis. First, at the gene level we observed that the total number of regulated genes was time and clinical score-dependent. Then, the predominant canonical pathways were identified at each time point to characterize the main physiopathological mechanisms taking place during disease progression. The next step involved identifying modulated pathways in the same model and tissues in animals receiving pharmacological treatment with recognized mode of action. Examples of such modulated pathways are discussed. **Conclusions:** We have developed a useful and valuable tool for monitoring pathways in disease models, which can be used to characterize the pharmacological modulation of candidate targets and profile compounds.

P206

APOE 4 polymorphism results in early cognitive deficits in the experimental autoimmune encephalomyelitis mouse

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Background: Recent studies have identified an association between APOE 4 and cognitive deficits in multiple sclerosis (MS), which is most striking in the youngest cohort of patients. Previous studies in transgenic APOE animal models have demonstrated cognitive deficits in aged mice, emphasizing its effect in diseases such as Alzheimer's. Understanding the unique role of APOE 4 in MS requires animal models which manifest cognitive deficits at an earlier age and stage. **Objective:** To determine the role of APOE 4 polymorphism in MS and to produce a model whereby the unique early effect can be studied, we measured cognitive performance in the transgenic APOE 4 mouse after experimental autoimmune encephalomyelitis (EAE) induction before the onset of motor symptoms. **Methods:** Cognitive function of APOE knockout (KO) and human APOE 4 knockin (E4) mice were compared with wild type on day 7-9 (age 6-8 weeks) after EAE induction prior to the onset of motor manifestations (day 13-15). Behavioral characteristics of the cognitive deficits were determined using the Morris water maze. Neurohistochemical studies of the hippocampus were performed to confirm the deficits. **Results:** After EAE induction, KO and E4 showed significant deficits in spatial learning and recall, suggesting deficits in cholinergic function. Evidence of neuronal damage in the hippocampal CA region was identified and correlated with regional increases in cholinesterase and decreased levels of acetylcholine in the E4 and KO groups relative to the wild type. **Conclusions:** Understanding the unique role of APOE in MS requires accurate animal models. We present evidence of the role of APOE in EAE prior to the onset of motor symptoms, suggesting that cognitive deficits may precede motor deficits in a predisposed population. Early identification of early cognitive deficits may broaden the diagnostic and therapeutic window and provide valuable information regarding the roles of inflammation and APOE in disease.

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P207

Prophylactic and therapeutic suppression of experimental autoimmune encephalomyelitis by a novel bifunctional peptide inhibitor

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Background: In our previous studies, we have designed proteolipid protein (PLP)-bifunctional peptide inhibitor (BPI) and glutamic acid decarboxylase (GAD)-BPI by conjugating an antigen peptide (i.e., myelin PLP 139–151 and GAD 208–217, respectively) with CD11a237–246 via a spacer peptide. Intravenous injections of PLP-BPI and GAD-BPI have been shown to suppress the disease progression in mouse experimental autoimmune encephalomyelitis (EAE) and in type-1 diabetes in non-obese diabetic mice, respectively. **Objective:** The objective of the present study is to optimize and evaluate the in vivo activities of our novel BPI, which alters immune response in autoimmune diseases by modulating the immunological synapse formation. **Methods:** In this study, PLP-BPI derivatives with capped N- (acetyl) and C-termini (amide) (i.e., Ac-PLP-BPI-NH2) and various spacer lengths were synthesized and evaluated in vivo in the EAE model. The in vivo activity of Ac-PLP-BPI-NH2 and its derivatives with different linkers were compared with the unmodified PLP-BPI in suppressing EAE. **Results:** Ac-PLP-BPI-NH2 prevented disease progression more efficiently than unmodified PLP-BPI. The derivatives of Ac-PLP-BPI-NH2 with different length spacers were active in suppressing EAE. Interestingly, Ac-PLP-BPI-NH2 treatment given after disease onset could dramatically ameliorate the disease. Treatment with BPI molecules had a lower incidence of anaphylactic responses than that with PLP139–151 peptide. **Conclusions:** In conclusion, Ac-PLP-BPI-NH2 can effectively suppress disease severity and morbidity of EAE by post-onset therapeutic treatment as well as prophylactic use. BPI could be a potential therapeutic agent for many autoimmune diseases in which antigenic epitopes are identified.

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P208

Rapid induction of VEGF by CD8 T cells precedes central nervous system vascular permeability

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Background: Disruption of the blood-brain barrier (BBB) is a hallmark feature of immune-mediated neurological disorders as diverse as viral hemorrhagic fevers, cerebral malaria and multiple sclerosis (MS). Although current models hypothesize that immune cells promote vascular permeability in human disease, the role CD8 T cells play in BBB breakdown remains poorly defined. Our laboratory has developed a novel murine model of CD8 T cell mediated central nervous system (CNS) vascular permeability using a variation of the Theiler's virus model of MS. Using this model, we have shown that in vivo stimulation of CNS infiltrating antigen-specific CD8 T cells initiates astrocyte activation, alteration of BBB tight junction proteins, and increased CNS vascular permeability in a non-apoptotic manner as assessed by active caspase-3 expression. **Objective:** To determine if antigen-specific CD8 T cells induce expression of VEGF and if this expression plays a necessary role in CNS vascular permeability. **Methods:** We have utilized FITC-albumin leakage assay, Western blotting, in situ hybridization and peptide inhibitor administration in these studies. **Results:** Our results demonstrate that VEGF is expressed by CNS cell types, with neurons being the major cellular source, as early as 4 hours after induction of CNS vascular permeability. We also demonstrate that a seven amino acid peptide VEGF inhibitor can partially block the occurrence of CNS vascular permeability in this model. **Conclusions:** Our novel findings are highly relevant to the development of therapies designed to control immune-mediated CNS vascular permeability. In particular, the results of this study implicate the use of VEGF inhibition as a putative therapy aimed at prevention of CD8 T cell-mediated CNS vascular permeability.

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P209

Neutrophil elastase inhibitor, sivelestat sodium hydrate reduces severity of experimental autoimmune encephalomyelitis

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Background: Recent progress suggested that activated T cells release soluble factors such as granzyme B (GrB) that participate in T cell-induced neurotoxicity. GrB is categorized as a serine protease that induces apoptosis by caspase activation, and its staining has been seen in multiple sclerosis (MS) brain tissue and implicated an important mediator of tissue damage. Sivelestat is a low molecule weight neutrophil elastase inhibitor that is used for treatment of systemic inflammatory response syndrome. **Objective:** It is not known whether sivelestat inhibits serine protease GrB. We have addressed this issue in vitro and used experimental autoimmune encephalomyelitis (EAE) as a read out. **Methods:** C57BL/6 female mice were immunized with MOG 35-55 (MEVGWYRSPFSRVVH-LYRNGK) peptide. SJL/J female mice were immunized either with PLP 139–151 (HSLGKWLGHDPDKF) or PLP 178–191 (NTWTTCQSIAPFSK) peptides. Peptides were injected subcutaneously 200 micrograms in complete Freund's adjuvant. These mice were intraperitoneally administered 4mg of sivelestat and the control group was infused with PBS two times a day for different period. Each injection was performed according to the animal experiment protocol at Juntendo University. Severity of EAE was graded 1 to 5 by clinical score. The effect of sivelestat on GrB was measured by 'GrB Assay Kit' (BIO-MOL). T cell proliferation assays were performed to examine the direct effect of sivelestat. GrB levels in the supernatant were measured by immunoblot. **Results:** Sivelestat inhibits enzyme activity of GrB in a dose-dependent manner. The clinical score of EAE was reduced in sivelestat-injected group. T cell proliferative response in each peptide was not inhibited by sivelestat. **Conclusions:** Sivelestat may be a good candidate for the new therapeutic compound for MS by blocking protease-induced neuronal death.

P210

Fish diet prevents impaired mobility in the murine cuprizone model for multiple sclerosisØivind Torkildsen¹, Linn Anne Brunborg², Anne Marita Milde³, Kjell-Morten Myhr¹, Lars Bø¹

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Background: Although many patients with multiple sclerosis (MS) use special diets intended to treat or prevent motor or non-motor deficits, the data available at present are insufficient to assess any potential benefit of diet modification. Cuprizone-induced demyelination is a commonly used animal model for central nervous demyelination; it is well suited for studying dietary factors that may influence neuropsychological dysfunction induced by this process. **Objective:** The aim of this study was to evaluate whether diets with n-3 polyunsaturated fatty acids (PUFAs) from two different sources could influence behavioral activity in cuprizone pre-treated mice, compared with a control diet rich in n-6 PUFAs. **Methods:** Sixty-three female C57Bl/6 mice were fed with 0.2% cuprizone on three different diets. The diets consisted of 1) salmon fillets rich in marine n-3 PUFAs, 2) cod liver oil rich in marine n-3 PUFAs, or 3) a control diet containing soybean oil rich in n-6 PUFAs. After 5 weeks of continuous cuprizone treatment, animal activity was assessed with the elevated plus-maze test. **Results:** The salmon-cuprizone group had less weight loss than the cod liver oil- (P<0.0001) and the soybean oil-cuprizone groups (P=0.001). Additionally, these mice showed more active behavior and more visits in both open and closed arms of the elevated plus maze than the other cuprizone treated groups (P's<0.0001). The salmon-cuprizone mice had also more entries in the open arms than both the cod liver oil-cuprizone (P<0.02) and the soybean oil-cuprizone treated mice (P<0.0001). **Conclusions:** A diet containing salmon seems to protect against the behavioral changes induced by cuprizone, indicating that a fish diet could have a protective effect in demyelinating diseases.

The present results provide a basis for further studies exploring the potential role of diet intervention in MS.

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P211

Cuprizone feeding in mice is a model for cortical demyelination
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Background: Cuprizone feeding is a commonly used model to study experimental de- and remyelination, with the corpus callosum and the superior cerebellar peduncles being the most frequently investigated white matter tracts. Previously, we have shown that cuprizone feeding also led to cortical demyelination. **Objective:** To analyze the dynamics of cortical demyelination in comparison to demyelination in the corpus callosum. **Methods:** To induce demyelination, 8-week old C57BL/6 male mice were challenged with 0.2% cuprizone feeding ad libitum for 6 weeks. At different time points (week 0=control, 3, 4, 4.5, 5, 5.5 and 6 weeks), mouse brains were investigated using histological and immunohistological staining methods. **Results:** Prominent and almost complete demyelination in the corpus callosum was observed after 5 weeks of cuprizone feeding, whereas complete cortical demyelination was only observed after 6 weeks of cuprizone feeding. Interestingly, remyelination in the corpus callosum started during cuprizone feeding and showed about 50% of re-expressed proteolipid protein at week 6. Accumulation of microglial cells in the corpus callosum started as early as week 3 and showed maximum infiltration at week 4.5 and still high numbers at week 6. Within the cortex, only scattered activated microglial cells were found at week 3, a small infiltrate at week 4.5 and only a few cells at week 5.5. No microglial cells were found at week 6 at highest demyelination. **Conclusions:** The present work demonstrated that cuprizone feeding is an excellent model to study cortical demyelination. The time course of demyelination is different in the corpus callosum and in the cortex, suggesting a different underlying mechanism.

P212

Treatment with a component of red wine, resveratrol, in a virus-induced murine model for multiple sclerosis

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Background: While axonal degeneration occurs in multiple sclerosis (MS) and the extent of axonal damage is correlated with clinical disability, there is no efficient treatment targeting axonal preservation. In Wallerian degeneration slow (Wlds) mice, axonal degeneration is delayed due to an increased nicotinamide adenine dinucleotide (NAD) biosynthetic enzyme Nmnat activity. SIRT1 is the downstream effector of the increased Nmnat activity. Preservation of axons in Wlds mice is beneficial in experimental autoimmune encephalomyelitis (EAE), an autoimmune model for MS. Resveratrol is a natural polyphenol compound of red wine used in human clinical trials to treat cancer and can cross the blood-brain barrier. Resveratrol can enhance SIRT1 activity, which confers axonal preservation, and exhibit anti-inflammatory and anti-viral activities. **Objective:** To test whether resveratrol could be therapeutic, possibly by limiting axonal damage in a viral model for MS, Theiler's murine encephalomyelitis virus (TMEV) infection. **Methods:** Female SJL/J mice were infected with the DA strain of TMEV. Infected mice were fed a diet containing 0.04% resveratrol (20 mg/kg/day) during the acute stage of infection (days 0 to 14) or during the chronic stage (days 21 to 35), or a control diet. **Results:** Clinically, the mice treated during the acute stage showed more weight gain than control mice at 2 weeks postinfection ($p < 0.001$, by ANOVA). Central nervous system (CNS) tissues and spleen mononuclear cells were harvested 5 weeks after infection. The mice treated during the chronic stage tended to have higher clinical

and pathological scores than control mice, although they did not reach statistical significance. There were no significant differences in viral persistence in the CNS or lymphoproliferative responses to the virus among groups. We speculate that the significant weight gain during the acute stage in the early resveratrol treatment group could be due to a neuroprotective property of resveratrol. **Conclusions:** Since degenerated axons do not regenerate in the CNS, axonal degeneration results in permanent clinical disability in MS. Thus, potential axonal sparing activity by resveratrol could be of great benefit.

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P213

Identification of the interacting proteins with the QUAKING RNA binding protein

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Background: The QUAKING (QKI) proteins are members of the heteronuclear ribonucleoprotein particle K (hnRNP K) homology (KH) domain family of RNA binding proteins essential for gliogenesis. QKI regulates several post-transcriptional processes including pre-mRNA splicing, mRNA export, mRNA stability and protein translation in oligodendrocytes. The post-transcriptional processes require constant individual mRNA transcripts bound by a dynamic array of different proteins involved in a messenger ribonucleoprotein (mRNP) complex. We hypothesize that QKI regulates post-transcriptional events in a complex with other proteins. **Objective:** To identify QKI interacting proteins and determine their biological relevance. **Methods:** Endogenous QKI was immunopurified from a glial cell line (U343) using QKI specific antibodies. The co-immunoprecipitated proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and identified using mass spectrometry. **Results:** A total of 15 proteins were identified by mass spectrometry that had a significant score. Of these, heterogeneous nuclear ribonucleoprotein Q (hnRNP-Q) and heterogeneous nuclear ribonucleoprotein R (hnRNP R) were RNA binding proteins implying that QKI resides in a ribonucleoprotein complex within the cell. Interestingly, we also identified the polyadenylate-binding protein (PABP), a protein that is known to associate with the poly(A) tail of mRNAs and regulate protein translation. We mapped the modular domains required for their association and these proteins were co-localized in oligodendrocytes and other glial cells. The functional significance of this and other interacting proteins will be presented. **Conclusions:** Our findings show that QKI exist in a ribonucleoprotein complex and these findings have implications for the role of QKI during oligodendrocyte function and myelination.

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P214

Antigen presentation by dendritic cells is sufficient for full induction of active experimental autoimmune encephalomyelitis with peptide but not protein

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Background: The antigen presentation requirements during autoimmune central nervous system inflammation are incompletely known. Dendritic cells are a special class of antigen presenting cell capable of priming naïve T cells and initiating a wide spectrum of effector functions. Dendritic cells may play several critical roles in the pathogenesis of experimental autoimmune encephalomyelitis (EAE), a model for multiple sclerosis (MS). **Objective:** To determine whether dendritic cells are sufficient as antigen presenting cells for the induction of active EAE. **Methods:** We utilized transgenic mice with major histocompatibility complex (MHC) class II expression restricted to

dendritic cells only, termed CD11cA β mice. Active EAE was induced in CD11cA β mice using MOG35-55 peptide or rMOG1-125. Clinical and pathological features of disease were measured, along with cytokine production by CD4 T cells following immunization. **Results:** We found that dendritic cells were a minimally sufficient APC for every phase of EAE following MOG35-55 immunization. Clinical and pathologic features of disease in CD11cA β mice were similar to those in wild-type mice. In addition, fractions of IFN- γ and IL-17 producing CD4 T cells were similar in both mice following MOG35-55 immunization. Furthermore, we found that a subset of dendritic cells, characterized by their susceptibility to lethal irradiation, is sufficient for peptide-induced active EAE. However, CD11cA β mice were not fully susceptible to EAE induction with rMOG1-125. **Conclusions:** In summary, dendritic cells are a minimally sufficient antigen presentation cell capable of inducing active EAE following peptide immunization. However, these results suggest that other antigen presenting cells are required for protein-induced disease.

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P215

Repair of myelin by glatiramer acetate following toxin-mediated demyelination in mice

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Background: In previous work (ECTRIMS, 2007), we described how T cells and monocytes exposed in culture to glatiramer acetate (GA) express a variety of neurotrophic factors that include insulin-like growth factor-1 (IGF-1), platelet-derived growth factor (PDGF) and brain-derived neurotrophic factor (BDNF). As these are growth factors described for the generation of oligodendrocyte precursor cells (OPCs), we tested and found that daily treatment of mice with GA for 7 days increased the number of OPCs in the demyelinated spinal cord. **Objective:** To test the hypothesis that GA would alter growth factor levels in the demyelinated spinal cord of mice and that a long-term outcome is the increased repair of myelin. **Methods:** We have tested the hypothesis that GA would alter growth factor levels in the demyelinated spinal cord of mice and that a long-term outcome is the increased repair of myelin. **Results:** Seven days after demyelination with lysolecithin, tissue around the lesion site had elevated levels of transcripts encoding IGF-1, ciliary neurotrophic factor (CNTF) and leukemia inhibitory factor (LIF), compared with intact spinal cord. Daily GA treatment for 7 days elevated the injury-induced rise of IGF-1, but did not affect CNTF and LIF. BDNF transcripts, which were not elevated by lysolecithin-demyelination, were raised by GA treatment in vivo. At 28 days, using myelin markers, we determined that GA increased indices of remyelination ($p < 0.05$). Moreover, staining profiles reminiscent of repairing shadow plaques were evident in the GA- but not vehicle-treated groups. **Conclusions:** These experiments highlighted the feasibility of promoting neural repair using medications such as GA.

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P216

The complete blockade of opioid receptors with naltrexone exacerbates experimental autoimmune encephalomyelitis in a mouse model

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Background: At least one native opioid peptide, opioid growth factor (OGF), methionine enkephalin, has a marked effect on preventing or attenuating experimental autoimmune encephalomyelitis (EAE) in mice. Intermittent opioid receptor blockade using low-dose naltrexone (LDN) has a similar action on EAE in mice. **Objective:** To determine whether complete opioid receptor blockade exacerbates EAE in mice. **Methods:** C57Bl/6 mice were inoculated with MOG35-55 peptide and

pertussis toxin. Immediately following MOG injection, mice were treated intraperitoneally once daily with 10 mg/kg naltrexone (high-dose naltrexone, MOG-HDN) or saline (MOG-vehicle) and disease scores and morphological parameters recorded. **Results:** By day 18, 100% of the animals in the MOG-HDN group demonstrated symptoms of the disease in comparison to 64% of the MOG-stimulated-vehicle mice ($p < 0.05$). By day 30, all animals in both groups had disease. Mean disease score for MOG-HDN mice was higher throughout the 30-day period relative to MOG-vehicle mice. A significant increase in the number of MOG-HDN mice with a behavioral score of 3 (paralysis of both hindlimbs) was noted relative to the MOG-vehicle group on day 20. The numbers of activated astrocytes in the MOG-HDN group were comparable to those in the MOG-vehicle group. **Conclusions:** Continuous opioid receptor blockade by naltrexone exacerbates the onset and course of EAE. These data stand in contrast to the results with LDN or OGF where EAE was prevented or delayed, and suggest that endogenous opioid pathways, rather than naltrexone itself, may be involved in the pathophysiology and treatment of EAE.

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P217

Hippocampal neurodegeneration in experimental autoimmune encephalomyelitis

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Background: Approximately 50 to 65% of multiple sclerosis (MS) patients experience cognitive deficits, including learning and memory dysfunction. Neuronal injury and synaptic loss have been shown to occur within the hippocampus in other neurodegenerative disease models, and these pathologies have been correlated with cognitive impairment. Whether hippocampal abnormalities occur in MS models is unknown. **Objective:** To determine whether inflammation and neurodegeneration occur in the hippocampus in an animal model of MS. **Methods:** Experimental autoimmune encephalomyelitis (EAE) was induced in C57BL/6 mice and motor disability was scored daily through out experiment. At early, middle and late time points, EAE mice were compared with normal control mice, using immunohistochemistry, Western blotting, confocal microscopy and unbiased stereology, to assess the inflammation and neurodegeneration within the CA1 hippocampal region. **Results:** Chronic inflammation occurred within and around the CA1 region of the hippocampus, which consisted of resident microglial activation with a relative paucity of infiltrating immune cells (T cells and macrophages). In addition, hippocampal neurodegeneration was observed. This included decreases in CA1 volume, loss of gamma-aminobutyric acid (GABA)-ergic interneurons, a reduction in synaptic protein expression and increased cell death of neurons and glia. **Conclusions:** Our results demonstrated that hippocampal inflammation and neurodegeneration occur during autoimmune-mediated demyelinating disease. This work established a preclinical model for assessing treatments targeted toward preventing hippocampal-dependent cognitive deficits in MS. **Supported by:** NIH Institutional Predoctoral Training Grant, 5 T32 HD07228: 25–26. National Multiple Sclerosis Society (USA) Center Award, CA1028. National MS Society Research Grant, RG3593.

Imaging – Part 1

P218

Magnetic resonance spectroscopy in neuromyelitis optica

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Background: Clinical findings, cerebrospinal fluid and magnetic resonance imaging (MRI) parameters in neuromyelitis optica (NMO) patients suggest that at least initially, inflammation does not involve the central nervous system apart from focal NMO lesions.

Inflammation seems to be confined to the blood-brain barrier and surrounding perivascular tissue. This is different to multiple sclerosis (MS), where the normal-appearing white matter (NAWM) may already be affected in an early stage. Damage of the NAWM can be shown by reduced N-acetyl aspartate (NAA) levels in magnetic resonance spectroscopy (MRS). **Objective:** To compare the MRS of patients with NMO or NMO spectrum disorder (NMOSD), relapsing-remitting MS (RRMS) and healthy controls to confirm the assumption that the NAWM shows differences between NMO/NMOSD and RRMS patients. **Methods:** We studied eight NMO-IgG-positive patients with NMO/NMOSD and compared the results with those of RRMS patients (n=10) and healthy controls (n=8). Patients were matched for age, gender, disease duration and lesion load, controls for age and gender. We used 2D 1H-MR-chemical shift imaging (TR=1500 ms, TE=135 ms, nominal resolution 1 cm) operating at 3 Tesla to assess the metabolic pattern in the fronto-parietal NAWM. Ratios of NAA to creatine and choline and absolute concentrations of the metabolites in the NAWM were measured in each voxel matching exclusively white matter on the anatomical T2-weighted MRI scans. **Results:** NAA levels of NMO patients and healthy controls did not differ significantly. The pattern in RRMS patients varied. Some RRMS patients did not show reduced NAA-levels, while NAA levels were markedly reduced in others. **Conclusions:** MRS allows the detection of axonal damage in NAWM. We did not find metabolic signs of damage in the NAWM in NMO/NMOSD. Contrary to our assumption, a clear distinction between NMO/NMOSD patients and RRMS could not be made but may become evident with higher numbers of patients in each group. **Supported by:** Multiple Sklerose Forschungsgesellschaft Wien (www.ms-ges.or.at).

P219

Tactile-associated recruitment of cervical cord is more altered in multiple sclerosis patients with more severe disability

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Background: Previous functional magnetic resonance imaging (fMRI) studies of the cervical cord have shown a significantly altered activity in patients with multiple sclerosis (MS) compared with controls. However, cord fMRI recruitment changes in patients with different clinical phenotypes have never been investigated. **Objective:** To compare tactile-associated cervical cord activations in patients with relapsing-remitting (RRMS) and secondary progressive (SPMS) MS. **Methods:** Cervical cord fMRI scans were acquired from 30 RRMS patients (12/18 males/females; mean age = 40.5 years; mean disease duration = 12 years; median Expanded Disability Status Scale (EDSS) score = 3.0), 20 SPMS patients (9/11 males/females; mean age = 50.8 years; mean disease duration = 21 years; median EDSS score = 6.0) and 19 healthy controls, while performing a tactile stimulation of the palm of the right hand. Using a general linear model, statistical maps of fMRI activity were generated. On these maps, activity in the right, left, anterior and posterior cervical cord at different levels was quantified for all subjects. An ANOVA model was used to compare the mean intensity signal change induced by the task among controls, RRMS and SPMS patients. A random effect logistic regression model was used to assess the within-group difference in the occurrence of fMRI activity in right vs. left and anterior vs. posterior cord. **Results:** The mean intensity signal change showed a significant trend of increase across the three groups (p=0.008). Healthy controls showed a higher occurrence of fMRI activity in the right vs. left (p=0.004) and posterior vs. anterior (p=0.02) cervical cord. RRMS patients showed a higher cord activity in the posterior vs. anterior cord (p=0.02) only. No significant difference, neither between right vs. left, nor between anterior vs. posterior cord, was found for SPMS patients. **Conclusions:** This study showed an abnormal pattern of activations in MS patients following tactile stimulation, with a progressive over-recruitment of cervical cord and loss of lateralization with increasing disability.

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P220

Diffusion tensor imaging in the evaluation of fine structure of giant white matter lesions

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Background: Enlarged giant white matter lesions (GWML) identified by brain imaging are a diagnostic challenge. Diffusion tensor imaging (DTI) is a novel technique that enables the evaluation of the lesion microstructure and thus may serve as a powerful tool to appraise giant lesions. **Objective:** To evaluate whether DTI data can distinguish between GWML related to multiple sclerosis (MS) or to glioma. **Methods:** DTI data were acquired on a 3.0 Tesla magnetic resonance using a single-shot echo-planar imaging sequence with axial slices of 2.6 mm and 31 gradient directions. Apparent diffusion coefficient (ADC) and fractional anisotropy (FA) measurements were obtained from seven GWML of patients with MS (five females, mean age 27.86 ± 10.33 years). Data were post-processed by DTIStudioV2.4 software (<http://lbam.med.jhmi.edu/>). The findings were compared with literature DTI data (Takashi I et al., 2005) similarly obtained from 41 GWML of patients with glioma (18 females, mean age 45.6 ± 21.8 years). **Results:** All DTI parameters were significantly increased in glioma compared with MS lesions. FA values of glioma-GWML (0.20 ± 0.05) were significantly higher than those of MS-GWML (0.11 ± 0.02), p=5.62*10⁻⁶. ADC values of glioma-GWML (1239.38 ± 313.26 10⁻⁶ mm²/sec) were significantly higher than those of MS-GWML (194.14 ± 33.88 10⁻⁶ mm²/sec), p=2.44*10⁻¹¹. **Conclusions:** FA and ADC values of gliomas-GWML were higher than those of MS-GWML. These findings implicated symmetrically organized brain tissue with higher cellularity and more water diffusivity within the tumors. In contrast, in MS, the inflammatory changes associated with edema restrict water movement and lead to decreased anisotropy. We suggest that DTI parameters can help in characterizing the underlying cause of GWML.

P221

Acute hemorrhagic leukoencephalitis with benign evolution: computed tomography and magnetic resonance imaging features

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Background: Acute hemorrhagic leukoencephalitis (AHLE) is a rare entity that represents a hyperacute form of acute disseminated encephalomyelitis. Rapid diagnosis is crucial because early aggressive management has proven successful in saving the lives of patients with AHLE. Early image findings can be useful. **Objective:** To describe a case of benign AHLE focusing on the radiological findings. **Methods:** A 29-year-old Japanese descent woman was admitted to our service for evaluation of headache, somnolence, fever, nausea and vomiting that had started 24 hours prior to admission. There were no relevant precedent events. Temperature was 38.1°C, pulse was 92 and blood pressure was 130/80 mm Hg. Neurologic examination revealed a sleepy patient without focal signs. Blood tests were normal. Cerebrospinal fluid (CSF) was unremarkable except for mild proteinorrhachia (54 mg %) and 237 intact red cells/mm³. Bacterioscopic analysis was negative. There were no oligoclonal bands or varicella-zoster virus IgM or IgG antibodies. CSF polymerase chain reaction for herpes simplex virus 1 and 2 were negative. Brain computed tomography at admission showed reduced density in the pons, thalami and right putamen, as well as blood within the right superior frontal sulcus. Magnetic resonance imaging (MRI) showed signal changes in the pons, cerebellar peduncles and white matter, thalami, lenticular nuclei, left occipital and right insular cortex (fluid-attenuated inversion recovery). Diffusion images showed tenuous signal changes more suggestive of vasogenic edema. Axial T2* images showed petechial hemorrhages in the pons. Broad treatment was started until investigation had been

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concluded. One week after her admission, the patient regained consciousness and demonstrated gradual neurologic recovery. MRI performed 30 days after her admission was normal except for axial T2* images with persistent petechial deposits. One month after the initial evaluation, the patient was completely recovered. **Results:** See 'Conclusions. **Conclusions:** Our patient had a complete recovery after a prompt suspicion of AHLE and rapid introduction of appropriate treatment. Radiological findings can be a useful tool in early diagnosis.

P222

Functional magnetic resonance imaging correlates of neuropsychological impairment in primary progressive multiple sclerosis
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Background: Cognitive impairment has been described in a variable percentage of patients with primary progressive multiple sclerosis (PPMS) and has been related to the extent and severity of brain macroscopic disease burden. **Objective:** To investigate the functional correlates of cognitive network dysfunction in patients with PPMS and their correlations with structural magnetic resonance imaging (MRI) parameters. **Methods:** From 16 right-handed PPMS patients and 17 sex- and age-matched healthy volunteers (HV), structural and functional MRI (fMRI) during the performance of the n-back task were acquired. Neuropsychological tests (NPT) exploring memory, attention and frontal lobe cognitive domains were administered. T2 lesion load (LL) and corpus callosum area (CCA) were measured. fMRI analysis of corrected answers was performed using statistical parametric mapping. **Results:** Six PPMS patients had an abnormal performance in three or more NPT, thus fulfilling pre-defined criteria for cognitive impairment (CI). Structural MRI metrics did not differ between CI and unimpaired PPMS patients. Compared with controls and CI patients, cognitively preserved patients had more significant activations of several regions in the frontal lobes, bilaterally. Conversely, compared with the other two groups, CI patients had more significant activations of several regions in the parietal lobes, the supplementary motor area, the cingulum and the primary motor cortex, bilaterally. In PPMS patients, significant correlation was found between decrease of activation in the frontal lobes and T2-LL. **Conclusions:** Increased activations of cognitive-related networks might represent a functional reserve with the potential to limit the onset of CI in PPMS. The progressive exhaustion of frontal lobe function, due to the accumulation of T2-visible lesion burden, is likely to be among the factors related to CI in these patients.

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P223

Conventional magnetic resonance and diffusion tensor imaging in recurrent neuromyelitis optica: analysis of 30 patients

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Background: Conventional magnetic resonance (MR) is the main test to identify central nervous system white matter lesions. New software called diffusion tensor imaging (DTI) has been used in the investigation of lesions on the normal-appearing white matter. **Objective:** To analyze brain and spinal cord white matter lesions by conventional MR and DTI in Brazilian patients with neuromyelitis optica (NMO). **Methods:** We selected 30 patients with NMO who attended at the Hospital da Lagoa (Rio de Janeiro city), from January 2005 to April 2006. The MR protocol included brain, optic nerve and spinal cord in T1 axial and sagittal, T2 axial and sagittal, fluid-attenuated inversion recovery (FLAIR) axial and sagittal, coronal STIR, T1 with gadopentate dimeglumine and sagittal sequences and DTI on a GE Exite 1.5T.

The DTI sequences was programmed for 23 directions defining the anisotropy map to detect lesions on the normal-appearing white matter on the brain (corona radiata, corpus callosum, posterior limb of the internal capsule) and brain stem (midbrain - crus cerebri, pons - anterior part, medulla oblonga - pyramidal tract). **Results:** The conventional brain magnetic resonance imaging (MRI) was normal in 85% of the cases; 15% had inflammatory lesions not fulfilling the MRI criteria for multiple sclerosis (MS) (McDonald, 2001). With the DTI technique, 47% showed brain abnormalities. The optic nerve showed abnormalities in 87.5% of the cases (STIR technique), 50% bilateral and 37.5% unilateral. The brain stem showed inflammatory lesions in 35% of the cases. **Conclusions:** The criteria for definite NMO (2006) allow clinical manifestations outside the optic nerve and spinal cord. All the radiological studies performed until 2006 included patients with exclusively optic nerve and spinal cord involvement. Our data indicated that NMO syndrome is characterized mainly by optic and spinal cord bouts but brainstem involvement analyzed by conventional MRI and DTI imaging showed 30% of brainstem lesions.

P224

Reduced cerebellar grey matter volume is associated with cerebellar dysfunction in multiple sclerosis

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Background: In multiple sclerosis (MS), brain atrophy is thought to mark neuro-axonal loss. Grey matter (GM) damage may be particularly relevant in determining irreversible disability and regional GM atrophy measures may offer useful information about the causes of specific clinical deficits. **Objective:** To determine the magnitude of cerebellar GM atrophy in patients with clinically isolated syndromes (CIS) and MS, and its relationship with clinical manifestations of cerebellar damage. **Methods:** Seventy-three patients presenting with a CIS were followed-up at 20 years (29 CIS, 33 relapsing-remitting MS (RRMS), 11 secondary progressive MS (SPMS)). Patients had T1-weighted volumetric magnetic resonance imaging (MRI) at follow-up. Their MRI was compared with 25 control subjects. GM masks were generated automatically from MRI using SPM5; the cerebellum was manually delineated from the resulting GM regions and normalized cerebellar GM volumes were determined (nCGMV). Clinical assessment at follow-up included the Kurtzke cerebellar functional system score, 25-foot timed walk and nine-hole peg test (9HPT). Linear regression analysis was used to investigate differences in nCGMV between groups, and the association of nCGMV with z-scores for the 25-foot timed walk and 9HPT, using age and gender as covariates. **Results:** Mean nCGMV was 95.0 ml in controls, 91.5 ml in CIS patients, 88.3 ml in RRMS patients and 82.8 ml in SPMS patients. A mean reduction in nCGMV of 4.9 ml (adjusted for age and gender) was observed in MS patients relative to controls (95% confidence interval (CI) -0.3-10.1, p=0.065). Twenty-five patients were assessed as having cerebellar dysfunction; mean nCGMV was 84.1 ml in these patients compared with 91.8 ml in patients who had no detectable cerebellar dysfunction (mean reduction adjusted for age and gender 7.6 ml, 95% CI 3.6-11.6, p<0.001). Significant associations of nCGMV with performance on both the timed walk and 9HPT were observed (p=0.015 and p<0.001, respectively). **Conclusions:** GM atrophy occurs in the cerebellum of MS patients and is related to clinical function. It may provide a sensitive marker of clinically relevant cerebellar damage, providing complementary data to other regional atrophy and intrinsic tissue measures.

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P225

Texture analysis of brain magnetic resonance imaging differs in pediatric clinically isolated syndrome patients at low risk and high risk for developing multiple sclerosis

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Background: Only a subset of children presenting with clinically isolated syndrome (CIS) will convert to multiple sclerosis (MS). Identifying children at high risk for conversion to MS at the time of initial CIS presentation may be useful for planning treatment, and may also provide insights into MS pathogenesis. Texture analysis quantifies changes in the spatial distribution of intensity levels within images and can detect changes on magnetic resonance imaging (MRI), which may be difficult to appreciate visually. **Objective:** To investigate differences in texture features of baseline brain MRI between pediatric CIS patients at high and low risk for developing MS. **Methods:** Pediatric CIS patients being followed with serial MRI as part of a larger longitudinal study were divided into two cohorts: high risk for conversion to MS or confirmed MS (defined as exhibiting new lesions on serial MRI or clinical evidence of relapse; N=20), and low risk for conversion to MS (defined as optic neuritis or longitudinally extensive transverse myelitis with otherwise normal brain MRI, or polylesional presentations with or without encephalopathy with subsequent MRI evidence of lesion resolution and no new lesions; N=25). Second-order gray level co-occurrence, matrix-based texture analysis was performed on baseline PD- and T2-weighted images. Average texture feature values were computed over normal-appearing white matter (NAWM) for all patients, and within white matter (WM) lesions and perilesional regions for patients exhibiting lesions at baseline (high-risk lesional: N=14; low-risk lesional: N=4). **Results:** The angular second momentum texture feature, which characterizes the regional consistency of intensity patterns, was significantly lower within WM lesions on baseline PD- and T2-weighted images in the high-risk group compared with the low-risk group. No differences were found within the NAWM or perilesional regions. **Conclusions:** In this preliminary work, texture analysis detected differences in the spatial distribution of intensity levels within WM lesions between the high- and low-risk groups on baseline MRI. Thus, texture features may have potential as prognostic indicators in children with CIS. **Supported by:** Canadian Institute for Health Research, Multiple Sclerosis Scientific Research Foundation, Multiple Sclerosis Society of Canada.

P226

Gray matter atrophy is present in patients with clinical isolated syndrome suggestive of multiple sclerosis

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Background: Previous magnetic resonance imaging (MRI) studies have demonstrated that gray matter (GM) atrophy is present in patients with clinically definite multiple sclerosis (MS) at different stages of the disease. However, the existence of GM atrophy in patients with clinically isolated syndrome (CIS) remains unknown. **Objective:** To assess global and regional GM atrophy in patients with CIS. **Methods:** Volumetric 3D T1 brain MRI and axial T2 brain MRI were performed in 62 patients with a CIS and 34 matched healthy controls. We assessed global GM volume using the GM fraction and regional GM volume using the optimized voxel-based-morphometry analysis method (SPM2). We also measured the T2 (T2LL) and T1 lesion loads (T1LL). Disability was assessed using the Expanded Disability Status Scale (EDSS). **Results:** In patients, median time between clinical episode and MRI was 4 months (1–6). Median age was 29 years (18–45) in patients and 28 years (19–44) in controls. In patients, median EDSS was 1 (0–4), median T2LL 2.21 cm³ (0–111) and median T1LL 0.5 cm³ (0–25). Patients showed significant GM fraction reduction (mean

0.499 + 0.046) compared with controls (mean 0.523 + 0.046). Voxel-based morphometry (VBM) demonstrated in patients GM atrophy located bilaterally in the thalamus, the caudate, the lenticular nucleus, the hippocampus, the inferior temporal gyrus and the orbitofrontal cortex (p<0.005 FWE correction). No correlations were found between GM fraction or regional GM atrophy and EDSS, T2LL and T1LL. T2LL was moderately correlated with EDSS (p: 0.002; R₂: 0.15). **Conclusions:** This study performed in a large cohort of subjects demonstrated that GM atrophy is present in CIS patients and is predominantly located in the deep GM.

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P227

Deep gray matter atrophy is associated with T2 hypointensity in secondary progressive multiple sclerosis

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Background: Gray matter atrophy is known to be present in multiple sclerosis (MS) even from the very early stages. However, the mechanisms contributing to this atrophy are not fully understood. One hypothesis maintains that excessive iron deposition leads to T2 hypointensity and tissue degeneration in the gray matter in MS. **Objective:** To test for a relationship between atrophy and T2 hypointensity in the putamen in patients with MS. **Methods:** Forty-eight MS patients, 39 with relapsing-remitting MS (RRMS) and nine with secondary progressive MS (SPMS), underwent magnetic resonance imaging (MRI) in this cross-sectional study. Baseline characteristics (mean±SD [range]) were: age = 41.4±9.1 (22.0–59.0) years, disease duration = 10.4±8.7 (0.5–41.0) years, and Expanded Disability Status Scale (EDSS) score = 3.2±2.0 (0.0–7.5). MRI was performed at 1.5T and included T2-weighted fast spin-echo and 3D T1-weighted (SPGR) images of the whole brain. The bilateral putamen was traced in whole by an experienced observer using a semi-automated technique. Intra-subject T2 intensity in the putamen was normalized to the cerebrospinal fluid using our previously established method. Using the Pearson's correlation coefficient, we studied the correlation between the normalized putamen volume and gray matter T2 intensity. **Results:** In the SPMS patients, we found a strong correlation between putamen volume and putamen T2 intensity (r=0.7, p=0.042), indicating an association between atrophy and T2 hypointensity. This correlation was not significant in the MS group as a whole or within the RRMS subgroup. We continue to accumulate additional patients for analysis to extend and confirm these findings and also assess clinical-MRI correlations. **Conclusions:** In this preliminary study, we found an association between T2 hypointensity and atrophy of the putamen in SPMS but not RRMS. These findings implicate a locally depending relationship between iron deposition and tissue atrophy in the gray matter playing a role in the development of progressive disease. **Supported by:** National Institutes of Health (1R01NS055083-01). National Multiple Sclerosis Society (USA) (RG4032A1/1, RG3705A1, RG3798A2).

P228

Association of regional gray matter volume loss and progressive white matter lesions in multiple sclerosis

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Background: Previous studies have established regional gray matter (GM) volume loss in multiple sclerosis (MS), but the association between development of white matter (WM) lesions and changes of regional GM volumes is unclear. **Objective:** To clarify this issue by the

use of voxel-based morphometry (VBM), a non-biased measure of regional differences in GM volumes. We predicted that regional GM volume reductions occur predominantly in patients with increasing WM lesion volumes. Additionally, we hypothesized that a core group of patients with both increasing T1- and T2-lesion burden would show volumetric GM reductions that are qualitatively similar but more pronounced than in patients with T2-lesion progression alone. **Methods:** T1-weighted, three-dimensional magnetic resonance imaging (MRI) data from 212 MS patients followed-up for 12 months were analyzed using VBM. An analysis of covariance (ANCOVA) model assessed with cluster size inference (all corrected $p < 0.05$) was used to compare GM volumes between baseline and follow-up while controlling for age, gender and disease duration. Volumes of T1 hypointense and T2 hyperintense lesions and the number of new T2 lesions were determined. **Results:** Comparing all MS patients ($n=212$) longitudinally, GM volume remained unchanged during a 1-year-follow-up (corrected $P=0.544$). In patients with relapsing-remitting MS (RRMS) and increasing T2-lesion burden ($n=67$), significant cortical GM volume loss occurred in the fronto-temporal cortex (e.g. anterior cingulate gyrus and cerebellum). In RRMS with increasing T2- and T1-lesion burden ($n=45$), additional clusters occurred in the insula bilaterally as well as in frontal areas. In contrast, patients lacking an increase in WM-lesion burden over time did not show GM changes. **Conclusions:** Overall, the progression of regional cortical GM volume reductions seems to require WM-lesion progression. **Supported by:** GlaxoSmithKline.

P229

Basal ganglic and thalamic volume change in relapsing-remitting multiple sclerosis

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Background: Multiple sclerosis (MS) is still regarded primarily as a white matter disease. Recent imaging and pathology studies have demonstrated that gray matter is an important pathophysiological feature in MS. **Objective:** We hypothesized that the basal ganglic compartments (caudate nucleus, putamen and globus pallidus) and thalamic volume is lower in MS patients with mild disability, that is, a low Expanded Disability Status Scale (EDSS) score, compared with patients with moderate disability EDSS. **Methods:** T1-weighted, three-dimensional magnetic resonance imaging (MRI) of 86 patients with relapsing-remitting MS (RRMS) were included. Patients were classified in 'mild disability' when their EDSS was < 2.5 and in 'moderate disability' when the EDSS was between > 3 and < 6 . The thalamic and basal ganglic components volumes were measured using a fully automated segmenting and volumetric method (individual atlas-based volumetry using statistical parametric mapping). **Results:** The statistical comparison of volumetric values of 'mild disability' and 'moderate disability' subgroups show significant ($p < 0.05$) volume differences in the thalamus and putamen. The 'mild disability' subgroup has higher mean thalamic (left 3.451 cm³, right 3.119 cm³) and putamic volumes (left 2.870 cm³, right 3.301 cm³) than the 'moderate disability' subgroup (thalamus left 3.087 cm³, right 2.746 cm³; putamen left 2.423 cm³, right 2.814 cm³). No significant changes were found in the caudate nucleus and globus pallidus. **Conclusions:** These findings suggest that thalamic and putamic atrophy is associated with the EDSS score in patients with RRMS. **Supported by:** GlaxoSmithKline.

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A diffusion tensor, magnetic resonance imaging study of cervical cord damage in benign and secondary progressive multiple sclerosis patients

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Background: Diffusion tensor (DT) magnetic resonance imaging (MRI) is able to quantify the severity of brain and cervical cord pathology in multiple sclerosis (MS), thus providing in vivo pieces of information about the extent of tissue damage that can go undetected when using conventional MRI. **Objective:** To investigate the DT MRI patterns of cervical cord damage in benign MS (BMS) and secondary progressive MS (SPMS) patients, in order to achieve a better understanding of the nature of disability in MS. **Methods:** Conventional and DT MRI scans of the cervical cord were acquired from 61 patients with BMS, 49 with SPMS and 18 healthy controls. After the creation of mean diffusivity (MD) and fractional anisotropy (FA) maps, histograms of MD and FA values were produced for the cervical cord tissue. Cross-sectional cord area (CSA) at C2 level was also computed. **Results:** The median Expanded Disability Status Scale (EDSS) score was 2.0 (range 0.0–3.0) for BMS and 6.0 (range 4.0–8.0) for SPMS patients. Compared with healthy controls, BMS patients had higher average cord MD ($p < 0.001$) and lower average CSA ($p < 0.03$). When compared with BMS patients, SPMS patients had a lower cord average FA ($p = 0.003$) and lower average CSA ($p < 0.001$). In MS patients, EDSS was significantly correlated with CSA ($r = -0.45$, $p < 0.001$) and average cord FA ($r = -0.37$, $p = 0.002$). Both CSA and average cord FA entered a multivariable model as independent predictors of EDSS in MS patients ($p = 0.001$). **Conclusions:** Our results are consistent with the presence of milder tissue damage in the cervical cord of MS patients without locomotor disability, which might be a hallmark of the benign course of the disease. DT MRI may complement atrophy measurement in a comprehensive MRI assessment of cervical cord abnormalities. **Supported by:** FISM (Fondazione Italiana Sclerosi Multipla), contract n. 2005/R/18.

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Neuro-axonal brain viability does not differ between benign and early relapsing-remitting multiple sclerosis patients

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Background: The term benign multiple sclerosis (BMS) indicates a favorable course of multiple sclerosis (MS), when patients have mild or no disability several years after the clinical onset of the disease. Axonal damage, which is considered a major determinant for the accumulation of disability in MS, can be measured in vivo using proton magnetic resonance spectroscopy (1H-MRS). **Objective:** To quantify the severity of 'global' axonal damage in BMS and early relapsing-remitting MS (RRMS) patients using whole brain N-acetyl aspartate (WBNA) 1H-MRS, to better elucidate the structural correlates of a non-disabling disease evolution. **Methods:** WBNA concentration was measured in 48 patients with BMS and 17 patients with early RRMS, using an unlocalized ad hoc 1H-MRS sequence. Conventional dual echo and T1-weighted scans were also obtained to measure T2-hyperintense lesion load (T2LL) and normalized brain volume (NBV). **Results:** T2LL was higher in BMS than in early RRMS patients ($p = 0.018$), while neither NBV nor WBNA concentration significantly differed between the two groups of patients. No

correlations were found between WBNA concentration and Expanded Disability Status Scale (EDSS), T2LL and NBV. **Conclusions:** The similarity in the WBNA concentration between BMS and early RRMS patients fits with the hypothesis that a non-disabling, long-term evolution of MS may be due, at least in part, to the preservation of axonal density and integrity. Such a condition seems to be independent from the burden of MRS-visible lesions.

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Diffusion tensor imaging of white matter in children with multiple sclerosis

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Background: Diffusion tensor imaging (DTI) has been used to interrogate white matter (WM) microstructure in adult multiple sclerosis (MS) patients, and has demonstrated reduced tissue integrity within lesions and normal-appearing white matter (NAWM). Applying DTI to a pediatric MS population provides a unique opportunity to identify potential disruption of myelinated pathways in early stages of disease pathology. **Objective:** To compare WM integrity between children with MS and healthy children using DTI. **Methods:** DTI data were analyzed for 17 children with MS and 17 healthy children (mean ages 15.0 and 11.7 years, respectively). Images were acquired using a GE Twin Speed Excite-HD 1.5T scanner. A T1-weighted SPGR image was acquired for defining regions-of-interest (ROI). Acquisition of a proton-density/T2-weighted sequence facilitated co-registration of anatomical and DTI images (25 directions, b=1000 s/mm² slice thickness (MS) 5 mm, (controls) 3 mm). Fractional anisotropy (FA) and apparent diffusion coefficient (ADC) were calculated in four corpus callosum (CC) regions: genu, anterior body, posterior body and splenium. Hemispheric ROI (frontal, temporal, parietal and occipital) were defined bilaterally using an anatomical mask. Tissue segmentation was performed in MS patients to obtain DTI and volume measures for lesions, NAWM and combined WM (lesion + NAWM), in all ROI. **Results:** Three CC and all eight hemispheric ROI showed significantly reduced FA and elevated ADC values in children with MS relative to controls (p<0.001). Hemispheric WM of MS patients had 36% lower mean FA values than the control participants (p<0.05). Marked FA reductions in the MS patients were not attributed to lesions alone, as lesion and NAWM FA values were not significantly different, and lesions accounted for less than 3% of regional volumes. The contribution of demyelination or axonal degeneration to pathological WM will be explored with eigenvalue analysis. Clinical feature correlation is underway. **Conclusions:** Marked differences in DTI measures suggest profound, widespread WM impairments, even in NAWM, in children with MS.

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Early corpus callosum damage predicts progression of disability over 3 years in primary progressive multiple sclerosis

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Background: Patients with early primary progressive MS (PPMS) have widespread reduction in fractional anisotropy (FA) when compared with controls. This includes the corpus callosum. **Objective:** To perform a longitudinal study on this cohort to identify brain areas of

white matter (WM) damage, which predict progression of disability over 3 years. We focused on two measures of white matter (WM) tract damage: FA, which reflects demyelination and axonal loss, and tract atrophy, which mainly reflects axonal degeneration. These two measures were obtained using two novel and unbiased diffusion tensor imaging (DTI) techniques: tract-based spatial statistics (TBSS), which localizes areas of FA reduction, and a recently introduced method which estimates the atrophy of WM tracts. **Methods:** Thirty-two patients with PPMS within 5 years of symptom onset underwent whole-brain DTI at baseline and were scored on the Expanded Disability Status Scale (EDSS) at baseline and after 3 years. EDSS step changes were calculated to assess disability progression over the follow-up. To investigate whether the DTI-derived measures predicted EDSS step change, a linear regression analysis was repeated for tract FA and atrophy at each voxel using FMRIB Software analysis tools and corrected for multiple comparisons using permutation-based inference (t>2, p<0.05). The white matter pathway atrophy was estimated on the basis of the Jacobian determinant of the deformation field. **Results:** Lower baseline FA along the entire corpus callosum (e.g. genu, body and splenium) predicted greater EDSS deterioration (p<0.05). A borderline association was found between the atrophy of callosal body and bilateral fornix and greater disability progression (p<0.07). **Conclusions:** This finding suggests the clinical relevance of inter-hemispheric callosal pathways in MS, as different measures reflecting their integrity predict the clinical evolution of the disease over 3 years.

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Association between regional gray matter volume loss and Expanded Disability Status Scale score in relapsing-remitting multiple sclerosis: a longitudinal voxel-based morphometry study

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Background: Recent immunohistochemical studies of multiple sclerosis (MS) have demonstrated gray matter (GM) demyelination in chronic relapsing-remitting MS (RRMS), whereas other studies suggested global GM volume loss in patients with MS. However, so far a comprehensive look at the GM regional changes in association with white matter (WM)-lesion load and clinical variables has not been carried out. **Objective:** To identify associations between longitudinal regional GM volume changes and clinical variables using magnetic resonance imaging (MRI). Our overall hypothesis was that clinical scores, that is, Expanded Disability Status Scale (EDSS) and subscores, will be associated with specific regional changes in GM volume. **Methods:** Prospective data from the Genetic Multiple Sclerosis Associations study (GeneMSA) were collected from 146 subjects who were followed-up longitudinally for up to 24 months. Patients were categorized according to their EDSS score (mild, moderate and severe). MRI data were acquired at baseline, 12 and 24 months follow-up using a 1.5T scanner. Data were analyzed using a voxel-based semi-automated method for segmentation and registration of each image dataset (SPM5). Analysis of covariance (ANCOVA) model was used to compare GM volumes between baseline and follow-ups while controlling for age, gender and disease duration. **Results:** MS patients with WM inflammation also showed GM volume reductions over time, whereas those without WM inflammation did not. Comparing all RRMS patients (n=148) longitudinally, GM volume remained unchanged during a 1-year follow-up. In RRMS patients and increasing T2-lesion burden (n=67), significant cortical GM volume loss occurred in the fronto-temporal cortex. EDSS scores were correlated with regional GM changes in those patients with ongoing WM destruction. **Conclusions:** Further development of image processing techniques should help elucidate the relationships between MRI findings and disease processes in MS.

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Is voxel-based morphometry an appropriate method to study associations between regional gray matter volume changes and white matter lesions in the time course of multiple sclerosis?

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Background: Previous magnetic resonance (MR) studies have established gray matter (GM) abnormalities in multiple sclerosis (MS) with the use of voxel-based morphometry (VBM). VBM will likely emerge as an important tool in understanding the topography, time course and clinical relevance of GM involvement in MS. **Objective:** To modify voxel-based morphometry (VBM) to compare on a voxel-by-voxel basis, GM volumes as measured by segmentation of conventional spin-echo MR images and white matter (WM) lesions to assess the longitudinal trajectory of regional magnetic resonance imaging (MRI) changes. A major challenge for the use of VBM in MS studies, however, is the potential misclassification of WM lesions as GM. **Methods:** In principle, we used a similar approach as in previous studies with some modifications: T1-weighted, three-dimensional MRI images and proton density images were acquired at baseline and a 1-year follow-up, preprocessed and analyzed using statistical parametric mapping software (SPM5). Preprocessing included the generation of three-dimensional binary MS lesion masks by outlining the lesions on the proton density scans using a semi-automatic software in order to avoid misclassification of WM lesions as GM. SPM5-processing included normalization and segmentation, smoothing and parametric statistics. Segmentation accuracy was assessed by examining axial slices of each subject's GM, WM and cerebrospinal fluid image in the individual's space. The warping accuracy was assessed by displaying axial slices from each subject with edges from the atlas image. Linear regression modeling (corrected for multiple comparisons at cluster level) was used for tissue density comparison. **Results:** Optimization of VBM allowed us to detect longitudinal changes of GM volume in a number of regions with small structures, such as the medial temporal lobes and the cingulate cortex. In addition, an association of GM volumes with individual WM lesion volumes was shown. **Conclusions:** VBM analysis of MS patients may serve as a suitable tool for the study of regional volume changes in MS-MRI images. **Supported by:** Glaxo-Smith-Kline.

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Correlation of disease severity and regional brain tissue loss in relapsing-remitting multiple sclerosis: a voxel-based morphometry study in a large population

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Background: Regional assessment of gray matter (GM) loss has shown a preferential involvement of frontal regions in multiple sclerosis (MS). Expanded Disability Status Scale (EDSS) score has been demonstrated to correlate with global brain atrophy; however, studies of correlation with regional cortical thickness have provided conflicting results. **Objective:** The aim of our study was to investigate possible correlations between disease severity and regional brain tissue loss using optimized voxel-based morphometry (VBM), in a large group of relapsing-remitting MS (RRMS) patients. **Methods:** 128 RRMS patients and 33 healthy subjects were enrolled. Brain magnetic resonance imaging studies were segmented using an unsupervised multispectral method. Resulting GM and white matter (WM) volumes were analyzed using optimized VBM analysis to assess correlations ($p < 0.05$ FWE-corrected at cluster level) with EDSS, total bouts and lesion load

(LL), independent of age and disease duration. **Results:** EDSS correlated with GM loss in primary motor and somatosensory areas bilaterally and in the left fusiform gyrus, and with WM loss in the sub-cortical region near the right primary motor cortex, extending through the pyramidal tract to the brainstem. Total number of bouts correlated with regional GM (and not WM) loss in the medial part of the supplementary motor area bilaterally. LL correlated to GM loss in both caudate heads, in parahippocampal and cingulate gyri, motor area and insula and to WM loss in the corpus callosum, in the posterior temporal region bilaterally and in the right anterior centrum semiovale. **Conclusions:** We studied, in RRMS patients, the correlation of disease severity in terms of disability (EDSS), clinical activity (total number of bouts) and radiological burden of disease (LL) with GM and WM volumes, showing in more disabled patients a preferential reduction in the primary motor cortex bilaterally, with an associated WM loss in the pyramidal tract down to the brainstem, and with supplementary motor area GM loss in patients with a higher number of bouts.

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Clinical and magnetic resonance imaging dissociation in patients with multiple sclerosis is not explained by loss of brain volume or the presence of spinal cord lesions

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Background: In most patients with multiple sclerosis (MS), disease severity as measured by magnetic resonance imaging (MRI) correlates reasonably well with clinical disease severity. The characteristics of patients with clinical/MRI dissociation are not well defined. **Objective:** To examine the effect of brain parenchymal fraction (BPF) and presence of spinal cord lesions on disability status and progression in two populations of patients with multiple MS showing clinical/MRI dissociation. **Methods:** Patients underwent clinical visits with determination of Expanded Disability Status Scale (EDSS) scores every 6 months, annual brain MRI scans with quantitative assessment of T2LV and BPF, and bi-annual imaging of the cervical and thoracic spinal cord. All patients (n=397) were placed into one of three categories based on EDSS and T2LV at the first observation: patients (n=12) with low T2 lesion load (LL) and high disability (LL/HD) had $< 2 \text{ cm}^3$ in T2 lesion volume and ≥ 3 on the EDSS; patients (n=31) with high T2 lesion load (HL) and low disability (HL/LD) had $> 6 \text{ cm}^3$ in T2 lesion volume and < 2 on the EDSS; remaining patients (n=354) were classified as non-dissociated (ND). **Results:** The baseline BPF did not differ across the groups ($p=0.22$). Longitudinal changes in BPF and T2 lesion volume were not significantly different across the groups ($p=0.77$ and $p=0.16$, respectively) over a mean duration of observation of 1.02 years. The LL/HD group had a slightly higher incidence of spinal cord lesions compared with the HL/LD group, but this difference was not statistically significant ($p>0.5$) for both the cervical and thoracic spine. **Conclusions:** Neither brain volume loss, as measured by BPF, nor the presence of spinal cord lesions explained the dissociation between EDSS and T2LV. These data suggest that factors other than structural pathology as measured by MRI play a role in the pathophysiology of disability in at least some patients with MS.

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Effects of actual and simulated Z-shifts on T2-lesion volumes in patients with multiple sclerosis

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Background: Precise quantification of T2-hyperintense magnetic resonance imaging (MRI) lesion volumes (T2-LV) in patients with multiple sclerosis (MS) is important. It can be affected, however, by local-volume changes related to scanner gradient-distortions (GD) and inconsistent patient-positioning - particularly along the magnet's

long (*i.e.*, Z) axis. **Objective:** To quantify the effect of Z-shifts on estimated T2-LVs. **Methods:** Over 2 years, 12 sets of MRI data were acquired in 6 MS patients on a 1.5T Siemens Sonata. A GD-correction field (GDCF) was generated using spherical harmonic expansion to map coordinates from an 'ideal' coordinate system (Lego-DUPLO® phantom) to the scanner's imaging coordinate system. T2-LV masks were generated using a manually-corrected, automated Bayesian-tissue-classification approach. We estimated: (i) the effect of the actual Z-shift-associated GD by applying the GDCF to each of the T2-LV masks; and (ii) the effect of a greater range of simulated Z-shifts (-50-mm to +50-mm, in 5-mm steps) by applying the GDCF to each of the T2-LV masks, Z-shifting these masks, applying the inverse-GDCF, and calculating the resulting volumes by integrating the intensities. **Results:** The actual locations of the centers-of-mass of the patients' heads on T2-imaging had a range of 44.7-mm (-15.1 to 29.6) along the Z-axis in the 12 scans; applying the GDCF decreased the observed T2-LVs in all of the scans [mean (range) = -1.9% (-0.7%, -2.6%)]. Simulated 50-mm Z-shifts into, and out of, the magnet had significant, but asymmetric, effects on mean T2-LVs [out: -2.0% (-5.3%, 0.6%), $p=0.003$; in: -7.1% (-10.1%, -3.7%), $p<0.0001$]; this effect was smaller and more symmetric in scans in which lesions were located closest to magnetic isocenter, reflecting a typical 'barrel-distortion' effect. **Conclusions:** Z-shift-associated GDs can have significant effects on T2-LVs in MS patients, increasing as the distance-from-isocenter of the lesions increases. Accordingly, inadvertent Z-shifts should be avoided or corrected for. Careful and consistent alignment of subject's brains to magnetic isocenter should result in decreased GD-effects, more-reliable T2-LV estimates, and increased statistical power.

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Z-shift effects on segmented brain-tissue volumes

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Background: Precise magnetic resonance imaging (MRI) quantification of whole-brain (WB), lateral-ventricle (LV) and hippocampal (HC) volumes can be affected by local volume changes related to scanner gradient-distortions (GD) - particularly along the magnet's long (*i.e.*, Z) axis. **Objective:** To quantify, and correct for, the effect of Z-shifts on WB, LV, and HC volumes. **Methods:** High-resolution T1-weighted MRI was acquired in 9 controls on a 1.5T Siemens Sonata: both with standard canthomeatal alignment and after a 50-mm Z-shift out of the magnet. A GD-correction field (GDCF) was generated using spherical harmonic expansion to map coordinates from an 'ideal' coordinate system (Lego-DUPLO® phantom) to the scanner's imaging coordinate system. WB, LV, and HC masks were generated for each MRI dataset using FreeSurfer v4.0.1. The effect of Z-shift on these masks was simulated in 5-mm steps (-50-mm to +50-mm) by applying the GDCF to each of the masks, z-shifting them, applying the inverse-GDCF, and calculating the resulting volumes by integrating the intensities. Each T1-image was treated similarly, and SIENAX v2.5 VSCALING factors were generated for each Z-shifted T1-image. **Results:** Volumes from actual and simulated 50-mm-out Z-shifts were highly concordant (ICC: WB=0.99; HC=0.92, LV=0.99, all $p<0.0001$). Simulated 50-mm Z-shifts out of, and into, the magnet asymmetrically decreased mean WB (out: -1.5%, $p=0.02$; in: -7.4%, $p<0.0001$) and LV (out: -0.8%, $p=0.16$; in: -8.2%, $p<0.0001$) volumes; and had smaller, symmetric effects on mean HC volumes (out: -5.2%, $p<0.0001$; in: -4.0%, $p<0.0001$). Normalizing with the VSCALING factor eliminated the Z-shift effect on WB volumes, and greatly reduced it for the LVs (which are located similarly to the WB relative to magnetic isocenter), but not for the HCs (which are not). **Conclusions:** Z-shift-associated GDs can have significant effects on brain volumes, with smaller, more-symmetric GD-effects being observed in structures that are closer to magnetic-isocenter (*e.g.*, the HC). Accordingly, inadvertent Z-shifts should be avoided or corrected for. Careful and consistent alignment of subject's brains to isocenter should result in decreased GD-effects, more-reliable volume estimates, and increased statistical power.

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High-field structural magnetic resonance imaging correlates of motor network dysfunction in primary progressive multiple sclerosis

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Background: Using 1.5 T scanners, previous studies of the sensorimotor system showed that, contrary to other disease phenotypes, patients with primary progressive multiple sclerosis (PPMS) have an increased recruitment of several brain areas outside the classical motor network. **Objective:** To confirm previous findings using a 3 Tesla (T) scanner and to investigate the correlation between movement-associated functional magnetic resonance imaging (fMRI) changes and measures of global and regional brain tissue damage. **Methods:** From 15 right-handed PPMS patients and 15 sex- and age-matched healthy volunteers (HV), we acquired dual-echo (DE), diffusion tensor (DT) MRI and fMRI during the performance of a simple motor task. DT tractography was used to calculate DT-derived metrics of the corpus callosum (CC) and bilateral corticospinal tracts (CST). fMRI analysis was performed using statistical parametric mapping. **Results:** Compared with HV, PPMS patients had more significant activations of the right primary sensorimotor cortex, the right cingulate motor area, the secondary sensorimotor area (SII), bilaterally, the basal ganglia, bilaterally, the insula, bilaterally, the left parahippocampal gyrus, the cuneus, bilaterally, and the left cerebellum. In PPMS, activation of the left cuneus and the left SII were significantly correlated with DT MRI metrics of damage in the CC and the left CST. **Conclusions:** This study confirms that in patients with PPMS movement-associated fMRI changes extend beyond the classical motor network. The analysis of correlations suggest that in these patients regional damage of selected white matter tracts influences motor function.

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Structural and functional magnetic resonance imaging abnormalities of the language network in primary progressive multiple sclerosis: a combined functional magnetic resonance imaging and tractography study

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Background: Impairment of several cognitive domains, including attention, memory, reasoning and verbal fluency, is frequently encountered in patients with primary progressive multiple sclerosis (PPMS). **Objective:** In this study, we combined functional magnetic resonance imaging (fMRI) and diffusion tensor (DT) MRI tractography to explore functional and structural abnormalities of the language network in PPMS patients. **Methods:** Using a 3 Tesla scanner, dual-echo, DT MRI and fMRI during the performance of a verbal fluency task were acquired from 15 right-handed PPMS patients and 17 sex- and age-matched healthy volunteers. Neuropsychological tests (NPT) exploring memory, attention, verbal fluency and reasoning were administered. DT MRI tractography was used to calculate DT derived metrics inside the left and right arcuate fasciculus. The corticospinal tracts (CST) were studied as 'control' white matter fiber bundles. fMRI analysis was performed using statistical parametric mapping. **Results:** None of the patients showed abnormal performance in NPT exploring verbal fluency. Compared with controls, PPMS patients had increased MD values in the arcuate fasciculus and CST, bilaterally. During fMRI, compared with controls, PPMS patients had significantly reduced activation of the left caudate nucleus and the left inferior frontal gyrus (IFG). They also showed increased activation of the left precuneus, and the inferior parietal lobule, bilaterally. In PPMS patients, MD increase in the left arcuate fasciculus was significantly related to increased activation of the left precuneus ($r=0.87$) and decreased activation of the left caudate nucleus ($r=-0.83$) and the left IFG ($r=-0.86$),

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while no correlations were found with CST damage. **Conclusions:** In PPMS patients without verbal fluency deficits, abnormal recruitment of language-related network occurs. The correlation found between measures of abnormal activation and selective damage of the left arcuate fasciculus suggests that functional cortical changes in patients with PPMS might represent an adaptive response driven by damage of specific white matter structures.

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Differential degree of blood-brain barrier permeability during experimental autoimmune encephalomyelitis measured by gadolinium-enhancement magnetic resonance imaging

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Background: Gadolinium-enhancement magnetic resonance imaging (MRI) is a well-recognized clinical tool for the detection of blood-brain-barrier (BBB) rupture in multiple sclerosis (MS). However, the number of enhancing lesions are generally quantified without a measure of the degree of vascular integrity within MS lesions. Kinetics of contrast agent diffusion may be used to extract quantitative information regarding the functional integrity of the BBB. The rat model of acute experimental autoimmune encephalomyelitis (EAE) is commonly used for therapeutic drug development, as reliable model of BBB leakage and CNS inflammation, mimicking some MS traits. **Objective:** The aim of this work was to set up an MRI method, optimized for animals, in order to quantify the degree of vascular integrity during the EAE progression in rats. **Methods:** From day 6 to 20 post-immunization (pi), in-vivo MRI on rat acute EAE was performed on a 7-Tesla scanner. Serial T1-weighted images were acquired at 3-minute intervals for 20 minutes after administration of Gadobenate-Dimeglumine (Gd-BOPTA, MultiHance®, BRACCO). The dynamic of Gd-enhancement was registered in successive slices covering the entire EAE brain. The Gd-enhancement intensity and dimension of the leakage were quantified. **Results:** Daily clinical observations showed classical EAE symptoms (score 1 to 3) starting from day 10 to day 14pi followed by a phase of remission (day 17–20pi). The first Gd enhancement (65%) was observed prior to any clinical signs (day 6–7pi) at the pons-medulla area with a rapid diffusion (~6-minutes). The maximum Gd-enhancement (111%) occurred at the first clinical signs (day 11pi) with a relatively slow uptake (~12 minutes) and it was followed by a low and slow enhancement (~50% in 18 minutes) at the peak and remission phases. **Conclusions:** This dynamic Gd-enhanced MRI approach reveals different patterns of BBB injury during EAE progression, adding further information on disease mechanisms to be used for therapeutic drug development. The translational application within the clinic will be explored.

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An investigation of repair mechanisms after spinal cord relapse in multiple sclerosis using magnetic resonance imaging

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Background: In a previous study, we described the latest developments of spinal cord imaging, including IHMR spectroscopy, on a 1.5T scanner, and its application to multiple sclerosis (MS) patients at the onset of an acute spinal cord relapse. **Objective:** We have now carried out a longitudinal study on the same patient cohort to assess the mechanisms of repair which contribute to clinical recovery. We focused on two spinal cord measures: the cervical cord cross-sectional area, which reflects axonal loss, and N-acetyl-aspartate (NAA) concentration, which is marker of axonal mitochondrial metabolism and axonal count. **Methods:** Fourteen patients with an acute cervical cord relapse and 13 controls were studied clinically at baseline and at one, three and six months. At each time point, the cross-sectional cord

area and NAA concentration were obtained from the same cervical region. Mixed-effect linear regression models were performed to investigate the temporal evolution of these measures and the association between them and clinical recovery. Ordinal logistic regression analyses identified predictors of recovery. **Results:** Patients showed a significant linear decline in spinal cord area during follow-up, whilst patients who recovered showed non-linear changes in NAA, which increased significantly between one and six months. A greater increase of NAA after one month was associated with a greater rate of recovery and a steeper decline in cord area. Disease duration at baseline predicted clinical recovery. **Conclusions:** The increase in NAA after one month indicates enhanced mitochondrial activity, presumably in an effort to maintain axonal conduction. This appears to be more evident in patients who had a greater rate of recovery, and to be related to progressive axonal loss. Repair mechanisms appear to be less efficient in patients with longer disease duration. These insights into the mechanisms of spinal cord repair highlight the need to explore therapies that enhance recovery by targeting mitochondria.

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Increase of cost-benefit ratio of magnetic resonance imaging: an adaptive design for identifying high inflammatory multiple sclerosis patients

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Background: A recent magnetic resonance imaging (MRI) stratification algorithm based on three monthly sequential MRIs classifies multiple sclerosis (MS) patients into four 'MRI phenotypes' based on the presence or absence of central nervous system inflammation and the presence or absence of focal and/or general tissue damage. **Objective:** To assess whether the MRI-based stratification algorithm can be adapted to a setting in which imaging resources are limited. **Methods:** We performed a meta-analysis with clinical trial placebo patients included in the Sylvia Lawry Centre database. 141 patients with three monthly sequential MRIs were available for this study. The stratification into low (≤ 1 Gd-lesion/month) and high inflammatory (>1 Gd-lesion/month) based on three MRIs was taken as gold standard and compared with stratification algorithms with limited imaging resources. Accuracy measures were compared among the stratification algorithms and put into relation to costs of MRIs. **Results:** The misclassification rate for a stratification into high or low inflammation based on two MRIs was 5.7% in comparison with the defined gold standard. Further reduction of imaging resources to a single scan results in misclassification rates ranging from 10.6% to 18.4%. All stratification algorithms were conservative and misclassification rates were lower in secondary progressive MS (SPMS) patients. Costs are reduced by more than 55% when (1) only one scan was performed in patients with SPMS and (2) a second scan was performed in relapsing-remitting MS patients with high inflammation in the first scan. The misclassification rate of the new adaptive design was 8.46%. **Conclusions:** As imaging resources are limited an adaptive design for the performance of MRIs provides a trade-off between classification accuracy into high and low inflammatory and imaging costs, yielding an improved cost-benefit ratio. The proposed adaptive design can be applied to enhance clinical trial designs and to provide evidence-based decision support to improve all aspects of patient care from prognostic estimates to treatment individualization.

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A genome scan for loci affecting the rate of brain atrophy in multiple sclerosis

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Background: Multiple sclerosis (MS) is an inflammatory and neurodegenerative disease that has an important genetic component. Recently, this has been clearly demonstrated by the identification and validation of susceptibility loci for MS at IL2RA and IL7R using a genome-wide association scan approach (IMSGC, NEJM, 2007). Yet, these loci have little or no effect on the course of the disease, and MS remains a disease with a great need for prognostic factors to help direct patient management. In addition, the neurodegenerative component of MS, which contributes the bulk of the disability in this disease, remains poorly understood today. **Objective:** To identify loci that affect the rate of brain atrophy in MS. Secondarily, to identify loci associated with the rate of T2 lesion volume (T2-LV) accumulation and the accumulation of clinical disability. **Methods:** We have analyzed 761 552 single nucleotide polymorphisms (SNPs) distributed throughout the genome in 565 subjects with volumetric data from 2168 magnetic resonance imaging (MRI) scans using a random effects model that incorporates age, disease duration and gender. The level of clinical disability is explored using the MS severity scale (MSSS) and a quantitative trait analysis approach. **Results:** The three genome scans have been run, with the most extreme results being in 10^{-6} to 10^{-7} range for each scan. For example, the top result for the rate of atrophy is rs1108268 ($P=3.3 \times 10^{-6}$). Results for the secondary analyses: We are currently replicating 200 SNPs selected from these analyses in an additional 550 subjects with similar data. These replication results will be presented at the conference. **Conclusions:** Our genome scans have yielded substantial evidence of association between certain loci and (1) the rate of brain atrophy, (2) the rate of T2-LV accumulation, or (3) MSSS. An independent set of subjects will validate true associations. **Supported by:** Affymetrix Inc.

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Retinal thickness and optic radiation in neuromyelitis optica: an optic coherence tomography and diffusion tensor magnetic resonance-tractography study

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Background: Neuromyelitis optica (NMO) involves spinal cord and optic nerves and related aspects of the brain. Conventional brain magnetic resonance imaging (MRI) is usually normal but we suspect that new MRI techniques could help to demonstrate tissue damage. **Objective:** To demonstrate the role of diffusion tensor MR-tractography (DTT) to evaluate optic radiations in patients with NMO and to look for correlation with retinal thickness. **Methods:** Twenty-four patients with NMO and 24 healthy volunteers (sex- and age-matched) were evaluated with DTT. Optic radiations were reconstructed and mean diffusivity (MD) and fractional anisotropy (FA) were measured. All patients were also tested with ophthalmological coherence tomography (OCT) in order to calculate retinal thickness. **Results:** MD was significantly higher in patients with NMO: $823 \times 10^{-6} \text{mm}^2\text{s}^{-1}$ vs $781 \times 10^{-6} \text{mm}^2\text{s}^{-1}$ ($p < 0.005$). There was no significant difference for FA: 480 vs 470, $p = 0.43$. MD modification was statistically correlated with retinal thickness on OCT ($p < 0.05$). In contrast, we found no correlation between FA and OCT results. **Conclusions:** Our results suggest increased water molecular motion induced by fiber destruction on optic radiations. The correlation with OCT results suggests a retrograde degeneration from the optic nerve into the optic tracts. In contrast, normal FA results suggest that remaining fibers keep a relative anisotropy and may be considered as normal. DTT in NMO patients helps to demonstrate destruction of white matter fiber tracts, especially optic radiations, with a good correlation with retinal atrophy.

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Magnetic resonance spectroscopy evaluation in patients with neuromyelitis optica

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Background: Neuromyelitis optica (NMO) is an inflammatory disease associating optic neuritis and myelitis. Although some studies reported multiple sclerosis (MS)-like lesions in 10 to 30% of NMO patients, brain magnetic resonance imaging (MRI) is usually normal. Several studies observed metabolic abnormalities on magnetic resonance spectroscopy (MRS) in MS, even in normal-appearing white matter (NAWM). To our knowledge MRS has never been studied in NMO. **Objective:** The aim of the study was to evaluate metabolic abnormalities in the NAWM and normal-appearing grey matter (NAGM) of NMO patients. **Methods:** We evaluate 25 patients (17 women and 8 men, with a mean age of 44 years). NMO was diagnosed following the recent criteria modified by Wingerchuck et al. (2006). All patients had a brain and spinal cord MRI including MRS sequences in both NAWM and NAGM. We compared these patients with 15 sex and age-matched normal subjects. **Results:** The NAA/creatinine ratio in NAWM (1.89 ± 0.26 in NMO compared with 1.91 ± 0.15 in controls) and NAGM (1.62 ± 0.21 in NMO compared with 1.59 ± 0.18 in controls) was normal; the choline/creatinine ratio in NAWM (1.03 ± 0.18 in NMO compared with 1.08 ± 0.14 in controls) and NAGM (0.89 ± 0.2 in NMO compared with 0.94 ± 0.2 in controls) was normal, and the myo-inositol value in NAWM was also normal (0.42 ± 0.12 in NMO compared with 0.41 ± 0.18 in controls). **Conclusions:** MRS is normal in both NAWM and NAGM of NMO patients for the main metabolisms (NAA, choline and myo-inositol) corresponding to axonal loss, inflammation and gliosis. This is clearly different to what is found in MS, where NAA is frequently decreased and choline increased even in NAWM. These results could have an impact in the understanding of pathophysiological differences between MS and NMO and could help to differentiate MS and NMO in some cases.

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Feature-space analysis of multi-contrast magnetic resonance imaging intensities in multiple sclerosis lesions

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Background: This study seeks to identify patterns of multiple sclerosis (MS) lesion heterogeneity apparent on magnetic resonance imaging (MRI), and from these patterns classify lesion subtypes. The heterogeneity of MS is characterized by a variable clinical outcome and different forms of tissue damage apparent in histology. Imaging markers to classify MS lesions according to histological subtypes and monitor their evolution in vivo are lacking. Although the pathological specificity of routine structural MRI is considered low, MS lesions appear heterogeneous on multi-contrast MRI, which we hypothesize might reflect pathological heterogeneity. Typically, lesion subcomponents are not quantified on MRI. **Objective:** This study addressed the pixelwise segmentation of individual lesions into components based on signal intensities. From these components we seek a preliminary classification of lesion subtypes. **Methods:** MRI included T2-, PD- and pre/post contrast T1-weighted images. A total of 550 T2-hyperintense lesions in a cohort of 28 MS patients were semi-automatically outlined. Feature-space analysis of multi-contrast MRI intensities was performed within all T2-outlined lesions. Cluster analysis was used to group pixels of damaged tissue that share similar intensity patterns into four classes. Lesions were then analyzed for their relative composition of these MRI tissue classes. **Results:** The most relevant feature observed in intensity space after the clustering was a separation between pixels along the PD-intensity axis. Mapping of the four resulting clusters of pixels within individual lesions showed consistent concentric patterns within the majority of lesions, reminiscent of histological heterogeneity patterns. As validation of concept we compared enhancing and non-enhancing lesions in their composition on

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PD/T2/T1/T1-post-contrast feature-space and found good discrimination. **Conclusions:** Classification of MS lesion subtypes based on MRI intensity clustering seems feasible and promising. We are extending the analysis to a larger cohort to test the method's specificity in identifying different patient subgroups.

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Early brain atrophy and lesion load measures relate to clinical status after six years in patients with clinically isolated syndromes

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Background: Conventional magnetic resonance imaging (MRI) lesions measures assist diagnosis in multiple sclerosis (MS) and clinically isolated syndromes (CIS) suggestive of MS, and modestly predict long-term disability in some CIS studies. Brain atrophy suggests neuroaxonal loss in MS with potential to reflect disease progression more than conventional MRI lesion measures. **Objective:** To investigate whether brain atrophy and lesion load, during the first year in patients presenting with CIS, independently predict clinical outcome (time to MS diagnosis and disability at 6 years). **Methods:** Ninety-nine patients presenting with CIS were included in the study. T1 gadolinium-enhanced and T2-weighted brain MRI had been acquired at baseline and approximately one year later. Percentage brain atrophy rate between baseline and follow-up scans was analyzed on T1-weighted images using SIENA. In addition baseline T2 (T2LL), T1 (T1LL), gadolinium-enhancing lesion (GdLL) volumes and the one-year change in T2LL were calculated. Clinical outcomes at six years were conversion to clinically definite MS (CDMS) and disability assessed by the Expanded Disability Status Scale (EDSS) and Multiple Sclerosis Functional Composite. Data were analyzed using Cox regression and ordinal logistic regression analyses. **Results:** Mean brain atrophy rates were -0.38% for all CIS patients, -0.49% in patients who had developed MS at six years and -0.25% in those who had not. In the statistical model including all the MRI variables, brain atrophy ($p=0.005$) and baseline T2LL ($p<0.001$) were independent predictors of CDMS, and one-year T2LL change ($p=0.007$) and baseline GdLL ($p=0.03$) were independent predictors of EDSS. **Conclusions:** The findings confirm that brain atrophy occurs during the earliest phases of MS and suggest that one-year longitudinal measures of MRI change, such as brain atrophy or T2LL change, if considered together with baseline MRI variables, might help to predict the clinical status six years after the first demyelinating event in CIS patients better than baseline MRI measurements alone.

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3T predicts better clinical and demographic outcomes in patients with multiple sclerosis than 1.5T. A preliminary case-control study

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Background: Evaluation of dissemination in time and space on 3T magnetic resonance imaging (MRI) increases sensitivity in diagnosing multiple sclerosis (MS) compared with 1.5T. It is yet unclear if the assessment of lesion injury and atrophy development on high-field MRI can account for better prediction of clinical and demo-

graphic outcomes. **Objective:** To investigate whether T2- and T1-lesion volumes (LVs), and brain atrophy assessments on 3T predict better clinical and demographic outcomes in MS patients and normal controls (NC) than on 1.5T. **Methods:** Forty-two (42) MS patients (33 relapsing-remitting and 9 secondary-progressive) and 38 NC were examined on both 1.5T and 3T within one week. The T2- and T1-weighted lesions were outlined semiautomatically. Fractions of brain parenchyma (BPF), gray matter (GMF) and white matter (WMF) were calculated using mfast program. Patients and NC were assessed clinically within 24 hours of MRI exam. **Results:** Mean T2-LV was 13ml and 18.5ml, and mean T1-LV was 3.1ml and 3.9ml on 1.5T and 3T, respectively ($p<0.0001$). Absolute brain volume was higher on 3T (1445.3ml) than on 1.5T (1300.6ml), $p<0.0001$. BPF was lower on 3T (0.789) than on 1.5T (0.793), $p=0.042$. There was no correlation between T2-LV and atrophy measures at 1.5T. At 3T, there was a strong trend for correlation of T2-LV and BPF with Expanded Disability Status Scale score ($r=0.3$, $p=0.063$ and $r=-0.43$, $p=0.069$). The relationship of T1-LV and disability was similar at both scanner strengths. All atrophy measures were more strongly related to age on 3T than on 1.5T. In NC, correlation of T2-LV and number with age on 3T was 10-fold higher than on 1.5T. **Conclusions:** Use of 3T is a promising tool for overcoming clinical-MRI paradox in patients with MS. These findings can be explained by the fact that 3T scanner is depicting more diffuse pathology in the brain than 1.5T. Higher hyperintense LV on 3T is biologically relevant.

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Lesion characteristics at 1.5 and 3 Tesla in multiple sclerosis patients and healthy controls: a morphological and topological quantitative comparison study

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Background: Previous studies in patients with multiple sclerosis (MS) showed higher number and volume of lesions on 3T compared with 1.5T. Morphological and topological lesion characteristics between 1.5 and 3T have not been examined. **Objective:** To investigate effect of scanner field strength on the number, volume, size and spatial distribution of T2- and T1-weighted lesions in a sample of MS patients and normal controls (NC), using lesion-wise and voxel-wise fully automated comparison procedures. **Methods:** Forty-two (42) MS patients (33 relapsing-remitting and 9 secondary-progressive) and 38 NC were examined on both 1.5T and 3T within one week in random order. The T2- and T1-weighted lesions were outlined semiautomatically in a blinded fashion on co-registered 1.5T vs 3T images by two operators. Discrepancies in lesion classification were resolved by a common agreement. Number, size and lesion volume (LV) were calculated. Spatial lesion distribution was assessed using T2- and T1- voxel-wise lesion probability maps (LPMs). Lesion-wise paired analysis examined proportion of lesions not simultaneously outlined on 1.5T and 3T. **Results:** In both MS and NC, for lesion-wise paired T2 analysis, 3T showed 36% more lesions and 55% more LV compared with 1.5 T ($p<0.0001$). In MS patients, for T1 lesion-wise paired analysis, 3T showed 25% more lesions and 20% more LV compared with 1.5 T ($p<0.0001$). Notably, categorical size analysis showed that likelihood to detect smaller lesions was significantly enhanced at 3T for both MS and NC groups. LPM analysis revealed significantly increased voxel-wise lesion probabilities at 3T than at 1.5T in both groups ($p<0.01$) that were most pronounced in the occipital lobes, periventricular and cortical regions for T2 lesions. **Conclusions:** This lesion- and voxel-wise comparison study between 1.5T and 3T scanners in MS patients and NC provides important information regarding morphological and topological differences between the two scanner field strengths.

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P252

Development of new magnetic resonance imaging lesions in clinically isolated syndrome patients: a single-center study
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Background: Monthly gadolinium-enhanced magnetic resonance imaging (MRI) is the most sensitive measurement to show the development of new lesions in patients with multiple sclerosis (MS). However, no studies have been performed so far to investigate the role of monthly follow-up MRI exams in patients with clinically isolated syndrome (CIS). **Objective:** The aim of this study was to investigate whether baseline clinical and MRI characteristics affect the outcome of new lesions in CIS patients during a six-month follow-up. **Methods:** Eighty-three consecutive CIS patients (31 males and 52 females, mean [±SD] age = 30.3± 7.1, median Expanded Disability Status Scale [EDSS] score = 1.0, mean time between the onset of symptoms and study enrollment = 3.7 ± 1.7 months), with at least two hyperintense brain lesions, underwent a monthly MRI (1.5 Tesla) for six months. No patients were treated with disease-modifying therapy during the six-month follow-up. **Results:** Out of the 83 patients, eleven (13.3 %) presented a relapse and 66 (79.5 %) developed new T2 lesions during the six-month follow-up. Patients who developed new T2 lesions had a greater mean number of gadolinium-enhancing and T2 lesions at baseline than patients who presented a stable MRI (1.1 vs 0; $p < 0.02$ and 21.1 vs 6.6; $p < 0.001$ respectively). No baseline differences were found in clinical and demographic characteristics between the two groups. Patients with more than six T2 lesions at baseline had higher risk of developing new T2 lesions during the six-month follow-up (odds ratio with 95% CI: 13.4; 3.8–46.5; $p < 0.001$). **Conclusions:** Most of the CIS patients develop new T2 lesions during the first six months. The risk of developing new lesions is related to the baseline number of T2 lesions and is higher in patients with more than six lesions.

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Evidence for cortical reorganisation in cognitive domains in multiple sclerosis: insights from a 'Go/NoGo' Task
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Background: Cognitive dysfunction in multiple sclerosis (MS) can occur even in the earliest disease stages and its prevalence increases with disease duration. Whether and how the neural correlates change in parallel is incompletely understood. **Objective:** We therefore created a functional magnetic resonance imaging (fMRI) paradigm with applicability across a large clinical spectrum of MS aiming to elicit activation in a widespread cognitive network. **Methods:** We investigated 40 MS patients [n=10 clinically isolated syndrome (Expanded Disability Status Scale(EDSS) score 0–2, age 23–50), n=10 relapsing-remitting MS (0–3.5, 24–49), n=10; secondary progressive MS (3.5–7.5, 25–57), n=10; long-term low disability MS (0–3.5, 33–58)] and 21 healthy age-matched controls using a 3T scanner and a 'Go/No Go' paradigm. Participants also underwent extensive behavioral and neuropsychological testing (including the Brief Repeatable Battery for Neuropsychological Tests, the Wisconsin Card Sorting Test, the 'Zahlen-Verbindungstest', the Nine-Hole Peg Test and the Maximal Finger Tapping Rate). **Results:** Behaviorally, reaction times and the number of correct responses were lower in patients than controls. This was mostly attributable to a worse performance of secondary progressive MS patients. On fMRI, both patients and con-

trols demonstrated robust activation of a frontoparietal cerebral network. Depending on task difficulty, differential recruitment of network components became evident. Also, brain activity increased with increasing cognitive demand in the pre- and supplementary motor area (SMA) in both groups. However, in contrast with healthy controls, MS patients showed additional activity in posterior parietal areas with the most difficult task. Differences in activation were more pronounced in patients with longstanding disease. **Conclusions:** Our results confirm previous studies on the role of pre-SMA/SMA in response inhibition. They also support the concept of increasing cortical recruitment with increasing cognitive demands which is supported by cortical reorganization in longstanding MS. **Supported by:** unrestricted research grant of Teva-Aventis.

P254

Magnetic resonance imaging results of a 3-year randomized trial comparing two therapeutic strategies in aggressive relapsing-remitting multiple sclerosis: mitoxantrone as induction for 6 months followed by interferon-b-1b versus interferon-b-1b
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Background: Interferon-beta 1b reduces relapses occurrence and magnetic resonance imaging (MRI) disease activity. **Objective:** To assess the impact of an induction treatment with mitoxantrone (MITOX), followed by a maintenance therapy with interferon-beta 1b on MRI metrics from very active relapsing-remitting multiple sclerosis (VARRMS) patients. **Methods:** 109 VARRMS patients (2 relapses with sequelae in the previous 12 months and at least 1 Gd+ lesion) were randomized: group 1 received MITOX 20 mg monthly combined with methylprednisolone (MP) 1 g for 6 months followed by IFN-beta 1b for the subsequent 27 months; group 2 received IFN-beta 1b for 3 years combined with MP 1 g monthly for the 6 first months. A total of 55 patients (group 1) and 54 patients (group 2) were compared using an intention-to-treat analysis. At baseline, the two groups were matched for age, MS duration, Expanded Disability Status Scale score, and annual relapse rate during the previous 12 months. A subgroup of 54 patients (33 from group 1 and 21 from group 2) had an MRI follow-up evaluation at different time points: 9 months (54 patients), 24 months (42 patients) and 36 months (34 patients). MRI secondary endpoints were: number of new T2 lesions, Gd + lesions, and new black holes. **Results:** The cumulative number of new T2 lesions over 36 months was significantly reduced in group 1 compared with group 2 (3.6 +/- 5 versus 9.9 +/- 10, $p < 0.041$), and this was also observed at each time point. At month 9, the mean number of Gd+ lesions (0.4 +/- 1 versus 2.1 +/- 4, $p < 0.012$) was significantly lower, and the number of patients without Gd+ lesions higher (88% versus 57%, $p < 0.01$) in group 1 compared with group 2. No difference was found between the two groups concerning the mean number of new black holes (cumulative and at each time-point). **Conclusions:** The MRI findings of this study support the use of MITOX as an induction treatment to improve the impact of Interferon-beta 1b in VARRMS patients. **Supported by:** Bayer-Schering, Immunex-Lederley .

P255

Fluctuations in brain parenchymal fraction: a longitudinal study
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Background: Lesion volumes are known to vary from one magnetic resonance imaging (MRI) exam to another due to multiple sclerosis (MS) disease activity. Scan-to-scan fluctuations in brain atrophy measurements have not been well described. **Objective:** To quantify short-term brain parenchymal fraction (BPF) fluctuations in MS patients and healthy controls and to determine their implications. **Methods:** Subjects with clinically isolated syndrome (CIS), relapsing-remitting MS (RRMS), secondary progressive MS (SPMS), and healthy controls (HCs) were enrolled in an observational protocol. MRI scans were

obtained semi-annually in MS and CIS and annually in HCs. Annualized BPF change was the percentage change from baseline divided by follow-up time in years. BPF Mean Square Error (MSE) was defined as the average squared deviation from the linear regression line of yearly or semi-yearly BPF measurements. MSE was calculated for individual patients studied over 4 years. **Results:** 17 HC, 7 CIS, 36 RRMS, and 26 SPMS completed the study. Scan-to-scan variation, expressed as the mean BPF MSE, was higher in MS compared with the combined HC and CIS groups ($P < 0.01$), and higher in SPMS than in RRMS ($P < 0.01$), indicating that BPF fluctuations were disease-related and greater in subjects with the most severe disease. Annualized atrophy rates observed during a 1-year interval were not correlated with annualized atrophy rates observed over a 4-year interval. **Conclusions:** The cause of short-term fluctuations in brain atrophy in MS is not known, but is thought to be biological as opposed to technical. The results indicate that the rate of brain atrophy over a 1-year period, even when measured under optimal imaging conditions, does not accurately estimate longer-term atrophy rates. This suggests that individual treatment decisions should not be based on two or three serial MRI studies performed over a 1-year period. Sample size estimates for clinical trials focused on brain atrophy need to consider short-term BPF fluctuations.

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Pathologic correlates of magnetic resonance imaging-detected diffuse tissue damage in multiple sclerosis brains

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Background: The pathologic substrates for reduced magnetization transfer ratio (MTR), decreased fractional anisotropy (FA), and increased mean diffusivity (MD) in normal-appearing white matter (NAWM) of multiple sclerosis (MS) brains is unknown. **Objective:** To determine pathologic correlates of magnetic resonance imaging (MRI)-based measurements of diffuse tissue damage in normal-appearing white matter in MS brains. **Methods:** MS brain tissue was obtained through a rapid post-mortem procurement protocol that included in situ MRI. Image characteristics were used to guide tissue sampling. Four types of MRI-defined regions were identified: (1) lesions, defined as regions that were abnormal on T2, T1, and MTR images; (2) NAWM regions with slightly-abnormal MTR that were close to lesions (saClose); (3) NAWM regions with slightly-abnormal MTR that were far from lesions (saFar); and (4) NAWM regions with normal MTR that were far from lesions (NAWM). Each region was stained for myelin, axons (neurofilament), activated microglia/macrophages (MHCII), and astrocytes (GFAP). Axonal pathology was visually graded (1–4) based on neurofilament density, quantity of swollen/convoluted axons, and transected axon ovoids. **Results:** Forty-eight regions from 4 MS brains were analyzed. Myelin density was low in lesions (47.8%), but there were no detectable differences in myelin density across the three types of non-lesion regions (80.0, 84.1, 82.5%, for NAWM, saFar, saClose regions respectively). Axonal pathology increased from NAWM, saFar, saClose, to lesion regions (median scores: 2, 2.5, 3, and 4, respectively). MHCII density was highest in saFar regions (24.5%) and otherwise relatively constant (18.3, 18.7, and 19.0%, for NAWM, saClose, and lesion regions, respectively). **Conclusions:** The pathologic substrate for MRI abnormalities in NAWM of MS brains varied based on distance from focal-demyelinating lesions. In regions close to lesions, axonal pathology extending beyond foci of demyelination may explain slightly abnormal MTR. In regions distant from lesions, axonal pathology and microglial activation are associated with decreased MTR.

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Changes in brain lesion load over 20 years and subsequent brain atrophy in clinically isolated syndrome patients

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Background: Magnetic resonance imaging (MRI) white matter (WM) lesions are prominent early in relapse onset multiple sclerosis (MS) whereas atrophy, although evident from disease onset, is more prominent in the later stages. The long-term relationship between these two processes is unclear. **Objective:** To evaluate the relationship of changing brain lesion load (LL) over 20 years with gray matter (GM) and WM volumes at 20-year time point, in a cohort of clinically isolated syndrome (CIS) patients followed-up for 20 years from disease onset. **Methods:** Forty subjects who had clinical and MRI follow-up at 5-yearly intervals were followed for a mean of 19.5 years from disease onset with a CIS suggestive of MS, [now 13 CIS; 21 relapsing-remitting and six secondary progressive MS (mean age 50.8; 26 women and 14 male)] along with 25 healthy controls (mean age 41.7; 14 women, 11 male). T2 LL was measured at baseline and at about 5, 10, 14 and 20 years later. GM and WM volumes were measured at 20 years on T1-weighted images of the brain using SPM2. GM and WM fractions (GMF and WMF) were calculated relative to the total intracranial volume. **Results:** At year 20, the median Expanded Disability Status Scale for the whole cohort was 2.5 and 3.0 for the MS patients only. GMF ($p=0.001$) and WMF ($p=0.01$) were lower in MS patients compared with controls. Change in T2 LL over the first 5 years was more closely related to GMF: 1SD higher LL in years 0–5 predicting 1.3SD lower GMF ($p<0.001$) versus 1SD higher LL in years 5–20 predicting 0.5SD lower GMF ($p<0.001$). Only the changes in LL over the first 5 years predicted WMF at 20 years: 1SD higher lesion growth predicted 1.03SD lower WMF ($p=0.01$). **Conclusions:** Earlier, more than later, lesion accumulation is related to subsequent GM and WM brain atrophy. Factors other than lesion accumulation may influence long-term atrophy.

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White matter lesion characteristics predict types of gray matter abnormality on magnetic resonance imaging in secondary progressive multiple sclerosis

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Background: Recent magnetic resonance imaging (MRI) studies have demonstrated that measures of gray matter (GM) abnormality correlate well with clinical disability. However, the pathogenesis of the GM abnormality is poorly understood. **Objective:** We aimed to investigate whether gray matter abnormality measured on MRI was associated with lesion characteristics. **Methods:** We assessed the relationship between white matter T2 lesion volume (T2LV), T1 lesion volume (T1LV), mean lesional magnetization transfer ratio (MTR) and MRI markers of tissue damage in the normal-appearing gray and white matter in 117 subjects with secondary progressive multiple sclerosis. Measures of brain T2LV, T1LV, normalized gray matter volume (NGMV) and normalized white matter volume (NWMV) were obtained. Mean MTR was calculated for T2 lesions, gray matter (GM), total white matter (WM) and normal-appearing white matter (NAWM). **Results:** NGMV and mean gray matter MTR were strongly associated with T2LV ($r=-0.6$, $p<0.001$; $r=-0.71$, $p<0.001$), T1LV ($r=-0.64$, $p<0.001$; $r=-0.71$, $p<0.001$) and mean lesional MTR ($r=0.59$, $p<0.001$; $r=0.72$, $p<0.001$). Weak to moderate associations were found between

white matter volume and MTR and the lesion measures, but multiple regression analyses suggested that this was only due a confounding association between the white and gray matter measures. T1LV was the only significant independent lesion predictor of NGMV in a step-wise regression model and explained 41% of the variance. Mean lesional MTR and T2LV were independent correlates of mean gray matter MTR and explained 61% of the variance. **Conclusions:** Axonal transection within lesions with secondary neuronal apoptosis may explain the relationship between T1LV and NGMV. A parallel accumulation of demyelinating lesions in white and gray matter may contribute to the association of T2LV and lesion MTR with GM MTR. The lack of independent correlation between lesions and WM abnormality suggests that these pathologies have a different pathogenesis. **Supported by:** Multiple Sclerosis Society of Great Britain .

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Altered functional adaptation of working memory performance in early relapsing-remitting multiple sclerosis

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Background: Cognitive deficits in multiple sclerosis (MS) are common and mainly affect information processing speed, attention, working memory and flexibility. These disturbances can already be present at the early stages of the disease and substantially influence quality of life. Previous functional magnetic resonance imaging (fMRI) studies on cognition in MS revealed changes in brain activation patterns. MS patients even at very early stages of the disease were shown to activate additional brain regions. **Objective:** The aim of the present study is to investigate functional changes related to cognitive performance. **Methods:** Six patients with relapsing-remitting MS (RRMS) (mean age: 39, mean Expanded Disability Status Scale score: 2.2, mean disease duration: 4) and six healthy controls (NC; mean age: 34) underwent a comprehensive neuropsychological examination. In addition, depression, fatigue and quality of life were assessed. During the fMRI investigation, subjects performed an alertness and a working memory task with increasing task complexity (1-back, 2-back, 3-back). fMRI was performed on a 3T head scanner (Allegra, Siemens). Data analysis was done using AFNI software. **Results:** The neuropsychological examination revealed significant differences between patients and HCs in information processing speed, verbal and spatial short-term memory. In contrast, performance in working memory measures did not differ significantly between the two groups. In addition, patients scored significantly higher on fatigue. Significant differences in brain activation patterns were found during 2-back and 3-back performance, when patients showed increased activation in the medial frontal gyrus (MFG). These changes however were not related to decreased task performance. While NC showed a significant and widely distributed change of activation for the contrast between 3-back and 1-back, only sparse change was found in patients. **Conclusions:** Our results indicate an altered functional adaptation to higher demanding working memory tasks (2-back and 3-back) in patients at early stages of RRMS despite the lack of obvious behavioral impairment.

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Automated quality control of brain magnetic resonance images

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Background: Magnetic resonance imaging (MRI) is routinely used in clinical trials to quantitatively assess the evolution and response to therapy of multiple sclerosis (MS) and other neurological diseases. In MS, outcome metrics include lesion volumes, lesion counts, and changes in brain magnetization transfer ratio (MTR) values. These

values are generally quantified with the help of image processing tools, such as tissue classification, image registration and segmentation. Accordingly, the evaluations are heavily dependent on the quality of the acquired MR image. **Objective:** To address this issue, we developed an automated method for assessing the quality of MRI data acquired in a clinical trials environment. **Methods:** The quality control (QC) procedures include: 1) patient brain identity verification, 2) alpha-numeric parameter matching with approved dummy run parameters, 3) signal-to-noise ratio estimation, 4) gadolinium-enhancement verification, 5) detection of ghosting due to head motion, 6) computation of head position relative to the magnet's iso-center, and 7) MTR verification. Each QC procedure produces a quantitative measurement which is compared against an acceptance threshold that was determined based on an analysis of traditional manual and visual QC performed by trained experts. **Results:** The results demonstrate that our automated QC checking procedures have high sensitivity and specificity compared to manual and visual QC performed by experts. ROC graphs for procedures 1, 4, and 5 resulted in the following: metric/threshold range/sensitivity values/specificity values: correlation/98.9%–99.2%/100%/100%; percent enhancement/5%–10%/100%/100%; and ratio of the variance in two noise regions/0.86–1.16/93.2%/92.3%. For procedures 2, 3, 6, and 7, strong correlations were found between the manual and automated results. **Conclusions:** Many QC procedures can be replaced by automatic, objective procedures to increase data throughput while reducing reader variability.

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Patterns of regional brain atrophy and growth in children with clinically isolated syndromes at high and low risk of conversion to multiple sclerosis

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Background: Studies in adult multiple sclerosis (MS) implicate brain atrophy, and atrophy of the thalami in particular, as magnetic resonance imaging (MRI) markers of the neurodegenerative processes in MS. **Objective:** To determine whether atrophy of the thalamus and other structures differs between pediatric patients with clinically isolated syndromes at high risk or low risk of conversion to MS. **Methods:** Serial MRI images from 22 children enrolled in the prospective Canadian Pediatric Demyelinating Study were analyzed. Patients were designated as either low risk (n=13) if they remained free of clinical relapse or MRI evidence of new lesions at 12 months, and as high risk (n=9), if new lesions developed on subsequent MRI scans or if they have been diagnosed with definite MS based on clinical relapses. Images were acquired using a standardized research protocol on a GE Signa Excite 1.5T scanner at baseline (within 7 days of onset of the initial demyelinating event) and at 3, 6 and 9 months. After pre-processing, region of interest segmentation was performed using FreeSurfer v4.0.3. **Results:** Patient ages were comparable, 10.8 ± 2.3 years for the high-risk and 10.2 ± 3.7 for the low-risk groups. Significant volume changes were apparent by 9 months from the baseline scan. Thalamic volumes increased by 2.5 ± 7.0 % (mean ± SD) in the low risk group and decreased by 6.1 ± 10.0 % in the high risk group (p=0.02). Corpus callosal volumes also increased in the low-risk group by 0.4 ± 11.6 % and atrophied by 6.5 ± 7.7% (p<0.05) in the high-risk group. Cerebral cortex and gray matter showed trends toward volume increase in the low-risk group and atrophy in the high-risk group. **Conclusions:** These findings suggest that MRI evidence of neurodegeneration may distinguish those children destined for MS diagnosis from children experiencing an isolated demyelinating event, and support a prominent and perhaps primary role for neurodegeneration even in the youngest MS patients. These findings raise grave concern regarding the implications for cognitive outcome in these children, given the selective degree of atrophy of structures implicated in cognitive performance.

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Pediatric patients with clinically isolated syndromes at high risk of conversion to multiple sclerosis exhibit brain atrophy while those at low risk of conversion exhibit brain growth

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Background: Accelerated brain atrophy has been observed in all phases of multiple sclerosis (MS), even following the first attack. Measurement of brain atrophy has been suggested as a marker of axonal loss and disease progression. **Objective:** To determine whether brain volume change is different among pediatric patients with clinically isolated syndromes (CIS) at high or low risk for progression to MS. **Methods:** Serial magnetic resonance imaging (MRI) images from 22 children enrolled in the prospective Canadian Pediatric Demyelinating Study were analyzed. Patients were designated as either low risk (n=13) if they remained free of clinical relapse or MRI evidence of new lesions at 12 months, and as high risk (n=9), if new lesions developed on subsequent MRI scans or if they were diagnosed with definite MS based on clinical relapses. Images were acquired using a standardized research protocol on a GE Signa Excite 1.5T scanner at baseline (within 7 days of onset of the initial demyelinating event) and at 3, 6 and 9 months. After pre-processing, percent brain volume change was calculated using SIENA 2.5 from FSL 4.0.1. Brain volume changes were calculated longitudinally relative to the baseline scan. **Results:** Patient ages were comparable, 10.8 ± 2.3 for the high-risk and 10.2 ± 3.7 for the low-risk groups. Significant differences in brain volume change between the groups were apparent at the 9 month time point relative to the baseline scan. By month 9, the percent brain volume changes were $-1.5 \pm 1.2\%$ (mean \pm SD) in the high-risk group and $0.3 \pm 1.1\%$ in the low-risk group. The differences between the low-risk and high-risk groups were statistically significant ($p = 0.002$). **Conclusions:** In contrast with children with an isolated episode of demyelination who demonstrate stability or age-expected brain growth, brain atrophy can be detected in children at high risk of MS. These findings implicate both a predictive and primary role for neurodegeneration early in the MS disease process and raise concern regarding the implication of brain atrophy on the long-term physical and cognitive outcome in these children.

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Normal findings on brain fluid attenuated inversion recovery magnetic resonance imaging scans at 3T

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Background: Fluid attenuated inversion recovery (FLAIR) magnetic resonance imaging (MRI) of the brain has become a routine tool for assessing lesions in patients with suspected neurologic disorders such as multiple sclerosis. FLAIR hyperintensities seen in normal subjects have been described at 1.5T, but the normative findings at 3T have not been studied. **Objective:** To describe brain hyperintensities detected in normal volunteers on FLAIR MRI using both 1.5T and 3T. **Methods:** Twenty-two adult normal volunteers [age (mean \pm SD): 44 ± 8 , range 30–53 years] were scanned with axial 2D FLAIR at 3T. Fifteen of these subjects also underwent 2D FLAIR at 1.5T, using similar optimized parameters and voxel size. **Results:** The 3T images commonly showed a range of hyperintensities such as discrete hyperintense foci (in 68%

of subjects), anterior and posterior periventricular capping, diffuse posterior periventricular white matter hyperintensity, septal hyperintensity, and ventricular cerebrospinal fluid flow artifacts. Regarding discrete foci, lesion volume ($r=0.72$ at 3T; $r=0.62$ at 1.5T) and number ($r=0.74$ at 3T; $r=0.63$ at 1.5T) strongly correlated with age on both platforms. 3T showed a higher lesion volume (170 ± 243 , range 0–872 vs. 93 ± 152 , range 0–536 mm³, $p < 0.01$) and number (9.4 ± 13 , range 0–47 vs. 5.5 ± 9.2 , range 0–33, $p < 0.01$) than 1.5T. All discrete hyperintense foci were confined to the supratentorial white matter. The other hyperintensities (e.g. diffuse posterior white matter hyperintensity) were generally more common and more prominent at 3T than 1.5T. **Conclusions:** Discrete and diffuse parenchymal brain white matter hyperintensities in normal volunteers are more common and prominent at 3T than 1.5T. Caution should be exercised when applying 3T FLAIR MRI to the study of neurologic disorders such as multiple sclerosis to determine the clinical relevance of detected hyperintensities.

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Visualization and quantification of iron in multiple sclerosis lesions using susceptibility-weighted imaging

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Background: Brain iron accumulation has been shown histologically in multiple sclerosis (MS) and has been specifically seen in the vessel wall of veins. T2W hypointensities have suggested iron deposition in the cortex as well as in the deep gray matter. Iron-mediated damage to the brain may be complex, and oxidative stress and generation of toxic free radicals are implicated. Susceptibility-weighted imaging (SWI) is a 3D flow-compensated gradient echo sequence which uses magnitude and phase data to enhance information about local tissue susceptibility independent of field strength. We have used the SWI phase images to map out putative iron content in the brain. **Objective:** In this work, we used SWI to visualize and characterize the iron content in MS lesions. **Methods:** Twenty-seven MS Patients were scanned on three field strengths: 14 patients at 1.5T, 7 patients at 3T and 6 patients at 4T. In addition to conventional magnetic resonance imaging (MRI) scans, 3D SWI were also obtained. The SWI phase images were used to quantify iron content. The correlation between T2 signal intensity (potential inflammation) and SWI (putative iron content) was examined. **Results:** 422 lesions were seen by combining both methods. 204/422 lesions were seen in common by both methods. 75/422 lesions were seen by conventional MRI sequences only while 143/422 lesions by SWI only. The average iron content in MS lesions was 47 mcg Fe/gm of brain tissue, and significantly higher than normal tissue. We also found a negative correlation of T2 signal intensity with SWI filtered phase images, and hence the putative iron content. **Conclusions:** SWI reveals iron deposition in MS lesions not seen with conventional MRI, independent of the field strength. Furthermore, SWI suggests that there is a correlation between T2 signal intensity and SWI phase changes (putative iron content). Quantifying iron in MS may introduce novel insight into MS pathology and serve as potential surrogate marker in exploratory studies.

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Improved radio frequency coils for 3T magnetic resonance imaging of the optic nerve

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Background: Magnetic resonance imaging (MRI) of the optic nerve has had limited diagnostic utility because of a lack of optic

nerve-specific imaging coils. Receiver coils designed specifically for imaging the optic nerve should provide an increased signal-to-noise ratio (SNR) and image quality over commercially available coils that are designed for general purpose MRI of the head. **Objective:** The purpose of this work was to develop a 3T MRI receiver coil array that would improve the quality of optic nerve imaging and improve the detection and study of optic neuritis. **Methods:** Two different radio frequency (RF) coil arrays have been constructed using 20 and 28 separate circular elements on fiberglass formers. The elements for each coil were arranged such that central elements were overlapped by five or six adjacent elements, depending on curvature of the mask. The mask shapes were optimized for optic nerve imaging from the orbit to the chiasm. The 28-channel coil also allowed for high-resolution imaging of the entire brain including the optic cortex. **Results:** The relative SNR of the dedicated coil arrays was 35–40% better at the chiasm, and approximately 300% better at the orbit, than that obtained with the commercially available 12-channel coil. In-plane images of the optic nerve were noticeably improved over commercial coil images, and patient images showed abnormalities that correspond well with patient disease histories. Diffusion tensor imaging using the improved coils also looked promising and may provide new information concerning optic neuritis. **Conclusions:** High resolution dedicated RF coils, designed for the specific purpose of 3T MRI of the optic nerve, provide improved SNR and image quality compared with commercially available general purpose coil arrays. Optic nerve lesions consistent with neuritis symptoms were identified. **Supported by:** The Cumming Foundation, Siemens Medical Solutions, NIH RO1 HL48223.

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Comparison of sample size requirements for treatment effects using gray matter, white matter and whole brain volume in patients with multiple sclerosis

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Background: Although clinical trials for neuroprotective treatments are being designed, the best outcome measure for such trials has not been determined. **Objective:** To compare the sample size requirements for a neuroprotection trial with change in cerebral gray matter volume (GMV), white matter volume (WMV) or whole brain volume (BPV) as the outcome measures. **Methods:** Four datasets with longitudinal magnetic resonance imaging measures of multiple sclerosis (MS) patients were investigated: 116 relapsing-remitting MS (RRMS) patients followed for 9 months, 26 patients RRMS patients followed for 1.5 years, 28 clinically isolated syndrome (CIS) patients followed for 3 years, and 13 secondary progressive MS (SPMS) patients followed for 2 years. In each dataset, normalized GMV, normalized WMV and normalized BPV were analyzed using a random intercepts and slopes model to estimate the variance components and percentage change. The required sample size to observe a 33%, 50% and 90% reduction in the percent change was calculated for each dataset using both a percentage change from external data and the estimated percentage change from each dataset. **Results:** For RRMS patients, the percentage change was the greatest in GMV, but the percentage change in WMV was the greatest in the CIS and SPMS patients. The variability in BPV was the lowest in each group vs. GMV and WMV. Therefore, BPV required the smallest sample size when the same external percentage change was assumed for each volume measurement. When the estimated percentage change was used, the sample sizes required for RRMS patients were similar for GMV and BPV (and both were lower than for WMV); conversely, the sample size for CIS and SPMS patients were similar for WMV and BPV (and both were lower than for GMV). **Conclusions:** Cerebral GMV may be a viable outcome measure for clinical trials investigating neuroprotection in RRMS patients, especially considering a treatment effect may be larger on GMV compared with BPV. However, the use of GMV or WMV is somewhat limited by increased variability in these measures vs. BPV.

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Evaluation of an automatic change detection method in serial scalar images characterizing diffusion properties of multiple sclerosis patients

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Background: Diffusion tensor imaging (DTI) has been widely used to investigate brain changes in pathological conditions. In particular, longitudinal changes for individual subjects can be used for monitoring progression of multiple sclerosis (MS) disease. Detecting these changes between two images remains a challenging problem, particularly when considering scalar indices computed from DTI. **Objective:** The goal of this work is to develop and evaluate a new automatic method for automatic change detection between scalar images characterizing diffusion properties. **Methods:** Twelve patients with MS (2 relapsing-remitting and 10 secondary progressive forms) (Mean Expanded Disability Status Scale score : 4.27) were enrolled. 3D DTI images (33 directions) were acquired at baseline and after 1 year on a 3 Tesla GE scanner. We applied a new automatic change detection system (Boisgontier et al., ISMRM 2008) on serial DTI sequences by considering the mean diffusion (MD), the fractional anisotropy (FA) and the radial diffusion (RD) indices. This method relies on the generalized likelihood ratio test, while accounting for the non-stationarity of noise that corrupts these images. The significance of the detected changes has been estimated by considering serial examinations from a healthy volunteer. **Results:** All patients present significant longitudinal modifications in MD and TR images, whereas only 7 present significant changes in FA. Changes detected by the automatic method mainly correspond to decreases in FA (70.8% of changes detected in FA, significance $p = 0.0079$), increases in MD (70.8% of changes detected in MD, significance $p < 0.0001$) and increases in RD (75.9% of changes detected in RD, significance $p < 0.0001$). These findings are consistent with the results reported in the literature by considering statistics on ROI (lesions or apparently normal white matter). **Conclusions:** This novel automatic method can be useful in detecting subtle and local DTI changes over time in a specific subject, especially in MS disease processes.

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Optical coherence tomography in patients under 21 years with inflammatory demyelinating conditions

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Background: Optical coherence tomography (OCT) has been shown to be a safe and useful tool in adults to quantify axonal loss in retinal nerve fiber layer (RNFL). **Objective:** To examine the RNFL thickness and macular volume (MV) in patients under 21 years and diagnosed with inflammatory demyelinating condition (IDC). **Methods:** RNFL thickness and MV of 32 patients with IDC (multiple sclerosis (MS), $n=24$; acute disseminated encephalomyelitis (ADEM), $n=5$; and transverse myelitis (TMO), $n=4$) and 19 controls between ages 9 and 21 was measured using OCT (Zeiss Meditec 3.0). The average of minimum RNFL thickness (worse eye) and minimum total MV (worse eye) were compared between the groups using the Wilcoxon rank sum test. **Results:** The study groups were identical for age, gender and ethnicity. History of optic neuritis was positive in 24% of patients with IDC. The minimum RNFL thickness in patients with IDC was 8 μm thinner compared with the controls (mean \pm SD: $95 \pm 15 \mu\text{m}$ vs. $103 \pm 7 \mu\text{m}$; $p=0.06$). Compared with controls the minimum RNFL thickness in MS patients was significantly thinner by 8.2 μm ($103 \pm 7 \mu\text{m}$ vs. $94 \pm 15 \mu\text{m}$; $p=0.05$). Though the minimum RNFL thickness in ADEM ($93.7 \pm 25 \mu\text{m}$ vs. $103 \pm 7 \mu\text{m}$; $p=0.14$) and TM ($96.8 \pm 6 \mu\text{m}$ vs. $103 \pm 7 \mu\text{m}$; $p=0.11$) patients was thinner than the controls it was not statistically significant, perhaps because of the small number of

patients in these groups. The minimum TMV was comparable among groups. **Conclusions:** Preliminary results from our study suggest that MS patients under 21 have lower RNFL thickness than matched controls. We demonstrate that OCT can be used to measure RNFL thickness and MV in children as young as 9 years to detect early changes in IDC. OCT can be particularly useful among young children in whom visual field tests and visual evoked potential tests can be challenging. We will provide additional data on pediatric patients with IDC.

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Blood flow parameters in the ophthalmic artery in acute and chronic phase of optic neuritis - ultrasound evaluation

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Background: Recently several authors described hemodynamic changes in orbital vessels during optic neuritis (ON). During acute ON higher peak systolic velocities (PSV) and resistance index (RI) in the ophthalmic artery (OA) were usually observed, as compared with the unaffected side. **Objective:** To describe acute and chronic hemodynamic changes in the OA in ON patients by measuring peak systolic and end diastolic velocities (EDV) and resistance index in the OA of both orbits. **Methods:** Ultrasound measurement of blood flow parameters (PSV, EDV and RI) in the OA of both orbits was performed in 46 patients during acute unilateral ON (these subjects were examined in the interval of maximum 7 days after onset of symptoms) and 118 patients who have ever experienced unilateral ON (these patients were examined later than 1 year after ON). The same measurements were also performed in 45 healthy controls. **Results:** The subjects examined during acute unilateral ON presented with significantly increased PSV and RI in the OA on the side affected with ON compared with the contralateral OA. In the chronic phase of ON (more than one year after acute symptoms of ON) we did not observe any difference in the blood flow parameters in the OA on the side affected with ON compared with the contralateral side, but generally blood flow velocities in the OA were lower in chronic group than in healthy controls. **Conclusions:** The changes in orbital hemodynamics during acute unilateral ON suggest the role of vasoconstriction of orbital vessels as one of the underlying pathophysiological mechanisms of acute ON. These changes, however, are short-lasting and are not observed in the chronic phase of ON.

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The motor corpus callosum as a marker of structural, functional and behavioral changes in early relapsing-remitting multiple sclerosis patients

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Background: The corpus callosum (CC) represents the principal anatomical structure connecting cortical areas of both brain hemispheres and is often and early affected in relapsing-remitting multiple sclerosis (RRMS). **Objective:** We sought to study the integrity of the motor CC on an anatomical, functional and behavioral level by combining functional magnetic resonance imaging (fMRI) and diffusion tensor weighted imaging (DTI) with paired transcranial magnetic stimulation and a bimanual motor task. **Methods:** Thirteen patients with early RRMS (age 32±6 years, Expanded Disability Status Scale (EDSS) score 1.3±0.65, disease duration, 17±15 months) and 12 healthy controls (age 31±9 years) participated. A combined fMRI/DTI fiber-tracking procedure was used to measure fractional anisotropy (FA) as parameter of axonal density of callosal motor fibers. Paired TMS was used to study interhemispheric inhibition (IHI) at an interstimulus interval of 12 ms according to an established protocol (Ferber et al., 1992). In the behavioral task, we tested the accuracy with which subjects were able to tap with their left and right

index finger on two lights flashing synchronously or at different time intervals (Tuller et al., 1989). **Results:** FA was significantly reduced in RRMS patients (0.81±0.06) compared with healthy subjects (0.85±0.02; p=0.03) and RRMS patients showed significantly less IHI than healthy subjects (p=0.04). In the motor task, patients showed a significantly better performance, as expressed by higher tapping accuracy compared with healthy controls (p=0.001). **Conclusions:** We show that in patients with early RRMS, microstructural alterations (decreased FA) and impaired functional interhemispheric connectivity (decreased IHI) are prevalent, compared with healthy controls. In contrast, on the behavioral level, RRMS patients even seem to perform better in bimanual motor tasks, possibly due to less temporal interference between the motor cortices of the two hemispheres if no longer connected by normally functioning CMFs.

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Ibudilast reduces conversion of new inflammatory lesions to persistent black holes in active relapsing multiple sclerosis

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Background: Black holes, or chronic T1-hypointense lesions, are regarded as the magnetic resonance imaging (MRI) equivalent of severe tissue destruction. Histopathological and magnetic resonance spectroscopy studies have provided evidence that the degree of T1 hypointensity reflects the extent of axonal loss. In particular, persistent black holes (PBH), i.e. lesions that persist to be hypointense over time, indicate definitive tissue loss. **Objective:** To determine the effect of ibudilast, a putative neuroprotectant, on the evolution of new (inflammatory) lesions to PBH compared with placebo. **Methods:** A post-hoc, assessor-blinded evaluation of MRI data collected during year 1 of the MN-166-CL-001 study was performed in a group of 297 relapsing multiple sclerosis (MS) patients, randomly assigned to placebo, 30 or 60 mg ibudilast/day orally. New active lesions were selected at the first on-study MRI (month 2) and were tracked on month 4 and month 10 for T1-signal evolution. Lesions that were hypointense at month 10 were considered PBH. The average pattern of lesion evolution per subject was compared between treatment arms. **Results:** Among patients with active (mostly new enhancing) lesions, 72 received placebo, 64 received 30 mg ibudilast/day and 56 received 60 mg ibudilast/day. The average proportions of active lesions evolving to PBH were 0.24 for placebo, 0.20 for 30 mg/d, and 0.16 for 60 mg/d treated patients. The relative risk for evolution to PBH was significantly lower for the 60 mg/day treated patients (RR 0.63, CI 0.44–0.90, p=0.011) and tended to be lower for 30 mg/day treated patients (RR 0.735, CI 0.52–1.03, p=0.074) compared with placebo. **Conclusions:** Ibudilast exerts an inhibitory effect on the development of PBH (with presumed axonal damage) in active MS lesions. This could indicate a neuroprotective effect and is consistent with the previously reported reduction of whole brain atrophy rates in patients treated with ibudilast.

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Brian iron quantification in primary-progressive multiple sclerosis: a magnetic field correlation study

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Background: Abnormal iron deposition has been reported in the brain of patients with relapsing-remitting multiple sclerosis (RRMS). Iron accumulation can cause oxidative tissue injury especially to the iron-rich cells in the basal ganglia, leading to neurodegeneration. Unlike patients with RRMS, patients with primary-progressive MS (PPMS) show a progressive accumulation of disability in the absence of clinical relapses. Occult tissue damage seems to play a major role in the disease progression; however, the mechanisms leading to

neurodegeneration are still unknown. **Objective:** To evaluate the presence and extent of iron accumulation in the deep gray matter of PPMS patients in comparison with RRMS patients and healthy controls using a quantitative magnetic resonance technique - magnetic field correlation (MFC). **Methods:** Eleven PPMS patients (mean age 52 ± 9.6 years), eleven RRMS patients (mean age 49.9 ± 8.7 years) and 10 healthy controls (mean age 50.6 ± 7.6 years) underwent the following magnetic resonance imaging (MRI) protocol on a 3.0 Tesla imager (Trio, Siemens, Erlangen Germany): axial pre- and post-contrast T1-weighted, T2-weighted and MFC images. MFC was acquired with a single-shot asymmetric echo planar imaging sequence. MFC maps were generated from these images. Regions of interest were selected for MFC measurements in both deep gray matter (thalamus, pallidus, putamen and caudate nuclei) and white matter regions (corpus callosum and normal-appearing frontal white matter), and the mean MFC values were compared among the three groups. **Results:** Compared with controls, MFC was significantly higher in the thalamus, pallidus, putamen and caudate nuclei of PPMS patients (p values <0.03) and in the pallidus of RRMS patients (p value = 0.01). In RRMS patients, a moderate correlation ($r=0.47$; $p<0.0001$) was found between the MFC value in the putamen nucleus and the expanded disability status scale score. **Conclusions:** Iron deposition as measured by MFC occurs in the deep gray matter of patients with PPMS. Although the MFC values were higher in PPMS patients compared with RRMS patients of matched age and disease duration, this difference did not reach statistical significance. MFC might complement conventional MRI when assessing PPMS disease activity. **Supported by:** NIH grants RO1 NS051623-01 and R37 NS 29029-11.

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Brain tissue sodium concentration in multiple sclerosis: a preliminary study

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Background: Axonal degeneration occurs progressively from the onset of the disease and is a significant cause of increasing disability. Several studies have shown that the accumulation of sodium in the axons can promote reverse action of the sodium/calcium exchanger which, in turn, leads to a lethal overload in intra-axonal calcium. Partial blockade of sodium channels protects axons from degeneration in experimental models of multiple sclerosis (MS), and it is currently under investigation in clinical trials. Sodium magnetic resonance imaging (²³Na MRI) provides an indicator of cellular and metabolic integrity and ion homeostasis. Sodium MRI at 3 Tesla provides high-quality images in acceptable acquisition time. This application is made feasible by a 3D radial gradient-recall-echo (GRE) sequence with extremely short echo time values. **Objective:** To demonstrate the feasibility of brain sodium MRI in patients with MS and to investigate the presence and extent of total sodium concentration (TSC) changes in lesions and in normal-appearing white matter and gray matter (NAWM and NAGM). **Methods:** Twelve patients with relapsing-remitting MS (RR-MS) (7 women, 5 men; mean age 35.4 ± 8.7 years) and thirteen healthy controls (9 women, 4 men; mean age 36.0 ± 9.2 years) were prospectively recruited. In patients, the mean disease duration was 6.2 ± 3.4 years and the median Expanded Disability Status Scale score was 2; range: 1–5. All subjects underwent MRI on a 3T imager. TSC values of Gd-enhancing, hypointense and isointense lesions, NAWM and NAGM in different brain regions were studied. **Results:** TSC values were significantly increased in lesions, NAWM and NAGM of RR-MS patients compared with healthy controls, with the highest values being in hypointense and Gd-enhancing lesions. In addition, TSC changes in lesions and normal-appearing brain tissue showed a moderate and significant association with the EDSS score suggesting that, at least in this preliminary study, TSC abnormalities are clinically significant. **Conclusions:** This study demonstrates the feasibility of sodium MRI in patients with MS. Sodium MRI may provide a potentially useful non-invasive evaluation of pathological changes at a cellular level. **Supported by:** Supported in part by NIH RO1 NS051623.

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Optic nerve axonal loss using optical coherence tomography in 93 patients with multiple sclerosis and comparison with healthy subjects

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Background: Axonal loss is thought to be a cause of persistent disability in multiple sclerosis (MS). Retinal nerve fiber layer (RNFL) imaging by optical coherence tomography (OCT) seems to be a non-invasive way of detecting optic nerve axonal loss following optic neuritis. **Objective:** To assess whether MS affects RNFL thickness. **Methods:** Prospective study including 93 patients. Two groups were studied: 1) patients with MS (diagnosis 2) healthy subjects. RNFL was measured using OCT3. Kruskal Wallis tests were performed for comparison between RNFL and MS type. A correlation (Spearman coefficient) was computed between RNFL (firstly using the mean of both eyes and secondly the minimum value between both eyes) and Expanded Disability Status Score (EDSS) score. Finally, we compared RNFL between subjects with and without MS. A sub analysis was done, restricting the group with MS to those without previous acute optic neuritis. **Results:** Of the 93 (186 eyes) included MS patients, 47 had presented previous unilateral or bilateral acute optic neuritis (74 eyes) and 46 (92 eyes) were free of optic neuritis; median EDSS score was 4. There was no significant relationship between RNFL and EDSS score or MS type. Comparison was made with 15 healthy subjects. Average RNFL thickness for whole MS patients was $90 \mu\text{m}$ (mean of both eyes) and $84 \mu\text{m}$ (minimum value of both eyes) versus $104 \mu\text{m}$ (mean of both eyes) and $101 \mu\text{m}$ (minimum value of both eyes) for the healthy subjects ($p<0,001$). Average RNFL thickness for MS patients without previous acute optic neuritis was $94 \mu\text{m}$ (mean of both eyes) versus $104 \mu\text{m}$ for the healthy subjects (mean of both eyes) ($p=0,004$). There were highly significant reductions of RNFL thickness in affected patients (with or without previous acute optic neuritis) compared with control eyes. **Conclusions:** Using OCT, we can evaluate the optic nerve axonal loss occurring in MS, which seems to be caused not only by previous acute optic neuritis episodes but also possibly by a chronic optic injury. There was no correlation between RNFL and EDSS score. We thus demonstrate that OCT seems effective for detecting optic nerve axonal loss. However, OCT seems to be unrelated to the disability in MS (rated by EDSS score).

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Magnetic resonance imaging long-term follow-up of patients with neuromyelitis optica

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Background: Neuromyelitis optica (NMO) predominantly involves optic nerves and spinal cord. Spinal magnetic resonance imaging (MRI) lesions in NMO frequently affect the grey matter extending over three or more vertebral segments, which is not characteristic for multiple sclerosis (MS), while MRI lesions of optic nerves cannot be distinguished from optic neuritis in MS. NMO may also be accompanied by MRI brain lesions showing an unspecific pattern in the majority of patients. Data documenting long-term follow-up of MRI lesions in NMO are scarce. **Objective:** Long-term analysis of spinal cord and brain lesions over the course of NMO. **Methods:** Repeated cranial and spinal MRI examinations from six patients with NMO or NMO-spectrum disorder conducted over a period of up to nine years. All patients were NMO-IgG-positive. **Results:** In the acute stage, all spinal lesions were located centrally in the grey and white matter. Marked swelling of the spinal cord and gadolinium enhancement were frequently detected. One patient showed an owl's eye appearance on axial sections shortly after onset of clinical symptoms, resembling MRI of spinal ischemia. In the chronic stage, centrally located high-signal alterations and spinal-cord atrophy were observed

in some patients. In other patients, we saw almost complete resolution of marked spinal MRI lesions without consecutive atrophy and with full clinical remission. Patterns of brain lesions varied considerably and showed an unspecific course. MRI follow-up was helpful to establish the correct diagnosis in a patient initially fulfilling diagnostic MRI-criteria for MS. In another patient a tumefactive lesion was the indication for brain biopsy. **Conclusions:** In our sample, the further course of spinal MRI lesions was not predictable during the acute stage. Brain lesions did not show a specific pattern or course.

P276**Using structural and functional magnetic resonance imaging to explain visual loss at the onset of acute optic neuritis**

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Background: Combining structural and functional magnetic resonance imaging (fMRI) may help to elucidate mechanisms of damage and repair in demyelinating diseases. **Objective:** To investigate how the anterior and posterior pathways contribute to clinical deficit at the onset of optic neuritis, using multi-modal MRI measures of structural damage of the optic nerve and radiations, and cortical functional responses to visual stimulation. **Methods:** 26 patients with acute unilateral optic neuritis within 5 weeks of presentation were assessed with logMAR visual acuity, visual evoked potentials (VEP), optic nerve Gadolinium-enhanced-T1, fast-spin-echo and fluid-attenuated inversion-recovery (FLAIR) imaging, brain diffusion-tensor imaging and visual fMRI. Parameters derived from these sequences included optic nerve lesion length and swelling, tractography-derived fractional anisotropy of the optic radiations, and functional activation maps. Firstly, separate linear regression models were estimated in Stata-9 to identify parameters which predicted visual loss. Secondly, significant structural measures were entered into a multiple regression model in SPMS, together with visual acuity, side affected, age and gender. This was to identify any functional activity associated with visual loss, after accounting for the extent of structural damage and demographic variables. **Results:** Whole-field VEP amplitude and optic nerve lesion length were significantly associated with visual loss. Bilateral activation in the extra-striate occipital cortex, adjacent to the parieto-occipital sulcus, correlated directly with visual acuity, after correcting for demographics, VEP, and optic nerve lesion length. **Conclusions:** Visual loss is predicted by measures of conduction block (VEP amplitude), and extent of inflammation (lesion length), but not by optic radiation parameters. The direct correlation between visual acuity and extra-striate cortex activation might reflect a smaller afferent input as vision worsens, independent from the structural predictors considered in our model. An alternative explanation is plasticity within the visual association cortex of the dorsal stream, helping minimise clinical deficit. Longitudinal studies are required to clarify this.

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P277**The utilization of corpus callosum atrophy as a marker for disease progression in multiple sclerosis**

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Background: Multiple sclerosis (MS) is an idiopathic inflammatory demyelinating disease. Axonal degeneration seems to play an important role for the pathogenesis of neuronal degeneration and neuronal loss with neurological dysfunctions. It is still disputed as to when axonal degeneration is initiated during the disease course, but it is believed to be a consequence of inflammation and demyelination and is recognized as the cause of disability and disease progression. Magnetic resonance imaging (MRI) has made MS diagnosis easier and

currently is the best means to show changes due to acute and chronic plaques. The most common MS plaque localizations are; periventricular white matter, internal capsule, corpus callosum (CC), pons, brachium pontis, optic nerves and spinal cord. **Objective:** Our aim is to search for the frequency of CC atrophy and correlation with Expanded Disability Status Scale (EDSS) in MS patients. **Methods:** 79 clinically definite MS patients and 50 controls were involved in the study and were screened for CC volumes. Patients were evaluated with routine neurological examination, EDSS scores were scored. Subjects were screened for CC volumes with the 1.5TGE Signo Excite MRI device. Measurements were performed on sagittal, coronal and axial planes, including relative hyperintense areas that separate the CC from surrounding tissue. Plaque areas in the CC that cause signal changes were excluded from volume measurements. **Results:** Evaluation of 79 patients revealed; 60 patients (75%) as relapsing-remitting MS and 19 patients (25%) secondary progressive MS respectively. CC volume evaluation revealed that 31 patients (39.2%) had CC atrophy. When evaluated for a correlation between EDSS and CC atrophy, 24 patients out of 60 patients with an EDSS ≤ 4 had abnormal CC measurements while 7 out of 19 patients with an EDSS ≥ 4 had CC atrophy ($p > 0.05$). **Conclusions:** Our results failed to show a correlation between EDSS and CC atrophy, suggesting that brain atrophy in MS might initiate at early disease stages, even before high EDSS scores. It is very important to have means to evaluate atrophy and disease progression, which might effect treatment options. Further studies concerning axonal degeneration and brain atrophy are needed.

P278**Distribution of multiple sclerosis plaques in the spinal cord**

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Background: Although much clinical and research attention is paid to the brain, the spinal cord is frequently involved in multiple sclerosis (MS) patients. Little is known about the specific localization of demyelinating plaques within the spinal cord. A thorough understanding of this topography could increase diagnostic specificity and may aid in our understanding of disease pathogenesis. **Objective:** To describe the distribution of MS demyelinating plaques in the spinal cord of all patients in an academic MS center. **Methods:** Retrospective review of the magnetic resonance imaging (MRI) reports of 803 consecutive MS patients at an academic MS Center. **Results:** The majority of plaques were located in the cervical region (C2). A retrospective review of 803 consecutive charts revealed 532 patients with a spinal MRI; 249 (46.8%) of these patients had at least one plaque in their spinal cord. Of the 532 patients who had a spinal MRI, 516 had at least one cervical MRI performed and 270 had at least one thoracic MRI (254 patients had both a cervical and thoracic MRI). Of the 516 patients who had received a cervical MRI, 228 (44.2%) had at least one plaque. Of the 270 patients who had received a thoracic MRI, 81 (30.0%) had at least one plaque located in that region. There were significantly less plaques located in the thoracic cord when compared with the cervical cord. Within the thoracic cord there appears to be a parabolic configuration to the data, with a minimum amount of plaques located at roughly the T4 region (with the exception of T12). Sixty-eight of the 81 plaques identified within the thoracic region were also associated with a cervical plaque; the remaining 13 patients had thoracic plaques alone. **Conclusions:** An increased understanding of demyelinating lesion predilection towards certain areas, specifically the spinal cord, may help to discern MS pathogenesis. Several explanations are examined, including; the effects of venous distribution, blood-spinal-barrier permeability to cytokines, and the existence of extracellular pathways allowing disease mediators into affected tissues.

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Can magnetization transfer data from different magnetic resonance imaging scanner/sequence/pulse types be standardized? an application of correction factor function method

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Background: Feasibility of magnetization transfer imaging (MTI) application in multi-center studies is still under intensive debate in patients with multiple sclerosis (MS). Different scanner, pulse and sequence types, and non-homogeneity of the scanners are the most limiting factors preventing combination of the raw data between different centers. **Objective:** To create and estimate predictive factor functions that correct magnetization transfer ratio (MTR) values obtained at individual magnetic resonance imaging (MRI) scanners on a voxel basis. To apply these functions to raw and calculated MTR data. **Methods:** Five healthy subjects were scanned on 5 different MRI scanners at two pulse rates using proton density and T1 gradient-echo sequence with and without magnetization pulse. A three-stage approach was designed that included Spatial Voxel Co-occurrence Matrices (SVCm) for dimension reduction of about 5 million voxel-based MTR values per scan. Five per cent trimmed means were applied. SVCms were constructed for each subject and for each pair of scanners/sequences/pulse rates. Predictive correction factor curves were estimated and fitted by low degree of freedom curve estimations functions through generalized linear regression models. Functions were aggregated into scanner-pair specific correction factor functions with model-averaging approaches in order to find optimal functions for future prediction purposes. **Results:** Functions were obtained in order to estimate voxel-based MTR values from any of the five scanner/sequence/pulse rates to any other scanner/sequence/pulse rates. The voxel by voxel reconstructed 'artificial MTR images' derived by applying predictive correction factor functions showed better fitting results than those transformed on already calculated raw MTR values. All individual curves fitted the robust means with adjusted $R^2 > 0.95$ for valid ranges of voxel-based MTR values. Adaptation of these functions to existing non-voxel-based statistics was also made. **Conclusions:** Accurate estimation of voxel-based MTR values using correction factor functions can overcome comparison problems between scanner/sequence/pulse differences in multi-center studies.

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Measurement of gray matter volume is less susceptible to pseudoatrophy effect than that of white matter or whole brain volume in patients with multiple sclerosis. results from Avonex-Steroids-Azathioprine combination study

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Background: Pseudoatrophy can be defined as an accelerated brain volume reduction with no associated loss of cell structures. Disease-modifying treatment decreases whole brain volume (WBV) in multiple sclerosis patients more rapidly than placebo in the first six months of treatment due to the shrinking effect on inflammation. **Objective:** To investigate whether gray matter volume (GMV) is less susceptible to pseudoatrophy effect than WBV or white matter volume (WMV). To explore if baseline T2-lesion volume (T2-LV) or the T2-LV change in the first six months of treatment is responsible for pseudoatrophy effect. To predict long-term atrophy and disability rates by estimating pseudoatrophy volume (PV) in the first six months of treatment. **Methods:** In the Avonex-Steroids-Azathioprine combination study, 181 patients were randomized 1:1:1 to receive mono or combination therapy over two and five years. The extension phase of the study reached eight years. Bi-monthly magnetic resonance imaging scans were acquired in the first and second years and annually thereafter. PV was estimated at two, four, and six months of the study from year

two percent brain volume change (PBVC), GMV, WMV and WBV changes by assuming linear rates over two years and applying those rates to baseline. Differences between estimated rates and the corresponding observed rates were defined as PV. **Results:** GMV change and PBVC were less susceptible to PV in 0–6 months in all three treatment arms. Decrease of T2-LV in the first six months reduced more WMV and WBV. WM PV in 0–6 months was predictive of WMV and Expanded Disability Status Scale changes after 0–3, 0–4, and 0–5 years, but not for GMV or PBVC. **Conclusions:** Measurement of GMV is less susceptible to pseudoatrophy effect than that of WMV, WBV or PBVC. WM PV is predictive of long-term atrophy and disability development. This suggests that pseudoatrophy does not reflect a beneficial process.

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Gadolinium enhancing lesions decrease over time in early primary progressive multiple sclerosis

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Background: Fewer gadolinium-enhancing lesions are seen on magnetic resonance imaging in primary progressive multiple sclerosis (PPMS) when compared with other MS subtypes, though this is less marked in early PPMS, where there appears to be more enhancement. **Objective:** To investigate if (1) baseline enhancement levels were maintained over five years in early PPMS (2) the number of enhancing lesions at baseline, or the change in their number over 6 and 12 months, affected clinical progression. **Methods:** Forty-five patients with PPMS (28 male, mean age 44.2 years, median Expanded Disability Status Scale (EDSS) 4.5) within five years of symptom onset were scored on the EDSS and multiple sclerosis functional composite subtests, including the time walked test (TWT), and scanned six monthly for three years, and at five years. We acquired T1-weighted brain and cord images before and after triple dose gadolinium (0.3mmol/kg), and T2-weighted brain images at each time-point. We modeled change in the proportion of patients with enhancement using a mixed-effect logistic model including a quadratic term, and predictors of clinical outcome using multiple linear and ordinal logistic regression, adjusted for age and brain T2 lesion load. **Results:** Forty per cent of patients had at least one enhancing lesion in the brain or spine at the start of the study, decreasing to a plateau of 10–15% by five years ($p=0.006$). Median EDSS increased throughout the study. EDSS deterioration over three years was predicted by the change in the number of enhancing brain lesions over the first six months ($p=0.002$). However deterioration in TWT performance at five years was predicted by more enhancing brain lesions at baseline ($p=0.015$), and the change in the number of enhancing lesions over the first six months and one year ($p<0.05$). **Conclusions:** Enhancement levels in early PPMS decrease over time, suggesting an inflammatory phase early in the disease course, and have some impact on subsequent disability.

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Spinal cord atrophy in early and established primary progressive multiple sclerosis: a two-year follow-up

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Background: Spinal cord atrophy has been described in both early and more established primary progressive multiple sclerosis (PPMS). **Objective:** To compare the evolution of spinal cord damage between early and established PPMS and examine its clinical relevance, we studied cervical cord cross-sectional area and volume over two years in two groups of patients, and in controls. **Methods:** Twenty-nine patients with early PPMS (15 male, mean age 47.3yrs, mean disease duration 3.4yrs), 19 with established PPMS (12 male, mean age

51.8yrs, mean disease duration 9.6yrs), and 13 controls (6 males, mean age 37.1yrs) underwent brain and spinal cord 3D-IR-FSPGR imaging at baseline and two years, and patients were scored on the expanded disability status scale and MSFC and its subtests including the timed walk test (TWT). Total intracranial volume (TIV) was calculated using SPM2. The cross-sectional upper cervical cord area (UCA) was calculated as described by Losseff, whilst the total cervical cord volume (TCV) measured from C1 to C7, and the lower cervical cord volume (LCV) from C4 to T1 were obtained as described by Coulon. Multiple linear regression models adjusted for age, gender and TIV investigated differences in atrophy development between each group. **Results:** UCA in both patient groups decreased significantly (by 3.12mm²/year in early PPMS, $p < 0.0001$, 95%CI -4.79,-1.46; by 2.52mm²/year in established PPMS, $p = 0.015$, 95%CI -4.54,-0.50), as did LCV (by 132mm³/year in early PPMS, $p = 0.007$, 95%CI -225.8,-38.25; by 197.45mm³/year in established PPMS, $p = 0.001$, 95%CI -311.27,-83.62) compared with controls. The decline in TCV was significant only in early PPMS compared with controls (by 155.08mm³/year, $p = 0.003$, 95%CI -253.88,-56.28). There were no differences in the reduction rate in any of the atrophy measures between patient groups. Decrease in LCV in all patients together correlated with increased time taken for the TWT ($p < 0.01$). **Conclusions:** In this study spinal cord atrophy occurred in early and established PPMS at a similar rate, and correlated with declining mobility. **Supported by:** MS Society of Great Britain and Northern Ireland.

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Magnetization transfer ratio in multiple sclerosis: axonal loss or demyelination?

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Background: While magnetisation transfer ratio (MTR) is claimed to be an important in vivo indicator of multiple sclerosis (MS) pathophysiological processes, its true nature is still obscure. While there is evidence that it correlates with both axonal density and demyelination, the extent of this correlation has not been defined. The optic nerve represents a good model to study MS due to the fact that it subserves aspects of vision which are easily identifiable and measurable. **Objective:** In this pilot study we studied the result of MTR in comparison with electrophysiological markers of neuronal injury after an episode of optic neuritis (ON). Latency of a newly developed multifocal visual evoked potential (mfVEP) was used as a marker of demyelination, while amplitude indicated axonal loss. **Methods:** Patients with single unilateral episode of ON were enrolled. Based on result of the mfVEP testing patients were divided into two groups: patients with no axonal loss but extensive demyelination (normal amplitude, but significantly delayed latency) and patients with axonal loss, but no demyelination in the remaining fibers (amplitude reduction, but no latency delay). Orbital magnetic resonance imaging was performed on the Phillips 3T machine. Asymmetry between MTR of affected and fellow eyes was analysed. **Results:** There was consistent asymmetry in MTR between affected and fellow eyes in all patients with axonal loss, with the former eye demonstrating considerably smaller values compare with the latter (average MTR asymmetry=0.05+/-0.008). Patients with demyelination (latency delay) but without axonal loss (fully recovered amplitude), on the other hand, did not demonstrate significant MTR asymmetry (average MTR asymmetry=0.001+/-0.019). **Conclusions:** Results of our pilot study suggest a possibility that axonal loss has strong relationship with MTR. Latency delay in cases where MTR was normal, on the other hand, may indicate that demyelination is not always reflected by MTR.

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Optic neuritis as a sentinel lesion to study neuroprotection and repair in a trial of autologous mesenchymal stem cells in multiple sclerosis

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Background: Optic neuritis (ON) is common in multiple sclerosis (MS). Assessment of the afferent visual pathway provides insights in to the pathophysiology of the demyelinating lesion, and has potential for investigating new therapies in ON and MS. **Objective:** Summarise the clinical, electrophysiological and imaging methods for assessing optic nerve structure and function and their potential role in testing neuroprotective and repair therapies in MS. **Methods:** Outline the design and assessment methods in an exploratory trial of autologous mesenchymal stem cells (MSC) in MS. **Results:** A trial of MSC in MS (MSCIMS) is underway. MSCIMS is a phase IIA pre (6 months) vs. post (12 months) single intervention comparison trial, using ON as a sentinel lesion in 20 patients with MS and previous ON to give insights into the potential efficacy of MSC. Outcome measures include: (i) Quantitative visual function assessment including low contrast acuity, colour vision using Farnsworth-Munsell 100 Hue test, Humphrey's visual perimetry; (ii) Optical coherence tomography to measure the thickness of the retinal nerve fiber layer and thus quantify axonal loss; (iii) Quantitative intrinsic magnetic resonance imaging (MRI) measures in the optic nerve including magnetisation transfer ratio (a marker of demyelination and remyelination) and diffusion tensor imaging (a marker of axonal integrity); (iv) functional MRI with visual stimulation to measure the contribution of cortical adaptation to functional improvement; (v) Visual evoked potentials to assess myelination and conduction block. **Conclusions:** Availability of sensitive imaging and functional assessment techniques makes ON an attractive model of a demyelinating lesion in vivo for evaluating experimental neuroprotective and repair therapies in MS. The utility of such techniques will be further clarified in the MSCIMS study.

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Diffusion tensor imaging detects clinical and structural alterations in optic neuritis

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Background: The magnetic resonance imaging (MRI) paradox in multiple sclerosis describes the poor correlation of clinical measures with conventional MRI. Diffusion tensor imaging (DTI) quantifies the microscopic diffusion of water. White matter tracts are directional, or anisotropic. Diffusion will preferentially occur parallel to axons (axial diffusivity), because myelin restricts diffusion in the perpendicular direction (radial diffusivity). Our group has correlated these directional diffusivities with histopathology in animal models. **Objective:** Correlate directional diffusivities in optic nerves to clinically relevant ophthalmologic tests. **Methods:** Twenty-eight subjects with a remote history of optic neuritis at least one year prior underwent high-resolution MRI. The region of interest included the nerve center along a 20mm longitudinal segment. Clinical measures included Snellen 20 ft visual acuity (VA), 5% Sloan contrast sensitivity (CS), optical coherence tomography (OCT), and visual evoked potentials (VEP). Twelve healthy controls were imaged for comparison. Spearman coefficients determined correlations between DTI and clinical measures. **Results:** Normal subjects had axial diffusivity $1.66 \pm 0.18 \mu\text{m}^2/\text{ms}$, radial diffusivity $0.81 \pm 0.26 \mu\text{m}^2/\text{ms}$, apparent diffusion coefficient (ADC) $1.09 \pm 0.21 \mu\text{m}^2/\text{ms}$, and fractional anisotropy (FA) 0.43 ± 0.15 . For nerves affected by optic neuritis ($n=38$), axial diffusivity (2.06 ± 0.19), radial diffusivity (1.30 ± 0.31) and ADC (1.57 ± 0.22) were significantly increased, and FA (0.31 ± 0.07) was significantly decreased. Clinical parameters were significantly correlated with all diffusion parameters. Strong correlations with radial diffusivity were observed: $r=0.68$ for CS; $r=-0.56$ for VA;

$r=-0.81$ for OCT; and $r=0.54$ for VEP. **Conclusions:** DTI provides important information about clinical function, structure, and physiology in remote optic neuritis. This information is not provided by conventional MRI. In remote injury, radial diffusivity increases, along with an increase in total diffusivity and a decrease in anisotropy. Future studies should determine the utility of DTI in clinical practice, and as a surrogate endpoint in trials.

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Short term validity of Barkhof and Swanton magnetic resonance imaging criteria for multiple sclerosis in patients with clinically isolated syndromes

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Background: Magnetic resonance imaging (MRI) evidence for dissemination in space (DIS) and dissemination in time (DIT) is used within the McDonald criteria for the diagnosis of multiple sclerosis. In order to make an earlier diagnosis of clinically definite multiple sclerosis (CDMS) in patients with clinically isolated syndromes (CIS), new criteria were proposed in which DIS requires at least one T2 lesion in at least two of four locations (juxtacortical, periventricular, infratentorial and spinal cord), and DIT requires a new T2 lesion on a follow-up MRI. **Objective:** To compare Barkhof criteria and the new proposed MRI criteria (Swanton) by use of conversion to CDMS. **Methods:** The former and latter MRI criteria were applied in a cohort of 49 patients with CIS, both at baseline and at least three months follow up MRI. The specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of both MRI criteria for the presence of a second relapse after one year of follow-up was performed. **Results:** The sensitivity of the new criteria was 86.66% (95% CI 59 - 98%), and of Barkhof criteria 80% (95% CI 51 - 95%). The specificity of the new criteria was 44.11% (95% CI 27 - 62%), meanwhile Barkhof criteria was 55.88 (95% CI 29 - 67%). The PPV was almost the same for both criteria (40.62% for the new criteria and 44.44% for Barkhof criteria). The NPV was slightly higher in the new criteria (88.23% vs 86.36%). The accuracy of the new criteria was 57.4% (95% CI 43.3 - 71%), and of Barkhof criteria 63.26% (95% CI 49.76 - 76.75%). **Conclusions:** The new MRI criteria for DIS and DIT are simpler than the former ones, and they are at least as reliable as Barkhof criteria. There are no significant differences between criteria in sensitivity and specificity, after one year of follow-up.

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Functional sensorimotor connectivity is preserved in patients with pediatric multiple sclerosis

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Background: Brain plasticity has been considered among the factors contributing to limit the clinical manifestations of multiple sclerosis (MS). **Objective:** To investigate changes of measures of functional connectivity within the motor network in patients with pediatric MS in comparison with those with the adult-form of the disease, and to assess the correlation between connectivity changes and structural damage within the corpus callosum (CC) and the corticospinal tract (CST). **Methods:** Diffusion tensor magnetic resonance imaging (MRI) and functional MRI scans during right hand movement were acquired in 17 pediatric MS patients, 16 adult patients with clinically isolated syndromes (CIS) suggestive of MS, 14 adult patients with relapsing-

remitting MS (RRMS), and 10 healthy controls. Whole brain, CC and CST T2 lesion load, as well as diffusivity metrics within the CC and the CST were measured. **Results:** Coefficients of connectivity of the sensorimotor network were similar between controls and pediatric MS patients, while in adult patients with CIS and in those with RRMS there was a progressive increase of functional connectivity between the left and the right primary sensorimotor cortex (SMC) and vice versa, the right cerebellum and the left SMC, the right cerebellum and the right SMC, the left secondary sensorimotor cortex and the left SMC, and the supplementary motor area and the left SMC. All these changes were more pronounced in patients with RRMS. The increase of coefficients of connectivity was correlated with disease duration and regional damage inside the CC and the CST. **Conclusions:** The preservation of brain adaptive properties might contribute to explain the more favorable clinical outcome of pediatric MS patients. The progressive recruitment of cortical networks over time in patients with the adult forms of the disease might result in a more precocious exhaustion of the their plastic reservoir, thus contributing to the clinical progression of the disease.

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The corpus callosum is a primary site of anisotropy changes in patients with optic neuritis

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Background: Diffusion tensor imaging (DTI) can detect microstructural brain damage in multiple sclerosis (MS) patients beyond the resolution of highly sensitive conventional magnetic resonance imaging (MRI) techniques such as fluid-attenuated inversion recovery. DTI quantifies the amount of non-random water diffusion and provides unique in vivo information about pathological processes even in the so-called normal appearing white matter (NAWM) of MS brain tissue. In clinically definite MS, occult injury has preferentially been detected in the corpus callosum. **Objective:** In this prospective study we aimed to identify structural changes in patients with optic neuritis (ON) as a clinically isolated syndrome. **Methods:** 24 patients and 15 control subjects were prospectively followed by clinical and MRI examinations. MRI was performed at baseline (MRI1), after 6 months (MRI2) and after one year (MRI3) and three years (MRI4), respectively. Apparent diffusion coefficient (ADC) and fractional anisotropy (FA) maps were derived from DTI. Four distinct regions of interest were defined in NAWM. **Results:** FA values decreased in the genu of the corpus callosum from MRI1 to MRI4 ($p=0.005$) and in the splenium of the callosal body (SCC) ($p=0.006$). Compared with controls, patients with ON already had lower FA values in the SCC at baseline ($p<0.01$). In patients developing definite MS during follow-up ($n=9$), we found a correlation of FA in the SCC with time ($R=-0.40$, $p=0.004$) that was not present in patients without progression to definite MS. **Conclusions:** Our data defines the corpus callosum as an early site of anisotropy changes in patients with ON. Whereas at baseline, lower FA values were found in all patients, only in those patients that developed definite MS did the FA changes further deteriorate.

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Infratentorial lesions are a marker for poor prognosis in multiple sclerosis

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Background: The number of baseline lesions predicts future attacks and disability in clinically isolated syndromes (CIS). The presence of two or more infratentorial lesions has been related to disability in a retrospective study with 42 CIS patients. **Objective:** To investigate the

role of baseline infratentorial lesions in long-term prognosis. **Methods:** From 1995 to 2001, subjects were included in a prospective cohort of patients with CIS. Patients underwent brain magnetic resonance imaging (MRI) within three months of their first attack and again 12 months and five years later. We prospectively studied the number and location of lesions at baseline and new lesions at follow-up. Retrospective scan analysis was conducted to specifically look at number and location of infratentorial lesions. We analyzed the time to a second attack and to reach Expanded Disability Status Scale (EDSS) score 3.0. **Results:** We finally included 246 patients with CIS followed for a median of 7.7 years (IQR: 76–114 months). As expected, the baseline number of lesions was related both to time to conversion and time to EDSS 3.0. Infratentorial lesions were present in 31% of the patients (10.5% had only brainstem lesions; 8% had only cerebellar lesions, and 12.5% had simultaneous lesions). Patients with infratentorial lesions had a higher risk of conversion (71% vs 30%; $p < 0.001$) and a higher risk of developing disability (32% vs 12%; $p = 0.0011$). Compared with the reference group with non-infratentorial lesions at baseline MRI, patients with only cerebellar lesions (but no brainstem lesions) showed a 3% increased risk of reaching EDSS 3.0 (n.s.) and patients with only brainstem lesion (but no cerebellar lesions) had a 22% increased risk of reaching EDSS 3.0 ($p = 0.014$). Simultaneous lesions in brainstem and cerebellum increased the risk by 30% ($p = 0.0003$). **Conclusions:** The presence of infratentorial lesions determines the risk for disability. Brainstem lesions, more than cerebellar lesions, are responsible for poor prognosis.

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Pediatric patients have a higher disease burden at time of multiple sclerosis onset compared with adults

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Background: Although pediatric multiple sclerosis (MS) is believed to have a more benign course than adult MS, this may not be true in an ethnically mixed population. **Objective:** To compare initial brain magnetic resonance imaging (MRI) characteristics of children and adults with MS. **Methods:** We evaluated initial brain MRI scans of pediatric (\leq age 18) and adult (>18) MS patients for lesions that were T2-bright, ovoid and well-defined, large (>1 cm), or enhancing. **Results:** We identified 31 pediatric-onset and 31 adult-onset MS patients; 42% of children vs 87% of adults were white. Median time to first brain MRI was shorter in children (8 versus 129 days, $p < 0.0001$). Children had a higher number of total T2- (median 21 vs 6, $p < 0.0001$) and large T2-bright areas (median 5 vs 1, $p < 0.0001$) than adults. Children more frequently had T2-bright foci in the posterior fossa (74% vs 48%, $p = 0.037$) and enhancing lesions (70% vs 25%, $p = 0.0008$) than adults. **Conclusions:** Children have a higher disease burden with more frequent posterior fossa involvement at MS onset than adults. While it is unknown if these differences are explained by age or ethnicity, these characteristics have been associated with worse disability in adults.

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Neuroimmunology

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Parameters of the immune system activation in patients with clinical diagnosis of neuromyelitis optica

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Background: Neuromyelitis optica (NMO) is an inflammatory, demyelinating disease of the central nervous system, characterized by

the occurrence of severe transverse myelitis and bilateral or sequential optic neuritis, initially considered monophasic (Devic, 1894), but recognized as a relapsing disease (Mandler, 1993). NMO patients usually have worse prognosis, more deficits after each bout and early incremental disability due to frequent and severe relapses (Wingerchuk et al, 1999) which distinguish them from those with multiple sclerosis (MS). Lennon et al (2004) detected a specific marker for NMO, a serum autoantibody, neuromyelitis optica immunoglobulin G (NMO-IgG), which distinguishes NMO from other demyelinating diseases. Neuropathology findings showed perivascular deposition of immunoglobulin, activation of the complement cascade and eosinophils in the inflammatory infiltrates of active lesions of NMO (Lucchinetti et al, 2002; Correale et al, 2004). Therefore, humoral markers have a role in NMO pathogenesis. **Objective:** In this study we analyzed the immunological profile of NMO patients from the Idiopathic Inflammatory Demyelinating Diseases department of Clementino Fraga Filho University Hospital and Santa Casa da Misericórdia do Rio de Janeiro General Hospital, considering IgG and IgA to interleukin-4 (IL-4) and interferon-gamma (INF-gamma). **Methods:** The plasma levels of cytokines interleukin-4 (IL-4) and interferon-gamma were assessed by enzyme-linked immunosorbent assay. The cohort consisted of twenty-eight NMO patients; twenty-one females and seven males, with age range 25 to 62 years. A control group was composed of twenty-six pair-matched patients. **Results:** NMO patients had no difference in levels of cytokine interferon-gamma ($p = 0.61$) but IL-4 ($P = 0.0084$) production, associated with activation of regulatory T cells, was significantly higher among these patients. **Conclusions:** Our results indicate that activation of humoral immunity has an important role in the physiopathology of NMO neuroinflammation.

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Serological tests for neuromyelitis optica: specificity of immunoprecipitation assay for AQP4 autoantibody enhanced by eliminating GFP-Reactive IgG

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Background: The diagnostic sensitivity and specificity of the immunofluorescence assay for AQP4-specific NMO-IgG autoantibody has been confirmed internationally for neuromyelitis optica (NMO) spectrum disorders. Several antigen-specific immunoprecipitation assays have been described for detecting AQP4-IgG. In optimizing our originally described green fluorescent protein (GFP)-tagged AQP4 immunoprecipitation assay for high throughput clinical testing, we encountered cases of false positivity wherein patients' IgG bound to free GFP in the absence of AQP4. **Objective:** To determine, in a clinical setting, the rate of false positive AQP4 immunoprecipitation in assays employing GFP-tagged AQP4. **Methods:** We evaluated sera from two patient groups for whom NMO-IgG immunofluorescence was requested on a service basis (October 1, 2007 - March 31, 2008): Group 1, immunofluorescence positive (n=557); Group 2, immunofluorescence negative (n=4943). Sera yielding positive immunoprecipitation results were re-assayed with GFP-AQP4 and with GFP alone. Values for GFP alone were subtracted to yield final results (normal value ≤ 10 nmol AQP4 bound/L serum). **Results:** Group 1: 331 patients had initial and final AQP4-IgG values >10 nmol/L (59%); none precipitated GFP alone. Clinical information was available for 16%; all had an NMO spectrum disorder. Group 2: 80 patients had initial AQP4-IgG values > 10 nmol/L (1.6%); 76 remained positive after subtracting GFP values (median 32.6; range 10.2 - 867 nmol/L). The four patients who were seronegative after subtracting GFP values had diagnoses of neuroretinitis, multiple sclerosis, neurosarcoidosis and paraneoplastic optic neuropathy. Of the remaining seropositive patients for whom clinical information was available, one had monophasic optic neuritis and the rest had an NMO spectrum disorder. **Conclusions:** False-positive results for AQP4-IgG, attributable to immunoprecipitation of GFP, were encountered in 4 of 80 patients (5%). Assay specificity assurance requires re-assay of apparently positive samples with both GFP-AQP4 and GFP alone.

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Antiaquaporin-4 antibodies and infectious myelitis

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Background: Testing for aquaporin-4 IgG antibodies (NMO-IgG) is highly recommended in the presence of centromedullary multi-segmental lesions at magnetic resonance imaging (MRI), a pattern considered to be highly suggestive of neuromyelitis optica (NMO). However, infectious and post-infectious disorders may display a similar pattern and it may be difficult to distinguish between them. **Objective:** To report the association between infectious myelitis, centromedullary multisegmental lesions and NMO-IgG. **Methods:** The cases of two patients exhibiting centromedullary multisegmental lesions at MRI who presented with infectious myelitis and NMO-IgG are presented. **Results:** Case 1: A 59-year-old man with lower limbs paraparesis, sensory level at D10, and urinary and fecal incontinence. MRI showed multisegmental high-signal intensities in the cervical and thoracic spinal cord segments and a cystic lesion on T2-weighted images. Cerebrospinal fluid and serological tests were positive for *Paracoccidioides brasiliensis*. Serum NMO-IgG examined at the Mayo Clinic was positive. Sulphadiazine and dexametasonone therapy was started. After 30 days there was no significant improvement. He was discharged wheel-chair bound, with urinary and fecal incontinence. Case 2: A 58-year-old woman was admitted with spastic paraparesis, left ptosis and sensory level at D2. MRI revealed high-intensity gadolinium-enhanced signal in the central portion over the entire length of the spinal cord on T2-weighted images. Laboratory examination revealed elevated anti-HTLV-I antibody titer and viral load. She was treated with 1000 mg methyl-prednisolone for three days, followed by monthly therapy for six months. She improved gradually and stabilization occurred after six months (expanded disability status scale was 6.5). Serum NMO-IgG examined at the Mayo clinic was positive. The myelopathy had worsened at the last follow-up visit, despite the viral load reduction. **Conclusions:** Although NMO-IgG has been primarily examined in autoimmune neurological disorders, it has not been routinely evaluated in infectious or post-infectious myelopathies. Our findings indicate the need for further studies on the association between NMO-IgG and infectious myelitis. **Supported by:** FAEPA.

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Glatiramer acetate reduces B cell survival factors in the central nervous system of experimental autoimmune encephalomyelitis induced mice

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Background: Renewed interest in the role of B cells in the pathogenesis of multiple sclerosis (MS) has recently been generated following the successful clinical trials in which B cells have been targeted and depleted. In addition to their precursor role for antibody production, B cells are important in development, regulation and antigen presentation. B cell activating factors of the tumor necrosis factor superfamily members BAFF and APRIL are the important molecules for several immune functions, including B cell survival. The specific function of these cytokines in MS has not been fully evaluated. **Objective:** To evaluate the effect of glatiramer acetate (GA) on the induction of B cells with regulatory properties in Experimental Autoimmune Encephalomyelitis (EAE) mice. **Methods:** Spleen, lymph node and brain tissues were obtained from EAE-induced and GA-treated mice, and then assayed for expression of BAFF and APRIL, IL-6, IL-17, IL-1b, TNF- α , and transcription factors for Th1 and Th2 cytokines. Using flow cytometry, we evaluated the expression of two BAFF-binding receptors, namely, BAFF-receptor and TACI in spleens and lymph nodes, and the expression of BAFF and APRIL in brain tissues by reverse transcriptase-polymerase chain reaction. Expression of specific molecules was evaluated by immunocytochemistry and

multiplex. **Results:** The expression profiles of B cells remain unchanged between GA-treated and untreated groups. The percentages of B cells expressing BAFF-R and TACI did not differ between EAE-induced and GA-treated mice. The mRNA for BAFF and APRIL was significantly reduced in brain tissues of GA-treated mice compared with EAE-induced mice. The average IgD+ B cell count per mouse spleen or cervical lymph node increases after GA treatment. Functional, phenotypic and cytokine analysis of B cells following GA treatment is currently being investigated. **Conclusions:** Our observations demonstrate that GA has a significant downregulatory effect on BAFF and APRIL that may be responsible for mediating an anti-inflammatory effect in EAE-induced mice. BAFF induction by target organs of autoimmune diseases may be a link between innate immunity and autoimmunity.

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Glatiramer acetate regulates retinoic acid enzymes and Th17 cells in the central nervous system of experimental autoimmune encephalomyelitis induced mice

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Background: Retinoic acid (RA) is a metabolic product of vitamin A (retinol) that induces the differentiation of various types of neurons and glia by activating transcription factors, cell signaling molecules and cell-surface receptors. Recent studies have demonstrated that all-trans RA in the presence of TGF β and IL-2 can induce differentiation of CD4+ effectors to FoxP3+ adaptive Treg cells that acquire a4b7 integrin+ and chemokine receptor 9 phenotype, conferring lamina propria homing. The function of RA in multiple sclerosis (MS) is presently unknown. **Objective:** To investigate the effect of glatiramer acetate (GA) in regulation of RA enzymes and Th17 cells in the central nervous system (CNS) of experimental autoimmune encephalomyelitis (EAE) induced mice. **Methods:** Tissue samples from spleens, lymph nodes and brains were examined for expression of RA synthesizing enzymes, cytokines IL-6, IL-17, Foxp3 and transcription factors for Th1 and Th2 cytokines and compared with untreated diseased mice. Detection of RA levels in tissues was performed by fluorescence-activated cell sorting using the F9 reporter cell line, and expression of RA-synthesizing enzymes was determined by reverse transcriptase-polymerase chain reaction. **Results:** A significant increase in RA-synthesizing enzymes (RALDH 1, 2,3) was observed within CNS and spleen of EAE mice following treatment with GA (p<0.001). RA levels in tissues from GA-treated mice were enhanced when compared to naive or EAE-induced mice. An increase in FoxP3 expression and decrease IL-6 and IL-17 expression (p<0.001) was observed following treatment with GA. Gene expression of IL-6, IL-17 and ROR γ t was profoundly reduced by GA within the CNS. **Conclusions:** Our observations demonstrate that treatment with GA can upregulate both the synthesis of RA enzymes as well as retinoid secretion in EAE mice. This increase in RA coincides with a profound increase in CNS-derived Foxp3 Tregs in response to GA treatment. Conversely, GA can downregulate IL-17 and IL-6 expression within the CNS, which may be responsible for mediating the anti-inflammatory effect in EAE-induced mice. Ongoing studies focused on the blockade or deficiency of RA on CD4+Foxp3 expression in this EAE model will be reported.

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The lymphocyte subpopulation in patients with primary progressive multiple sclerosis and secondary progressive multiple sclerosis

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Background: Multiple sclerosis (MS) is a chronic, inflammatory, autoimmune, demyelinating disease of central nervous system. The pathological process underlying MS involves dysregulation of the immune system and it is a predominantly T cell-mediated immune disorder. **Objective:** The aim of our study was the evaluation of select T-cells subpopulations CD4, CD8, CD25, CD4+45RA+, CD4+45RO+ and NK cells in patients with primary progressive multiple sclerosis (PPMS) and secondary progressive multiple sclerosis (SPMS) **Methods:** We investigated 22 patients (4 men and 18 women with mean age 42, 9±6, 6) with SPMS, 10 patients (6 men and 4 women in mean age 42, 9±8, 8) with PPMS, and 20 healthy control patients (7 men and 13 women). Peripheral blood samples were collected and expression of CD4, CD8, CD25, CD4+45RA+, CD4+45RO+ and NK cells were evaluated by the flow cytometry method. **Results:** Compared with the controls, the PPMS patients showed statistically significant lower expression of CD8 (28, 9±8, 0% vs 20, 9±7, 3%), CD25 (5.3±2, 7% vs 3, 1±2, 4%) and NK cells (22.3±8, 4% vs 10, 1±3, 8%), and they had statistically significant higher expression of CD4 (42, 1±9, 2 vs 51, 9±13, 7) and CD4+45RO+ (1, 5±2, 6 vs 24, 7±10, 7). Compared with the controls the SPMS patients showed statistically significant lower expression of CD8 (28, 9±8, 0% vs 22, 2±4, 4%), and NK cells (22.3±8, 4% vs 5, 6±4, 5%), and they had statistically significant higher expression of C4 cells (42, 1±9, 2% vs 50, 5±6, 7%) and CD4+45RO+ (1, 5±2, 6 vs 24, 9±9, 5). There were no statistically significant differences in CD4, CD8, CD25, CD4+45RA+, CD4+45RO+ and NK cells expression between SPMS and PPMS patients. **Conclusions:** Patients with PPMS and SPMS show disturbances in expression of CD4, CD8, CD25, CD4+45RO+ and NK cells, but there were no statistically significant differences in CD4, CD8, CD25, CD4+45RA+, CD4+45RO+ and NK cells expression between these two groups.

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Clonally-expanded plasma cells in neuromyelitis optica cerebrospinal fluid produce IgG against aquaporin-4

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Background: Neuromyelitis optica immunoglobulin G (NMO-IgG) binds the aquaporin-4 (AQP-4) water channel of astrocytes that are abundant in the perivessel and subpial areas of the brain. NMO-IgG appears specific for NMO and is now included as a diagnostic criterion for disease. The source of central nervous system NMO-IgG and its role in disease pathogenesis are uncertain. **Objective:** To characterize the germline distribution and antigenic specificity of the intrathecal plasma cell response in early NMO. **Methods:** Heavy- (VH) and light-chain (VL) variable regions from single-antibody-secreting CD138+ plasma cells sorted from the cerebrospinal fluid (CSF) of an NMO-IgG seropositive patient with recurrent optic neuritis were analyzed by single-cell reverse-transcriptase polymerase chain reaction. Human recombinant antibodies (rAbs) were produced from the paired VH and VL sequences of clonally-expanded and non-clonal CD138+ cells and assayed for immunoreactivity against AQP-4. **Results:** The CSF of the early NMO patient demonstrated a dynamic, clonally-expanded plasma cell population with features of an antigen-driven response. In distinction to the strong VH4 germline bias in multiple sclerosis (MS) CSF repertoires, VH2 family germline sequences dominated the NMO CSF CD138+ repertoire. Patient sera, CSF and two of

three rAbs generated from expanded CSF plasma cell clones bound to an AQP-4-transfected human glial cell line. In addition, one rAb also bound to AQP-4 on mouse cerebellar sections and human fetal astrocytes. RAbs recognized conformational epitopes on the AQP-4 protein. **Conclusions:** The CSF plasma cell repertoire in early NMO demonstrates features of an antigen-directed humoral immune response. VH family germline usage is distinct from that in MS, and CSF plasma cell clones produce IgG targeted against AQP-4. Characterization of the CSF plasma cell repertoire in early NMO strongly suggests that NMO and MS are distinct and that an immune response against AQP-4 plays a role in NMO pathogenesis.

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Envelope protein from human endogenous retrovirus W serum antigenemia in multiple sclerosis patients and controls: a multicenter and blind-tested study on large series of multiple sclerosis patients and controls

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Background: Eight per cent of the human genome consists of human endogenous retrovirus (HERV), disseminated within the genomes of many species over millions of years, but relatively little is known about the function and pathological potential of these retrotransposable elements within the human genome. A novel virus, named multiple sclerosis associated retrovirus (MSRV), belonging to the HERV-W family was identified. Several studies provide evidence for a significant pathogenic contribution of MSRV and its HERV-W genetic family to MS pathogeny. For example, the ENV recombinant protein was found to display pro-inflammatory activities both in vitro in human mononuclear cell cultures and in vivo in a humanized severe combined immuno-deficient mouse model. MSRV virions were detected in MS patients' cerebrospinal fluid (CSF) and plasma, with significant differences with controls. Moreover the retroviral load in CSF at disease onset was found to predict MS evolution. The recent emergence of confirmatory epidemiological and experimental data from independent groups with various techniques provides a solid base for future scientific progress. **Objective:** Complementary tools were required for immunodosage and further studies in patients. **Methods:** We have developed an enzyme-linked immunosorbent assay for the detection of HERV-W antigens in human blood. **Results:** An ongoing multicenter study on HERV-W envelope antigenemia has already evidenced significant antigenemia in MS patients. Standardisation of the test is now being pursued in order to evaluate the antigen prevalence in different populations. We now think this protein is linking the virion particle and the nucleic acid detection in MS patients with the immunopathological properties of this human endogenous retroviral family (HERV-W). **Conclusions:** The technical development of the test presented here could be directed towards therapeutic monitoring and clinical prognosis, and may be useful in determining the suitability of patients for specific therapeutic approaches.

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AQP4 polymorphism in neuromyelitis optica

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Background: Neuromyelitis optica (NMO) is characterized by extensive myelitis, optic nerve neuritis and, for some patients, anti-AQP4 antibodies. There is no data regarding the AQP4 polymorphism in NMO. **Objective:** In order to assess the role of AQP4 in Devic

syndrome, a population of 43 patients with Devic syndrome, controls and multiple sclerosis (MS) populations were screened for AQP4 mutations. **Methods:** The five exons and the flanking splice sites were amplified from genomic DNA and sequenced on an ABI PRISM 3100 Genetic analyser (Applied Biosystems). The frequency of each variant in patients with Devic syndrome was compared to that in controls and MS populations, and further included AQP4 antibody status, using the conditional form of the 2-tailed Fisher exact test, assuming independent inheritance of each variant. **Results:** No mutations were found. This indicates that mutations of AQP4 are not a common cause of Devic syndrome. We identified only single nucleotide polymorphisms and sequence variants without pathogenic significance in our population of patients with Devic syndrome. No statistical differences were found between the patients with Devic syndrome and the control group. On the other hand, one variant was significantly decreased in the MS population (SNP rs35248760, $P = .048$) compared with patients with Devic syndrome, with an increased significance by performing statistical analysis between MS patients and AQP4 antibody-positive patients with Devic syndrome (rs35248760, $P = .035$). **Conclusions:** There appear to be different polymorphisms in MS and NMO. Future collaborative studies may include an association study in a larger population of patients with Devic syndrome, controls and MS populations to confirm and determine whether specific genetic variation in AQP4 increases susceptibility or modifies disease in Devic syndrome.

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IgM oligoclonal bands predict an earlier second relapse in patients with clinically isolated syndromes

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Background: IgM oligoclonal bands predict an aggressive course in multiple sclerosis (MS). **Objective:** To assess the ability of IgM oligoclonal bands (OCB), IgG-OCB and the Barkhof magnetic resonance imaging (MRI) criteria to predict an earlier second relapse in patients presenting with a clinically isolated syndrome (CIS). **Methods:** Sixty-one patients with CIS, 72% females, median age 28 years, median evolution time 14 months, were included in the study. Basal MRI was performed within the first month, and the second and three months later. The presence of Barkhof criteria (BC) and the conversion to MS according to the McDonald criteria were analyzed. Paired cerebrospinal fluid and serum samples were tested for the presence of IgG and IgM OCB within the first three months. Variables included in the analysis were the presence or absence of IgG-OCB and IgM-OCB; and the number of BC fulfilled (0–2 BC, 3–4 BC) in the first MRI. The time to the second relapse was used as outcome for the survival analysis. **Results:** 42.3% of patients converted to MS according to the McDonald criteria and 26.2% patients presented a second relapse. IgG and IgM OCBs were absent in 38.5% patients; in 61.5% IgG-OCBs were present, and in 33.3% IgM-OCBs were present. 33.9% accomplished 3–4 BC, and 66% accomplished 0–2 BC. The Kaplan-Meier survival analysis showed that the only variable significantly associated with an earlier second relapse was IgM-OCB (17 vs. 45 months, $p=0.003$). Moreover, the multivariate Cox regression analysis corrected for age and sex only selected the presence of IgM-OCB as a predictor variable (Hazard Ratio for presenting a second relapse 3.57, 95% CI 1.10–11.49). **Conclusions:** The presence of IgM-OCB in cases with CIS is associated with an earlier time to a second relapse. These results support the previously reported higher disease activity in relapsing-remitting MS patients with IgM-OCB.

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Screening for aquaporin-4 antibodies in French-Canadian patients with unusual myelitis, optic neuritis, or both

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Background: Refinement of diagnostic criteria for Devic's neuromyelitis optica (NMO) was initiated in 1999 by Wingerchuk et al. A serum autoantibody marker for NMO was identified by the same group in 2004, and the NMO-IgG binding to the aquaporin-4 water channel test was made available. The range of clinical and magnetic resonance imaging (MRI) manifestations has since considerably increased. **Objective:** To review the use of this test in Quebec in the last two years, and to determine its usefulness in a French-Canadian adult population. **Methods:** Five Quebec multiple sclerosis clinics participated in this new technology evaluative study. They had access to the commercially available test at the Mayo Clinic, Rochester (MN). The database was closed on March 31, 2008. **Results:** All patients (74) who were tested for NMO-IgG were analysed. In the course of the disease, 52 have experienced a myelitis (70.3%), 33 an optic neuritis (44.6%), and 19 have presented both (25.7%). All but one (Semite) were Caucasian. There were 53 women (71.6%) of whom 8 were positive (15.1%) for serum NMO-IgG. No male was found positive for the test. Age at testing was 43+/-12y. (median :45) for the whole cohort and 44.5+/- 7.9 (median : 42) for the confirmed NMO patients. Of these, two have experienced myelitis only, two had optic neuritis only, and four had both in the course of the disease (only one at presentation). **Conclusions:** Strict adherence to the revised NMO clinical and imaging criteria could have improved the sensitivity of the test. Middle-aged women with repeated bouts of optic neuritis and/or myelitis, and MRI findings of low lesion burden of the brain in the presence of a longitudinally extensive lesion (>2 segments) of the spinal cord should be tested for NMO-IgG in our population.

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Absence of evidence for varicella zoster virus as a disease-relevant antigen in multiple sclerosis

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Background: Varicella zoster virus (VZV) virions and VZV DNA have been reported in the cerebrospinal fluid (CSF) of relapsing-remitting multiple sclerosis (MS) patients within one week of relapse. **Objective:** (1) to determine whether real-time polymerase chain reaction (PCR) detects VZV DNA in cell-free CSF obtained from MS patients within one week of exacerbation, or in acute plaques; and (2) to determine whether VZV is the primary target of the MS intrathecal IgG response. **Methods:** DNA extracted from high-speed pellets (70,000 x g) of cell-free MS CSF collected within one week of exacerbation and from acute MS plaques was used in quantitative real-time PCR with primers for VZV open reading frames 63 or 21. CSF from 53 MS patients, from 15 patients with non-MS central nervous system inflammatory disease, and recombinant IgG derived from clonally expanded plasma cells in six MS CSFs were also analyzed for binding to VZV in multiple immunoassays. **Results:** VZV DNA was not amplified from MS CSF DNA ($n = 7$), from three inflammatory neurologic disease control CSFs, or from DNA extracted from two acute MS plaques. Of 53 MS CSFs examined by enzyme-linked immunosorbent assay, 43 (81%) were positive (three times background), compared to 5 of 10 (50%) control CSFs. Six of the 53 MS CSFs were tested for VZV by immunoblotting, and all six were positive, compared with two of four inflammatory control CSFs. Forty-three recombinant antibodies (rAbs) were prepared from these six MS patients, and none were positive by immunoblotting. One of 40 rAbs immunostained VZV-infected cells. **Conclusions:** IgG in MS CSF that reacts with VZV reflects serum antibody. The overwhelming absence of recombinant

antibody prepared from clonally expanded plasma cells in MS CSF to react with VZV combined with lack of amplified VZV DNA from acute MS CSF or from acute MS plaques indicate that VZV is not a disease-relevant antigen in MS.

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Probable pathophysiological association between chronic Chlamydia pneumoniae infection and multiple sclerosis (allergic encephalomyelitis)

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Background: Multiple sclerosis is a disease of the central nervous system thought by most to be autoimmune, as it is clear that nerve tissue is being damaged and that immune system activity is present at the actual lesion location. No current laboratory model of multiple sclerosis, in particular, is known with these features, which raises the possibility of some novel process underlying new lesion formation and activity. There have been conflicting results concerning an association between Chlamydia pneumoniae and multiple sclerosis in the last decade. The ability of Chlamydia pneumoniae to infect and persist in macrophages makes it a likely candidate to disseminate in a number of different tissues, including those of the central nervous system. **Objective:** A 26-year-old woman with relapsing-remitting multiple sclerosis had two severe attacks in her 18th and 24th weeks of pregnancy, and three severe attacks in her fifth and sixth months, with the last attack continuing 15 days into the post-partum period. Different antibody titres of Chlamydia pneumoniae IgG, IgM and IgA were determined in the serum during pregnancy and in the post-partum period. **Methods:** Magnetic resonance imaging, serology of immune response of responsible bacteria, and neurophysiological tests (electroencephalogram, visual evoked potential, electromyoneurography) were used. **Results:** The magnetic resonance imaging revealed tumefactive lesions on the frontal, parietal, cerebellar, pontine and mesencephalic regions and the serology demonstrated a Chlamydia pneumoniae-mediated immune response during pregnancy and the post-partum period prior to reactivation of attacks. **Conclusions:** With the global reality of medical science where 'every allergic reaction has an allergen', and based on a review of our serologic results, radiologic and clinical diagnosis of our patient during her attack activation and remission periods, Chlamydia pneumoniae may indeed be the one of the allergens associated with the pathophysiology of multiple sclerosis.

P304

Functional role of brain-derived neurotrophic factor in axon protection during autoimmune demyelination

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Background: Brain-derived neurotrophic factor (BDNF) plays a key role in neuronal survival, differentiation and plasticity and was also shown to be axon-protective. Whilst neurons are its main cellular source in the central nervous system (CNS), bioactive BDNF is also expressed in immune cells in vitro and in multiple sclerosis (MS) lesions. Thus, infiltrating immune cells in the CNS may not only be detrimental, but can also mediate neuroprotective effects, e.g. by BDNF secretion. **Objective:** We investigated the effect of immune cell derived BDNF in myelin oligodendrocyte glycoprotein induced experimental autoimmune encephalomyelitis (MOG-EAE), an animal model mimicking many aspects of MS. **Methods:** The Cre/loxP system was used to generate conditional knock-out mice which lack BDNF in

T cells/myeloid cells. Mice were immunized with MOG35-55 and analyzed histologically at different time points of the disease. In further studies, a lentiviral complementation approach was used. MOG-specific transgenic T cells were infected with a lentivirus in which a cDNA encoding for BDNF was inserted. These cells were injected intravenously on day 7 after active immunization with MOG. **Results:** Mice deficient for BDNF in T cells and myeloid cells displayed an attenuated immune response in the early phase, but an incremental disability and increased axonal loss in the late phase of MOG-EAE. Injection of BDNF-overexpressing T cells led to a less severe course of MOG-EAE in comparison with injection of T cells infected with a GFP-expressing lentivirus alone. Histological analyses confirmed axonal preservation in lesions and perilesions of mice treated with transduced cells expressing bioactive BDNF (axonal density in lesions 7.8 ± 0.76 axons/mm²) compared with GFP-expressing cells (4.5 ± 0.57 axons/mm², $p < 0.01$). Amyloid precursor protein-positive profiles were decreased by about 36% in mice injected with BDNF-overexpressing cells, while inflammatory infiltration was similar in both groups. **Conclusions:** Immune cell-derived BDNF has a functional role in axon protection during EAE, thereby placing this neurotrophin into the focus as a potential therapeutic target in MS.

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P305

Immunomodulatory aspects of 1,25 Dihydroxyvitamin D3 in multiple sclerosis

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Background: Although Vitamin D is best known as a calcium homeostasis modulator, it also has immune modulating potential, as shown by its protective effect against multiple sclerosis (MS) development, supported by reduced disease risk associated with sun exposure and Vitamin D supplement use. Elevated Vitamin D blood levels have also been associated with lower risk of MS. **Objective:** To study the immunomodulatory effects of Vitamin D in MS. **Methods:** Serum 25(OH)D3 and 1,25(OH)2D3 levels were measured using enzyme-linked immunosorbent assay in 58 relapsing-remitting MS (RRMS) patients during remission, 34 RRMS patients during relapses, 40 primary progressive MS (PPMS) subjects and 60 healthy controls. Cell proliferation was measured using [3H]-thymidine incorporation assays. Vitamin D receptor (VDR), alpha1-hydroxylase, and indoleamine 2,3-dioxygenase (IDO) expression was measured by reverse transcriptase polymerase chain reaction, while cytokine production was evaluated using enzyme-linked immunosorbent spot assay. CD4+CD25+ regulatory T cells were studied using flow cytometry. **Results:** 25(OH)D3 and 1,25(OH)2D3 levels were significantly lower in RRMS patients than in controls, more so during relapses than remissions. By contrast, PPMS patients showed similar levels to controls. Proliferation of both freshly isolated CD4+ T cells and myelin basic protein-specific T cells was inhibited by 1,25(OH)2D3. Activated Vitamin D also enhanced IL-10 producing cell development, and reduced IL-6 secreting cell numbers. 1,25(OH)2D3 induced VDR expression in both activated and resting cells. Interestingly, T cells metabolized 25(OH)D3 into biologically active 1,25(OH)2D3, since they constitutively express alpha1-hydroxylase. Finally, 1,25(OH)2D3 also increased expression and biological activity of IDO, triggering significant increase in the number of CD4+CD25+ regulatory T cells. **Conclusions:** 1,25(OH)2D3 plays an important role in T cell homeostasis during RRMS. Correction of its deficiency may prove useful in the treatment of the disease.

P306**The role of CD8+ CD25+FoxP3+ regulatory cells in multiple sclerosis**Jorge Correale¹, Andrés Villa²¹Neurology, Institute for Neurological Research Dr Raul Carrea, FLENI, Buenos Aires, Argentina; ²Jose Maria Ramos Mejia Hospital, Buenos Aires, Argentina

Background: Previous studies have shown that CD8+ regulatory T cell clones (TCCs) lysed target CD4+ autoreactive T cells. Here, we studied the characteristics of non-cytotoxic TCCs, and identified a population of CD8+CD25+FoxP3+ regulatory T cells. These cells have been previously identified in the thymus, and in a trial using modified anti-CD3 mAb in type 1 diabetes patients, clinical responders showed increased numbers of peripheral blood (PB) CD8+ T cells expressing CD25 and FoxP3. **Objective:** To study the role of CD8+CD25+FoxP3+ cells during the course of multiple sclerosis (MS). **Methods:** PB and CSF (cerebrospinal fluid) CD8+ TCCs recognizing MBP 83-102 and MOG 63-87-specific CD4 T cells were isolated from 20 patients during exacerbations, 15 patients in remission and 15 controls. CD8+ regulatory cell phenotype and FoxP3 expression were studied using flow cytometry and reverse transcriptase polymerase chain reaction (RT-PCR). Cytokine production was evaluated using enzyme-linked immunosorbent assay. Bcl2 expression was measured by RT-PCR, and apoptosis was determined by flow cytometry using Annexin V. **Results:** Five to twelve-fold inhibition of CD4+ self-reactive T cell proliferation, IFN-gamma, and IL-17 secretion was observed when irradiated CD8+CD25+FoxP3+ cells were added to cultures. Suppression was abrogated by silencing FoxP3 using small interfering RNA. Cells were CTLA-4+, GITR+, CCR7+ and CD62L+, producing IL-10 and TGF-beta. Interestingly, in addition to up-regulating Bcl2 expression, thus becoming more resistant to apoptosis, their cloning frequency was substantially decreased in CSF and PB of MS patients during exacerbations compared with patients in remission and controls. **Conclusions:** CD8+CD25+FoxP3+ cells are a novel subset of regulatory cells exerting a key role in regulating self-reactive CD4+ T cells during the course of MS. Induction of these cells may provide a new alternative to either eliminate or inhibit self-reactive T cells during the course of autoimmune diseases.

P307**Promoting differentiation of human fetal oligodendrocyte progenitors**Qiao Ling Cui¹, Guillermina Almazan², Jack P. Antel¹¹Montreal Neurological Institute, Montreal, Quebec, Canada; ²Department of Pharmacology and Therapeutics, Montreal, Quebec, Canada

Background: Previous studies have shown that human fetal oligodendrocyte progenitor cells (OPCs) differentiate more slowly than their adult counterparts. **Objective:** Therefore, the objective of this study was to identify factors which promote differentiation of human fetal OPCs into mature myelin producing cells. **Methods:** A2B5, a monoclonal antibody that recognizes gangliosides expressed on the surface of OPCs, was used to select potential OPCs from human fetal brain cells. **Results:** We observed that (1) addition of platelet-derived growth factor (PDGF) and fibroblast growth factor (FGF) to OPC cultures maintained in DMEM+F12+N1+T3 increased the numbers of Olig2+ cells by 36% after two days, and subsequently increased the numbers of O4+, GC+ and O1+ cells after six days of treatment by 3.5, 4.0 and 2.9 fold, respectively and by 20, 17, and 12 fold, respectively by day 10 of treatment. Addition of IGF-1 and brain-derived neurotrophic factor further increased the numbers of Olig2+ cells by 13% compared with PDGF and FGF-treated levels after two days, and subsequently increased the numbers of O4+, GC+ and O1+ cells after six days by 1.4, 3, and 2.9 fold, respectively. A relative increase in numbers of O4+, GC+ and O1+ cells continued to be observed with more prolonged culture periods (10-14 days). (2) Treatment of OPCs with peroxisomal proliferator-activated receptor (PPARs) ligands under all the above culture conditions for two days increased both Olig2+ and O4+ cells (overall mean increase of 1.6 and 1.5 fold, respectively). This pulse treatment continued to enhance differentiation of OPCs into GC+, O1+ and MAG+ cells over longer culture periods (up to 14 days)

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under both basal and growth factor supplemented culture conditions. **Conclusions:** In conclusion, our results indicate that human fetal OPCs require multiple growth factors to differentiate. PPAR further promotes OPC differentiation even under growth factor supplemented conditions. Further unraveling the mechanisms that control differentiation of oligodendrocytes would provide insight as to how to enhance myelin repair by OPCs in multiple sclerosis.

P308**Relevance of the study of oligoclonal bands as a prognostic factor of the conversion of clinically isolated syndrome into clinically definite multiple sclerosis in daily practice**

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Background: In daily practice, when we are in presence of a clinically isolated syndrome (CIS), magnetic resonance imaging (MRI) is currently the most important supplementary exam to establish the risk for developing clinically definite multiple sclerosis (CDMS). In this perspective the cerebrospinal fluid study seems to have been relegated to the background. **Objective:** Evaluate as a prognostic factor of the conversion of CIS into CDMS the presence of oligoclonal bands (OB) in the lumbar puncture. **Methods:** For this study we have selected patients having presented a CIS between January 1st 2003 and December 31st 2005, and who have undergone, at a teaching hospital, a lumbar puncture so as to identify potential presence of OB. All the patients have undergone medullar and cerebral MRI, to which we have retrospectively applied the Barkhof criteria (BC). We diagnosed the conversion to CDMS when a second attack occurred before March 2008. **Results:** We studied 72 patients with CIS (sex ratio women/men=4/5; mean age=35 years. In the group of patients presenting the BC (n=33) in the baseline MRI, the presence of OB was predictive of CDMS in 74% of them. However, a CDMS occurred for three patients who were OB negative. In the group of patients not presenting the BC (n=39), the presence of OB was predictive of CDMS in 59% of them. However, a CDMS occurred for six patients who were OB negative. For our center we obtained a sensitivity of 51% for BC versus 65% for presence of OB, and a specificity of respectively 64% versus 32%. **Conclusions:** In the presence of a CIS, combining OB and an MRI that does not meet the BC improves the accuracy of our forecast of CDMS. Nevertheless, our results remain lower than those of prospective studies on selected populations, probably due to the short timescale of the study.

P309**Astrocyte-secreted Sonic Hedgehog bears central nervous system anti-inflammatory activity and promotes optimal human blood-brain barrier functioning**Aurore Dodelet-Devillers¹, Igal Ifergan¹, Monique Bernard¹, Hania Kebir¹, Romain Cayrol¹, Karolina Wosik¹, Frédéric Charron², Alexandre Prat¹¹Neuroimmunology, CHUM - Université de Montréal, Montréal, Quebec, Canada; ²Institut de Recherches Cliniques de Montréal (IRCM), Montréal, Quebec, Canada

Background: The blood-brain barrier (BBB), composed of tightly bound endothelial cells (ECs), regulates the entry of blood-borne molecules and immune cells into the central nervous system. During neuroinflammatory conditions such as in multiple sclerosis, the BBB is compromised and infiltrating immune cells partake in lesion formation. Perivascular astrocytes regulate BBB permeability by secreting essential factors, the identity of which remains unclear. Recent studies indicate that the Hedgehog (Hh) signaling pathway in adult tissues plays an important role in vascular proliferation, differentiation and tissue repair. **Objective:** To demonstrate that astrocyte-secreted Sonic Hh (Shh) contributes to the maintenance and repair of BBB functions. **Methods:** Using primary cultures of human BBB-ECs treated in vitro with exogenous Shh as well as with agonists and antagonists of the Hh pathway, migration and permeability experiments were performed on a modified Boyden chamber assay. Surface marker expression and chemokine secretion was evaluated by flow cytometry and

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enzyme-linked immunosorbent assay. **Results:** Our study shows that human astrocytes express and secrete Shh and conversely, that human BBB-ECs bear the Hh receptor Patched-1 (Ptch-1), the signal transducer Smoothed (Smo), and transcription factors of the Gli family. Furthermore, we show that activation of the Hh pathway in BBB-ECs restricts the passage of soluble tracers, decreases the surface expression of ICAM-1, and decreases BBB-ECs secretion of pro-inflammatory chemokines IL-8/CXCL8 and MCP-1/CCL2. The migration of CD4+ lymphocytes is also reduced after BBB-EC treatment of Shh and Hh pathway agonists. In vitro treatment of BBB-derived ECs with inflammatory cytokines TNF- α and IFN- γ , down-regulates the mRNA expression of Ptch-1, Smo and Gli-1, thus deregulating the Hh signaling pathway and preventing the barrier-stabilizing properties of Hh. **Conclusions:** Our data provide strong evidence for an anti-inflammatory and BBB-promoting effect of astrocyte-secreted Shh and suggest that a pro-inflammatory environment disrupts the BBB by impacting, at least in part, on Hh signaling in brain ECs.

P310

Cerebrospinal fluid screening for evidence of Epstein Barr virus presence in multiple sclerosis

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Background: Epstein-Barr virus (EBV) infection is increasingly considered as the initial trigger of autoimmunity in multiple sclerosis (MS). Evidence of prior systemic EBV infection is present in almost all patients who develop MS, and more recently EBV has been found in B cell follicles in the meninges. **Objective:** The aim of this study was to identify whether there was cellular evidence of EBV-associated genes in cerebrospinal fluid (CSF). We also screened an EBV cDNA library with CSF to identify putative EBV-specific autoantigens. **Methods:** We analysed CSF cells from 300 MS patients (with Institutional Review Board informed consent) by nested polymerase chain reaction (PCR) for the presence of four EBV latent gene transcripts (EBER-1, EBER-2, EBNA-1 and LMP-1). CSF samples from 108 MS patients were also tested for the presence of the human herpes virus-6 (HHV-6). CSF cells from a subset of MS patients were sorted by fluorescent activated cell sorter into B and T cell pools to identify the cells harboring the virus. cDNA from sorted single B cells from five CSF samples were tested for EBER-1. In addition, CSF cells were chemically treated to transform EBV latency into the lytic cycle to increase PCR detection. Pooled CSF samples that were positive for EBV were subsequently used to screen a phage displayed cDNA library constructed from EBV-infected B lymphoblastoid cells (IM9 cells). **Results:** EBER-1 was the most frequently detected viral transcript with 11.7% positivity, while the other transcripts were detected at much lower frequencies (EBER-2: 4.5%, EBNA-1: 1.9%, LMP-1: 0.9%) and there were no positive HHV-6 samples found. EBV latent transcripts were found in both sorted B and T cell pools. Single B cells, in all of which heavy and light chain IgG sequences were detected, were EBER-1 negative. In EBV-positive samples reactivation of EBV using phorbol ester and sodium butyrate did not increase sensitivity of the nested PCR for EBER-1 compared with untreated cells. The cDNA library screening did not identify any EBV-specific proteins. **Conclusions:** We failed to find that EBV transcripts are universally present in CSF and we also did not find a central nervous system immune response to EBV proteins. This does not rule out EBV as the initial systemic trigger causing a centrally directed aberrant immune response.

P311

Peroxisome proliferator-activated receptor acts as an endogenous brake on the formation of Th1 and Th17 myelin-reactive cells

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Background: Recently, peroxisome proliferator-activated receptor (PPAR) nuclear receptors (PPAR α , PPAR δ , and PPAR γ) have received

attention as potential molecular targets for therapeutic intervention in multiple sclerosis (MS). Treatment of mice with ligand-activators of these receptors ameliorates clinical symptoms in experimental autoimmune encephalomyelitis (EAE), an animal model of MS (for review see Racke et al, J. Nutr. 136: 700, 2006). Previous work has shown that PPAR γ and PPAR α attenuate central nervous system (CNS) inflammation by inhibiting the formation of Th1, but not Th17 pathogenic CD4+ cells. **Objective:** To investigate whether the delta isoform of PPAR (PPAR δ) has a similar immunosuppressive function in mice. **Methods:** We contrasted the cytokine profile of PPAR δ -/- versus wild-type (WT) immune cells activated in the presence and the absence of a synthetic ligand activator of PPAR δ . We also contrasted the clinical course of EAE in WT versus PPAR δ -/- mice. **Results:** We found that PPAR δ deficiency resulted in higher proliferation and IFN- γ and IL-17 production by CD4+ cells in response to TCR and CD28 stimulation and higher production of IL-12p40 in antigen presenting cells in response to lipopolysaccharide. Upon induction of EAE, PPAR δ -/- mice exhibited a more severe clinical course of disease than WT counterparts, which was associated with a higher frequency of IFN- γ and IL-17-producing CD4+ cells in the CNS. Treatment of CD4+ cells with GW0742, a ligand activator of PPAR δ , inhibited the production of IFN γ and IL-17 by CD4+ cells. However, this effect was only observed when CD4+ cells were cultured under serum-free conditions. Addition of oleic acid to serum-free media resulted in a reduction of IL-17 production by WT, but not PPAR δ -/- CD4+ T cells, suggesting that PPAR δ may well bind this or other fatty acid ligands. **Conclusions:** These data suggest that PPAR δ binds an endogenous ligand in immune cells and acts to limit CNS inflammation during EAE by inhibiting the formation of Th1 and Th17 myelin-reactive CD4+ cells. Since PPAR δ can inhibit both Th1 and Th17 pathogenic responses, it may be an important molecule to target in the design of therapies to control CNS inflammation in EAE and MS. **Supported by:** NIH and National Multiple Sclerosis Society (USA) grant to Lawrence Steinman; National Multiple Sclerosis Society (USA) and Canadian MS fellowships to Shannon Dunn and Roopa Bhat.

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Ready for a new saga? Th17 and not Th1 are selectively increased in active multiple sclerosis and undergo apoptosis with interferon β

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Background: Although deregulation of Th1 responses has been linked to autoimmune disorders, the pathogenic T cell population for experimental autoimmune encephalomyelitis (EAE), the murine model of multiple sclerosis (MS), involves interleukin-17-producing T cells (Th17). **Objective:** Analysis of peripheral blood Th17 and Th1 cells in active (i.e. within 10 days of a relapse) or inactive MS (i.e. without clinical or magnetic resonance imaging signs of disease activity), and of the expression of interferon (IFN) receptors and of the sensitivity of Th1 and Th17 cells to IFN β . **Methods:** Active MS (AMS) (n = 29), inactive MS patients (IMS) (n = 31), and healthy subjects (HS) (n=22). Patients free from any immunosuppressive therapy. Fluorescent activated cell sorter (FACS) analysis with specific monoclonal antibodies of intracellular cytokines, surface type I receptor chain1 (IFNAR1), and STAT1 phosphorylation with or without IFN β . On CD4 lymphocytes, study of cell cycle or apoptosis with or without IFN β -1a with RNase A and propidium iodide or with AnnexinV/7-Amino-Actinomycin D (AV/7-AAD) double staining; and of IFN β -induced STAT1 phosphorylation with Western blot. **Results:** Th17 cell % increased by about 7-fold in AMS both compared with IMS or HS. Th1 cell % did not change. A one-year longitudinal study of 15 (4 started IFN β -1b) patients confirmed that Th17 and not Th1 cell % decreased strikingly in patients changing from AMS to IMS. IFN β reduced in a dose-dependent manner in vitro proliferation of Th17 and not Th1 cells. Hypodiploid and early apoptotic (AV+ 7-AAD-) cell % significantly increased by IFN β in Th17 and not in Th1 cells. IFNAR1 expressing cell % significantly greater in Th17 than in Th1

cells in all study groups (AMS, IMS and HS). IFNAR1 expression at FACS analysis and IFN β -dependent STAT1 response at Western blot progressively increased with a highly significant positive correlation in developing Th17 and not in Th0 or Th1 cells. **Conclusions:** This is the first evidence of an expansion of Th17 and not Th1 cells associated with disease activity in MS. Differently from Th1, Th17 cells express higher levels of IFNAR1 and are sensitive to IFN β apoptotic effect. The higher levels of IFNAR1 might account for the higher sensitivity of Th17 cells to IFN β , making them a selective target of this therapy.

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Muscle fatigue resistance during experimental autoimmune encephalomyelitis in rats

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Background: Muscle weakness and fatigue are important (functional) symptoms in patients with multiple sclerosis (MS) resulting from the pathological events in the central nervous system. **Objective:** We examined muscle fatigue resistance following the development of acute experimental autoimmune encephalomyelitis (EAE). **Methods:** Following familiarization (1w) female Lewis rats were divided in a control (CON, n=12) and EAE (n=12) group, the EAE group determined by the injection of purified myelin basic protein in combination with mycobacterium tuberculosis inducing (hind limb) paralytic disease after 12–14d. Approximately 3d after development of maximal clinical signs, fatigue resistance of the foot extensor muscle group (tibialis anterior (TA), extensor digitorum longus (EDL)) was examined using an isokinetic dynamometer and simultaneous stimulation of the common peroneal nerve. Hence, maximal muscle torque was registered during 120 (3s rest intervals) maximal (1mA, 250ms) isokinetic contractions at 150Hz and 100°/s. Hereafter, TA and EDL were removed for immunohistochemical type I, IIa and IIb muscle fiber cross sectional area (CSA) determination. **Results:** Maximal muscle torque in CON was 98±14mN declining (p<0.05) to 64±10mN following 120 contractions. In EAE, however, maximal muscle torque remained stable at 53±9mN following 120 contractions. In EDL (preliminary data), type I and IIa CSA did not differ between CON (I:520±53 μ m², IIa:538±58 μ m²) and EAE (I:635±78 μ m², IIa:572±73 μ m²) animals. Type IIb CSA, however, was lower (p<0.05) in EAE (1163±85 μ m²) vs. CON (1461±113 μ m²). Similarly, in TA (preliminary data), type I and IIa CSA did not differ between CON (I:643±140 μ m², IIa:692±102 μ m²) and EAE (I:753±147 μ m², IIa:696±123 μ m²) animals. Type IIb CSA, however, was lower (p<0.05) in EAE (869±133 μ m²) vs. CON (1750±208 μ m²). **Conclusions:** EAE decreases maximal muscle torque yet does not affect muscle fatigue resistance. Preliminary data indicate that this might be due to decreased type IIb muscle fiber CSA. **Supported by:** IWT grant #50078, Flanders, Belgium.

P314

De Seze criteria: application to a series of 14 patients who presented a first acute severe demyelinating event

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Background: The application of De Seze criteria to patients presenting a first severe acute demyelinating event helps to distinguish an acute disseminated encephalomyelitis (ADEM) from other central nervous system inflammatory diseases, with a sensitivity of 83% and a specificity of 95%. **Objective:** We applied these criteria to 14 patients who presented a first severe acute demyelinating event and whose later clinical evolution permitted to clearly identify the neurological diagnosis. **Methods:** This study concerned 14 patients who presented a first acute demyelinating event. Our series initially included 16 patients but two were excluded because of their initial clinical condition in order to respect the exclusion criteria of the orig-

inal article. **Results:** We identified 11 women (78.6%) and 3 men (21.4%) with a mean age of 33.7 years +/- 12.5 years, the follow-up of the patients being from 3 months to 11.5 years after the initial episode (4 years average follow-up). At the end of the follow-up, 7 patients were diagnosed with ADEM (50%) and 7 with multiple sclerosis (MS) (50%). Five patients out of 7 in the MS group have a tumor-like presentation (71.4%), this parameter partly explaining the initial discrepancy in diagnosis. After application to our series, De Seze criteria for ADEM have a sensitivity of 85.7% and a specificity of 71.4%. **Conclusions:** The application of the new criteria does not enable us to achieve the same values as in the original article, in terms of sensitivity, specificity, positive and negative predictive values. The lack of specificity is due to the misclassification of MS patients with a tumor-like presentation, for which there are 2 out of 5 false positives. One explanation is that the clinical criteria used can encompass atypical forms of MS, in particular in its tumor-like presentation. De Seze criteria represent an invaluable aid for the clinician in the diagnosis of a first severe demyelinating event. Considering our results, a tumor-like form of MS could be an exclusive situation to the application of these criteria. A prospective study on a larger cohort is necessary to confirm or invalidate these preliminary results.

P315

Genetic predisposition of interaction of HLA-DRB with heat shock protein 70-1 gene polymorphism in Iranian multiple sclerosis patients

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Background: Multiple sclerosis (MS) is a polygenic inflammatory disorder of the central nervous system most common in young women. Heat shock protein 70 (HSP70) is essential for maintenance of normal cellular function and recovery after insult. To date, there are some reports regarding the association between HSP70 gene polymorphisms and autoimmune disease, including MS. More recently, several researchers have focused on HLA-DRB alleles as a potential candidate. Most of them have shown that HLA-DRB1*15 allele is a higher genetic predisposition risk for development of MS. **Objective:** The aim of this study was to determine whether variation in the HSP70-1 gene at position +190 itself and amalgamation with HLA-DRB alleles is associated in MS patients and healthy individual controls. **Methods:** Genomic DNA was extracted from whole blood of 128 patients with MS and 137 age, ethnically and geographically matched control subjects. Patients were divided into two groups according to their expanded disability status scale score with mild to moderate and severe disease. The genotypes were determined by single specific primer-polymerase chain reaction. STATA software was used for analysis and Fisher's exact test was applied to determine differences between groups. **Results:** Frequencies of HSP70-1 genotypes were significantly different between MS patients and control subjects (P =0.004; OR= 2.87; CI=1.55–5.33). In addition, G/G genotype was protective when come with HLA-DRB1*13, *14, *16, DRB3*01 and DRB5*01 alleles. Conversely, G/G genotype along with DRB1*0103 allele was more frequent in MS patients. Furthermore, age at onset and course of the disease were influenced by interaction of HLA-DRB alleles and HSP70-1 gene polymorphism. **Conclusions:** Our results have shown that HSP70-1 gene (G/C) polymorphism was strongly associated with development of MS in this population. Interestingly, the combination of G/G genotype with DRB1*13, *14, *16, DRB3*01 and DRB5*01 alleles enhanced protection against MS; in contrast, combination of DRB1*0103 allele with G/G genotype increased susceptibility to MS.

P316

A novel assay to measure effector B cell responses in pediatric clinically isolated syndrome/ multiple sclerosis

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Background: We previously showed that purified human B cells reciprocally express down-regulatory (IL-10) and pro-inflammatory (TNF α , LT) cytokines when activated through CD40-alone or combined B-cell receptor and CD40-stimulation (Duddy et al, J Immunol, 2007). More recently, we discovered that similarly purified B cells from adult multiple sclerosis (MS) patients exhibit a deficiency in IL-10 and a propensity to over-produce LT and TNF α (Bar-Or et al, AAN, 2008). **Objective:** Whether these B cell abnormalities are relevant to early/initiating events in MS pathogenesis could be best addressed in childhood-onset MS. This, however, requires developing assays applicable to limited blood volumes obtainable from children. **Methods:** We developed a flow cytometry-based intracytoplasmic-cytokine staining (ICS) technique that detects IL-10, LT and TNF α produced by CD19+ B cells, activated within whole peripheral blood mononuclear cells (PBMC) and validated on cryopreserved samples. **Results:** As in our established system using purified B cells, our novel assay confirmed significantly greater induction of normal B-cell IL-10 upon isolated CD40-stimulation, compared with dual-stimulation carried out within whole cryopreserved PBMC (7.5% \pm 0.8%, versus 2.5% \pm 1.0%, respectively; n = 4, p = 0.02). Reciprocally, LT was significantly more induced upon dual-stimulation compared with isolated CD40-stimulation (MFI 760 versus 86, respectively; p < 0.02). We are currently applying the novel assay in a blinded fashion to cryopreserved PBMC samples collected from children with MS (n = 10) and neurological disease controls (n = 9) and will report on B cell cytokine abnormalities in pediatric MS, as well as on the potential relationship between such B cell abnormalities and Epstein Barr virus serology and titers. **Conclusions:** We successfully established a novel assay measuring disease-relevant effector B cell cytokine responses in cryopreserved PBMC, applicable to multi-center pediatric MS samples. Our study should provide insights into B cell contributions to early events in the MS disease process.

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P317

Atorvastatin interference with interferon-beta signaling in human immune cells and in multiple sclerosis

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Background: Statins block HMG-CoA reductase and inhibit cholesterol synthesis. Atorvastatin suppresses pro-inflammatory Th1/Th17 responses in experimental autoimmune encephalomyelitis. Interferon-beta ameliorates multiple sclerosis (MS). A combination of statins with interferon-beta was expected to be therapeutic in treatment of MS. Recent studies with different designs, however, showed different effects of statins during interferon (IFN) therapy. **Objective:** To determine whether statins have an inhibitory effect on IFN-induced activation in human T cells and monocytes, and in MS immune cells. **Methods:** Phosphorylation (activation) of the phospho-tyrosine-STAT1 that is essential for IFN signaling was quantitated with Western blots and intracellular flow cytometry in IFN-induced Jurkat (T) cells and U937 cells (monocytes), as well as in mononuclear cells from MS-affected and unaffected individuals, pre-treated in vitro with atorvastatin (1, 2, 5, 10, and 20 μ M) from 1 to 24 hours. **Results:** Atorvastatin significantly blocked IFN-induced signaling in a dose-dependent manner in Jurkat and monocyte cells, and in immune cells from controls and from stable and active relapsing-remitting MS

(RRMS) patients. Statins appeared to affect adherence-purified human monocytes more than T cells, block induction by IFN-beta-1b more than by IFN-gamma, and block P-Y-STAT1 more than P-S-STAT1. Pre-treatment with statin, beginning as early as 1h, suppressed STAT1 activation in cell lines induced with IFN-beta-1a (Avonex), IFN-beta-1a (Rebif), and IFN-beta-1b (Betaseron). Activation of STAT1 was restored with addition of Mevalonate, a product of HMG-CoA reductase. **Conclusions:** High dose statin (10 μ M, equivalent to high in vivo doses) blocks IFN-induced signaling in immune cells. This suggests statins potentially prevent some of the therapeutic effect of IFN-beta in RRMS.

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Serum cytokine profile and disease activity in multiple sclerosis and neuromyelitis optica

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Background: Multiple sclerosis (MS) and neuromyelitis optica (NMO) are two distinct central nervous system demyelinating diseases, with differences both in pathology of lesions and magnetic resonance imaging distribution of disease. MS pathology is due to demyelination resulting from inflammation or oligodendrocyte death. NMO pathology is due to a vasculopathy causing secondary demyelination, which is typically severe with necrosis, a finding not observed in MS. **Objective:** Describe serum cytokine profile in MS and NMO patients during disease activity and stability. **Methods:** Multiplexed fluorescent bead-based immunoassay (Bio-Rad Laboratories, Hercules, CA) was used to detect 17 cytokines in the serum of MS, NMO, and normal control patients. Analyses were performed for IL-2, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12(p70) and IL-13. Serum IL-4, GM-CSF, IFN-gamma, and IL-17 were below the detection limit for this assay. **Results:** The pattern of cytokine elevation was compared between active MS versus active NMO patients to see if there were distinguishing immune markers between the two diseases. The pattern of elevation was similar for IL-2, IL-6, IL-7, and IL-8, with higher levels of these cytokines in most patients with active disease versus recovering or stable disease. However, IL-5, IL-10, IL-12, and IL-13 were elevated in most patients with active MS but not in active NMO, stable disease, or controls. **Conclusions:** Preliminary results showed differences in the two demyelinating processes, MS vs. NMO. Among the cytokines analyzed, a specific immune cytokine profile was not established for NMO. However, different cytokine profiles between the two diseases corroborate differences in pathology.

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Strain- and sex-specific relocation of CXCL12 at the blood-brain barrier may contribute to a relapsing-remitting phenotype during experimental autoimmune encephalomyelitis

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Background: Leukocyte extravasation into the central nervous system (CNS) is an essential component of multiple sclerosis (MS) and its mouse model, experimental autoimmune encephalomyelitis (EAE). Studies in C57BL/6 mice with monophasic-EAE indicate that polarized expression of the chemokine CXCL12 at the blood-brain barrier (BBB) prevents leukocyte entry into the parenchyma and that loss of this polarity occurs at the peak of EAE and in postmortem specimens specifically from MS patients. This altered pattern of CXCL12 expression was significantly correlated with the level of perivascular egress of immune cells into CNS parenchyma. **Objective:** Because altered CXCL12 distribution correlates with severity of disease in MS patients, we examined the role of CXCL12 in the only existing relapsing-remitting EAE model, which is elicited in female SJL mice. **Methods:** Brain and spinal cord microvasculature of C57BL/6 and SJL male and female mice were examined for CXCL12 expression throughout their

respective courses of EAE. Peripheral lymphoid and CNS production of CXCL12, CXCR4, CXCR7, Th17, IL-23, and IL-1B were also examined. **Results:** CXCL12 BBB expression in naïve and diseased male C57BL/6 and SJL mice was identical: basolateral CXCL12 re-localized to luminal surfaces prior to disease onset and persisted throughout the peak of disease. In contrast, naïve female SJL mice exhibited luminal CXCL12 expression specifically in white matter regions, while basolateral CXCL12 expression was detected predominantly within the grey matter. Female SJL mice with EAE exhibited complete loss of CXCL12 staining within inflamed vessels while non-inflamed vessels continued to demonstrate luminal CXCL12. Lymphoid and CNS levels of IL-17, IL-23, and IL-1B increased with disease exacerbations and were similar between both sexes. **Conclusions:** These experiments suggest that the type or course of autoimmune inflammation may correlate with alterations in CXCL12 expression within specific white matter regions that occur in a strain- and sex-specific manner.

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The use of CD39 as a marker for functional regulatory T cells in multiple sclerosis

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Background: CD4+CD25+ Foxp3+ regulatory T (Treg) cells are essential to maintain tolerance and prevent autoimmune disease via suppression of autoreactive T cells. The suppressive function of Treg appears defective in multiple sclerosis (MS) patients when compared with healthy controls, although total numbers of Treg cells are similar to those found in controls. The ectonuclease CD39 was recently found to be expressed on a subset of Treg cells, and exerts suppressive function by conversion of ATP to AMP, with consequent anti-inflammatory effects. Furthermore, the percentage of CD39+ Treg cells was reduced in MS patients compared with controls. **Objective:** Functional Treg studies require relatively large numbers of cells and are time consuming to perform on large clinical cohorts. Thus, given the importance of Treg cells in human autoimmune and other diseases, a robust cell surface marker to identify functional Treg cells would be extremely valuable. Here we aimed to determine the relative suppressive capacity of both CD39+ and CD39- Treg in comparison with the total Treg population, and to phenotypically compare Treg populations in MS patients and controls. **Methods:** Total Treg cells as well as the CD39+ and CD39- Treg subsets were FACs sorted, and their suppressive capacity compared using a direct suppression assay with stimulated CD4+ T cells as responders. The phenotype of Treg cells was determined by flow cytometry, using CD4, CD25, FoxP3, CD127 and CD39 as markers. **Results:** Our data indicates that the suppressive capacity of Treg lies within the CD39+ population. CD39+ Treg suppressed proliferation and modulated cytokine production by responder T cells stimulated with anti-CD3 or antigens including myelin oligodendrocyte glycoprotein. Furthermore we demonstrated a reduction in the percentage of CD39+ Treg in a cohort of 50 relapsing-remitting MS patients compared with healthy controls. **Conclusions:** These data suggest that CD39 represents a cell surface marker that can be used to identify functional Treg cells, and that Treg cells identified using this marker are depleted in MS.

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Determination of patterns of oligoclonal bands specific to multiple sclerosis stage by digital image processing

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Background: Early diagnosis of multiple sclerosis (MS) is very important in treatment of patients and evolution of disease. Obtaining oligoclonal bands (OCB) from cerebrospinal fluid samples is a common procedure useful for diagnosis. However, bands are not always easy to define and are subject to observer variability and interpretation. **Objective:** To present the fundamentals and preliminary results of a method for generating patterns of OCBs corresponding to different stages of MS by digital image processing and clustering by advanced statistical tools. To show how these patterns are different and may be employed for classification. **Methods:** Digital images from OCBs of 87 patients are obtained and classified into 4 groups, according to stage of disease: 1= clinically isolated syndrome, 2=relapsing-remitting, 3=secondary progressive and 4=primary progressive. Image-processing software is used to extract and normalize bands. 'Average patterns' of bands in each group are defined, counting the relative weight of each band in each group. These patterns are analyzed by i) standard spectral (Fourier) techniques to measure spectral contents in a generalized frequency axis and ii) by generation of specific spectrograms. Relative distances among patterns are measured by Euclidean metrics. **Results:** Spectral analysis shows an increasing area under the spectral curves of each pattern, according to its disease stage, in range (0.5–4)e-3 cycles/mm. Spectrogram images show how these spectral contributions have a stronger contribution in the region of 'longer' bands (more separate from origin). Relative distances among patterns define a symmetrical matrix, with greater distances among patterns corresponding to groups with different diagnosis. Closer, increasingly similar diagnoses have smaller relative distances. **Conclusions:** Though still preliminary, these results show a promising approach to classification of OCBs of MS patients. Combined with proper metric evaluation procedures, currently under investigation, these procedures may yield a tool for patient classification and staging.

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Auto-reactive antibodies in primary progressive multiple sclerosis patients

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Background: Primary progressive multiple sclerosis (PPMS) has distinct pathological features, clinical course and response to treatment as compared with the more common relapsing MS. It has been proposed that autoantibody mediated immune attack and lack of central nervous system repair could be responsible for the gradually increasing disability in PPMS. We studied various autoreactive antibodies (ARAB) including anticardiolipin, antinuclear, anti-SSA and anti-SSB, and anti-thyroid antibodies in PPMS patients. **Objective:** To study the frequency of ARAB in patients with PPMS and to characterize clinical and magnetic resonance imaging (MRI) features of patients with positive ARAB. **Methods:** This is a retrospective study of PPMS patients evaluated at the UMass Memorial MS Center over the last five years. Data on patient characteristics, clinical presentation, disease duration and progression, associated autoimmune diseases and ARAB were collected. Brain and spinal cord MRI images were reviewed in a blinded fashion to examine the presence of atrophy and to categorize T2 lesion load as low or high. **Results:** ARAB data were available in 23 patients with a confirmed diagnosis of PPMS as per McDonald's criteria. Twelve (52.2%) patients showed presence of one or more ARAB. Anticardiolipin antibody was the most common ARAB. There was no significant difference between ARAB positive and ARAB negative patients for age (mean \pm SD) (56.2 ± 6.8 vs. 55.4 ± 4.4 years respectively), age of onset of MS (45.9 ± 9.7 vs. 47.3 ± 6.7 years), disease duration (9.5 ± 5.8 vs. 9.1 ± 6 years) and expanded disability status scale (3.8 ± 1.4 vs. 4 ± 2.2). Associated autoimmune diseases, family history

of MS or autoimmunity, spinal fluid IgG index and oligoclonal bands were also similar in the two groups. Eighty two percent of ARAB-positive patients had high T2 lesion load as compared with 27% of ARAB negative patients ($p = 0.03$). No significant difference was found in presence of brain atrophy (45.5% vs. 30%, NS) or T1 black holes (45.55 vs. 40%, NS). **Conclusions:** ARAB are present in about 50% of the PPMS patients. ARAB-positive patients are more likely to have a high T2 lesion load, suggesting a possible pathogenic role of auto-antibodies in T2 lesions in PPMS.

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Regulatory T cells and Th17 cells in healthy individuals and in patients affected by multiple sclerosis

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Background: Th17 cells have been recently shown to have a crucial role in autoimmune diseases, such as multiple sclerosis (MS). These pro-inflammatory cells have been strongly associated with the expression of CCR6. We have previously defined a distinct subset of CCR6+ T reg cells which accumulate in the central nervous system after induction of experimental autoimmune encephalomyelitis. This subset represents a population of 'regulatory effector-memory' T cells, destined to control potentially destructive immune responses directly in inflamed tissues, functioning as a natural counterbalance to 'conventional' effector-memory T cells. More recently, we have described a strong immunosuppressive function within the CD4+CD25highCCR6+CD39+ subpopulation of T reg cells, also showing that this subset is significantly decreased in MS patients in the stable phase of the disease. **Objective:** To assess the role of Regulatory T cells and Th17 cells in healthy individuals and in patients affected by MS **Methods:** In the present study we have characterized both the CCR6+ Th17 and CCR6+ T reg cells in relapsing-remitting MS patients and in age- and sex-matched healthy individuals. **Results:** Within CD25high T cells we found several subsets of cells: a small fraction produced IL-17 or IFN γ . Th17 cells were CCR6+, while Th1 IFN γ -producing cells were consistently CCR6-. Moreover, IFN γ and IL-17 positive cells were confined to the CD39 negative T cell population, thus confirming that CD39 represents a valuable marker for T reg cells within the CD25high subset. In the attempt to define phenotypic and functional correlations with the clinical state of MS patients we then monitored CD25highCD39+ and CD25highCD39- cells in patients in different phases of the disease and in healthy donors. Patients in the stable phase of the relapsing-remitting form of the disease had reduced numbers of CD39+ cells within the CD4+CD25high cell population. Interestingly, the distribution and frequency of this T cell subset in MS patients in the acute phase of disease was comparable with that of healthy individuals **Conclusions:** The implications of our findings will be discussed.

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Seven cases of relapsing myelitis with no identified cause

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Background: Despite extensive investigation, a significant number of cases of relapsing myelitis remain without an etiological diagnosis. Although the diagnostic criteria for idiopathic myelitis are well known the natural history regarding recurrence is not as well defined. **Objective:** To describe a series of idiopathic relapsing myelitis. **Methods:** Cases of idiopathic myelitis were collected from the Santa Maria Neurology department registries from 1996–2007. **Results:** Seven cases were gathered. Six patients were females, mean age of 41.7 years old (25–58), with an average of 3(\pm 1) relapses. Five patients

showed changes in spinal magnetic resonance imaging (MRI) but all had normal brain MRI and evoked potentials. Infection and vasculitis were excluded. Three of the seven cases had high titers of anti-thyroid antibodies and a single patient had oligoclonal bands in the cerebrospinal fluid. Aquaporin-4 antibodies were not detected by a fluorescence based immunoprecipitation assay nor by an EGFP-AQP-4 transfected cell-based assay. They all had a clinical improvement with steroids and three patients became corticoiddependent. **Conclusions:** The present work adds seven more cases to the few series of relapsing myelitis described in the literature and highlights the importance of a systematic investigation of these cases. Although the determination of new serological markers (such as anti-aquaporin-4 antibodies) has reduced the percentage of unclassified cases, those that remain without a diagnosis still need to be managed. In our cases all the patients responded well to steroids.

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Estriol treatment reduces matrix metalloproteinase-9 activity in multiple sclerosis and experimental autoimmune encephalomyelitis

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Background: Matrix metalloproteinases (MMP) play a crucial role for transmigration of inflammatory cells into the central nervous system. Elevated levels of MMP-9 have been described in serum and cerebrospinal fluid of multiple sclerosis (MS) patients and predict the occurrence of new active lesions on magnetic resonance imaging (MRI). **Objective:** To investigate if in vivo treatment with estriol affects MMP-9 levels from immune cells in patients with MS and mice with experimental autoimmune encephalomyelitis (EAE). **Methods:** Ex vivo stimulated peripheral blood mononuclear cells (PBMCs) from three female MS patients collected before and during six months of oral estriol treatment were analyzed for levels of MMP-9 and its inhibitor TIMP-1. MMP-9 biological activity from immune culture supernatants was assessed using zymography. Active EAE was induced in female C57BL/6 mice with MOG 35-55. Animals were treated with estriol (E3), estrogen receptor (ER) alpha or ER beta agonist or vehicle. MMP-9 levels in splenocyte culture supernatants were measured during the peak of clinical disease and the chronic phase. **Results:** Supernatants from PBMCs obtained during estriol treatment in female MS patients showed significantly lower MMP-9 levels, decreased MMP-9/TIMP-1 ratios and decreased MMP-9 activity as determined by zymography compared with before treatment. Three months after the end of treatment, MMP-9 and MMP-9/TIMP-1 ratios returned to pretreatment levels. These changes coincided with a decrease in enhancing lesion volume on MRI. Similarly, estriol treatment reduced MMP-9 levels in supernatants from splenocytes during the peak of EAE and during the chronic phase. This was confirmed by decreased MMP-9 activity in zymography. Experiments with selective ER alpha and ER beta agonists revealed that this effect was mediated via ER alpha but not ER beta. **Conclusions:** Estriol acting via ER alpha to reduce MMP-9 from immune cells is one mechanism potentially underlying the estriol-mediated reduction in enhancing lesions in MS and inflammatory lesions in EAE.

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Dendritic cells express increased osteopontin in both experimental autoimmune encephalomyelitis and multiple sclerosis and amplify IL-17 production by CD4+T cells

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Background: Osteopontin (Opn) is a pleiotropic cytokine that has been linked to autoimmune disease conditions including multiple

sclerosis (MS) and its animal model experimental autoimmune encephalomyelitis (EAE). Although it has been reported that Opn may exacerbate EAE by affecting IFN- γ producing Th1 cells, Opn expression in Dendritic cells (DCs) and its role in IL-17 induction from T cells during EAE or MS is unknown. **Objective:** To examine Opn in DCs and its role on IL-17 induction in T cells during EAE and MS. **Methods:** To investigate whether Opn expression is altered in DCs during EAE, we isolated DCs and T cells both from periphery and the central nervous system (CNS) of EAE animals and analyzed them for expression of Opn and Opn receptors. The effect of Opn on IL-17 production from T cells was tested by culturing DCs from EAE mice with T cells or by adding exogenous Opn. The receptors involved in Opn-mediated cytokine induction from T cells were confirmed by specific receptor blocking antibodies and knockout animals. To examine Opn in MS patients, we used ex vivo DCs and T cells. The expression of Opn in DCs and the effect of Opn on MS-derived T cells were tested by polymerase chain reaction and enzyme-linked immunosorbent assay. **Results:** We found that during EAE, Opn expression was elevated in DCs both in the periphery and in the CNS. In addition, there was increased expression of Opn receptor on T cells, Opn-induced IL-17 production by CD4+ T cells via the $\beta 3$ integrin receptor and Opn-inhibited IL-10 production via the CD44 receptor. Furthermore, anti-opn treatment reduced clinical severity of EAE by reducing IL-17 production. Analogous to EAE, in subjects with MS we found increased expression of Opn in DCs and increased expression of the Opn receptors on T cells. Furthermore, Opn-stimulated CD4+ T cells from MS patients produced significantly higher amounts of IL-17. **Conclusions:** Our results demonstrate a central role for DC-produced Opn both in EAE and MS that is linked to the production of IL-17.

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Influence of Tysabri® treatment on immune functions in multiple sclerosis patients

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Background: Natalizumab (Tysabri®) is an effective new treatment for patients with relapsing-remitting multiple sclerosis (MS). Natalizumab strongly reduces the invasion of leukocytes into the central nervous system (CNS) by blocking VLA-4. During the clinical phase III trials with Natalizumab, three patients receiving additional immunomodulatory agents developed progressive multifocal leukoencephalopathy (PML). PML is a JC virus-mediated, usually fatal infection of the CNS which typically occurs in severely immunocompromised patients. **Objective:** The aim of this study was to analyze lymphocyte subpopulations and in vitro reactivity to various antigens of peripheral blood mononuclear cells (PBMC) derived from MS patients treated with Natalizumab alone and controls. **Methods:** Thirty-nine MS patients before and under Natalizumab therapy (duration of treatment 1–12 months), 10 healthy donors and seven patients with rheumatoid arthritis treated with Rituximab were included in the study. Quantitative changes in lymphocyte subpopulations during the course of treatment were measured by fluorescent activated cell sorter (FACS) analysis of fresh blood samples. For in vitro stimulation of PBMC, bacterial and viral antigens were used. The cell division index (CDI) of CD4+ and CD8+ T-cells was determined via a carboxyfluorescein succinimidyl ester labeling method and FACS analysis. The virus status for JC-, BK- and CM-Virus was tested by real time polymerase chain reaction in PBMC and urine samples. **Results:** As previously described, the total number of lymphocytes/ μ l blood increased during Natalizumab treatment. Correlated to treatment duration, we especially observed an increase of CD19+ B and CD8+ T lymphocytes. Treatment duration was significantly correlated with an increase of the CDI of CD4+ T cells in response to different stimuli. **Conclusions:** Our results indicate that there is a positive correlation between treatment duration and a) the increase of CD8+ T and CD19+ B cell count, and b) the immunoreactivity of CD4+ T cells.

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HLA and KIR genotyping in Italian patients with relapsing-remitting multiple sclerosis

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Background: Given the role of Killer Immunoglobulin-like Receptors (KIRs) in the immune response, its specificity for HLA class I allotypes, and its extensive genomic diversity, it is conceivable that KIR gene variations might modulate susceptibility to a number of diseases. Several diseases have indeed been associated with KIR locus variability, indicating the functional relevance of KIR polymorphism in the development and progression of some autoimmune diseases. **Objective:** We analysed the KIR and HLA Cw gene distribution in multiple sclerosis (MS) patients to evaluate any possible association with MS susceptibility or protection. **Methods:** We report herein results obtained by PCR-SSP molecular typing for KIR and HLA-Cw genes in 109 relapsing-remitting MS patients. These data were compared to those obtained in 44 healthy subjects and 217 historical healthy controls (HC). Chi-square analysis Yates corrected and Fisher exact test were evaluated when appropriated; Bonferroni correction for multiple tests was applied. **Results:** A significant lower frequency of KIR2DS4*003 was observed in MS patients (74%) as compared both with historical results (89%) ($py=0,0007$ OR=0.34) and with the internal HC group (91%) ($py=0,04$ OR=0.29). In addition, molecular analysis of HLA-Cw genes' distribution revealed a significant decrease of Cw*12 allele, which was found in 6.0% of patients but in 15.9% of HC ($py=0,01$ OR=0,34). Interestingly, 6.7% of the MS patients were positive for both Cw*12 and 2DS4*003; this association was significantly lower than what was observed in HC (29.5%) ($py=0,008$ OR=0,19) increasing the statistical significance observed for these two markers separately. **Conclusions:** Because natural killer cells were shown to have a role in experimental autoimmune encephalomyelitis, our results suggest that the KIR2DS4*003/HLA-Cw*12 combination may be relevant to protection against the development of MS or that, alternatively, a strong linkage disequilibrium within the KIR gene complex can account for disease association.

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ATP production in CD4+ positive cells as a measure of immune function in multiple sclerosis patients - possible impact of interferon beta on bioenergetics of immune effector cells

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Background: Progressive multifocal leukoencephalopathy (PML) occurs in the context of decreased cell-mediated immunity such as HIV infection. Pathogenesis of PML during combined natalizumab/interferon beta treatment has not yet been elucidated. **Objective:** To examine intracellular ATP values of CD4+ T cells of treatment-naïve or treated multiple sclerosis (MS) patients and healthy volunteers as a measure of cell-mediated immunity. **Methods:** After approval by the local ethics committee, healthy volunteers (n=41), untreated MS patients (n=29) and MS patients receiving either beta interferons (n=27) or immunosuppressive therapy (mitoxantrone n=15) were tested. Whole blood was either unstimulated or stimulated with phytohemagglutinin (PHA). After 15h CD4+ cells were immunoselected with anti-human CD4-coated magnetic beads

following cell lysis and ATP concentrations measured in a luciferase activity based assay by luminescence in an FDA-approved assay (ImmunoKnow™). CD4/CD8 cell counts were determined by flow cytometry. **Results:** Neither untreated (medians, 559 ± 30.4 ng/ml) nor mitoxantrone-treated MS patients (three months prior to blood sampling, 599 ± 46.8 ng/ml) exhibited significant differences of ATP concentrations in PHA-stimulated CD4+ cells in comparison with sex and age-matched controls (511 ± 23.9 ng/ml). PHA-stimulated CD4+ cells of beta interferon-treated MS patients revealed significantly reduced ATP concentrations (365 ± 44.3 ng/ml, $p = 0.0008$) when compared with untreated MS patients and healthy controls. Reduced ATP-concentrations were largely independent of CD4-cell number. **Conclusions:** This data indicates an effect of beta interferon therapy on a bioenergetic measure of cellular immune function in CD4-cells of MS patients, with no long-term effect of immunosuppressive mitoxantrone therapy. Possible implications for individualized treatment strategies in the context of natalizumab side-effects e.g. PML will be discussed.

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Role of the p38 mitogen-activated protein kinase (MAPK)/MAPK-activated protein kinase 2 (MAPKAP-K2) signaling cascade in oligodendrocyte differentiation

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Background: Oligodendrocytes (OLGs), the myelinating cells in the central nervous system, are the primary target of destruction in multiple sclerosis. The molecular mechanisms that underlie myelin formation remain poorly understood. p38 mitogen-activated protein kinases (MAPKs) are a kinase family that regulate multiple cellular functions including proliferation, survival and differentiation. Previous work in our laboratory demonstrated that p38 is necessary for both OLG and Schwann cell myelination. **Objective:** To determine the mechanisms of p38 and MAPK-activated protein kinase 2 (MAPKAP-K2) regulation of OLG differentiation. **Methods:** OLGs from neonatal rat brains were obtained from mixed primary glial cultures using well-established methods. Purified OLGs were maintained in serum-free media and treated with p38 α/β and MAPKAP-K2 inhibitors, or small-interfering RNAs (siRNAs). Effects on OLG differentiation were assessed using immunological and biochemical techniques. **Results:** We found that OLGs abundantly express p38 α , and its decrease by siRNA, or with a selective inhibitor resulted in significant reduction in the expression of galactosylceramide, sulfatide, and myelin-specific proteins: myelin basic protein (MBP), myelin-associated glycoprotein (MAG), and proteolipid protein (PLP). Maximal effect of p38 inhibitors on OLG differentiation was early in their development. Quantitative analysis of mRNA transcripts using real-time PCR revealed significant decreases in MBP, MAG, and PLP. Furthermore, inhibition of MAPKAP-K2, a downstream effector of p38, also blocked OLG differentiation. **Conclusions:** Our results show a critical role for p38 α and MAPKAP-K2 in the regulation of key steps in OLG differentiation. Studies are underway to further understand the mechanisms of how these kinases regulate this process.

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Cathepsins and their endogenous inhibitors cystatins in multiple sclerosis

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Background: Cathepsins are involved in a variety of physiological processes such as antigen processing and presentation, as well as extracellular matrix (ECM) degradation and cell migration, and thus

may have significant implications in immunity to infection and in autoimmune diseases. Notably, in our recent pharmacogenetics study an association was detected between the gene encoding the lysosomal cysteine protease - Cathepsin S and response to glatiramer acetate (GA) immunotherapy for relapsing multiple sclerosis (MS). **Objective:** A. To compare levels of cathepsin S (CTSS) and cathepsin B (CTSB) and their inhibitors cystatin C (CSTC) and B (CSTB) in peripheral blood leukocytes (PBLs) of healthy individuals versus MS patients at relapse and remission. B. To examine the effect of MS treatments - methylprednisone and interferon beta - on cathepsin and cystatin expression. **Methods:** Expression levels of the cathepsins and cystatins were analyzed in RNA of PBL from MS patients and matched healthy individuals, using real-time polymerase chain reaction, and in their serum proteins, using enzyme-linked immunosorbent assay. **Results:** CTSS RNA expression was significantly elevated both in remitting and relapsing MS patients in comparison with healthy controls; CTSS protein expression was significantly elevated only in the relapse state of MS patients compared with healthy controls. Following steroid treatment of relapsing MS patients, RNA and protein levels of CTSS were significantly reduced, and a reduction in the serum protein levels of pro-cathepsin B was observed. In parallel, a significant increase in the protease inhibitor cystatin C serum protein levels was observed. Following interferon beta treatment, protein levels of cathepsin S were significantly reduced, and PBL RNA levels of CSTC and CSTB were significantly increased. **Conclusions:** We observed increased expression levels of cathepsin S in PBLs and serum samples from MS patients. Glucocorticoids caused a decrease in cathepsin levels, concomitant with an increase in their inhibitors. We suggest cathepsin activity may contribute to MS pathology and may serve as a target for therapy.

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Phenotype of cerebrospinal fluid T cells induced by glatiramer acetate

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Background: Treatment with glatiramer acetate (GA) induces GA-reactive T cells which secrete anti-inflammatory cytokines and neurotrophic factors. To be relevant in multiple sclerosis (MS), such T cells must penetrate the blood brain barrier and exert their effector functions within the central nervous system (CNS). **Objective:** To compare GA-reactive T cells from cerebrospinal fluid (CSF) and blood before and during GA treatment. **Methods:** We compared the cytokine profile, cross-reactivity and HLA restriction of GA-reactive T cells from the CSF and blood of three MS patients before and during 3–6 months of GA treatment, and in two patients during long-term GA treatment. T cell lines were generated by GA stimulation of T cells from CSF and blood, and GA-reactive T cell lines were cloned by limiting dilution. **Results:** GA-reactive T cells displayed a predominantly anti-inflammatory phenotype characterized by high levels of interleukin 5 and low levels of interferon gamma, which was more pronounced in CSF. GA treatment induced a more pronounced anti-inflammatory cytokine profile in CSF compared with blood. Both HLA-DR and DP-restricted GA-reactive T cells were cloned from CSF, whereas only DR-restricted T cell clones were cloned from blood. GA-specific T cells from CSF did not cross-recognize complete myelin basic protein or a panel of synthetic peptides spanning seven myelin proteins. **Conclusions:** GA treatment induces a profound anti-inflammatory T cell phenotype in the CSF, supporting an anti-inflammatory mechanism for GA in the CNS. Cross-recognition of myelin proteins may not be necessary for the mechanism of GA.

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Oligoclonal bands in multiple sclerosis: differences in repeated cerebrospinal fluid examinations

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Background: Generally, oligoclonal bands (OBs) are found in a very high percentage in definite multiple sclerosis (MS) cases and MS patients rarely lack OBs. Nevertheless, OBs can be less frequent at the onset of MS, and over time OBs do appear. Moreover, in MS patients, OBs can appear months after the first negative result. **Objective:** In our study, we retrospectively analyzed serial cerebrospinal fluid (CSF) obtained from MS patients to determine the effects of repetition of CSF examination on OB positivity. **Methods:** We investigated the serum and CSF from 276 clinically definite MS patients. Demographic and clinical features were recorded. OBs were detected with isoelectric focusing. Mean age was 35.1, and mean disease duration at first CSF examination was 3.6 years. **Results:** OB was positive in 199 patients (72.1%) in the first testing. In 41 out of 77 remaining patients OB determination was repeated, and 26 (63.4%) were found to have positive OB. The total percentage of OB-positive patients was 81.5 after second lumbar puncture. OB testing was repeated in 15 of the OB-negative patients, and four of them (26.6%) were found to have OB. Total OB-positive patients was 82.9% after the third testing. **Conclusions:** The data obtained from this study ascertained that a second OB test is sufficient, following a negative first test, but a third examination adds no further information about the appearance of OBs. In addition, once detected, OBs are permanent in MS patients.

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Inflamed blood-brain barrier promotes recruitment of effector memory CD8⁺ T lymphocytes and partakes in the pathogenicity of multiple sclerosis

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Background: Dysregulation of the blood-brain barrier (BBB) and transendothelial migration of immune cells are among the earliest central nervous system (CNS) changes partaking in lesion formation in multiple sclerosis (MS). T lymphocytes found in MS lesions encompass CD4⁺ (T helper-T_H) and CD8⁺ (T cytotoxic-T_C) lymphocytes. The number of CD8⁺ T lymphocytes present in MS lesions reaches or surpasses that of CD4⁺ T lymphocytes. Clonally expanded CD8⁺ T lymphocytes have been found in MS lesions, and its animal model experimental autoimmune encephalomyelitis (EAE) as well as in the cerebrospinal fluid (CSF) of MS patients. However the phenotype of migrating CD8⁺ T lymphocytes and the mechanism by which such cells cross the BBB remain largely unknown. **Objective:** To evaluate the phenotype of and mechanism by which CD8⁺ T lymphocytes access the CNS. **Methods:** We used 1) an *in vitro* model of the human BBB 2) CNS material from EAE animals and 3) CSF from MS patients, to evaluate the mechanism by which CD8⁺ T lymphocytes can migrate to the CNS. **Results:** Using CSF obtained from MS patients and spinal cord material from MOG₃₅₋₅₅-induced EAE, we demonstrate that CD8⁺ T lymphocytes are mostly of the effector memory (EM) phenotype (CD8⁺ CD62L⁺ CCR7⁺ CD28⁺ GranzymeB^{hi}). We further show that purified human CD8⁺ TEM lymphocytes transmigrate more readily across human BBB endothelial cells than *ex vivo* unfractionated CD8 lymphocytes and that BBB endothelium promotes the selective recruitment of CD8⁺ TEM lymphocytes. Furthermore, we provide evidence for an active and selective recruitment of IFN- γ and IL-17-secreting CD8⁺ lymphocytes by human and mouse BBB endothelium, *in vitro* and *in vivo*. Finally we show that the migration of CD8⁺ T lymphocytes across BBB-ECs is dependent on VLA-4, but independent of ICAM-1/LFA-1, and P-selectin. **Conclusions:** Our study thus provides evidence for an active role of the BBB in the recruitment of potentially auto-aggressive CD8 TEM lymphocytes to the CNS.

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Decreased T-bet expression in circulating T cells from women affected by multiple sclerosis during pregnancy

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Background: During pregnancy, women affected by multiple sclerosis (MS) usually experience a decrease in clinical disease activity and the rate of MS relapse declines over the course of pregnancy. Following delivery, the rate of relapse increases before returning to pre-pregnancy levels 3–6 months postpartum. The expansion of the maternal CD4⁺CD25⁺ regulatory T cell pool seems to contribute to the maternal tolerance to fetus in mice and human and to be necessary for pregnancy success. **Objective:** To investigate the mechanisms by which pregnancy suppresses clinical disease and to examine the cause for the increase in disease activity during the postpartum period in MS. **Methods:** We evaluated by cytofluorimetry the mean expression of pSTAT1, pSTAT3 and T-bet in circulating CD4⁺, CD8⁺ and monocytes and the number and mean expression of Foxp3 in circulating CD4⁺CD25⁺ T cells (T-regs) from 10 pregnant relapsing-remitting MS patients (PMS) during every trimester of gestation (12th, 24th and 36th week) and one month after delivery and from 20 non-pregnant MS patients in remission (NPMS) and 20 non-pregnant healthy women (NPHC). **Results:** PMS at the 1st and 2nd trimester of pregnancy showed lower T-bet mean fluorescence intensity (MFI) in CD4⁺ T cells than NPMS and NPHC. pSTAT1 and T-bet MFI were lower in CD8⁺ T cells from PMS at the 2nd trimester of pregnancy than in ones from NPMS and NPHC. Foxp3 MFI in CD4⁺CD25⁺ T cells and the percentage of CD4⁺CD25⁺Foxp3⁺ T cells were higher in PMS at the 2nd trimester of pregnancy than in NPMS and NPHC. **Conclusions:** In women affected by MS the decrease in clinical disease activity over the course of pregnancy might be due to the expansion of circulating CD4⁺CD25⁺Foxp3⁺ regulatory T cells and to the lower expression of T-bet in T cells.

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Intrathecal anti-Epstein Barr virus responses in distinct multiple sclerosis subgroups

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Background: Several studies suggest enhanced intrathecal responses against Epstein Barr virus (EBV) in multiple sclerosis (MS). **Objective:** To measure viral capsid antigen (VCA) and Epstein-Barr nuclear antigen (EBNA1) antibody levels in serum and cerebrospinal fluid (CSF) from MS patients and controls. **Methods:** CSF and serum was collected from a total of 134 patients with relapsing-remitting MS (RRMS) (21), primary progressive MS (PPMS) (10), clinically isolated syndrome (CIS) (44) and other neurological diseases (OND) (59). Anti VCA and EBNA1 IgG responses were quantified using enzyme-linked immunosorbent assay, as well as with a semi-quantitative immunoblot assay measuring EBV core antigens. Correcting for a possible dysfunction of blood-CSF barrier, we used the formula (IgG CSF/IgG serum)/(albumin CSF/albumin serum). The results were statistically tested using the Kruskal Wallis, Mann-Whitney U and χ^2 -square tests. **Results:** All MS and CIS sera tested anti-VCA and EBNA1-positive, whereas between 10 and 20% of OND controls were negative. Anti-VCA and -EBNA1 IgG serum levels were significantly higher in MS and CIS patients compared with OND (p<0.0001). Anti-EBNA1 CSF levels were specifically elevated in MS and CIS patients, and this finding remained after correction for blood-CSF barrier dysfunction (p=0.021). The specificity of higher EBNA reactivity in CSF was strengthened by testing CMV and VCA IgG levels within the same serum and CSF samples, which showed no difference. PPMS patients showed higher, but non-significant intrathecal EBNA levels compared with RRMS. In the 44 CIS patients tested, intrathecal EBNA1 responses were not associated with skewed progression to definite MS. **Conclusions:** Humoral responses against the latent EBV antigen

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EBNA1, but not VCA are elevated in CSF of MS patients, and perhaps higher in PPMS. After CIS, there was no correlation with a second attack.

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Antibody to aquaporin-4: serum levels correlate with clinical disease activity

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Background: Neuromyelitis optica (NMO) is a severe inflammatory central nervous system (CNS) disorder of putative autoimmune etiology, which predominantly affects the spinal cord and optic nerves. Recently, a highly specific serum reactivity to CNS microvessels and subpial was described in patients with NMO (called NMO-IgG). Subsequently, aquaporin-4 (Aqp4), the most abundant water channel in the CNS, was identified as its target antigen. Support for a pathogenic role of the antibody would come from studies demonstrating a correlation between Aqp4-Ab titers and the clinical course of disease. **Objective:** In this study, we determined Aqp4-Ab serum levels in 96 samples from eight NMO-IgG positive patients (median follow-up 62 months). **Methods:** Sera were tested in a newly developed fluorescence-based immunoprecipitation assay employing recombinant, full length human Aqp4. **Results:** We found that Aqp4-Ab serum levels correlate well with clinical disease activity, with relapses being preceded by an increase in Aqp4-Ab levels. Moreover, Aqp4-Ab levels were found to correlate with CD19 cell counts under therapy with rituximab. Treatment with immunosuppressants such as rituximab, azathioprine, and cyclophosphamide resulted in a marked reduction in antibody levels and relapse rate. **Conclusions:** Our results support the hypothesis of Aqp4-Ab being involved in the pathogenesis of NMO.

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Evaluation of biomarkers for the response to interferon-β treatment in multiple sclerosis

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Background: One biological response to treatment with IFN-β is the induction of MxA, but the development of neutralizing antibodies (NABs) may abolish the biological response and treatment effect of IFN-β. **Objective:** The objective was to identify new biomarkers for response to IFN-β in multiple sclerosis (MS) patients. **Methods:** A series of potential biomarkers were analysed by gene expression on DNA arrays (Affymetrix GeneChip® Human Focus Array) and RT-PCR (ABI PRISM 7500 Real Time PCR System). Blood samples were collected in PAXgene™ Blood RNA tubes. **Results:** After three and six months therapy 1200–1600 genes had lower expression levels and

290–315 had higher expression levels 9–12 hours after injection of IFN-β as compared with pre-injection. The most strongly induced genes were IFI27 (interferon α-induced protein 27), and the chemokines CCL2, CCL8, and CXCL10. The MxA-encoding gene was also among the strongly induced genes. Analysis of samples taken 36–48 hours after IFN-β injection demonstrated that the gene expression for the twenty strongest induced genes was reduced compared with the 9–12 hours time point, except for IFI27, which retained a similarly high level of expression. Using GAPDH as reference gene, the indices of IFI27, CCL2, CXCL10 and MxA mRNA expression were significantly higher in IFN-β-treated MS patients than in healthy controls. However, untreated MS patients demonstrated higher indices than healthy controls, thus defining a grey-zone of uncertainty for mRNA induction by IFN-β in MS patients. In NAB-negative IFN-β-treated patients IFI27, CCL2, CXCL10 were more strongly induced than MxA, and a reduced overlap was observed between treated and untreated MS patients. Analysing IFN-β-treated patients grouped by the varying presence of NABs, similar discrimination of the patients was seen for each of the tested markers as compared to MxA. **Conclusions:** IFI27 is identified as an attractive candidate biomarker for assessing the biological response to IFN-β in MS patients. Compared with MxA, IFI27 appears superior in terms of degree and duration of induction.

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VEGF-A induces blood-brain barrier permeability via down-regulation of endothelial CLN-5

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Background: Permeability of the blood-brain barrier (BBB) is an early and significant event in lesion pathogenesis in multiple sclerosis (MS), but the mechanisms involved remain elusive. The barrier exists at the level of brain microvascular endothelial cells (BMVEC) which restrict paracellular diffusion via tight junctions containing claudins (CLN), occludin, and junction adhesion molecules (JAM), with selective barrier properties dependent on CLN-5. Formation and regulation of the endothelial barrier depend on other lineages, notably astrocytes and pericytes. **Objective:** Here, we investigated mechanisms underlying BBB permeability in models of MS. **Methods:** These experiments used a functional genomics-based approach. **Results:** We found that BBB permeability is accompanied by coordinated changes in tight junction proteins in both BMVEC and reactive astrocytes. In cultures of BMVEC, both CLN-5 and occludin were lost in response to the astrocyte-derived factor VEGF-A. In primary human astrocyte cultures, IL-1β or TGFβ1 induced VEGF-A, and also triggered expression of CLN-1, CLN-4, and JAM-1. In experimental autoimmune encephalomyelitis, an animal model of MS, barrier permeability was associated with reduced endothelial CLN-5 and induction of astrocytic CLN-1 and CLN-4. Parallel functional studies showed that IL-1β-treated astrocytes induced HBMVEC permeability via VEGF-A. HBMVEC cultures expressing CLN-5 under the control of a VEGF-insensitive promoter were protected from VEGF-induced permeability. **Conclusions:** Taken together, these data implicate VEGF-mediated downregulation of CLN-5 as a significant mechanism in BBB permeability in MS. They further suggest that loss of the endothelial barrier is accompanied by organization of surrounding astrocytes to form a structure that may regulate macromolecular traffic in the lesions of the disease. **Supported by:** The authors thank Dr. Bradford Poulos, Director of the Human Fetal Tissue Repository at the Albert Einstein College of Medicine, for tissue collection, and the Mount Sinai School of Medicine Microscopy Shared Resource Facility. Supported by USPHS Grants NS46620 and NS056074 (both GRJ). Supported by National Multiple Sclerosis Society (USA) Postdoctoral Fellowship FG1739 (YZ) and Research Grants RG3874 (GRJ), and RG3827-A5 (CFB), and by the Jayne and Harvey Beker Foundation (GRJ). The MSSM-Microscopy Shared Resource Facility is supported, in part, with funding from NIH-NCI shared resources grant R24 CA095823.

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Interferon- β treatment of multiple sclerosis patients induces SHP-1 expression and modulates pro-inflammatory gene expression in peripheral blood mononuclear cells in vivo

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Background: Recent studies in mice have demonstrated that the protein tyrosine phosphatase SHP-1 is a crucial negative regulator of cytokine signaling, inflammatory gene expression, and demyelination in central nervous system. Furthermore, in mice SHP-1 is induced by interferons resulting in increased modulation of inflammatory gene expression (Christophi et al., *J Neurochem.* 2008). Recently, we demonstrated that peripheral blood mononuclear cells (PBMCs) of multiple sclerosis (MS) patients have a stable deficiency in SHP-1 expression relative to normal control subjects, which we believe is responsible for augmented STAT6-activation and STAT6-responsive proinflammatory gene expression in these cells (Christophi et al., *Lab Invest.* 2008). **Objective:** Investigate whether IFN- β modulates the expression and activity of the phosphatase SHP-1. **Methods:** Relapsing-remitting MS patients received a three-month treatment with IFN- β (Rebif, Serono/Pfizer) and the expression of SHP-1 and inflammatory genes was quantified with real time polymerase chain reaction, Western immunoblots, and flow cytometry. **Results:** IFN- β treatment resulted in a significant increase in SHP-1 protein and mRNA expression in freshly drawn PBMCs. Two promoters drive the expression of two distinct transcripts of the SHP-1 gene. Interestingly, promoter II transcripts, but not promoter I transcripts, were increased in PBMCs of MS patients following the 3-month treatment with IFN- β . In order to examine functional consequences of IFN- β -mediated SHP-1 induction in PBMCs, we analyzed STAT6-activation and STAT6-responsive genes in freshly isolated PBMCs before and after the 3-month therapeutic treatment period. As expected from in vitro studies, in vivo treatment of MS patients with IFN- β effectively reduced STAT6-activation and STAT6-inducible gene expression in MS patients to normal levels. **Conclusions:** IFN- β treatment normalizes SHP-1 deficiency seen in MS patients and results in a downregulation of STAT6-responsive genes that may play an important role in disease pathogenesis. **Supported by:** Serono/Pfizer, the National Multiple Sclerosis Society (USA) (RG2569CS) the NIH (NS041593).

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Suppression of experimental autoimmune encephalomyelitis by selective inhibition of Notch3 receptor in myelin-reactive T cellsMaciej Jurynczyk¹, Anna Jurewicz¹, Cedric S. Raine², Krzysztof Selmaj¹¹Department of Neurology, Medical University of Lodz, Lodz, Poland; ²Albert Einstein School of Medicine, Bronx, New York, USA

Background: Notch signaling, which is critical for many developmental processes, also regulates peripheral T cell responses. The activation and differentiation of Th1 cells are essential steps in the pathogenesis of multiple sclerosis (MS) and experimental autoimmune encephalomyelitis (EAE). It has been previously shown that γ -secretase inhibition of all four Notch receptors blocks Th1 type polarization and ameliorates the clinical course of EAE. **Objective:** In this study we have examined the role of individual Notch receptors in peripheral responses of myelin-reactive T cells. **Methods:** Using CFSE assay, quantitative PCR, immunoblotting, ELISA and EAE models we have assessed proliferation, cytokine production and encephalitogenic potential of proteolipid protein (PLP)-reactive T cells. **Results:** We have found that specific antigenic stimulation of PLP-reactive T cells induces specifically transcription of Notch1 and Notch3 mRNA. However, using γ -secretase inhibitor and antibodies neutralizing distinct Notch receptors, we have observed that selective inhibition of Notch3, but not Notch1, receptor decreased proliferation, Th1- and Th17-type responses of PLP-reactive T cells. Moreover, suppression of Notch3 signaling in T cells downregulated expression of Protein Kinase C θ , an important regulator of mature T cell functions. **Conclusions:** Thus, by reducing encephalitogenicity of myelin-reactive T cells, selective inhibition of Notch3 receptor may offer a new attractive target in treating autoimmune diseases, including MS.

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Glatiramer acetate treated B cells suppress experimental autoimmune encephalomyelitis

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Background: Multiple Sclerosis (MS) is a T cell-mediated autoimmune disease characterized by inflammatory and demyelinating lesions throughout the central nervous system (CNS). However, recent evidence indicates that B cells are present in the CNS and play an important role in MS. B cells can present antigen to T cells, regulate T cell differentiation and their immune responses through differential production of regulatory cytokines. Glatiramer acetate (GA) is one of the FDA-approved drugs used for MS treatment and is characterized by an excellent safety profile. The mechanism of action of GA in MS has been elusive. **Objective:** We hypothesize that GA exerts its beneficial effect in MS by: i) modulating the antigen-presenting property of B cells, ii) modulating effector B cells to differentiate into regulatory B (Reg) cells and iii) mediating its effect through other regulatory cells. **Methods:** To test this hypothesis we purified B cells from control mice or mice injected with GA. These B cells were then transferred to naive mice followed by induction of experimental autoimmune encephalomyelitis (EAE). **Results:** GA-treated B cells suppressed EAE. Also, B cell-deficient mice pre-treated with GA did not suppress EAE compared with wild-type mice. GA-treated B cells had a decreased cell surface expression of CD80 and CD86 co-stimulatory molecule as well as reduced MOG33-55 peptide presentation to MOG TCR transgenic T cells when compared with untreated B cells. Although GA did not modulate differentiation of B cells to regulatory B cells, there was a significant increase in the number of NKT and NK cells in mice with adoptively transferred GA-treated B cells. **Conclusions:** We conclude that GA-specific B cells may, at least in part, be responsible for the suppression of EAE resulting from treatment with GA by inhibiting activation of encephalitogenic T cells and expansion of NK and NKT cells with regulatory function.

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Defective regulation of BDNF, NGF and NT3 mRNA production after CD40 stimulation in immune cells of patients with relapsing-remitting multiple sclerosisNatali Urshansky¹, Karin Mausner², Arnon Karni¹¹Neurology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; ²Sackler Medical School, Tel Aviv University, Tel Aviv, Israel

Background: Recent studies have suggested a neuroprotective activity of lesion-immune cells in multiple sclerosis (MS) by neurotrophins. We have reported that peripheral blood mononuclear cells (PBMCs) from relapsing-remitting MS (RRMS) patients secrete low brain-derived neurotrophic factor (BDNF) levels, and its up-regulation after CD40 stimulation is defective. **Objective:** To study the production and regulation of BDNF, nerve growth factor (NGF) and neurotrophin 3 (NT3) mRNAs in immune cells of patients with RRMS. **Methods:** PBMCs from 25 patients with RRMS (13 untreated, 12 treated with interferon- β) and 17 matched healthy controls (HC) were incubated for 1 hr with anti-CD40 monoclonal antibodies (mAb) or its isotype controls (IC). mRNA quantity of BDNF, NGF and NT3 in PBMCs was studied by real-time qRT-PCR reactions with appropriate primers, while GAPDH gene was run in parallel as internal control. Results are expressed by the production ratio of each neurotrophin mRNA vs. GAPDH mRNA. **Results:** Basal BDNF mRNA expression was similar in patients (3.6 ± 1.0) and HC (3.9 ± 0.9). NGF mRNA tend to be lower in patients (1.4 ± 0.4) vs. HC (2.9 ± 0.8 , $p=0.11$) and NT3 mRNA was lower in patients (2.2 ± 0.8) vs. HC (4.6 ± 1.2 , $p<0.05$). CD40 stimulation of PBMCs of HC up-regulated mRNA expression of BDNF, NGF and NT3 as compared with the effect of IC (BDNF mRNA: 9.5 ± 3.1 vs. 3.0 ± 0.7 , respectively, $p=0.04$; NGF mRNA: 6.0 ± 0.8 vs. 2.8 ± 0.8 , respectively, $p<0.05$; NT3 mRNA: 9.6 ± 2.9 vs. 4.1 ± 1.2 , respectively, $p<0.05$). No significant effect of CD40 stimulation on the expression of neurotrophins mRNA was found in the patients. We compared the up-regulatory capacity of CD40 stimulation using the ratio of mRNA production after CD40 stimulation vs. IC between HC and patients.

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BDNF mRNA: 2.7 ± 0.5 vs. 1.1 ± 0.1 , respectively, $p < 0.001$, NGF mRNA: 4.1 ± 1.3 vs. 1.4 ± 0.4 , respectively, $p = 0.008$, NT3 mRNA: 3.8 ± 1.0 vs. 1.0 ± 0.3 , respectively, $p = 0.005$. No significant differences in the expression of neurotrophins' mRNA were found between untreated and interferon- β treated patients. **Conclusions:** The neuroprotective potential of PBMCs seems to be defective in patients with RRMS as there is a trend for reduced production of neurotrophins' mRNA and a lack of an up-regulatory effect of CD40 stimulation on their production.

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Immediate broad spectrum immunomodulating effects induced by bone marrow derived stem cells (MSC), in multiple sclerosis patients transplanted with MSC in a phase I/II clinical trial

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Background: Bone marrow (BM) mesenchymal stem cells (MSC) possess strong neurotrophic effects and have become the focus of research as a potential method for inducing neuroprotection in diseases such as multiple sclerosis (MS). MSC were also proven to exert strong in vitro immunomodulatory effects. **Objective:** To evaluate the immediate immunomodulatory effects of MSC in MS patients in a phase I/II clinical trial with intravenous and intrathecal administration of MSC. **Methods:** A total of 14 MS patients were injected with autologous MSC, following their culture for 40–90 days. All patients received intrathecally a mean of 64.4, and 10 of them intravenously, a mean of 22 million cells. We performed an immunological analysis in 6 of the patients before the injection of the stem cells at 4 and 24 hours later. Peripheral blood monocytes were obtained and the following tests were performed: a) FACS analysis for the expression of markers of regulatory cells (CD4/CD25/FoxP3), myeloid dendritic cells activation markers (CD86, CD83 and HLA-DR), T-cell activation markers (CD69), b) lymphocyte proliferations assay and c) cytokine production. **Results:** All tested patients showed significant changes in all the above immunological tests, starting as early as 4 hours following the injection of MSC, including a 30–50% increase in the proportion of CD4+CD25+ regulatory T cells and 30–60% reduction of CD83 expression on myeloid dendritic cells. In addition, in vitro a significant decrease was noted in the proliferative responses upon stimulation of the lymphocytes with anti-CD3 or the PHA mitogen as well as a significant decrease in IL-17 secretion. **Conclusions:** This is the first trial with intrathecal and intravenous injection of MSC in MS and revealed the strong immediate immunomodulatory effects induced by the injection of these cells. Further controlled studies and longer observation periods are needed to evaluate possible efficacy and long-term clinical and immunological effects of MSC transplantation.

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Identification of double negative T cells expressing IL-17 in multiple sclerosis

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Background: IL-17 plays a critical role in autoimmune and allergic conditions: disease progression of experimental allergic encephalitis is severely attenuated in IL-17-deficient mice and IL-17-secreting lymphocytes have been detected in the cerebrospinal fluid of multiple sclerosis (MS) patients, but their identity and properties remain elusive. **Objective:** To characterize and evaluate the function of IL-17-producing cells in MS. **Methods:** Peripheral blood was obtained from patients with relapsing-remitting MS, other neuro-inflammatory diseases (ONID), and healthy volunteers (HS). We analyzed the expression of several chemokines and chemokine receptors on the cell surface of peripheral mononuclear cells (PBMCs) prior to stimulation. After PBMCs were stimulated with PMA and ionomycin, we detected

intracellular IL-17 and IFN- γ by flow cytometry. **Results:** Within the CD4 T cell population (CD3+CD4+CD8-), the frequency of IFN- γ -producing cells (TH1), IL-17-producing cells (TH17), and Foxp3-expressing Treg cells in PBMCs did not differ between MS, ONID, and HS. However, within the double negative T (DNT) cell population (CD3+CD4-CD8-), IFN- γ -producing cells decreased in frequency in PBMCs from MS as compared with HS. More strikingly, IL-17-producing cells were more frequent in DNT cells from MS as compared with ONID and HS. These results suggest that the distinct pool of IL-17-producing DNT cells in MS might be larger than that in ONID and HS, whereas the pool of IFN- γ -producing DNT cells might be smaller. Innate T cells, including $\gamma\delta$ T cells, V α 24iT and V α 7.2iT cells constitute a large percentage of DNT cells. In mice, it has been shown that innate T cells such as NK1.1negV α 14iT have the ability to produce IL-17 in airway neutrophilia and other disorders. Therefore we suspect that not only TH17 *per se*, but also innate T cell production of IL-17 might be critical elements contributing to autoimmune disorders such as MS. **Conclusions:** We identified the distinct population of DNT cells expressing IL-17 in MS.

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Human T_H17 memory lymphocytes promote blood-brain barrier disruption and infiltration of inflammatory T cells into the central nervous system

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Background: The blood-brain barrier (BBB) plays a crucial role in protecting the central nervous system (CNS) by restricting entry of cells and molecules into the brain. In the CNS disorder multiple sclerosis (MS), breakdown of the BBB allows activated leukocytes to infiltrate the brain parenchyma, leading to the formation of the characteristic demyelinated lesions. Interleukin (IL)-17-secreting lymphocytes (T_H17) appear to be essential in the pathogenesis of numerous inflammatory diseases, including MS. **Objective:** To determine the contribution of human T_H17 lymphocytes to the disruption of the BBB and leukocyte infiltration into the CNS, both important early events in the development of MS. **Methods:** We developed and optimized a method to successfully generate human T_H17 lines *in vitro* from peripheral blood CD4⁺CD45RO⁺ memory lymphocytes of healthy donors and MS patients. We measured IL-17, IL-22 and IFN- γ levels by intracellular cytokine staining/flow cytometry analysis. We studied T_H17 lymphocyte migration across the endothelium using *in vitro* and *in vivo* models of trans-BBB migration. **Results:** We demonstrate that in response to IL-23, human T_H17 memory CD4⁺CD45RO⁺ lymphocytes, but not naive CD4⁺CD45RA⁺ lymphocytes, produce IL-17 and IL-22, with a subset of cells co-expressing IL-17 and IFN- γ . We show that IL-17 and IL-22 disrupt BBB tight junctions *in vitro* and *in vivo* and that receptors for these cytokines are upregulated on human endothelium during inflammation. Moreover, human T_H17 cells transmigrate efficiently across brain endothelial cells and promote CD4⁺ lymphocyte recruitment across the BBB, through the concerted action of IL-17 and IL-22. Interestingly, when generating T_H17 lines from the peripheral blood of women with active MS, we note an increase in the percentage of IL-17⁺, IL-22⁺ and double IL-17⁺ IFN- γ ⁺ lymphocytes compared with those generated from healthy men and women controls. **Conclusions:** Our study further refines the phenotype of human T_H17 lymphocytes and emphasizes the importance of T_H17 lymphocyte infiltration into the CNS and their consequent involvement in lesion formation in MS.

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B-cell driven humoral immune response in clinically aggressive African-American multiple sclerosis: a rational disease model for B-cell depleting therapy

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Background: Several studies have suggested that compared with Caucasians (CAC) with multiple sclerosis (MS), African-Americans (AA) with MS experience more aggressive disease course and acquire greater disability in a shorter period of time. The role of B-cell driven humoral immune responses in MS pathogenesis is complex. Preliminary data suggest that cerebrospinal fluid (CSF) humoral immune responses may be elevated in AA MS although it is unclear if they contribute to the aggressive disease course observed in AA MS patients. **Objective:** We conducted a study to examine the CSF humoral immune response in AA with MS, including correlation with clinical outcomes and multi-parametric magnetic resonance imaging (MRI) measures of inflammatory tissue injury. **Methods:** We examined the CSF data, clinical demographics, and MRI scans of AA and CAC MS patients. MRI data included conventional metrics as well as magnetization transfer ratio, magnetic resonance spectroscopy, and C2 cervical cord volume. **Results:** CSF data of 150 consecutive AA were compared with 150 CAC MS patients. The mean CSF IgG index was 1.5 in AA and 1.08 in CAC ($p < 0.0001$). 93.4% of AA and 92.5% of CAC MS patients were on disease-modifying therapy (DMT). Mean time from disease onset to initiation of DMT was 3.55 in AA and 3.45 years in CAC. Mean expanded disability status scale was 4.32 in AA and 3.15 in CAC MS patients ($p < 0.01$). Mean NAA/Cr, NABT MTR, and C2-cord volume were all significantly ($p < 0.0001$) reduced in AA. Gadolinium-enhancing lesions were significantly ($p = 0.04$) greater in AA (2.21) than CAC (1.03). CSF IgG index demonstrated robust inverse correlation with MTR and gadolinium-enhancing lesions in AA. Further statistical analysis including analysis of oligoclonal banding is pending. **Conclusions:** This study provides evidence for B-cell driven humoral effector mechanisms that may be responsible for exaggerated inflammatory tissue destruction in AA MS. This may provide the rationale for using therapies that modify B-cell behavior and also explain why first-line DMT therapies are relatively less effective in AA. Large scale therapeutic trials targeting B-cell depletion are warranted in AA with MS as well as non-AA MS patients with clinically aggressive disease.

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Increased severity of experimental autoimmune encephalomyelitis in B cell activating factor receptor-deficient mice is associated with elevated B cell activating factor expression

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Background: B cell activating factor (BAFF) is critical for B cell survival, a function that is mediated through the BAFF receptor, (BAFF-R). Whether BAFF and its receptors are important in central nervous system (CNS) inflammation is unclear. **Objective:** To study the role of BAFF-R on B cells, T cells and macrophages in the development of experimental autoimmune encephalomyelitis (EAE). **Methods:** BAFF-R knockout (KO) mice and B6 controls were immunized with MOG p35-55 peptide and compared for the development of weakness and CNS inflammation. T cells, activated macrophages and BAFF-expressing cells were determined using immunostaining and confocal microscopy. BAFF and cytokine secretion were assayed by enzyme-linked immunosorbent assay. **Results:** BAFF-R KO mice had earlier onset of disease (mean day of onset 11.4 ± 0.4 (SE), $n = 7$ vs. 16.1 ± 1.1 , $n = 7$, $p = 0.0014$) and larger inflammatory foci compared with controls (mean area 101 ± 23 (SE) $\times 10^3$ square microns, $n = 29$ vs. $19.3 \pm 6.7 \times 10^3$, $n = 16$, $p = 0.007$) as described previously.

Immunostaining revealed elevated levels of activated macrophage marker IBA-1, which co-localized with increased BAFF expression in inflammatory foci of BAFF-R KO mice compared with controls. Cultured splenocytes from MOG-immunized BAFF-R KO mice, compared with immunized B6 controls, showed increased secretion (pg/mL) of BAFF (223 ± 49 (SE) vs. undetectable, $p = 0.0014$), IL-2 (435 ± 26 (SE) vs. 172 ± 6 , $p = 0.0006$), and IL-6 (166 ± 6 (SE) vs. 51 ± 7 , $p = 0.0003$). **Conclusions:** MOG p35-55 peptide-induced EAE in BAFF-R KO mice is characterized by earlier onset of disease and more extensive CNS inflammation. The latter consisted of larger numbers of activated macrophages, many of which expressed BAFF, which correlated with increased BAFF secretion in the periphery. Our results suggest that BAFF-R may be important in down-regulating EAE, possibly by influencing macrophage function through a mechanism that appears to involve modulation of BAFF expression.

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Distinct cerebrospinal fluid cytokine/chemokine profiles in opticospinal MS, atopic myelitis and other causes of myelitis

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Background: We reported the emergence of a distinct myelitis in patients with atopic diathesis (atopic myelitis, AM) by a nationwide survey throughout Japan. Similar cases have recently been reported in Caucasians. Pathological studies of biopsied spinal cord specimens revealed chronic active inflammation with eosinophilic infiltration. In addition, antibodies to aquaporin-4 (AQP4) are found in a fraction of Japanese patients with opticospinal multiple sclerosis (OSMS). **Objective:** To clarify the cerebrospinal fluid (CSF) cytokine /chemokine profiles in AM and OSMS with and without anti-AQP4 antibody, and other causes of myelitis. **Methods:** We measured 27 cytokines, chemokines and growth factors simultaneously in CSF from 22 patients with AM, 20 with OSMS, 11 with HTLV-1-associated myelopathy (HAM), 9 with Sjögren syndrome-related myelitis (SM) and 20 with other non-inflammatory neurological diseases (OND), using a fluorescent bead-based immunoassay. **Results:** In AM patients, CCL11 (eotaxin) and IL-9 were significantly increased as compared with OND and other myelitis patients, while in OSMS patients IFN- γ and granulocyte-colony stimulating factor levels were significantly higher than patients with OND and other causes of myelitis. Significant increase of IL-17 in comparison with OND patients was found only in OSMS patients, irrespective of presence or absence of anti-aquaporin-4 (AQP4) antibody. In the anti-AQP4 antibody-positive OSMS patients, IFN- γ and CXCL10 (IP-10) were markedly upregulated even compared with the antibody-negative OSMS patients. In HAM patients, CXCL10 and CCL5 (RANTES) were higher than OND and other myelitis patients. In SM patients, CCL3 (macrophage inflammatory protein (MIP)-1 α) and CCL4 (MIP-1 β) were higher than in OND patients. In AM patients, CCL11, IL-9 and IL-1ra showed positive correlations with the final Kurtzke's Expanded Disability Status Scale scores while IL-1ra and IL-12(p70) had positive correlations with disease duration. **Conclusions:** Intrathecal upregulation of CCL11 and Th2 cytokines is characteristic of AM, which is distinct from IL-17/IFN- γ -related autoimmune condition of OSMS.

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P350 **$\gamma\delta$ T cell-derived IL-1 β alters the pattern of expression of CXCL12 at the blood-brain barrier during central nervous system autoimmunity**

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Background: Prior studies indicate that central nervous system (CNS) expression of the chemokine CXCL12 normally occurs along basolateral surfaces of the microvasculature, thereby controlling leukocyte access to the parenchyma. In patients with multiple sclerosis (MS) and in mice with experimental autoimmune encephalomyelitis (EAE), however, CXCL12 is relocated to luminal surfaces of CNS venules, allowing egress of autoreactive leukocytes from perivascular spaces. **Objective:** As the relocation of CXCL12 highly correlates with the extent of perivascular infiltrates, we hypothesized that leukocyte-derived inflammatory mediators relocate CXCL12 via altered expression of CXCL12 receptors. **Methods:** We examined CXCL12 relocation after intravenous administration of cytokines to naive mice and in cytokine receptor-deficient mice immunized with myelin oligodendrocyte glycoprotein (MOG). We also identified perivascular leukocyte subsets during induction of EAE and determined whether leukocyte-derived cytokines affect the endothelial cell expression of the CXCL12 receptors CXCR4 and CXCR7. **Results:** Examination of CNS specimens from MOG-immunized mice at preclinical time-points revealed that venular CXCL12 relocation occurs prior to the onset of clinical disease and that 100% of these venules contain perivascular CD3+, but not CD11b+, cells, which expressed IL-1 β and were CD4+, CD8+ and $\gamma\delta$ TCR+. Administration of IL-1 β , but not TNF- α , induced CXCL12 relocation in 90.1% of vessels and mice with targeted deletion of IL-1R did not relocate CXCL12 at the microvasculature. In contrast, MOG-immunized, TNFR1-/- mice exhibited both relocation of CXCL12 and a predominance of perivascular $\gamma\delta$ T cells, suggesting a role for these cells in relocating CXCL12 at the blood-brain barrier (BBB). In addition, bone marrow chimeric experiments revealed that loss of CNS IL-1R determines the severity of EAE. Finally, in vitro treatment of brain endothelial cells with IL-1 β induced the expression of CXCR7 mRNA. **Conclusions:** These results suggest that the CNS trafficking of $\gamma\delta$ T cells during autoimmunity leads to altered expression of CXCL12 at the BBB via increased endothelial cell expression of CXCR7.

P351**Ablation of peroxisome proliferator-activated receptor gamma in CD4+ T cells results in enhanced central nervous system autoimmunity caused by an increase in antigen specific T helper 17 cells**Luisa Klotz¹, Indra Dani², Waldemar Kolanus³, Ari Waisman⁴, Thomas Klockgether¹, Percy Knolle²¹Dept. of Neurology, University of Bonn, Bonn, Germany; ²Institute of Molecular Medicine and Experimental Immunology, University of Bonn, Germany; ³The Limes Institute, University of Bonn, Germany; ⁴Department of Internal Medicine, University of Mainz, Germany

Background: The peroxisome proliferator-activated receptor gamma (PPAR γ) belongs to a group of ligand-activated transcription factors involved in the regulation of metabolism and inflammation. PPAR γ is expressed in cells of the peripheral immune system, but also within the central nervous system (CNS). **Objective:** Interestingly, oral administration of PPAR γ agonists ameliorates the clinical course and histopathological features in experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis. However, the molecular mechanisms of PPAR γ -mediated suppression of (auto)-immunity are still unclear. **Methods:** We generated mice with a T cell-specific PPAR γ knock-out employing a Cre-recombinase mediated gene ablation which is limited to CD4+ cells. In these mice (CD4-PPAR γ -ko) and their Cre negative wild-type littermates, we investigated T cell responses and disease course during actively induced MOG-EAE. **Results:** CD4-PPAR γ -ko mice exhibited both an earlier disease onset and a significantly increased disease severity

during the induction phase of EAE when compared with wildtype mice. Accordingly, effector T cell infiltration of the CNS was significantly increased, whereas the frequency of local regulatory T cells was significantly decreased. Recall assays employing CD4+ T cells derived from inflamed brains of CD4-PPAR γ -ko animals yielded significantly augmented T helper(H)1 and TH17 responses when compared with wildtype littermates. **Conclusions:** Our data demonstrate that PPAR γ activation impairs T cell differentiation into potentially autoreactive TH1 cells and TH17 cells in vivo. Selective targeting of PPAR γ in T cells therefore represents a promising future strategy for control of T cell mediated autoimmunity.

P352**Prohibitin: prohibiting inflammation in glatiramer acetate-treated multiple sclerosis patients?**

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Background: One approved therapy of patients with relapsing-remitting multiple sclerosis (RRMS) is glatiramer acetate (GA), which among other effects induces a shift in the cytokine profile of CD4+ and CD8+ T cells. As the mechanisms involved are largely unknown, we investigated the regulated proteome of CD4+, GA-specific T cell lines of RRMS patients. Among other proteins we found prohibitin (PHB) upregulated during GA therapy. **Objective:** To validate upregulation of PHB in GA-specific T cell lines and to investigate PHB expression in freshly isolated peripheral blood mononuclear cells (PBMCs) of untreated and GA-treated RRMS patients. **Methods:** GA-specific T cell lines of RRMS patients were generated before (n=4) and during GA therapy (n=4) by the split well technique. Additionally, PBMCs were obtained from untreated (n=12) and GA-treated (n=7) RRMS patients and healthy controls (n=10). We established a FACS staining protocol to quantify the intracellular amount of PHB in T cell lines and PBMCs. **Results:** The FACS analysis of GA specific T cell lines showed a significantly higher amount of PHB in T cells generated during GA therapy than in T cells generated before GA therapy (p < 0.008, t-test). In PBMCs we found an upregulation of PHB in GA-treated MS patients compared with untreated RRMS patients (p < 0.003) and healthy controls (p < 0.01). No difference was found between untreated RRMS patients and healthy controls. **Conclusions:** Our results show that intracellular PHB expression is upregulated during GA treatment in freshly isolated PBMCs and in GA-specific T cells. As PHB is strongly involved in cell differentiation and proliferation and was lately found upregulated in TH2 cells compared with TH1 cells, PHB may be involved in anti-inflammatory mechanisms operating during GA treatment.

P353**Evidence of specific T-cell priming from the first-in-man single ascending dose study of peptide copolymer PI-2301 for the treatment of multiple sclerosis**Joseph Kovalchin¹, Jeffrey Krieger¹, Kathy Collins¹, Kristen Rafuse¹, Tony Avril², Gwenola Gandon², Alain Patat³, Nicolas Fauchoux³, Uday Patel¹, Edward Mascioli¹, Eric Zanelli¹¹Pharmacology/Immunology, Peptimmune, Cambridge, Massachusetts, USA; ²Centre Eugène Marquis, Rennes, France; ³Biotrial S.A., Rennes, France

Background: PI-2301 is a second-generation compound in a class of immunomodulators called peptide copolymers which has shown efficacy in various preclinical models of autoimmune disease. Copolymers are random mixtures of peptide sequences comprised of a limited number of amino acids. Copaxone® is a copolymer which has been approved as a treatment for relapsing-remitting multiple sclerosis (RRMS). PI-2301, like Copaxone, promiscuously binds MHC Class II molecules and induces a skewed TH2, anti-inflammatory immune response. **Objective:** The present study is aimed at evaluating the safety, tolerability and early immunological effect(s) of PI-2301 in a single ascending dose, first-in-man study involving

healthy, male adult volunteers. **Methods:** The clinical study was designed in accordance with recommendations as defined in the Committee for Human Medicinal Products guidelines issued in July 2007 for potentially immunomodulating therapeutics. Fifty-six subjects (eight cohorts of seven individuals; 5 active and 2 placebo) were given a single subcutaneous injection of PI-2301 of either 0.03, 0.1, 0.3, 1, 3, 10, 30 or 60 mg. The parameters evaluated were safety, pharmacokinetics, in vitro T-cell recall responses, antibody response to PI-2301 and changes in serum cytokines and chemokines. **Results:** PI-2301 was generally well tolerated. The only consistent side-effect was transient injection site reactions, first seen with the 1 mg cohort, and observed to be approximately dose-related. Circulating levels of PI-2301 were detected in serum of subjects in the 10, 30 and 60 mg cohorts. No significant anti-PI-2301 IgG antibody titer was found in the serum of treated subjects. Evidence of immune priming (as shown by specific T-cell proliferative response, and IFN γ and IL-13 production) was observed at the 1 mg and higher doses. **Conclusions:** This study represents the initial clinical study in the development of an improved peptide copolymer with immunomodulatory properties targeted for the treatment of multiple sclerosis.

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Changes in adhesion molecule expression induced by natalizumab treatment in multiple sclerosis

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Background: Natalizumab (TysabriTM) treatment is the first monoclonal antibody therapy approved for the treatment of multiple sclerosis (MS). The therapeutic mechanisms of the humanized monoclonal antibody natalizumab is the blockade of α 4-integrin, which leads to an inhibition of immune cell extravasation into the central nervous system. An understanding of effects on further components of the immune system may help to prevent potential side effects or to determine patients who are non-responders to natalizumab treatment early. **Objective:** To investigate changes in the expression of a panel of adhesion molecules as induced by natalizumab treatment in MS. **Methods:** Quantitative expression levels of cell surface-bound intercellular adhesion molecule-1, -2, -3 (ICAM-1, -2, -3), leukocyte function antigen-1 (LFA-1) and α 4-integrin on mononuclear cells (CD3+ T-cells, CD 19+ B-cells, CD 14+ monocytes) in the peripheral blood of ten MS patients were measured by two color flow cytometry (Beckman Coulter) immediately before the first and the fourth infusions of natalizumab. **Results:** We found a significant decrease of unblocked α 4-integrin cell surface expression on all investigated mononuclear subsets (B cells -62%, $p < 0.0001$; T cells -54%, $p < 0.0001$; monocytes -31%, $p < 0.005$) in the blood of MS patients after three months of natalizumab treatment as compared with baseline levels. Moreover, we obtained a consistent decrease of the expression of LFA-1 on mononuclear cells (B cells -7%, $p < 0.05$; T cells -15%, $p < 0.0001$) after three months of natalizumab treatment. **Conclusions:** We obtained impressively consistent results of a sustained decrease of unblocked α 4-integrin expression not only in all patients but also in all investigated leukocyte subgroups. These findings indicate that a significant blockade of cell surface-bound α 4-integrin can be found four weeks after the last natalizumab infusion. Moreover, our data indicate that natalizumab treatment in MS leads to further changes in the expression of cell surface-bound AM, probably as a secondary effect. **Supported by:** Biogen Idec, Austria.

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Low-dose subcutaneous alemtuzumab effectively reduces key lymphocyte subpopulations in patients with severe relapsing-remitting multiple sclerosis

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Background: Recently alemtuzumab was shown to be highly effective in the treatment of relapsing-remitting and secondary chronic progressive MS when used intravenously in dosages between 96mg and 192mg annually in two treatment cycles. The strong clinical efficacy, however, goes along with a broad and partly severe adverse event profile including thrombocytopenic thrombopenic purpura, severe thyroid dysfunction, reactivation of infectious disease and many more. **Objective:** In order to improve its adverse event profile it has been suggested alemtuzumab could be used subcutaneously and in lower dosages. **Methods:** Here we present the - to our knowledge - first case of relapsing-remitting MS (21y, female, Caucasian) treated with alemtuzumab subcutaneously with a reduced dosage of 3mg - 3mg - 6mg and 12mg (=24mg total) for the first treatment cycle. **Results:** Frequent analysis of the lymphocyte subpopulation clearly showed an almost complete reduction of B cells (CD19), T-Cells (CD3+), CD4+ and CD8+ cells, activated T cells (CD3+ HLA DR) and NK cells (CD 3/16+56+) for at least 3–4 months. The patient did not suffer from significant adverse events, stabilized clinically and improved from expanded disability status scale 7.5 to 7.0 within the first 6 months. **Conclusions:** Alemtuzumab for the treatment of MS can be safely administered subcutaneously and even in low dosages suppresses key lymphocyte subpopulations almost completely for several months. This modified regime may retain the high clinical efficacy of the drug, may prove to be safer for patients and may therefore allow treatment of a broader spectrum of patients with MS.

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Cerebrospinal fluid B cells correlate with early brain inflammation in multiple sclerosis

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Background: There is accumulating evidence from immunological, pathological and therapeutic studies that B cells are key components in the pathophysiology of multiple sclerosis (MS). Recent studies detected B cells in the cerebrospinal fluid (CSF) of patients with MS and other neurological inflammatory diseases (IND), but not in non-inflammatory neurological diseases (OND). **Objective:** We started a prospective study in order to gain new insights into differences concerning the nature of the inflammatory response between relapsing and progressive MS. We decided to compare CSF cell profiles from patients at the onset of the disease (clinical isolated syndrome, CIS), relapsing-remitting (RR) and chronic progressive MS. As controls we have used IND and OND patients. **Methods:** CSF was obtained by standard diagnostic lumbar puncture. CSF cells were immediately stained with fluorochrome-labeled antibodies to human leukocyte surface antigens and analyzed via three-color flow cytometry. Matrix metalloproteinases (MMP)-9 and chemokine levels were measured using a commercially available ELISA. CSF B cells were correlated with clinical and magnetic resonance imaging (MRI) data, inflammatory CSF parameters (CSF cell counts, IgM and IgG production) and with MMP-9 and CXCL13 levels. **Results:** We report an accumulation of CSF mature B cells (CD19+CD138-) in early and relapsing MS and IND, as well as of plasma blasts (CD19+CD138+) in CIS and RRMS. Furthermore, this accumulation of B cells correlated with acute brain inflammation measured by MRI and with inflammatory CSF parameters such as the number of CSF leukocytes, intrathecal IgM and IgG synthesis and intrathecal production of MMP-9 and the B cell chemokine CXCL13. **Conclusions:** Our results demonstrate the accumulation of mature B cells (CD19+CD138-) and plasma blasts

(CD19+CD138+) in the CSF of patients with a CIS and RRMS, which is associated with paraclinical indicators of acute brain inflammation. We thus conclude that the occurrence of B cells in the CSF is an important step for acute brain inflammation in early MS.

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IL-7 receptor alpha chain discriminates between a normal regulatory and a hyperproliferative proinflammatory T cell subset in patients with relapsing-remitting multiple sclerosis

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Background: Regulatory CD4+CD25high T cells have a key role in controlling autoimmunity. In humans, CD4+CD25high regulatory T cells have recently been implicated in multiple sclerosis (MS), an inflammatory and demyelinating disease of the central nervous system. However, because CD4+CD25high T cells can also include activated cells, we revisited the potential role of this subset depleted of CD127, a marker expressed on activated and memory but not regulatory T cells. **Objective:** To test the suppressive function of CD4+CD25high regulatory T cells depleted in CD127+ cells. **Methods:** Thirty-six untreated relapsing-remitting MS patients and 25 healthy individuals were included in this study. Regulatory T cells were tested for their ability to inhibit the proliferative response and cytokine production of autologous CD4+CD25- cells to anti-CD3 stimulation. **Results:** First, a defective suppressive function of CD4+CD25high T cells in MS patients was confirmed (39% in MS patients vs 69% in age- and sex-matched healthy individuals, $p < 0.05$, Mann-Whitney test) when the CD25high cells within the CD4+ subset were sorted using a 4% gating stringency. However, the regulatory properties of CD127-depleted CD4+CD25+ T cells were similar in MS patients and controls. Further, CD4+CD25high T cells also had the same level of regulatory function in MS patients and healthy individuals when the CD25 gate used to sort the cells was more stringent (2%) and eliminated most of the CD127+ T cells. Finally, CD127+ cells within the CD4+CD25high cell subset appeared to be more proliferative and produced more proinflammatory cytokines (TNF α , IFN γ and IL2) in MS patients than in healthy individuals ($p < 0.05$, Mann-Whitney test). **Conclusions:** Taken together, these experiments suggest that the decreased inhibitory function observed in CD4+CD25high T cells from MS patients is due to the presence of a hyperproliferative CD127+ T cell subset. CD4+CD25highCD127+ cells expressing both the IL2 and IL7- α chain receptors, displayed hyperproliferative and proinflammatory properties and therefore could be of importance in MS.

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Posterior reversible encephalopathy syndrome in neuromyelitis optica spectrum disorders

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Background: Posterior reversible encephalopathy syndrome (PRES) is characterized by vasogenic subcortical edema without infarction. It is associated with hypertensive syndromes and immunosuppressive medications, especially calcineurin inhibitors. PRES has not been associated with neuromyelitis optica spectrum disorders (NMOSD). **Objective:** To report the clinical and neuroimaging findings of four patients with NMOSD who developed PRES. **Methods:** Patients were identified by review of Mayo Clinic's NMO clinicoserologic database. All patients were evaluated by one of the authors and medical records and neuroimaging were reviewed. **Results:** All patients were Caucasian women. Two satisfied criteria for NMO and two had relapsing longitudinally extensive transverse myelitis. All were NMO-IgG

seropositive. The median duration of NMOSD before onset of PRES was 7 years (range 2–24). Two patients had documented supine hypertension preceding PRES onset. The third received plasmapheresis less than 24 hours before PRES onset; the fourth received intravenous immune globulin (IVIG) seven days before PRES onset. Abrupt confusion and depressed consciousness ensued in all cases. One developed aphasia and agitation. Visual abnormalities included diplopia (n=2), cortical blindness (n=1) and nystagmus (n=1). Brain magnetic resonance imaging in all patients revealed T2W hyperintense signal abnormalities, primarily parieto-occipital and infratentorial, with gadolinium enhancement. All patients recovered clinically (median 24 hours; range 1–7 days). Follow-up neuroimaging revealed marked improvement in all patients (median interval 10 days; range 4–23 days). **Conclusions:** Brain lesions in patients with NMOSD are heterogeneous and include inflammatory lesions with similar neuropathology to cord lesions. However, PRES may occur in some patients. We hypothesize that the alteration in central nervous system water flux accompanying AQP4 autoimmunity is a predisposing factor for PRES, especially in clinical settings that alter systemic fluid balance. Recognizing this association may expedite the diagnosis and warrants consideration when treating NMOSD patients with plasmapheresis or IVIG in the face of steroid-resistant exacerbations of NMOSD.

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Pregnancy as a natural modulator of innate immunity in multiple sclerosis: Data from the Rotterdam Pregnancy MS Study

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Background: Pregnancy can attenuate multiple sclerosis (MS) disease activity, especially in the third trimester, with a 70% decrease of the annualized relapse rate. Yet in the first three months after delivery about one in every three women with MS has a relapse. **Objective:** The overall aim of the Dutch pregnancy MS study is to analyze fluctuations of both adaptive and innate responses in different pregnancy stages of MS patients and healthy controls. In this specific substudy on innate signals, we focused on gene expression in blood-derived CD14+ monocytes. Furthermore, we investigated whether pregnancy induced fluctuations of serum leptin levels differed between MS patients and controls and whether serum leptin level correlates with periods of enhanced and diminished disease activity and pregnancy outcome. **Methods:** Women with relapsing-remitting MS (RRMS) were longitudinally studied before, during and after pregnancy and compared with healthy women. Using Affymetrix gene expression arrays, genome-wide RNA expression was evaluated in purified CD14+ cells from pregnant MS patients, using stringent statistical analysis. The microarray data are being validated by qPCR analysis. Serum leptin levels were measured by ELISA. **Results:** During pregnancy several genes in CD14+ cells were significantly downregulated, including a subset of chemokines and genes involved in the JAK/STAT pathway. Furthermore, several other genes were significantly upregulated, such as CD64. The serum leptin level (mean \pm SD) in the women with MS before pregnancy was 22.9 \pm 12.8 ng/ml. The serum leptin level significantly increased during pregnancy in MS patients. Serum leptin levels after delivery dropped significantly in both MS patients and healthy controls. Interestingly, the women with the highest decrease in serum leptin level after delivery more often had a postpartum relapse (p -value:0.008). **Conclusions:** Our data shows important differential gene expression in CD14+ cells in MS patients during pregnancy. Furthermore, we demonstrated an association between leptin decrease after pregnancy and risk of postpartum relapse.

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Aquaporin-4 autoantibodies define autoimmune optic neuritisAxel Petzold¹, Sean J. Pittock³, Vanda A. Lennon³, Cosimo Maggione¹, Brian G. Weinschenker³, Gordon Plant²¹UCL, London, United Kingdom; ²Moorfields Eye Hospital, St. Thomas' Hospital, The National Hospital for Neurology and Neurosurgery, London, United Kingdom; ³Mayo Clinic, Rochester, Minnesota, USA

Background: The spectrum of immune-mediated optic neuropathies (ON) ranges from mild reversible visual symptoms, typically for patients with multiple sclerosis (MS), to frequently irreversible progression to blindness in severe conditions such as chronic inflammatory ON. Some of the severe cases will require rigorous immunosuppression to preserve visual function. Identification of high-risk patients in the acute phase is relevant to guide these early important patient management decisions. **Objective:** To investigate whether aquaporin-4 (AQP4) autoantibodies (NMO-IgG) may be of diagnostic value in patients with newly diagnosed ON. **Methods:** This prospective study recruited 114 patients presenting with optic neuritis in London between 1995 and 2007. Patients were classified by clinical, magnetic resonance imaging and cerebrospinal fluid features in five categories: (1) neuromyelitis optica (NMO), (2) ON typical for multiple sclerosis (MSON), (3) chronic relapsing inflammatory optic neuropathy (CRION), (4) relapsing isolated optic neuritis (RION), and (5) isolated episode of ON (ION). Blood samples were tested for NMO-IgG at the Mayo Clinic laboratories by indirect immunofluorescence on a substrate of mouse cerebellum, midbrain and renal medulla. **Results:** The study patients included 28 with MSON, 42 with ION, 16 with RION, 19 with CRION and 9 with NMO. NMO-IgG was detected in the serum of 56% of patients with NMO and none (0%) of the patients presenting with MSON. The proportion of seropositivity was 5% for the remaining diagnostic categories (CRION, RION and ION). **Conclusions:** Consistent with previous reports, the proportion of NMO-IgG seropositive NMO patients was in the 50–60% range. The proportion of NMO-IgG positivity in patients with CRION, RION or ION was small, but importantly all patients with MSON were seronegative. We conclude that testing for NMO-IgG in patients with recurrent or severe ON who lack convincing evidence of MS is justifiable to identify those patients at high risk for relapse (or transverse myelitis), given the established risk of severe relapse and development of myelitis in seropositive patients. Seropositive individuals may benefit from immunosuppression rather than MS-directed immunomodulatory therapies.

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Natural naive regulatory T cell development and function are disturbed in multiple sclerosis patientsKoen Venken¹, Niels Hellings¹, Karen Hensen², Jean-Luc Rummens², Piet Stinissen¹¹Biomedical Research Institute, Hasselt University, Diepenbeek, Belgium; ²Virga Jesse Hospital, Hasselt, Belgium

Background: Myelin-reactive T cells may play a pathogenic role in multiple sclerosis (MS). We and others have shown that regulatory CD4+CD25+FOXP3+CD127low T cells (Tregs) are functionally disturbed in relapsing-remitting (RR) MS patients but not in secondary progressive (SP) MS patients. **Objective:** To clarify this difference in Treg activity between early and chronic disease stages in MS. **Methods:** We analyzed the functional capacity and homeostatic parameters of (precursor) naive Tregs (nTregs) and memory Tregs (mTregs). We also measured myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG) proliferative responses of CD4total T cells (including Tregs), naive (CD45RAhigh) and memory (CD45RA-) CD4+CD25-CD127high T cells (no Tregs included) of RRMS and SPMS patients and healthy controls (HC) by means of a CFSE dilution assay and measured cytokine production of the myelin-reactive T cells. **Results:** The suppressive capacity of FACS-sorted nTregs was impaired in both early and chronic MS patients, whereas only the latter group showed a restored mTreg function. Chronic MS patients had increased numbers of mTregs as compared with age-matched early MS patients, whereas nTreg frequencies did not differ.

T cell receptor excision circle (TREC) numbers were reduced in nTregs of early MS patients, indicating a diminished nTreg thymic output. MBP and MOG proliferative responses of CD4total T cells, but not of Treg-depleted naive and memory CD4+CD25-CD127high T cells, were significantly higher in RRMS patients as compared with HC and SPMS patients. A significantly higher proportion of myelin reactive memory T cells from MS patients as compared with HC produced IL-17. **Conclusions:** These data provide evidence for a disturbed thymic nTreg development and function in early MS disease stage. This may play a role in the peripheral activation of myelin antigen-reactive T cells leading to central nervous system pathology. Future therapeutic immune interventions for MS may focus on an early restoration of Treg function and suppression of pathogenic myelin-reactive Th17 cells.

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Immunological and clinical status 14 months after cessation of natalizumab therapyOlaf Stuve¹, Petra D. Cravens², Katherine Treadaway², Theodore J. Phillips³, Gina M. Remington², Gloria von Geldern⁴, Sabine Cepok⁴, Mahendra P. Singh², Jan W. Cohen Tervaert⁵, Marc De Baets⁵, David G. MacManus⁶, David H. Miller⁶, Ernst-Wilhelm Radue⁷, Elizabeth Cameron², Nancy Monson², Richard Kim⁸, Bernhard Hemmer⁹, Michael K. Racke¹⁰¹Dallas VA, Dallas, Texas, USA; ²Neurology, UT Southwestern, Dallas, Texas, USA; ³Texas Neurology, Dallas, Texas, USA; ⁴Heinrich Heine University, Dusseldorf, Germany; ⁵University Hospital Maastricht, Maastricht, Netherlands; ⁶Institute of Neurology, Queen Square, London, United Kingdom; ⁷University Hospital Basel, Basel, Switzerland; ⁸Biogen-Idec, Cambridge, Massachusetts, USA; ⁹Technical University of Munich, Munich, Germany; ¹⁰The Ohio State University Medical Center, Columbus, Ohio, USA

Background: Natalizumab (@Tysabri) is a humanized recombinant monoclonal antibody against very late activation antigen-4 (VLA-4) approved for the treatment of patients with multiple sclerosis (MS). A phase II study failed to demonstrate a difference between natalizumab treatment groups and the placebo group with regard to gadolinium-enhancing lesions on magnetic resonance imaging (MRI) three months after discontinuation of therapy. **Objective:** The objective of this study was to assess clinical MS disease activity, surrogate disease markers on MRI, immunological parameters in peripheral blood and cerebrospinal fluid (CSF), as well as safety in MS patients after discontinuation of natalizumab therapy. **Methods:** This study is a serial cross-sectional assessment, in which 23 patients who were treated with natalizumab in the context of two phase III clinical trials were followed over 14 months. The annual relapse rate, neurological disease progression assessed by the Expanded Disability Status Scale, disease surrogate markers on MRI, cellular and humoral immune markers in peripheral blood and CSF, and adverse events of the drug were monitored. **Results:** With regard to clinical disease activity, neuroimaging, and immune responses, the majority of patients in our cohort were stable. Decreased lymphocyte cell numbers and altered cell ratios returned to normal 14 months after cessation of natalizumab. No infectious complications were observed. The occurrence of clinical relapses was associated with high lymphocyte numbers in the CSF. **Conclusions:** This is the first long-term follow-up of patients who discontinued natalizumab. We did not observe a clinical, radiographic, or immunological rebound phenomenon after discontinuation of natalizumab therapy.

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FOXP3 and cytokine profile in patients with optic neuritis

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Background: Acute mono-symptomatic optic neuritis (ON) is a common first manifestation of multiple sclerosis (MS). The pathogenesis of ON involves a myelin antigen T-cell mediated autoimmune response (as in MS) which leads in the demyelination of the optic nerve. The immunological response is closely controlled by cytokines and T-regulatory cells. A disturbance in the net balance between pro-inflammatory and anti-inflammatory cytokines may play an important role for the progression from mono-symptomatic ON to clinically definite MS. Recent studies suggest that the expression of FOXP3 is mainly by T-reg cells and that the expression of FOXP3 is reduced in patients with relapsing-remitting MS (RRMS). **Objective:** The aim of the study was to investigate the FOXP3 mRNA expression profile along with the transcript expression level of both pro and anti-inflammatory cytokines in patients with ON compared with a sex and age-matched healthy control (HC) group. **Methods:** In this study we examine 1) a group of untreated patients with acute ON, 2) a group of HC matched in age and gender, for the expression of FOXP3, pro-inflammatory (IFN- γ , TNF- α , IL-12), immune suppressive (IL-10, IL-4, TGF- β) and other cytokines (IL-2, IL-5, IL-6) with real time PCR. T-student parametric test was used to evaluate the statistical significance. **Results:** IFN- γ (1.30 fold; P=0.0162), TGF- β (0.48 fold; P=0.01) and IL-4 (2.30 fold; P=0.0472) were statistically significantly elevated in ON. No significant alteration between the groups was observed regarding the expression levels of FOXP3 mRNA and the above mentioned cytokines. **Conclusions:** The elevation of IFN- γ , TGF- β and IL-4 transcription supports the theory that ON is an inflammatory condition where pro- and anti-inflammatory mechanisms are involved in the down-regulation of the disease. FOXP3 gene expression in ON is not different from the HC, which may suggest that already in the early stages of demyelinating disease, patients are not able to mobilize FOXP3 and are therefore unable to down-regulate the inflammation.

Rehabilitation and Quality of Life

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A comparison of two community-based leg strengthening programs for persons with multiple sclerosis

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Background: Leg weakness affects mobility and quality of life in persons with multiple sclerosis (MS). Resistance training has been demonstrated to improve leg strength and walking endurance in this population; however, circuit training has not been examined in this population. In Thunder Bay, the need for a community exercise program for persons with MS was identified. **Objective:** To develop and compare the effectiveness of two community-based strength training programs on walking endurance, balance, quality of life and fatigue for persons with MS. **Methods:** 14 women with MS were randomly allocated to one of two treatment groups: resistance training (n=6) or circuit training (n=8), attending one-hour sessions three times weekly for six weeks in a community fitness facility. Classes were instructed by a physiotherapist. Adherence was 84%. Pre and post outcome measures used were: Berg Balance Score (BBS), Physiotherapy Clinical Outcome Variables Scale (COVS), lower extremity muscle strength, 6 Minute Walk Test (6MWT), 12-item Short form Health Survey (SF-12) and Fatigue Severity Scale (FSS). **Results:** T-test scores indicated significant increase in the 6MWT (p=0.001), BBS (p=0.039) and ankle plantar flexor strength (p=0.012, p=0.004, left and right respectively). The circuit group (6/8 had a weaker left leg) demonstrated significant increase in left hip abductor and extensor strength (p=0.038, p=0.029, respectively). Two falls occurred in the circuit group. **Conclusions:** Both exercise programs were effective in

increasing strength for some muscle groups, improving walking endurance and balance. The circuit group required additional supervision and participant mental acuity. Given safety and resource concerns, the resistance program is recommended for community venues. **Supported by:** Northern Ontario Heritage Fund Corporation through McMaster University and the Northern Ontario School of Medicine.

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Comparison of fatigue and quality of life in relapsing-remitting multiple sclerosis, primary progressive multiple sclerosis and neuromyelitis optica: analysis of 100 Brazilian patients

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Background: Fatigue is considered one of the most common symptoms of relapsing-remitting multiple sclerosis (RRMS). In primary progressive multiple sclerosis (PPMS) there are contradictory data. In neuromyelitis optica (NMO), a study developed at the Mayo Clinic suggest that this symptom is less common than in MS. **Objective:** To compare fatigue and quality of life (QoL) among patients with RRMS, PPMS and NMO. **Methods:** A descriptive observational study was conducted at the Hospital da Lagoa, Rio de Janeiro (Brazil) between March 2006 and December 2007. From a convenience sample of 100 subjects, 20 patients with PPMS, 61 with RRMS and 23 with NMO were selected to be assessed regarding fatigue (fatigue severity scale - FSS) and quality of life (SF-36). In FSS, fatigue presence is indicated for a score ≥ 28 and the absence of fatigue is indicated for a score ≤ 28 . The SF-36 is a questionnaire with an ending scoring of 0 (worse overall health condition) and 100 (best overall health condition). We compared the dimensions of SF-36 and fatigue among the three groups of patients. **Results:** In the selected sample a shorter average time of disease was observed in PPMS (6.2 years) than the other diseases, while 75% of these patients had a time of disease shorter than 10 years. Women were predominant among the three groups: RRMS (70.7%), PPMS (65%) and NMO (87%). Fatigue was observed in 66.7% of the RRMS population, 80% in PPMS and 78.3% in NMO, with no significant difference (p=0.39). Comparing the SF-36 dimensions, a higher impact on Physical Function and Role-Emotional dimensions was observed in PPMS (p<0.05). The Vitality function was the most affected in RRMS (p=0.06-ANOVA). **Conclusions:** High levels of fatigue were observed in the three groups, not confirming previous studies with NMO patients. There was a tendency for a worst quality of life in PPMS, mainly in the Physical Health and Role-Emotional dimensions.

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Pulmonary function in relapsing-remitting multiple sclerosis without respiratory complaints

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Background: Little is known about the development of respiratory muscle dysfunction and its association with disability and fatigue during the course of multiple sclerosis (MS). Recent studies have demonstrated pulmonary dysfunction in relapsing-remitting MS (RRMS), primary progressive MS (PPMS) and secondary progressive MS (SPMS) patients in the absence of respiratory symptoms. **Objective:** We investigated here the predictive value of respiratory muscle functions and the change in forced vital capacity [FVC], lung volumes, total lung capacity (TLC), residual volume (RV), and diffusing capacity for CO (DLCO), in detecting deterioration of respiratory muscle function in RRMS patients who were selected for a study of objective fatigue by the six minute walk test. **Methods:** Twenty one patients were studied, 15 females, 6 males, aged between 19 and 43 year (mean=30.43), with a time of duration of disease of 1 to 17 years (mean= 7.23). The median expanded disability status scale was 1 (0-3.5). 9/21 (42%) patients reported fatigue. No patients had respiratory complaints and none smoked. Clinical data, spirometry, lung volume, diffusion test and maximal static respiratory pressures were analyzed. Predicted values

(%P) were calculated for FVC, FEV1 and FEV1/FVC (Pereira 2007); TLC, RV and RV/TLC (Crapo); DLCO/VA (DL/VA) (Miller); PI max and PE max (Black and Hyatt). **Results:** All patients had normal TLC (97.5% P, mean predicted values) and FEV1/FVC (85%). Abnormalities were identified in three patients with DLCO (83.7% P), four with PImax (102% P) and 13 with PEmax (64%). Eight patients had borderline values for TLC, 14 for DLCO only, one after DLCO/VA correction; two for PImax. Gender did not affect the results. Fatigue was reported by 7/14 patients (50%) with pulmonary abnormalities and 2/7 patients (28.5%) with normal tests. **Conclusions:** Our results indicated that respiratory force was the first to be affected in RRMS patients even in the absence of respiratory symptoms. These results should be taken into account in tests for evaluation of objective fatigue.

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Fatigued patients with multiple sclerosis have impaired central motor activation and timed walking compared with non-fatigued patients

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Background: Patients with multiple sclerosis (MS) have reduced central motor activation compared with healthy controls. However, the relation to fatigue is uncertain. We studied fatigue and motor function in three groups of MS patients with mild physical disability. **Objective:** To examine the relation between central motor function and fatigue in patients with relapsing-remitting MS. **Methods:** Patients with relapsing-remitting MS and Expanded Disability Status Scale (EDSS) ≤ 3.5 were grouped as fatigued (Fatigue Severity Scale (FSS) ≥ 5.0) or non-fatigued (NF) (FSS ≤ 4.0). Participants were secondary fatigued (SF) in cases of depression, poor sleep, pain, spasticity, infection or treatment side effects. Otherwise, patients were defined primary fatigued (PF). Motor function was evaluated with the Six Minute Walk Test (6-MWT) and isometric dynamometry. Maximal Voluntary Contraction (MVC), Central Activation (CA) and Peripheral Activation (PA) were determined by percutaneous twitch interpolation of the right quadriceps muscle. The Multidimensional Fatigue Inventory (MFI-20) with five subscales was administered: General-, physical- and mental fatigue, reduced activity and reduced motivation. **Results:** 19 PF, 20 SF and 21 NF patients with equal demographic data, disease duration and EDSS score corrected for fatigue were studied. Baseline MVC and PA were not different between groups (MVC (Nm): PF 148(32), SF 159(78), NF 173(49) (mean(SD)), ($p=0.34$); PA: PF 1.02(0.08), SF 1.04 (0.11), NF 1.05(0.14), ($p=0.67$)). In contrast, the CA and the 6-MWT were lower in the PF and SF group compared with the NF group ((CA (%): PF 95.9(5.1), SF 95.8(4.4), NF 99.2(0.98) ($p=0.013$)); (6-MWT (m): PF 524(85), SF 562(69), 623(74) ($p<0.001$))). MVC was inversely related to FSS ($p=0.04$) and physical fatigue ($p=0.01$). CA correlated inversely to FSS ($p=0.05$), general and physical fatigue ($p=0.01$, $p=0.01$) and the 6-MWT was inversely related to FSS ($p=0.001$) and all MFI-20 subscale scores ($p<0.05$). **Conclusions:** Primary and secondary fatigued MS patients have reduced central motor activation and walking distance compared with non-fatigued MS patients.

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The effects of high-voltage pulsed galvanic stimulation on fatigue in multiple sclerosis patients

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Background: In the clinical setting, there are several ways to assess central fatigue in multiple sclerosis (MS) patients. However, the assessment and treatment of fatigue is still difficult and variable in MS patients. **Objective:** This study proposed to determine the effects of High Voltage Pulsed Galvanic Stimulation (HVPGS) on fatigue in MS

patients. HVPGS was applied to hip and knee flexors and ankle dorsiflexors in the first group, and exercise was prescribed to the second group. **Methods:** Fatigue was examined with Visual Analogue Scale (VAS), Fatigue Severity Scale (FSS), and Fatigue Impact Scale (FIS). 33 MS patients were randomised in two groups; HVPGS group (N:16, mean age 41.81 \pm 8.43 years, Expanded Disability Status Scale (EDSS) score:4.84 \pm 1.21) and exercise group (N:17, mean age 36.24 \pm 10.08 years, EDSS:3.94 \pm 2.27). **Results:** VAS, FSS and FIS scores reduced significantly in both HVPGS and exercise groups after 18 sessions, while the changes were greater in the HVPGS group. In HVPGS group VAS in activity was reduced 7.91 \pm 1.39 to 4.42 \pm 1.59, while it was reduced 7.16 \pm 2.23 to 5.10 \pm 1.77 in the exercise group. VAS in rest scores were also decreased significantly in both groups, but the reduction was less than in activity. FSS scores were 46.25 \pm 9.15 and 35.88 \pm 7.37 in HVPGS and 43.53 \pm 14.45 and 36.94 \pm 13.79 in the exercise group. FIS scores were 82.06 \pm 15.21 and 75.31 \pm 15.16 in HVPGS, 80.41 \pm 19.64 and 76.65 \pm 19.86 in the exercise group. Significant differences were noted before and after both treatments for VAS, FSS ($p<0.001$) and FIS ($p<0.005$). **Conclusions:** It was concluded that HVPGS is an effective method that could be used safely in MS patients, especially for decreasing fatigue levels. It was thought that if specific therapeutic managements such as HVPGS and exercise were combined with other rehabilitation programs, this may be the most appropriate approach.

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From efficacy to effectiveness: does a nurse-led cognitive behavior therapy intervention work in patients with multiple sclerosis in the real-life clinical setting?

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Background: Mental health issues such as depression and anxiety are common in multiple sclerosis (MS). Lack of effective treatment can lead to reductions in perceived quality of life, social support, reduced cognitive functioning, increased perception of disability and suicide. **Objective:** Our aim was to assess the effectiveness of a community psychiatric nurse-led service in managing and treating mental health issues in people with MS. A variety of treatment modalities were used including cognitive behavior therapy (CBT), other psychological approaches and medication. This abstract focuses on the CBT intervention. **Methods:** The clinic provided screening of emotional and mental distress and treatment in the form of anti-depressant medication, CBT or both. CBT was based on individual formulations utilising Beck's Model of Cognitive Therapy and patients were seen at their home address or in the clinical setting. **Results:** 45 patients out of 118 referrals have been referred for CBT. 12 (26.7%) have completed therapy, 9 (20%) declined therapy, 7 (15.6%) dropped out of therapy, 7 (15.5%) are currently in therapy and 6 (13.3%) are on the waiting list. The mean age of patients is 41 (range 24–62) with the majority of patients having relapsing-remitting MS ($n=37$, 82.2%). The majority of patients are referred for treatment of depression (60%) but other primary issues such as anxiety, anger and treatment of fatigue are also prevalent. The average number of sessions was 7 (range 2–20). Themes arising from therapy included dealing with loss and a sense of being a burden, effective communication with family/carers, overcoming subjugation, low self esteem, dealing with uncertainty and shame. A Wilcoxon signed rank test was conducted to evaluate the impact of CBT with the 21 completers on the HADS scale. There was a statistically significant decrease in scores ($p<0.05$) suggesting that CBT was useful for this population. **Conclusions:** A nurse-led intervention treating common mental health issues in MS is acceptable to patients and referrers and appears to reduce anxiety and depressive symptoms. **Supported by:** Teva Pharmaceuticals, MS Society (UK).

P370**Expanding the role of exercise in the lives of persons with multiple sclerosis**Adelheid Blumer¹, Garry Wheeler¹, Scott Rattray¹, Keith Johnson²¹Client Services, MS Society of Canada, Edmonton, Alberta, Canada;²University of Alberta, Physical Therapy, Edmonton, Alberta, Canada

Background: Until recently, physical activity and exercise were contraindicated for persons with multiple sclerosis (MS) due to thermosensitivity, fatigue and vision-related issues. The importance of regular physical activity in the lives of persons with MS is now increasingly recognized as an important adjunct to traditional medical and other therapeutic interventions. As such, physical activity programming extends the concept of combinational therapeutic interventions. **Objective:** Therefore, we have developed the MSACTIVEnow program at Alberta Division and Edmonton Chapter to increase community capacity for active living for persons with MS. **Methods:** We explored the 'meaning' of regular physical activity in 37 persons with moderate to severe Expanded Disability Status Scale MS in a qualitative study comprised of a series of personal interviews. We assessed sense of identity, self esteem, sense of personal control, dignity, psychological and physical functioning. No negative affects of exercise were reported although barriers cited included lack of facilities, and lack of programs and expertise associated with exercise and MS. **Results:** A full time MSACTIVEnow coordinator and MS Society-funded, university based, MS ACTIVEnow fellow, are responsible for developing and delivering programs, developing training (multi-media) materials, and conducting community education sessions for professionals. The MSACTIVEnow program will also access medical professionals as part of a referral network with regard to access to appropriate sites. To date we have (i) Developed a full set of educational materials for persons with MS; health fitness and lifestyle professionals, and medical professionals, (ii) conducted several pre-initiative launch instructional sessions for persons with MS and health fitness and lifestyle professionals, (iii) (will have) completed a formal launch of the MS Active NOW initiative. **Conclusions:** This program now moves into the critical stage of health promotion programs, that of sustaining the messaging for stakeholders. The message is that appropriate exercise regimens are beneficial for people with MS, enhancing quality of life and establishing community.

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P371**www.msinfowiki.ca: stay connected online**

Adelheid Blumer

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Background: Newly diagnosed with multiple sclerosis (MS)? Family or friend? Front-line service provider? Student doing research? You've got great questions... and we've got good basic answers. However, we know that finding reliable, current and relevant information can be confusing in a world of information overload. **Objective:** Being able to access trusted information is key to the successful management of living with MS, critical to making decisions around treatment and very important when establishing a support network. Our goal was to ensure MS information is only a click away. **Methods:** Listening to questions people with MS have about the disease and hearing their need for accurate and easy to understand information became the impetus for this project. A team undertook the tremendous task of perusing MS database articles, publications, and online sources to bring together clear, basic, easy to understand information. After testing by focus groups and service providers the page was connected to our Alberta MS Society website at www.mssociety.ca. Users not only find information but also orientation tools and access to many quality supports for their families. **Results:** This three-year MS information standardizing project in Alberta has resulted in a user-friendly, searchable information database highlighting over 600 MS topics with 2,500 links to definitions, related subjects, studies and sources. Plus, a cross-reference feature connects users to ASK MS, a comprehensive MS Society of Canada database with over 2,000 educational articles available through a 1-800 information line.

Conclusions: Knowledge is power and the foundation for enhanced quality of life, and a future free of MS. Got a question about MS? Get online today at www.msinfowiki.ca - and stay connected.

P372**Analysis of an intensive neurorehabilitation therapy in MS: a clinical trial**Jose A Cabrera-Gomez¹, Yanelly Real-Gonzalez², Roberto Diaz-Marquez², Alexander Echemendia Del Valle², Alina Gonzalez-Quevedo¹, Gilda Martinez-Aching², Mayda N Lopez-Hernandez², Jorge Luis Torres Izquierdo², Reinaldo Gomez-Perez², Neivis Torriente-Herrera², Yamile Valle-Ramos²¹Cuban Multiple Sclerosis Society, Havana, Cuba; ²Multiple Sclerosis Clinic, International Neurological Restoration Center, Havana, Havana City, Cuba

Background: Disease-modifying therapies have not eliminated the need for neurorehabilitation in multiple sclerosis (MS). **Objective:** To evaluate the efficacy of an intensive neurorehabilitation program in MS. **Methods:** 30 patients with MS (McDonald et al) received an intensive neurorehabilitation program six hours daily for four weeks. Each patient had one-to-one physiotherapist/occupational/speech-language therapists. The evaluations were made by a laboratory team, who do not participate in the program, by: Scripps Neurological Rating Scale (SNRS), Expanded Disability Status Scale (EDSS), Ambulatory Index, 9 hole-peg-test, PASAT-3, Fatigue Impact Scale (FIS), Environmental Status Scale & quality of life scale (MSQLI-54). **Results:** 30 MS patients (22 progressive and 8 relapsing), 19 female/ 11 male, mean age 40.43 ±11.46 years, 13.40± 7.76 years of evolution, completed the program. The evaluation of impairment/disability was: SNRS initial 53/final 68.08 (p=0.000; EDSS initial 5.8(±1.51)/final 5.08 (±2.10) p=0.0001. Functional Systems demonstrated; pyramidal initial 3.97/final 3.15 p=0.001; Cerebellar initial 2.57/final 1.26 p=0.000; brain stem initial 1.57/final 0.78 p=0.001; Sensorial initial 1.40/final 1.00 p=0.023; bowel and bladder initial 1.53/final 0.44, p=0.000; visual initial 0.70/final 0.48, NS; cerebral initial 0.47/final 0.23, NS; and others (spasticity) initial 1.07/final 0.38, p=0.000. Ambulatory Index initial 27.40(±28.54)/final 23.95(±24.82) p=0.393; 9 hole-peg-test dominant hand initial 31.38(±8.26)/final 27.54(±6.71) p=0.008 and 9 hole-peg-test hand non-dominant initial 38.01/final 30.39 p=0.014; PASAT-3 initial 21.14/final 29.29 p=0.018. Environmental Status Scale initial 17.06/final 16.53 NS; Fatigue initial 47.18/final 30.58 p=0.005. MSQLI-54 demonstrated an improvement in Physical Health, initial 46.11(±12.32)/57.32(±14.37) p=0.008 and Mental Health, initial 51.64(±21.65) /62.46(±20.50) p=0.026. **Conclusions:** An intensive one-to-one neurorehabilitation program demonstrated in 4 weeks an improvement of impairment, disability, fatigue and QoL, in MS not reached by any of the disease-modifying therapies.

P373**Are there relationships between fatigue, subjective sleep quality and depression in MS patients?**Rafael Montenegro-Rodrigues², Leandro C. Calia¹, Gisele C. Calia², Juliana R. Santos², Aginaldo A. Carlesse²¹Neurology, Universidade Santo Amaro, São Paulo, São Paulo, Brazil;²Ambulatório de Especialidades de Interlagos - General Hospital of Grajau - Associação Congregação de Santa Catarina, São Paulo, São Paulo, Brazil

Background: There is much evidence regarding the causal connection between fatigue and depression in multiple sclerosis (MS) patients. Poor sleep quality could be considered as a cause or effect of physical and cognitive impairment, and could worsen fatigue levels. However, there are still doubts about the profile of sleep quality of MS patients and its relationship with fatigue and depression. **Objective:** Our aim was to verify the relationship between fatigue, subjective sleep quality and depression in MS patients. **Methods:** Eighteen (12 women) relapsing-remitting MS (RRMS) and secondary progressive MS (SPMS) patients clinically defined as mild to moderate disability (EDSS scores 1 to 6.5) were interviewed and answered the Pittsburgh Sleep Quality Index (PSQI), Beck Depression Inventory (BDI), Fatigue Severity Scale (FSS) and Epworth Sleepiness Scale. Descriptive statistics (frequency, median, mean and standard deviation) and Spearman correlations

were used to analysis ($p < 0.05$). **Results:** Mean PSQI, FSS and BDI global scores were 6.89 ± 3.51 , 36.94 ± 16.54 and 12.5 ± 7.71 , respectively. Fatigue and depression have shown good positive correlation ($r = 0.814$ $p < 0.001$). There was no correlation between sleep quality and fatigue; however, poor subjective sleep quality (PSQI > 5) was found in 12 patients (66.6%) and mild or moderate depression (BDI > 9) was found in 10 patients (55.6%). **Conclusions:** The relationship between fatigue and depression corroborates results found in other studies. Despite the absence of a significant correlation between sleep quality and fatigue/depression, it is important to highlight the high incidence of poor sleep quality in this sample. Symptoms linked to sleep deprivation can negatively influence the quality of life in MS patients.

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Racial disparities in adherence to disease-modifying agents and quality of life in patients with MS

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Background: Multiple sclerosis (MS) has increased prevalence among those of Caucasian race. However, studies have shown a more aggressive course with increased disability among African-Americans (AA). While clinical trials have shown disease modifying agents (DMAs: glatiramer acetate, interferon-beta-1a and interferon-beta-1b) to reduce relapse rate, magnetic resonance imaging burden, and short-term disability, little is known about DMA adherence by race and the impact on patient-centered outcomes in practice. **Objective:** To examine racial differences in adherence to DMA, health status, quality of life and work disability among patients with MS. **Methods:** MS patients were identified for study in a large, integrated health care system serving residents of southeastern Michigan. The study population consisted of 181 HMO-insured patients over 2 study years (2004–2006). Pharmacy claims data were used to construct measures of medication adherence. Disability (Expanded Disability Status Scale; EDSS), health status (SF-36) and quality of life (MS Quality of Life Inventory; MSQLI) along with patient socio-demographic characteristics were captured using a patient survey. Racial differences were assessed using GEE methods which accounted for the non-independence of patients being treated by the same physician. **Results:** 111 patients with a dispensed DMA responded to the survey. AA patients ($n = 44$) were younger (46 vs 49 years), less likely to be married (44% vs 78%) with lower income and less adherent to DMAs (66% vs 87% adherent). Although disease duration was shorter among AAs (6.8 vs 10.5 years), work disability was significantly higher (32% vs 13%) among AAs with no statistically significant differences by race in EDSS, MSQLI or SF-36 scores, although AAs reported slightly worse scores. **Conclusions:** Lower DMA adherence and greater work disability were found among AA patients. Larger, prospective studies are needed to more fully understand the implications of medication adherence on work disability and other patient outcomes among racially diverse populations of patients with MS.

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Sociological characteristics of MS patients

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Background: Multiple sclerosis (MS) affects different parts of life such as married state, child bearing, education, employment and retirement. **Objective:** We tried to find out sex differences in sociological characteristics of MS patients. **Methods:** We interviewed 106 MS patients, 32 male and 74 female and analyzed married state, child number before and after diagnosis, level of education, employment, duration of employment and retirement. **Results:** We found out that 24 (75%) males are married and 8 (25%) are not married, 51 (68.9%) females are married, 9 (12.2%) are not married, 9 (12.2%) are divorced and 5 (6.7%) are widows. 11 (34.4%) males have no children, 9 (28.1%) have 1 child, 8 (25.0%) have 2 children and 4 (12.5%) have more than 2 children. 14 (18.9%) females have no children,

18 (24.3%) have 1 child, 28 (37.8%) have 2 children and 14 (18.9%) have more than 2 children. 17 (80.9%) males and 50 (83.3%) females had children before the diagnosis was established. 21 (65.6%) males finished high school, 5 (15.6%) are college-trained and 6 (18.7%) are without education. 44 (59.4%) females finished secondary school, 6 (8.1%) are college-trained and 24 (32.4%) are without education. 8 (25%) males are still working, while 24 (75%) are retired. 30 (40.5%) females are still working, while 44 (59.5%) are retired. MS was the main cause of retirement in the male group, and for 32 (72.7%) in the female group. **Conclusions:** The results show that MS mainly affects child bearing ages in both groups, meaning that only 19.1% of males and 16.7% of females have children after disease onset. MS shortens working age and causes early retirement, especially in male group.

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DELPHI-QOL: a study of physicians' opinions about the diagnosis, treatment and quality of life management of multiple sclerosis

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Background: To describe physicians' opinions about the diagnosis (evoked potentials, cerebrospinal fluid examination, magnetic resonance imaging), treatment (use of corticosteroids, disease-modifying drugs, early treatment) and quality of life (use of QoL scales) of multiple sclerosis (MS). **Objective:** 1. To determine importance of ratings of diagnostic, clinical management and quality of life management options. 2. To determine differences in ratings between subgroups of physicians. **Methods:** This is a descriptive study of physicians' opinions following a survey, review and discussion at a workshop. A statistically representative sample of 60 Belgian neurologists has been invited to participate in the survey and workgroup. The methodology used for this study is a Delphi method with a survey, and scoring round following presentation and group discussion, using a computer-based system. **Results:** A three-round Delphi study was used to gain insight into opinions. In Round 1, 35 questions were grouped, and fed back in Round 2 at the workshop, scored from Yes/No or Multiple Choice answers. Round 2 responses were fed back for scoring in Round 3 following a clinical case. Diagnosis: Would you perform Visual EPs? Round2/3: Yes 87/79% No 13/21%; Would you perform All EPs? Round2/3: Yes 57/68% No 43/32%; Would you do a CSF exam? Round2/3: Yes 88/83% No 12/17%. Early treatment: Would you insist that the patient begins a treatment? Round2/3: 15/13%; Would you propose a treatment? Round2/3: 28/30%; Would you wait for signs of clinical and/or MRI evolution? Round 2/3: 57/57%. Quality of Life: would you use a user-friendly questionnaire? Round 2/3: Yes 61/71% No 39/29%; Would a user-friendly questionnaire influence the patient management?: Round 2/3: Yes 53/72% No 47/28%. Discussion: There was only little change in the neurologists' opinion despite the discussion. The use of CSF examination remained high and the proposal of early treatment to MS patients remained low. The neurologists' opinion did change after the discussion in favor of the use of QoL scales. **Conclusions:** The combination of this survey and study's findings will enable a better understanding of physicians' approaches to MS. It is believed that this study will yield findings that may be generalised to other countries.

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Evaluation of a computerized cognitive training program in patients with impaired working memory functions

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Background: The most commonly impaired cognitive functions in multiple sclerosis (MS) are memory, attention, processing speed and mental flexibility. Frequently, these impairments are related to a core

deficit in working memory. **Objective:** The aim of the study was to evaluate a recently developed computerized working memory training program (BrainStim) in MS patients with deficits in working memory. **Methods:** 15 MS patients with impaired working memory functions allocated from our outpatient clinic were included. 9 patients (mean age: 43.9, mean Expanded Disability Status Scale (EDSS): 3.6, mean disease duration: 11.1) were trained with BrainStim during one month while 6 patients (mean age: 47.0, mean EDSS: 3.5, mean disease duration: 17.3) were assigned to the control group without training. The outcome measures consisted of a neuropsychological assessment including tests for memory, attention, and processing speed as well as of self-report measures for fatigue, depression and quality of life. The assessment at the beginning was performed twice at an interval of two weeks to control for possible learning effects. A retest was performed after one month. **Results:** Compared with baseline the intervention group improved after training in working memory measures as well as in tasks of short-term memory, attention and processing speed. Further, the intervention group reported improvements in fatigue, depression and quality of life. In contrast, as compared with baseline, patients without training performed worse on working memory tasks and showed worsening of fatigue and quality of life. **Conclusions:** The results of the current study indicate positive effects of training with BrainStim. These effects seem to be specifically related to working memory functions. Further, the additional improvements in other cognitive domains, and the observed beneficial effect on subjectively experienced fatigue, depression and quality of life suggest that training with BrainStim may have a positive impact on cognitive function in general and may thereby improve quality of life.

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Occurrence of restless legs syndrome in multiple sclerosis

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Background: Restless legs syndrome (RLS) is one of the most common sleep and movement disorders. It is characterized by four essential diagnostic criteria: an urge to move the limb, usually associated or caused by unpleasant sensations in the legs (parestheses or disestheses); symptoms that start or become worse during the period of rest; at least partial relief of symptoms with physical activity; and worsening of the symptoms in the evening or in the night. (Prevalence in the primary care population in Macedonia is 8.2%). These symptoms often result in disturbance of sleep, daytime tiredness and impact on quality of life. **Objective:** The objective of the study is to assess the prevalence of RLS among patients with multiple sclerosis (MS). **Methods:** An RLS questionnaire was completed by 143 patients (104 female and 39 male) with MS (diagnosed according McDonald Criteria). Each patient underwent neurological investigation, Expanded Disability Status Scale (EDSS), magnetic resonance imaging. The RLS questionnaire consisted of four parts; the first part asked diagnostic questions to screen for RLS; the second part ascertained demographic characteristics and co-morbidity; the third part consisted of questions to assess symptoms of RLS in the RLS group (patients with positive answers for questions in the first part); and the final part asked questions about previous treatment. **Results:** Out of 143 patients, fifty (34.96%) responded positively to all four questions in the first part of the questionnaire (screen for RLS), and were defined as RLS cohort. EDSS scores ranged between 0.5 and 8.0 (mean 5.6). The EDSS score was higher in RLS+ group, compared with the RLS- group. RLS occurred frequently in patients with spinal cord lesions, as seen in magnetic resonance imaging. **Conclusions:** RLS affects almost one third of MS patients, patients with higher EDSS (higher disability) and with longer duration of the disease.

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Psychometric validation of daily life scale aimed at evaluating living abilities in multiple sclerosis patients

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Background: Multiple sclerosis (MS) substantially decreases working and living abilities. Scales evaluating impairment of daily life due to MS are still lacking **Objective:** To evaluate the psychometric properties of a self-administered rating scale evaluating impairment of living activities due to MS (DL-scale for Daily Life scale). **Methods:** A 20-item scale was built by MS experts and reduced to a 17-item scale after first validation studies. The DL-scale evaluates various living abilities (self-care, house keeping, transport, leisure) on a 5-point rating scale (0=no difficulty, 5=not possible due to MS). The scale was proposed to 124 patients with relapsing-remittent MS (females 73%, age 44±12, median Expanded Disability Status Scale (EDSS) score=4). Internal consistency (standardized Cronbach's alpha) and construct validity (principal component analysis using Varimax rotation) were assessed. Convergent validity was judged against EDSS score. Reproducibility was assessed by a test-retest within a 15-day interval in 29 further patients (reliability coefficient). Scores of patients who answered to be not involved in a given activity were considered as missing data. For occupational activities 10 points were given if the patient had to give up working or studying, 5 points if he had to change his profession/studies. For patients pursuing their usual working activity the maximum score of 3 questions regarding difficulties was retained. A global score was computed. **Results:** The DL-scale was well accepted (<5 minutes). The global score was 26±21 (min 0, max 80, theoretical max=80) and strongly correlated with EDSS (r=0.73). Internal consistency was excellent (Cronbach α =0.96). First axe explained 63% of the global variance. Test-retest reproducibility was excellent (reliability coefficient 0.97). **Conclusions:** The DL-scale is an easy-to-use self-administered reliable unidimensional tool assessing impairment of living abilities in MS patients. A large phase IV study is ongoing to address responsiveness.

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Working memory in multiple sclerosis: methodological discussion of a cognitive rehabilitation intervention

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Background: Impairment of working memory (WM) is commonly reported in patients with multiple sclerosis (MS). Although WM deficits may impact severely on functional status, there are only few studies designed to evaluate the effectiveness of rehabilitation approaches in MS patients. We report the preliminary results of a rehabilitation study and will discuss methodological issues specific to this population. **Objective:** The objective of the present study was to evaluate two cognitive interventions for rehabilitation of WM in MS patients. **Methods:** Patients with relapsing-remittent MS and showing a deficit in backward digit span were randomly assigned to two cognitive therapies, ecological versus laboratory intervention. Both interventions were designed to train each of the three components described in Baddeley's model of WM (visual sketchpad, phonological loop and central executive). Participants were engaged in eight 60-minute training sessions. The effectiveness of these interventions was assessed a) on WM abilities (with other tasks than the ones implied in interventions); b) on other functions soliciting WM processing (i.e. episodic memory). A neuropsychologist blind to rehabilitation techniques conducted these evaluations. Five evaluations were performed: two pre-intervention assessments (to control non-specific learning), one after the first four sessions, one after the eighth session and one on long-term follow-up (3 months post treatment). **Results:** We present preliminary results on 14 patients. Only one task (letter-digit sequences) improved between the second evaluation and the fourth evaluation (p<.05) for both intervention groups. For the ecological group however, this benefit disappeared on long-term

follow-up. For the dual-task, a significant benefit was found ($p < .05$) only for the laboratory group but this improvement was no more observed on the long-term evaluation. **Conclusions:** The small size of the sample prevents any definite conclusion being drawn regarding the efficacy of the proposed therapy. However, several methodological flaws specific to MS patients can be discussed.

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Iranian network of multidisciplinary clinics for multiple sclerosis, a view of existing experiences and future horizons

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Background: To approach to complexities in diagnosis, treatment and follow-up of multiple sclerosis (MS), there is a progressive need to establish a national network of multidisciplinary clinics (MCs) for MS with input from a range of specialists. **Objective:** In this article we attempt to define different dimensions of MCs in a national context, presenting positive experiences in this way in a collaborative atmosphere in Iran. **Methods:** Clinical, imaging and cognitive wings as the main parts along with supplementary biochemical and rehabilitation facilities should be brought together in MCs. National unified semi-structured clinical data-gathering forms, checklists, scores, rating-scales and self-reports should be prepared. Quantitative neuroimaging pipelines and digital-analysis methods with unified protocols are the essentials needs for MCs. Neurocognitive assessments of MS patients act an important role in diagnosis, treatment and follow-up. Persian-validated computerized and paper-pencil neuropsychological assessment tasks for main cognitive domains with normative-data for Farsi-speaking subjects based on our cultural-contexts are basic needs. **Results:** Progressive steps are obtained in the design and validation of national clinical registration forms. The next phases are a digital archiving network with a unified technical platform and data sharing facilities. Some functional and structural brain imaging studies are in progress. Validated neurocognitive assessment tools in different domains for Farsi-speaking subjects are provided. Unified reporting protocols and suggested rehabilitation techniques or treatment modifications are under discussion. Specialized neurocognitive laboratories with qualified staff are or will be founded for patients' assessment. **Conclusions:** A national network of MCs for MS makes it possible to confront this disease with a comprehensive registry, joint national and international research projects and a digital database management system with data-sharing facilities on a common platform. The lack of previous joint-collaborative works, and sophisticated imaging and rehabilitation facilities have been the main limitations for development of MCs in Iran.

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Walking and Expanded Disability Status Scale: are self-estimated walking distances accurate?

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Background: The Expanded Disability Status Scale (EDSS) is often used for impairment and disability assessments in multiple sclerosis (MS) patients. **Objective:** The aim of this study was to compare self-estimated walking distances (SEWD) and real walking distances (RWD) of MS cases and to evaluate the effect of subjective and motor fatigue on SEWD and RWD. **Methods:** In this study thirty MS patients [Group I: N:16, mean age:36.75, EDSS:1.0 (1-3.5), Group II: N:14, EDSS: 4.5 (4-5), mean age: 38] and twenty healthy controls (N: 20, mean age: 37.2) were included. Disease duration of patients in group I, II was 8.94 11.64 years respectively. Patients were assessed using Fatigue Severity Scale (FSS) for subjective fatigue, Six Minutes Walking Test (SMWT) for walking distance, and Walking Fatigue Index (WFI) for motor fatigue. **Results:** FSS scores of groups were 36.12±10.51, 50.35±12.17, 9.75±1.83 in group I, II and the control group respectively. There was a significant difference between groups ($p < .05$). There was no significant difference between SEWD and RWD of group I and control group subjects ($p > .05$). In group II patients could not accurately estimate their walking distances and RWD were longer than SEWD ($p < .05$). There were significant differences between groups' WFI scores ($p < .05$). Group II had the highest score. The FSS score was related to RWD only in the control group ($p < .05$). There was a negative correlation between RWD and WFI scores in Group II ($p < .05$). **Conclusions:** EDSS is more reliable in patients with low disability level. Maximum walking distance and motor fatigue are well correlated. Therefore real walking distance assessment for patients whose pyramidal systems are explicitly affected seems essential, rather than relying on the patient's subjective interpretation. This is important if the EDSS is between 4-5.

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Sensory re-education reduces sensory deficits in multiple sclerosis patients

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Background: Multiple sclerosis (MS) patients suffer from various sensory impairments that can significantly interfere with daily tasks performance, especially those associated with fine motor movements. We have recently developed a sensory training tool (STT) to improve sensory skills in impaired MS patients. **Objective:** To evaluate the effects of STT in a large group of sensory impaired MS patients and to assess the difference between individual and independent training procedures. **Methods:** Thirty-five MS patients (Mean±SD; Age 46.2±11.7 years, disease duration 14.2±8.6 years, Expanded Disability Status Scale 4.7±1.6) with sensory impairment were evaluated using the Semmes-Weinstein monofilaments test (MFT) and the 2 point discrimination test (2PDT) before and after STT training. In 16 patients the Nine-Hole-Peg Test (9-HPT) and the Functional Dexterity test (FDT) were additionally assessed. None of the participants had severe cognitive impairment that could interfere with learning. A subgroup of 16 patients were trained either individually for 3 weeks by a daily session with an occupational therapist (N=8), or for 4 weeks independently, instructed only at baseline and at 1 week follow-up (N=8). **Results:** STT training resulted in significant improvement in performance of the 2PDT (N=340 fingers, $p < .001$) and the MFT (N=340 fingers, $p = 0.013$). In fine-motor coordination significant improvement was demonstrated only in the speed of performance on the FDT (30 hands, $p = 0.012$). No differences were found between the individual and independent training programs. **Conclusions:** Sensory training based on re-education principals using the STT has beneficial effects on the sensory and fine-motor performance of MS patients.

P384**The use of acupuncture in multiple sclerosis patients with pain: a retrospective review**

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Background: Multiple sclerosis (MS) is a chronic autoimmune disease associated with inflammatory demyelination of the central nervous system and axonal damage. Pain is one of the main symptoms found in patients with MS (more than 70% of them). Acupuncture has been used for 2500 years to treat diseases. It is safe and can be an effective tool in the treatment of pain caused by multiple sclerosis. It produces a physiological and neural response in the patients. **Objective:** Evaluation of whether pain symptoms improve after acupuncture. **Methods:** A retrospective study of 124 patients (22 male, 102 female) treated for 12 weeks (once a week) from 2006 to 2007. They were between 22 and 67 years old. The disability score was measured by the Kurtzke Expanded Disability Scale (EDSS) at the beginning and end of the treatment. The improvement of the symptoms was evaluated through the Verbal Analogical Scale (scores 0 to 10) at the end of the program. Neuropathic pain was found in 20.96% of patients and skeletal muscle pain in 52.41%. **Results:** The EDSS average of 4.19 was unchanged. Improvement of less than 30%: neuropathic pain - 30.77%, skeletal muscle pain - 10.77% Improvement of between 40% and 60%: neuropathic pain - 7.70%, skeletal muscle pain - 10.77% Improvement of more than 70%: neuropathic pain - 6.15%, skeletal muscle pain 78.46% **Conclusions:** Acupuncture might be an effective alternative for improving the symptoms of MS. It can improve quality of life, although it does not decrease disability measured through the EDSS.

Supported by: Multiple Sclerosis Brazilian Society.**P385****Effects of a high-intensity resistance training program on strength, mobility and fatigue in moderately severe individuals with multiple sclerosis**

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Background: Muscle weakness from fatigue, an inactive lifestyle and de-conditioning may magnify mobility impairments in individuals with multiple sclerosis (MS). Lower extremity resistance training has demonstrated improvements in strength and mobility in varied populations. However, in individuals with MS results have been variable for improvements in function and strength, though fatigue is consistently improved. Individuals with MS of moderate severity with impaired mobility and resistance exercise via negative, eccentrically-induced work (RENEW) that induces high muscle forces at a low metabolic cost has not been investigated. **Objective:** The purpose of this study was to compare the results of a standard exercise (STAND) and RENEW exercise program on strength, mobility and fatigue in individuals with moderately severe MS. **Methods:** Nineteen individuals with MS (8 males, 11 females; age mean=49±11.1 years; Expanded Disability Status Scale mean=5.2±0.9 (range 3.5–6.5) were randomized into one of two training groups. Measurements included: 1. Knee extension strength, Quantitative Muscle Assessment (QMA); 2. Mobility, Timed Up and Go (TUG), all individuals demonstrated mobility impairment, TUG, mean 14.95 seconds; 3. Fatigue Severity Scale (FSS). Statistical tests to determine differences and relationships were performed with a $p < 0.05$. **Results:** No significant interactions or main effects of time or group for the strength and mobility measures were observed. A significant improvement of FSS was observed over time, ($p < 0.01$, 21% decline) but no difference was seen between groups. TUG and knee extension strength demonstrated no significant changes and only minimal trends toward improvement, 1.9% and 3.1% respectively. **Conclusions:** Results did not demonstrate significant differences in strength and mobility, and only a minimal trend toward improvements in strength. Fatigue improved with exercise. Results demonstrate that individuals with moderately severe MS and impaired mobility do not respond to high-intensity resistance training the same as other populations, e.g. frail elders and Parkinson's disease.

Supported by: National Multiple Sclerosis Society (USA) PP #1150.**P386****Safety and feasibility of a high-intensity resistance training program for individuals with multiple sclerosis**

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Background: Endurance exercise and moderate intensity concentric resistance training can be beneficial for individuals with multiple sclerosis (MS). A program of high-intensity resistance exercise via negative, eccentrically-induced work (RENEW) that induces high muscle forces at a low metabolic cost may mitigate weakness and fatigue in individuals with MS. However, since negative consequences have been associated with eccentric exercise, including muscle damage, decreased force producing capacities and pain, safety and feasibility of RENEW need to be addressed. **Objective:** The purpose of this study was to determine if RENEW was safe and feasible in individuals with MS. **Methods:** Nine individuals with MS, (4 males, 5 females; age mean=48 ±7.7 years; Expanded Disability Status Scale mean=5.0±1.0; months since diagnosis mean=150±90.7). All underwent standard of care training and RENEW (3x/week, 12 weeks). Measures of safety included: monitoring of exacerbations; daily fatigue (10 cm visual analog scale (VAS)); overall fatigue (Fatigue Impact Scale (FIS)); thigh muscle pain (VAS); and whether weekly total work (kj) production decreased. Feasibility measures included: a progression in weekly total work over the training period. **Results:** No exacerbations occurred. Fatigue decreased progressively from week 1 (mean=4.2±2.3) to week 12 (mean=2.6±2.2) ($p=0.01$, Cohen's $d=0.75$). FIS decreased from pre-(mean=2.76±.53) to post-training (mean= 2.18±.83) ($p=0.02$, $d=0.88$). Total work per week increased from week 1 (mean=11,725±9715kj) to week 12 (mean=146,275±108,355kj) ($p < 0.01$, $d=1.86$). Muscle pain never exceeded 1.1 cm. **Conclusions:** RENEW was safe and feasible in this cohort of individuals with MS based on the lack of exacerbations, improvements in fatigue, low muscle pain and progressively increasing work abilities. These results provide a solid foundation for examining the efficacy of RENEW on muscle size, muscle force production and functional mobility in individuals with MS.

Supported by: National Multiple Sclerosis Society (USA) Pilot Project 1150.**P387****Motivational interviewing intervention to preserve employment in multiple sclerosis: promoting self-efficacy in the workplace**

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Background: People with multiple sclerosis (MS) in the US are unemployed at higher rates than expected given their average age, education, and level of occupational attainment. Research indicates barriers to maintaining employment can be addressed through self- and employer-initiated accommodations for physical and cognitive limitations. However, individuals with MS have been unwilling to use existing accommodation services due to concerns about disclosure, or because access to available resources is difficult. Motivational interviewing (MI) is a brief evidence-based intervention designed to enhance self-management of a variety of symptoms. We have applied MI in this study to assist employed people with MS maintain employment. **Objective:** Evaluate the efficacy of MI delivered by telephone to assist people with MS to develop and implement accommodation plans and preserve employment. Here we report on preliminary results with respect to participant satisfaction immediately following the intervention. **Methods:** Sixty subjects with MS who were working and concerned about their employment were randomly assigned to immediate or delayed MI intervention delivered by telephone. Thirty-four participants completed a 2-week follow-up survey to assess satisfaction with and perceived behavior change attributed to the MI process. **Results:** 85% of subjects were satisfied or very satisfied with the intervention. 64% changed their evaluation of accommodations in the workplace and half reported that their employment situation had improved as a result of the telephone counseling. 83% reported they expected the counseling to help them change in the future and

88% reported they were confident they could adjust their employment or accommodations as necessary in the future. **Conclusions:** From this preliminary data, it appears that participants were satisfied with the intervention, perceived it to be effective, and perceived that they had enhanced self-efficacy with respect to their employment status. Given the cost-effectiveness of the telephone-based MI and subject satisfaction, further research on efficacy is warranted.

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Development of International Classification of Functioning, Disability, and Health Core Sets for multiple sclerosis

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Background: With the approval of the World Health Organization's (WHO) International Classification of Functioning, Disability, and Health (ICF) there is now an universally accepted framework to classify and describe functioning, disability and health in individuals with health conditions. For its application in clinical practice, tools such as disease-specific ICF Core Sets are needed. ICF Core Sets are agreed-on lists of ICF categories relevant for the description of functioning of individuals with a specific health condition. **Objective:** The objective of the project is the development of ICF Core Sets for multiple sclerosis (MS) to specify functioning and disability of individuals with MS. **Methods:** The ICF Core Sets for MS were decided during an international consensus conference. Twenty-one experts from different WHO world regions and from different health professions took part in this conference. The participants of the conference decided on the ICF Core Sets for MS according to a multistage and established decision-making and consensus process which integrates evidence from four so-called preparatory studies (empirical study, systematic review, expert survey, qualitative study). **Results:** The requirements to describe functioning in MS are different for a comprehensive multidisciplinary clinical assessment and a research setting. Therefore, a Comprehensive and a Brief ICF Core Set for MS were developed. The ICF Core Sets for MS include as few categories as possible to be practical, but as many as necessary to be sufficiently comprehensive to describe the prototypical spectrum of limitations of functioning in a multidisciplinary assessment (Comprehensive ICF Core Set) and in clinical studies (Brief ICF Core Set), respectively. **Conclusions:** With the ICF Core Sets for MS we can now rely on an international standard of ICF categories to be relevant in patients with MS to specify functioning.

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Fatigue and depression in pediatric multiple sclerosis and monophasic variants

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Background: Fatigue and depression are well-known problems in adults with multiple sclerosis (MS). It is not yet clear whether this is also prevalent in children with clinically definite MS or after an episode of monophasic demyelination of the central nervous system (CNS). It is important to consider and recognize these problems in teens with a history of a demyelinating disease of the CNS, because of its impact on the quality of life. Previous studies in children with MS focused particularly on cognitive problems. **Objective:** To study the presence and severity of fatigue and depression in a group of children with MS and its monophasic variants between age 11–17 years. Furthermore, we investigated the health-related quality of life.

Methods: Validated questionnaires were used in patients with definite MS or after a monophasic demyelinating attack. Questions concerned fatigue- and depression-related symptoms (CIS-20 and CDI), as well as health-related quality of life (TACQOL). Healthy children of the same age were used as controls. **Results:** 25 Children were analysed ('patient group'), 8 with a diagnosis of MS and 17 with a history of a demyelinating event. Depression-related symptoms occurred more often in the patient group than in the control group ($p=0.038$). Children with MS reported more overall fatigue-related symptoms in comparison with controls ($p=0.005$) and the group after a monophasic demyelinating event ($p=0.033$). In the patient group scores for fatigue and depression were significantly correlated. The patient group reported a significantly lower quality of life compared with the controls. This was mainly due to the low quality of life scores in the MS patients. **Conclusions:** Children with MS or a history of a demyelinating event reported more depression-related symptoms and a lower quality of life, compared with healthy teenagers. Furthermore, fatigue was more prevalent in children with MS in relation to both other groups.

Supported by: MS Research.

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Bladder and bowel dysfunction in persons with multiple sclerosis

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Background: Bladder and bowel dysfunction is common in persons with multiple sclerosis (pwMS), impacts their quality of life (QoL) and is frequently underestimated. **Objective:** To describe the prevalence, factors impacting bladder and bowel dysfunction and QoL in pwMS. To study relationships between incontinence outcome measures used in this population. **Methods:** Prospective, observational cohort with definite MS ($n=73$) in the community (Expanded Disability Status Scale 2–8). Structured interviews for continence assessment for Urological disability: Neurological Disability Scale, American Urological Association Symptom Index (AUA), Urogenital Distress Inventory (UDI6), Incontinence Impact Questionnaire (IIQ7), and Fecal Incontinence Score for bowel dysfunction. Participation restriction: Multiple Sclerosis Impact Scale, General Health Questionnaire. **Results:** Patients mean age 50 years, 73% female and 56% with progressive MS. 42% were asymptomatic (on medication or intermittent catheterization), 21% reported urinary frequency, and a further 10% urinary incontinence (UI). 41% were bothered by frequent urination and UI due to urgency (39%), or with activity (26%). Bowel incontinence was less frequent (14%). Continence problems impacted ability to perform household chores (22%), physical recreation (28%), entertainment activities (23%), and emotional health (31%). A detrimental impact on QoL was reported with urinary (47%) and bowel problems (32%). There was a significant relationship between level of symptoms (AUA) and the level of urogenital distress (UDI $\rho=0.74$, $p<.001$) and impact of incontinence (IIQ $\rho=0.68$, $p<.001$). Patients with UI reported greater urogenital distress and impact of incontinence ($\rho=0.82$, $p<.001$). The single AUA item assessing impact of bladder symptoms on QoL showed highly significant correlations with all other bladder scales ($\rho=.60$ to $.74$), making it a potential 'screening tool' to identify patients for further assessment. **Conclusions:** This preliminary study suggests that continence issues cause significant disability in pwMS. Improved awareness of currently available treatment options and clinically robust trials are needed to assess outcomes of continence intervention.

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Effects of pregnancy on the disease course in Iranian multiple sclerosis patients

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Background: Multiple sclerosis (MS) is a debilitating disease that mainly affects young women of child-bearing age. The influence of

pregnancy and delivery on MS and the effects of the disease on birth outcome have always attracted the attention of patients and caregivers. **Objective:** This study aims to assess the possible influence of pregnancy and delivery on the relapse rate of MS and to evaluate the factors that may affect the relapse rate in the postpartum course in Iranian MS patients. **Methods:** We retrospectively determined the relapse rate (RR) per woman per year in the year before pregnancy, each trimester of pregnancy and first trimester after delivery in 92 women with clinically definite MS who have had pregnancy in their disease course and between years 2001–2006. The RRs in each trimester of pregnancy and the first three months postpartum were compared with those of the last pre-pregnancy year. **Results:** The results showed that compared with the pre-pregnancy year, there was a significant reduction in RR in each trimester of pregnancy, especially the third one (P value <0.01). Also, the increment of RR in the first three months after delivery compared with the pre-pregnancy year was statistically significant (P value <0.01). There was no significant relationship between RR in first three months postpartum and the type of delivery, type of analgesia used during cesarean section, maternal age at pregnancy, MS duration at pregnancy, or number of relapses in pre-pregnancy year or during pregnancy. **Conclusions:** Our study confirms that in MS, the RR decreases throughout pregnancy and increases in the first three months postpartum. This increment is not affected by the type of delivery, type of analgesia used during cesarean section, maternal age at pregnancy, MS duration, or number of relapses in pre-pregnancy year or during pregnancy.

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Health-related quality of life in 1269 patients with multiple sclerosis: dissociation between physical and psychological wellbeing
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Background: Measuring and improving health-related quality of life (HRQoL) has made crucial contributions for the management of multiple sclerosis (MS). However, previous studies, typically performed in small cohorts of highly selected patients from specialized treatment centers, yielded inconsistent results. **Objective:** The aim of this study was to investigate relationships between HRQoL and disease characteristics, socioeconomic and demographic factors in a large cohort of persons with MS. **Methods:** A 53-item survey was mailed to all 5475 members of an MS patient organization in the German state of Baden-Wuerttemberg. This questionnaire included demographic and socioeconomic parameters, items on severity, course, and duration of illness, level of impairment, and a questionnaire on physical and psychological health to assess HRQoL (SF-12). **Results:** Completed questionnaires of 1269 patients (47±11 yrs., 74% women, duration of illness 17±10 yrs., 45% relapsing-remitting MS) were analyzed. No or minor impairment (Expanded Disability Status Scale (EDSS) 0–3.5) was reported by 38% of the respondents, 29% stated moderate (EDSS 4–6), and 24% severe (EDSS 6–10) impairment (9% = no answer). As measured with the SF-12, respondents showed markedly impaired physical ($M = 37.99 \pm 11.17$) and psychological ($M = 45.95 \pm 11.40$) wellbeing. We found strong associations between impairment on EDSS analog scores ($r = .72$), duration of illness ($r = .30$), and 'physical quality of life' assessed with the SF-12 ($p < .01$). 'Psychological wellbeing and quality of life' was only weakly correlated with EDSS analog scores ($r = .10$) and duration of illness ($r = .10$), although these associations were still significant ($p < .01$). HRQoL was not related to socioeconomic factors, sex, course of MS, and religiosity. Educational level was positively correlated with HRQoL ($p < .05$), although the strength of this correlation was low ($r = .09$). **Conclusions:** In patients with MS, physical and psychological HRQoL are severely impaired. Physical and psychological wellbeing are dissociated: impairment and physical wellbeing are closely linked whereas psychological wellbeing is only weakly correlated with disease progression. HRQoL is not associated with socioeconomic factors.

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Effect of a single dose of intrathecal baclofen on spasticity, muscle strength and walking speed in multiple sclerosis
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Background: In the context of multiple sclerosis (MS), intrathecal baclofen (ITB) has been shown to improve severe spasticity refractory to other treatments, but its effects on strength and motor function have not been well studied. This is particularly important when ITB is used in ambulatory patients. **Objective:** Evaluate the effect of a single dose of ITB on spasticity and related symptoms, muscle strength, and gait speed, and to determine if changes in strength and gait speed parallel changes in spasticity. **Methods:** Retrospective analysis of data collected in an IRB-approved clinical registry. All patients diagnosed with MS and severe spasticity undergoing a test injection of ITB. Modified Ashworth scale in select lower extremity (LE) muscle groups, pain rating, spasm frequency, manual muscle testing, and timed 25-foot walk (for ambulatory patients) were assessed before and 3 hours after injection. **Results:** 34 patients were included (22 F; 15 ambulatory). All patients improved in LE Ashworth score, pain score and spasm frequency ($p < 0.0001$). LE strength improved in 11% of non-ambulatory and 47% of ambulatory patients. Of the ambulatory patients, a different but overlapping 47% improved in walking speed. In these patients, the degree of spasticity improvement predicted neither the improvement in walking speed, nor the gain in strength. However, gain in strength predicted gain in speed ($p = 0.024$). In patients with decreased walking speed, strength loss did not predict speed loss, but the degree of spasticity improvement did ($p = 0.026$). **Conclusions:** A bolus of ITB improved LE spasticity and associated symptoms in all patients, yet only some gained strength and/or improved walking speed. The magnitude of change in resistance to passive movement did not always predict the magnitude of change in strength or function. These findings suggest a complex relationship between relief of limb spasticity, motor strength and motor control, and underscore the need to evaluate all of these parameters when treating spasticity. Further studies are needed to explore the relationship between spasticity and motor control, and the effects of ITB on motor control and function in the MS population.

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Health care needs of patients with multiple sclerosis in the first year after diagnosis

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Background: Much is known about treatment in multiple sclerosis (MS), less about health care needs of the patients in the first year after diagnosis. **Objective:** We focussed on these needs by developing a special questionnaire to optimize physician-patient relationship. **Methods:** We fit an established health care need scale to the special situation of MS patients. Three scales (I) Need for information and co-management (17 items), (II) Need for socio-emotional aid (15 items) and (III) Need for physician availability (19 items) were defined. Patients answered the questions with respect to the individual importance (1 = not important to 5 = very important). Furthermore, current mood (MStF-K), quality of life (HALEMS) and socio-demographic data were analyzed. Six MS centers of the MS-Net of the DMSG Rhineland-Palatinate sent the questionnaire to 107 patients. **Results:** The questionnaires of 60 patients could be analysed (41 female, mean age 36 years). Most of the patients lived in a partnership, 15 were single, 3 divorced and 2 widowed. Just 4 patients were unemployed. The three most severe symptoms were sensory disturbances (64%) fatigue

(61%) and cognitive impairment (40%). Immunomodulatory therapy was started by 39 patients; 2 patients had stopped because of averseness to syringes and 37 had continued, mostly driven by fear of relapse. Fear of side effects and missing information were the main reasons not to start therapy. The most important need was (I) ($m=4.37$), followed by (III) ($m=4.23$) and (II) ($m=3.16$). Anxious depressed mood correlates with more need for (III) and (I), whereas morose mood or to be in high spirits had no relevance. **Conclusions:** Fatigue and cognitive impairment as two of the three top-ranking problems should be taken more into account in patients with early MS. Detailed disease and treatment information as well as being available is very important for physicians to optimize the physician-patient relationship.

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Assessment protocol for music therapy in patients with optic neuritis

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Background: Music therapy (MT) is a recently introduced tool for improvement of the quality of life of patients with a wide range of disorders. **Objective:** To describe a protocol for the assessment of effectiveness of MT on the visual quality of life (VQL) of patients. **Methods:** The protocol was designed according to demographic and clinical data usually observed in optic neuritis (ON). Inclusion and exclusion criteria were clearly defined. The validated Brazilian Version of the National Eye Institute Visual Function Questionnaire 25 Items (NEI-VFQ 25) was chosen to assess the visual quality of life. Standardized MT techniques were used. **Results:** The protocol comprised a form with demographic data including case number, date of birth, gender, race, age at disease onset, presence of multiple sclerosis (MS) or only isolated ON, Snellen visual acuity, visual field features, optic edema or atrophy, color function and contrast sensitivity abnormalities, interval between ON and examination, data about treatment. VQL subscales and specific scores were included for time intervals (prior to MT and after 3, 6, 9 and 12 months of MT). At each interval all visual tests were repeated. MT intervention comprises activities involving music time, tempo and rhythm to be associated to appropriate spatial configuration exercises to focus patient's attention to possible neglected or unattended visual functioning. **Conclusions:** The present protocol may be a valid and objective tool to evaluate the effects of MT on the visual quality of life of patients with ON and MS.

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Early effects of music therapy on the visual quality of life in optic neuritis

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Background: Visual Quality of Life (VQL) has been studied in many conditions including optic neuritis (ON). Music therapy (MT) has been employed in a number of neurological disorders. We investigated the effects of MT on the VQL of patients with ON. **Objective:** To investigate the effects of MT on the VQL of patients with ON. **Methods:** Patients with ON for at least six months were divided into two paired groups. One group received MT intervention whereas the other was used as a control. Both groups underwent a complete ophthalmologic evaluation, including visual fields, contrast sensitivity test and color vision assessment. The National Eye Institute Visual Function Questionnaire (VEI-VFQ 25) was employed. Patients were assessed by the NEI-VFQ 25 immediately before the MT (T0), at three (T1) and six months (T2) thereafter. The MT techniques employed aimed to promote changes in self-esteem and self-confidence through patients' audition and active involvement. **Results:** There were

20 patients in each group (ages 15 to 40 years), presenting ON for at least six months either as an isolated syndrome or in the course of MS. In the treated group higher scores were obtained for all subscales at T1 and T2 as compared with T0, whereas no substantial change occurred in the control group. The most sensitive subscales to change were Vision Specific Social Functioning, Dependency, Mental Health, Role Difficulties, General Health and General Vision. **Conclusions:** Music therapy intervention produced a significant and early improvement in the VQL of patients with demyelinating ON.

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Self-reported fatigue and quality of life in pediatric multiple sclerosis

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Background: The frequency and risk factors of fatigue have not been systematically studied in pediatric multiple sclerosis (MS). Moreover, the relationships between fatigue and quality of life (QOL) in pediatric MS have not been assessed. **Objective:** To assess the frequency of clinically significant fatigue in pediatric MS, and its relation to clinical factors and QOL. **Methods:** Fifty-one pediatric MS patients seen at the National Pediatric MS Center at Stony Brook were studied with the general fatigue subscale of the Multidimensional Fatigue Scale. Scores were considered significant if they fell 1.5 standard deviations from normative values. QOL was evaluated via the PedsQOL Inventory. Pearson correlations were used to examine the relations between patient fatigue and Expanded Disability Status Scale scores (EDSS), disease duration, and relapse rate. Further, correlational analyses assessed the relations between fatigue and the QOL factors of physical functioning, school functioning, emotional functioning, and social functioning. **Results:** Patients ranged in age from 9–17 years (mean=14.86, SD=2.07) and had a mean EDSS score of 1.86 (SD=1.63; range 0–6.5). Disease duration ranged from 0.5–90 months (mean=19.41, SD=20.60) and patients had an average relapse rate of 0.25 relapses per month (SD = 0.30, range = 0.03 - 2.0). A total of 43.1% (22/51) reported significant fatigue. No significant relations were observed between EDSS ($r=0.037$, $p=0.797$), disease duration ($r=0.144$, $p=0.315$), or relapse rate ($r=0.181$, $p=0.203$). Fatigue correlated with the following QOL variables: physical functioning ($r=0.598$, $p<.001$), school functioning ($r=0.630$, $p<.001$), and emotional functioning ($r=0.474$, $p<.001$), though not with social function ($r=0.219$, $p=0.123$). **Conclusions:** Fatigue is a major symptom for nearly half of pediatric MS patients and may be present without significant neurologic impairment, after short disease duration, and low relapse rate. Fatigue is of concern as it can adversely affect physical function, school performance, and emotional function.

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Does an innovative automated recruitment process generate an unbiased study sample from a pool of eligible patients?

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Background: A randomized, controlled clinical trial was designed to assess a web-based system designed to guide self-management of multiple sclerosis (MS) symptoms. Subjects were recruited from a large pool of MS patients identified using computerized algorithms on data derived from electronic health records. **Objective:** To assess whether an automated recruitment process can generate a representative sample from a large pool of potential subjects and multiple sclerosis functional composite (MSFC) reference population. **Methods:** Patients with definite MS and residence in a specified geographic area were entered into a potential subject pool (PSP) following queries of the electronic record database. Routinely, patients randomly drawn from

the PSP were issued study invitation letters. Upon close of enrollment we compared two groups, 1) those who completed informed consents (IC) and 2) those in the PSP minus those who completed IC or actively declined participation (PSP-R(educated)). Comparability between groups was assessed based on gender, age, ethnicity, marital status, and Charlson weighted index of co-morbidity. MSFC scores for the IC group were compared with the MSFC Manual reference population. **Results:** Of 2041 patients in the PSP, 213 patients declined participation and were excluded from further analysis. 220 individuals completed the informed consent (IC). Thus 1608 remained active in the PSP-R. Comparative results for the IC and PSP-R groups are. Female: 78.2%(IC), 74.2%(PSP-R) Non Significant (NS); Age: 48.6 years(9.7) (IC), 47.2(11.3) (PSP-R) NS; Caucasian: 77.9% (IC), 75.9% (PSP-R) NS; Marital Status: 61.4% (IC), 57.7%(PSP-R) NS; Charlson Score: 0.90(1.2) (IC), 0.95(1.3) (PSP-R) NS. MSFC: Overall disability is, on average, greater (0.64 SD) in the IC group than the MSFC Manual reference population; this was driven by longer 25 foot walk times. **Conclusions:** These data indicate the recruitment process used in this RCT (N=219) generated a sample representative of patients potentially eligible but did not respond (N=1611) for sex, age, race, marital status and disease severity. The level of disability as measured by the MSFC in the IC group compares relatively well to an external reference population.

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The Neuro-QoL project: implications for multiple sclerosis research

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Background: Neuro-QoL is an NIH (National Institute for Neurological Disease and Stroke) funded project. This 5-year study is designed to construct a clinically relevant and useful health-related quality of life (HRQL) measurement system for major neurological conditions using item response theory and computer adaptive testing. Phase 1 includes 1) Defining criteria for acceptance by neurology researchers 2) Selecting 5 adult and 2 pediatric conditions for field testing 3) Selecting domains and sub-domains to generate generic item banks and disease targeted scales, 4) Creating item pools for generic and targeted scales and 5) Translation into Spanish. Phase 2 involves field testing the Neuro-QoL instrument and create/test item bank-derived generic short forms and disease targeted scales. **Objective:** Describe the rationale for including multiple sclerosis (MS) in the first phase of this project as a key neurological condition for which item banks are developed and tested. **Methods:** Phase 1 methods included a comprehensive methodology involving qualitative and quantitative approaches. Identification of neurological conditions was accomplished using literature review, interviews with 44 experts, and a consensus panel of 13 neurology experts. Generic domains and disease-specific scales were selected using literature reviews, expert interviews and focus groups. **Results:** Selection of conditions: literature review identified MS as a major neurological condition; 33 of 44 experts identified MS as having an important impact on HRQL; using criteria established by the consensus group, MS was identified as meeting criteria by 12 of 13 experts. The generic domains selected for the Neuro-QoL measurement platform include Physical, Mental and Social. Each Domain has multiple domains. MS targeted scales include Weakness/Fatigue, Sexual Function, Sleep Disturbance, Personality/Behavioral Change, and Bowel/Bladder Function. **Conclusions:** Neuro-QoL will enable the MS clinical and research communities to precisely assess the impact of treatment on HRQL (both generic and MS-specific) as well as compare the relative impact and burden of this disease with other major neurological conditions, using a standard metric.

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Oral fingolimod (FTY720) in relapsing multiple sclerosis: impact on health-related quality of life in a phase II study

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Background: Multiple sclerosis (MS) has been shown to negatively impact health-related quality of life (HRQL). During a 6-month, phase II study in relapsing MS, oral fingolimod (FTY720), a sphingosine-1-phosphate receptor modulator, reduced relapse rates by >50% and MRI activity by up to 80% vs placebo. **Objective:** To assess the effect of oral fingolimod on HRQL in this 6-month study and in an extension up to 24 months. **Methods:** 281 patients were randomized to oral fingolimod 1.25mg (n=94), 5mg (n=94), or placebo (n=93). Hamburg Quality of Life Questionnaire in MS (HAQUAMS) scores were recorded at baseline, Month 3 (M3), and M6 during the core study, and M12 and M24 during the extension. The HAQUAMS consists of 38 items, 28 of which contribute to five sub-domains. Domain scores range from 1 to 5, with lower scores indicating improved HRQL. A total score is generated by averaging the five sub-domain scores. **Results:** Mean total scores at baseline were 1.9 for oral fingolimod 1.25mg, 5mg and placebo; corresponding scores at M6 were 1.9 (mean change from baseline, -0.02), 1.9 (mean change, -0.01) and 2.0 (mean change, +0.12). Between-group comparisons of change in total scores from baseline to M6 were favorable for oral fingolimod 1.25mg-treated patients compared with placebo (p=0.044). HAQUAMS scores for specific domains were generally stable from baseline to M6 across all three treatment arms, though patients receiving oral fingolimod 1.25mg showed improvement in the Fatigue/Thinking domain compared with those on placebo (-0.05 vs +0.14; p=0.030). Stable total HAQUAMS scores were maintained for oral fingolimod-treated patients through M24. **Conclusions:** Evaluation of HRQL adds to the assessment of the overall effects of MS disease-modifying therapies. Analysis of this 6 month Phase II study suggests that oral fingolimod may have HRQL benefits for patients with MS. Further assessment of the impact of oral fingolimod on HRQL and MS-related fatigue is planned in Phase III trials.

P401

Familiarity of family physicians with relapse evaluation and treatment in patients with multiple sclerosis

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Background: Multiple sclerosis (MS) is a common disabling neurological disease and 85% of cases are relapsing-remitting at onset, with an overall prevalence in Canada of 240/100000. MS clinics are located at tertiary care centers that are not always easily accessible for all patients during an acute relapse. Many relapses are thus evaluated by family physicians (FP) in private clinics or in the emergency department. **Objective:** This survey was designed to determine the knowledge and experience of FP in the diagnosis and treatment of MS relapses. **Methods:** All College of Physicians and Surgeons of Ontario (CPSO)-licensed FP were identified in our catchment area, and mailed a two-page anonymous survey, with no reminders. **Results:** Of 1283 CPSO listed FP, 272 surveys (21%) were returned; only 216 (17%) were practicing as FP, while others had moved, specialized or retired. 37% identified their location as rural. Although 27% had a combined practice, 91% worked as primary care physicians in an office setting. 9% of physicians stated they had no MS patients in their practice, while 70% saw between 1-5, 17% between 6-10 and 2% greater than 10 (others did not respond). While 49% of respondents identified corticosteroids as a treatment for MS patients, only 43% knew corticosteroids were used for acute relapses. Of this 43%, only 16% were able

to correctly identify the dose range, while the remainder did not know or identified low doses (25–150mg) as the preferred regimen. Additionally, 31% stated they did not know how to identify a relapse; only 37% identified new signs or symptoms of neurological dysfunction as an indicator of a relapse. **Conclusions:** Despite the high prevalence of MS in Canada, family physicians are not familiar or comfortable with the identification and treatment of MS relapses in their patients.

P402

Health-related quality of life is reduced in children with multiple sclerosis

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Background: The impact of pediatric multiple sclerosis (MS) on health-related quality of life (HRQOL) is unknown. Since MS has the potential to disrupt a child's developmental trajectory, its impact on HRQOL may be substantial. **Objective:** To describe HRQOL in a well-characterized population of children with MS. **Methods:** The Pediatric Quality of Life Inventory Version 4.0 (PedsQL 4.0) was administered to children with MS and clinically isolated syndrome (CIS) and/or their parents, who are followed prospectively at the UCSF Regional Pediatric MS Center. Scores were compared with those reported for healthy and chronically ill children in a validation study of the PedsQL 4.0 (historical controls) using Student's t test. **Results:** PedsQL 4.0 scores were available for 20 ethnically diverse children; 15 children and 16 parents completed the survey. The mean age (+/- SD) of the children at symptom onset was 13 +/- 4 years; 15 had MS and 5 had CIS. Children with MS/CIS reported a mean PedsQL 4.0 total score of 71.56 +/- 18.61, which was substantially lower (worse) than scores for healthy controls (83.00 +/- 14.79; p=0.004) and for chronically ill children (77.19 +/- 15.53; p=0.17). Parents of children with MS/CIS reported a mean PedsQL 4.0 total score of 74.00 +/- 19.76, which was also lower than the mean for healthy children (87.61 +/- 12.33; p<0.0001) but was similar to that for chronically ill children (74.22 +/- 18.40, p=0.96). The differences in mean scores between MS/CIS and healthy children exceeded the established minimal clinically important differences for the PedsQL 4.0 (4.36 points for the child self-report; 4.50 points for the parent proxy-report). Both physical and psychosocial (emotional, school) summary scores were substantially lower for children with MS/CIS than for healthy children. **Conclusions:** Children with MS/CIS experience reduced HRQOL compared with historical healthy controls. Further investigation of predictors of HRQOL in pediatric MS is warranted.

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P403

Single treatment with inflatable pressure splint improves short distance gait velocity in patients with multiple sclerosis

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Background: Gait impairment is a highly prevalent symptom in multiple sclerosis (MS) and is associated with considerable disability related to motor weakness, increased muscle tone, sensory impairment and imbalance. The inflatable pressure splint (IPS), an air-tight double-sleeved plastic envelope that when fully inflated causes pressure around the limb, has been reported to reduce spasticity and improve gait in children with spastic cerebral palsy, and was also used for rehabilitation of knee flexion contractures in patients with MS. **Objective:** To assess the effects of short-term use of IPS on gait velocity and balance in patients with MS. **Methods:** MS patients with lower limb spasticity that interfered with their ambulation skills were

randomly assigned into the IPS treatment group (long leg IPS applied uni- or bilaterally for 20 minutes during rest), or to the rest group (lying supine for 20 minutes). In both groups gait was assessed by measurement of 10 and 20 meters walking time (TW10, TW20) and by the timed up and go test (TUG) before and immediately after the procedure. **Results:** Patients in the IPS treatment group (N=30, 21 females, mean age 48.2±10.1 years, disease duration 12.6±5.6 years, mean EDSS 5.4±1.2) significantly improved their gait and balance performance after treatment. TW10, TW20 and TUG decreased by 3.80 (p=0.0132), 9.65 (p=0.0138) and 3.16 (p=0.0016) seconds, respectively. In the rest group (N=30 patients, 22 females, mean age 47.1±12.3 years, disease duration 11.9±10.9 years, EDSS 4.6±1.6) no changes were recorded in gait performance. **Conclusions:** Single session of IPS treatment significantly improved short-distance gait velocity and stability. Both MS patients and their caring physiotherapists can benefit by the application of the IPS procedure that is inexpensive, easy to use and enables better performance of exercises.

P404

Socioeconomic impact of multiple sclerosis in the Mississippi Delta

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Background: The Mississippi Delta is one of the most medically underserved and economically disadvantaged regions of the South. The high treatment costs of multiple sclerosis (MS) puts tremendous burden on the finances and the quality of life in patients with this disease. Since MS mostly affects people of the working age group, the financial and personal impact becomes even greater. **Objective:** In the present pilot study, we evaluated the racial, gender and age susceptibility as well as the economic outlook of MS patients in this region. **Methods:** The data was obtained from The Greenwood Neurology Clinic where altogether 46 patients have been diagnosed with MS. **Results:** Out of the 46 patients there are 37 African-Americans and 9 Caucasians. 29 patients are female and 17 male, whereas 25 of the females are African-American. Most of the patients are under 50 years of age. There are 6 patients that are 30 years of age and under, 5 of them being African American females. A majority of the patients are on the Copaxone, Tysabri or Avonex regimen. **Conclusions:** The cost of these medications average from \$11,000 to \$23,000 dollars annually. There are also often costs not calculated such as earnings lost or informal care. The economic, social and medical costs seem significant for minority patients with a chronic disease such as MS.

P405

Experience of an out-patient multidisciplinary approach in disabled patients with multiple sclerosis

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Background: In our multiple sclerosis (MS) clinic, we propose to treat disabled patients with an outpatient multidisciplinary approach (OMA) by a team composed of a neurologist, physical therapist, psychiatrist, neuro-urologist, speech therapist, occupational therapist, social worker and nurse. Evaluation of outcome and long-term follow-up of patients included in this OMA is required to establish its effectiveness. **Objective:** To evaluate the effectiveness of OMA in disabled MS patients. **Methods:** In this prospective clinical study, MS patients from two departments of neurology were screened by INTERMED. This score, assessing psychological, social, health care, is a screening instrument to identify patients in need of multidisciplinary treatment (Hoogervorst et al, JNNP, 2003). Forty consecutive MS patients with an INTERMED Score >25 were included. In group 1 (n=28) OMA was proposed. In group 2 (n=12) patients were followed with the usual outpatients clinic. Expanded Disability Status Scale (EDSS), anxiety and depression (HAD scale), and quality of life (MS29 scale) were quantified at M0, M6, and M12. Evaluation of each group was performed by two independent neurologists. **Results:** The mean

INTERMED Score was 30 [25–33]. 16 women and 24 men, with a mean age of 49 years [SD 14], mean disease duration of 17 years [range 10–25], and mean EDSS of 6.3 [SD: 0.9] were included. Demographical and clinical characteristics did not differ between the two groups. Disability assessed by EDSS and HAD remained stable at each evaluation in the two groups. A trend to improvement in quality of life was observed at M6 in the group 1 (n=28) but this effect was not sustained at M12. In contrast, a trend to lower quality of life was observed at M6 and M12 in group 2. **Conclusions:** These results suggest a transitory benefit of OMA on quality of life in disabled MS patients, and might suggest the need for yearly OMA. This yearly follow-up will be evaluated using the same scales.

P406**Quality of life in multiple sclerosis: the role of social support**

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Background: An analysis of the scientific literature on multiple sclerosis (MS) shows a remarkable and increasing interest in the debate on Quality of Life (QoL). However, little information is available at this time on the factors meaningfully affecting QoL in patients with relapsing-remitting MS (RRMS). Social Support (SS) is considered as a protective factor right from the delivery of the diagnosis, since it fosters a better reaction to stress, a better adaptation to illness and a greater treatment compliance. **Objective:** Based on these preliminary remarks, a retrospective observational study is proposed, whose main goal is the analysis of the interrelation between QoL and SS in patients with RRMS. **Methods:** The study has observed a sample of 60 individuals with the following characteristics: at least 2 years elapsed since diagnosis, at least 2 relapses since onset, Expanded Disability Status Scale (EDSS) score between 1 and 5.5 and stable clinical picture at enrolment. For the assessment of QoL and of SS both quantitative and qualitative instruments have been adopted: MSQoL-54, Social Provisions Scale, Sense of Community scale, a semi-structured interview, and the semi-projective test Family Life Space. The quantitative data have been analysed by means of suitable statistical indexes, and the qualitative data have been analysed by means of content analysis. **Results:** A meaningful correlation has been found between QoL and SS, and particularly high levels of SS are correlated with high scores in the physical and mental health subscales (MSQoL-54). It was found that the main support is provided by the family. The Sense of Community assessment has been problematic, as the choice of family as the main reference point has invalidated the Sense of Community test results. **Conclusions:** We have demonstrated that social support must be considered as a crucial element in the individual's adaptation process to chronic illness.

P407**Transcultural adaptation and validation of the Six Spot Step Test in a Brazilian population**

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Background: The clinical manifestations of multiple sclerosis (MS) patients are variable with no linear evolution, marked by fluctuations of symptoms, disability progression and variable periods of stability and improvement. These variations are very difficult to measure and in clinical practice some instruments may help to verify the progression, improvement and stability of clinical symptoms. **Objective:** Adaptation and validation of Six Spot Step Test (SSST) in MS patients, comparing with healthy controls. **Methods:** The SSST was applied in 57 MS patients and 92 controls to verify the average of time to complete the test. The test field measures 1x5 meters (width x length). At the middle of each end-line a circle, with a diameter of 20cm, is marked. At each side-line two circles of the same size are marked with a distance from the end-line of 1 and 3m on one side and 2 and 4 meters on the other side. Five wooden cylindrical blocks with a

diameter of 8cm and a height of 4cm, weighing 134g, are placed in the center of the circles. The groups are instructed verbally and are shown by the examining technician how to perform the SSST. The subject walks criss-cross from one circle to the next while pushing the blocks out of the circles and the test fields. The groups perform the test twice with each leg, and the time is measured to reach an average of times. **Results:** There was no difference in the SSST relating to age and gender. In the MS patients group the average time was 14 seconds, with a range 6.93 to 59.12 seconds. In the control group the average of time was 7 seconds, with a range of 4.63 to 38.8 seconds. Comparing the dominant leg with non-dominant leg in the control group we found a difference of 20%, but this depends on which leg is impaired in the MS patients group. **Conclusions:** We validated the SSST in Brazil and we believe that this test may be useful to evaluate ambulation in MS patients.

P408**Association between the number of years with the diagnosis of multiple sclerosis and perception of autonomy and participation in persons with the disease**

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Background: Multiple sclerosis (MS) is a chronic, often disabling disease of the central nervous system that affects women twice as often as men. Recent evidence increasingly suggests that rehabilitation focusing the improvement of physical activity, and participation in social activities, improves the lives of persons with the disease. **Objective:** The aim of the present study is to examine the relationship between years diagnostic of disease and participation/autonomy in patients with MS. **Methods:** PARTICIPANTS: 280 patients with MS were recruited via their physician at a neurology department of a central hospital in Lisbon. They were eligible for inclusion in the study if they met the following criteria: diagnosis according to relevant medical criteria, between 18 and 65 years, being diagnosed at least 1 year ago, Expanded Disability Status Scale (EDSS) score under 7. The mean age was 40 years (range 18–65), 71.3% were women, 61.1% were currently married, 63% active workers, mean school level of 12 years, and mean score of EDSS is 2.8 **METHODS:** the study is correlational and cross-sectional. **MATERIAL:** The questionnaire Impact on Participation and Autonomy (IPA) addresses autonomy and participation in 5 domains: autonomy indoors, family role, autonomy outdoors, social relations, and work and educational; an additional question was 'for how many years have you had the diagnosis of MS?' **Results:** The correlations between years diagnostic of disease (DD) and IPA dimensions controlling for age are all statistically significant but moderate: between DD and Autonomy Indoors ($r=0.23$ $p<0.003$), between DD and Family Role ($r=0.25$ $p<0.001$), between DD and autonomy outdoors ($r=0.21$ $p<0.001$), between DD and social relations ($r=0.09$ $p<0.05$), DD and Work and Educational Opportunities ($r=0.14$ $p<0.001$). **Conclusions:** The study shows that there is a statistically significant correlation between these variables, suggesting that when the years of diagnosis increase participation and autonomy reduce. Autonomy and perception of functioning can be an important buffer for patients with MS.

P409**Visuo-proprioceptive rehabilitation improve postural control in patients with multiple sclerosis**

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Background: Lack of balance is among the most disabling symptoms of multiple sclerosis (MS). It may affect about 70% of patients over the course of the disease. **Objective:** Our purpose was to evaluate the effects of visuo-proprioceptive training in a cohort of MS patients with a predominant balance disorder due to sensory and/or cerebellar ataxia. **Methods:** Thirty patients (18 females, 12 males) with

unrestricted walking ability (median Expanded Disability Status Scale (EDSS) score of 3.0, range 1.5–5.5) and 14 healthy age- and sex-matched controls were recruited to participate in this pilot study. To avoid the ‘learning effect’, patients performed a baseline visit (T0) followed by a run-in period without any rehabilitative intervention, and other two assessments before (T1) and at the end (T2) of the period of rehabilitation (12 sessions twice in week for 3 consecutive months). All patients underwent a neurological examination (EDSS and multiple sclerosis functional composite (MSFC) score) and a stabilometric test aimed at assessing the stability in bipodalic stance by a computerised postural recorder device (Delos Postural Proprioceptive System, DPPS). The Dizziness Handicap Inventory (DHI) was also administered. **Results:** When compared with controls, MS patients had a greater degree of trunk sway, measured by DPPS, both with open eyes ($p=0.001$) and closed eyes ($p=0.01$). Longitudinal analysis showed no significant differences between T0 and T1 assessment. No changes in EDSS score and in the stabilometric test (open eyes) were recorded. In contrast, after the visual and proprioceptive rehabilitation there was an improvement in MSFC score ($p<0.001$), in DHI score ($p=0.05$) and in the stabilometric test (closed eyes) ($p=0.05$). Patients with an EDSS score ≤ 4.0 showed the best improvement after the rehabilitation protocol. **Conclusions:** Preliminary results of the present study showed that visuo-proprioceptive rehabilitation had a positive impact on the balance disorders caused by MS, particularly in the early phase of the disease.

P410

Quality of life in 1000 patients with relapsing-remitting multiple sclerosis and the impact of treatment initiation with intramuscular interferon beta-1a

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Background: Multiple sclerosis (MS) can have considerable impact on patients’ quality of life (QoL), but little is known about QoL and social-demographic parameters related to QoL in early MS. QoL has recently become a relevant outcome parameter in phase III trials, while such data was not evaluated in phase III trials for IFN-beta. **Objective:** To examine quality of life (QoL) in a large cohort of untreated patients with relapsing-remitting multiple sclerosis (RRMS) and to investigate the impact of intramuscular interferon beta-1a (IFNβ-1a i.m.) treatment. **Methods:** A prospective, observational, multicenter study conducted in Germany. Untreated patients with RRMS were included at participating centers. Patients were treated with intramuscular IFN beta-1a (AVONEX®) 30 ug once weekly. Five follow-up examinations were scheduled between baseline and 12 months. Main outcome measures: Quality of life (QoL) as measured by the EuroQol questionnaire (EQ-5D). **Results:** 1,157 patients were included (mean age 37.61 years, median disease duration 13 months, mean relapse rate 1.65, mean Expanded Disability Status Scale score 2.07). Baseline relapse rate was reduced to 0.6 at 12 months ($p<0.0001$). QoL was considerably lower in MS patients compared with previous experiences in the general German population. QoL improved after treatment initiation (utilities of EQ-5D 0.77 versus 0.75 at baseline, $p=0.0032$). 15.5% were incapable of working due to MS (compared to 1.8% in the age matched German population). Higher disease activity and incapability of working were negative predictors of QoL. **Conclusions:** QoL is considerably impaired even in the early stages of MS. Treatment initiation with self-injectable IFN beta attenuates MS disease activity and improves QoL. Frequent incapability of working early in the course of MS is a major challenge for the social security systems.

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P411

Changes of clinical functions after neurorehabilitation and aerobic training correlate with changes of the brain activity pattern as a reaction to complex motor stimulation in multiple sclerosis

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Background: This research looks for links between brain activity changes caused by rehabilitation and the changes that indirectly characterize brain adaptation processes. **Objective:** The aim of this work was to find whether there are correlations between the changes in clinical functions and changes of the brain activity pattern as a reaction to complex motor stimulation in multiple sclerosis (MS). **Methods:** 12 patients with MS (EDSS from 3.0 to 4.5) and 12 healthy controls were examined twice (clinical functions and event related fMRI on complex motor stimulation - active movement by the right hand immediately followed by the left hand and vice versa) in two months. In this time 6 patients underwent neurorehabilitation and 6 aerobic training. Changes of the proportion of the number of activated voxels at a given statistical level in the primary motor cortex for each hand as ‘tandem’ (the second one during complex motor stimulation) to the ‘navigator’ hand (the first one) were correlated with changes in clinical functions. **Results:** After neurorehabilitation the change of the proportion of activated voxels for the right hand as ‘navigator’ to ‘tandem’ show high correlation with the improvement of speed of walking (Timed 25 - Foot Walk, -0.91), spasticity on lower extremities (Modified Ashworth Scale, -0.92) and tremor on upper extremities (0.85). The proportion for the left hand as ‘navigator’ to ‘tandem’ correlates with the change of weakness of upper extremities (Motoric Index, 0.84). After aerobic training changes of the proportion of activated voxels for the right hand as ‘navigator’ to ‘tandem’ show high correlation with the improvement of cognitive functions (PASAT 3, -0.88). Changes of the proportion for left hand correlate with changes of spasticity of upper extremities (0.88). **Conclusions:** Both therapeutic programs show the correlations between changes in clinical functions and changes in the brain activity pattern. **Supported by:** Grant of Czech Ministry of Health 1A/8628-5.

P412

Patient perspectives on quality in mental health care for people with multiple sclerosis

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Background: Although the existence and prevalence of mental health problems among people with multiple sclerosis (MS) has been well documented, little is known about the quality of mental health care provided to MS patients. **Objective:** This study was designed to explore the patient’s experience of mental health care, to add to our understanding of the nature of quality mental health care for people with MS. **Methods:** A multi-center survey of MS patients’ experience of mental health care, described elsewhere (Ding, L. et al, 2008, submitted for publication) was utilized to identify MS patients who reported high and low-quality mental health care. Focus groups were convened at each site. All patients in the focus groups had received mental health treatment in the past two years. Data were summarized and analyzed by allocating and coding comments by participants under specific themes and categories. **Results:** The need for mental health intervention, according to participants, begins soon after diagnosis. There was wide variability in the provision of mental health support at that critical time. This may have occurred because of limited and inconsistent screening for mental health problems at all of the MS Centers. Knowledge about MS, and experience working with people with MS were important provider characteristics described by participants. Many stated that the mental health provider should

understand that MS is not the only stressor in their lives. Participants reported positive results when mental health services were available at the MS Center. They believed that communication and collaboration between neurologists and mental health providers was an essential aspect of quality care. Participants whose family members were included in their treatment reported that it had been very helpful. Community referrals by Center staff led to good results for many participants. **Conclusions:** Patients indicated quality mental health care for MS patients includes early screening at MS Centers, care availability soon after diagnosis and beyond, by providers familiar with the issues faced by people with MS and their families, and collaboration between health care and mental health providers.

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P413

Employment discrimination experiences of adults with multiple sclerosis

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Background: This presentation reviews results of research on discrimination allegations and resolutions pertaining to employees with multiple sclerosis (MS) in the American workforce. The research was conducted with the support of the National Multiple Sclerosis Society and the United States Equal Employment Opportunity Commission (EEOC). **Objective:** Presenters will discuss the results of investigations a) comparing the employment discrimination encountered by workers with MS and workers with other types of disabilities; b) comparing the discrimination allegations and resolutions of women and men with MS; c) describing the predictors and patterns of perceived employment discrimination encountered by employees with MS; and d) analyzing the rate, type, and predictors of merit resolutions (i.e., allegations verified by the EEOC). **Methods:** With permission of the EEOC, the research team analyzed data that included all charges of employment discrimination resolved by the EEOC since implementation of the ADA in 1992 through 2003. **Results:** Selected conclusions from the research include: a) adults with MS were more likely than the comparison disability group to allege discrimination related to reasonable accommodations, terms of employment, constructive discharge, and demotion; b) women and men with MS reported similar background characteristics and patterns of employment discrimination, although some evidence suggested that women were more likely to file allegations of intimidation and harassment; c) the EEOC found no cause for discrimination in the majority of allegations filed by women and men with MS, although both groups had higher rates of merit closure than the comparison group; and d) merit closures were more likely to occur for reinstatement, reasonable accommodation, and terms of employment than for discharge. **Conclusions:** Findings support the need for early workplace intervention to help employees with MS identify and respond to instances of discrimination. Similarly both women and men with MS require additional information clarifying how to document allegations of discrimination and file such allegations with the EEOC.

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P414

A prospective study on clinical outcome of inpatient versus outpatient rehabilitation in subjects with multiple sclerosis

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Background: Several data support the utility of rehabilitation to improve clinical and functional performance of patients with multiple sclerosis (MS). It is still debatable whether intensive inpatient

treatment results in a more evident benefit than outpatient treatment. **Objective:** In this study we evaluate the clinical and functional outcome of inpatient and outpatient rehabilitation in two different cohorts of patients with MS. **Methods:** We considered a group of 21 consecutive patients with both relapsing-remitting (RRMS) and secondary-progressive (SPMS) course of disease in two different region of Italy. All patients had worsening of neurological condition of at least 1.0 point on the Expanded Disability Status Scale (EDSS) in the last 12 months without superimposed relapses in the previous 3 months, and had an EDSS score of 3.5 to 6.5. Nine subjects (3 RRMS, 9 SPMS) underwent an intensive inpatient rehabilitation program in a Neurorehabilitation Department in Northern Italy and 12 patients (6 RRMS, 6 SPMS) followed the same program in an outpatient clinic in Southern Italy. As outcome measures we evaluated EDSS, Barthel Index (BI), time to walk 15 feet (t15F) and 9-Hole-Peg-Test (9HPT). Both groups are similar in basal data such as age, sex, duration of disease, EDSS, BI, and 9HPT. **Results:** We found that inpatient and outpatient rehabilitation gave a significant improvement in EDSS score ($p < 0.0001$), 9HPT, BI ($p < 0.02$), while there seemed to be no effective in t15F ($p = 0.09$). Comparing inpatient vs outpatient outcome, we found that first group had significantly more improvement in EDSS, 9HPT and BI with respect to the outpatient group. We did not find any differences in outcome measures with respect to course of disease. **Conclusions:** Our data demonstrate that both inpatient and outpatient rehabilitation gave significant results in terms of clinical and functional improvement in MS patients regardless of their clinical course. Moreover, intensive inpatient rehabilitation provided greater benefit than outpatient rehabilitation.

P415

The relationship between baseline clinical measures and quality of life in patients with relapsing multiple sclerosis: analyses from the phase 3 trial of intramuscular interferon beta-1a

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Background: There is evidence that multiple sclerosis (MS) patients' quality of life (QoL) correlates with clinical measures such as the Expanded Disability Status Scale (EDSS) and relapse rate. **Objective:** To further evaluate the relationship between baseline QoL and clinical measures as well as the impact of intramuscular (IM) IFN β -1a on QoL in relapsing-remitting MS (RRMS). **Methods:** In the pivotal phase 3 clinical trial, it was hypothesized that IM IFN β -1a would improve QoL as measured by the Sickness Impact Profile (SIP) in patients with poorer baseline QoL. Hence, patients were stratified based on their baseline SIP score: intact QoL (SIP <10) or poorer QoL (SIP \geq 10); SIP of 10 is \geq 2 standard deviations away from values observed in healthy controls. The SIP, consisting of Total, Physical, and Psychosocial scores, was administered at baseline and every 6 months over 2 years. Correlation between baseline SIP and clinical measures (EDSS, relapse rate) was assessed using t-test and Pearson correlation analysis. **Results:** SIP data from 158 patients (Total <10, n=94; Total \geq 10, n=64; Physical <10, n=117; Physical \geq 10, n=41; Psychosocial <10, n=82; Psychosocial \geq 10, n=76) were analyzed. Patients with higher Total ($P=0.017$) and Physical ($P < 0.0001$) SIP scores had significantly higher baseline EDSS scores. Total SIP correlated with Cerebellar ($P=0.005$) and Bowel/Bladder ($P=0.003$) functional systems (FS). Physical SIP correlated with Pyramidal ($P=0.008$), Cerebellar ($P < 0.0001$), Sensory ($P=0.0009$), and Bowel/Bladder ($P=0.0006$) FS. Psychosocial SIP correlated with Bowel/Bladder ($P=0.01$) FS only. In contrast, prestudy relapse rate did not correlate with SIP scores. EDSS progression (worsening by \geq 1 point, sustained for 6 months) was associated with significant worsening in Physical scores over 2 years ($P=0.031$) compared with non-progression. Over 2 years, IM IFN β -1a therapy significantly improved Physical scores in patients with poorer baseline QoL ($P=0.045$) compared with placebo. **Conclusions:** MS patients with greater disability and FS impairment had lower baseline QoL. Treatment with IM IFN β -1a significantly improved QoL in these patients.

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Hippotherapy for multiple sclerosis - pilot study assessing effects on balance, spasticity, ability to walk and quality of lifeAnja Sager¹, Marion Drache², Bettina Schaar¹, Dieter Pöhlau²¹*Institute of Rehabilitation and Sport for the Disabled, German Sport University Cologne, Cologne (Köln), Germany;* ²*Kamillus Clinic, Asbach, Germany;* ³*Johannisberg Therapeutic Riding Center, Windhagen, Germany*

Background: Hippotherapy is a physiotherapeutic neurophysiological treatment that uses the movement of horses. The oscillating impulses of the three-dimensional equine movement are communicated to the patient sitting upright on the horse's back. **Objective:** To assess the effects of hippotherapy on multiple sclerosis (MS) patients with respect to balance, spasticity, ability to walk and quality of life. **Methods:** Single-group, A-B-A experiment with initial, interim and final testing. During the three-month treatment, 16 MS patients came twice weekly to individual thirty-minute hippotherapy sessions. Tests included the Berg Balance Scale (BBS), the modified Ashworth Scale (MAS), the 10-meter walking test and the SF-36 health status questionnaire. The test group comprised 13 female and three male subjects (N = 16, age: 44.81 (± 9.18) years; disease duration was 10.44 (± 8.66) years; Expanded Disability Status Scale score was 4.5 - 6.5). **Results:** Before and after test scores differed significantly or very significantly: difference in BBS scores (p=0.002). Decrease in the subjects' lower extremity spasticity (p<0.001). Difference in walking speed, measured before and a half hour after hippotherapy (p=0.009). Results of the SF-36 questionnaire showed significant differences in general perception of health status (p=0.003), physical ability to function (p<0.001), physical role function (p=0.007), bodily pain (p=0.046), vitality (p<0.001), social functioning (p=0.009) and physical well-being (p=0.004). **Conclusions:** Hippotherapy has positive effects on MS patients with respect to balance, spasticity, ability to walk and quality of life. A long-term study with a larger number of subjects and accompanying control group should be conducted; this is already in the conceptual phase.

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Efficacy of a computer-based cognitive training program on the cognitive performance of patients with multiple sclerosisEvelyn Shatil¹, Omer Horowitz¹, Avishag Metzger², Ariel Miller²¹*Cognitive Science, CogniFit, Yokneam Ilit, Israel;* ²*Department of Neurology, Carmel Medical Center, Haifa, Israel*

Background: Cognitive impairment, including impaired memory, attention, processing and executive functions, affects up to 65% of patients with multiple sclerosis (MS). **Objective:** To assess the efficacy of a computer-based cognitive training program (MindFit®) on the cognitive performance of patients with MS. **Methods:** Participants assigned to the training group completed a training program over a period of 12 weeks. A control group received no training. All participants were evaluated by the Neuropsychological Examination - MindFit® (NEM) on 14 different cognitive abilities, and on the digit-symbol (WISC), the REy and on a semantic fluency test, the Zung Depression Scale, the Expanded Disability Severity Scale and the Fatigue Severity Scale at baseline and at the end of the 12-week study. **Results:** The groups were similar in age, gender, education (p>.05), in fatigue and depression scores at time 1 (p>.14). To examine the differences in cognitive performance between the groups analysis of covariance was used. For each analysis the dependent variable was the score on the ability at time 2, and the independent variables were group and the score on the ability at time 1. The MindFit-trained group was superior to the control on three memory measures: visual memory (F[1,42] = 13.60, p < .001; general memory F[1,42] = 13.80, p < .001; and working memory F[1,42] = 12.65, p<.001). No differences were found on any of the other cognitive abilities measured or on the depression scale. An additional finding is that the fatigue scores increased for the trained group F(1,42) = 5.45, p<.05. Women composed 66% of subjects in each group and although not significant, the degree of improvement was consistently higher for men on the three memory measures (1.21 vs 0.65; 1.29 vs 0.76; 1.35 vs 0.78). **Conclusions:** Specific cognitive training using the MindFit® program can improve memory skills in patients with MS. Further

research is required on the interaction between fatigue and cognitive training in MS as well as on interrelations between MS, gender, and benefit from cognitive training in larger samples.

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P418

Development and evaluation of a self-management cognitive program for multiple sclerosis

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Background: Multiple sclerosis (MS)-related cognitive impairments negatively affect many aspects of functioning and participation. Therefore, it is critical that cognitive interventions be an essential component of MS rehabilitation. Cognitive rehabilitation can be enhanced through the use of compensatory and self-management training programs to circumvent the impact of cognitive impairments and increase levels of functioning and self-efficacy. **Objective:** This study included the development and evaluation of a self-management program that addresses cognitive impairments commonly found in MS. During the program, participants learned how to apply compensatory strategies to cognitive tasks. The goals of the program were to increase participants' knowledge of cognitive impairments, increase use of compensatory strategies, and increase levels of self-efficacy to manage cognitive difficulties. **Methods:** The cognitive program can be classified as a complex health intervention because it includes a variety of content and structural components that can impact participants' ability to successfully manage cognitive impairments. Therefore, a mixed-methods, time series design was used and included an outcomes and process evaluation in order to understand the mechanisms that contributed to change among the participants. **Results:** Data from 35 participants, who attended one of five program cycles, were analyzed. Participants reported benefiting from compensatory strategies they learned in a variety of daily activities. Quiz scores indicated that all participants increased their knowledge of cognitive impairments. In addition, participants significantly increased their levels of self-efficacy (F=19.70, df=2,32, p<0.001). The process evaluation identified components that facilitated participants' learning and contributed to making behavior changes such as learning to problem-solve through cognitive challenges, practicing strategies in between sessions, and being part of a group. Participants recommended modifications for future programs, namely the incorporation of caregivers. **Conclusions:** The results of this study point to the benefits of self-management, group-based programs in the rehabilitation of cognitive impairments in MS.

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Quality of visual life outcomes in Brazilian multiple sclerosis patients

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Background: Patients with multiple sclerosis (MS) frequently complain of visual abnormalities. Many MS patients precisely define their visual complaints as the presence of spots or loss of part of the visual field, others vaguely refer to blurred vision or imprecise visual discomfort. Presumably all these abnormalities may impair patients' ability to perform their daily living activities, and the quality of visual life may be compromised. **Objective:** To study the quality of visual life in a group of Brazilian MS patients. **Methods:** A cross-sectional study in a sample of 69 MS patients and 71 healthy controls was conducted. We applied the Brazilian version of the 25-Item National Eye Institute Visual Function Questionnaire (NEI VFQ-25) and the following visual function tests: Snellen visual acuity (VA), Farnsworth-Munsell 100-Hue color vision (100Hue), Low-Contrast Sloan Letters Charts (LCSLC) and Humphrey visual field (HVF). The Expanded Disability Status Scale score (EDSS) was also recorded. The t test for an

independent sample between the MS cohort and controls was performed for comparison of the visual questionnaire scales. Spearman rank correlation was used in MS group between NEI VFQ-25 scores and the visual function tests. **Results:** A significant correlation between the MS cohort and controls was observed in the composite score and five subscales of the visual questionnaire ($p < 0.05$). The composite score of the NEI VFQ-25 correlated well with vision function tests including VA ($r = -0.25$, $p = 0.04$), LCSLC 1.25% ($r = 0.35$, $p < 0.01$), HVF ($r = 0.35$, $p < 0.01$) and EDSS ($r = -0.32$, $p = 0.01$). The 100-Hue test did not show statistically significant correlation with the composite score. **Conclusions:** MS patients have a deteriorated quality of visual life as shown by the NEI VFQ-25. Abnormalities of visual function tests may explain the subjective visual complaints in these cases.

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Gait and balance improved in patients with multiple sclerosis after inpatient physiotherapy

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Background: Multiple sclerosis (MS) may lead to gait and balance problems, and appropriate treatment is needed. Physiotherapy has been shown to improve and maintain gait and balance in various populations. **Objective:** To investigate whether gait and balance improved after inpatient physiotherapy for patients with MS, both in short and long-term perspectives. **Methods:** 56 patients with MS (34 relapsing-remitting MS, 18 secondary progressive MS and 4 primary progressive MS) with clinical stable disease and Expanded Disability Status Scale score between 4.0 and 6.5 received individualized physiotherapy according to the Bobath concept, for four weeks. Gait and balance were measured by: six-minute walk test (6MWT) (main outcome), 10m timed walk (10MTW), Timed Up&Go (TUG), Trunk Impairment Scale (TIS) and Berg Balance Scale (BBS) at screening, baseline (6 weeks after screening), immediately after treatment and after three and six months. Changes were analyzed by general linear model for repeated measures (GLM) with one within subject factor (time). If the time factor was significant ($p \leq 0.05$), Bonferroni-adjusted paired t-tests were used for pair wise comparisons between the different time periods. **Results:** GLM showed significant change for all measures ($p < 0.05$). Stability from screening to baseline was demonstrated for all measures, except TIS which demonstrated decline (mean -1.7, $p < 0.001$). Improvement from baseline to after treatment was demonstrated for all measures; 6MWT (mean 54.5m, $p < 0.001$), TIS (mean 4.5, $p < 0.001$), BBS (mean 3.2, $p < 0.001$), TUG (mean -1.3, $p < 0.001$) and 10MTW (mean -1.0, $p = 0.019$). Improvement was still significant after three and six months for TIS ($p < 0.001$), 6MWT ($p < 0.001$ and $p = 0.003$ respectively), and for BBS ($p = 0.015$ and $p = 0.001$ respectively). For 10MTW improvement was significant after three months ($p = 0.029$), but not after six. For TUG it was not significant after three and six months. **Conclusions:** Gait and balance improved after four weeks with inpatient physiotherapy. Short and long-term improvement was demonstrated.

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P421

Factors associated with change in quality of life, disease level and functional status since natalizumab initiation

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Background: Natalizumab is indicated as monotherapy for the treatment of patients with relapsing forms of multiple sclerosis (MS) and is available under the manufacturer's restricted distribution program (TOUCH). **Objective:** To identify factors associated with patient-

reported quality of life (QoL), disease level and functional status change after 3 months on natalizumab. **Methods:** MS patients who were enrolled in the TOUCH program and had received their 3rd natalizumab infusion participated in a 20-minute telephone/internet survey. Outcome measures included change in overall QoL and pre/post disease level and functional status since natalizumab initiation. Age and gender-adjusted logistic regression models were used to determine the relationship between changes in QoL, disease level or functional status and years since MS diagnosis. **Results:** Of 451 survey participants, 73% were female; mean age was 46 ± 11 years. Most patients ($\geq 96\%$) did as well or improved in QoL, disease level and functional status with significant associations ($p < .001$) between QoL and disease level and functional status change. Since few patients ($\leq 4\%$) indicated worsening of QoL, disease level or functional status, they were excluded from the analysis and only the likelihood of improvement vs. doing as well was analyzed. Compared with younger patients (< 35 years), patients > 65 were 87% less likely to report overall QoL improvement ($p = .017$); patients 35–44 were 61% less likely ($p = .006$) to report functional status improvement and patients 45–54 were 57% less likely ($p = .031$) to report disease level improvement. After adjusting for age and gender, patients receiving natalizumab earlier in the disease course had a higher likelihood of overall QoL improvement ($p = .022$). A similar association was found between improvement in functional status and number of years since MS diagnosis ($p = .058$). **Conclusions:** After only 3 months on natalizumab, patients reported improvement in overall QoL, disease level and functional status. Results suggest that early natalizumab intervention may be important, since disease duration was significantly associated with improved patient-reported QoL.

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P422

Longitudinal individualized analysis of computer-based cognitive rehabilitation in multiple sclerosis

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Background: The majority of patients with multiple sclerosis (MS) will have some degree of cognitive impairment (CI). CI is mainly seen in areas of memory, attention, information processing, visual-spatial abilities and executive function. Currently, there are limited options for its treatment. Here, we study a novel therapeutic approach, based on the use of a proven computerized program (NeuroPsychOnline) in cognitive rehabilitation. **Objective:** To use a computer-assisted cognitive rehabilitation program in patients with MS and mild to moderate CI, and to assess possible changes in quality of life after completing the program. **Methods:** This is a 30 week, prospective study of 12 patients with MS and CI determined by a baseline neuropsychological evaluation. Patients will be administered the MicroCog as a baseline and every 6 weeks to monitor progress. All the patients use the internet to complete activities at home and throughout the study. **Results:** There are currently nine active participants. Four of these patients have been enrolled in the study for less than six weeks, and consequently, follow-up MicroCog data points are not yet available for these participants. In addition, one participant has been minimally compliant, and therefore will not be included in the following analyses. Of the four compliant patients who have participated for more than six weeks, results from MicroCog testing indicate that General Cognitive Functioning increased an average of 16 percentile points, Attention/Mental Control increased an average of 14 percentile points, and Memory increased an average of 12 percentile points. In addition, although Information Processing Speed decreased an average of 9 percentile points, Information Processing Accuracy increased an average of 34 percentile points. **Conclusions:** In general, these preliminary results suggest that participating in this online cognitive rehabilitation program produced a trend toward improvements in cognitive functioning, especially with regard to attention, memory, and accuracy of information processing.

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WHO/MSIF Atlas of MS: multiple sclerosis resources across the world

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Background: Despite the considerable medical, social and economic impact of multiple sclerosis (MS), information about its occurrence, frequency and distribution (epidemiology) in all countries with a significant prevalence of MS and the resources available to diagnose, inform, treat, rehabilitate and support people with MS is lacking. To address this need, the largest ever international survey of its kind was undertaken, within the framework of the World Health Organization (WHO) Project Atlas, representing a major collaborative effort between WHO and the Multiple Sclerosis International Federation (MSIF). **Objective:** The Atlas of MS aims to raise awareness of the global MS situation, encourage further research and data collection and be a key tool to support initiatives to develop public policy, service provision and support. **Methods:** A questionnaire was developed and validated. Country respondents were identified by MSIF, World Federation of Neurology, WHO Project Atlas contacts and literature search. Received data were standardized, coded, entered into a database and analyzed (globally, by six WHO regions and four World Bank income categories). Data are organized in themes and presented as charts, maps and text. **Results:** Information from 113 WHO Member States, areas and territories representing 88% of the world population was collected. Despite limitations to the data collection, the results clearly indicate that no one country provides adequate resources; in many countries the resources that are available are grossly inadequate; and the availability of resources varies widely between countries both within all regions and across the world. **Conclusions:** The value of the Atlas of MS is in replacing impressions and opinions with facts and figures. The findings have wide implications for the work of health professionals, patient groups, the health industry and governments - and will inform national and regional advocacy and development policies.

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Quality adjusted life years: quantifying the impact of disease progression on the quality of life of persons with multiple sclerosis

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Background: Quality-adjusted life years (QALYs) adjust for years lived in state of disability, making a year lived with a disability worth less than a full year of health. QALYs can thus illustrate the societal impact of diseases such as multiple sclerosis (MS). **Objective:** This study compared the QALYs of MS patients living in four situations: 1) at home, independently; 2) at home, with home care; 3) in an assisted living facility; 4) in long term care (LTC). **Methods:** A purposive sample of 89 patients, from the register of a University-based MS clinic, completed the EQ5D, a health utility questionnaire used for calculating QALYs. **Results:** Although lower than a full health score of 1.0, the average EQ5D utility score for those living independently was significantly better than all of the other groups at 0.72 compared with home care at 0.45, assisted living at 0.36, and LTC at 0.22 ($F=29.6$, $p=.00$). The average age of the sample was 54. If all lived to 75 in their current state, a total of 21 years, the QALYs lost to those living at home independently would be 5.88 years. In other words, living for a quantity of 21 years, but only at a 0.72 degree of quality because of MS, would equal to living only 15.12 years at full health. The QALYs lost in the other three groups were increasingly more severe: home

care - 11.55 years, assisted living - 13.44 years, and LTC - 16.38 years, translating into only 4.62 years of full health for this group. **Conclusions:** The steep incline in QALYs lost as MS patients progress from independent living to facility living is striking. These data illustrate the need for therapies and treatments that delay progression so that patients may live at a higher level of quality for a longer period of time.

P425

Is fatigue a predictor of the efficacy of rehabilitation in multiple sclerosis patients?

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Background: Fatigue is a symptom frequently reported in patients with multiple sclerosis (MS). It often determines a severe impact on motor and social activities of patients and it is believed that it could interfere in the rehabilitation outcome of MS patients. **Objective:** In this study we evaluate if rehabilitation is able to improve fatigue in MS patients, and if the presence of fatigue could be considered as a negative predictor in clinical and functional outcome of rehabilitation in MS patients. **Methods:** We recruited 64 MS patients who underwent a program of intensive rehabilitation in our Neurorehabilitation Unit. We measured the entity of fatigue symptom with the Fatigue Severity Scale (FSS) administered at the beginning and at the end of rehab treatment. We defined Fatigue MS (FMS) subjects with a FSS score equal or over 36 points and Non-Fatigue MS (NFMS) all with a FSS score below 35. As a clinical outcome measure of rehabilitation we considered Expanded Disability Status Scale (EDSS) and FIM for the functional evaluation of these patients. **Results:** In our cohort of patients, rehabilitation produced a significant improvement on FSS score in 39 patients, with a strong statistical significance ($p<0.0001$). However, the most interesting data is that fatigue seems to have no impact in clinical and functional outcome of rehabilitation. In fact, despite both EDSS and FIM improving significantly in our 64 subjects, Mann-Whitney analysis highlighted that fatigue is not able to influence the efficacy of rehabilitation: $Z=-0.725$ for EDSS with $p=0.468$ and $Z=-0.838$ with $p=0.402$ for FIM. **Conclusions:** These data support the evidence that fatigue does not impact on the efficacy of rehabilitation, despite its subjective clinical impact on the daily life of MS patients. Moreover, our data underline that rehabilitation could significantly reduce fatigue reported by subjects with MS.

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Quality and needs assessment for collaborative palliative care in the progressive multiple sclerosis population

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Background: The individual medical and psychosocial needs of progressive multiple sclerosis (MS) patients may not reflect the perceived needs of the physician or allied health care team. The new World Health Organization mandate of palliative care aims to improve the quality of life of patients. This approach may be further enhanced if patient needs are better identified early during the disease process. **Objective:** To identify whether perceived psychosocial and medical concerns vary between MS specialists and progressive MS patients. **Methods:** 10 psychosocial and 12 medical concerns were identified by MS nurses as indicated by the patient population of the FHMS clinic. A Likert-scale impact questionnaire was constructed. 86.9% of patients ($n=20$) attending the 'Spasticity, Pain and Ambulation clinic' had progressive MS and were presented with this scale. Quantitative and qualitative data was collected. Five MS experts were asked to rank their top 5 concerns from the impact questionnaire. Data was compared. **Results:** The 56% ($n=13$) returned the questionnaire (male:female=4:9). Mean time from MS onset to completion of the questionnaire was 15yrs. Expanded Disability Status Scale scores

ranged from 5.5 to 9.0 (mean=6.7). Frequency analysis for patients' responses revealed: Independence and privacy issues were rated as the number one psychosocial concern, followed by concerns pertaining to loss of ability to provide or earn income, frustration/boredom, lifestyle changes and decreased social capability. Anxiety, depression and financial medical costs did not feature prominently. MS specialists were more likely to be concerned with different psychosocial needs, rating anxiety and depression higher. Concerns regarding spasticity and neuropathic pain were the primary medical concern for both groups, followed by bowel and bladder, mobility and fatigue. **Conclusions:** This pilot study has identified that there are important patient issues not always addressed by physicians and allied care in the management of patients with progressive MS. Awareness of these discrepancies may lead to an integrated, multidisciplinary palliative care approach to the MS patient, carer(s) and family members.

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The functional index for living with multiple sclerosis: development and preliminary results

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Background: The adoption rates are low for most existing disease-specific quality of life (QOL) measures in the multiple sclerosis (MS) practice setting. A simple to administer, easy to score and interpret scale would greatly enhance the experience and level of care that the MS patient receives. The Functional Index for Living with Multiple Sclerosis (FILMS) is a 125-point 25-item self-report scale designed to be comprehensive, easily administered, rapidly scored by clinic staff. The instrument consists of a concise set of questions to yield the information across six separate domains (pain, cognition, emotional functioning, fatigue, physical functioning, and general QoL). Higher scores correlate with better QOL. **Objective:** A 100-subject validation study of the FILMS is underway in a community neurology practice. Preliminary data are presented here. **Methods:** Subjects are patients diagnosed with clinically definitive MS and deemed stable at the time of enrollment. Subjects complete the following questionnaires on two separate occasions, separated by 2–6 weeks: FILMS, SF-36, Modified Fatigue Impact Scale, the Beck Depression Inventory-II, a Visual Analog Pain Scale, and the MS Neuropsychological Questionnaire. The data collected in FILMS subscales are correlated with control instruments and re-test reliability is validated. **Results:** A total of 47 subjects have been enrolled to date. 29 subjects completed the re-test. Current subject age averages 52.4 years and time since diagnosis averages 13.4 years. Analyses to date show an average total subject score of 87.45 (SD=20.697, range 48–119), good test-retest reliability at 0.928, subscale internal consistencies in the range of 0.653 to 0.895, and FILMS subscale-control instrument correlations in the range of 0.712 to 0.855. **Conclusions:** The FILMS survey promises to demonstrate strong correlation with comparison instruments and test-retest reliability. This survey promises to simplify acquiring data reflecting QOL of MS patients.

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Women's health and pregnancy in patients with multiple sclerosis

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Background: Multiple sclerosis (MS) often affects females during reproductive years. Although generally no negative impact on fertility is assumed, only limited data are available. In addition, little is known about family decision-making and medical guidance during pregnancy in this population. We surveyed 172 female MS patients on women's health and MS issues related to fertility and pregnancy.

Objective: To explore women's health and pregnancy related topics in women with MS. **Methods:** Self-report cross-sectional survey of MS patients in Washington State. To date, 412 completed questionnaires were received (return rate 94%) and data from 202 questionnaires have been entered (172 females). **Results:** Data from 172 female MS patients have been analyzed. The women reported irregular menstrual cycles during child-bearing years (20%), diagnosis of endometriosis (13%) and polycystic ovarian syndrome (4%). 121 women had biological children, although most had their children prior to MS diagnosis. 25 women became pregnant after being diagnosed with MS. 60% of these were not on disease-modifying treatment (DMT), the remainder discontinued DMT before conception (20%) or during pregnancy (20%). 12% of these women reported relapses during pregnancy and 57% within 6 months of delivery; 65% breast-fed. MS patients received pregnancy-related information from MS specialists (18%), MS organizations (14%) or primary care physicians (7%). **Conclusions:** Our preliminary analysis suggests that the non-parity rate is increased in comparison with the national average (30% versus 18%). Whether this is the result of biological factors or choice is unknown. At this point, our data indicate that the rate of fertility-related health issues in women with MS may not be much different than in the general population. In agreement with the literature, the risk of relapse post-delivery is high. Extended periods off DMT due to pregnancy and breast-feeding may compromise optimal MS management. Our study highlights the reciprocal influence between women's health issues and MS. The definitive data will be presented and discussed.

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First results from a randomized-controlled trial of a group-based intervention intended to promote knowledge and self-management strategies in patients with multiple sclerosis

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Background: Multiple sclerosis (MS) is a chronic disorder with an unpredictable course and an unknown etiology. MS is associated with a pattern of heterogeneous symptoms which frequently require a complex and lifelong treatment regime. In the face of such a challenge, MS patients quite often feel overwhelmed and experience a great deal of emotional distress and feelings of uncontrollability after they have been confronted with the diagnosis. **Objective:** Here we wanted to test whether a structured group intervention has an impact on emotional distress, knowledge and self-management strategies in patients with MS. **Methods:** A total of 39 MS patients (mean Expanded Disability Scale Status = 2.03; mean age: 31.28) were randomly assigned to either an immediate structured group intervention (N = 22) or a waitlist control group (N = 17). The intervention involved a total of 12 group sessions (90 minutes each) with basic information on medical treatment, self-management strategies and stress reduction techniques. Perceived stress, affective symptoms and disease related self-management strategies were assessed using questionnaires prior and after the group intervention/wait list control period. **Results:** After the intervention, patients who took part in the structured group intervention reported an increase in disease-related knowledge (p = 0.006), a decrease in affective symptoms (anxiety: p = 0.011; depression: p = 0.035), reduced stressful experience (p = 0.036) as well as increased overall well-being (p = 0.012). There was also a tendency for a reduction in cognitive avoidant disease management strategies (p = 0.004). No such effects were observed for patients in waitlist control group. **Conclusions:** A structured group intervention that promotes knowledge and self-management has a positive effect on affective wellbeing in MS patients. In the future, structured group interventions might therefore add supplementary benefit to standard neurological treatment.

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Symptom Management

P430

Gait apraxia in multiple sclerosis

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Background: Impairment of gait is a common disability resulting from multiple sclerosis (MS). MS-related gait impairment is primarily due to corticospinal tract dysfunction, or due to sensory or cerebellar ataxia. Impairment rating scales are designed on this principle and do not separately evaluate apraxia. Gait ignition failure or apraxia is defined as gait disorder not attributable to significant motor, cerebellar, or sensory dysfunction. **Objective:** To describe disabling gait apraxia in 13 MS patients. **Methods:** The Mayo MS clinic database (1994–2007) was queried for patients with MS and gait disorders including apraxia or gait ignition failure. Thirteen patients had MS with severe gait apraxia in the absence of other contributing impairments. No alternative cause for gait apraxia (e.g. normal pressure hydrocephalus, Binswanger's disease, extrapyramidal disease) was apparent. **Results:** Seven of 13 patients were women; 23% had relapsing-remitting, 46% secondary progressive and 31% primary progressive MS. Gait apraxia was evident a median of 8 years (range 0–34) following MS symptom onset. 77% patients had cognitive dysfunction and 92% had neurogenic bladder dysfunction. When apraxia was first diagnosed, median EDSS was 6 (range 5–7.5). Confluent periventricular white matter T2 lesions, generalized cerebral atrophy and symmetrical enlargement of the ventricles were the commonest magnetic resonance imaging findings. Immunomodulatory medications were used in 6 patients, (beta interferons 5, glatiramer acetate 2, mitoxantrone 1 and azathioprine 1), and levodopa in one patient without significant improvement in apraxia. **Conclusions:** Gait apraxia can cause significant functional impairment without other gait-impairing neurological deficits. The appreciation of gait apraxia may be limited by coexisting deficits in other MS patients. The natural course of the neurological deficit in such patients is unknown, and potentially their behavior in clinical trials may differ from that of other patients with other ambulatory disabilities.

P431

Menstrual cycle and menopause in patients with multiple sclerosis. How do these affect the symptoms of disease?

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Background: There is evidence supporting the pathogenic influence of sex hormones on immune system activity in multiple sclerosis (MS). Changes in sex steroid levels affect the Th-1/Th-2 lymphocyte balance which may explain why MS activity can change between different phases of the menstrual cycle, pregnancy and menopause. Pregnant patients with MS show a significant decrease in the rate of relapse followed by a significant increase during the first 3 months postpartum. **Objective:** To document clinical changes in MS symptoms during the menstrual cycle, menopause and pregnancy. **Methods:** A prospective questionnaire-based study for women with MS aged 18 to 65. Age, MS type, date of diagnosis and initiation of symptoms, parity, menstrual and contraceptive history were documented. Changes in MS symptoms during menstrual period, pregnancies, menopause or with the use of hormone therapy were assessed. We differentiated MS symptoms from symptoms of premenstrual syndrome. Patients with hysterectomy, amenorrhea or postmenopausal onset of MS were excluded. **Results:** A total of 100 patients will be evaluated. At the present time 71 patient have been included (94% relapsing-remitting MS, 6% secondary progressive MS) mean age of 41, 66% Caucasians, 17% Hispanic, 12% African American and 5% other ethnic background. 42% complained of worsening of symptoms while menstruating, and 21% worsening prior to the menstrual period. Twenty-eight patients had at least one pregnancy after their MS diagnosis; of these 57% reported improve-

ment of symptoms during pregnancy, 19% had a relapse in a 12-month period after delivery and one patient had a relapse during pregnancy. In our menopausal group of patients (n=13), 77% had worsening after menopause and 46% had increase in their number of clinical relapses after menopause. **Conclusions:** Female hormones play an important role in MS. This may have therapeutic implications in the future.

P432

Anxiety in multiple sclerosis: prevalence and associated factors in a large community sample

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Background: Mood disorders are prevalent in individuals with multiple sclerosis (MS), though unlike depression, little is known about anxiety disorders in this population. To date, no large-scale population-based studies have examined the prevalence of anxiety disorders in MS, though small studies estimate the prevalence could be as high as 41%. **Objective:** To determine the prevalence of anxiety disorder and the association with other demographic and health characteristics, including depressive symptoms, in a large community sample of individuals with MS. **Methods:** Information on anxiety, depression, demographics, and other health characteristics was collected through a self-report cross-sectional survey of 1,271 individuals with MS in Washington State. Anxiety was assessed using the Hospital Anxiety and Depression Scale (HADS) and depression using the Patient Health Questionnaire (PHQ-9). Multiple logistic regression using a cut-off of 9 or greater on the HADS was completed to examine factors associated with significant anxiety. **Results:** 25% of individuals (n=313) met criteria for likely anxiety disorder. Of those, 75% also met criteria for likely depressive disorder on the PHQ-9. Regression results suggest anxiety is associated with difficulties in thinking, higher depressive symptoms, more pain, increased stress, more sleep problems, not using a wheelchair, and with better health on the SF8 physical subscale. **Conclusions:** Anxiety disorders affect a large percentage of individuals with MS and co-occur with depressive symptoms in 75% of patients. Given the high prevalence and association with other mood and disease symptoms, clinicians should screen for anxiety disorders in individuals with MS and provide treatment when possible to reduce overall disability in this population.

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P433

Use of antidepressants for treatment of depression, pain, fatigue, and sleep in multiple sclerosis

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Background: Individuals with multiple sclerosis (MS) often experience a variety of symptoms related to their disease, including depression, pain, fatigue, and difficulties sleeping. A previous study found that 35% of individuals with MS report taking antidepressant medications, though reasons for taking these medications could have been unrelated to depressive symptoms. **Objective:** To examine prevalence of current use of antidepressant medications and explore alternative reasons for antidepressant use in a community sample of individuals with MS. **Methods:** A self-report cross sectional survey of 487 community-dwelling individuals with MS in Washington State assessed current use of 20 different antidepressant medications including selective serotonin reuptake inhibitors (SSRIs) and bupropion. Individuals were also asked to report whether the medications were used to help manage their depression, pain, fatigue, sleep, or if unsure. Participants were allowed to indicate if the medication was being used to manage more than one symptom. **Results:** 236 individuals (48%) reported current use of at least one antidepressant medication. Of these, 71% (n=168) reported using it for treatment of depression, 20% for pain (n=47), 29% (n=68) for sleep

management, 11% (n=27) for fatigue, and 2% (n=5) were unsure. The majority of individuals on antidepressants were taking SSRIs and/or bupropion. Antidepressant medications most often indicated as being used for pain were amitriptyline, duloxetine, and nortriptyline. Those most often used for sleep were trazodone and amitriptyline and the only one commonly used for fatigue was bupropion.

Conclusions: Use of antidepressants is higher here than in some previous reports. Participants report that antidepressant medications have been prescribed for conditions other than depression, making it difficult to isolate the prescription practices related to depression itself.

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P434

Clinical and radiological findings in patients with multiple sclerosis and pain

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Background: Despite pain being a disabling symptom in patients with multiple sclerosis (MS), the reported prevalence of pain in MS differs considerably, and clinical or radiological findings related with pain are not well established. **Objective:** To describe characteristics and prevalence of pain in patients with MS, and to assess the associated clinical variables and radiological findings. **Methods:** We prospectively studied patients with MS. A structured questionnaire which evaluated depressive symptoms, type of pain, localization, intensity (defined according to a visual analogic scale (VAS) as severe (VAS 7–10), moderate (VAS 4–6) and mild (VAS 0–4)) and pain therapy was recorded in patients who reported pain at the time of interview. Protocol variables were demographic data, MS clinical forms (relapsing-remitting, progressive secondary and progressive primary), neurological dysfunction (defined according to Expanded Disability Status Scale (EDSS)), symptoms at onset, attack frequency, illness duration, disease modifying treatment, fatigue, spasticity, oligoclonal bands in cerebrospinal fluid, visual evoked potentials, depressive symptoms (Hamilton test) and presence of lesions in spinal cord magnetic resonance imaging (MRI). **Results:** 134 patients were included, and MRI was performed in 104. Pain was reported by 74 (55%) patients. Pain was most frequently neuropathic, located in limbs, severe and burning/spiky. Only 38% of patients received therapy for their pain, predominantly anti-inflammatory drugs. Patients with pain had a worse functional state (EDSS score, 4.5[3–6] vs 1.5[1–2], p<0.001), a higher number of relapses (7.13±3.4 vs 3.75±2.9, p<0.001), more frequent progressive MS (86.7% vs 13.3%, p<0.001), depression (91.9% vs 8.1%, p<0.001), spinal cord involvement at onset (79.2% vs 20.8%, p=0.009), spinal cord lesions in MRI (84.3% vs 15.7%, p<0.001) and longer duration of disease (14.6±7.8 vs 8.43±5.9 months, p<0.001). In a logistic regression model, the presence of lesions in spinal cord MRI (OR 3[1.5–24.5]) and higher EDSS score (OR 1.7[1.1–2.7]) were independently associated with pain. **Conclusions:** Pain is a frequent disabling symptom in MS that requires a better attention and treatment. Pain is associated with greater disability and spinal cord lesions.

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Urodynamic profile and urinary symptoms in multiple sclerosis population

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Background: About 75%-84% of multiple sclerosis (MS) patients develop urinary symptoms, and these may have a serious impact on quality of life. Therefore it is important to diagnose bladder dysfunction at an early stage and initiate appropriate treatment symptoms.

Previous data about the relationship between objective and subjective bladder disturbances in MS are not conclusive. **Objective:** To correlate the prevalence of objective bladder dysfunction in relation to urinary symptoms in a MS population. **Methods:** During a period of about 1 year, 109 consecutive patients with clinically definite MS, with an Expanded Disability Status Scale (EDSS) score ≤7, underwent the following urinary assessments: maximum urinary flow rate (Qmax), postvoid residual urine volume (PVR), and bladder Electromyography (b-EMG). Furthermore the patients were asked about the relevant urinary complaints as part of the bowel/bladder Functional system score: urgency, frequency, hesitancy, urge incontinence and incomplete emptying. **Results:** The mean age of the patients was 44.6 (SD 10.1) years; 73% were female, 91% had relapsing-remitting MS and 10% secondary progressive MS. The mean disease duration was 12.5 (SD ±8.7) and EDSS score ranged from 0.0 to 7.0 with a median of 2.6 (SD±1.7). The median Qmax was 19.8 ml/sec (range 0 to 65; normal values were considered >15 ml/sec); the median PVR was 79 ml (range 0 to 1000; normal values were considered <60 ml). In 37 patients (34%) a detrusor-sphincter dyssynergia was recorded by b-EMG. 58 patients (53.5%) complained of at least one urinary symptom; the most frequent was urgency (41%), then frequency (16.1%), hesitancy (8.9%), incomplete emptying (12.5%), urge incontinence (3.6%). Statistical correlation between objective urinary measurements and subjective complaints, analysed by logistic regression analysis, was significant for Qmax respect to the following symptoms: urgency (Chi-Sq 0.005), frequency (Chi-Sq 0.0117), and hesitancy (Chi-Sq 0.0203). In these preliminary results no other significant correlation was observed. **Conclusions:** These preliminary results confirm the value of objective assessments of bladder dysfunction in every MS patients. Further statistical analyses will be carried out.

P436

Use of a self-reported questionnaire to describe the impact of multiple sclerosis on patients' disability and daily functioning

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Background: Screening patients before their appointment with a carefully constructed questionnaire can help healthcare professionals address patients' most pressing concerns and optimize their disease management plan. **Objective:** To identify the most important issues, impact of multiple sclerosis (MS) on disability and daily functioning of patients from a multidisciplinary outpatient clinic using a self-reported questionnaire. **Methods:** The clinic at The Montreal Neurological Institute follows over 2500 MS patients with an average of 3500 visits/year. A cross-sectional sample of all patients with a diagnosis of definite or probable MS who filled out a questionnaire just prior to their appointment between April 2007 and April 2008 were included in this study. The patient's self-evaluated health status was compared with their same-day neurological exam. The differences in questionnaire metrics assessing MS impact on disability and daily functioning were estimated according to patient demographics, disease characteristics and Disability Status Scale (DSS) extracted from the clinic's longitudinal database. Chi square test was used to analyse all categorical data. **Results:** The study included 635 patients, with 74.5% females. The disease course was relapsing-remitting for 68.2% of the patients, secondary progressive for 26.1%, and primary progressive for 5.7%. The mean age was 47.4 yrs + 11.4 (mean + SD). Fifty-three percent of the patients reported symptoms as their main concern followed by wanting more knowledgeable information on MS and therapies. Men were more willing to participate in research than women (p<0.006). Men reported more problems with sexual function than women (p<0.0001). There were significant relationships between increasing DSS and increasing difficulty with each of the reported deficits and each of the metrics of daily functioning. **Conclusions:** Final results will be presented at the meeting.

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Investigation of retinal nerve fiber layer in multiple sclerosis with optical coherence tomography

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Background: Optic neuritis (ON) can cause thinning within the retinal nerve fiber layer (RNFL), which can be quantified using optical coherence tomography (OCT). Changes in RNFL principally represent axonal damage, due to the absence of myelin. **Objective:** We aimed to evaluate the axonal damage in relapsing-remitting multiple sclerosis (RRMS) and secondary progressive multiple sclerosis (SPMS) among relationships between RNFL thickness and visual acuity, visual field, colour vision and visual evoked potentials (VEP). **Methods:** 30 RRMS (22 eyes with ON history, 38 without ON), 11 SPMS (4 eyes with ON history, 18 without ON) and 36 control subjects (72 healthy eyes) were analyzed. OCT testing was used to quantify mean overall, quadrant (superior, inferior, nasal and temporal) RNFL thickness. Humphrey automated perimetry, VEP, colour vision (100 hue test) and Snellen visual acuity were evaluated. **Results:** There was no significant difference in RNFL thickness between overall MS patients and controls. It was significantly lower in the ON-positive group compared with controls ($p < 0.05$). No significant difference between ON-negative MS patients and controls was found. In RRMS, RNFL thickness showed no significant difference from controls; however, when we compared ON-positive patients in RRMS with controls, it was significantly lower ($p < 0.01$). In comparison of SPMS patients with controls, RNFL thickness in the inferior quadrant of OCT was found to be lower than the controls ($p < 0.001$). There was a significant difference in colour vision score, between ON-positive and negative groups in RRMS and SPMS ($p < 0.001$) while visual field and visual acuity showed no difference. **Conclusions:** In our study we found that, ON-positive eyes showed significant RNFL thinning compared with controls and unaffected eyes. In contrast to previous studies we could not find any RNFL thinning in the unaffected eyes of MS patients.

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Myelopathy associated with Sjögren's syndrome: comparison with neuromyelitis optica and longitudinally extensive transverse myelitis

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Background: Central nervous involvement in Sjogren's syndrome has a variety of manifestations include focal deficits, optic neuritis, and myelopathies. Myelopathies associated with Sjogren's syndrome (SS) are similar to progressive multiple sclerosis (MS) or neuromyelitis optica (NMO). MSS, NMO, and longitudinally extensive transverse myelitis (LETM) are usually associated with a lesion in the spinal cord extending over three or more vertebral segments. **Objective:** To compare the clinical and immunological characteristics of myelopathy in SS, NMO, and LETM. **Methods:** Eight patients with SS, nine patients with LETM, and eight patients with NMO were included. Clinical, laboratory, magnetic resonance imaging (MRI), and NMO IgG antibody status in each group were evaluated. **Results:** Five SS patients had brain lesions and five had optic neuritis. Four of the five SS myelopathy patients who underwent serum NMO IgG antibody testing by immunoprecipitation of EGFP-tagged Aquaporin-4 showed positive results, and one patient showed a borderline result. Most SS myelopathy and NMO patients experienced relapsing and remitting courses throughout their entire disease courses. Both patients with SS myelopathy and NMO had similar spine and brain MRI findings, and showed a higher annual relapse rate and more extensive spinal cord lesions than LETM patients. The mean age at onset and cerebrospinal fluid protein level were higher in SS myelopathy than in NMO. Four

of the 8 patients with SS myelopathy fulfilled the revised diagnostic criteria for definite NMO. Two SS patients who failed to fulfill the diagnostic criteria because they had no optic neuritis also showed positive NMO IgG autoantibody results. **Conclusions:** SS and NMO showed similar clinical characteristics and autoantibody statuses, and they differed from LETM in terms of sex ratio, disease severity, and autoantibody status. These two diseases might share a common humoral immunological reaction, but further studies are needed to elucidate the common mechanisms.

P439

Bone mineral density, motor function, and vitamin D status in fully ambulatory persons with relapsing-remitting multiple sclerosis - population based cross-sectional data

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Background: Several studies have shown that bone mineral density (BMD) at the femoral neck decreases with increasing physical handicap (Expanded Disability Status Scale (EDSS)) in multiple sclerosis (MS) patients. **Objective:** To estimate determinants of BMD in fully ambulatory persons with MS based on screening data from a randomised controlled trial ('Can supplementation with vitamin D prevent bone loss in persons with relapsing-remitting MS?'). **Methods:** Examinations at screening (performed in February and March 2008) included EDSS, multiple sclerosis functional composite, 10 ft timed tandem walk, strength of grip, BMD (total hip, femoral neck, lumbar spine, forearm), and blood tests including 25(OH) vitamin D measurements. **Results:** Sixty-one women and 26 men aged 19–50 years (median 31) were examined. Median duration of disease was 9 years from first symptom, median EDSS 2.5 (range 0–5.0). High dose intravenous methylprednisolone had been used in the treatment of 38% of the participants; none had received a cumulative dose of more than 1 g oral prednisolone. Abnormal BMD (z -score ≤ -2.0) was found in 12/84 individuals at one ($n=10$), two ($n=1$) or three ($n=1$) of the following sites: total hip and/or femoral neck; ultradistal radius; lumbar spine. Vitamin D status was considered deficient in 35% of participants (serum 25(OH) vitamin D 25–49 nmol/L), and only 15% had optimal values (>75 nmol/L). Preliminary analyses show statistically significant correlations between motor function and BMD in both lower and upper extremities. Linear regression analysis will be applied to estimate the effects of motor function, serum 25(OH) vitamin D, disease duration, cumulative corticosteroid treatment, use of disease modifying treatment, smoking, age, and sex on BMD. **Conclusions:** Low BMD is likely to develop into osteoporosis with increasing age and duration of MS. Prevention of osteoporosis is a high priority, because treatment of the established disease remains sub-optimal. Results from the 2-year trial will show whether fully ambulatory persons with MS benefit from supplementation with 20,000 IU cholecalciferol weekly, a dose expected to increase 25(OH) to optimal levels (>75 nmol/L).

P440

A randomized placebo-controlled double-blind study reconsidering modafinil for treatment of fatigue in multiple sclerosis

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Background: Fatigue is one of the most disabling symptoms in multiple sclerosis (MS). **Objective:** To assess the effect of modafinil, a wakefulness-promoting psychostimulant, on fatigue in patients with MS. **Methods:** 121 MS patients with a baseline score of ≥ 4 on the Fatigue Severity Scale (FSS) and an Expanded Disability Status Scale (EDSS) score of < 7 were eligible for the 8-week randomized, double-blind placebo-controlled study. Modafinil was dosed up to 200 mg/day within one week. Assessments were performed at baseline and after 4 and 8 weeks. The primary outcome parameter was the mean change of the FSS total score. A decrease of 0.5 points was

considered clinically relevant. Secondary outcome variables were assessed by other questionnaires covering fatigue, daytime sleepiness and sleep quality as well as changes on the Symbol Digit Modalities Test (SDMT) and the Paced Auditory Serial Addition Test (PASAT). Furthermore, a substudy investigated transcranial magnetic stimulation (TMS) paradigms before and about 3 hours after first dose as well as at the end of the study period in a subgroup of 23 patients. **Results:** Preliminary results: 121 patients (70% female, 30% male, demographic means: age 40 years, EDSS 3.3, FSS 5.9 at baseline) were included in the study. 56% had a relapsing-remitting disease course, 26% a secondary chronic progressive and 18% had a primary progressive disease course. Dropout rate was 8%. First analysis of neuropsychological data shows a significantly improved performance in SDMT and a positive trend in PASAT. Interim analysis of the TMS subgroup shows a significant improvement of attention, motor function as well as an increase of the intracortical excitability in treated patients compared to controls. Final study results will be presented. **Conclusions:** In some contrast to previous studies, data analysis so far does indicate a positive effect of modafinil on fatigue, cognitive impairment and motor function in MS. **Supported by:** Merckle GmbH, Ulm Germany.

P441

Symptom cluster as a predictor of physical activity in multiple sclerosis: preliminary evidence
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Background: Previous studies have identified symptoms as a correlate of physical activity in multiple sclerosis (MS). Importantly, those studies all suffered from a common set of limitations, namely the general focus on the overall frequency or intensity of symptoms, lack of a guiding symptom-based theoretical framework, and a cross-sectional research design. **Objective:** The present study examined the symptom cluster of fatigue, pain, and depression and its direct and indirect prediction of physical activity behavior in a sample of individuals with MS using a prospective research design and the Theory of Unpleasant Symptoms. **Methods:** The sample included 292 individuals with a definite diagnosis of MS. The participants completed self-report measures of fatigue, depression, pain, self-efficacy, and functional limitations at baseline and six-months later wore an accelerometer for seven days and then completed a self-report measure of physical activity. The data were primarily analyzed using confirmatory factor analysis and structural equation modeling in Mplus 3.0. **Results:** Our results indicated that (1) fatigue, depression, and pain represented a symptom cluster based on bivariate correlations, confirmatory factor analysis, and cluster analysis; (2) this symptom cluster had a strong and negative predictive relationship with physical activity; (3) those who reported greater depression and pain had more severe fatigue, and more severe fatigue was associated with lower levels of physical activity; and (4) functional limitations, but not self-efficacy, accounted for the predictive relationship between the symptom cluster and physical activity. **Conclusions:** Such findings provide preliminary support for the importance of considering symptom clusters as a meaningful correlate of physical activity in persons with MS.

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P442

Complementary and alternative therapy use in multiple sclerosis: counting the cost
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Background: Complementary and alternative medicine (CAM) use is common in patients with multiple sclerosis (MS), despite limited evidence of efficacy. There is little data on cost of CAM to patients. **Objective:** To compare rates of CAM use in patients with MS and those with other neurological disorders. To determine the cost of regular CAM use. **Methods:** All patients attending the neurology out-

patient department were asked to complete a standardised questionnaire which contained demographic information, details of the underlying neurological diagnosis, use and cost of CAM. **Results:** Five hundred patients were included, 182 male, mean age 46.7 years. Of these, 90 (18%) patients had MS. Patients with MS were significantly more likely to have used CAM, 75.6 % vs 55.4 % ($p < 0.05$). Fifty-three percent reported a beneficial effect from CAM. Most patients had not informed their doctor of their CAM use. The mean annual cost of regular CAM use was 1233.18 with a range of 0 (from friends) to 9360. There was a trend for patients with MS to spend more on CAM than patients without MS (1694.36 vs 1226.79 per annum) but this did not reach statistical significance. **Conclusions:** The majority of patients with MS have used CAM despite a lack of scientific evidence of efficacy. Patients with MS have significantly higher rates of CAM use than patients with other neurological disorders. There is a significant personal cost to patients even though many are not in stable employment. There is a non-significant trend for patients with MS to spend more on CAM than other patients.

P443

Efficacy results of a study of THC:CBD in central neuropathic pain due to multiple sclerosis

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Background: THC:CBD oromucosal spray (Sativex®, an endocannabinoid system modulator), is approved in Canada for the relief of central neuropathic pain in multiple sclerosis (MS) and cancer pain. **Objective:** Presentation of the efficacy results from the largest study of cannabinoids in MS neuropathic pain conducted in Europe and Canada. **Methods:** This 14-week randomized, placebo-controlled, double-blind, parallel group study was undertaken in patients still experiencing pain despite available treatment. Each spray of THC:CBD (100ul), delivers 2.7mg delta-9-tetrahydrocannabinol (THC) and 2.5mg cannabidiol (CBD). Patients self-titrated up to a maximum of 24 sprays/day. Numerical Rating Scale pain scores (0–10) were collected daily. **Results:** 339 patients were enrolled. At baseline, mean duration of pain was 5.5 years, mean Expanded Disability Status Scale score was 5.0, and mean pain score was 6.6. In the THC:CBD group 50% (84/168) of patients reported improved pain scores of 30% or greater. However, in the placebo group 45% (77/171) of patients reported an equivalent improvement; the comparative analysis did not reach statistical significance at 14 weeks (although the difference did reach significance at 10 weeks). In post-hoc analysis, patients with a shorter duration of pain (<4 years, n = 168) were noted to respond significantly ($p = 0.028$) better than those on placebo. Placebo group patients who titrated to the maximum dose had disproportionate improvements in their pain scores. Analysis of the influence of concomitant medication is under way. **Conclusions:** In this treatment-resistant patient population, the THC:CBD group showed a high response rate, statistically different to placebo at the 10th follow-up week but not at the 14th week primary endpoint. Self-titration in combination with a subjective endpoint seems to impact the placebo response. Moreover, patients with an extensive history of neuropathic pain might have lost the capacity to respond to treatment. Efforts to control for these aspects should be made in future studies. **Supported by:** GW Pharma Ltd.

P444

Relationship between fatigue, sleep and symptoms of multiple sclerosis

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Background: Poor sleep is a recognised clinical problem in multiple sclerosis (MS) and may directly impact fatigue. Sleep disturbance in the context of complex disability poses a particular challenge to

assessment and treatment. **Objective:** To investigate how sleep experience and other MS symptoms relate to fatigue, using standardised measures. **Methods:** Sixty-one patients (mean age 45 years; 45 female; 43 with relapsing-remitting MS and 18 with progressive disease) were interviewed using standardised measures: Fatigue Severity Scale (FSS), Guys Neurological Disability Scale (GNDS), Hospital Anxiety and Depression Scale (HADS), Multiple Sclerosis Spasticity Scale (MSSS-88), Epworth Sleepiness Scale (ESS), International Consultation on Incontinence Questionnaire Overactive Bladder Module (ICIQ_OAB), McGill Pain Questionnaire (MPQ), Restless Leg Syndrome (RLS) Single Question and Pittsburgh Sleep Quality Index (PSQI). **Results:** Thirty-nine (64%) reported clinically significant fatigue (mean FSS 4.45). Pearson's correlation coefficients were used to assess interrelationships between variables. Fatigue correlated strongly with disability (0.63) and moderately with sleepiness (ESS, 0.57), depression (0.53), anxiety (0.46) and overactive bladder (0.43). Fatigue correlated with overall sleep experience (PSQI, 0.51). Fatigue also correlated with the following PSQI components: daytime dysfunction (0.59), subjective sleep quality (0.53), sleep disturbances (0.51), and sleep latency (0.37); but interestingly not with use of sleeping medication (0.02), sleep duration (0.14), or habitual sleep efficiency (0.23). There was a weak correlation with muscle stiffness (0.38) and no significant correlation with pain (0.13) or RLS (0.29). **Conclusions:** This study used standardised measures to demonstrate that fatigue significantly correlated with disability, mood, bladder function and some components of sleep quality. These related factors should be formally assessed as part of a sleep audit, to identify potential contributors to poor sleep experience and appropriate treatment strategies. The efficacy of treating related factors to improve fatigue requires investigation. **Supported by:** King's College MS Trust Fund.

P445

Can head and neck cooling improve symptoms of multiple sclerosis?

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Background: Multiple Sclerosis (MS) is a permanent and progressive neurological disease. Heat can exacerbate symptoms of MS whereas cooling can provide symptomatic relief. One caveat of cooling studies is the lack of a true control conditions; a condition that gives the perception of cooling but does not change core temperature. **Objective:** Since the head and neck areas are particularly sensitive to cold and cooling interventions, we investigated the effects of cooling the head and neck for 60 minutes on symptoms of MS while including a true control condition (sham cooling). **Methods:** To isolate the effects of perceived versus physiological cooling, a sham cooling condition was incorporated, in which subjects perceived the sensation of being cooled without any actual physiological cooling. Six heat-sensitive, ambulatory females with MS and Expanded Disability Status Scale scores ranging from 2.5–6.5 participated in the study. They visited the clinic three times for 60 minutes of true, sham, or no cooling, followed by evaluation of ambulation, visual acuity, and muscle strength. Cooling conditions were implemented using a custom head and neck cooling hood. Rectal and skin temperature, heart rate, and thermal sensation were measured throughout cooling and testing. **Results:** The true cooling condition significantly decreased core temperature by 0.37°C and elicited a colder thermal sensation compared to the no cooling group, but perceptions of cooling were similar to the sham cooling group. True cooling improved performance in the 6-minute walk test and the timed up-and-go test but not visual acuity or hand grip strength. **Conclusions:** We conclude that head and neck cooling may be an effective tool in increasing ambulatory capacity in individuals with MS.

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Premature ovarian failure prevention during mitoxantrone treatment in women with multiple sclerosis

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Background: Premature ovarian failure (POF) with consequent irreversible amenorrhea and infertility is a common long-term consequence of chemotherapy. Preliminary studies on LES women treated with cyclophosphamide pulse therapy showed that GnRH-analogue treatment significantly prevented POF. **Objective:** To prevent POF in mitoxantrone-treated women of reproductive age with multiple sclerosis (MS) with GnRH-analogue. **Methods:** A monthly intramuscular depot injection of 3.75 mg D-TRP6-GnRH-a (Decapeptyl C.R. IPSEN SpA) was administered after informed consent to 11 relapsing or progressive MS women with the clinical features required for mitoxantrone treatment. Hormonal treatment was continued for all the mitoxantrone cycle. Mitoxantrone was given at the dose of 12 mg/m² every month for 6 months (to be adapted to individual leukocyte count) in relapsing-remitting patients or at the dose of 12 mg/m² every 3 months for 18 months in secondary progressive patients. Kaplan-Meier survival estimates was used to calculate the cumulative preservation of ovarian function. Significance is tested with a Cox regression-based model. Cox proportional hazards regression is used to estimate the hazard ratio of ovarian failure. **Results:** To assess the preservation of ovarian function we measured the levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), 17-beta estradiol and progesterone every month, before, during and after the injection of GnRH, during the therapy with mitoxantrone and/or from conception. FSH levels are the objective measure of POF in women with oligo-amenorrhoea. In case of FSH levels suggestive for POF we confirmed the diagnosis with the documentation of amenorrhoea during at least 12 month and FSH levels ≥ 40 mIU/ml. All the women are under neurological and gynecological control periodically and none have any side effects from the administered therapies, except for transient weight gain and rash in the injection site. Six of the patients treated with GnRH-analogue had finished the therapy with mitoxantrone and returned to normal menstrual cycles, with hematological exams testing fertility. **Conclusions:** GnRH-analogue treatment in women with MS in therapy with mitoxantrone can prevent POF.

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Influence of 5-year glatiramer acetate therapy on prevalence of lower urinary tract dysfunction in relapsing-remitting multiple sclerosis

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Background: Lower urinary tract symptoms/dysfunction (LUTS) have a high prevalence in multiple sclerosis (MS). These disabling symptoms decrease quality of life and require symptomatic treatment. Influence of current disease-modifying immunomodulating therapy including glatiramer acetate (GA) on LUTS in RRMS has not been investigated. **Objective:** To investigate influence of long-term GA therapy on lower urinary tract dysfunction in relapsing-remitting MS (RRMS) patients. **Methods:** RRMS patients (n=85; age 29 \pm 7.3 years; 52 females, 33 men; annual relapse rate 2.6 \pm 0.5) were randomly divided into 3 groups: Group 1 - patients with LUTS according to the International Prostate Symptom Score (IPSS) scale, receiving 5-year GA therapy (n=34); Group 2 - patients without LUTS, receiving 5-year GA therapy (n=29); Control Group - patients without LUTS receiving no immunomodulating therapy (n=22). Dynamic urologic examination, Expanded Disability Status Scale (EDSS), IPSS-score and relapse rate were evaluated every 6 months during the 5-year therapy period (10 follow-up visits). **Results:** Initially in Group 1 the EDSS score was 2.0 \pm 0.6, IPSS score 13.5 \pm 4.3. Irritative symptoms predominated in 11 (32%) patients, obstruction symptoms in 8 (24%) patients; 15 (44%) had mixed dysfunction. In Group 2 the EDSS score was 2.1 \pm 0.4, and in Group 3, 2.2 \pm 0.5. On 5-year GA therapy EDSS decreased in Group 1

(1.6±0.4, $p<0.01$) and in Group 2 (1.54±0.4, $p<0.05$). In the control group the EDSS score significantly increased (3.5±1.1, $p<0.01$). Annual relapse rate decreased in Group 1 (0.9±0.9, $p<0.01$) and Group 2 (1.1±0.05) and increased in the control group (3.2±0.9 $p<0.01$). In patients receiving GA after 5 years of treatment, changes in IPSS score were not observed in Group 1; in Group 2 LUTS developed in 8 (28%) patients, and the IPSS score was 11.3±3.2. In contrast, in the control group already on the 5-year of observation LUTS was diagnosed in 16 (73%, $p<0.01$) patients (IPSS-score was 18,4±6,3). **Conclusions:** 1) In RRMS patients suffering from LUTS 5-year GA therapy result in parallel decrease of annual relapse rate and LUTS stabilization. 2) In RRMS patients without bladder dysfunction long-term GA therapy delays development of LUTS.

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Clinical characteristics of multiple sclerosis associated with uveitis

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Background: Uveitis is an autoimmune disorder associated with multiple sclerosis (MS) in a subset of patients. To date, this association has been considered as a chance association, as autoimmune diseases are known to potentially coexist in a same patient. **Objective:** To describe the clinical profile MS patients with associated uveitis. **Methods:** Uveitis was identified in five patients of our clinic on a retrospective basis. We describe clinical characteristics of both MS and uveitis in that group. **Results:** There were four women and one man. One had antecedent thyroiditis and 2 patients had frequent mouth ulcers. Out of the 5 cases, 3 experienced uveitis before the onset of MS but one of them had recurrent episodes until 3 years after MS onset. Uveitis was of various types: anterior and intermediate (n=2), anterior (n=1), panuveitis (n=1, bilateral), posterior (n=1). In 4 cases uveitis was unilateral and on the left side. Only one patient had recurrent episodes. Mean age of onset of uveitis was 44.6 (range 20–62). Out of the 5 patients, 1 had primary progressive MS and 4 a relapsing-remitting course but uveitis in one of those cases begun during the secondary progressive phase. Mean age of onset of MS was 44.8 yrs (range 24–52), but 4/5 patients had MS onset after 45yrs. Optic neuritis (ON) was noted before uveitis (n=1, left side) or after (n=2, one left sided and one bilateral). The case with bilateral ON also had bilateral uveitis. None of the patients had active MS at the time of uveitis and none of them received disease modifying drugs (DMD). Magnetic resonance imaging characteristics were not noteworthy but this examination was not performed at the time of uveitis. **Conclusions:** Despite the small number of patients, it could be noted that patients of this cohort have some common characteristics: onset of MS was after 45yrs, in 4/5 patients. None of them received DMD at the time of uveitis. This last point is interesting as it has been suggested that DMD, and particularly interferon, could treat uveitis. Except for age of onset, it could not be noted that MS had a specific profile. Given the size of this group, we cannot draw any conclusions regarding uveitis or about the potential relationships with ON.

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Sleep abnormalities in multiple sclerosis patients

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Background: Subjective sleep difficulties are common and correlate with fatigue in multiple sclerosis (MS) patients, but there are few studies which objectively evaluate sleep disorders with overnight polysomnography (PSG). **Objective:** To evaluate 1) sleep characteristics in MS using a comprehensive structured assessment and complete overnight PSG, and 2) objective daytime somnolence using a multiple sleep latency test (MSLT). **Methods:** MS patients are being recruited from a specialty clinic irrespective of fatigue level or subjective sleep abnormalities. Included patients have an Expanded Disability Status Scale (EDSS) score of 0 to 7.0. Exclusion criteria include major depression, treatment for a known sleep disorder, and forced vital capacity of <60%. All patients complete structured sleep questionnaires and two consecutive overnight PSG studies followed by MSLT. The following definitions are used: obstructive sleep apnea/hypopnea (OSAH) with an apnea-hypopnea index score of ≥15 events per hour of sleep, periodic leg movement disorder (PLMD) with a periodic leg movement index score of ≥15, and hypersomnolence and borderline hypersomnolence with a mean sleep latency of ≤5 minutes and 5 to 10 minutes on MSLT. **Results:** To date, 33 MS patients have completed the study. Mean patient age is 47.3 ± 10, range 26 to 71 years, and mean EDSS score is 3.6 ± 1.9, range 1.0 to 6.5. A variety of sleep disorders have been found: OSAH in 23 (70%) of patients, a positive response to the Restless Legs Syndrome (RLS) questionnaire in 16 (48%), PLMD in 14 (42%), and narcolepsy in 1 (3%). MSLT revealed hypersomnolence in 6 (18%), and borderline hypersomnolence in 6 (18%). **Conclusions:** Objective sleep abnormalities and hypersomnolence appear to occur commonly in MS patients. A variety of sleep disorders can be found including OSA, PLMD, RLS, and narcolepsy. These observations suggest that MS patients should be routinely screened for sleep disorders.

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Service provision and symptom management for multiple sclerosis patients in a British supraregional multiple sclerosis service

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Background: In 2003 the UK National Institute for Clinical Excellence issued guidelines for the management of MS. These included as key priorities auditing the quantity of service provided and the prevalence of 'hidden' symptoms. **Objective:** We report the annual frequency and type of visits, and estimate the prevalence of symptoms and their treatment as recorded contemporaneously by health professionals. **Methods:** The data presented here is part of an ongoing audit (Feb 08 - May 08) of 500 patients seen in 2007. **Results:** We present the results from the first 151 audited records. Patient were categorised according to the number of attendances to the hospital as low (<3; 52%), medium (3–4; 21%), high (5–6; 15%) and very high (>6; 13%) frequency attenders. Patients were almost always followed-up by a physician. More than half of the patients accessed a nurse, physio-therapist or occupational therapist. A third of patients were referred to the orthoptist and other health allied professions accounted for less than a fifth of referrals. We looked for symptoms being sought for (S) and treatment initiation or changes (T). Bladder symptoms were the

most commonly questioned and needed the most treatment adjustments (S=81%;T=21%). Spasticity (S=54%; T=19%), fatigue (S=46%; T=14%) and pain (S=49%; T=13%) followed. Bowel (S=40%; T=5%), mood (S=17%; T=3%), cognitive disturbances (S=14%; T=nil), sexual problems (S=5%; T=2%) and anxiety (S=5%; T=nil) were infrequently questioned and even less frequently managed. **Conclusions:** These data confirm that MS care requires a multidisciplinary team. It supports a model of a combined clinic with physician, nurse, occupational therapist, physiotherapist and orthoptist. The number of attendances with this model can be kept low. When bladder symptoms are sought there appears to be a high rate of clinical need requiring treatment. Anxiety, cognitive disturbances and sexual problems are under-reported and may therefore be undertreated.

Poster Session 2 Disease Modifying Therapy – Part 2

P451

An open-label multi-center safety and efficacy study of oral recombinant ovine interferon tau (IFNt) administered daily in relapsing-remitting multiple sclerosis

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Background: No oral agents are currently FDA-approved for use in multiple sclerosis (MS). **Objective:** To determine the safety and obtain preliminary evidence of efficacy of a novel oral interferon tau (IFNt) in subjects with relapsing-remitting MS (RRMS). **Methods:** This was a Phase II, open-label, multi-center study to evaluate the safety and preliminary efficacy of oral IFNt 3.0 mg TID for nine months in RRMS. All subjects had at least one gadolinium-enhancing lesion on at least one of three monthly run-in brain magnetic resonance images taken prior to treatment. Magnetic resonance imaging was performed monthly for nine months on treatment and then repeated after an additional three months off treatment. The primary analysis compared the mean number of gd-enhancing lesions of each 3-month treatment period to the pre-treatment mean using Friedman's test; post-hoc analyses compared the pre-treatment mean to each 3-month period. **Results:** Twenty-five (25) subjects, 20F/5M, with a clinical diagnosis of RRMS according to McDonald criteria were enrolled; 22 completed the study protocol. The mean number of new lesions in the pretreatment interval (median of pretreatment means=1.5) was significantly higher ($p=0.0003$) than the means of the on-treatment intervals (median of month 1-3 means=0.67; median of month 4-6 means=0.42; median of month 7-9 means=0.67). The largest differences were observed for the month 1-3 and 4-6 intervals ($p<0.001$); a marginally significant difference was observed for the month 7-9 interval ($p=0.06$). Five patients experienced an MS exacerbation on treatment (ARR= .23). Clinical adverse events were generally mild and did not result in discontinuation of the study drug. No serious laboratory adverse events were reported. **Conclusions:** Tauferon 3mg TID appeared to be effective over 9 months in decreasing the mean number of new gadolinium-enhancing lesions compared to baseline and appeared to be safe and well tolerated.

Supported by: This trial was sponsored by PEPGEN Corporation, Alameda, CA, USA. We wish to thank Dennis P. Gilman, Ph.D. 6, Dawn Maguire, and CP Liu, Ph.D, all of PEPGEN Corporation.

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MxA and MMP-9/TIMP-1 ratio as biomarkers of treatment efficacy and disease activity in multiple sclerosis patients

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Background: The importance of neutralizing antibodies (Nabs) in the decreasing of clinical effectiveness of interferon beta therapy is well known. However, the cytopathic effect assay presents some disadvantages, such as the variability inherent in viral-based assays and the long time required to perform it. Mixovirus resistance protein (MxA) expression and the measurement of the ratio between Matrix Metalloproteinase 9 and its tissular inhibitor (MMP-9/TIMP-1) have been suggested as being useful tools in controlling therapy with interferon beta. **Objective:** To analyze the roles of MxA and MMP-9/TIMP-1 ratio as markers of bioavailability and clinical effectiveness of interferon beta treatment. **Methods:** Pairs of blood and serum samples were collected from 54 multiple sclerosis patients during seven programmed visits for two years: one before the start of the treatment and six during interferon beta treatment. In case of relapse, another pair of samples was collected. Expression of MxA, MMP-9 and TIMP-1 was analyzed by qRT-PCR and presence of NAbs was determined by cytopathic effect assay. **Results:** As might be expected, MxA expression is correlated with the presence of NAbs in serum, with the mean of MxA significantly lower in the group of patients without NAbs ($p=0.004$). Also, we found that the percentage of patients suffering from relapses was higher in the group of patients who presented NAbs in serum (36.8%) compared with the group of patients without NAbs (22.8%) ($p=0.03$). On the other hand, we found that the MMP-9/TIMP-1 ratio was increased in the samples collected during relapses compared with samples in remission. **Conclusions:** NAbs significantly decrease the clinical efficacy of interferon beta treatment and MxA could be a good marker of bioavailability. Measurement of MMP-9/TIMP-1 ratio could be useful in prediction of relapses.

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Cladribine tablets in relapsing-remitting multiple sclerosis: study design of the 2-year, Phase IIIb CLARITY (CLAdRibine tablets Treating multiple sclerosis orally) extension study

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Background: The importance of early treatment interventions with improved efficacy, safety and tolerability profiles in multiple sclerosis (MS) has driven the ongoing development of several new products including oral cladribine tablets, which are under Phase III investigation as first-line therapy in patients with relapsing-remitting MS (RRMS). **Objective:** To investigate the long-term safety, tolerability, and efficacy of oral cladribine tablets in patients with RRMS. **Methods:** Patients who have completed 2 years in the CLARITY study will be eligible to enter this Phase IIIb, double-blind, placebo-controlled, multi-center, parallel-group, 2-year extension study. Patients previously randomized to placebo in the first 2 years of the CLARITY study will receive a 5-day course for two consecutive months per year of once-daily cladribine tablets; patients who previously received cladribine will be randomized (2:1) to two 5-day courses/year, in consecutive months, of once-daily cladribine tablets or placebo in their respective groups. Rescue treatment with interferon beta-1a (44 mcg subcutaneously three times weekly) or another disease-modifying drug will be available. **Results:** Primary analyses will be based on safety endpoints. These will include assessment of hematological and hepatic function, incidence of all treatment-emergent adverse events (AEs) and serious

AEs. Efficacy endpoints will include the proportion of relapse-free patients, relapse rates, and measures of disability progression. Magnetic resonance imaging endpoints will include: total burden of disease, transformation of T1 gadolinium (Gd)-enhancing lesions to hypointense lesions, brain volume changes, and new T1 Gd-enhancing and T2 lesions. Health-related quality of life, pharmacoeconomic and pharmacogenetic/genomic endpoints will be assessed. **Conclusions:** The CLARITY extension study is designed to provide information on long-term safety, tolerability, and clinical benefit of extended annual short-course administration of oral cladribine tablets for up to 4 years in patients with RRMS. A well-tolerated and effective oral therapy with short-course annual dosing may improve adherence and overall patient satisfaction.

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Glatiramer acetate reduces multiple sclerosis severity: analysis of patients from the US pivotal study using the Multiple Sclerosis Severity Score

Joseph Herbert, for the US pivotal glatiramer acetate study group.

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Background: Glatiramer acetate (GA) is effective in reducing relapse rate, magnetic resonance imaging activity and slowing accumulated disability as measured by Expanded Disability Status Scale (EDSS) in relapsing-remitting multiple sclerosis (RRMS). MS Severity Score (MSSS) is a more sensitive outcome to assess disease progression, as it takes into consideration both disability (EDSS) and disease duration. Thus, MSSS may better assess the effect of GA on disability. **Objective:** To assess the effects of GA on disease severity in RRMS patients using the MSSS. **Methods:** 251 patients with RRMS (EDSS scores 0–5) were randomized to GA 20 mg/d or placebo in a multicenter, double-blind trial of approximately 35 months follow-up. Patients were assigned to 1 of 6 disease severity groups based on MSSS score at baseline and study completion. Analyses were conducted using baseline EDSS scores and those assessed at study completion, and disease durations assessed from time of first symptom. **Results:** At baseline, median MSSS scores for GA-treated and placebo patients were similar (4.59 vs. 4.29, $p=0.1$). Patients in both treatment groups were similarly distributed among the 6 MSSS severity groups. At the study completion, median MSSS change from baseline was greater in GA-treated patients compared with placebo (-0.73 vs. -0.19, $p=0.0019$). More GA-treated patients compared with placebo shifted to a lower severity group (49% vs. 31%) and fewer to a higher severity group (16% vs. 26%) during treatment ($p=0.0014$). **Conclusions:** GA effectively and significantly reduced disease severity on the MSSS in RRMS patients. The value of MSSS, as a tool to measure disease progression, highlights the fact that EDSS scores and change in EDSS must be considered in the context of the baseline EDSS and the duration of the disease.

Supported by: Teva Neuroscience.

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Interferon- β upregulates brain-derived neurotrophic factor secretion from peripheral blood mononuclear cells of relapsing-remitting multiple sclerosis patients through a CD40-dependent mechanism

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Background: We have reported that immune cells from relapsing-remitting multiple sclerosis (RRMS) patients secrete low levels of brain-derived neurotrophic factor (BDNF) and that there is defective regulation of its secretion via CD40. **Objective:** To study the effect of interferon- β 1a (IFN β 1a) on secretion and regulation of BDNF from immune cells of patients with RRMS. **Methods:** Peripheral blood mononuclear cells (PBMCs) from 13 untreated patients (UNTX) with RRMS and 20 matched IFN β 1a (Rebif)-treated RRMS patients were incubated with anti-CD40 monoclonal antibody (mAb) and its iso-

type control (IC). PBMCs of 4 UNTX and 4 matched healthy controls (HC) cultured in the presence and absence of 20 ng/ml IFN β 1a. BDNF supernatants levels were studied by enzyme-linked immunosorbent assay. Expression of CD14 and CD40 on PBMCs was studied by flow cytometry. **Results:** PBMCs of IFN β 1a-treated patients secreted higher BDNF vs. UNTX (1438.1 \pm 142.0 vs. 771.0 \pm 109.5 pg/ml, $p<0.001$). CD40 stimulation of PBMCs from IFN β 1a-treated patients increased the BDNF vs. IC (1751.6 \pm 163.81167.8 \pm 132.4 pg/ml, $p<0.001$). No such effect of anti-CD40 mAb was found in UNTX. Furthermore, %CD40+ cells of CD14+ cells (monocytes) was higher in IFN β 1a-treated patients vs. UNTX (81.4 \pm 3.6% vs. 59.3 \pm 8.0%, $p=0.04$), the mean fluorescence intensity (MFI) of CD40 on monocytes was higher among IFN β 1a-treated patients vs. UNTX (78.1 \pm 13.8% vs. 38.3 \pm 1.6%, $p=0.02$). In vitro addition of IFN- β 1a to PBMCs from both HC and UNTX increased %CD40+ monocytes (HC: 80.9 \pm 5.8% vs. 45.3 \pm 2.8%, $p=0.01$; UNTX: 75.9 \pm 7.9% vs. 36.7 \pm 9.7%, $p=0.003$). A similar effect of additional IFN β 1a was found on MFI of CD40+ monocytes (HC: 76.7 \pm 11.0% vs. 38.4 \pm 3.5%, $p=0.04$; UNTX: 79.0 \pm 5.1% vs. 36.5 \pm 1.4%, $p=0.008$). The addition of IFN- β 1a to CD40-stimulated PBMCs of UNTX restored the up-regulatory effect of CD40 stimulation on BDNF secretion (1229.45 \pm 307.56 vs. 937.26 \pm 57.27 pg/ml, $p=0.03$). **Conclusions:** The reduced BDNF secretion of PBMCs and the defective effect of CD40 stimulation on BDNF in UNTX is reversed by IFN- β 1a therapy. Among the other known mechanisms of its action, IFN- β 1a may exert a neuroprotective effect via BDNF secretion from immune cells which is related to an increased CD40 expression on monocytes.

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Absolute metabolite concentrations in cerebral white matter of multiple sclerosis patients with beta interferon treatment

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Background: A few investigations concern interferon (IFN)-treated multiple sclerosis (MS) patients using proton spectroscopy, however not with an absolute quantitation or during extended treatment. **Objective:** To quantify metabolite changes during IFN therapy using magnetic resonance spectroscopy. **Methods:** We included 14 MS patients, (9 men, 5 women, mean age 41.8 years, mean disease duration 10.9 years, 9 with relapsing-remitting MS, 6 with secondary progressive and bouts) scheduled for immunomodulatory treatment (5 IFN1A, 9 IFN1B) as well as 14 healthy controls, (8 men, 6 women, mean age 40.2 years). All patients had clinically definite MS (Poser criteria). Measurements were performed in white matter (four voxels). **Results:** Longitudinal results: N-acetylaspartate + N-acetylapartylglutamate (NAA+NAAG) showed a trend to higher values before treatment. Myo-inositol concentrations were significantly and increasingly elevated ($p=0.03$). Glutamine and glutamate concentrations dropped significantly ($p=0.009$) after treatment started but raised later. MS patients/ healthy controls: Creatine and myo-inositol concentrations were significantly higher in MS patients before and after treatment. NAA + NAAG concentrations were significantly lower before and after treatment. Glutamine and glutamate concentrations were higher before therapy, later equal to healthy controls. **Conclusions:** IFN-treated patients demonstrate increasing myo-inositol, a marker of progressive glial proliferation. Also, decreasing concentrations of total NAA derivatives despite IFN therapy suggest ongoing progressive pathology and constant neuronal loss in the course of MS. MS patients compared with matched healthy controls show highly significant differences regarding metabolites (Cr, myo-Ins, NAA) that increase during the therapy period, also indicating that the medication can only moderately influence the metabolites. The most interesting finding is related to the excitatory molecules, glutamine and glutamate. Before IFN therapy statistical analysis showed significant elevation in the concentrations; however after IFN therapy this difference is no longer observed. This finding underlines a possible role of IFN in the expression of down-regulating excitotoxic molecules.

Supported by: Biogen Idec.

P457**Safety, tolerance and efficacy of Pravastatin in MS-STEP in multiple sclerosis: a randomized double-blind placebo controlled pilot study**

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Background: HMG-CoA reductase inhibitors, the so called statins, have demonstrated anti-inflammatory properties besides their well-known cholesterol lowering effects. In recent open-labeled or retrospective studies, simvastatin or atorvastatin decreased the magnetic resonance imaging (MRI) activity in multiple sclerosis (MS). In this placebo-controlled randomized pilot trial we investigated the safety, tolerance and MRI efficacy of 40 mg/d of pravastatin in MS patients. **Objective:** To assess the safety, tolerance and efficacy of oral daily administration of pravastatin in MS. **Methods:** 16 untreated relapsing-remitting MS patients with at least one gadolinium-enhancing lesion (GEL) on the selection MRI were enrolled in a six-month trial with a monthly MRI evaluation and randomized for 40 mg/d of pravastatin or placebo. Clinical and biological assessments were performed at entry, M3 and M6. Adverse events were recorded every month. **Results:** Demographic and disease-related parameters were not different between the two groups at entry. Adverse events were recorded with the same frequency in both groups, viral infections being the most frequent. Only one serious adverse event was recorded during the study (cytolytic hepatitis), but in the placebo group. The percentage of reduction of GEL was of 0%, 26%, -10%, 11% 44% and 44% for M1, M2, M3, M4, M5 and M6 respectively in the placebo group and 56%, 46%, 75%, 70%, 70% and 85% at M1, M2, M3, M4, M5 and M6 respectively in the treated group. When the number of GEL for each month is compared to the number of GEL at entry, the difference is only significant in the treated group from M3 to M6 ($p=0,0004$, $p=0,0047$, $p=0,0047$, $p=0,0018$ for M3, M4, M5 and M6 respectively, paired t test). **Conclusions:** This first randomized placebo-controlled pilot study of the use of pravastatin in MS suggests a good tolerance and an efficacy of the drug on inflammatory MRI parameters.

P458**Lupus-like syndrome induced by interferon beta-1b treatment- a case report**

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Background: Lupus-like syndrome (synonym: drug-induced systemic lupus erythematosus, DILE) is a rare and very serious complication of treatment with some drugs and clinically imitates systemic lupus erythematosus (SLE). It is assumed that the process is triggered by oxidative metabolites of a drug. There are about known 80 drugs which may cause lupus-like syndrome at the present time (hydralazine, procainamide, quinidine, isoniazid, diltiazem, minocycline, etc.) The most common symptoms and signs of this syndrome are arthralgia, arthritis, myalgia, fever, serositis, hepatosplenomegaly, exanthema and presence of antinuclear antibodies (ANAs). Complications of DILE that affect the kidneys and central nervous system are generally considered rare. In contrast to SLE, all symptoms fade away after removal of the causative agent. Lupus-like syndrome is treated by stopping administration of causative drug. **Objective:** We present a case of 43-year-old woman with multiple sclerosis (MS) treated with interferon beta-1b, who presented with lupus-like syndrome after 8 years of treatment. Interstitial nephritis with renal insufficiency and sicca syndrome were found. **Methods:** The following laboratory abnormalities were found: elevation of erythrocyte sedimentation rate, presence of ANA Ig. **Results:** After stopping treatment with INF beta 1b, renal function slowly recovered, and ANAs are still present. Our patient was switched to glatiramer acetate, but had to stop this

therapy because of intolerance. At this time she is treated with natalizumab and she is in good clinical condition. **Conclusions:** SLE is an autoimmune disease that can affect the skin, joints, heart, lungs, kidneys and brain. DILE is a variant of autoimmune disease that can arise after months to years of treatment the likelihood of its occurrence increases with time of application and cumulative dose of drug.

P459**The effect of BG00012 on conversion of gadolinium-enhancing lesions to T1-hypointense lesions**

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Background: BG00012 is an oral formulation of dimethyl fumarate that may have a distinct dual anti-inflammatory and neuroprotective mechanism of action. BG00012 240 mg TID significantly reduced the number of new Gd+ lesions from Weeks 12–24 by 69% ($P<0.001$) and new T1-hypointense lesions by 53% ($P=0.014$) vs placebo in a phase 2b study in relapsing-remitting multiple sclerosis (RRMS). Persistent T1-hypointense lesions are associated with greater tissue damage including axonal loss. **Objective:** To evaluate and compare the probability of conversion of new Gd+ lesions into T1-hypointense lesions between BG00012 and placebo groups in the phase 2b study. **Methods:** In this retrospective, rater-blinded analysis, each new Gd+ lesion at Weeks 4, 8, and 12 was assessed to determine if it evolved into a T1-hypointense lesion at Week 24. Logistic regression was used to compare the probability of conversion from new Gd+ lesion into T1-hypointense lesion between the BG00012 240 mg TID and placebo groups. The odds ratio (OR) of conversion was computed using the placebo group as the reference. **Results:** Fifty-six subjects (BG00012, $n=18$; placebo, $n=38$) were included in the analysis. At the subject level, the mean proportion of new Gd+ lesions that converted into T1-hypointense lesions was lower in the BG00012 group compared with placebo (0.29 vs 0.41). At the lesion level, 29% of new Gd+ lesions converted into T1-hypointense lesions in the BG00012 group vs 44% in the placebo group. The OR comparing the BG00012 group with placebo showed a reduced probability of conversion of Gd+ lesions into T1-hypointense lesions (OR: 0.53; 95% CI: 0.46 to 0.62; $P<0.0001$). Results were consistent when analyses were performed on small (≤ 5 mm) and large (> 5 mm) Gd+ lesions. **Conclusions:** The evolution of new Gd+ lesions into T1-hypointense lesions over the period studied (12–20 weeks) was reduced in BG00012-treated subjects. This finding may indicate a role for BG00012 in tissue preservation. **Supported by:** Biogen Idec, Inc.

P460**A profile of multiple sclerosis patients treated for at least five years with the same disease-modifying agent at the Syracuse MS Clinic**

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Background: Development of disease-modifying agents (DMAs) significantly impacted the disease process in multiple sclerosis (MS) by decreasing the relapse rates, magnetic resonance imaging disease burden and activity. Clinical trials looking at the effect of DMAs in delaying disability in either relapsing-remitting or secondary-progressive MS have shown modest and questionable benefit respectively. One reason for compliance with DMAs is perceived disease stability as measured by Expanded Disability Status Scale (EDSS). There are six FDA-approved DMAs in the US: Avonex, Rebif, Betaseron, Copaxone,

Novantrone and Tysabri. Our analysis is limited to first four agents. **Objective:** To evaluate MS patients treated with same DMA continuously for at least five years in terms of disease progression. **Methods:** We followed approximately 1500 patients at the MS clinic in Syracuse. Treated patients were also registered with the NYSMSC and were prospectively followed by yearly EDSS evaluations. Only patients staying continuously on the initial DMA for five years were selected and analyzed for disease progression as reflected by the initial and 5-year EDSS scores. Patients who switched therapies were excluded. For the convenience of analysis, we categorized them into relapsing (relapsing-remitting, progressive-relapsing) and progressive (secondary-progressive). **Results:** Our analysis included a total of 156 patients, of which 68 were on Avonex (42 with relapsing and 26 with progressive), 17 were on Betaseron (10 with relapsing and 7 with progressive), 55 were on Copaxone (40 with relapsing and 15 with progressive), 16 were on Rebif (12 with relapsing and 4 with progressive). When average initial EDSS scores were compared with the average EDSS scores at 5 years, disease progression was statistically significant in the relapsing group on Copaxone ($P=0.007$) and in progressive groups on Copaxone ($P=0.002$) and Avonex ($P=0.001$). **Conclusions:** Statistical significant progression was seen more in patients on Copaxone and Avonex and more in females on these two DMAs. Further analysis is underway to stratify by EDSS levels. **Supported by:** Serono/Pfizer.

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Oligoclonal band pattern during interferon beta therapy of patients with multiple sclerosis

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Background: The finding of alkaline oligoclonal bands (OCBs) in the cerebrospinal fluid (CSF) supports the diagnosis of multiple sclerosis (MS). According to some studies, the decrease of OCB can demonstrate decreased intrathecal synthesis of IgG and inflammatory reaction in the central nervous system. The aim of this study was to assess OCB in the CSF of patients with relapsing-remitting MS (RRMS) treated with interferon beta therapy. The possible influence of steroid and immunosuppressive therapy on the OCB changes was also assessed. **Objective:** To assess OCB changes in the CSF in the group of patients with RRMS during interferon therapy. **Methods:** The authors examined a group of 19 RRMS patients treated with interferon beta therapy (beta-interferon 1a, 1b), 5 males (aged 25–44, mean 29.8 ± 6.5 years) and 14 females (aged 26–51, mean 36.3 ± 6.4 years). CSF samples were taken 0–42 months before and 1–16 months after the initiation of interferon therapy. The number of alkaline OCBs in the CSF was assessed by isoelectric focusing. Paired sample t-test, Wilcoxon signed-rank test and analysis of variance were applied when assessing statistical significance. **Results:** In the patient group, the number of OCBs at follow-up decreased significantly - paired t-test showed a statistically significant decrease of OCB number ($p=0.004$). Analysis of variance showed no differences between the group of patients with interferon-1a and interferon beta-1b therapy. Mann-Whitney test did not show any statistically significant differences in the group of patients treated together with steroid or immunosuppressive therapy compared with the patients treated only with interferon therapy. **Conclusions:** These results can demonstrate the immunomodulatory effect of interferon beta therapy as decreased intrathecal synthesis of IgG and inflammatory reaction in the central nervous system.

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Skin lesions in multiple sclerosis patients on immunomodulatory treatment

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Background: Immunomodulatory treatment (IMT) reduces disease activity in patients with multiple sclerosis (MS). This is generally a well-tolerated treatment. Mild reactions to IMT at the injection site such as transient erythema are a common side effect. However, some reactions can evolve into potentially serious lesions culminating in infection, necrosis, and in some circumstances requiring surgical repair. **Objective:** To describe clinically and histologically the cutaneous reactions induced by different immunomodulatory treatments (IMTs). **Methods:** Six patients with cutaneous reactions to different IMTs were evaluated clinically and by biopsy. **Results:** Six patients, 5 female and 1 male. Mean age 33,6 (range 23–46), all of them with relapsing-remitting MS. Four in interferon beta 1b treatment, one on subcutaneous interferon beta 1a and another on glatiramer acetate therapy. Mean months of treatment on different IMT=41 (range 2–96). Two of 6 patients presented panniculitis without evidence of vasculitis secondary to subcutaneous injection; both needed surgical repair and interrupted the treatment. Cellulitis was diagnosed in another 2 patients, the lesions slowly improving after treatment with oral antibiotics. The patients were discharged and it was not necessary to stop IMT. One patient presented a dermatofibroma which required surgical repair and the interruption of interferon beta 1b treatment. The last patient on glatiramer acetate treatment presented only subcutaneous nodules with intensive pruritus at different injection sites. The lesions improved with antihistamine and oral corticosteroids. **Conclusions:** Therapy with IMT is associated with a spectrum of cutaneous reactions. In some patients it is not necessary to interrupt the treatment and in some circumstances surgical repair is required. Patients must be warned about these possible side effects and should have periodic medical check-ups for their detection and early treatment.

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A retrospective analysis in patients with relapsing-remitting multiple sclerosis

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Background: Multiple sclerosis (MS) is a chronic inflammatory disorder of the central nervous system that mainly affects young adults. This retrospective analysis evaluated the efficacy of disease-modifying therapies (DMT) (interferon betas and copaxone) in a large cohort of relapsing-remitting MS (RRMS) patients. **Objective:** Documentation of the course of MS in patients treated with DMT or untreated patients to assess unmet therapeutical needs. **Methods:** The analysis was conducted at approximately 400 sites in Germany and included more than 5000 patients with RRMS treated with DMT or no DMT for at least 12 months. Data on demographics, treatment history, Expanded Disability Status Scale (EDSS), number of relapses, and magnetic resonance imaging (MRI) findings were collected. **Results:** Data from 5483 patients has been analysed. Patients had a mean age of 41 years, 73% were female, median duration of disease was 7 years, EDSS (median) was 2.0 at the beginning of the 12 month period. 81.3% of patients received DMTs within the last 12 months. A majority (64%) of patients receiving DMTs were stable. However, 36% of patients on treatment had at least one relapse within the last 12 months. MRI data were available for 2382 patients. Of the group of patients with MRI data available, 27.5 % on DMTs had ≥ 1 gadolinium-enhancing

lesion. Data collection is on-going and data on approximately 8000 patients will be presented. **Conclusions:** Although DMTs stabilized disease in 64% of patients, approximately 30% of treated patients continue to have clinical and MRI disease activity. According to current definitions, these patients are not sufficiently controlled by DMTs. Some of these patients may require further intervention to control disease activity.

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Spasticity in multiple sclerosis: a pilot study to evaluate the efficacy of glatiramer acetate

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Background: Multiple sclerosis (MS) patients present different degrees of spasticity during their disease evolution. Spasticity is characterized by the imbalance of muscle contraction and relaxation that leads to a state of rigidity and involuntary muscle spasms. Among the drugs that can worsen spasticity the most important is interferon beta. The percentage of patients treated with IFN-beta that report increased spasticity varies from 13 to 18% depending on the study, with no clear differences between interferons. **Objective:** To evaluate the spasticity evolution on relapsing-remitting MS patients treated with glatiramer acetate. **Methods:** We prospectively followed 11 patients with MS previously treated with beta-interferon who switched to glatiramer acetate due to adverse effects or inefficacy. The total follow-up was 18 months and all were treated with glatiramer acetate 20 mg subcutaneously once daily. The spasticity was assessed through the Penn Frequency Scale of Spasms, the Ashworth Scale Modified, Adductor Tone and the Global Pain Rating Scale. **Results:** At the end of the follow-up a significant improvement in the majority of the variables studied was demonstrated. The frequency of spasms were reduced from 2 to 0.27 ($p=0.0020$). The rating of the Ashworth scale on the right side was reduced from 1.85 to 1.18 ($p=0.0020$); on the left side, from 1.86 to 1.2 ($p=0.0449$). Pain was decreased from 47.69 to 24.51 ($p=0.0020$). **Conclusions:** Treatment with glatiramer acetate may improve spasticity in patients previously treated with beta interferons.

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Assessing cost-effectiveness determinants between 2 monoclonal antibodies and interferon beta for relapsing multiple sclerosis

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Background: Monoclonal antibodies appear to be especially potent immunomodulatory treatments for relapsing multiple sclerosis (MS), but their relative costs and benefits are uncertain. **Objective:** To perform a cost effectiveness analysis comparing alemtuzumab, natalizumab, and interferon beta-1a in patients with relapsing MS. **Methods:** We developed a static decision tree model to estimate the costs and effects of natalizumab, alemtuzumab, and high dose interferon beta 1a for patients with relapsing MS with an Expanded Disability Status Scale (EDSS) score of 2.0–2.5. The time horizon was 2 years with a payer's perspective. Main outcome measures were quality-adjusted life years (QALYs) and incremental cost effectiveness ratios in US dollars per QALY. Probabilities of relapse, disability progression, and side effects were derived from published trial results. Utilities for different EDSS states and costs associated with treatment and side effects were derived from published data. **Results:** Alemtuzumab was more cost effective and was the completely dominant strategy over both natalizumab and interferon beta. There was a higher incremental cost with interferon beta (\$1,000 US) and natalizumab (\$19,000 US). Alemtuzumab provided a greater benefit over interferon-beta (0.00298 QALYs or 1 quality-adjusted life day in 2 years) and natalizumab (0.00192 QALYs or 0.7 quality-adjusted life day in 2 years). When natalizumab and interferon beta were compared with each other in a separate CEA analysis, no strategy was

dominant. Sensitivity analyses demonstrated that the most responsive variable was the cost of each agent. Relative benefits were less sensitive to changes in probabilities of developing idiopathic thrombocytopenic purpura, autoimmune thyroiditis, and relapses that occur with each agent. **Conclusions:** Alemtuzumab is more cost effective in relapsing MS patients compared with natalizumab and high-dose interferon beta-1a. Despite higher efficacy with monoclonal antibodies in reducing relapse rates and disease progression, costs associated with these treatments are an important determinant in assessing overall cost effectiveness.

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Recurrent granuloma annulare occurring during treatment with daclizumab

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Background: Daclizumab is a humanized monoclonal antibody specific for the interleukin 2 (IL-2) receptor alpha chain that has shown promising effects in multiple sclerosis (MS). Granuloma annulare (GA) is a self-limited chronic, benign dermatosis of unknown cause. Previous studies have suggested that high amounts of IL-2 are present within GA lesions. GA has not been previously reported to occur as a response to daclizumab. **Objective:** To report a unique observation of recurrent GA in a patient during daclizumab therapy. **Methods:** Case report. **Results:** A 34-year-old woman with a nine-year history of worsening relapsing-remitting MS was enrolled in an open-label study assessing the efficacy of monthly daclizumab infusions and weekly interferon beta injections. After receiving her ninth dose of daclizumab, the patient developed a generalized erythematous, papular and non-pruritic rash. This rash was distributed over her lower back, abdomen and forearms. She denied any prodrome viral symptoms or systemic complaints. Microscopic examination of biopsies demonstrated granulomatous foci containing multinucleated giant cells. The lesions improved with the administration of oral corticosteroid therapy, which was gradually tapered over three weeks. The patient resumed her daclizumab infusions. She then developed a second occurrence of GA one year later, which resolved over three weeks. **Conclusions:** It is unclear as to why our patient developed GA during therapy. This finding raises questions over the long-term implications of prolonged IL-2 blockade with monoclonal antibodies. Future studies are needed to further assess the relationship between GA and daclizumab.

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Treatment of multiple sclerosis with natalizumab and interferon-beta-1a subcutaneous: a healthcare utilization study

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Background: The relative economic impact of natalizumab and interferon beta (IFN β)-1a subcutaneous (SC) for the treatment of multiple sclerosis (MS) has not been previously studied. **Objective:** To compare annual health care resource utilization and costs after initiation of natalizumab with those of IFN β -1a SC in MS patients. **Methods:** MS patients treated with natalizumab or IFN β -1a SC during 2006–2007 were identified from the Thomson MarketScan commercial and Medicare claims database. Eligible patients had ≥ 30 days of enrollment before and after the first claim for natalizumab or IFN β -1a SC. Severe MS relapse indicators used as covariates in the regression analysis were MS hospitalization or emergency room visit, intravenous or injectable steroid use, oral steroid use, and intravenous immunoglobulin therapy. Total annual costs were calculated based on number and costs of healthcare services categorized into medical and pharmacy costs from the year following drug initiation. All costs were adjusted to 2007 dollars. **Results:** 1354 eligible patients were identified

(natalizumab, n=253; IFN β -1a SC, n=1101). Patients had a mean age of 46 and 42 years ($P<0.001$) and 71% and 77% ($P=0.04$) were female for the natalizumab and IFN β -1a SC groups, respectively. At baseline, indicators of a severe MS relapse were found in 58% and 44% of natalizumab and IFN β -1a SC patients, respectively ($P<0.001$). After initiation of the study drug, mean total medical costs per person per year were \$60,221 and \$32,772 for natalizumab and IFN β -1a SC, respectively ($P<0.001$). Multivariate regression analysis adjusting for baseline characteristics confirmed the difference in medical costs between the treatments ($P<0.001$). Other significant predictors of higher costs were increasing age, baseline costs, and any indication of severe MS ($P<0.001$, for all). **Conclusions:** These findings suggest that total costs for treatment with natalizumab are nearly double those of IFN β -1a SC. Additionally, the cost to administer and monitor patients treated with natalizumab are highly variable.

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Mycophenolate mofetil in MS: a retrospective study

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Background: Mycophenolate mofetil (MMF) is an immunosuppressive treatment, proposed as an adjunctive therapy for multiple sclerosis (MS). Several studies have reported the relative safety of this treatment but to date, its efficacy has rarely been described. We performed a retrospective open study on 26 MS patients treated by MMF. **Objective:** To assess safety and efficacy of monotherapy with MMF in patients with MS. **Methods:** Twenty-six patients from our MS center (20 females, 6 males) treated with MMF (14 secondary progressive MS, 12 relapsing-remitting MS) were included. All patients were previously treated by immunomodulatory or immunosuppressive drugs. Tolerance of MMF, clinical status, annualized relapses rate (ARR), evolution of the Expanded Disability Status Scale (EDSS) score, and number of Gd-active lesions were assessed, before treatment and at last neurological visit. Twenty patients who received Mitoxantrone before MMF (MTX-MMF) were compared with 20 control patients treated with Mitoxantrone followed by an immunomodulatory drug (MTX-IM). **Results:** Mean age at baseline was 42.7 ± 8.5 yrs, mean disease duration was 15.6 ± 6 yrs. Treatment was interrupted in 2 patients because of diarrhea and asthenia. Adverse events (occurring in 19%) were a transitory lymphopenia and digestive disorders. At baseline, ARR was 0.90 ± 0.66 , EDSS score was 4 ± 1.78 and mean number of gadolinium-enhancing lesions was 1.21 ± 1.91 . After 20.4 ± 12.8 months of treatment, AAR was significantly reduced (0.38 ± 0.61 , $p<0.009$). EDSS score and number of Gd-enhanced lesions were stable. There were no statistically significant differences between the MTX-MMF and MTX-IM group. **Conclusions:** Our study confirms that MMF is a well-tolerated treatment in MS. Although this study was uncontrolled, our results suggest that MMF can improve or stabilize MS patients as a second line therapy.

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Effectiveness of beta interferon on the first attack after confirmed multiple sclerosis in childhood: a comparative cohort study

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Background: In the absence of randomized controlled trials to support therapeutic decisions in pediatric multiple sclerosis (MS), comparative observational studies based on the real practice of physicians are important tools. The studies of efficacy assessing the relapse rate in pre and post-treatment periods had limitations because they did not take into account the varying rate of relapse over time, which could have biased the results. **Objective:** To assess the effectiveness of beta interferon (IFN) in preventing the first attack and severe disability

after confirmed MS diagnosis in a pediatric cohort. **Methods:** A cohort of 197 relapsing-remitting pediatric MS patients was studied (1990–2005). Patients were followed from MS diagnosis until the first subsequent attack or severe disability occurrence (DSS score of ≥ 4) or were censored. The Cox model, with time-dependent IFN exposure to account for the varying times of starting this treatment, was used to estimate the effect of IFN on the risk of this attack or severe disability, adjusting for potential confounding factors. **Results:** During cohort follow-up (mean 5.5 years), 70.5% of the 197 children had a first attack (80% within the first two years) and 24 started IFN (mean delay 3.6 months; mean duration 17.1 months). The use of IFN was associated with a significant reduction in the rate of the first attack during the first year of treatment (hazard ratio: 0.31, 95% confidence interval: 0.13–0.72) as well as the first two years (0.40, 0.20–0.83). This effect was less significant over the entire follow-up of up to four years of treatment (0.57, 0.30–1.10). The use of IFN suggests a reduction of the occurrence of severe disability, although not statistically significant (HR 0.78; 95% CI: 0.25–2.42). **Conclusions:** The use of IFN, given after the diagnosis of MS, significantly reduces the risk of relapse during the first two years.

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MS and risk of venous thromboembolism: a 30-year population-based cohort study

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Background: Immobilization due to neurological disability may increase the risk of venous thromboembolism (VTE) in patients with multiple sclerosis (MS); however, few data exist on the risk of either deep vein thrombosis (DVT) or pulmonary embolism (PE) in these patients. **Objective:** To assess the risk of VTE in Danish patients with MS compared with the general population. **Methods:** The Danish National Registry of Patients, which covers all Danish hospitals since 1977, was used to identify a cohort of patients with MS and no history of VTE prior to MS onset (N=15,366). A matched (sex, age, municipality) population control cohort (N=76,212) was selected from the Danish Civil Registration System. Controls were excluded if they had a history of VTE prior to the date when the corresponding patient in the MS cohort was first hospitalized for MS. Registry data were used to record hospitalizations for DVT or PE. We computed risks of VTE for MS and control patients and used Cox regression analysis to calculate gender-adjusted hazard ratios as a measure of relative risk (RR) for VTE over a follow-up period of up to 30 years. **Results:** Twenty-nine (0.19%) patients with MS and 35 (0.05%) control patients had a DVT registered within 1 year of MS diagnosis; RR=2.98 (95% CI: 2.05–4.34). Fifteen (0.10%) patients with MS and 16 (0.02%) control patients were registered with PE; RR=3.33 (95% CI: 1.98–5.62). During the subsequent 29 years, 242 (1.57%) patients with MS and 728 (0.96%) control patients were registered with DVT; RR=2.35 (95% CI: 2.04–2.73), and 98 (0.64%) patients with MS and 439 (0.58%) control patients were registered with PE; RR=1.72 (95% CI: 1.38–2.14). The elevated RRs were similar for unprovoked and provoked VTE. The excess risk for VTE among patients with MS increased with repeated hospitalizations for MS. **Conclusions:** MS is a strong risk factor for VTE.

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Seven years of mitoxantrone therapy follow-up in an Italian multiple sclerosis centre: evaluation of clinical, magnetic resonance imaging aspects, side effects and impact on disability
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Background: Mitoxantrone (MTX) is one of the treatments for worsening relapsing-remitting, secondary progressive and primary progressive multiple sclerosis (MS). Cardiac toxicity and malignancies are more serious adverse events associated with MXT treatment. **Objective:** To examine the drug impact on relapse rate and Expanded Disability Status Scale (EDSS) progression, magnetic resonance imaging (MRI) (T2 and T1Gd+ number lesions), serious adverse events such as cardiac toxicity and malignant potential, and disability evolution after therapy conclusion. **Methods:** Between 2000 and 2007 we followed-up 158 patients (101 females, 57 males; mean age of 41±10 years old, mean EDSS=4) with relapsing-remitting or secondary progressive MS (73RR/85SPMS), treated with 5 (83 patients) or 10 (75 patients) mg/m² once a month for 5 times, thus once every 3 months. 34 patients have dropped out, 124 are still followed-up. At least 110 of 134 patients underwent mean cumulative dose=110±20 mg/m². RRMS patients had presented at least 2 relapses with sequelae or 3 relapses in the year before; SPMS patients had MRI inflammatory activity; Seventy-five patients were previously treated with interferon. We examined relapse rate and EDSS through 3 months clinical evaluation, MRI yearly change and monitored serious adverse events, cardiac toxicity with 3 monthly echocardiography plus, by 2005, myocardial performance index (MPI) and presence of malignancies. **Results:** MTX is efficient in reducing relapse rate and MRI inflammatory lesions. It is also able to establish EDSS progression, but only when there is inflammatory activity and resolution in mean 2±1 years after the therapy ending; by this time both EDSS progression and MRI atrophy became evident. The mitral deceleration time was significantly increased. The mean value of MPI was >0.47 in all patients. We had several adverse events during therapy (1 gastric cancer, 1 acute leukemia) and following therapy (1 ovarian cancer, 2 mammalian cancers, 1 acute leukemia, 1 melanoma). **Conclusions:** Drug efficacy is related to anti-inflammatory action (relapse rate and T1 Gd+ reduction); there were no effects on disability and atrophy. MPI is correlated with cumulative dose of mitoxantrone: some patients have pathological MPI alterations when they arrived at the cumulative dose of >30 mg/m² and, from this point, the MPI alteration rises with each dose. The drug is related to a malignant potential that must be observed also for a considerable time after therapy has ended.

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CoMPaRe - comparing MusiQoL and MSQoL-54 in multiple sclerosis patients on long-term Rebif® therapy

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Background: Monitoring quality of life (QoL) is becoming increasingly important to neurologists treating multiple sclerosis (MS) patients. Several instruments are available; however, the Multiple Sclerosis International QoL (MusiQoL) questionnaire is the only disease-targeted questionnaire constructed based on formal interviews of MS patients. **Objective:** To evaluate the usefulness of the MusiQoL instrument in comparison with the MSQoL-54 questionnaire in relapsing MS patients on Rebif® therapy. **Methods:** This is a Canadian observational, multi-center, phase IV study of approximately 360 patients with relapsing forms of MS. All patients completed the MusiQoL, MSQoL-54 and questions about preferences for ease of use and content of the two measures. Patients also received a neurologi-

cal examination including Expanded Disability Status Scale (EDSS) at baseline and at 6-month intervals through 24 months. **Results:** Baseline results are presented here for the initial 236 enrollees. The MusiQoL took less time to complete than the MSQoL-54 (6.5±7.4 minutes vs 12.9±9.3, respectively, p<0.0001). In terms of missing data, 22 patients (9.3%) did not complete >2 items for MSQoL-54 compared with three patients (1.3%) for the MusiQoL. Component scores anticipated to correlate highly with the EDSS did have strong associations with both measures: r=-0.57 for the EDSS with the MSQoL-54 Physical Health Composite and r=-0.56 for the MusiQoL Activity of Daily Living scale (both p<0.0001). The MusiQoL was rated as easier to read, understand, and answer (p<0.05), and a higher proportion of patients rated it as 'very easy to complete,' compared with the MSQoL-54. In contrast, the MSQoL-54 was rated as having questions more closely related to daily quality of life, questions more specific to MS, and as the QoL measure the patient would recommend to others (all p<0.005). **Conclusions:** Based on these preliminary baseline data, MusiQoL may be another QoL instrument that is comparable with MSQoL-54, but easier and quicker to complete. **Supported by:** EMD Serono Canada Inc.

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Efficacy of mycophenolate mofetil in the treatment of multiple sclerosis: a retrospective analysis

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Background: Treatment options for secondary progressive multiple sclerosis (SPMS) and primary progressive multiple sclerosis (PPMS) are limited. Mycophenolate mofetil (MMF) has been used to prevent of allograft rejection in patients who have undergone transplants. More recently, MMF has been used in the treatment of several neurological diseases. **Objective:** To report our experience using MMF in the treatment of MS. **Methods:** We conducted a retrospective evaluation of 90 MS patients followed in our clinic, who were started on MMF. Response to treatment was evaluated in 52 patients that were on MMF for at least 12 months and 20 that had completed 24 months. Expanded Disability Status Scale (EDSS) scores were calculated at four time points; 12 months before initiation of MMF, at the time of MMF initiation, and 12 and 24 months later. In ambulatory patients, the recorded 25-foot walk times were also noted. EDSS scores and times to walk 25ft at the different time points were compared using Wilcoxon Rank-Sum test. **Results:** Of the 90 patients who started MMF, 7 stopped because of intolerance. For ambulatory patients (EDSS 0-4), EDSS score increased between visit 1 (2.67 ± 1.04) and visit 2 (2.84 ± 0.8) (p=0.258), but decreased significantly after 1 year of treatment (2.55 ± 0.87) (p=0.009). It decreased further to 2.30 ± 0.9 after another year of treatment (p = 0.074 vs visit 2). For patients with EDSS scores of 6 or above at visit 1, there were no significant changes in the disability status during the treatment period. Five of the patients had at least one relapse during the study period. **Conclusions:** Our results suggest that MMF is well tolerated, and shows efficacy in the treatment of MS. It may be an option in the treatment of progressive MS.

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Natalizumab significantly increases the cumulative probability of sustained improvement in physical disability

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Background: In the AFFIRM study, natalizumab significantly reduced the risk of sustained disability progression in patients with relapsing multiple sclerosis (MS) by 42%-54% (P<0.001) and significantly

increased the proportion of disease-free patients (36.7% vs. 7.2% for placebo; $P < 0.0001$). A Kaplan-Meier analysis of disability progression does not detect sustained improvements in disability because improved and unchanged patients are grouped together and compared with those who deteriorated. Therefore, further analyses were conducted to evaluate whether natalizumab can reverse neurological disability, thus improving patient clinical status. **Objective:** To evaluate the effects of natalizumab on sustained improvement in physical disability as measured by Expanded Disability Status Scale (EDSS) over 2 years in patients with relapsing MS. **Methods:** Post hoc analyses of AFFIRM patients with an EDSS score ≥ 1.0 ($n = 596$ natalizumab, $n = 293$ placebo), an EDSS score ≥ 2.0 ($n = 417$ natalizumab, $n = 202$ placebo), and highly active disease with an EDSS score ≥ 1.0 (≥ 2 relapses and ≥ 1 gadolinium-enhanced lesion; $n = 141$ natalizumab, $n = 56$ placebo) at baseline were conducted. Improvement in disability was defined as a 1-point decrease in EDSS score sustained for 12 weeks. The cumulative probabilities of sustained improvement at 2 years were estimated using the Kaplan-Meier method, and treatment effects were analyzed using a Cox proportional hazards model adjusted for baseline EDSS score. **Results:** Natalizumab significantly increased the cumulative probability of sustained improvement in disability over 2 years compared with placebo in patients with a baseline EDSS score ≥ 1.0 (hazard ratio [HR] = 1.58; 95% confidence interval [CI], 1.13–2.21; $P = 0.008$), a baseline EDSS score ≥ 2.0 (HR = 1.69; 95% CI, 1.16–2.45; $P = 0.006$), and in patients with highly active disease at baseline and an EDSS score ≥ 1.0 (HR = 2.81; 95% CI, 1.20–6.63; $P = 0.018$). **Conclusions:** Natalizumab significantly improves neurological function as measured by a sustained decrease in EDSS score over 2 years in patients with relapsing MS. **Supported by:** Biogen Idec, Inc. and Elan Pharmaceuticals, Inc.

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Cinnovex vs. Avonex: a double-blind, randomized, non-inferiority trial, preliminary results

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Background: Cinnovex® is a biogener form of INF β -1a manufactured by CInaGen Inc. This study was conducted to compare the efficacy and safety of Cinnovex® and Avonex®. **Objective:** To compare the effects of Cinnovex® and Avonex® in relapsing-remitting multiple sclerosis (RRMS) by relapse rate, Expanded Disability Status Scale (EDSS) change, adverse effects, brain magnetic resonance imaging (MRI) changes, and neutralizing antibodies (NABs). **Methods:** A randomized, double-blind non-inferiority controlled trial in RRMS patients as defined by McDonald criteria. Patients were 18–50 yrs old at the time of entry with EDSS ≤ 5.5 and not receiving any disease-modifying therapy. Patients were randomly assigned to receive either Cinnovex® or Avonex® 30?g IM QW for 104 weeks. Adverse events and laboratory tests were recorded every week for the first 4 weeks and every 4 weeks afterwards. Patients were examined monthly by a neurologist and brain MRI and NABs was performed every 6 months. This preliminary analysis was performed after 21 months from the beginning of the study. The study was approved by ethics committee of University of Tehran. **Results:** Sixty-one patients have been enrolled and randomly assigned to study groups and 12 patients have dropped out of the study. EDSS change from baseline to last measurement, probability of sustained disability progression, number of relapses and number of patients with relapse was not significantly different in study groups. The only adverse events with a significant difference in the two groups was a greater incidence of headache in the Avonex® group and more urinary infection in the Cinnovex® group. Increased SGPT was seen in 8 patients (5/Avonex®)/3(Cinnovex®), and in 2 patients, one in each group, this had increased by more than 5 times; both were excluded from the study. The number of T2 lesions, T2 lesion volume and number of enhancing brain MRI lesions were also not significantly different between groups. **Conclusions:** Twenty-one months after study onset, with a mean follow-up of 28 weeks in each arm, no significant difference was seen in clinical outcome measures (EDSS, relapse rate), adverse events, number and volume of T2 lesions and number of enhancing brain MRI lesions

between Cinnovex® and Avonex®. The study will be continued until each patient has been followed for two years.

Supported by: CInaGen Inc.

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Rituximab as add-on therapy for breakthrough disease: clinical effects over 24 weeks

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Background: Data indicate that B cells and antibodies are involved in the pathogenesis of multiple sclerosis (MS). Rituximab is a monoclonal antibody that depletes circulating CD20+ B cells and has been shown to be a potential agent in the treatment of relapsing-remitting MS. We report clinical data from an investigator-initiated trial of rituximab in relapsing MS patients with breakthrough disease activity despite treatment with immunomodulatory drugs (IMD). **Objective:** To assess the clinical effects of add-on rituximab. **Methods:** Twenty-six relapsing MS subjects with continued clinical activity despite treatment with an FDA-approved IMD were enrolled. Enhancement on any of 3 monthly pre-treatment magnetic resonance images (MRIs) was required for inclusion. Total enhancing lesions on the 3 pre-treatment MRIs ranged from 1–17. Subjects received four doses of 375mg/m² rituximab while continuing to take IMD. Multiple Sclerosis Functional Composite (MSFC) and Expanded Disability Status Scale (EDSS) were performed prior to and after B cell depletion. Three non-scored 'practice' MSFC tests were performed prior to the baseline MSFC. Pre- vs. post-rituximab results were compared using Wilcoxon matched pairs signed ranks test. **Results:** Eighteen female and 8 male subjects (mean age 42 \pm 6yrs) with mean disease duration of 9.5 \pm 7.3 years were treated and followed for >24weeks. Circulating B cells remained depleted for the duration of the study period. Mean EDSS at baseline was 4.7 \pm 1.5 (median 4.0, range 2.0–6.5), and did not change post-treatment. At 24 weeks post-treatment, MSFC was improved by a mean z-score increase of 0.15 over baseline ($p = 0.031$). Improvement in MSFC was driven by performance on the PASAT, with a mean z-score increase of 0.21 ($p < 0.004$). The 25 foot walk and 9 hole peg test remained stable over this time period. **Conclusions:** Rituximab as add-on therapy in relapsing MS is associated with an improvement in the MSFC over 24 weeks.

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A comparison of fatigue and cognition in relapsing-remitting MS patients receiving interferon medication

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Background: Interferon- β (IFN- β) disease-modifying drugs reduce relapse rates and slow disease progression in relapsing-remitting multiple sclerosis (RRMS). Early treatment with these therapies may also prevent fatigue and slow cognitive decline. **Objective:** The goal of this study was to quantify the change in patient fatigue and/or cognitive status following the onset of treatment with IFN- β therapy. **Methods:** A single center, phase IV, non-randomized, open-label study involving 39 patients diagnosed with RRMS was conducted to assess fatigue and/or cognition at 4 predetermined study visits (month 0, 3, 9, 15). A baseline clinical history was conducted at visit 1. Patients were allocated to treatment with IFN β -1a intramuscular (IM), IFN β -1a subcutaneous (SC) or IFN β -1b at visit 2 according to physician recommendation. Fatigue was measured using the Modified Fatigue Impact Scale (MFIS), while cognition was measured using the Brief Repeatable Battery of Neuropsychological Tests (BRB) at each study visit.

Results: The results of a multivariate analysis for 39 patients (IFN β -1a (SC), 18; IFN β -1a (IM), 9; IFN β -1b, 12) are reported. Significant improvements were observed in cognition from visits 2 through 4, in 3 of 5 BRB cognitive domains, in each treatment group. Patient fatigue remained stable for patients on IFN β -1a (SC) (average adjusted MFIS score (standard error) = 57.8 (4.2), 54.8 (4.1) and 52.2 (4.4) at visits 2, 3 and 4, respectively) and IFN β -1a (IM) (average adjusted MFIS score = 60.4 (5.9), 54.1(5.9) and 58.3 (6.0) at visits 2, 3 and 4, respectively), but worsened significantly between visits 3 and 4 with IFN β -1b treatment (average adjusted MFIS score = 57.6 (4.7), 51.9 (4.7) and 61.6 (4.9) at visits 2, 3 and 4, respectively) ($p < .05$). **Conclusions:** This research has identified a potential role of IFN- β therapy to attenuate MS-induced cognitive deficits with preferential merit for using IFN β -1a over IFN β -1b in patients that suffer MS-induced fatigue at the onset of their disease.

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Fatigue in relapsing-remitting multiple sclerosis - assessment of clinical, neuropsychological and immunological parameters in patients treated with glatiramer acetate

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Background: The pathomechanisms of fatigue which is a common symptom in multiple sclerosis (MS) are incompletely understood. Glatiramer acetate (GA), an immunomodulatory agent approved for treatment of relapsing-remitting MS (RRMS), has been shown to exhibit beneficial effects on MS fatigue (Metz et al., 2004). **Objective:** To correlate clinical, neuropsychological and immunological parameters in RRMS patients with fatigue before and during treatment with GA. **Methods:** In a prospective, open-label, multi-center trial, 30 patients with RRMS and fatigue were treated with GA for 12 months. Inclusion criterion was the presence of fatigue as one of the most frequent and disabling symptoms. Before and during treatment, fatigue was assessed using the Fatigue Severity Scale (FSS), the MS-FSS, and the Modified Fatigue Impact Scale (MFIS). Laboratory assessments included screening of 190 parameters using real-time PCR microarrays in blood samples obtained from 12 patients (baseline and month 12) followed by further analysis of several cytokines, chemokines and neurotrophic factors. **Results:** Fatigue self-assessments were completed in 25 patients. After 12 months of treatment with GA, thirteen of these patients had improved in all three scales (with the most prominent effects on the MFIS), whereas five patients had deteriorated (the remaining patients exhibited mixed effects within the three scales). Laboratory assessments revealed heterogeneous mRNA levels of cytokines, chemokines and neurotrophic factors. Detailed statistical analyses and sub-analyses will be presented. **Conclusions:** GA may exhibit beneficial effects in some patients with RRMS and fatigue. Eventual correlations between fatigue and immunological parameters will be discussed.

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Daclizumab exhibits efficacy in multiple sclerosis subjects positive for interferon-beta neutralizing antibodies

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Background: The Phase 2 CHOICE study demonstrated that daclizumab at a dose of 2 mg/kg every two weeks, but not 1 mg/kg every 4 weeks, resulted in a decrease in new or enlarged Gd+ lesions when used in combination with interferon-beta (IFN-beta) therapy. To test the hypothesis that daclizumab would have similar efficacy when used as a monotherapy, its effect in the subset of patients who had neutralizing antibodies (NAb) to IFN-beta was analyzed. **Objective:** To evaluate if daclizumab demonstrated efficacy in the subset of subjects

that were positive for IFN-beta NAb in the CHOICE study. **Methods:** Serum samples were analyzed for the presence of anti-IFN-beta NAb using a viral inhibition assay following WHO/NIH guidelines. Subjects classified as positive for IFN-beta NAb had to have a positive response (titer >25) both at baseline and week 20, bracketing the daclizumab dosing period. Subjects were stratified according to their IFN-beta NAb status and the clinical effect of daclizumab was evaluated. The primary endpoint assessed was the sum of new or enlarged Gd+ lesions between weeks 8 and 24 in daclizumab treated groups vs. placebo. **Results:** The incidence of IFN-beta NAb positive subjects was 9/68 (13%) for placebo, 7/63 (11%) for 1 mg/kg, and 7/65 (11%) for 2 mg/kg treatment groups. For subjects positive for IFN-beta NAb, both the 1 mg/kg and 2 mg/kg daclizumab groups had significantly fewer new or enlarged lesions compared to placebo ($p = 0.0002$ and 0.0043 respectively). Furthermore, in both daclizumab treatment groups, the mean number of new or enlarged lesions was lower in subjects who were IFN-beta NAb-positive compared with those who were NAb-negative. **Conclusions:** These results indicate that the efficacy of daclizumab is not dependent on the presence of functional IFN-beta. This finding supports the further study of daclizumab as a monotherapy for MS.

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New non-myeloablative conditioning regimen for multiple sclerosis patients undergoing autologous hematopoietic stem cell transplantation

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Background: During the last decade myeloablative autologous hematopoietic stem cell transplantation AHSTCT has been used with increasing frequency as a therapeutic option for multiple sclerosis (MS) patients. Taking into account the information about risk of transplant-related mortality and severe side effects of myeloablative conditioning regimens, the rationale to use non-myeloablative regimens is reasonable. **Objective:** The goal of our research was to study safety and efficacy of non-myeloablative AHSTCT in MS patients. **Methods:** Thirty-six patients with MS (secondary progressive - 15 patients, primary progressive - 8, progressive-relapsing - 2, and relapsing-remitting - 11) were included in this study (mean age - 33.0, range: 17-51; male/female - 15/21). The non-myeloablative conditioning regimen included BCNU (300 mg/m²) alone or combined with melphalan (50 mg/m²) on day 1 before transplantation. In vivo T-cell depletion was performed through an infusion of 30 mg/kg of horse anti-thymocyte globulin on day 1 and 2 after stem cell reinfusion. Median Expanded Disability Status Scale (EDSS) score at baseline was 4.0 (range 1.5 - 7.0). The mean follow-up duration was 6 months (range 3 - 12 months). Neurological and quality of life (QoL) evaluation was performed at baseline, at discharge, at 3, 6, 9, 12 months, and every 6 months following AHSTCT; magnetic resonance imaging examinations - at baseline, at 6, 12 months, and at the end of follow-up. **Results:** Notably, no transplant-related deaths were observed. The transplantation procedure was well tolerated by the patients with no unpredictable severe adverse events. All of 28 patients included in the efficacy analysis experienced clinical stabilization (n=17) or improvement (n=11). Ten patients showed improvement by 0.5 points on EDSS and one patient by 1.0. All the patients included in QoL analysis (n=13) exhibited QoL response at 6 months post-transplant and improved QoL during the follow-up. **Conclusions:** In conclusion, the new non-myeloablative regimen of AHSTCT appears to be safe and effective treatment for MS. The collection of further data on long-term follow-up is worthwhile to confirm the efficacy of the new treatment regimen.

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Immunoabsorption plasma pheresis for the treatment of neuromyelitis optica spectrum disorderTakashi Ohashi¹, Kohei Ota², Yuko Shimizu¹, Kuniko Ohara¹, Chisen Takeuchi¹, Sachiko Kubo¹, Shinichiro Uchiyama¹¹Tokyo Women's Medical University, Shinjuku, Tokyo, Japan; ²Tokyo University of Science, Shinjuku, Tokyo, Japan

Background: Anti-aquaporin 4 (AQP4) antibody is specifically detected in the sera from patients with neuromyelitis optica (NMO) spectrum disorder. In these patients, acute exacerbations are often fulminant and unresponsive to high-dose intravenous methylprednisolone pulse (HIMP) therapy. Immunoabsorption plasma pheresis (IAPP) has therefore been performed for these cases, but the efficacy of IAPP for treating an acute relapse of NMO spectrum disorder has not yet been well evaluated. Therefore, an accumulation of therapeutic experience on this topic is considered to be important. **Objective:** To investigate the efficacy of IAPP as a treatment for acute relapses of NMO spectrum disorder. **Methods:** IAPP was performed on four patients suffering from NMO spectrum disorder with a severe exacerbation, which had proven to be unresponsive to HIMP. IAPP was performed 3 - 7 times every other day, using the tryptophan column (Immusorba TR-350®) through the double lumen catheter inserted in either a jugular vein or a femoral vein. The plasma volume processed through the column during each session ranged from 2 - 3,000 ml. In addition, the serum anti-AQP4 antibody and cytokine levels (IL-2, IFN- γ , TNF- α , IL-10) were measured before and after IAPP. **Results:** IAPP was well tolerated and effective in all the patients. A significant reduction of Expanded Disability Status Scale score (from 7.9 to 6.3) was observed during the treatment period. The serum anti-AQP4 antibody titer decreased from 4096x to 512x during three sessions of IAPP therapy. The serum IFN- γ level also decreased, however, the IL-10 level was observed to increase. **Conclusions:** IAPP may be a therapeutic option for the patients with a fulminant attack of NMO spectrum disorder that are unresponsive to the conventional therapy. Our data suggest that IAPP shows its effect by removing anti-AQP4 antibody, and by altering the T cell subfunction from Th1 dominant to Th2 dominant.

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Central nervous system vasculitis in a subject with multiple sclerosis on daclizumab monotherapy

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Background: Daclizumab is a humanized monoclonal antibody specific for IL-2R α receptor and has been approved for the prevention of allograft rejection in renal transplant patients. Two phase II studies of daclizumab in multiple sclerosis (MS) have reported significant reduction in contrast enhancing lesions (CELs) and improvement in clinical scores. **Objective:** To report the development of central nervous system (CNS) vasculitis in a subject with MS treated with daclizumab. **Methods:** Subjects with MS participated in a phase II study of daclizumab monotherapy in MS at the National Institutes of Health (NIH). Subjects received 1mg/kg of daclizumab IV monthly (total of 15 infusions) and were monitored by monthly magnetic resonance imaging (MRI) scans and clinical assessments. At the completion of the study, several subjects who elected to continue on daclizumab under the supervision of their private neurologists were followed at the NIH. **Results:** A 42-year-old woman with MS completed the phase II daclizumab monotherapy study at the NIH with an optimal response as evidenced by significant decrease in CELs and stable clinical exams. She elected to continue daclizumab following the trial. Daclizumab was stopped after a total of 21 doses due to the onset of new clinical symptoms and evidence of aggressive CNS inflammation based on brain and spine MRI. In particular, the brain MRI was remarkable for a vascular pattern of contrast enhancement. Because of continued clinical deterioration, stereotactic brain biopsy was performed showing marked inflammatory changes associated with small vessel destruction in the brain parenchyma consistent with CNS vasculitis. The subject began treatment with methylprednisolone followed by a regimen of cyclophosphamide. **Conclusions:** The

etiology of CNS vasculitis in this subject is uncertain but may have been associated with the use of daclizumab. While two phase II studies have reported that daclizumab is effective in reducing CELs, continued studies are necessary to acquire additional safety data on its use as a treatment for MS.

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Demographic and clinic characteristics of patients treated with Tysabri in the real situation of prescription compared with AFFIRM study

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Background: Natalizumab is the first $\alpha 4$ integrin in new class of selective adhesion-molecule inhibitors. Available in France since April 2007, natalizumab is indicated in monotherapy for treatment of very active forms of relapsing multiple sclerosis (MS) for patients whose treatment by beta interferon has failed. **Objective:** The objective of this work is to analyze demographic, clinic and tolerance data in the first patients treated by Natalizumab in two French regions (Alsace and Nord-Pas-de Calais) in the real situation of prescription compared with AFFIRM Study. **Methods:** Since April 2007, 259 patients were included in the study, 79 males (30.5%) and 180 females (69.5%). Analysis criteria include: evaluation of handicap (Expanded Disability Status Scale (EDSS) score), preliminary rate of relapse, previous treatment before Tysabri, first results of tolerance, and number of relapses after six month of treatment with Tysabri. **Results:** Before treatment of Tysabri, the demographic characteristics data of our patients are not significantly different from those of the AFFIRM Study. Our patients have a median diagnosis age of 7 years (1-34) vs. 5 years (0-34). The EDSS Score before Tysabri is 4 (0-6.5) in our patients compared with 2 (0-6) for AFFIRM Study. After 6 months of treatment the EDSS score remains stable. Twenty-six percent of the patients presented at least one adverse event of treatment and 6.1% stopped treatment. **Conclusions:** The results of this study show the demographic profile of our patients is not different from that of patients in the AFFIRM study. Our patients presented a higher median EDSS score, the tolerance seems identical to that reported in pilot studies. These results are very encouraging and must be confirmed on long-term evaluation.

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Therapeutic efficacy of natalizumab (Tysabri) in MS patients with high disease activity: a Danish nationwide studyAnnette Oturai¹, Thor Petersen², Nils Koch-Henriksen³, Poul Erik Hyltdgaard Jensen¹, Finn Sellebjerg¹, Per Soelberg Sorensen¹¹The Danish Multiple Sclerosis Center, Department of Neurology, Copenhagen University Hospital, Copenhagen, Denmark; ²Department of Neurology, Aarhus University Hospital, Aarhus, Denmark; ³Department of Neurology, University Hospital in Aalborg, Aalborg, Denmark

Background: Previous studies on the effect of Tysabri in multiple sclerosis (MS) patients have included patients with moderate disease activity. According to Danish regulatory provisions, Tysabri can only be administered to patients with two or more relapses on disease-modifying therapy (DMT) in the previous year. The effect of Tysabri in a patient population with high disease activity has not previously been reported. **Objective:** To evaluate the therapeutic effect of Tysabri in MS patients with high disease activity. **Methods:** Since 1996 the Danish MS Treatment Register has prospectively collected clinical data on all patients on DMT. We analyzed data from the first 173 consecutive Tysabri-treated patients, observed for at least 24 weeks. We measured Tysabri-binding antibodies at month 3, 6 and 12. **Results:** Baseline data at onset for the 173 Tysabri treated patients were female:male ratio 2.15:1, median age 40 (range 18-64) years, disease duration 8.0 (0-36) years, and Expanded Disability Statuas Scale score 4.5 (0-8). A total of 167 patients (96.5%) had previously received DMT, of whom 35 (21%) had been treated with mitoxantrone. An interim analysis after a mean observation time of 6.2 months (range 2.1 - 14.6) showed an annualized relapse rate of 0.76 compared to a rate of 2.4 ($p < 0.0001$) in the 12 months preceding Tysabri treatment.

We did not observe any opportunistic infections. Four allergic reactions were reported, but there were no severe reactions. Of 110 patients 9 (8%) were tested positive for Tysabri-binding antibodies at week 12. **Conclusions:** Tysabri appears to be effective in MS patients with high disease activity as the annual relapse rate dropped from 2.40 to 0.76, but the relapse rate was higher than the 0.26 reported in the AFFIRM study after the first treatment year. This is likely to reflect differences in disease activity before the initiation of Tysabri treatment.

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Atorvastatin combined with interferon beta 1a in relapsing-remitting multiple sclerosis: preliminary results of a 24 month randomized open-label clinical trial

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Background: Statins seem to have therapeutical potential in immune-mediated disorders such as multiple sclerosis (MS) suggesting that combination therapy with interferon (IFN) and statins may provide additional therapeutic benefit in MS. **Objective:** To evaluate the efficacy and safety of IFN beta 1a in combination with atorvastatin in multiple sclerosis (MS) and whether it increases therapeutic benefits. **Methods:** Twenty-two relapsing-remitting MS patients aged 20–55 years with disease activity while on IFNB-1a 44microg subcutaneous (SC) 3 times weekly for 6–12 months were randomized to receive either IFNB-1a 44 microg SC alone or in combination with atorvastatin 20 mg /day for 24 months. Expanded Disability Status Scaale (EDSS) scores and laboratory parameters were assessed every 3 months and magnetic resonance imaging (MRI) and neuropsychological evaluation were assessed at screening, 6, 12, 18, and 24 months. The primary outcome was MRI activity. Secondary outcomes were clinical relapses, EDSS variation, and safety laboratory parameters. Data were analysed with Chi-square and Student T test analysis. **Results:** Of the 22 patients randomized, 18 patients have completed 18 months on study: combination therapy (n=7) or IFNB-1a alone (n=11). Baseline characteristics were similar between treatment groups. The same proportion of patents in both group had no gadolinium-enhancing lesions at 12 months. One enhancing lesion and relapse (1/7) was found in the combination group, whereas 4 enhancing lesions (4/11) and 5 relapses were observed in the Rebif alone group. EDSS variation at 18 months was not significant in either group. No clinically relevant blood analysis alteration was observed in either group. **Conclusions:** Combination therapy with atorvastatin 20 mg/day and IFNB-1a was safe and well tolerated with compatible efficacy on disease activity during the 18 month follow-up period. Clinical and MRI data at the end of 24 months will probably clarify the beneficial effect of the association. As we used a lower dose we did not observe a loss of efficacy in IFNB-1a, and this may be attributed to the higher doses used (40–80 mg/d)as add-on statins in these studies.

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The effects of interferons and copaxone on MMP-9/TIMP-1 ratio and TIMP-2 levels and on new enhancing lesions on magnetic resonance imaging in patients with relapsing-remitting multiple sclerosis

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Background: MMP-9 plays a key role in the disruption of the blood-brain barrier(BBP), while MMP-2 seems to be associated with the chronic progressive phase of the disease. TIMP-1 (the tissue inhibitor of MMP-1) is the natural inhibitor of MMP-9 and the balance between them may play a role in stabilizing the BBB. Several studies have

shown that high serum levels of MMP-9 and low levels of TIMP-1 predict the appearance of new gadolinium enhancing lesions in relapsing-remitting multiple sclerosis(RRMS). **Objective:** The primary aim of the study is to determine serum levels of MMP-2, MMP-9, TIMP-1 and TIMP-2 in patients with RRMS and to evaluate the effects of interferon and copaxone therapy on these measures. The secondary aim to determine the relationship between these serum levels and magnetic resonance imaging (MRI) activity. **Methods:** 80 patients with clinically definite RRMS were enrolled to the study and randomized into 4 equal groups, each receiving 2 different doses of interferon beta 1-a (Avonex), (Rebif 44 mgms), interferon beta 1-b (Betaferon) and glatiramer acetate (Copaxone). Measures of serum MMP-2, MMP-9, TIMP-1 and TIMP-2 measures and gd-enhanced MRIs were obtained initially and every 3 months during the 12-month follow-up period and are analysed with enzyme-linked immunosorbent assay. MMP-9/TIMP-1 and MMP-2/TIMP-2 ratios were calculated. The numbers of Gd-SD, Gd-TD, new Gd-SD, new Gd-TD and new T' lesions were counted. A control group was not used. **Results:** Serum MMP-9/TIMP-1 ratio (P<0.0001) decreased in all treatment groups during the 12-month follow-up period. The number of active lesions also decreased in all treatment groups(p ranging from 0.0005 to 0.005). Serum MMP-9/TIMP-1 ratio showed to be a good positive predictor of the number of MRI Gd-TD active lesions. **Conclusions:** The preliminary results of this study show that interferon beta and copaxone therapy is beneficial in restoring the MMP-9/TIMP-1 ratio balance, and this ratio can be considered as a reliable marker and may be predictive of MRI activity in RRMS.

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The UK long-term cost-effectiveness study of disease-modifying therapies in multiple sclerosis: lessons from the first analysis

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Background: Data is unavailable on the long-term cost-effectiveness of disease-modifying therapies (DMTs) for multiple sclerosis (MS). The short-term nature of clinical trials and the marginal and variable changes in disability reported require questionable assumptions to be made in attempting to model long-term outcomes and cost-effectiveness. **Objective:** The UK MS risk-sharing scheme aims to collect 10 year outcomes on a large cohort (>5,000) patients to assess the long-term cost-effectiveness of DMTs. **Methods:** The scheme was open to patients initiating any of the four licensed DMTs between May 2002 and April 2005 (Rebif 22 or 44, Avonex, Betaferon, Copaxone) according to UK guidelines. This observational study with annual assessments allowed the choice of therapy to be at the discretion of the neurologist and patient. Current comparator datasets, consisting of disability transition probabilities, are drawn from the Ontario (natural history) cohort and a (predicted) outcome dataset derived from 2–3 year DMT trial data for individual therapies. The first analysis was performed when all patients had the opportunity to complete 2-year follow-up. **Results:** Of consented patients 75% were female, the mean baseline age was 39yrs and >900 patients were in each therapy group. 5009 had treatment onset and baseline Expanded Disability Status Scale (EDSS) within 3 months, 4301 (85%) had relapsing-remitting disease and 3686 had 2-year data eligible for primary analysis (missed appointments, switchers, withdrawn consent, death, emigration, secondary progression, all exclusions from this analysis). Confirmed disability outcomes were available from 1479 patients with 3-year data. A range of outcomes will be presented and these are influenced by early fluctuations in EDSS and assumptions made in modeling EDSS data to match the comparator natural history dataset. **Conclusions:** Early results from this 10-year study identify many of the difficulties implicit in long-term observational studies. Lessons learned from this analysis will inform and strengthen future analyses of what will be the largest long-term study of cost-effectiveness of DMT.

P488**Natalizumab utilization and safety in patients with relapsing multiple sclerosis: updated results from TOUCH™ and TYGRIS**

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Background: Natalizumab (TYSABRI®) is the first $\alpha 4$ -integrin antagonist available for the treatment of relapsing multiple sclerosis (MS). In a pivotal phase 3 study, natalizumab monotherapy achieved a 68% reduction in annualized relapse rate and a 42% and 54% reduction in disability progression sustained for 12 and 24 weeks, respectively, compared with placebo. Reports of 2 cases of progressive multifocal leukoencephalopathy (PML) in MS patients taking natalizumab prompted the establishment of two risk management programs, TYSABRI Outreach: Unified Commitment to Health (TOUCH™) and the TYSABRI Global ObseRvation Program in Safety (TYGRIS), to monitor and evaluate the long-term safety of natalizumab. **Objective:** To report the current exposure and safety data for patients with relapsing MS treated with natalizumab. **Methods:** The TOUCH™ Prescribing Program ensures the appropriate and informed use of natalizumab and monitors patients for signs and symptoms of serious opportunistic infections, such as PML. All prescribers, infusion sites, and patients receiving natalizumab in the United States are required to enroll in TOUCH™. TYGRIS is a voluntary global observational study investigating the long-term safety of natalizumab in a clinical practice setting. Approximately 5000 patients are expected to enroll in TYGRIS, including those in TOUCH™. Patient assessments occur at 6 month intervals for up to 5 years. Medical histories and details of prior use of natalizumab, immunomodulatory, antineoplastic, and immunosuppressive agents are collected, and serious adverse events, including PML and other opportunistic infections, are evaluated. **Results:** As of March 29, 2008, 17,883 patients have been enrolled in TOUCH™ and as of February 23, 2008, 2100 patients have been enrolled in TYGRIS. The most current safety and exposure data will be presented. **Conclusions:** The results from TOUCH™ and TYGRIS demonstrate a favorable benefit-risk ratio for natalizumab and will continue to increase our understanding of the long-term safety of this treatment.

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P489**Epigallocatechin-gallate in relapsing-remitting multiple sclerosis (SunIMS study)**

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Background: Recent work from our department showed a beneficial effect of epigallocatechin-3-gallate (EGCG), the major polyphenolic compound of green tea, on disease course in experimental autoimmune encephalomyelitis (EAE), the animal model of multiple sclerosis (MS). We could show that EGCG reduced clinical severity when given at initiation or after the onset of EAE by both limiting brain inflammation and reducing neuronal damage. **Objective:** To investigate the safety, tolerability and efficacy of orally administered EGCG in patients with relapsing-remitting MS. **Methods:** A randomised, placebo-controlled multi-center trial was designed and has started enrollment in September 2007. Patients aged 18–60 are randomised to receive either EGCG or placebo twice daily. Primary endpoint is the number of new T2 lesions on brain magnetic resonance imaging after a 12-month treatment period in the EGCG group compared with placebo. Secondary endpoints include the progression of brain atrophy (decrease of brain parenchymal fraction), changes in the NAA/Cr ratio on magnetic resonance spectroscopy, the number of relapses and the progression of disability. A sample size of 150 patients is scheduled. **Results:** To date, 80 patients have been randomised. Thirty patients have been on treatment for more than 6 months. No serious adverse events have since been observed. Side effects are mostly mild and include headache, abdominal discomfort, insomnia, and

clinically insignificant elevation of liver enzymes in 3 cases. Enrollment is ongoing. **Conclusions:** This is the first study with orally administered EGCG in MS. Our preliminary data suggest a good tolerability of EGCG in our patients. Further data on the study design, the enrollment status and tolerability of the study medication will be presented.

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P490**Intense immunosuppression as the initial disease-modifying therapy in clinically active relapsing multiple sclerosis**

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Background: Intense immunosuppression (IIS) is often used as rescue or disease-stabilizing therapy in clinically active relapsing multiple sclerosis (MS) patients with sub-optimal response to immunomodulating therapy (IMT). However, there is little data on the use of IIS as initial therapy in MS. **Objective:** We conducted a study to examine the feasibility, efficacy, and safety of IIS as the initial therapy in clinically active relapsing MS. **Methods:** Relapsing MS patients who received IIS as the initial therapy at our Center were studied. None of the patients had received FDA-approved disease-modifying therapy (DMT) prior to the use of IIS. All but two patients had received IV methylprednisolone (IVMP) for the treatment of relapses as well as pulsed IVMP to stabilize clinically active disease. All patients had experienced at least two relapses in the year prior to therapy with IIS. All patients received monthly IV cyclophosphamide (CTX) for 6 months followed by initiation of therapy with DMT including interferon beta (IFNB) or glatiramer acetate (GA). All patients underwent brain magnetic resonance imaging scans prior to IIS and at year 1 i.e. 6 months after the completion of IIS therapy. **Results:** 26 consecutive patients with clinically active MS received IV CTX for 6 months. Mean age, disease duration, Expanded Disability Atatus Scale, relapse rate, gadolinium-enhancing lesions (GEL) per patient at baseline were 34.8 years, 3.08 years, 3.61, 3.42, and 3.55, respectively. At year 1, mean EDSS, relapse rate, and GEL were significantly reduced to 2.22, 0.47, and 0.17, respectively. Mean percentage brain volume change at year one was -2.37%. Additional data beyond one year of follow-up will be presented. All patients tolerated IIS well and no opportunistic infections or unusual toxicity was observed. **Conclusions:** This study provides evidence that IIS may be used as the initial therapy in clinically aggressive disease. Larger, randomized studies are warranted to assess the long-term efficacy and safety of this therapeutic approach followed by standard therapies such as IFNB or GA.

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P491**Evaluation of the myocardial performance index for early detection of anthracycline-induced cardiotoxicity in patients with multiple sclerosis: a three-year follow-up**

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Background: Mitoxantrone (MTX) was approved by the FDA in 2000 for the treatment of multiple sclerosis (MS). One of the most serious adverse events associated with the drug is cardiac toxicity. **Objective:** The purpose of this study is to evaluate the early alterations follow-up in left ventricular(LV) systo-diastolic function in patients with MS receiving MTX, by the use of this new index. **Methods:** The myocardial performance index (MPI) is a non-invasive Doppler measurement of global (systolic/diastolic) ventricular function. It is defined as the sum of isovolumic contraction time and isovolumic relaxation time, divided by ventricular ejection time. MPI >0.47 is a marker of LV dysfunction. We are following-up the same patients previously studied; in particular, at the moment, we had obtained data from twenty of them (mean cumulative dose 50±10.3 mg/m² of MTX), during a monitoring time of 36±3 months. **Results:** The trend of mitral flow

pattern showed a significant decrease of Epv and Evti in these MS patients. Nevertheless, no significant decrease of Epv/Apv and Evti/Atvi was observed. In addition, the mitral deceleration time was significantly increased. The mean value of MPI was >0.47 in all patients. One patient had finished the therapy by 2005 and four in 2007: the MPI index is nevertheless pathological. **Conclusions:** The MPI represents an index of combined systolic and diastolic myocardial performance, strongly correlated with the given cumulative dose of MTX. Once more, significant correlation between the given cumulative dose of MTX and MPI and mitral deceleration time respectively was demonstrated. A complete MPI patients' evaluation will be carried out to confirm this parameter trend.

P492

Medical and indirect costs of employees with multiple sclerosis: effect of multiple sclerosis therapies

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Background: Few studies have examined the impact of multiple sclerosis (MS) therapies on the total medical and indirect costs of MS in the workplace. **Objective:** To compare the annual total medical and indirect costs of newly treated vs untreated employees with MS. **Methods:** Employees 18–64 years of age with ≥ 1 MS diagnosis (ICD-9-CM: 340.x) after January 1, 2002 were selected from a privately insured claims database comprising 17 US companies. Employees with ≥ 1 MS therapy claim composed the newly treated group; employees with no MS therapy claim at any time composed the untreated group. The index date was the day after the most recent claim (treated, MS therapy claim; untreated, MS claim) meeting the following requirements: continuous health coverage for 3 months before (baseline period) and 12 months after (study period) the index date and active for ≥ 1 day during baseline. Total medical costs (all-cause inpatient, emergency room, outpatient, and labs) and indirect costs (disability and medically-related absenteeism) were compared for treated MS vs untreated MS employees, adjusting for baseline characteristics, including co-morbidities. Costs were reported in 2006 US dollars. **Results:** Treated MS employees ($n=258$) were on average younger (40.9 vs 44.4 years, $P<0.0001$) and more likely to be female (72% vs 62%, $P=0.007$) than untreated MS employees ($n=322$, $P=0.007$). Three-month baseline MS-related medical costs were higher among treated MS employees (\$2520 vs \$1012, $P<0.0001$), and untreated MS employees had a higher mean Charlson Comorbidity Index (0.4 vs 0.2, $P<0.003$). There was a non-significant trend toward higher baseline non-MS-related medical costs in untreated vs treated MS employees. Risk-adjusted total annual medical costs (\$4393 vs \$6187, $P<0.0001$) and indirect costs (\$2252 vs \$3053, $P<0.0001$) were significantly lower for treated MS employees than for untreated MS employees. **Conclusions:** MS therapies significantly lower total medical and indirect costs in employees with MS.

Supported by: EMD Serono, Inc., and Pfizer Inc.

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Design of a four-arm, randomized, placebo-controlled phase II study of 36 weeks of atacept monotherapy in relapsing multiple sclerosis

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Background: B cells are key players in autoimmune diseases and there is increasing clinical evidence for their contribution to multiple sclerosis (MS) pathology. Atacept, a recombinant fusion protein,

impedes B cell and plasma cell function by blocking BlyS (B-Lymphocyte Stimulator) and APRIL (A Proliferation-Inducing Ligand), important regulators of B-cell function and survival. The clinical development program of atacept in MS comprises two Phase II studies to address the potential anti-inflammatory and neuroprotective properties of atacept. Here we present the ongoing ATAMS (ATacept in Multiple Sclerosis) study. **Objective:** The ATAMS study (IMP28063) will assess the safety and tolerability of atacept and its effects on central nervous system inflammation in relapsing MS (RMS), and determine a minimally effective dose. **Methods:** Eligible patients (target: $n=300$) must be 18–60 years old with a diagnosis of RMS, an Expanded Disability Status Scale score of 0–5.5; and either ≥ 2 documented relapses during the previous 2 years, ≥ 1 documented relapse in the year prior to enrollment, or ≥ 1 T1 gadolinium-enhancing lesion at screening. Patients will be randomized 1:1:1:1 to receive either one of three doses of atacept (initial loading dose of 25, 75, or 150mg SC BIW for 4 weeks, followed by weekly SC injections of 25, 75, or 150mg) or matching placebo for a total of 36 weeks. **Results:** The primary endpoint is the mean number of T1 gadolinium-enhancing lesions/patient/scan from weeks 12–36 inclusive. Secondary endpoints include the number of new T1 hypointense lesions and proportion of relapse-free patients. Immunogenicity will be assessed as a proportion of patients developing anti-atacept antibodies. **Conclusions:** The ATAMS study will provide information on the safety, tolerability, and immunogenicity of atacept over 36 weeks of treatment, its ability to reduce brain inflammation, as measured by frequent magnetic resonance imaging, and a minimally effective dose. The complementary Phase II ATON (ATacept in Optic Neuritis) study will explore the neuroprotective potential of atacept in patients with optic neuritis.

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P494

Safety and effectiveness of natalizumab re-dosing and treatment in the STRATA study

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Background: Natalizumab (TYSABRI®) has demonstrated significant efficacy on relapse rate and disability progression in patients with relapsing multiple sclerosis (MS). The Safety of TYSAARI Re-dosing and Treatment (STRATA) study is an ongoing open-label, multinational study with the primary goal of evaluating the long-term safety of natalizumab. Preliminary results (24-week data) from STRATA support the safety of switching to natalizumab from other MS therapies and its favorable tolerability profile. **Objective:** To report safety and efficacy results from STRATA based on up to 48 weeks of treatment. **Methods:** STRATA consists of a 48-week initial treatment period and a 4-year extension treatment period. All patients receive natalizumab 300 mg intravenously once every 4 weeks. Enrolled patients had completed the AFFIRM, SENTINEL, or GLANCE studies as well as a dosing suspension safety evaluation, and had not been antibody-positive to natalizumab during prior evaluations. Efficacy assessments included annualized relapse rates (ARRs) and Expanded Disability Status Scale (EDSS) scores at weeks 24 and 48. **Results:** As of 26 February 2008, 1089 patients have enrolled in STRATA and have received a median of 12 (min, max: 1, 14) natalizumab infusions after a median treatment gap of 84.9 weeks (min, max: 57, 147). The cumulative probability of relapse and ARR over time remained low. The unadjusted ARR were 0.249 and 0.191 at weeks 24 and 48, respectively. There was no EDSS progression from baseline to week 48. Among the 5% of patients with a serious adverse event, MS relapse was the most common. No opportunistic infections have occurred. **Conclusions:** To date, no clinical worsening of MS has been observed

during STRATA, and updated safety results show that natalizumab continues to be generally well tolerated. Continuation of STRATA will further characterize the long-term efficacy and safety of natalizumab. **Supported by:** Biogen Idec, Inc. and Elan Pharmaceuticals, Inc.

P495**Monitoring of natalizumab therapy with cerebrospinal fluid activation markers**

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Background: Natalizumab is a FDA-approved medication for the treatment of active multiple sclerosis (MS). Currently, response to the medication is assessed by clinical assessment and brain magnetic resonance imaging (MRI) findings. Analysis of cerebrospinal fluid (CSF) activation markers may aid decision-making in difficult cases. **Objective:** To determine the effect of natalizumab treatment on the levels of a panel of putative disease activity markers in the CSF. **Methods:** CSF samples were obtained with informed, IRB-approved consent from 60 MS patients at our center undergoing treatment with natalizumab. Levels of fetuin-A, osteopontin, and nitric oxide samples in the CSF at baseline, post treatment at 6 months and 12 months were determined using their respective ELISA kits following the manufacturer's instructions. Values of activation markers were read off a standard curve. In addition to activation markers, the total cell counts of all samples were noted. Pre and post-treatment values were statistically analyzed by a paired t-test. **Results:** Natalizumab reduced total cell counts, and levels of CSF osteopontin, nitric oxide, and fetuin-A in approximately 60% of all patients. There was, however, no statistical significance in pre- and post-treatment values (at one year) for cell count, osteopontin, and nitric oxide. Fetuin-A had a mean pre-treatment level of 1676 (ng/mL) and this was reduced at six months to 1371 (ng/mL; $p < 0.0001$) and at a year to 1255 (ng/mL; $p < 0.00001$). In addition, CSF fetuin-A levels in individual patients correlated with disease activity as determined by clinical relapses and brain MRI activity. Overall, at six months 74% of treated patients had reduced fetuin levels and by one year 81% were affected. This suggests that the maximal therapeutic effect of natalizumab may take several months. **Conclusions:** Of the CSF disease activity biomarkers investigated, fetuin-A appears to be significantly reduced by natalizumab treatment. This may have utility in therapeutic decision-making.

P496**Interferon beta therapy and pregnancy outcomes in patients with multiple sclerosis**

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Background: Little is known on pregnancy outcome after in utero exposure to beta-interferon (IFNB) therapy. **Objective:** To assess the impact of IFNB therapy on pregnancy outcomes in an Italian cohort of multiple sclerosis (MS) patients. **Methods:** We recruited MS patients (both women and men), followed-up in 14 Italian MS Centres, for whom a pregnancy was recorded in the period 1996–2007. Patients

were divided into 3 groups: drug-exposed pregnancies (EP: suspension of the drug <1 month prior to conception); non-exposed pregnancies (NEP: suspension of the drug >1 month prior to conception) and never treated pregnancies (NTP). All the patients were administered a structured interview which gathered detailed information on pregnancy course and outcomes, as well as on possible confounders. Multivariate logistic and linear models were used for treatment comparisons. Furthermore, propensity score (PS) adjusted logistic and linear models were assessed. **Results:** Data on 432 pregnancies were collected (364 in women, 68 in men). Among women, 82 were classified as EP to IFNB, 97 as NEP, and 160 as NTP. Pregnancies resulted in 72 live births and 10 non-live births in the EP, 90 live births and 7 non-live births in the NEP, 155 live births and 5 non-live births in the NTP. IFNB exposure was not significantly related to spontaneous abortion (OR=0.99;95%CI 0.29–3.37;p=0.98) whereas it was associated with a higher frequency of preterm delivery (OR=1.94;95%CI 1.04–3.61;p=0.037), and lower weight ($p < 0.0001$) and length ($p < 0.0001$) of the child. These findings were confirmed in the PS-adjusted models. Considering pregnancy and fetal outcomes in the 63 men (31 exposed to IFNB, 32 non-exposed), we did not observe significant differences between EP and NEP. **Conclusions:** Data in our cohort show that the mother's IFNB exposure is associated with a lower child's weight and length, whereas IFNB does not seem to be associated with a higher frequency of spontaneous abortion.

P497**Post-marketing reports of acute leukemia in mitoxantrone-treated multiple sclerosis patients**

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Background: Mitoxantrone has been an approved oncology treatment for acute non-lymphocytic leukemia since 1987. In 2000, mitoxantrone gained US approval for worsening forms of multiple sclerosis (MS), and remains the only medication approved for these MS subtypes. Post-marketing cases of acute leukemia in mitoxantrone-treated MS patients have been reported. **Objective:** To evaluate the characteristics of acute leukemias in MS patients who received mitoxantrone therapy in the United States. **Methods:** Spontaneously-reported cases of leukemia in mitoxantrone-treated MS patients between 2003 and 2007 were used for this analysis. Due to the spontaneous nature of these reports, an incidence rate can not be determined. **Results:** In the post-marketing setting, 39 cases of leukemia in mitoxantrone-treated MS patients were reported through 2007. Patients had a mean age of 48.2 years (range, 29–68 years) and 28 (71.8%) were women. Various dosing regimens were reported; however, the mean (SD) total dose of mitoxantrone, available for 18 patients, was 83.2 (24.1) mg/m² (range, 48–135 mg/m²). The most frequent diagnoses among the 39 cases were acute myelogenous leukemia (38.5%) and acute promyelocytic leukemia (33.3%). Refractory secondary leukemia is more common in mitoxantrone-treated patients with concomitant or previous use of DNA-damaging antineoplastic agents, cytotoxic drugs, or escalation of anthracycline dose. Relative to this, selected cases will be presented. **Conclusions:** Mitoxantrone is the only medication approved for worsening forms of MS. Physician and patient awareness of the risks and characteristics of acute leukemia in mitoxantrone-treated MS patients should allow for informed decision making and adequate monitoring in patients in whom mitoxantrone treatment is deemed appropriate.

Supported by: EMD Serono, Inc. and Pfizer Inc.

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Glatiramer acetate following mitoxantrone induction in relapsing-remitting multiple sclerosis: extended experience

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Background: Extensive trial, clinical and magnetic resonance imaging (MRI) experience confirm the profound anti-inflammatory effect of mitoxantrone in relapsing-remitting MS (RRMS). Enthusiasm for its use has however been tempered by the potential for significant, but dose-related, toxicity. This combination of efficacy but toxicity favors use as a short-term 'induction' agent. **Objective:** Several groups have reported preliminary experience in this setting, though there is no consensus on follow-on therapy. We report our extended experience with glatiramer acetate (GA) as follow-on therapy to mitoxantrone in RRMS. **Methods:** All RRMS patients treated with mitoxantrone/GA, with >1 year follow-up, were identified. In general patients had experienced two or more disabling relapses with apparent sequelae in the two years prior to treatment. In most patients additional factors identified them as having high-risk disease (frequent relapses, motor involvement, MRI lesion load). Data on relapse, disability (Expanded Disability Status Scale (EDSS)) outcomes and adverse events were recorded. **Results:** Seventy-seven patients were treated, 58 were treatment-naïve. Mean disease duration was 4.05 years, 19 were treated within 1 year of onset. The majority (58) received 48mg/m² mitoxantrone over 8 months, overlapping with GA for the final 3 months. Mean follow-up was 44 months (range 20–90), 33 months on GA alone. There was a single case of therapy-related leukemia. Annualised relapse rate fell from 1.85 to 0.16 or less sustained up to 6 years of follow-up. Seventy patients remain on GA to date. EDSS is improved or stable in 69 (91%). Three patients (4%) developed secondary progressive MS. No patient has been retreated with mitoxantrone, 2 have subsequently received CAMPATH-1H. **Conclusions:** In active RRMS short-term induction with mitoxantrone followed by GA results in profound and sustained suppression of clinical disease activity and minimises dose-related risks of immunosuppression. Treatment is well tolerated and no novel side effects of this combination have emerged over prolonged follow-up.

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Clinical and magnetic resonance imaging measures in the assessment of the response to interferon betaJordi Río¹, Alex Rovira², Elena Huerga², Lara Nonell¹, Manuel Comabella¹, Jaume Sastre-Garriga¹, Joaquín Castillo¹, Alejandro Horga¹, Mar Tintoré¹, Xavier Montalban¹*¹Neuroimmunology Unit, Vall d'Hebron University Hospital, Barcelona, Spain; ²IDI-Vall d'Hebron University Hospital, Barcelona, Spain*

Background: Several criteria for treatment response to interferon (IFN) beta have been proposed. Nevertheless, there is no consensus among different investigators. **Objective:** The aim of this study was to investigate magnetic resonance imaging (MRI) and clinical predictors of response during the first months of therapy with IFN beta in relapsing-remitting multiple sclerosis (RRMS) patients. **Methods:** This is a prospective and longitudinal study of patients with RRMS treated with IFN beta. All patients included underwent brain MRI before the onset of therapy with IFN and 12 months after; a neurological assessment every 3 or 6 months. Patients were classified based on the presence of new lesions, presence of relapses, disability increase or combinations of all these variables after one year of therapy. Regression analysis was performed in order to identify clinical and MRI variables of response after a follow-up of three years. **Results:** We included 222 RRMS patients with a clinical and MRI examination and with a follow-up of three years. After one year of therapy 23%, 15%, and 32% of the patients had relapses, increase of disability, and new T2 lesions respectively. The logistic model including relapses, progression and new lesions demonstrated that only the combination of new T2 lesions with the presence of relapses (OR 4.4 ; IC 1.6–12.5) or disability progression (OR 7.1 ; IC 1.6–33.9), or both (OR 6.5 ; IC 1.9–23.4) achieved significant values to identify those patients with a poor outcome after three years of therapy. **Conclusions:** In RRMS

patients treated with IFN beta the combination of measures of disease activity and the presence of new T2 lesions may have a prognostic value for identifying patients with a poor outcome during the ensuing years of therapy.

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RENEW study update XVIII: ongoing evaluation of the safety and tolerability of mitoxantrone in worsening multiple sclerosisVictor Rivera¹, Ahmad AL-Sabbagh², Randy Bennett², Patricia Coyle³, Stanton Elias⁴, Daniel Mikol⁵, Hillel Panitch⁶, Loren A. Rolak⁷, William Sheremata⁸, Bianca Weinstock-Guttman⁹, Edward Fox¹⁰, for the RENEW Study Group.*¹Baylor College of Medicine, Houston, Texas, USA; ²EMD Serono, Inc., Rockland, Massachusetts, USA; ³Stony Brook University Hospital, Stony Brook, New York, USA; ⁴Henry Ford Health Science Center, Detroit, Michigan, USA; ⁵University of Michigan Multiple Sclerosis Center, Ann Arbor, Michigan, USA; ⁶Fletcher Allen Healthcare, Burlington, Vermont, USA; ⁷Marshfield Clinic, Marshfield, Wisconsin, USA; ⁸University of Miami School of Medicine, Miami, Florida, USA; ⁹The Jacobs Neurological Institute, Buffalo, New York, USA; ¹⁰Central Texas Neurology Consultants, Round Rock, Texas, USA*

Background: The Registry to Evaluate Novantrone Effects in Worsening multiple sclerosis (RENEW) study is a multi-center, open-label, observational study designed to evaluate the safety of mitoxantrone in worsening relapsing-remitting multiple sclerosis (WRRMS), secondary progressive multiple sclerosis (SPMS), and progressive-relapsing multiple sclerosis (PRMS) in the post-marketing setting. **Objective:** To provide an update on the ongoing RENEW study. **Methods:** The RENEW study included patients aged 18–65 years with WRRMS, SPMS, or PRMS who had initiated treatment with mitoxantrone 12 mg/m² and were expected to follow package-insert dosing and monitoring recommendations. Patients were evaluated before treatment initiation and monitored for up to 5 years. During the treatment period, complete blood cell and platelet counts and liver function tests were conducted every 3 months and left ventricular ejection fraction (LVEF) was measured at baseline, before each dose, and whenever clinically indicated. After completion of treatment, LVEF and laboratory parameters were measured annually for up to 5 years after treatment initiation. **Results:** 509 patients were enrolled in the RENEW study at 46 US centers. The current 18th update includes new data collected between July 17, 2007 and January 25, 2008 and an update from the cumulative study which began in April 2001. Through January 25, 2008, treatment has concluded in 470 patients and continues in 39 patients. Of those who have discontinued treatment, 174 patients continued to be followed-up. During the current reporting period, no new cases of leukemia, congestive heart failure, treatment-phase serious adverse events, amenorrhea, or serious infections were reported; however 2 deaths (unrelated to treatment, n=1; unknown relationship, n=1), 2 patients with decreases in LVEF relative to baseline but remaining ≥50%, and 6 relapses were reported. Cumulative data for the entire study period will be presented. **Conclusions:** The RENEW study continues to provide important data on the use and safety profile of mitoxantrone in patients with worsening forms of MS in the post-marketing setting. **Supported by:** EMD Serono, Inc.

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Treatment with glatiramer acetate reduces T1 and T2-weighted magnetic resonance imaging activity in patients with clinically isolated syndrome suggestive of multiple sclerosis

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Background: Glatiramer acetate (GA) reduced relapse rate and magnetic resonance imaging (MRI)-monitored disease activity in relapsing-remitting (RR) multiple sclerosis (MS) patients. This three-year, randomized, placebo-controlled, multi-center trial, assessed the efficacy of GA therapy initiated shortly after the first clinical event in patients with clinically isolated syndromes (CIS) suggestive of MS. **Objective:** To evaluate the efficacy of early treatment with GA (COPAXONE®) on MRI metrics in patients with CIS suggestive of MS. **Methods:** Patients at presentation with CIS and at least two T2-weighted brain lesions, with a size of at least 6mm were enrolled into the study. Only patients with a unifocal disease manifestation were included. They were randomized to receive either 20mg/day GA sc or placebo. The primary efficacy outcome was time to clinically definite (CD) MS, based on a second clinical attack. MRI metrics served as secondary and exploratory end-points. A preplanned interim analysis was performed on data acquired from approximately 80% of the three-year study exposure. **Results:** A total of 481 patients were randomized to receive GA (n=243) or placebo (n=238). At baseline, the two study groups did not differ in terms of clinical (age, time from first event to randomization, corticosteroid use for first attack, Expanded Disability Status Scale) and MRI (number and volume of T2-weighted and gadolinium-enhancing lesions) metrics. Further to the reduction in the risk of developing CDMS by 45% compared to placebo (hazard ratio of time to CDMS 0.55, p=0.0005), patients receiving GA also experienced a reduced number of new T2-weighted lesions by 58% (p<0.0001) and of exposure-adjusted T2-weighted lesions volume (p value=0.0002). GA was also effective in reducing number of both new T1 Gd-enhancing and new T1 hypointense lesions (p=0.0001 in both case). GA was well tolerated, with 16% overall withdrawals to the time of the interim analysis, and a safety profile similar to that observed in RRMS. **Conclusions:** The results establish efficacy of early treatment with GA in both delaying time to CDMS and in reducing MRI activity in patients at presentation with CIS.

WITHDRAWN

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Treatment with disease-modifying drugs does not alter disability in relapsing-remitting multiple sclerosis

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Background: Numerous trials confirm that disease-modifying drugs (DMDs) such as interferons or glatiramer can mitigate relapses of multiple sclerosis (MS), though the trials have been brief and the benefits have been modest. Less clear is whether sustained DMD treatment can prevent long-term disability. Extended placebo-controlled trials are impractical, so longitudinal databases have been proposed as a tool for assessing long-term disability **Objective:** To determine whether sustained treatment with DMDs over periods up to 12 years alters the development of disability in MS. **Methods:** We analyzed the

Expanded Disability Status Scale (EDSS) scores of 573 relapsing-remitting MS patients in a longitudinal database treated with DMDs (224 with glatiramer and 349 with various interferon preparations) for 1–12 years. Two control groups were used for comparison: (1) the patient's own rate of progression prior to starting treatment, and (2) patients who were advised to start treatment but refused, and so were followed without therapy. EDSS scores were calculated at one year intervals for all patients. **Results:** There was no difference in disability between patients treated with any of the DMDs, versus the control groups (which were equally matched), at any time up to 12 years of follow-up. A sub-group of patients who initiated treatment early (within 6 months of initial symptoms) fared no better than those who started later. Few patients in any group developed EDSS>4 unless they entered a secondary progressive phase, which occurred in 14% of treated and 15% of control patients. **Conclusions:** Acknowledging that there are limitations to analysis of longitudinal databases, we conclude that DMDs, over a span up to 12 years, do not delay disability and do not prevent the onset of secondary progression. (Genetic analysis of these patients will also be presented.)

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Predictors of long-term disability in relapsing multiple sclerosis: 8- and 15-year analyses from the phase 3 clinical trial of intramuscular interferon beta-1a

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Background: Expanded Disability Status Scale (EDSS) score worsening by 1 point, sustained for 6 months, was the primary endpoint in the pivotal phase 3 clinical trial of intramuscular (IM) IFNβ-1a in patients with relapsing multiple sclerosis (MS). However, little is known about the long-term prognostic value of disability progression and other parameters of disease activity. **Objective:** To evaluate the relationship between progression status and disease activity over 2 years, and long-term disability in MS patients. **Methods:** Post hoc analyses of data from the pivotal phase 3 study were performed and included patients who received ≥2 years of treatment with IM IFNβ-1a. Relationships between sustained EDSS progression, annualized relapse rate (ARR), and mean T2 lesion volume over 2 years and EDSS milestones ≥4.0, ≥5.0, ≥6.0, and ≥7.0 at 8 and 15 years were analyzed. For deceased patients the last known EDSS score was carried forward. **Results:** There were 160 and 116 patients included in the 8-year and preliminary 15-year analyses, respectively. Patients with EDSS progression over 2 years were significantly more likely to reach all EDSS milestones at 8-year (all P<0.001) and 15-year (all P≤0.001) follow-up. Compared with placebo, treatment with IM IFNβ-1a significantly reduced the probability of reaching EDSS milestones 4.0 (P=0.007) and 5.0 (P=0.01) at 8 years. Patients with an ARR >1 were significantly more likely than those with an ARR ≤1 to reach all EDSS milestones at 8-year and 15-year follow-up (all P<0.003). T2 lesion volume also significantly correlated with reaching all EDSS milestones at 8-year follow-up (all P<0.05). **Conclusions:** Six-month sustained EDSS progression over 2 years in clinical trials is a meaningful predictor of long-term, clinically relevant disability in patients with relapsing-remitting MS. These results suggest that sustained disability progression is the most appropriate measure for evaluating the efficacy of potential MS therapies.

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JC and BK virus in natalizumab-treated patients

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Background: Natalizumab treatment in combination with other disease-modifying agents is associated with progressive multi-focal leukoencephalopathy (PML). The risk of PML with natalizumab

monotherapy is not known. It is important to identify natalizumab-treated patients who are at greater risk of developing PML. **Objective:** To detect JC virus (JCV) DNA, the causative agent of PML, and a closely related virus (BKV) in natalizumab-treated patients prior to the development of PML. **Methods:** We screened plasma and cerebrospinal fluid (CSF) of 200 natalizumab-treated patients at our center receiving monthly natalizumab infusions. Screening was done at baseline, and after 6 and 12 months of treatment. In addition, all patients had neurological evaluations and brain magnetic resonance imaging (MRI) at 0, 6, and 12 months. Natalizumab treatment was discontinued in any patient with positive viral DNA and repeat testing was done monthly in this cohort. **Results:** After six months of treatment, no patient developed clinical or brain MRI evidence of PML. However, seven of the 200 patients had detectable JCV/BKV DNA. All seven had undetectable viral DNA at baseline. Five patients were positive for BKV DNA in the CSF and the other two patients were positive for JCV DNA (one in plasma and the other in CSF). After cessation of natalizumab treatment, all seven patients converted to undetectable viral DNA within 6 months. The results of the 12 month plasma/CSF screening for JCV/BKV will be presented. **Conclusions:** At 6 months the detection rate of JCV/BKV virus in CSF or plasma is 7 of 200 (3.5%) natalizumab-treated patients. Despite this finding, no cases of PML have occurred. Ongoing 12-month analysis in patients who had undetectable CSF/plasma JCV/BKV after 6 months, will allow for more definitive conclusions.

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Ten-year experience of bone marrow transplantation for multiple sclerosis

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Background: Autologous bone marrow transplantation (ABT) is considered a potential therapeutic option to treat severe multiple sclerosis (MS) refractory to conventional treatment. **Objective:** The aim of this work is to describe demographical, clinical and magnetic resonance imaging (MRI) features and outcome of a series of all our MS patients who underwent ABT for refractory illness. **Methods:** We retrieved, reviewed and analyzed data from our database, medical records and MRI images of twelve patients who were grafted from May 1998 to May 2008. **Results:** Demographical variables: 75% females, mean age at MS onset 27.4 years (SD 6.30), mean disease duration at ABT 10.1 years (SD 3.89). All the patients were resistant to other therapies (including Mitoxantrone in 75% of patients) and had an aggressive disease: 7 patients (58.3%) had secondary-progressive MS with a worsening of at least 1 point in the Expanded Disability Status Scale (EDSS) during the last year, 2 of which had superimposed relapses and 5 patients (41.7%) had relapsing-remitting MS with 2 or more relapses during the last year in spite of conventional treatments. No major complications happened after transplantation. Mean EDSS at ABT was 5.4 (SD 1.27). After an average of 33 months of follow-up after grafting, all but two patients (83.3%) stopped having clinical relapses and progressing on disability measured with EDSS. Neuroimaging signs of activity were lacking in all patients in control MRI after the procedure, when available (83.3%). **Conclusions:** The bone marrow transplantation is a feasible, secure and effective therapy for aggressive MS resistant to other treatments.

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Oral fingolimod (FTY720), 0.5 or 1.25 mg, for 14 days has no effect on cardiac function

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Background: Oral fingolimod (FTY720), a sphingosine-1-phosphate receptor modulator, is therapeutically active in multiple sclerosis (MS) and is currently being assessed at doses of 0.5 and 1.25mg in phase III pivotal trials of patients with relapsing-remitting MS. Consistent with the mechanism of action of fingolimod, pharmaco-

logical studies have also reported a reversible decrease in blood lymphocyte count and a transient decrease in heart rate after initiating therapy. **Objective:** To assess cardiac function in healthy volunteers receiving the lower doses of oral fingolimod (0.5 or 1.25mg) currently being evaluated in phase III trials. **Methods:** In this randomized, double-blind, parallel-group, multiple-dose study, subjects received daily oral fingolimod 0.5mg (n=12), 1.25mg (n=12), or placebo (n=12) for 14 days. Absolute lymphocyte count (ALC) and M-mode, two-dimensional echocardiograph imaging were assessed on Days -1, 1, 7, and 14. Cardiac output (CO), stroke volume (SV), and systemic vascular resistance (SVR) were evaluated at all time points. **Results:** Fingolimod was well tolerated and all 36 subjects completed the study. Treatment with oral fingolimod resulted in a significant, 70% decrease in ALC from baseline (Day -1) to Day 14. No significant change from baseline in CO, SV, or SVR was seen in any group during the study; the maximal decrease in CO was 12% (p=0.26) and occurred in the placebo group on Day 1. **Conclusions:** Because oral fingolimod retains a subset of lymphocytes in the lymph nodes, the decrease in ALC is an expected manifestation of therapy. Importantly, at doses of 0.5 or 1.25mg, no significant effect on CO was detected either immediately post-dose or after 14 days of treatment. These results provide evidence that oral fingolimod will not affect cardiac function at doses planned to treat MS.

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Design of an exploratory two-arm, randomized, placebo-controlled Phase II study of 36 weeks of atacept treatment in patients with optic neuritis as clinically isolated syndrome

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Background: B cells are key players in autoimmune diseases and there is increasing clinical evidence for their contribution to the pathology of multiple sclerosis (MS) and related central demyelinating disorders such as optic neuritis (ON). Atacept, a recombinant fusion protein, impedes B cell and plasma cell function by blocking BlyS (B-Lymphocyte Stimulator) and APRIL (A Proliferation-Inducing Ligand), important B-cell regulators. The clinical development program of atacept in MS comprises two Phase II studies to address the potential anti-inflammatory and neuroprotective properties of atacept. The ongoing ATON (ATacept in Optic Neuritis) study uses the neurodegenerative changes of the retina and optic nerve occurring during ON as a model system to test atacept as a neuroprotective treatment strategy in MS. **Objective:** The ATON study (IMP28156) will assess the safety and tolerability of atacept in patients with ON and explore its efficacy to preserve retinal nerve fiber layer (RNFL) thickness as assessed by optical coherence tomography (OCT). **Methods:** Patients (target: n=100) diagnosed with unilateral symptomatic ON as clinically isolated syndrome will be randomized 1:1 to receive either atacept (150mg SC QW, including a 4-week loading dose) or placebo for a total of 36 weeks. OCT will be used to assess degenerative changes of the optic nerve and RNFL. **Results:** The primary endpoint is the change in RNFL thickness in the affected eye of patients with ON from baseline to week 36. Secondary endpoints include: difference in RNFL thickness between affected and fellow eye; change in macular thickness and volume; visual function; and incidence of treatment-emergent adverse events. **Conclusions:** The ATON study will provide information on the safety and tolerability of atacept over 36 weeks of treatment and on its ability to preserve RNFL thickness in ON as a measure of neuroprotective properties. The complementary Phase II ATAMS (ATacept in Multiple Sclerosis) study will assess the anti-inflammatory potential of atacept in relapsing MS.

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Clinical and magnetic resonance imaging analysis of dietary intervention in early relapsing-remitting multiple sclerosisPushkar S. Shah¹, Louise Gold², Jonathan I. O'Riordan²¹Institute of Neurosciences, Southern General Hospital, Glasgow, Scotland, United Kingdom; ²Ninewells Hospital, Dundee, Scotland, United Kingdom

Background: Epidemiological studies suggest that dietary factors may influence the risk of developing multiple sclerosis (MS). In addition there is high usage of complementary therapies amongst MS patients, and in particular dietary modifications are popular. There is currently no significant trial data regarding dietary interventions in MS. **Objective:** To assess the effect of dietary intervention, in particular the Best Bet Diet (BBD) on disease progression in early relapsing-remitting MS. **Methods:** Patients with a diagnosis of relapsing-remitting MS by MacDonald criteria with Expanded Disability Status Scale (EDSS) scores of ≤ 3.5 were randomised to one of the two study diets (BBD and the diet advised by the MS society of UK) for a period of 12 months. The assessor was blinded to the treatments. EDSS scores, multiple sclerosis functional composite (MSFC) scores, body mass index (BMI) and volumetric magnetic resonance imaging (MRI) brain were assessed at baseline and 6 monthly. Fatigue severity scales and global health visual analogue scales (VAS) were monitored. Subjects completed a 7-day food diary every 3 months. **Results:** 28 patients consented, and 22 completed the trial. There were no adverse effects in any groups. There was no significant difference in BMI at baseline and at 12 months in either group. There was trend of lower rate of brain atrophy and change in EDSS in the group on BBD but this was not statistically significant. MSFC score was significantly better in BBD group ($P < 0.05$), with difference of means at 2.08. There was a significant improvement in VAS in the BBD group ($P < 0.05$). **Conclusions:** Dietary factors may be important in MS pathology. A hypoallergenic diet with high dose Vitamin D3 and fish oils, such as the BBD, may have a role in slowing disease progression in MS. **Supported by:** MS charity - "Direct-MS".

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Effects of glucosamine sulfate on multiple sclerosis progression: a randomized, placebo-controlled trialVahid Shaygannejad¹, Fereshteh Ashtari¹, Mohsen Janghorbani²¹Isfahan Neuroscience Research Center (INRC), Isfahan, Iran; ²Isfahan University of Medical Sciences (IUMS), Isfahan, Iran

Background: Glucosamine, a natural glucose derivative and an essential component of glycoproteins and proteoglycans, has been safely used to relieve osteoarthritis, but there is no evidence to support its use in multiple sclerosis (MS). **Objective:** The aim of this study was to evaluate the effectiveness of glucosamine sulfate in the prevention of progression of MS. **Methods:** One hundred and twenty definite MS patients age 17 to 55 years were randomly allocated to receive a 6-month treatment course of either oral glucosamine sulfate (1500 mg/day) or placebo. Response to treatment was assessed at 6 months after start of therapy. **Results:** The results of the study demonstrated that a slight but significant improvement in relapse rate occurred in the glucosamine sulfate group. Of the 60 patients treated with glucosamine sulfate, the mean (SD) of relapse rate decreased from 1.2 (0.7) at baseline to 0.5 (0.6) at the end of the study period ($P < 0.01$). Correspondingly, in the 60 patients treated with placebo, the mean (SD) of relapse rate did not change. After 6 months, 53.3% of patients receiving glucosamine sulfate remained relapse-free compared with 48.3% of those given placebo. The average Expanded Disability Status Scale (EDSS) score at the end of trial did not change between glucosamine sulfate and placebo group (mean difference, 0.2; 95% CI, -0.4, 0.8). **Conclusions:** Thus, this study suggests that treatment with glucosamine sulfate significantly reduces relapse rate in patients with MS. No significant differences were seen between glucosamine sulfate and placebo on the mean (SD) EDSS.

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High-dose immunosuppressive therapy with autologous hematopoietic stem cell transplantation for multiple sclerosis treatment: results of prospective phase II multi-center studyYury L. Shevchenko¹, Andrei A. Novik¹, Alexey N. Kuznetsov¹, Boris V. Afanasyev², Igor A. Lisukov³, Oleg A. Rykavichin⁴, Alexandr A. Myasnikov⁵, Tatiana I. Ionova⁶, Vladimir Y. Melnichenko¹, Denis A. Fedorenko¹, Artur E. Zdorov⁵, Anton V. Kishitovich⁶, Ruslana V. Kruglina¹, Andrei V. Kartashov¹, Roman A. Ivanov¹, Gary J. Gorodokin⁷¹Pirogov National Medical Surgical Center, Department of Hematology and Cellular Therapy, Moscow, Russian Federation; ²Pavlov State Medical University, Department of Bone Marrow Transplantation, St. Petersburg, Russian Federation; ³Institute of Clinical Immunology, Siberian Branch of Russian Academy of Science, Department of Hematology, Novosibirsk, Russian Federation; ⁴Burdenko Central Military Hospital, Department of Hematology, Moscow, Russian Federation; ⁵Republic Hospital, Department of Hematology, Petrozavodsk, Russian Federation; ⁶Multinational Center of Quality of Life Research, St. Petersburg, Russian Federation; ⁷New Jersey Center for Quality of Life and Health Outcome Research, New Jersey, New Jersey, USA

Background: During the last decade High-dose immunosuppressive therapy (HDIT)+ autologous hematopoietic stem cell transplantation (AH SCT) has been used with increasing frequency as a therapeutic option for multiple sclerosis (MS) patients. Comprehensive analysis of long-term outcomes of HDIT+AH SCT is worthwhile. **Objective:** The goal of our research was to study long-term treatment outcomes in MS patients after HDIT+AH SCT. **Methods:** Fifty-eight patients with MS (secondary progressive - 28 patients, primary progressive - 10, progressive-relapsing - 1, and relapsing-remitting - 19) from 6 medical centers were included in this study (mean age - 32.0, range: 17-51; male/female - 23/35). Median Expanded Disability Status Scale score at base-line was 5.0 (range 1.5 - 8.0). The mean follow-up duration was 22 months (range 9 - 90 months). Neurological evaluation was performed at baseline, at discharge, at 3, 6, 9, 12 months, and every 6 months thereafter following HDIT+AH SCT; magnetic resonance imaging (MRI) examinations - at baseline, at 6, 12 months, and at the end of follow-up. **Results:** Notably, no transplant-related deaths or unpredictable severe adverse events were observed. All of the 50 patients included in the efficacy analysis experienced improvement ($n=31$) or clinical stabilization ($n=19$). Two patients deteriorated to a worse score after 18 months of stabilization; 2 others progressed after 12 and 30 months of improvement, respectively. Results of MRI scans were available in 42 patients. Seventeen patients (40.5%) had active lesions at baseline and all turned to inactive status except two cases. Of the 25 patients without active lesions pre-transplant 24 remained inactive; one patient showed disease activity after transplantation. No active, new or enlarging lesions were registered in patients without disease progression. All the patients without disease progression were off therapy throughout the post-transplant period. **Conclusions:** In conclusion, the results demonstrate high efficacy of HDIT+AH SCT in MS patients: clinical improvement or stabilization was achieved in all MS patients included in the analysis. Further studies should be done to establish the best timing for transplantation and to validate treatment regimens.

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Discontinuation of interferon beta therapy in multiple sclerosis leads to prompt return to previous disease activity

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Background: Recommendation for duration of interferon beta (IFN β) treatment in multiple sclerosis (MS) patients with good response to this therapy is not defined. **Objective:** The goal of this study was to assess neurological disability and magnetic resonance imaging (MRI) results in MS patients who discontinued IFN β treatment after about 2 years of treatment. **Methods:** We included in the study 43 MS patients with the average time of IFN β treatment 25.4 months. The follow up was 34.1 months. Neurological progression was assessed by Expanded

Disability Status Scale (EDSS) score at the time point of IFN β discontinuation and at the time entry to the study. Ten patients were subjected to MRI examination during baseline, shortly after treatment termination and at 1-year post IFN β discontinuation. MRI data analysis included assessment of total lesion volume on T2- and T1-weighted images **Results:** In the follow-up period 30 patients (69.8%) experienced relapse, given an annual relapse rate (ARR) 0.64 per patient. There was no strong rebound syndrome after IFN β discontinuation. However, first relapse was more frequently within first 6 months post cessation of IFN β (n=16) than within subsequent 28 months (n=12). During the follow-up EDSS progressed on average by 1.36 point per patient. Analysis of MRI results showed that T2-lesion volume was significantly higher in the follow-up period compared with T2-lesion volume progression during treatment (p=0.001). Similarly, T1-lesion volume significantly increased during follow-up compared with treatment period (p=0.03) **Conclusions:** Our analysis of clinical and MRI activity in patients after discontinuation of treatment with IFN β showed that IFN β did not induce an extended disease remission and after 25.4 months disease activity returned to its pre-treatment rate. These results confirmed that MS patients with good response to IFN β therapy should remain on treatment for an extended time.

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REFORMS: the Rebif new formulation versus Betaseron tolerability study

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Background: A new formulation of interferon beta (IFN β)-1a subcutaneous (SC) has been developed with an improved local tolerability profile compared with the previous formulation of IFN β -1a SC. **Objective:** To compare the tolerability of the new formulation of IFN β -1a SC with that of IFN β -1b SC. **Methods:** REFORMS was a 12-week, open-label, multi-center, randomized study of IFN β -naive patients aged 18–60 years with relapsing-remitting multiple sclerosis. Patients were randomized to receive either the new formulation of IFN β -1a 44 μ g SC 3 times weekly or IFN β -1b 250 μ g SC every other day for 12 weeks. The primary endpoint was the mean change in patient-reported pain score on visual analog scale (VAS) from pre-injection to 30 minutes post-injection over the first 21 full injections for IFN β -1a SC (weeks 5–11) and IFN β -1b (weeks 7–12). Mean change in patient-reported pain score on VAS from pre-injection to immediately and 10 minutes post-injection, and the proportion of pain-free patients immediately and 10 and 30 minutes post-injection, were assessed. **Results:** 129 patients were evaluated (new formulation IFN β -1a SC, n=65; IFN β -1b, n=64). There were no statistically significant differences in patient baseline characteristics between treatment groups. The mean \pm standard deviation (SD) age was 40.3 \pm 9.8 years for the new formulation IFN β -1a SC and 40.8 \pm 9.6 years for IFN β -1b. Women represented the majority (~70%) of the study population. The mean (SD) time since diagnosis was 1.01 \pm 2.35 and 1.93 \pm 4.02 years in patients receiving the new formulation IFN β -1a SC and IFN β -1b, respectively. Findings at 12 weeks will be presented. **Conclusions:** Findings from this 12-week study will provide important comparative information for the new formulation of IFN β -1a SC and IFN β -1b on measures of tolerability and treatment satisfaction.

Supported by: EMD Serono, Inc., and Pfizer Inc.

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The efficacy of cladribine in patients with relapsing-remitting multiple sclerosis but without the need for ambulatory assistance: magnetic resonance imaging data from a phase II trial

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Background: Cladribine is a purine nucleoside analog with preferential lymphocyte-depleting properties. The Scripps-C study previously evaluated parenteral cladribine in relapsing-remitting multiple sclerosis (RRMS) also including some patients who required ambulatory assistance at baseline (Expanded Disability Status Scale (EDSS) score \geq 6.0). **Objective:** To reassess the efficacy of cladribine on magnetic resonance imaging (MRI) outcome measures in a population of patients with RRMS and ambulatory without assistance, similar to that enrolled in an ongoing RRMS study of an oral tablet formulation of cladribine. **Methods:** In this double-blind study, 52 patients were randomized to receive either 5-day courses of subcutaneous cladribine at 0.07mg/kg/day or saline placebo, monthly for 6 months (cumulative dose: 2.1mg/kg), and were followed for an additional 12 months. Brain MRIs were completed at: baseline; monthly intervals (first year); and months 15 and 18. Final evaluation (FE) was the last assessment available up to month 18. Efficacy was assessed statistically in those patients (cladribine n=19; placebo n=18) with baseline EDSS $<$ 6.0. **Results:** At baseline, the proportion of patients with T1 gadolinium-enhanced (Gd+) lesions was 47% and 44% in the cladribine and placebo groups; at FE, 11% and 50% of patients had T1 Gd+ lesions (p=0.0128). The mean (SD) number of T1 Gd+ lesions in the cladribine and placebo groups was 1.6 (2.3) and 0.9 (2.1), respectively, at baseline, and 0.1 (0.3) and 1.4 (2.6) at FE (p=0.0050, Wilcoxon Rank Sum Test). Mean (SD) T1 Gd+ volume decreased by 278.3 (706.2)mm³ in the cladribine group and increased by 88.6 (350.8)mm³ in the placebo group (p=0.0305). Mean (SD) T2 lesion volume decreased by 0.7 (1.8)cm³ in the cladribine group, and increased by 1.9 (2.9)cm³ in the placebo group from baseline (p=0.0043). **Conclusions:** Parenteral cladribine significantly reduced MRI disease activity in patients with RRMS who did not require ambulatory assistance. The current Phase III RRMS study is designed to attempt to confirm these data using oral cladribine at various doses.

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Oral fingolimod (FTY720) pharmacokinetics and pharmacodynamics are similar between Caucasian and Asian subjects

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Background: Oral fingolimod (FTY720) is a sphingosine-1-phosphate receptor modulator currently in phase III clinical trials for the treatment of multiple sclerosis (MS). While concerns of ethnic differences in its pharmacokinetics (PK) and pharmacodynamics (PD) are not borne out by studies comparing Caucasians and African-Americans, little is known about its PK and PD in Asians. **Objective:** To compare the pharmacological features of oral fingolimod in healthy Caucasian and Asian (Japanese) subjects for potential ethnic differences. **Methods:** Subjects demographically matched for sex, age, and weight were given a single dose of oral fingolimod 1.25 mg (6 pairs), 2.5 mg (7 pairs), or 5 mg (6 pairs), or multiple doses of fingolimod 5 mg/day for 7 consecutive days (6 pairs). The PK of fingolimod and its main metabolite fingolimod-phosphate, and its effect on lymphocyte count and heart rate (HR) were characterized over 1 month after single-dose administration and 2 months after multiple-dose administration. **Results:** After single-dose administration, C_{max}, AUC, and elimination t_{1/2} were similar between Asian and Caucasian subjects. After multiple-dose administration there were no clinically relevant differences in fingolimod accumulation ratio (7.0 \pm 0.7 for Asians, 6.6 \pm 0.4 for Caucasians), AUC (382 \pm 106 versus 390 \pm 73 ng.h/mL), or elimination t_{1/2} (7.9 \pm 2.0 versus 7.4 \pm 0.8 days). Fingolimod-phosphate PK was

similar between the two ethnic groups after single and multiple administrations. Acute decreases in lymphocyte counts from pre-dose were similar in both ethnic groups (75% after a 5 mg single dose versus 85% after 5 mg/day multiple dose). The lymphocyte recovery rate to baseline after single- and multiple-doses of 5 mg was reduced by 36% and 15% in Asian subjects compared with Caucasian subjects. The transient, acute decrease in HR after the first dose of fingolimod and subsequent return to baseline were similar in both ethnic groups. **Conclusions:** No marked differences were observed between healthy Caucasian and Asian subjects in fingolimod pharmacokinetics or pharmacodynamics, supporting use of a standard dose of oral fingolimod in Asian as well as Caucasian subjects.

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Natalizumab utilization and safety in the TYGRIS program in the European Union

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Background: The major identified risks of treatment with natalizumab during multiple sclerosis (MS) clinical trials were hypersensitivity reactions, antibody formation, and a rare risk of progressive multifocal leukoencephalopathy (PML). TYGRIS is a global observational program planned in 5000 patients (2500 in the European Union [EU]). It is designed to assess the long-term safety profile of natalizumab in clinical practice. **Objective:** The primary objective of TYGRIS is to determine the incidence and pattern of serious infections, malignancies, and other serious adverse events (SAEs) in patients with MS treated with natalizumab. **Methods:** The EU TYGRIS population includes patients who are naïve to natalizumab treatment. Demographic information, MS history, and treatment history are collected at baseline. Patients will be followed for 5 years regardless of whether they continue natalizumab, with periodic collection of any SAEs. **Results:** As of 10 April 2008, 2034 patients have been enrolled in TYGRIS in the EU and 47 (2%) have permanently discontinued therapy. The data analyzed for 1400 patients indicate a median age of 39 (range, 18–71) years. The majority of patients (70%) were women and 93% had received prior immunomodulatory or immunosuppressant treatment. Median duration of MS symptoms was 8 (range, 8–47) years. Median duration of exposure to natalizumab was 24 (range, 1–84) weeks. Cumulatively, there were reports of at least 1 SAE in 24 patients; these were considered related to natalizumab in 13 patients. No drug-related fatalities, confirmed cases of PML, or unexpected SAEs have been reported. **Conclusions:** To date, natalizumab has been generally well tolerated and has shown a safety profile in clinical practice that is consistent with the clinical trial data, with no unexpected events. A full analysis of all enrolled patients will be presented.

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P517

Intracerebral hemorrhage during treatment with natalizumab in a patient with multiple sclerosis and tumefactive demyelinating lesions

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Background: Natalizumab is a humanized monoclonal antibody against the alpha4beta1-integrin and has been proven highly effective and safe in monotherapy of multiple sclerosis (MS). **Objective:** To report co-occurrence of treatment with natalizumab and intracerebral haemorrhage in a patient with MS. **Methods:** Case report **Results:** A 41-year-old woman presented with acute right-sided hemiparesis due to an atypical intracerebral haemorrhage located in a previously ring-enhancing demyelinating lesion. Diagnosis of MS associated with tumefactive cerebral demyelination in magnetic resonance imaging

was established 21 months before. Demyelination was confirmed at that time by biopsy without evidence for neoplasm or vasculitis. Spontaneous intracerebral hemorrhage occurred 22 days after the third infusion of natalizumab. Further work-up including cerebral digital subtraction angiography revealed no signs of vasculitis, intracerebral aneurysms, vascular malformations, coagulopathy or arterial hypertension. JC virus RNA in cerebrospinal fluid and HIV serology were negative. **Conclusions:** Here we report the first case of an intracerebral hemorrhage in MS coinciding with natalizumab treatment. The mechanisms of leukocyte recruitment to the sites of inflammation via interaction of leukocyte alpha4beta1-integrin and endothelial vascular cell adhesion molecule-1 (VCAM-1) are well known. However, alpha4beta1-integrins are also expressed on endothelial cells and CD34+ bone marrow-derived progenitor cells, controlling several key pathways in angiogenesis. Association of natalizumab and hemorrhage could be by chance, although occurrence of spontaneous intracerebral haemorrhage in MS is most unusual. Whether angiogenesis in MS is beneficial or involved in the pathogenesis, remains unclear. However, neovascularisation may contribute to tissue repair, particularly in large inflammatory cerebral lesions with increased vascular fragility. Conceivable interaction of natalizumab with angiogenesis during tissue repair necessitates further studies in animal models to explore the risk of hemorrhage, particularly in extensive cerebral lesions with increased vascular fragility.

P518

Induction of IL-27 in dendritic cells treated with IFN β

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Background: Multiple sclerosis (MS) patients treated with IFN β have been reported to have reduced levels of pro-inflammatory cytokines such as IFN β and increased levels of anti-inflammatory cytokines such as IL-10. However, the precise mechanism of action of IFN β is unclear. Antigen presenting cells, particularly dendritic cells (DC), play a crucial role in directing T-cell responses via the production of regulatory cytokines including the IL-12 family members and IL-10. IL-12 promotes the induction of IFN β -secreting T helper (Th) 1 cells, whereas IL-10 promotes the development of IL-10-secreting regulatory T cells. Recently, the IL-12 family member IL-23, together with IL-6 and IL-1, has been shown to promote the development of Th17 cells, which have been demonstrated to play a pathogenic role in autoimmune disorders. A protective role for IL-27, another IL-12 family member, has been demonstrated in the experimental autoimmune encephalitis (EAE) model, where it antagonises the induction of Th17 cells by up-regulating IL-10. **Objective:** In this study we investigated the effect of IFN β treatment on production of IL-27 by human DC. **Methods:** DC were stimulated with recombinant IFN β . After 24 hours, cytokine concentrations were assayed by enzyme-linked immunosorbent assay. Cells were stained for expression of ILT3 and analysed by flow cytometry. Alternatively, DC were cultured in the presence of IFN β , activated with TLR ligands for 24 hours, and used to stimulate allogeneic CD4+ T cells. After 5 days, supernatants were assayed by ELISA for cytokines. **Results:** We demonstrated that IFN β enhanced production of IL-27 and IL-10 by DC. Furthermore, stimulation of allogeneic CD4+ T cells with DC grown in the presence of IFN β increased T-cell IL-10 and decreased IL-17 production. Treatment of DC with IFN β also up-regulated the expression of ILT3, a cell surface inhibitory molecule and marker of tolerogenic DC. **Conclusions:** These data suggest that IFN β may exert some of its protective effects by modulating the cytokine profile of both DC and T cells and by expanding or activating tolerogenic DC.

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P519

Interferon β 1b treatment in neuromyelitis opticaMasami Tanaka¹, Mika Komori¹, Keiko Tanaka²¹Neurology, Utano National Hospital, Kyoto, Japan; ²Brain Inst, Niigata Univ, Niigata, Japan

Background: The effects of interferon β 1b (IFN β 1b) treatment in multiple sclerosis (MS) patients has been confirmed; however, those in neuromyelitis optica (NMO) patients have not been shown. **Objective:** To evaluate the effects of IFN β 1b treatment on disease exacerbation and disability progression in MS or NMO patients. **Methods:** We reviewed of 104 consecutive patients with relapsing-remitting MS (RRMS) (69) or NMO (35) treated with IFN β 1b in an MS clinical center of a national hospital in Japan. **Results:** The decrease of annualized relapse rates in each RRMS patient after treatment (0–4) was significant ($p < 0.01$), but that in each NMO patient (0–6) was not ($p > 0.05$). In the patients with NMO, the decrease in exacerbation number after treatment with IFN β 1b was not significantly different between the anti-AQP-4-ab positive group and the anti-AQP-4-ab negative group. The Expanded Disability Status Scale (EDSS) scores of the patients with RRMS and NMO before treatment with IFN β 1b were 3.85 ± 2.47 and 5.02 ± 2.27 , and those after the treatment were 4.10 ± 2.63 and 5.61 ± 2.76 , respectively. Both patient groups showed increased EDSS scores after the treatment; however, NMO patients showed more pronounced worsening ($p < 0.0001$) than the RRMS patients ($p < 0.0008$). In the patients with NMO, the change in EDSS score was not significantly different between the anti-AQP-4-ab-positive group and the anti-AQP-4-ab-negative group. **Conclusions:** IFN β 1b treatment of NMO patients did not suppress disease exacerbation and disability progression.

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P520

Three-year favorable outcome of relapsing Devic disease after 6-monthly infusions of mitoxantroneRadu Tanasescu¹, Marina Ticmeanu¹, Inimioara M. Cojocaru¹, Dimela Luca¹, Adriana Nicolau³, Cristian Baicus², Marc Debouverie⁴¹Neurology, Colentina Hospital, Bucharest, Romania; ²University of Medicine & Pharmacy Carol Davila, Epidemiology Unit, Bucharest, Romania; ³Colentina Hospital, Department of Internal Medicine, Bucharest, Romania; ⁴Central Hospital, Nancy, France

Background: There are few studies addressing neuromyelitis optica (NMO) treatment and long-term evolution after therapy. Since NMO is considered to have a B-cell-induced pathogenesis, and mitoxantrone (MITO), a synthetic anthracenedione approved for worsening multiple sclerosis (MS), has been shown to primarily suppress the humoral response, it seem possible that MITO could be an efficient therapy in NMO. **Objective:** We previously reported a 9-month follow-up of four cases of relapsing-type NMO after MITO therapy. Here we report the three-year outcome of these patients. **Methods:** All four patients fulfilled the Wingerchuk revised diagnostic criteria. They were treated, at baseline, with MITO, 12mg/m², monthly, 6 months (maximum cumulative dose 120mg). Two patients had non-specific minor brain lesions, not specific for MS. No NMO-IgG was performed. All four had previous immunomodulatory therapy. Two patients received previous monthly intravenous methylprednisolone and three patients plasma exchange. The patients were evaluated clinically every 3 months and with magnetic resonance imaging (MRI) every 6 months after baseline. Treatment was well tolerated, with hematological and cardiac monitoring before each infusion and every 6 months during the follow-up. **Results:** Three of four patients were relapse-free during the three years of follow-up, the fourth had two relapses during the first 16 months from baseline and he remained stable during the remaining follow-up period under azathioprine. Patients without relapses experienced a degree of recovery of neurological function at 9 months that remained stable at the three-year interval. The pretreatment median Expanded Disability Status Scale score was 7 (all patients included), at 9 months was 5.5 and after 3 years was 6. The MRI activity of the spinal cord lesions correlated with clinical outcome. **Conclusions:** The observed median outcome compares favorably with the known natural history of NMO. MITO appears to be an efficient therapy in patients with

relapsing NMO, although further studies are needed, including randomized controlled trials of immune-suppression therapy.

P521

Exposure-response relationship of daclizumab added to interferon-beta for treatment of multiple sclerosis during a phase 2 study

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Background: The Phase 2 CHOICE study evaluated daclizumab, a humanized monoclonal antibody against the alpha subunit of the IL-2 receptor (CD25), added to ongoing IFN-beta therapy. This placebo-controlled study met the primary efficacy endpoint of significantly reduced total new or enlarged gadolinium contrast-enhancing lesions (Gd-CELS) between Weeks 8 and 24 in subjects receiving subcutaneous daclizumab 2 mg/kg every 2-weeks, but not 1 mg/kg every 4-weeks. **Objective:** To explore the relationship of daclizumab exposure to clinical efficacy and changes in exploratory safety biomarkers in the CHOICE study. **Methods:** Individual daclizumab exposure characteristics from a subset of subjects, including steady state trough (C_{ss}, min), peak (C_{ss}, max) and AUC_{ss}, were used, separately, as predictors to model total new or enlarged Gd-CELS between Weeks 8 and 24 using negative binomial regression. Daclizumab effects on absolute B, T, and NK cell counts and cell-mediated immunity (Cylex® ImmuKnow™ assay) were also evaluated. **Results:** A statistically significant inverse relationship was observed between daclizumab exposure and total Gd-CELS between Weeks 8–24. The total Gd-CELS between Week 8 and Week 44 was the lowest in subjects with C_{ss}, max >40 mcg/mL, or C_{ss}, min > 33 mcg/mL. Expansion of absolute NK cell counts (50–100% above baseline) was observed in both daclizumab dosing groups. There was no apparent reduction in measured cell-mediated immunity (ATP units) of stimulated CD4⁺ T cells in either daclizumab dosing groups when compared with the placebo group. **Conclusions:** These results suggest that daclizumab elicits exposure-dependent reductions in total or new Gd-CELS through Week 24 without any major impact on exploratory safety biomarkers. A Phase 2 study evaluating daclizumab as monotherapy in multiple sclerosis is underway to confirm these findings.

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Immunoablation with high-dose cyclophosphamide for refractory multiple sclerosis

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Background: High-dose cyclophosphamide (HD-CTX) has demonstrated efficacy in arresting multiple sclerosis (MS) and other autoimmune diseases. The aldehyde dehydrogenase mediated resistance of CD34+ cells to CTX allows for immune reconstitution without hematopoietic stem cell rescue. **Objective:** To assess safety and efficacy of HD-CTX for patients with refractory MS, defined as having failed standard therapies. **Methods:** We evaluated patients with recurring relapses, worsening disabilities, and increasing magnetic resonance imaging (MRI) lesions over 12 months for immunoablation with HD-CTX (200 mg/kg over 4 days), followed by filgrastim for reconstitution. Clinical, laboratory and MRI monitoring was scheduled at 3–6 month intervals over 24 months. **Results:** Six patients were treated since 2005 without lasting complications and monitored over 24 to 36 months. All adverse events resolved using standard therapies within 2 weeks of HD-CTX, and included neutropenic fevers (5 patients), diarrhea (3), hematuria (2), acute dystonia from prochlorperazine (1), urinary tract infection (1), fungal rash (1), and anemia and thrombocytopenia (4). Full hematologic recoveries occurred within 4 weeks of HD-CTX. All 6 patients demonstrated clinical and MRI evidence of improvement with effects ranging from stabilization to dramatic attenuations of disabilities, up to 4.5 Expanded Disability Status Scale points in 5 patients, and 1 to 14 SDs on Multiple Sclerosis Functional Composite in 3 patients. After

20 months of stability 2 patients had a milder than pre-HD-CTX relapse; one by MRI only, and the other clinically and by MRI. The latter patient received intravenous corticosteroids. **Conclusions:** Immunoablation with HD-CTX in our patients with intractable MS had realistic safety and tolerability, and resulted in significant improvements ranging from amelioration to stabilization of the disease course, and reduction of disabilities.

P523

A multivariate analysis of factors that influence adherence with disease-modifying therapy in multiple sclerosis

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Background: Long-term adherence to disease-modifying therapy (DMT) for multiple sclerosis (MS) is affected by many factors. Knowledge of the factors associated with decreased adherence may allow health care providers to intervene with patients at the greatest risk of poor adherence and encourage them to remain on therapy long term. **Objective:** The goal of this study was to identify factors associated with adherence to DMTs in patients with MS. **Methods:** A multivariate analysis of data from this multi-center, retrospective, observational (three-wave) study using internet-delivered patient surveys was conducted in 798 patients. Adherence was defined as not missing a DMT injection in the 4 weeks prior to survey completion. Univariate analyses of all questionnaire responses were conducted and then questions univariately related to adherence ($p \leq 0.10$) were included in the multivariate analysis. Two complementary analyses, stepwise regression (SWR) and all possible regression (APR), were performed and the results of both analyses were combined (CAS [combining results of APR and SWR]) to select variables associated with adherence. **Results:** An older age at diagnosis (Odds ratio [OR] = 1.032, $p = 0.0004$), satisfaction with treatment (OR = 2.085, $p = 0.0003$), and self-reported excellent or very good general health (OR = 1.425, $p = 0.0284$) were associated with better adherence. Patients taking prescription medicine for fatigue were less likely to adhere (OR = 0.642, $p = 0.0067$). After adjusting for potential confounders, significant differences in adherence among the DMTs were observed. Patients on interferon beta (IFN β)-1b (OR = 0.362, $p < 0.0001$) or glatiramer acetate (OR = 0.322, $p < 0.0001$) had significantly lower odds of adherence than patients on either subcutaneous or intramuscular IFN β -1a. **Conclusions:** Patients who are diagnosed with MS at a younger age, who perceive their health as fair or poor, or who are taking medication for fatigue may need more counseling on the importance of adherence. Focusing on factors associated with decreased adherence may enable health care providers to effect a favorable change in adherence.

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P524

Safety and tolerability of oseltamivir in patients with multiple sclerosis: a pilot study

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Background: Viral infections, especially influenza, are well known to be a trigger of multiple sclerosis (MS) relapses. Oseltamivir is a member of a relatively new class of drugs which inhibit the neuraminidase of these viruses, preventing release of virions by infected cells. The drug both prevents and improves the symptoms of influenza. By suppressing influenza virus infection, the drug may have a particular use in MS patients. **Objective:** The objective of this pilot study is to demonstrate the feasibility of testing safety and tolerability of oseltamivir in MS patients by measuring Expanded Disability Scale (EDSS) score before and after administration of the drug, and comparing incidence of side effects in patients on drug with two weeks off drug. **Methods:** Fifteen relapsing-remitting MS patients were recruited into this open-label study of oseltamivir and fourteen completed it. The EDSS score was ascertained at baseline and at the next regular clinic visit. Patients were contacted by telephone and both MS and non-MS side effects were ascertained on and off drug. **Results:** There was no statistically significant difference in EDSS between baseline and next regular clinic visit. There was no statistically significant difference in the frequency of MS and non-MS symptoms on drug and off drug. **Conclusions:** In this small open-label study, oseltamivir was well tolerated in MS patients with no difference on EDSS and side-effects not different on and off drug. Larger studies will be needed to confirm this and to ascertain whether there are any effects of the drug on disease course.

P525

Marked amelioration of experimental autoimmune encephalomyelitis in mice by a PEGylated murine recombinant interferon- β with improved pharmacokinetic properties

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Background: Recombinant interferon- β (IFN- β) has been the most common therapy for multiple sclerosis (MS). But the current IFN- β products have relatively short half-lives in circulation, which may require frequent administration to achieve significant efficacy. PEGylation of bioactive peptide is considered to improve its pharmacokinetic profile and therapeutic efficacy. We have developed a proprietary technology to introduce 43kDa polyethylene glycol (PEG) into the unique amino acid of human IFN- β without loss of its specific activity. Here we applied this technology to murine IFN- β and produced the PEGylated murine IFN- β (PEG-mIFN- β). **Objective:** We evaluated pharmacokinetic properties of PEG-mIFN- β and its therapeutic efficacies in experimental autoimmune encephalomyelitis (EAE), an animal disease model of MS, in mice. **Methods:** Female SJL or C57BL/6J mice were immunized intradermally with PLP139-151 or MOG35-55, respectively, emulsified in complete Freund's adjuvant. Mice were observed daily for signs of clinical diseases. Either vehicle, mIFN- β (100,000 U) or PEG-mIFN- β (10,000 or 100,000 U) was subcutaneously administered daily to the mice in each group. For pharmacokinetic analyses, blood was collected from the mice administered with mIFN- β or PEG-mIFN- β , and the serum mIFN- β concentration was determined by enzyme-linked immunosorbent assay. **Results:** PEG-mIFN- β showed markedly improved pharmacokinetic parameters compared with unmodified mIFN- β . Daily subcutaneous administration of PEG-mIFN- β significantly suppressed disease progression of both PLP-induced EAE in SJL mice and MOG-induced EAE in C57BL/6J mice. The effect of PEG-mIFN- β at 10,000 U/day was greater

than that of unmodified mIFN- β at 100,000 U/day in MOG-induced EAE. Furthermore, PEG-mIFN- β significantly suppressed inflammation and demyelination in the central nervous system of EAE-induced mice. **Conclusions:** These results suggest that PEGylation of mIFN- β markedly altered its pharmacokinetic properties resulting in significant efficacies in the amelioration of EAE symptoms. Therefore, PEGylation of human IFN- β may provide an efficient therapy for MS with infrequent administration.

P526

Lack of interferon beta bioactivity is associated with the occurrence of relapses in multiple sclerosis

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Background: Poor response of multiple sclerosis (MS) patients to interferon beta (IFN β) treatment can only partly be explained by neutralising antibodies (NAb). However, because NAb disappear in the majority of patients with continuous treatment and no consensus has been established on how to use NAb testing in clinical practice, evaluating IFN β bioactivity is being studied as an alternative for measuring response to IFN β . Myxovirus resistance protein A (MxA) messenger ribonucleotid acid (mRNA) expression reflects absolute IFN β bioactivity and MxA mRNA induction indicates the rise in expression level due to the IFN β administered. Whereas the MxA mRNA response to IFN β injection is clearly associated with NAb status and NAb status to efficacy of the drug, the direct relationship between IFN β bioactivity and clinical disease activity is still largely unknown. **Objective:** To determine if a lack of IFN β bioactivity is associated with a decrease in efficacy of IFN-beta. **Methods:** In this study we evaluated IFN β bioactivity in 126 multiple sclerosis patients treated with IFN β for at least 6 months, by measurement of MxA mRNA expression and MxA mRNA induction, to determine biological non-responders. Relapse rate and corticosteroid use one year before testing were scored retrospectively to reflect clinical disease activity. **Results:** The biological non-responders showed a significantly higher relapse rate compared with adequate or suboptimal biological responders ($p = 0.016$). Furthermore, the percentage of relapse-free patients was significantly lower in biological non-responders ($p = 0.019$). **Conclusions:** In our study a lack of IFN β bioactivity is associated with the occurrence of relapses. Our results should be confirmed in future studies, ideally prospectively designed and with larger patient numbers. These studies could identify which combination of diagnostic tests has most relevance for identifying clinical non-responders to IFN β .

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P527

Neutralising antibodies against IFN β persist long after cessation of therapy

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Background: Chronic use of protein-based therapeutics such as interferon beta (IFN β) in multiple sclerosis (MS) treatment can lead to the generation of neutralising antibodies (NAb). In some patients NAb persist and have been associated with a decrease in treatment efficacy. However, the behavior of NAb after cessation of IFN β therapy and their possible effect on disease course is largely unknown. **Objective:** First, to confirm the long-term persistence of NAb after cessation of therapy and to see if predisposing factors can be identified. Secondly,

to determine if persisting NAb after cessation of therapy are associated with a worse clinical outcome. **Methods:** A total of 71 MS patients treated with IFN β for at least 12 months and who had ceased treatment for at least 3 months were tested for NAb, using a previously described cytopathic effect assay (CPE). Clinical outcome was evaluated at the start of IFN β treatment, at the time of NAb testing and at the most recent visit to the out-patient clinic by using the Kurtzke's Expanded Disability Status Scale (EDSS) and the global Multiple Sclerosis Severity Score (MSSS). Relapse rate and corticosteroid use were assessed one year before NAb measurement to reflect clinical disease activity at the time of testing. **Results:** 54 (76%) patients were NAb negative, 6 (8%) patients tested low-titer NAb-positive and 11 (16%) patients were high-titer NAb-positive. NAb were shown to persist after a median follow-up of 1.4 years and up to 5.1 years (61 months). Of the high-titer NAb-positive patients, 4/11 (36%) had been treated with Betaferon and 7/11 (64%) with Rebif. No effect was seen of persisting NAb on EDSS and MSSS ($p = 0.926$ and $p = 0.589$ respectively). Furthermore, no differences were found with respect to clinical disease activity. **Conclusions:** NAb can persist long after cessation of IFN β therapy and their occurrence differs between IFN β products. In this study, no effect of persisting NAb on clinical disease course was found, although numbers were too small to exclude a meaningful effect.

Supported by: Joep Killestein and Laura F. van der Voort worked with companies that market drugs for MS (Biogen Idec, Serono, Schering, Teva) and with some companies that have development programmes for future drugs in MS. Both authors are partially funded by NABIN-MS, a specific targeted research project on neutralising antibodies to interferon beta in MS, established by the European Commission under its 6th Framework Programme.

P528

Early treatment of multiple sclerosis with alemtuzumab significantly improves patient functioning and self-reported quality of life compared with subcutaneous interferon beta 1a

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Background: CAMMS223, a randomized, open-label, rater-blinded, phase 2 study compared the safety and efficacy of annual alemtuzumab (alem) with 3 times/week subcutaneous (SC) interferon beta-1a (IFN β -1a) in treatment-naïve patients with relapsing-remitting multiple sclerosis (RRMS). **Objective:** A prior 3-year analysis of the study's co-primary endpoints, sustained accumulation of disability and relapse rate, showed alem was significantly more effective than SC IFN β 1a. Analysis of 2 other endpoints, patient functioning, measured by the Multiple Sclerosis Functional Composite (MSFC), and health-related quality of life, measured by the Short Form 36 (SF-36), are presented. **Methods:** 334 patients were randomized 1:1:1 to: 3 times/week SC IFN β -1a 44mcg; 24mg/day alem; or 12mg/day alem. Alem was intravenous, administered once daily for 5 days at Month 0, 3 days at Month 12, and in some patients, 3 days at Month 24. Entry criteria included disease onset within 3 years of randomization, baseline Expanded Disability Status Scale score ≤ 3.0 , ≥ 2 MS episodes within 2 years, and ≥ 1 enhancing lesion on screening magnetic resonance imaging. The SF-36 was self-administered twice a year; the MSFC was administered by treatment-blinded assessors quarterly. Data for the 12 and 24mg alem groups were examined separately and pooled. **Results:** Alem-treated patients generally showed significantly greater improvements from baseline on the SF-36 Mental Component at months 6 through 36, and Physical Component at months 6 through 30 (p -values < 0.05) than patients treated with SC IFN β -1a. Alem-treated patients also showed significantly greater improvements from baseline on the MSFC at months 9 through 36 (p values < 0.05). Notable alem-related adverse events included infusion reactions, immune thrombocytopenic purpura, thyroid dysfunction, and mild to moderate infections. **Conclusions:** Alem was significantly better than SC IFN β -1a at improving self-reported mental and physical well-being, as measured by the SF 36, and overall functioning, as measured by the MSFC, in patients with RRMS. These effects developed during the first year of the study and were generally sustained into the third. **Supported by:** Genzyme Corporation.

P529

TYSEDMUS: National cohort of multiple sclerosis patients treated with natalizumab using the French EDMUS (European Database for Multiple Sclerosis) network

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Background: Natalizumab, a monoclonal antibody, has shown efficacy in reducing the relapse rate and delaying disability progression in multiple sclerosis (MS) patients. However, severe opportunistic infections and infusion reactions have been observed in clinical trials. Furthermore, its high cost requires a rational use. In this context, the benefit/risk ratio (BRR) of natalizumab must be studied in real-life settings. **Objective:** The main objective of the TYSEDMUS study is to characterize the safety profile of natalizumab in real-life settings. Secondary objectives are to describe the clinical evolution of patients, the utilisation patterns of the treatment, and to compare the occurrence of malignancies and serious infections in the natalizumab-exposed population versus a non-exposed one. **Methods:** TYSEDMUS is a multicentric observational cohort study promoted by AFSSAPS (French Medicines Agency) and involving French neurologists prescribing natalizumab. It aims to include all patients exposed at least once to natalizumab. A comparison group of non-exposed patients, never treated with natalizumab, but receiving or not other immunosuppressive therapies, will be extracted from the EDMUS databases. Patients' characteristics under natalizumab (including clinical evolution), utilization patterns of natalizumab and occurrence of adverse events will be described. Incidence rates of adverse events of special interest will be provided with two-sided confidence intervals. Kaplan-Meier analysis will be used to estimate the time interval to the occurrence of adverse events. Comparison of the occurrence of serious infections and malignancies between patients treated or not with natalizumab will be done by Log-rank tests and Cox proportional hazards models/Poisson regression. **Results:** The follow-up duration is planned to be at least five years. Study methodology and descriptive results in the first patients will be presented. On April 1st 2008, more than 1000 patients had starting natalizumab in France. **Conclusions:** The TYSEDMUS study should allow to evaluate the BRR of natalizumab and monitor its use in everyday medical practice.

Supported by: AFSSAPS (French Medicines Agency).

P530

A phase II study of neuroprotective agent riluzole in early relapsing-remitting multiple sclerosis

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Background: Multiple sclerosis (MS) is associated with neurodegenerative processes including excitotoxicity. Inhibiting these processes early in the disease course may alter MS progression. **Objective:** To evaluate safety and preliminary efficacy of riluzole 50 mg twice daily versus placebo in early relapsing-remitting MS (RRMS). **Methods:** This is a 2-year randomized double-masked phase II trial of riluzole versus placebo in 40 patients naïve to disease-modifying therapy who developed their first MS symptoms within the past year. Patients initiate riluzole or placebo 50 mg daily for one month and subsequently increase to 50 mg twice daily for 2 months. At month 3, all patients initiate intramuscular interferon beta-1a weekly in addition to oral drug until month 24. Brain magnetic resonance imaging (MRI) and 1H-MRSI scans, neuropsychological, health-related quality of life (HRQOL) and neuro-ophthalmological measures are collected at months 0, 3, 6, 12 and 24. The primary end point is progression of brain atrophy over 2 years (SIENA). Secondary endpoints include normalized white and grey matter volumes (SIENAX), changes in metabo-

lite concentrations (NAA, glutamate, myo-inositol), optical computed tomography, Expanded Disability Status Scale, Multiple Sclerosis Functional Composite, neuropsychological and HRQOL measures. **Results:** 17 patients have been enrolled within a mean of 6 months after MS onset (mean age 39 years, 69% females). Baseline brain MRIs showed a mean of 16 T2-bright and 1.25 gadolinium-enhancing foci per scan. Ten patients have completed > 3 months on combined therapy (e.g. have been on study drug for > 6 months). All but 1 patient are on 50 mg twice daily of study drug. A few patients have reported transient mild dizziness after taking study medication. No laboratory adverse events have been reported. **Conclusions:** The combination of riluzole and IFNB-1a is well tolerated in this small cohort. This on-going phase II trial of neuroprotection in early MS will provide preliminary data on MS progression using multiple outcome measures that will allow designing future trials of promising neuroprotective agents.

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P531

Natalizumab use in a large multiple sclerosis center: ascertaining the therapeutic benefit and the causes for treatment discontinuation

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Background: Natalizumab, a recombinant, humanized anti- α 4 integrin monoclonal antibody, was recently approved as a new treatment agent for multiple sclerosis (MS), being considered as a second-line therapeutic intervention. **Objective:** To evaluate the characteristics of patients initiated on natalizumab therapy in a large MS center and assess their clinical and magnetic resonance imaging (MRI) outcome following a first year of therapy. **Methods:** This is a retrospective study based on chart review of consecutive MS patients who received or are presently receiving natalizumab therapy within a specialized MS center. Clinical information including previous disease-modifying agents (DMA), clinical status and MRI parameters before and during natalizumab therapy was recorded. Patients previously exposed (received 1-2 infusions) during first natalizumab approval and resumed therapy were defined as the rechallenged group. **Results:** Data on 166 patients (F/M: 132/ 34; age: 43.3 \pm SD 9.4 years; disease duration: 12.2 \pm SD 7.6 years) were available for analysis; 128 continued the therapy and 38 discontinued. Initiation of natalizumab and discontinuation of previous DMA was related to: limited efficacy (70%), chronic side effects (22%) and/or noncompliance (8%). The percentage of patients free of relapses increased from 38.5% before natalizumab to 83.3% after natalizumab and MRI activity also decreased: the percentage of patients with new T2 or active Gad-lesions decreased from 26.5% to 7.2% after natalizumab treatment. The rechallenged group consisted of 79 patients (47.6%); 22/79 (28%) discontinued the drug at the time of the second infusion because hypersensitivity/allergic reactions; all but one had confirmed anti-natalizumab antibodies (NatAb). Four patients discontinued because active disease while on natalizumab (3 had NatAb). Out of the previous natalizumab-naïve patients, 6/85 (7%) developed NatAb, and 5/6 had hypersensitivity reactions. **Conclusions:** Our data support the beneficial effects of natalizumab seen in the pivotal studies, but raises an increased awareness on the high risk of NatAb development and hypersensitivity/allergic reactions following isolated 1-2 natalizumab infusions.

P532**Volumetric analysis of change in FLAIR burden of disease over 2 years in patients with early forms of multiple sclerosis randomized to interferon β -1b or glatiramer acetate in the BECOME study**

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Background: BECOME (Betaseron® vs. Copaxone® in multiple sclerosis (MS) using 3-dose Gadolinium and 3-Tesla magnetic resonance imaging (MRI) Endpoints) is the first head-to-head comparison of IFN β -1b (IFN) and glatiramer acetate (GA) based primarily on MRI. The primary outcome, based on blinded reading of the number of enhancing lesions + the number of new non-enhancing FLAIR/T2 lesions (monthly scans up to 24 months) has been reported as demonstrating no significant difference between treatment groups (L Wolansky et al, ECTRIMS 2007). **Objective:** The purpose of this communication is to report on quantitative analysis of the FLAIR burden of disease between the treatment arms. **Methods:** Subjects with relapsing-remitting MS (n=61) and clinically isolated syndromes (n=14) were randomized to receive 250 μ g IFN every other day vs. 20 mg GA daily. Randomization was stratified based on presence of gadolinium enhancement at screening. Up to 24 on-treatment monthly MRIs were carried out. FLAIR hyperintense burden of disease on baseline vs. final scans (median 24 months) was determined utilizing ANALYZE software (Mayo Clinic) by a single examiner blinded to treatment group. T2-weighted images were used for reference. **Results:** We observed a per subject median reduction in the fluid-attenuated inversion-recovery (FLAIR) burden of disease of 206 mm cubed from baseline to the final scan. This change was statistically significant (Wilcoxon signed rank two-sided p<0.001). In the GA group, the median decrease in FLAIR burden of disease was 216.2 mm cubed (significant at p<0.01). The mean decrease was 365.6. In the IFN group, the median decrease in FLAIR burden of disease was 181.9 mm cubed (significant at p<0.01). The mean decrease was 580.0. The differences between the groups were not statistically significant (Wilcoxon Rank Sum p-value=0.77). **Conclusions:** Both IFN and GA demonstrated a significant improvement in FLAIR burden of disease from baseline to the final scan. There were no significant differences between the two treatment groups.

Supported by: Bayer Healthcare Pharmaceuticals. The intellectual property is that of UMDNJ.

P533**ATX-MS1467, a therapeutic peptide vaccine for treatment of multiple sclerosis**

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Background: Our laboratory has shown that soluble myelin peptides can prevent and treat autoimmune encephalomyelitis. **Objective:** To test a therapeutic peptide vaccine for safety and tolerability in patients with secondary progressive multiple sclerosis (MS). **Methods:** MS patients were screened for T-cell recognition of myelin basic protein (MBP). Peptide epitopes were identified and 4 of these were combined in a cocktail (ATX-MS-1467). Six secondary progressive (SPMS) patients received escalating doses of ATX-MS-1467 from 25 to 800 micrograms and were assessed clinically for safety and tolerability. Peripheral blood mononuclear cells (PBMC) were collected from each patient prior to, during and at one and three month time points after the final dose. PBMC were stimulated *in vitro* to assess the immune response to MBP and PPD, a component of the BCG vaccine. **Results:** There were no treatment-related adverse events or serious adverse events reported following administration of ATX-MS1467. There were no changes in clinical score among the patients and magnetic resonance imaging activity remained stable in all patients. One patient who had suffered from visual impairment for over 2 years prior to the trial demonstrated a significant improvement in visual acuity (6/24 right and 6/9 left eye prior to; 6/9 right and 6/6 left eye following the trial). Four of six patients displayed a significant T cell response to MBP prior to the trial and this response was suppressed at

the one month follow-up (P=0.0313). Suppression of response to MBP was antigen-specific since there was no significant change in the response to PPD (P=0.3438). **Conclusions:** Treatment of SPMS patients with ATX-MS1467 was safe and well tolerated. Preliminary evidence from this safety trial indicates that ATX-MS-1467 can suppress the immune response to myelin antigen and may therefore find utility in treatment of MS.

Supported by: Apitepe Ltd.

P534**Varicella zoster meningitis in a multiple sclerosis patient after twenty doses of natalizumab**

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Background: Natalizumab is a monoclonal antibody, which effectively treats multiple sclerosis (MS) with a reduction of relapse rate of 67%, progression of disability, 42%. Treatment has rarely been associated with opportunistic infection. Progressive multifocal leukoencephalopathy (PML) was reported in two patients receiving combination therapy with natalizumab and interferon beta 1a once weekly in the SENTINEL trial, but with 24,800 patients treated worldwide as of 3/08, 3,600 for 18 months or more, no recurrence of PML has occurred. Since resumption of therapy, herpes meningitis and encephalitis have been reported. **Objective:** Watchful management of patients receiving prolonged therapy with natalizumab every 4 weeks is emphasized. **Methods:** A 51-year-old man with relapsing-remitting MS received his 20th treatment of natalizumab on 4/3/08. Two days after treatment, he developed back pain, which he attributed to exertion; the next day, headache, progressive in severity and associated with myalgia and chills. He had a small erythematous area over his right biceps, not vesicular. His headache became severe. A lumbar puncture performed 2 days after headache onset showed white blood cells, 63: 70% monos, 30% polys, glucose, 39, protein >150. Bacterial and fungal cultures, herpes, varicella PCRs, JC virus titers and cryptococcal antigen were sent. Brain magnetic resonance imaging did not show meningeal enhancement or additional lesions. Cognition was minimally affected. **Results:** He was admitted and started on broad spectrum antibiotics, including IV acyclovir, until further results from LP. He received 3 days of plasmapheresis. ID was consulted. Varicella zoster PCRs in cerebrospinal fluid were positive. He received a total of 14 days of IV acyclovir, 12 mg/kg q8. His headache improved on the third day of plasmapheresis. He continues to recuperate at home. **Conclusions:** Although rarely reported, prolonged treatment with natalizumab can be associated with opportunistic and recurrent chronic infection. Vigilance in monitoring these patients is prudent.

P535**Osteonecrosis in multiple sclerosis patients**

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Background: Multiple sclerosis (MS) patients are frequently exposed to pulse corticosteroid therapy (PCT) during the course of disease. Osteonecrosis is a complication observed in steroid treatment, especially in long-term oral steroid medication, but the role of PCT is not clear. **Objective:** To study the frequency of osteonecrosis and its causes in MS patients. **Methods:** The treatment group consisted of 60 MS patients who received PCT at least 3g in the last five years and 22 MS patients who were not given any corticosteroid were the first control group. 25 healthy people were taken as a second control group. Treatment group was divided into three subgroups. 20 of them received both interferon and PCT, 20 received both glatiramer acetate and PCT, while the rest had only PCT. There was no statistical difference between groups for age, sex and disease duration. Patients with hemoglobinopathy, alcoholism, trauma, cytotoxic drug usage, caisson disease were not participated. All groups were evaluated by bilateral femoral magnetic resonance imaging. **Results:** No femoral head osteonecrosis (FHO) was detected in either control group. There were

4 FHO cases (6.7%) in the treatment group. 2 FHO cases in glatiramer acetate-PCT group, 2 FHO cases in PCT only group and no FHO case was detected in interferon-PCT group. **Conclusions:** According to our study, FHO was detected in 6.7% of MS patients who received PCT, while no FHO was found in control groups. Our results support that the main cause of osteonecrosis formation in MS patients is PCT rather than the pathogenesis of MS itself. We could not find a significant effect of interferon and glatiramer acetate treatment on FHO formation. 6.7% is a remarkable morbidity and neurologists must be careful about this complication, since early diagnosis can prevent bone destruction.

P536

Interferon therapy in Japanese multiple sclerosis and AQP4-antibody-associated neuromyelitis optica syndrome

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Background: It is reported that interferon beta-1b (IFN β) treatment exacerbates neuromyelitis optica (NMO). The precise clinical course profile of certain number of patients with AQP4-antibody-associated NMO syndrome who are treated with IFN β in one hospital is unclear. Early treatment for clinically isolated syndrome (CIS) is approved in the BENEFIT study, but in some cases NMO patients might be misdiagnosed with CIS and be treated with IFN β . **Objective:** In this study we compare the effect of IFN β on both multiple sclerosis (MS) and AQP4-antibody-associated NMO syndrome, retrospectively, and assess the effect of steroid and immunosuppressive therapy in the long run. **Methods:** A total of 38 patients with MS and 13 patients with definite NMO were treated with IFN β . Definite MS and NMO were diagnosed according to McDonald's and Wingerchuk's criteria, respectively. Three patients, who did not fulfill the definite NMO criteria but were diagnosed with AQP4-antibody-associated syndrome introduced IFN β , were included in this study. Age, gender, symptoms, Expanded Disability Status Scale and the number of clinical relapses before and after treatment of IFN β were compared. **Results:** Among 38 MS patients treated with IFN β , 16 patients were compared over a two-year observation period. AQP4-antibody-associated NMO syndrome has been treated with IFN β . We were able to compare only three patients out of 13 definite NMO patients with IFN β treatment in more than a one-year period. All of them halted IFN β therapy due to positive tests for anti-AQP4 antibody (5), worsening (4), no response (1), adverse drug effects (3). In AQP4-antibody-associated NMO syndrome 2 patients experienced a severe attack. Annual attack rates of MS patients decreased from 0.48 to 0.32; however, in definite NMO as well as in AQP4-antibody-associated NMO syndrome, only one out of 13 patients showed a decrease in relapse rate. **Conclusions:** In definite NMO, all patients were already being treated with either steroid or immunosuppressant therapy, resulting from frequent relapse or steroid dependency. Despite the unfavorable effect of IFN β , Azathioprin and/or steroid treatment in AQP4-antibody-associated NMO syndrome is highly effective in the long run. AQP4-antibody-associated NMO syndrome should be ruled out before initiation of IFN β because of the severe side effects during steroid withdrawal.

P537

Glatiramer acetate-specific T lymphocytes regulate oligodendrocyte progenitor cell numbers in vitro

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Background: Glatiramer acetate (GA) is a synthetic copolymer approved for the therapy of relapsing-remitting multiple sclerosis (MS), a demyelinating disease in which remyelination is typically limited. Work on the mechanism of action of GA in MS has focused on its immunomodulatory effects, but recent findings have suggested that GA-specific T lymphocytes may confer neuroprotection in addition to bystander suppression. Human GA-specific T lymphocytes

produce neurotrophic factors including brain-derived neurotrophic factor (BDNF), and GA treatment of experimental autoimmune encephalomyelitis (EAE) is associated with elevated levels of BDNF, NT-3 and NT-4 in the centralnervous system. In addition to their effects on neurons, these factors are important regulators of the survival and differentiation of oligodendrocytes, the main target cell type in early MS. **Objective:** Here, we tested the hypothesis that factors produced by GA-specific T lymphocytes regulate the viability, mitosis and/or maturation of oligodendrocyte progenitor cells (OPCs). **Methods:** We examined the effects of supernatants from Th1- or Th2-polarized human GA-specific lines on rodent immunopanned OPC cultures (A2B5+ Ran2-), and human OPC-enriched spinal cord cultures. Results were defined using confocal imaging for lineage and differentiation markers, and effects were compared with autologous medium control over a 5-day period. **Results:** In rodent OPC cultures, supernatants from GA-specific lines were associated with significant increases in the number of Olig2+ and O4+ oligodendrocytes, and a significant decrease in GFAP+ astrocytes. The effects of Th2-polarized supernatants were stronger than those of Th1-polarized lines. Similar effects were observed in human OPC-enriched cultures: supernatants from Th2 polarized lines were associated with a significant increase in the number of Olig2+ oligodendrocytes compared with control. **Conclusions:** Taken together, these results suggest that GA-specific T lymphocytes produce factors that potentiate OPC survival, mitosis and/or differentiation. We are currently investigating the mechanisms underlying these effects, and their specificity.

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Epidemiology/Genetics – Part 2

P538

The role of aquaporin-4 antibodies in longitudinally extensive transverse myelitis in Brazil

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Background: Longitudinally extensive transverse myelitis (LETM), a spectrum of neuromyelitis optica (NMO), is associated with aquaporin-4 IgG antibodies (NMO-IgG) in more than 50% of cases. However, infectious and post-infectious disorders may display a similar radiological pattern. In South America, transverse myelitis must raise suspicion of schistosomiasis, a parasitic infection caused by *Schistosoma* sp. *Schistosoma mansoni*, endemic in Brazil, is an underdiagnosed cause of ataxia telangiectasia (ATM). **Objective:** To evaluate clinical, radiological and prognostic factors in LETM, according to NMO-IgG status. **Methods:** During 3 years follow-up, 31 ATM patients fulfilled the inclusion criteria: 1) Transverse Myelitis Syndrome 2) LETM at magnetic resonance imaging (MRI), 3) cerebrospinal fluid (CSF) analysis and indirect immunofluorescence (IFI) for *S. mansoni*, 4) serum NMO-IgG by IFI, 5) normal brain MRI. Exclusion Criteria: 1) Previous diagnosis of any disease associated with TM. Both the assessment of spinal fluid and serum samples for IgG status were carried out blinded to the diagnosis. MRI patterns were classified according to topographical distribution. Prognosis was measured by Expanded Disability Status Score (EDSS). **Results:** 31 LETM: 20 female. 26 Afro-Brazilian. Mean age at onset was 35.6 years. NMO IgG status was positive in 11 (35.5%): 8 female, 9 Afro-Brazilian, the median EDSS was 6.5. CSF pleocytosis was present in all. MRI pattern showed cervico-thoracic predominance. 81% (9/11) relapsed: Recurrent LETM (6) and ON (3). No one was IFI-positive to *S. mansoni*. NMO IgG status was negative in 20 patients: 8 female, 9 Afro-Brazilian, the median EDSS 4.0. CSF pleocytosis was present in 17. MRI had no preferential pattern. 10% (2/20) developed recurrent LETM. One of them had a diagnosis of arterial-venous fistula. 5/20 (25%) were IFI-positive to *S. mansoni*. Thoracic and thoraco-lumbar

LETM was associated with *S. mansoni*. **Conclusions:** NMO-IgG showed lower sensitivity (35.5%), but higher specificity (81%) to relapse. However, in our sample all thoracic and thoraco-lumbar LETM was negative for NMO-IgG antibody. Even so, the finding of thoracic or thoraco-lumbar LETM supports the suspicion of schistosomiasis. Epidemiological evidence of exposure to *S. mansoni* provides further evidence.

WITHDRAWN

P540

EDSS Natural History Atlas: multiple sclerosis disability progression in Nova Scotia 1979 - 2004

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Background: The study population includes all Nova Scotians who attended the Dalhousie MS Research Unit (DMSRU) clinic, Halifax, Nova Scotia, Canada, in the period 1979–2004 and who were diagnosed as definite multiple sclerosis (MS) but were never-treated (NT) or not-yet treated (NYT) with disease-modifying drugs (DMDs). The DMSRU provides Nova Scotia's only specialized MS referral service and has delivered Nova Scotia's publicly funded DMD program since 1998. **Objective:** To describe the natural history (NH) of MS disability progression, measured by Extended Disability Status Scale (EDSS), by years since onset (YSO), final MS class and sex. **Methods:** The DMSRU served 2,290 Nova Scotia residents from 1979–2004. Final MS diagnosis and class were reassessed in 2004. 1,751 persons had definite MS. 1732 had data on EDSS, sex, age at onset, and final MS class. Natural history paths were described for persons NT or NYT with DMDs as of a visit date. 1606 patients had 5,244 clinic visits with EDSS scores of 1.0 - 9.5. Time from onset to 18 EDSS endpoints (1.0 to 9.5) was described using Kaplan-Meier life-table survival methods. A fixed effects model estimated mean EDSS progression by YSO. Progression was graphed for each of nine definite MS class-sex groups: ALL (1,606), primary progressive (PPMS, 274), relapsing-remitting (RRMS, 856), RRMS females (RRMSf, 677), RRMS males (RRMSm, 179), secondary progressive (SPMS, 476), SPMS females (SPMSf, 351), SPMS males (SPMSm, 125) and relapsing-onset (R-onset, 1,332). **Results:** Mean EDSS progression by YSO and time from onset to 18 EDSS endpoints (by YSO, class and sex) was described using Nova Scotia population-based data. **Conclusions:** Disability progression speed may vary across MS populations, after standardizing for YSO (or age), class and sex. This confounds the assessment of Phase III DMD efficacy study results and Phase IV effectiveness and cost-effectiveness study results. Reporting YSO to 18 EDSS endpoints facilitates comparisons with natural history studies that report diverse endpoints.

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P541

Towards a web-based analysis tool with access to important observational studies

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Background: Regulatory bodies have identified the urgent need for a 'critical path toolkit' to streamline drug development and the move towards individualised therapies. More objective tools to support deci-

sion making based on advanced mathematical models using comprehensive data collections are needed. **Objective:** To develop a web-based analysis tool, that enables an intuitive comparison of observational studies based on individual patient data. **Methods:** Within the scope of the project 'Evidence-based Decision Support in MS' (EBDiMS), the Sylvia Lawry Centre (SLC) collaborates with investigators of observational studies to compare some major multiple sclerosis (MS) cohorts regarding demographic, clinical and survival outcomes. Currently the investigators of cohorts in London Ontario, British Columbia, EDMUS Rennes, Gothenburg, Dublin, Pittsburgh and Danish MS Registry are providing access to the individual databases. According to the SLC validation policy parts of the data are held back for validation. **Results:** Currently 3076 patients of the three cohorts from London Ontario (LO,1972–1997), British Columbia (BC,1980–1993) and EDMUS Rennes (ER,1962–2003) have been processed and a web-based analysis tool was developed to analyse data in a pooled or comparative format. The female:male ratio in the pooled cohort is 2.2:1 (LO 2.0:1,BC 2.3:1,ER 2.3:1). Mean follow-up times after onset of first MS symptoms are 24.3 (LO), 13.2 (BC), 11.5 (ER) and 16.3 years for the pooled cohort. Overall 77.3% of all patients reached Expanded Disability Status Scale (EDSS) score 3 (LO 85.7%,BC 70.3%,ER 76.6%). Proportions of patients that reached EDSS 6 during follow-up are overall: 61.2%, LO 74.2%, BC 54.2%, ER 55.5%. Disability levels were confirmed during the whole follow-up. **Conclusions:** The developed web-based analysis tool provides an overview of different cohorts and the opportunity to interactively compare those cohorts based on individual data. Subsequent analyses will address subpopulations and heterogeneities in time, geography, ascertainment and treatment. The tool is continuously updated with new variables and high-quality datasets. We invite all institutions, investigators and neurologists to participate in this program by providing data and ideas.

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P542

The clinical features of serum anti-aquaporin 4 antibody-positive patients

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Background: Anti-aquaporin 4 (AQP4) antibody is a specific serum autoantibody detected in neuromyelitis optica (NMO). However, the characteristics of the patients with anti-AQP4 antibody remain to be fully elucidated. It is therefore considered important to further accumulate such cases and review the clinical features of these patients. **Objective:** To clarify the clinical features of anti-AQP4 antibody-positive patients. **Methods:** We retrospectively investigated 127 consecutive multiple sclerosis (MS)/neuromyelitis optica patients, and evaluated the clinical features of 11 patients with anti-AQP4 antibody. Next, we analyzed the distribution of magnetic resonance imaging (MRI) lesions, and the effects of various disease-modifying therapies. **Results:** Eleven out of the 127 patients (8.7%) were anti-AQP4 antibody seropositive. The sex ratio was 1:10. All investigated patients were relapsing-remitting, and the average Expanded Disability Status Scale (EDSS) score during remission was 5.0. Eight patients experienced both myelitis and optic neuritis during the disease course, although 3 patients had only recurrent myelitis. MRIs revealed cerebrum lesions in 9 patients, cerebellum lesions in 2 patients and brainstem lesions in 1 patient. Longitudinally extensive spinal cord lesions were seen in all patients. High dose intravenous methylprednisolone pulse therapy (HIMP) administered for acute relapses was effective in 8 patients. The other 3 patients, who were unresponsive to HIMP, underwent immunoadsorption plasma pheresis (IAPP), and all showed a significant clinical improvement. As disease-modifying therapies, interferon β -1b was applied to 6 patients, but withdrawn in 4 patients, either because adverse events developed or a sufficient therapeutic effect was not achieved. **Conclusions:** Compared with classical MS, these anti-AQP4 antibody-positive patients tended to be predominantly females, and the relapse rate and EDSS were higher. A certain number of anti-AQP4 antibody-positive patients demonstrated recurrent myelitis with no episodes of optic neuritis.

The treatment with IAPP was thus suggested to be effective, while the use of IFN β -1b was found to require a reexamination.

P543**Ambulation-related disability in Devic's neuromyelitis optica**

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Background: Devic's neuromyelitis optica (NMO) is a severe demyelinating disease causing motor and visual dysfunction in most affected individuals. Accumulating evidences suggest that NMO and multiple sclerosis (MS) are independent conditions, with different demographic, immunopathological, cerebrospinal fluid and imaging features. Whereas disability in MS is strongly related to disease duration, the effects of time on NMO has not been well established. **Objective:** To analyze the effect of disease duration on disability in the course of NMO. **Methods:** Medical records of NMO patients were reviewed. The Disability Status Scale (DSS) was used to assess permanent ambulation-related disability. Cross-section analysis was done at 1, 2, 3, 5, 10, 20 and more than 20 years of disease. **Results:** Out of 321 patients selected for the study 265 fulfilled Wingerchuk's revised criteria for diagnosis of NMO. There were 212 females and 53 males with ages at onset ranging from 5 to 71 years (median 29). Isolated optic neuritis was the first index event in 52%, transverse myelitis in 40%, and simultaneous optic neuritis and myelitis in 8%. The disease was relapsing in 90% of the cases. Median disease duration was 7.0 years (7 months to 60 years). The DSS in all groups with different disease duration ranged from 0 to 10 (median 4), except in patients with disease longer than 20 years (median 6). **Conclusions:** Sustained ambulation-related disability in NMO is widely variable and does not depend on disease duration. Severe disability results from attacks and is probably related to intensity of the inflammatory response. This observation adds further evidence to the view that NMO and MS are different disorders.

P544**Comorbid autoimmune and allergic conditions in multiple sclerosis: a comprehensive population-based case-control study**

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Background: There is conflicting data on the prevalence of autoimmune and allergic conditions (AICs) in multiple sclerosis (MS) and lack of reliable estimates of most AICs in the general population. Previous MS studies were limited by small samples and absent or biased control groups. Understanding whether AICs are associated with MS (or its treatments) may have important implications for etiology, prognosis and treatment of MS. **Objective:** To obtain prevalence estimates AICs in a large sample of MS cases compared with controls. **Methods:** We conducted a population-based case-control study of MS in the Northern California Kaiser Permanente Medical Care Program. Electronic medical records through 2005 were used to ascertain MS cases and identify 47 AICs. Controls were matched 5:1 for gender, year of birth (+/-2 yrs), facility and duration of Kaiser membership. **Results:** We identified 5318 MS cases and 26,588 matched controls. We found significant association of MS with ulcerative colitis (UC; OR=1.81 95%CI 1.20-2.73), uveitis/iritis (OR=2.21 95%CI 1.67-2.92), Guillain-Barre syndrome (GBS; OR=6.25 95%CI 2.47-15.85) and bullous pemphigoid (OR=6.66 95%CI 1.49-29.75). We found no significant association of MS with rheumatoid arthritis, lupus, autoimmune diabetes, asthma, eczema, psoriasis, Crohn's, Graves' or Hashimoto's disease despite prevalence in controls ranging from 0.29% to 3.28%. No apparent excess of idiopathic thrombocy-

topenic purpura or Raynaud's phenomenon was seen in MS cases. Distribution of the AICs in treated and untreated MS cases will be presented. **Conclusions:** Our findings suggest that patients with MS are more likely to have UC, uveitis/iritis, GBS, and bullous pemphigoid but are not more or less likely to have other AICs when compared with people of similar age and gender. This is the largest, most comprehensive study to date and may provide insight into MS etiology and important information for assessing the potential safety risks of emerging MS immunotherapies.

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P545**Prevalence of vitamin D-, herpesvirus family- and hormonally-related diseases in multiple sclerosis: a comprehensive population-based case-control study**

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Background: Vitamin D deficiency, Epstein-Barr Virus infection and hormonal factors are risk factors for multiple sclerosis (MS) as well as select cancers and other illnesses. In addition some MS treatments may be associated with an excess of malignancies, menstrual disorders and herpesvirus family illnesses. **Objective:** To obtain prevalence estimates of vitamin D-, herpesvirus family- and hormonally-related illnesses in patients with MS compared with controls. **Methods:** We conducted a population-based case-control study of MS in the Northern California Kaiser Permanente Medical Care Program. Electronic medical records through 2005 were used to ascertain MS cases and identify vitamin-D-, herpesvirus-family- and hormonally-related diseases. Controls were matched 5:1 for gender, year of birth, facility and duration of Kaiser membership. **Results:** We identified 5318 MS cases and 26,588 controls. We found a significant increase in select herpes illnesses among MS cases: mononucleosis (OR=6.25 95%CI=1.68-23.28), Varicella zoster (OR=1.36 95%CI=1.08-1.71) and Bell's palsy (OR=2.72 95%CI=1.68-4.38); osteoporosis in women and men with MS (OR=1.85 95%CI=1.53-2.23 and OR=8.75 95%CI=4.16-18.42, respectively), and pelvic adhesions in women with MS (OR=1.43 95%CI=1.08-1.89). We found no significant difference in breast, prostate, colon, ovarian, uterine or testicular cancer; malignant melanoma, lymphomas, hematological malignancies; herpes simplex infections or infertility amongst MS cases compared with controls. **Conclusions:** Our findings suggest that shared immunological defects and/or environmental triggers may be responsible for susceptibility to MS and primary infection/reactivation of select herpes family viruses. With the exception of osteoporosis (which is often caused by disability), we found no differences in vitamin D- or hormonally-related illnesses, suggesting that these are less important MS risk factors. The increase in pelvic adhesions in women with MS warrants further investigation. This is the largest, most comprehensive study to date and may provide insight into the pathophysiology and treatment of MS.

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P546**Analysis of relapse frequency and severity in relation to single and multiple pregnancies among women with multiple sclerosis**

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Background: There are no good predictors of post-pregnancy disease activity to guide preventive relapse treatment. **Objective:** To verify if multiple sclerosis (MS) activity in the pre-pregnancy period predicts disease activity during and after pregnancy. **Methods:** Women in our

clinic becoming pregnant after MS onset were prospectively observed during and 1 year after pregnancy, the year before pregnancy constituting the index period. As pregnancies are not independent events, we created subsets comprising: (i) first ever live birth; (ii) multiparous but first live birth post-MS and (iii) two deliveries (after MS onset). **Results:** Among 90 women, 120 pregnancies occurred. Age was the same in all subsets (mean ≈30 years). Only one post-pregnancy relapse was severe, none was severe prior; average Expanded Disability Status Scale score remained at 1.0–1.3 in all subsets. For group (i) (n=68), 40% had a relapse pre-pregnancy, and 19% of these relapsed during and 37% after pregnancy. For those without a pre-pregnancy relapse, (n=27; 60%), 15% relapsed during and 37% after. For group (ii) (n=22), 60% (n=13) had a relapse before, and 15% of these relapsed during and 60% after. The proportions for those relapse-free pre-pregnancy were 11% during and 44% after. For group (iii) (54 pregnancies), 35% had a relapse pre-pregnancy, and 35% of these relapsed during and 40% after. For those relapse-free pre-pregnancy, 16% relapsed during and 21% after. In this group, the concordance of relapse pattern between 1st and 2nd pregnancy was 89% ($\kappa=0.73$; CI: 0.44–1.0). There was no significant relationships with pre-pregnancy relapse. **Conclusions:** The reduction in relapse frequency was significant during pregnancy. After pregnancy it was similar to the low pre-pregnancy activity, suggesting there is no indication for preventive treatment after delivery. Hierarchical linear modeling will be carried out to estimate the proportion of variance in pregnancy and post-pregnancy relapse frequency explained by factors related to the woman, and factors related to the pre-pregnancy period.

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The intake of selected foods in multiple sclerosis: a possible interaction with late childhood infections

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Background: In several studies an increased odds ratio (OR) for multiple sclerosis (MS) was reported after infectious mononucleosis and the later occurrence of other childhood infections. Some other investigations pointed to the increased risk of MS after the consumption of smoked meat and sausages, in particular during childhood. **Objective:** To investigate the interaction of later childhood infections and the intake of smoked meat varieties as risk factors in population-based MS patients and hospital controls. **Methods:** A special interviewer-applied food-frequency questionnaire, along with the common questionnaire on exogenous factors until age 15, was applied to 136 MS patients (108 females, 28 males) and 109 hospital controls (66 females, 43 males) having low-back pain or minor general surgery, all born after 1949. Analyses were made by logistic regression. Year of birth and gender were generally adjusted, whereas all further variables were entered in a univariate or multivariate fashion. **Results:** In univariate analysis, the occurrence of any childhood infection after age 5 was not associated with MS. Consumption of animal fat (OR=2.25; p=0.011), cold-smoked meat (e.g. ham, bacon)(OR=1.86; p=0.069), and cold-smoked (OR=1.89; p=0.024) and hot-smoked (OR=2.75; p=0.0006) sausage (e.g. frankfurter, hot dog) during childhood were associated with MS, whereas oat flakes were protective (OR=0.52; p=0.015). In multivariate analysis, only hot-smoked sausage was significant (OR=3.01; p=0.001). When the interaction terms of all meat variables with any one childhood infection after age 5 were tested, the OR for hot-smoked sausage increased (OR=4.71; p=0.000028), whereas it remained in the same range as for the single items for the other foods. Subanalyses revealed particularly high increases for non-benign and chronic progressive MS, and for males. **Conclusions:** The risk of different smoked varieties ingested during childhood, in particular hot dogs, was confirmed. The interaction with late childhood infections might point to one or several very narrow time window(s) of an increased risk during childhood.

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The mortality rate of multiple sclerosis 1975–1995 in the State of Lower Saxony, northern Germany

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Background: In previous investigations, the multiple sclerosis (MS) rates, including mortality, showed correlations with a number of exogenous factors. **Objective:** To test some of these features in an additional geographical area and to search for new correlations. **Methods:** The MS mortality rate taken from death certificates was calculated for the whole State (51°–54° northern latitude; 47,390 sq.km; population 7.256 million in 1980), three calendar subperiods, and 47 counties (9 cities; 38 rural counties) in 1975–1995. The crude MS mortality rate was tested for inhomogeneity by the approximate chi-square test of Kurtzke (1966), and age-adjusted rates were used in correlation analyses. More than 50 exogenous variables were taken from different sources. Univariate tests according to Spearman and multivariate regression analyses were done. **Results:** The crude MS mortality rate for the whole State was 1.47 per 100,000. There was a continuous decline from 1.60 in 1975–1981 to 1.34 in 1989–1995 (per 100,000; chi-square for trend = 11.92; p = 0.0006). The age-adjusted MS mortality rate was unevenly distributed, with significantly (p < 0.05) elevated rates in 5 counties which were located predominantly in the southeastern mountainous part, and low rates in 5 counties in the northwestern lowland district closer to the sea coast. Univariate correlations (p<0.05; agriculture excluded) were found for 10 variables (not shown). In the forward inclusion method of multiple linear regression, only wood-processing (beta=0.396; p=0.003) and kali mining (0.268; p=0.041) industries made a significant contribution to MS mortality. Backward exclusion analysis confirmed the association with the wood-working industry. In univariate testing, the mortality of M.Hodgkin in females in 1976–1980 and in 1981–1985 was also related to MS. **Conclusions:** The relationship of MS frequency with altitude was confirmed. In addition wood processing made a prominent contribution that supports data from other countries. The relation between M.Hodgkin and MS remains to be defined. **Supported by:** Gemeinnuetzige Hertie-Stiftung, Frankfurt, Germany.

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Human leukocyte antigen class I risks in multiple sclerosis: A rather than C

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Background: Genes within the Human Leukocyte Antigen (HLA) complex play a significant role in multiple sclerosis (MS) etiology. Interest has mainly been focused on the class II genes HLA-DRB1 and HLA-DQB1 but recently, HLA class I genes have been shown to influence risk of disease. We reported an association with HLA-A whereas others recently suggested an importance of HLA-C alleles. **Objective:** To investigate, in a Nordic MS population, whether the class I gene HLA-C confers risk to MS in addition to HLA-DRB1 and HLA-A. **Methods:** Our study population consists of 1500 MS patients and 1600 controls from Sweden and Norway typed for HLA-A, -C and -DRB1 loci. The analysis of the HLA-C effect was done through logistic regression and stratification using R. We also used a tree based recursive partitioning method to visualize risk genotype groups. **Results:** Logistic regression analysis showed that models including HLA-DRB1 plus either HLA-A or HLA-C explained the risk of MS better than a model with HLA-DRB1 alone, confirming the additional influence of class I genes in MS. Of these models, HLA-DRB1 plus HLA-A was somewhat superior to HLA-DRB1 plus HLA-C. A model including HLA-DRB1, HLA-A and HLA-C was not superior to the

model of HLA-DRB1 and HLA-A ($p=0.2$). A logistic regression analysis including all major alleles of the three loci confirmed effects of HLA-DRB1*15 ($p=4.57e-12$) and HLA-A*02 ($p=0.003$). However, no allele of HLA-C showed significant association. Although when included, HLA-C*08 (freq 0.02) showed a significant association with risk of MS ($p=0.03$). HLA-C*05, previously reported to be associated with MS in a British-American MS cohort, failed to contribute significant risk. **Conclusions:** In this material, we confirm an independent role of HLA-class I for the risk of MS. The class I effect is more readily explained by HLA-A, or variants in linkage disequilibrium with HLA-A, than with HLA-C. However, an effect of minor HLA-C alleles, particularly HLA-C*08, cannot be ruled out.

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Protection against multiple sclerosis conferred by carriage of the inhibitory killer immunoglobulin-like receptors (KIRs) ligand HLA-Bw4

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Background: Combinations of killer immunoglobulin-like receptors (KIRs) and HLA class I ligands that reduce natural killer (NK) cells inhibition may increase the risk of autoimmune diseases. Multiple sclerosis (MS) affects the central nervous system and is presumed to be an autoimmune disease. There are significant genetic associations to genes in the HLA class II region (DRB1*1501-haplotype), but associations to genes in the HLA class I region have also been reported (for instance A2, A3 and Cw*05). **Objective:** We investigated whether there might be associations to given KIRs and/or their HLA class I ligands. **Methods:** 631 Norwegian MS patients and 555 Norwegian controls were typed for the presence or absence of genes encoding inhibiting KIRs (KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL5, KIR3DL1, KIR3DL2 and KIR3DL3) and activating KIRs (KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5 and KIR2DL4) using sequence specific primers. HLA-A, -B, -C and -DRB1 typing was performed by direct sequencing or sequence specific primers. **Results:** We found no significant differences in gene carrier frequencies of inhibitory and activating KIRs in MS patients as compared with controls when p-values were adjusted for number of KIR genes investigated. However, the frequency of the HLA-Bw4 specificity was significantly reduced in MS patients as compared with controls (41.5% vs. 55.1%, $p=4.9*10^{-6}$), even in individuals who did not carry any of the known HLA class II risk alleles (DRB1*1501, DRB1*03 and DRB1*01, $p=0.002$). No HLA class II independent associations were observed to genes at the HLA-C locus. **Conclusions:** The presence of the HLA-Bw4 specificity seems to protect against MS in an HLA class II-independent manner. Ongoing analyses aim to characterize possible interaction effects between HLA-Bw4 and KIR3DL1 and KIR3DS1, as well as between HLA-C1 and C2 groups and their corresponding receptors.

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Association between actinic damage, a biomarker of lifetime sun exposure, and first clinical diagnosis of central nervous system demyelination: the Ausimmune Study

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Background: In Australia, most of human vitamin D stores are ultraviolet radiation-derived and a strong positive latitudinal gradient of multiple sclerosis (MS) prevalence has previously been described. Past studies indicate a possible protective role for higher levels of sun exposure and/or vitamin D. Actinic damage on the back of the hand is an established biomarker of lifetime sun exposure. **Objective:** To examine whether cumulative past sun exposure, as measured by actinic damage, differs between persons with a first clinical diagnosis of central nervous system demyelination and population controls. **Methods:** The Ausimmune Study is an incident case-control study examining the role of environmental factors in the development of MS. We recruited cases with a first clinical diagnosis of central nervous system demyelination through neurology and radiology clinical notification systems. Community controls were age and sex-matched to cases in four centers down the eastern seaboard of Australia (from latitude 27 to 43 degrees South), between 1 Nov 2003 and 31 Dec 2006. Cumulative past sun exposure was measured by microscopic scoring of the texture of silicone rubber casts of the back of the hand for all participants. **Results:** In all regions, increasing age was associated with increasing actinic damage. Preliminary analyses indicate that cases had lower sun damage scores and the magnitude of this effect seemed to increase with latitude. We are now proceeding with multivariate analyses, adjusting for skin type and other possible confounding factors. **Conclusions:** These results, if confirmed in the full analysis, would be consistent with previous work that has shown that higher levels of cumulative sun exposure are protective against the development of central nervous system demyelination.

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Latitudinal variation in incidence of first demyelinating events: descriptive analyses of case participants in the Ausimmune Study

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Background: Increasing multiple sclerosis (MS) incidence and geographic patterns of occurrence suggest an environmental role in the causation of this multifactorial disease. Previous work has shown positive latitudinal gradients in MS prevalence - a finding consistent with

individual-level studies indicating a possible protective role for higher levels of sun exposure and/or vitamin D. **Objective:** To describe relevant features of the case participants of the Ausimmune Study that may provide clues to MS etiology. **Methods:** The Ausimmune Study is an incident case-control study examining environmental risks in the onset of first demyelinating events (FDEs), and possible progression to MS, down the eastern seaboard of Australia. We recruited cases with a first clinical diagnosis of central nervous system demyelination in four centers across a range from 27 to 43 degrees South latitude, between 1 Nov 2003 and 31 Dec 2006. **Results:** Analysis of the FDE cases revealed a strong latitudinal gradient in incidence, increasing by 9.2% (95% CI 6.7–11.6, $p < 0.001$) per higher degree of latitude. This association varied according to the presenting FDE type: for optic neuritis cases, there was an 11.4% increase in incidence (95% CI 6.6–16.4, $p < 0.001$) per higher degree of latitude, but only a 3.9% (95% CI 0–8.4, $p = 0.07$) increase in incidence for spinal cord syndrome cases. Primary progressive MS (PPMS) did not demonstrate latitudinal variation in incidence, but this analysis was based on small numbers (total $n = 18$). In the FDE group, the female to male ratio was 3.3:1, with a progressive decrease in this ratio with increasing latitude: from 6:1 at lowest latitude to 2.5:1 at the highest latitude (p for trend = 0.07). For PPMS, the overall sex ratio was 2:1. **Conclusions:** These descriptive case analyses show intriguing patterns that may be important for understanding the etiology of MS. Our findings suggest that the relative contributions of environmental and genetic factors to the clinical expression of MS vary with latitude. **Supported by:** Multiple Sclerosis Research Australia, The National Health and Medical Research Council of Australia, National Multiple Sclerosis Society (USA).

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Vitamin D Status in children with multiple sclerosis

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Background: Multiple sclerosis (MS) prevalence has been correlated with residence in regions with low ambient sunlight and with reduced serum 25-hydroxyvitamin D (25(OH)D) levels in adult MS patients. Little is known regarding vitamin D status in children with MS. **Objective:** To evaluate vitamin D status in a cohort of children with MS. **Methods:** We retrospectively reviewed the 25(OH)D levels in children with MS cared for in the Multiple Sclerosis clinic at the Hospital for Sick Children between January 1999 and April 2008. 25(OH)D levels were obtained at routine clinic visits as an exploratory evaluation. **Results:** We identified 35 children who had serum 25(OH)D levels measured. Overall 11.4% (4/35) of children were vitamin D deficient (0–30 nmol/L), 54.3% (19/35) were vitamin D insufficient (31–69 nmol/L), and 34.3% (12/35) had adequate levels (> 70 nmol/L). Sixteen children were taking oral vitamin D supplements at doses between 400 and 1200 IU daily and had higher serum 25(OH)D levels than those not supplemented (67.1 nmol/L \pm 26.8 vs 48.1 nmol/L \pm 20.5, $p = 0.02$). 50.0% of children taking oral supplements had 25(OH)D levels in the insufficient or deficient ranges compared with 78.9% of those who reported no vitamin D supplementation ($p = 0.15$). All vitamin D deficient children (4/4) had serum levels checked in winter months (October–February), further analyses on seasonal influence are pending. **Conclusions:** Approximately 65% of children with MS living in a northern climate have serum 25(OH)D levels below 70 nmol/L. Insufficient and deficient vitamin D levels occurred even in children reporting oral vitamin D supplementation at conventional or slightly higher than conventional daily doses. These findings suggest that vitamin D levels should be monitored in children with MS and that optimal vitamin D dosing in this population requires further study. Future prospective studies will be needed to determine whether vitamin D supplementation affects disease severity or progression.

P554

Prevalence of JCV viral sequences in cerebrospinal fluid cells of multiple sclerosis patients

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Background: The possible involvement of JCV in multiple sclerosis (MS) was first postulated years ago; an interesting scientific debate has emerged after the introduction of natalizumab treatment for MS patients and the development of progressive leukoencephalopathy (PML) in two of such patients. **Objective:** Evaluation of the presence of neurotropic viruses including JCV, varicella zoster virus (VZV), human herpesvirus 6 (HHV6) and Epstein-Barr virus (EBV) in cerebrospinal fluid (CSF) of MS patients and controls, and investigation on whether viral sequences are present in cell-free CSF or associated to CSF-compartmentalized cells. **Methods:** Eighty-one CSF samples (51 MS; 30 samples from patients with other neurological diseases (OND)) were analysed. Cells pelleted from CSF were lysed and CSF DNA extracted with spin-columns. Viral genome were detected by real-time PCR in CSF cell-free and cells separately. Viral loads were quantified for cell-free CSF viruses, whereas for the cells only qualitative examination was obtained. **Results:** 1) JCV was isolated in 5/51 (9.8%) MS patients and 1/30 OND individual. In all but one case viral sequences were cell-associated; JCV viral load was 2.26x10⁴ copies/ml in the only instance in which cell-free virus was isolated. 2) VZV DNA was isolated in only one patient (OND); EBV was not detected in any of the samples examined. 3) HHV6 DNA was detected in 1/46 (2.2%) cell-free CSF obtained from MS patients. Finally, 4) no double infections were found. **Conclusions:** JCV viral sequences are detected in a limited number (9.8%) of MS patients and are mainly associated with CSF cells; cell trafficking from the periphery to the CNS can result in the transport of this virus to the CNS, where local immunosurveillance can control viral replication.

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Smokers with multiple sclerosis are more likely to report comorbid autoimmune diseases

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Background: Studies suggest an association of multiple sclerosis (MS) and autoimmune disease. Smoking is a risk factor for MS and autoimmune diseases such as rheumatoid arthritis. Smoking could potentially explain an increased risk of comorbid autoimmune disease in MS. **Objective:** Using the North American Research Committee on Multiple Sclerosis Registry, we investigated the frequency of comorbid autoimmune disease in MS, and whether smokers with MS had an increased risk of comorbid autoimmune disease as compared with non-smokers. **Methods:** Registry participants report demographic and clinical information regarding their MS at enrollment and semi-annually thereafter. In 2006, 8,983 registry participants reported autoimmune comorbidities and smoking status. We classified participants as having any comorbid autoimmune disease if they had one or more of the following: rheumatoid arthritis, Sjogren's syndrome, systemic lupus erythematosus, inflammatory bowel disease, autoimmune thyroid disease, or uveitis. Using multivariable logistic regression, we examined the cross-sectional association of comorbid autoimmune disease with smoking status (ever vs. never). Using Cox proportional hazards regression, we examined the risk of developing an autoimmune comorbidity after MS onset using time until autoimmune diagnosis according to smoking status (current vs. never) among participants without a pre-existing comorbidity ($n = 6840$). **Results:** Responders were predominantly white (94%), women (75%) with mean (SD) age 52.7 (10.4) years. 1649 (18.5%) participants reported a questionnaire-specified comorbid autoimmune disease.

Thyroid disease was reported most frequently (871, 10%), followed by rheumatoid arthritis (318, 3.6%), and inflammatory bowel disease (307, 3.5%). In a multivariable logistic regression model, ever smokers had nearly 25% increased odds of reporting a comorbid autoimmune disease (OR 1.22; 1.08–1.38). After adjustment for sex, age of symptom onset, and year of symptom onset, smokers had an increased risk of developing any autoimmune disease (HR 1.23; 1.08–1.41). **Conclusions:** Smokers with MS have an increased risk of comorbid autoimmune disease.

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P556

Changing referral patterns in a tertiary multiple sclerosis clinic

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Background: Patients referred to multiple sclerosis (MS) clinics are frequently diagnosed with conditions other than MS. There is also an increasing awareness that MS is not confined to patients of European descent. **Objective:** We sought to retrospectively assess the demographic, clinical and magnetic resonance imaging (MRI) findings of patients referred to our clinic over a 6 month period. We hypothesize that: (a) the availability of MRI is increasing the number of patients referred with nonspecific MRI findings, (b) MRI availability is conversely also resulting in referrals for 'preclinical' MS and (c) demographic changes in the Canadian population are reflected in an increased number of non-Caucasians diagnosed with MS. **Methods:** A retrospective review of all new referrals to the St. Michael's Hospital MS Clinic in Toronto, Ontario from July–December 2007 was performed. The characteristics of patients diagnosed with MS were contrasted with those of patients not felt to have MS. Our data was compared with historical Canadian and contemporary American MS clinic results. **Results:** Over the 6-month period, a total of 158 new patients were referred. Seventy-one patients had MS and 25 had a clinically-isolated syndrome. One-third of the 30 patients who did not have MS were referred because of nonspecific MRI changes. An additional 10 patients had MRI features strongly suggesting demyelination in the absence of any clinical features of MS. Ten percent of patients with either MS or a clinically-isolated syndrome were not of European descent. **Conclusions:** Nonspecific MRI findings lead to a number of referrals of patients who do not have MS. However, a significant subset of asymptomatic patients had MRIs suggestive of demyelination. A sizable minority of MS patients were not of European descent. This study suggests that the referral patterns to MS clinics are being altered by both advances in neuroimaging and demographic shifts in the Canadian population.

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Multiple sclerosis and pregnancy: relationship with progression of disability

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Background: The effect of pregnancy on multiple sclerosis (MS) course in the short term (24 months after delivery) is well known. No definitive data are available on the long-term effects on progression of disability. **Objective:** To investigate a possible long-term effect of pregnancy on MS course and a possible influence of pregnancy after MS onset on disability progression. **Methods:** A retrospective study was performed on 274 women with MS (27.4% without pregnancies, 40.2% with pregnancy before and 32.4% with at least one pregnancy after MS onset) to investigate a possible relationship between pregnancy and progression of disability. Features of the examined population were: Relapsing-Remitting form 72.3%, Expanded Disability

Status Scale (EDSS) mean score 3.6 (range 0–9), mean disease duration 15.9 years (range 4.5–52); use of disease-modifying therapies for at least one year in 65.7% of patients. No significant differences in these features were observed between women with and without pregnancies. A Kaplan-Meier Survival Analysis was conducted. Two clinical endpoints were considered: time to reach irreversible EDSS score of 4 and 6. Time from MS onset was used as survival time variable. **Results:** Women with pregnancies after disease onset showed a longer time to reach EDSS score of 4 and 6 when compared with patients without pregnancies or with pregnancies before MS onset (respectively 16.8 years vs 11.2 and 10.8 years, $p=0.015$, for EDSS 4 and 21.3 years vs 15.7 and 14 years, $p=0.02$, for EDSS 6). **Conclusions:** These results could support a long-term positive effect of pregnancy on MS prognosis, particularly in women who had pregnancies after MS onset.

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Familial neuromyelitis optica

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Background: Few individual instances of neuromyelitis optica (NMO) familial aggregation have been previously reported comprising individuals of only two different ethnicities. **Objective:** To report a series of familial cases of NMO and to estimate the frequency of familial cases from a large clinical-serological database. **Methods:** We studied 15 affected individuals in 7 families with NMO (Wingerchuk 2006 criteria) or NMO-spectrum disorders (e.g. recurrent transverse myelitis NMO-IgG seropositive). Patients (index cases) ascertained through Mayo Clinic practice ($n=6$) were used to estimate the frequency of familial cases. **Results:** Fourteen patients (93%) were women. Three families were from Latin America (1 Hispanic, 1 Mulatto, 1 Native Brazilian), 2 were Caucasian, 1 African-American and 1 Asian. Six families had two affected individuals while one had three: 2 sisters ($n=3$); a niece and paternal aunt ($n=1$); a father and daughter ($n=1$); a mother and daughter ($n=1$); a daughter, mother and a maternal aunt ($n=1$). The age of onset (mean±SD) was 36.3 ± 15.7 years. The difference between ages of onset between the affected pairs was 18.9 ± 15.6 years and between year of onset was 16.7 ± 15.6 . NMO-IgG serology was positive in 12 of 15 (80%) individuals. Familial cases are estimated to represent at minimum of 2% of a series of 359 cases. **Conclusions:** NMO occurs separately from multiple sclerosis in families, consistent with it being a distinct disease. Only 1 or 2 generations were affected in any family, and affected individuals represented a small fraction of family members. The age of onset and the year of onset differed considerably within affected pairs. Taken together, these data suggest a complex genetic susceptibility in NMO.

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Osteoporosis in veterans with multiple sclerosis

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Background: Vitamin D insufficiency has been reported in patients with multiple sclerosis (MS) and linked to other diseases such as osteoporosis. However, no large studies have measured the prevalence of osteoporosis and the factors associated with the presence of this disease in MS patients. **Objective:** (1) Examine the prevalence of osteoporosis in veterans with and without MS. (2) Examine demographic and clinical factors (e.g. MS subtype, age at onset, disability, and comorbidity) that are associated with osteoporosis in MS patients. **Methods:** We included 1,210 MS patients from the Multiple Sclerosis Surveillance Registry, which was established through a mail survey to a nationally representative, stratified, random sample of veterans with

MS. We then randomly selected age-, sex- and race-matched controls from the Veteran's Health Administrative database. Prevalence for osteoporosis was calculated for the MS patients and their matched controls. Chi-square test was used to examine difference in prevalence between the two groups. Logistic regression was applied to examine factors associated with osteoporosis in MS patients. **Results:** MS patients (mean age: 54.1 years, ranged from 25–74 years) have about a two times higher prevalence of osteoporosis compared with matched controls (8.7% vs. 4.4%, $p < .0001$). Specifically, osteoporosis is more prevalent in middle-aged MS patients (age 45–64 years) compared with controls ($p < .001$). Within MS patients, female, older age, more severe disease and higher number of comorbid diseases are associated with osteoporosis. **Conclusions:** Osteoporosis is twice as prevalent in middle-aged MS patients compared with matched controls. Screening osteoporosis in high-risk MS patients is needed and should begin from the early course of the disease. Future studies should focus on the impacts of osteoporosis on disability and investigate the interventions that effectively prolong or prevent the onset of osteoporosis in MS patients.

P560

Sex differences related to marital status in patients with multiple sclerosis

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Background: Multiple sclerosis (MS) is a disabling disease affecting young adults and has strong social impact and consequences. Research has shown that marital status is disrupted by MS and suggests that the odds of male patients becoming divorced are significantly higher compared with females. **Objective:** To assess the relationship between clinical characteristics and marital status in a large cohort of MS patients. **Methods:** Analysis was based on longitudinal data of the New York State Multiple Sclerosis Consortium registry comprised of patients from 17 MS centers in New York State organized to prospectively collect demographic and clinical data. Included in this study were 2,092 patients (74.5% female) with three completed follow-up points of data collection during 1999–2006. We used Mantel-Haenszel Chi-Square for categorical factors and Mann-Whitney U tests for continuous factors, stratified for adjustment. **Results:** Among participants 15% were single, 67.8% married/cohabiting, and 17.2% divorced or separated. The mean Kurtzke Expanded Disability Status Scale score was 3.8 (SD 2.4) and 65% had relapsing-remitting disease at third follow-up (28% secondary progressive). The mean age of symptom onset was not significantly different between sexes (31.2 and 32.7 females and males respectively). However, age at either the time of MS symptom onset or at diagnosis had a significantly stronger effect for males related to marital status. The odds of male patients being married/cohabiting when age of onset was 31 or older were 1.8 ($p < .000$) compared with females. **Conclusions:** Age at symptom onset modifies the effect of male sex related to the likelihood of maintaining marital status or becoming divorced. Further

understanding of how older age of onset positively influences marital status differently for men compared with women may help inform psychoeducational interventions to reduce marital disruption and the emotional, psychosocial burdens of MS patients and family members.

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A comparison of the characteristics of elderly multiple sclerosis patients to younger patients at a tertiary multiple sclerosis center

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Background: Although usually a disease of young adults, multiple sclerosis (MS) can begin at older ages. Because it only modestly impacts life expectancy, most patients can anticipate living beyond age 60. This older population likely has unique needs. **Objective:** In order to plan ways to provide better care to this older population, we characterized the differences between patients older or younger than 60 years attending a single tertiary MS center. **Methods:** Patients seen during one year were sorted into those $>$ or $<$ 60. Charts of all MS patients 60+ ($n=150$) were reviewed and compared with a sample ($n=151$) of 60- patients selected by applying a random number sequence to an alphabetized list. **Results:** The center population included 9.4% who were 60+, mean age 67.9 compared with 41.6 for the 60- sample. Older patients were much more disabled (mean Expanded Disability Status Scale (EDSS) score 5.22) than the younger (mean EDSS 1.98, $p < .0001$); more likely to have reached EDSS >6.0 (63.8% vs. 16.0%, $p < .0001$); more likely to be completely non-ambulatory (EDSS >7.5 ; 22.1% vs. 5.3%, $p < .0001$). They more often required paid health aides (14.4% vs. 4.2%, $p=0.004$), but were not significantly more likely to be living alone. More older patients had primary progressive MS (21.4% vs. 5.4%, $p < .0001$) and secondary progressive MS (49.6% vs. 9.9%, $p < .0001$). Older patients did not differ significantly from younger in cerebrospinal fluid profiles, but had less disease activity on recent brain magnetic resonance imaging. The 60+ patients were significantly less likely to be using injectable disease-modifying therapy (53.3% vs. 81.4%) and less likely to be taking glatiramer acetate (23.3% vs 43%). The 60+ group had significantly more co-morbidities (2.52 vs. 0.97, $p < .0001$) and used more medications (median 5.00 vs. 1.00, $p < .0001$). Insurance status was comparable in that 84–85% of both groups had private insurance, often combined with Medicare in the 60+ group. **Conclusions:** Elderly patients at a tertiary MS center are significantly more disabled by a combination of MS and additional co-morbidity. They are likely to require greater time and services and warrant unique consideration of their needs.

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The presentation and long-term prognosis of a first demyelinating event, optic neuritis, evaluated by neuro-ophthalmologists

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Background: According to the Optic Neuritis Treatment Trial (Beck et al 2003) the 10-year risk of conversion to multiple sclerosis (MS) after acute demyelinating optic neuritis (ON) is 38%, and can be as high as 60% by 40 years in population-based studies (Rodriguez et al 1995). Although neurologists most likely see ON patients within the MS population, neuro-ophthalmologists are often the first specialist to manage ON patients. **Objective:** We reviewed the presentation, demographics and long-term prognosis of ON as a first demyelinating event, a clinically isolated syndrome (CIS) presenting to the neuro-ophthalmologists in our region. **Methods:** We surveyed data on first clinical presentation, treatment, magnetic resonance imaging (MRI) results and long-term follow-up for patients seen between January 1990 and March 2008. **Results:** We reviewed 208 (76.9% female) patients with an average age at presentation of 34 years (12 to 62). Ophthalmologist/optometrist referred 57.7% of patients. 30.8% of referrals were seen within one week of symptom onset and 50% within two weeks. At presentation 38.5% had vision 20/200 or worse

and 37.5% were treated with corticosteroids. While 17 had been previously diagnosed with MS and one with acute disseminated encephalomyelitis, 190 cases were diagnosed as CIS. In follow-up, 54.7% have since been diagnosed with MS. Of those who converted to MS, 80% had a presenting MRI suggestive of demyelination, while 64.9% of those who remained a CIS had a normal brain MRI. **Conclusions:** ON is a common first demyelinating event, or CIS, in MS patients seen by neuro-ophthalmologists. The 'real life' conversion rate in our region is 54.7%, and compares well with previously reported population-based studies. Clinically silent MRI lesions suggestive of MS at the time of ON presentation were detected in a majority of those presenting with ON with future conversion to MS.

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Age, race, and initial demyelinating events with fewer affected functional systems predict an increased hazard of early relapse in multiple sclerosis

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Background: Factors associated with increased risk of an early second demyelinating event in multiple sclerosis (MS) are poorly characterized. **Objective:** To determine predictors of having a second demyelinating event within the first year of MS onset. **Methods:** Patients with MS/clinically isolated syndrome (CIS) seen at the UCSF MS Center within one year of disease onset were identified. Univariate and multivariate Cox models were used to analyze predictors of having a second event within one year of the initial demyelinating event. **Results:** Of 330 adults with MS/CIS, 112 had a second event within one year. In univariate analyses, non-white race (HR=2.35, 95% CI [1.56, 3.55], p<0.0001), younger age (for each ten-year decrease of age, HR=1.53, 95% CI [1.29, 1.81], p<0.0001) and fewer functional systems (FS) involved (HR per one less FS=1.33, 95% CI [1.08, 1.63], p=0.008) were associated with an increased hazard of having a second event within one year of onset. The hazard ratios were unchanged in a multivariate model. **Conclusions:** Non-white race, younger age, and a lower number of affected FS all herald an increased hazard ratio for a second demyelinating event within one year.

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Does acute disseminated encephalomyelitis without encephalopathy exist?

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Background: Diagnosis of multiple sclerosis (MS) in children can be more difficult than in adults. More frequently than in adults, the first attack of inflammation cannot be distinguished from acute disseminated encephalomyelitis (ADEM). Classically, encephalopathy is obligatory for the diagnosis of ADEM, next to polyfocal onset and the presence on magnetic resonance imaging (MRI) of lesions in the white matter, thalamus or basal ganglia. **Objective:** To study whether the presence of encephalopathy in children with a polyfocal onset independently influenced the course of the disease regardless of MRI

abnormalities. Therefore, we avoided the term ADEM, but split the polyfocal onset group into subgroups with and without encephalopathy. **Methods:** In this nationwide retrospective multicenter study in the Netherlands, 117 children below the age of 16 years were included. Fifty-four children presented with a monofocal clinically isolated syndrome (CIS) and 63 children with a polyfocal CIS (PCIS). Out of these 63 patients, 36 also had encephalopathy. **Results:** A second MS defining attack occurred in 43% of the CIS cases, compared with 21% of the patients with PCIS onset (P <0.006). This second attack occurred in 17% of the PCIS cases with encephalopathy and in 26% of the PCIS cases without encephalopathy. Basal ganglia, thalamic lesions and lesions larger than 2 cm on MRI (considered typical of ADEM) were observed during PCIS, irrespective of the presence of encephalopathy. **Conclusions:** We showed that a clinical spectrum exist with children with MRI abnormalities indicative of ADEM without encephalopathy and who still remain monophasic.

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P565

The clinical features, prognosis and magnetic resonance imaging of Japanese patients with anti-AQP4 antibody seropositivity

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Background: Diagnostic criteria for definite neuromyelitis optica (NMO) are required, they are optic neuritis (ON), myelitis and at least two of three supportive criteria. Anti-AQP4 antibody seropositivity was 95–100% specific for NMO. There are some patients with anti-AQP4 antibody seropositivity who lack ON, myelitis or both. **Objective:** To elucidate the clinical features, prognosis and magnetic resonance imaging (MRI) of Japanese patients with anti-AQP4 antibody seropositivity. **Methods:** We found a total of 55 patients with chronic multiple sclerosis (CMS), NMO, ON and myelitis at Yokohama University Hospital and analyzed their clinical features, prognosis and MRI features. **Results:** Anti-AQP4 seropositivity was found in 32% of all the patients examined. Definite NMO was found in 33% of patients with anti-AQP4 antibody seropositivity. Around 33% of patients had myelitis and 11% had ON. Three patients without myelitis and ON had only brain lesions that did not meet the diagnostic criteria for MS. Around 77% had a long cord lesion in myelitis and NMO. The Expanded Disability Status Scale (EDSS) of patients with NMO was 5.4 and 2.8 for patients with anti-AQP4 antibody seropositivity except for NMO. Two NMO patients took the benign course (EDSS less than 3 and more than 10 years from onset.) **Conclusions:** Anti-AQP4 antibody seropositivity is highly sensitive and specific for NMO. Many patients with NMO had a poor prognosis. We showed that there are incomplete types of NMO and two patients with anti-AQP4 antibody seropositivity had only small brain lesions without ON and myelitis. Anti-AQP4 antibody seropositivity may be a high risk factor with NMO, but the clinical features and findings of brain MRI in patients with anti-AQP4 antibody seropositivity were varied. The patients with ON, myelitis and brain MRI finding that do not meet the diagnostic criteria for MS have to be examined for the anti-AQP4 antibody. If the anti-AQP4 antibody is detected in that patient, they should be carefully observed because of prevention of NMO.

P566

OAS genotype predicts responsiveness to beta-interferon therapy

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Background: Over one-third of patients treated with beta interferons have a suboptimal response and are at risk of accumulating fixed disability. Currently, there are no sensitive, validated markers of

response. IFN β upregulates OAS1, which induces RNaseL, increasing anti-viral gene activity. A single nucleotide polymorphism (SNP) in exon 7 of OAS1 influences OAS1 enzyme activity; the G allele confers high OAS1 activity, while the A allele confers low activity. We have shown that the OAS1 AA genotype is over-represented in multiple sclerosis (MS) patients compared with controls. We hypothesized that the AA genotype would also be over-represented in people with a sub-optimal response to IFN β therapy. **Objective:** To show that responsiveness to IFN β therapy in relapsing-remitting MS (RRMS) relates to the OAS1 genotype (GG or AG more likely in responders; AA more likely in suboptimal responders). **Methods:** We examined the occurrence of a functional SNP at exon 7 of the OAS1 gene in 319 MS patients, 125 of whom had RRMS treated with IFN β . Thirty of these patients had highly active MS requiring natalizumab therapy. **Results:** The AA genotype, associated with low OAS1 enzyme activity, was found in 28% of MS patients vs. 23% of controls; the GG genotype was present in 6.3% of MS patients vs. 19.6% of controls. Based on disease activity, 52 patients were designated as suboptimal responders and 75 as responders to IFN β . Of suboptimal responders, 23 out of 52 (44.2%) were homozygous AA, while only one was homozygous GG. This compares to 20% (15/75) AA and 5% (4/75) GG in responders. The distribution of OAS genotypes was significantly different between non-responders and both responders ($P = 0.011$), and the rest of the MS cohort ($P = 0.0097$). **Conclusions:** A functional SNP in the OAS gene may predict both susceptibility to MS and response to treatment; a GG genotype may predict optimal response. This could be invaluable in avoiding expensive therapy, and adverse effects, in those unlikely to respond.

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Ten years outcome of the South Atlantic project on multiple sclerosis in Rio de Janeiro (Brazil)

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Background: Brazil is a continental South American country with a great ethnic diversity and is located in tropical-equatorial areas with a low prevalence of multiple sclerosis (MS). The Grupo de Neuro Imunologia da Academia Brasileira de Neurologia (1994–1998) organized the first MS population survey (South Atlantic project) analyzing 602 MS patients, most of them from the southeast region, classified by the criteria of Poser et al. (1983). In the last decade, new diagnostic criteria for MS (McDonald et al., 2001), neuromyelitis optica (NMO) (Wingerchuk et al., 1999) and primary progressive MS (PPMS) (Thompson et al., 2000) have been proposed based on clinical, radiological and laboratory data. **Objective:** To review the diagnosis of patients included in SIAPEM (Brazilian MS data base) from 1995 to 1998, treated at three MS referral centers in Rio de Janeiro (Hospital da Lagoa, UNI-RIO and Clinica Bambina). **Methods:** The medical records of 208 patients were analyzed. The patients were classified into the different diseases of the group of demyelinating inflammatory demyelinating diseases (DDII) of the central nervous system (Weisshenker, 2005). Clinical and laboratory data were used for the application of the new criteria. **Results:** Loss of follow-up occurred in 19 out of 208 (9%) patients. Of the remaining 189 patients (62, 5% white Brazilian and 37, 5% African Brazilian) in the MS centers, DDII was confirmed in 182 (96%). The patients were classified as: MS (102 relapsing-remitting MS, 24 PPMS), NMO (28 with a relapsing-remitting clinical course and three monophasic), transverse myelitis (2), optic neuritis (2), acute disseminated encephalomyelitis (6), MDEM (3) and cases with large cerebral lesions (2). The diagnosis was changed in seven cases (3%): Arnold Chiari (1), tropical spastic paraparesis (2), lacunar stroke (1), vasculitis by cocaine (1), CMS ischemic spinal cord syndrome (1) and psychiatric syndrome (1). **Conclusions:** The Poser criteria did not allow a differential diagnosis between MS and other DDII. Nowadays, the diagnosis of MS, although still based on clinical data, has stronger support from magnetic resonance imaging. Two out of three of the Brazilian patients remained with the MS diagnosis. The second most frequent disease was NMO (16.4%).

The estimated prevalence of NMO in Caucasians is 1%. MS epidemiological studies in tropical areas need to apply both criteria (MS and NMO).

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The influence of the pregnant-puerperal cycle in the natural history of recurrent neuromyelitis optica

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Background: Neuromyelitis optica (NMO) is a rare disease of the central nervous system immune mediated related to humoral immunity. It is characterized by acute index events of transverse myelitis (ATM) and optic neuritis (ON), with variable remission, followed by a monophasic or relapsing clinical course. Since there is an enhancement of humoral immunity during pregnancy, it may be expected that there is a worsening in the condition of patients with the diagnosis of recurrent NMO (RNMO) during gestation. **Objective:** To describe the influence of the pregnant-puerperal cycle in the natural history of RNMO. **Methods:** A retrospective descriptive study was conducted in patients with the diagnosis of RNMO treated at the Hospital da Lagoa (HL) in Rio de Janeiro, Brazil. A questionnaire regarding neurological manifestations in the pregnant-puerperal cycle was applied to 25 patients. **Results:** Of the 25 patients, six were not pregnant, eight had pregnant-puerperal cycle before the diagnosis of NMO and 11 were selected for the study. The series studied corresponds to the cohort of 87 patients from HL since there was no difference with reference to demographic and clinical features. The mean age of onset was 22 years and the mean age of first pregnancy was 26 years. Analyzing the first gestations of the 11 patients, we identified two patients (18.18%) that presented the pregnant-puerperal cycle after NMO diagnosis, six (54.55%) that presented the pregnant-puerperal cycle after an isolated index event (ATM or ON), two patients (18.18%) with first manifestations at the postpartum period and one (9.10%) with simultaneous index events at the puerperal period. Bouts during the pregnancy-puerperal cycle were present in nine patients (81.82%), three (27.27%) during pregnancy associated with ATM and six (54.55%) at the postpartum period with ON. Two (18.18%) miscarriages were registered and one (11.11%) neomortality in the gestations at term was observed. **Conclusions:** The neurologic event occurred at the puerperal period in almost all patients. There was an aggravation of the disease at the postpartum period in nearly half of the patients studied with a worsening on the scale of incapacity.

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HLA-DRB1 allelic frequencies in multiple sclerosis patients in Argentina

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Background: The main contribution to multiple sclerosis (MS) susceptibility in various populations from distinct ethnic backgrounds seems to be the presence of certain major histocompatibility complex (MHC) genes, particularly the class II alleles. The HLA-DRB1*1501 allele is strongly associated with MS in Caucasian patients but there is a lack of data for Argentinian patients. **Objective:** To determine the frequency of HLA-DRB1 alleles in a population of Argentinian MS patients and to estimate the risk for developing MS. **Methods:** A total of 53 consecutive patients assisted at the Italian Hospital MS Center (Buenos Aires, Argentina) with clinically defined MS (Poser criteria) were typed for HLA-DRB1 allele groups. The control group was 1216 healthy blood donors. All subjects gave their informed consent, which was approved by the local Ethics Committee. DNA was extracted from peripheral blood cells, and HLA-DRB1 alleles were typed employing polymerase chain reaction-amplified genomic DNA sequence-specific primers, by means of commercial kits. Allelic frequencies were compared between groups ('Z' test) and the odds ratio (OR) was estimated. The Stata 8.0 program was used to analyze the

data. **Results:** When each allele group was analyzed separately, HLA-DRB1*1501 was the only one that significantly differed between patients and controls. The allelic frequency for HLA-DRB1*1501 was 0.072 (13.49%) in the control group and 0.172 (33.96%) in MS patients ($P < 0.0001$). The increased frequency of HLA-DRB1*1501 conferred a higher risk ($OR = 2.51$, $P < 0.001$) for developing MS, as seen in other countries. **Conclusions:** Our findings are closely similar to other reports for Caucasian populations, emphasizing the role of this allele group in MS susceptibility. A major population is currently under study to explore the association of this allele with MS phenotypes.

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Incidence and prevalence of multiple sclerosis in Corsica: a 3-year study

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Background: The distribution of multiple sclerosis (MS) in southern Europe is not yet well known. Recent studies demonstrated that the incidence and prevalence of MS in Sardinia are higher than expected compared with those found on continental Italy. **Objective:** To determine the annual incidence and prevalence rates of MS in Corsica in a 3-year study. **Methods:** The study area (Corsica) has a population of approximately 250,000 people. The primary sources for the case study were the neurologic and motor rehabilitation departments, the French MS centers, the Corsica MS Association, private neurologists and family doctors. All patients who satisfied the Poser and MacDonald criteria for clinically definite MS, possible MS and clinically isolated syndrome (CIS) were analyzed. The study started on April 1, 2003. **Results:** Two hundred and thirty two (160 females and 72 males) were identified at the end of the third study year. The prevalence rate was 52 per 100,000 during the first year, 82 per 100,000 for the second year and 93 per 100,000 for the third year. One hundred and seventy four patients were identified for definite MS and 58 patients for the first demyelinating event (CIS). The mean annual incidence rate was 8.4 per 100,000 for the first year, 7.6 per 100,000 for the second year and 7.6 per 100,000 for the third year. **Conclusions:** These results give support to the consideration of Corsica as an area of high prevalence and incidence rates for MS.

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Comparison of optic-spinal multiple sclerosis with conventional multiple sclerosis: clinical and laboratory features

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Background: The so-called optic-spinal form of multiple sclerosis (OSMS), which is characterized by the relatively severe and selective involvement of the optic nerves and spinal cord, is relatively common in Orientals, especially Japanese, although cases of multiple sclerosis (MS) similar to those of Caucasian MS patients do exist. **Objective:** In the present study, we examined some demographic and clinical features of OSMS, and compared these features with conventional MS patients, on the basis of demographic, clinical and immunological features. **Methods:** From a total of 1706 MS patients, 2.2% had OSMS. Sixteen of these OSMS patients, who had been followed-up for at least 2 years, were included in the study, and compared with 76 patients with conventional MS. **Results:** There was a statistically significant female predominance in OSMS patients (the female to male ratio was 16 to 3.4, $P = 0.004$). There were no significant differences in age (31.94 and 33.4, respectively) and disease duration (4.4 years and 4.7 years, respectively). On the basis of oligoclonal band (OCB) positivity, there was a very significant difference between groups: 4 out of 16 patients in the OSMS group had OCBs in the cerebrospinal fluid (25%), but the ratio was significantly higher in conventional MS

patients (82%, $P = 0.002$). Disability is almost equal in the groups: the Expanded Disability Status Scale (EDSS) scores were 1.83 and 1.96, respectively). **Conclusions:** Most of the OSMS patients had comparable disability with a low EDSS score, which might have been due to short disease duration. However, the OSMS patients might have a more benign course of the disease than in the Asian population, and they may be similar to patients with the benign form of chronic MS. This is very important in understanding the heterogeneous features of MS. Extended follow-up results may help to understand the difference between OSMS and conventional MS.

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Acceptability and cross-cultural feasibility of a self-administered questionnaire on past exposure to putative environmental risk factors for multiple sclerosis

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Background: The use of self-administered questionnaires to reliably assess exposures that occurred prior to disease onset in an essential data collection component in international multi-center case control studies. **Objective:** To test the acceptability and cross-cultural feasibility of a self-administered questionnaire to assess past environmental exposures, including childhood infections and infectious mononucleosis, vitamin D through diet and sunlight, and smoking in multiple sclerosis (MS) patients and healthy subjects (HS) in Norway, Italy, Serbia and Sweden. **Methods:** A six-page questionnaire was developed in English and then translated into Norwegian, Italian, Serbian and Swedish. MS patients and HS were asked to complete it and evaluate each question using scores of 1 ('easy to understand, easy to answer'), 2 ('easy to understand, difficult to answer'), 3 ('easy to understand, impossible to answer') and 4 ('difficult to understand'). **Results:** Eighty one subjects from Norway (26 MS patients, 55 HS), 104 from Italy (23, 81), 40 from Serbia (11, 29), and 32 from Sweden (20, 12) completed and evaluated the questionnaire. Gender was known for 244 subjects, 72 men and 172 women (mean age 37.6 ± 10.5 years). Scores of 1 were reported by over 53% for recalled diet, over 70% for sun exposure, over 73% for childhood infections/comorbidity/familial disorders, and over 94% for smoking. Neither sex nor subject group was found to be related to the reported level of understanding/difficulty. On some questions, older subjects reported more difficulty. Questions on recalled sun exposure were perceived to be more difficult by Norwegians. **Conclusions:** The questionnaire has proven to be cross-culturally acceptable and feasible to complete to the same degree in MS patients and HS, and is an appropriate tool for assessing the association between MS risk and past environmental exposure in large international case-control studies. Funding sources: Fondazione Italiana Sclerosi Multipla/Banco Sardegna, Italy (grant # 14/R/2007), Helse Vest (grant # 210870/2008), University of Bergen, Norway (grant to T. Riise, 2007) **Supported by:** Fondazione Italiana Sclerosi Multipla/Banco Sardegna, Italy (grant # 14/R/2007), Helse Vest (grant # 210870/2008), University of Bergen, Norway (grant to T. Riise, 2007).

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An international case-control study of risk factors for multiple sclerosis

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Background: Recent research has raised the level of confidence in a limited number of putative environmental risk factors for multiple sclerosis (MS). While some risk factors found in early case-control studies have been confirmed in more rigorous prospective studies, these studies have not had the statistical power to examine interactions amongst these risk factors. **Objective:** To examine the independent and joint role of Epstein-Barr virus infection, vitamin D through diet and sunlight exposure, and smoking on the risk of MS. **Methods:** A case-control study that will include more than 3000 MS cases and 15,000 population controls from Norway, Italy, Sweden, Serbia and Canada is underway. A standardized questionnaire with common content for all countries that is flexible enough to accommodate the variability in risk factor distributions (for example, diet) in the different countries has been developed. **Results:** The questionnaire has been piloted in Norway, Serbia, Sweden and Italy on a combined total of 80 MS patients and 177 healthy subjects. Preliminary results indicate that both groups found the questions easy to understand. Findings from this pilot will be used to improve the questionnaire, reducing misclassification of exposures. **Conclusions:** This case-control study is an important step forward in MS epidemiological research and is the first of a new generation of MS etiological research focusing on selected risk factors that are individually supported in the literature and incorporating a conceptual model of their interaction. The conduct of the study in both high-risk and medium-risk countries using a common methodology is novel. The questionnaire developed in this study could be eventually adopted by researchers in other countries allowing comparison (and pooling) of data with this international initiative. Financial support: Fondazione Italiana Sclerosi Multipla/Banco Sardegna, Italy (grant # 14/R/2007), Helse Vest (grant # 210870/2008), University of Bergen, Norway (grant to T. Riise, 2007) **Supported by:** Fondazione Italiana Sclerosi Multipla/Banco Sardegna, Italy (grant # 14/R/2007), Helse Vest (grant # 210870/2008), University of Bergen, Norway (grant to T. Riise, 2007).

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Increased disease severity among African-Americans in a pediatric multiple sclerosis cohort

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Background: Although African-Americans (AA) have a lower prevalence of multiple sclerosis (MS) than Caucasians (CA), some reports suggest AA have an increased risk of MS-associated disability. Descriptions of interethnic differences in disability focus on adult populations with long disease duration, while differences in early MS remain unknown. A pediatric MS population is well-suited to the investigation of the early acquisition of disability, since long-term end-points, such as fixed gait impairment, have not been reached. **Objective:** To compare measures of disability, disease severity and relapse activity between CA and AA in a pediatric MS cohort.

Methods: A retrospective chart review was conducted in a south-eastern US pediatric MS cohort. Measures of disability (Expanded Disability Status Score (EDSS), functional system (FS) score), disease severity (MS severity score), and relapse activity (frequency, severity and recovery) were recorded and compared. **Results:** Patients had definite MS (n = 42) or clinically isolated syndrome with magnetic resonance imaging suggestive of MS (n = 4). The cohort included 25 AA (54.3%) and 21 CA (45.7%). AA patients were disproportionately female (AA = 20/25 vs. CA = 11/21, P <0.05). Mean disease duration did not significantly differ between the groups (AA = 2.9 vs. CA = 1.8 y, P = 0.065). At last follow-up, AA had significantly higher mean scores in brainstem (0.52 vs. 0.05, P = 0.035) and pyramidal (0.68 vs. 0.05, P = 0.005) FS, with a trend towards significantly higher cerebellar FS (0.67 vs. 0.19, P = 0.067). Median final EDSS also trended higher among AA (2.0 vs. 1.5, P = 0.097). The MS severity score permits comparison when disease duration differs. Mean severity scores trended higher among AA (4.28) than CA (3.14). Relapse frequency in the first 2 years was equal between the groups. **Conclusions:** In an ethnically mixed pediatric MS cohort, AA exhibited greater impairment in brainstem, pyramidal and cerebellar FS. These results suggest early impairment within FS commonly associated with physical disability. Relapse frequency does not appear to account for these differences.

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Apolipoprotein E genotypes and rapid progression on the expanded disability status score in multiple sclerosis

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Background: Multiple Sclerosis (MS) is a neuro-inflammatory and neurodegenerative disorder. A large body of research supports a multifactorial etiology for MS, with an underlying complex genetic component likely acting in concert with undefined environmental factors. The results of genome screen studies have shown that genetic susceptibility to MS is conferred by multiple genes. MS is characterized by chronic inflammation, demyelination and repair by remyelination. The role of apolipoprotein E (APOE) in lipid transport might influence the patient's ability to remyelinate after inflammation and thus, their recovery after MS attacks. The ApoE gene is located on human chromosome 19. The expression of any of the three alleles form gives rise to six phenotypes. **Objective:** To determine whether there is an association between APOE polymorphisms and MS severity. **Methods:** One hundred twenty seven patients with clinically definite MS were genotyped for the APOE genotypes. Kaplan-Meier analysis with the log rank test was used to evaluate the influence of APOE genotypes on the time from disease onset to Expanded Disability Status Scale (EDSS) scores 4.0 and 6.0 in all patients. The APOE genotype was determined from blood samples using validated polymerase chain reaction methods. **Results:** In this sample, 68.5% were female and 33% were DRB1*15 allele carriers. The EDSS scores 4.0 and 6.0 were reached, respectively, by 57.5% and 16.5% of patients. Frequencies for APOE genotypes were 5.5%, 1.6%, 76.4% and 16.5% for e2/e3, e2/e4, e3/e3 and e3/e4, respectively. In this sample, there was no significant effect of APOE genotypes on the latency to reach EDSS 4.0 and 6.0, not even stratifying for sex or DRB1*15 allele carriers. **Conclusions:** The association between APOE polymorphisms and disease severity in MS has been studied with conflicting results. In our sample, the APOE genotypes were not associated with a significantly faster progression of disability in MS as reported by others.

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The occurrence of angiotensin-converting enzyme polymorphism in patients with multiple sclerosis and optic neuritis

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Background: In addition to its effect on homeostasis, angiotensin-converting enzyme (ACE) has potent inflammatory potential. A polymorphism in the ACE gene characterized by insertion (I) or deletion (D) in intron 16 has been identified by Rigat et al. This polymorphism accounts for approximately 50% of the variance of serum ACE concentration. **Objective:** To investigate if the occurrence of ACE polymorphism is associated with multiple sclerosis (MS) and further, to investigate if ACE polymorphism is related to measures of disease severity, to age at onset or to disease progression. **Methods:** A total of 533 patients were recruited for genetic testing from the MS out-patient clinic in the Department of Neurology in the University Hospital of Glostrup. ACE was genotyped from DNA isolated from peripheral blood drawn in k2EDTA tanks using a commercially available DNA extraction kit. The isolated DNA was amplified using two primer sets, one covering the whole gene and the other specific for the I allele. Polymerase chain reaction products were visualized in agarose electrophoresis. **Results:** We found no difference in the occurrence of polymorphisms in patients with MS compared with healthy controls. Patients with the D/I polymorphism had a slightly lower age at onset of MS as did the other groups. Patients carrying the D/I allele more often had T2 lesions on magnetic resonance imaging (MRI) compared with patients in the D/D group, and patients carrying the I/I allele more often had IgG oligoclonal bands occurring in the cerebrospinal fluid (CSF) compared with patients carrying the D/D allele. When comparing patients with I/I, D/D and D/I genotypes in patients with monosymptomatic optic neuritis, there was no difference in the occurrence of IgG oligoclonal bands in CSF or T2 lesions on MRI. **Conclusions:** We propose a possible impact of the ACE polymorphism on disease susceptibility expressed by the lower age of onset in the D/I group, the higher frequency of patients with IgG oligoclonal bands in the CSF in the I/I group and the higher frequency of patients with MRI lesions in the brain in the D/I group.

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Epidemiological characteristics of multiple sclerosis in Peru

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Background: There is little information on the epidemiological and clinical characteristics of patients with multiple sclerosis (MS) in Peru. **Objective:** To present a study of the most important epidemiological and clinical characteristics of patients with MS in Peru. **Methods:** We studied information on epidemiological and clinical characteristics in 360 patients at 10 hospital centers and patients' associations related to MS. **Results:** Around 61% of patients studied were women. The pro-medial age was 40.17 years. Around 90% were Hispanic-Indo-American (Mestizos). Around 60% were born in Lima and 67% lived in there, the capital of Peru. Around 48% had secondary education and 46% were professionals. Some 46% had recurrent-remittent forms of the disease, 44% were secondary progressive, 6% were primary progressive while 4% were other types. Around 52% had 6 to 10 years of disease, 48% had been diagnosed between 1 and 5 years ago. Some 54% had motor symptoms in the first episode. **Conclusions:** Most of the patients were born and lived in Peru. The epidemiological characteristics of patients with MS in Peru are similar to those in other countries.

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The role of relapses affecting the long-term disability outcome in multiple sclerosis in the London Ontario database

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Background: The prevention of relapses is now a therapeutic reality. The relationship of this measure to long-term disability needs to be better understood. **Objective:** To reassess the effect of relapses on the development of advanced disability with an additional follow-up of the original cohort. **Methods:** We used the updated version of the London Ontario database comprising 25,000 patient-years of follow-up. Life table analysis was used to compare survival in patients stratified according to the number of relapses in the first year (1; 2; >2), in the second year (0; 1; >1), and total number of relapses in the first 2 years (1; 2; >2), using the following end-points: time to Expanded Disability Status Scale (EDSS) score 6–8–10, time to onset of the progressive phase and time from onset of the progressive phase to EDSS 6–8–10. Patients were also stratified according to the total number of relapses before onset of the progressive phase (1–2; 3–4; >4) to compare times from onset of the progressive phase to EDSS score 6–8–10. **Results:** Significant differences between patients stratified according to the total number of relapses in the first, second and first 2 years of the disease were observed. For the latter, mean times to reach EDSS 6 were 21.4 years; 17.9 years; 14.1 years (P < 0.001), EDSS 8: 32.3 years; 27.8 years; 20.3 years (P < 0.001), and EDSS 10: 40.0 years; 36.7 years; 28.7 years (P = 0.040), time to onset of the progressive phase: 19.6 years; 16.2 years; 14.3 years (P = 0.006), time from onset of the progressive phase to EDSS 6: 6.1 years; 5.3 years; 2.4 years (P < 0.001) and EDSS 8: 16.4 years; 14.3 years; 9.7 years (P < 0.001). Significant differences were also observed in time from onset of the progressive phase to EDSS 6 (6.9 years; 4.7 years; 3.7 years; P < 0.001) and EDSS 8 (17.0 years; 13.0 years; 13.7 years; P = 0.007) between patients stratified according to the number of relapses before the onset of the progressive phase. **Conclusions:** Relapses during the first 2 years are important determinants of time to the onset of progression. They influence time to the high EDSS levels characterizing the evolution of the progressive phase. These results confirmed and extended what was seen after 12 years of observation.

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The London Ontario Database: a descriptive analysis of 25 years of follow-up

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Background: Previous studies of the London Ontario multiple sclerosis (MS) database extensively described the disease characteristics of the patient population after 12 years of follow-up. Twenty-five years of follow-up data have now become available and a larger number of subjects have reached high levels of disability, that is, Expanded Disability Status Scale (EDSS) scores 6–8–10. **Objective:** To describe the general demographic and clinical features of the population and to assess long-term disability outcomes. **Methods:** The updated raw data underwent extensive quality check procedures at the Sylvia Lawry Centre and any inconsistent data were corrected or purged from the final dataset. We reviewed the demographic and clinical characteristics of the population and carried out a survival analysis of disability. All the analyses were performed using SPSS and R software. **Results:** The updated database comprised 1023 patients. The most common presenting symptom was sensory disturbance followed by optic neuritis and gait impairment. The mean disease duration was

24.3 years and the population at the end of follow-up was mainly composed of secondary progressive MS patients (52.2%). The rate of conversion of relapsing-remitting MS patients to progressive disease expectedly increased with duration of follow-up: more than 80% of patients converted after 25 years of disease. The mean time from onset of disease to onset of the progressive phase was 21.4 years. With increased follow-up, the distribution of disability has shifted towards higher levels with more than 50% of patients having an EDSS score between 8 and 10. The mean time to reach EDSS 3, 6, 8 and 10 was 10.9, 18.5, 28.8 and 38.4 years, respectively. **Conclusions:** The updated dataset provided valuable information on a large MS population. The large number of patients reaching high levels of disability will allow us to better elucidate the role of clinical and demographic factors affecting the long-term outcome. In-depth statistical analyses are underway.

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Nutritional status in patients with acute exacerbation of multiple sclerosis: a controlled study

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Background: Malnutrition, various vitamin and mineral deficiencies have been repeatedly reported in patients with multiple sclerosis (MS), whereas high intake of saturated or omega-6 unsaturated fats has been suggested to play an etiological role. Previous studies have yielded inconsistent results. **Objective:** The multimodal assessment of the nutritional status in patients with acute MS exacerbations compared with a group of matched healthy control persons. **Methods:** We prospectively evaluated 20 patients (16 women, 33.6 ± 10.7 years, median expanded disability status scale (EDSS) score 1.5 (1.0–7.5)) with acute MS exacerbations and a group of 20 healthy controls. In the majority of patients (n = 15), MS had been recently diagnosed. Dietary information was collected using a standardized food questionnaire. Body composition was analyzed with Bioelectrical Impedance Analysis (BIA). Numerous laboratory analyses were performed including serum calcium, zinc, copper, vitamins, hormones and fatty acids using gas chromatography. **Results:** No patient followed a specific diet. Except for one underweight woman, signs of malnutrition were absent (BMI 25.0 ± 4.3 kg/m², unremarkable BIA results). Generally, patients had an intake of saturated fatty acids exceeding current dietary recommendations, although, this was not different from the control group as well as findings in the general population. Otherwise, analysis of the nutritional intake revealed no differences between the two groups. MS patients had lower levels of linoleic acid (772 ± 200 mg/liter vs. 978 ± 184 mg/liter; P = 0.00006) and dihomo-gamma-linolenic acid (40.9 ± 15.3 mg/liter vs. 57.9 ± 23.3 mg/liter; P = 0.029), while arachidonic acid levels were comparable. Other fatty acid, vitamin, mineral and hormone levels were not different. However, in four patients, zinc, and in six patients, vitamin B12, were slightly below normal limits. **Conclusions:** In contrast to data from more severely affected MS patients, in recently diagnosed patients, malnutrition is infrequent. There were only a few differences in the nutritional status between patients and healthy controls. MS patients showed lower levels of linoleic and dihomo-gamma-linolenic acid, and a proportion of patients had abnormal low vitamin B12 and zinc levels.

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Risk factors for short-term prognosis in the first decade of relapsing-remitting multiple sclerosis

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Background: Determining which patients are at greatest risk for multiple sclerosis (MS) progression, even over the short term, is important in both clinical and research settings. We previously demonstrated a short-term association between an increased number of risk factors and progression in patients with early relapsing-remitting MS, examining age, early relapse rate, severity of initial attack and mode of initial attack. **Objective:** Using a modified set of risk factors similar to our previous work, and a larger sample of patients, with longer follow-up, we again attempted to show an increased risk of progression associated with having a greater number of risk factors in this sample. **Methods:** All patients were seen within one year of their second attack of MS (or following the first attack and diagnosis based on magnetic resonance imaging (MRI)). They were seen by the lead investigator (TS) at least twice and were followed for at least 2 years after their first attack. Data were collected on the following risk factors: (1) age greater than 40 at first attack; (2) more than two attacks in 2 years; (3) an Expanded Disability Status Scale (EDSS) score greater than 1.5 after a second attack; (4) male gender; and (5) motor symptoms at onset. Groups were defined as having low (0 or 1), medium (2), or high (3 or greater) numbers of risk factors. EDSS scores were recorded for each visit. A final sustained EDSS score was used to measure progression. **Results:** Two hundred and ten patients (160 females and 50 males) were followed for an average of 94 months (SD = 44). Final EDSS scores were rated, on average, 116 months after first attack (SD = 51), and 90 months (SD = 43) following MS diagnosis. Around 30% were over 40 at onset, 38% had more than two attacks in 2 years, 21% had an EDSS score greater than 1.5 after a second attack, 24% were male and 58% had motor symptoms at onset. Kruskal-Wallis analysis found a significant group effect (P < .0001) on final EDSS. Low, medium and high-risk group mean ranks were 74, 108, and 132, respectively. **Conclusions:** This study, using a modified group of risk factors and following patients for a longer period of time, supported our previous findings, which suggest an additive effect of individual risk factors for increasing the risk of MS progression in the short term.

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The role of interleukin-2 in the development and course of multiple sclerosis is influenced by HLA-DRB alleles in the Iranian population

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Background: Recent genome-wide association studies have shown that cytokine gene polymorphisms may play an important role in the pathogenesis of multiple sclerosis (MS). Several studies have revealed that alleles at or near the class II loci HLA-DRB1 and HLA-DQB1 contribute significantly to genetic risk. In particular, DRB1*15 is a significant predisposing factor for MS development and may influence the role of other genes. **Objective:** To study the impact of interleukin (IL)-2/-330 single nucleotide polymorphism and its combination with HLA-DRB alleles. **Methods:** We used sequence-specific, primer-polymerase chain reaction to profile the genetic polymorphism of 130 MS patients and 139 healthy controls from the Iranian population. HLA-DRB alleles were determined by standard manufacturer's protocol in all cases. Data were analyzed by STATA software with two-sided Fisher's exact test. P values less than 0.05 were assumed to be significant. To identify the influence of these genotypes on the course of the disease, patients were divided into subgroups according to their age at onset, progression index (Expanded Disability Status Scale (EDSS)/years) and type of MS. **Results:** We observed a significant increase of the T allele and T/T genotype in the -330 position of the

IL-2 gene (43 and 169 vs. 29 and 156, respectively in patients and controls, $P < 0.05$). However, this polymorphism did not affect onset, type or severity of disease. Besides the HLA-DRB alleles, the T/G genotype was less frequent among HLA-DRB1*01, DRB1*16, DRB1*07, DRB1*11, DRB1*13, DRB1*14, DRB3*01, DRB4*01 and DRB5*01 positive patients ($P < 0.05$). In addition, the DRB1*0103 allele besides the T/T genotype was significantly associated with MS (15 patients vs. 0 controls, $P < 0.0003$). Furthermore, the T/G genotype was associated with early onset in DRB1*10 positive patients and with non-relapsing-remitting disease in DRB1*15 positive and DRB5*01 positive patients. **Conclusions:** Our results suggest that IL-2 is a key cytokine in the pathogenesis of MS, particular when associated with HLA-DRB alleles in the Iranian population.

P583

Features of multiple sclerosis in Iran

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Background: Contrary to many other Asian countries, Iran has a relatively large population of patients with multiple sclerosis (MS), which during last two decades has reached almost epidemic proportions due to still unidentified factors. **Objective:** Considering differences in disease prevalence and characteristics in other Asian countries (for example, Japan), in comparison to high prevalence western countries (such as, in Europe and North America), this study aims to define the characteristics of the disease in this part of Asia. **Methods:** Data from the Iranian MS Society and local research work on MS in Iran including data on demographics, vaccinations, family history, type of disease, signs and symptoms, findings on brain and spinal cord imaging, cerebrospinal fluid abnormalities, human leukocyte antigen characteristics, treatment modalities, course and prognosis were collected and analyzed. **Results:** Prevalence was found to be around 40 in 100,000, and the mean age of onset was 26 ± 7 years; the female/male ratio was around 1.8 to 1; more than three-quarters of patients had relapsing forms of MS, and the most common presenting symptoms were optic neuritis, sensory symptoms, weakness, imbalance and vertigo. Almost 70% of patients with relapsing-remitting forms received one of the three kinds of beta-interferons; mitoxantherone was relatively widely used, but glatiramer acetate and natalizumab were not routinely available. **Conclusions:** MS in Iran has virtually the same characteristics of classic forms of the disease in western countries, and other forms like optic-spinal MS (for example, in Japan), are not seen with noticeable frequency. A sharp recent rise in the incidence and prevalence of MS in Iran suggests that the country is possibly passing through a period of high etiological factor/factors activity, which may have occurred in western countries. We hope that MS scientists, epidemiologists and clinicians from all over the world will collaborate with us to define the nature of this ambiguous illness.

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Smoking as an independent risk factor for multiple sclerosis: considering possible combined effects with the human leukocyte antigen DR15 genotype and anti-EBNA antibody titers

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Background: The human leukocyte antigen (HLA) DRBQ*1501 risk haplotype, anti-EBNA antibody titers and smoking have been consistently associated with an increased risk of multiple sclerosis (MS). Previous work has shown that HLA DR15 and anti-EBNA titers are independent risk factors, but whether the observed association with

smoking is independent of these two factors is unknown. **Objective:** To examine the interplay between smoking, anti-EBNA antibody titers and HLA DR15 on MS. **Methods:** Individual and pooled analyses were conducted among 439 cases and 861 controls from three MS case-control studies: a nested case-control study in the Nurses' Health Study/Nurses' Health Study II, the Tasmanian MS study and a Swedish MS study. Conditional logistic regression models were used to calculate odds ratios (ORs) and 95% confidence intervals (95% CIs) for the association between the three factors and risk of MS and the significance of pairwise interactions was evaluated using likelihood ratio tests. Study estimates were pooled using inverse variance weights to determine a combined effect and p-value. **Results:** Among MS cases, anti-EBNA antibody titers were significantly higher in ever smokers compared with never smokers. The positive association between anti-EBNA antibody titer and MS was stronger among ever smokers (OR = 3.9, 95% CI = 2.7, 5.7) compared with never smokers (OR = 1.8, 95% CI = 1.4, 2.3) (p for interaction=0.001). No modification or confounding was observed by HLA DR15. The positive association between ever smoking and MS was only observed among those who had high anti-EBNA antibody titers (OR=1.6, 95% CI=1.2, 2.2) and the association was not modified or confounded by HLA DR15. **Conclusions:** In this pooled study, smoking enhanced the increased risk of MS associated with Epstein Barr virus infection. A better understanding of the underlying mechanisms is required.

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Gene expression profiling from T cells in patients with a first demyelinating episode

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Background: Genetic factors are known to play a role in the pathogenesis of multiple sclerosis (MS). Gene expression profiling (GEP) gives an opportunity to identify pathological pathways, proteins and molecules in immune cells that may be involved in MS pathogenesis. We have conducted a GEP analyses of patients during their first demyelinating episode (FDE) and matched healthy controls, and also assessed for GEP differences during the first attack and first remission. **Objective:** To compare and contrast the pattern of genes expressed by peripheral blood T cells in patients with an acute FDE and in appropriately matched healthy controls. To assess the change in gene expression profile in individual patients as the FDE remits. **Methods:** We analyzed gene expression of immunopurified peripheral blood CD3+ cells in patients during, and 3 months after, an FDE and matching healthy controls. Eleven patients with FDE prior to treatment with steroids and age and sex-matched controls were recruited through Melbourne hospitals. All patients also underwent magnetic resonance imaging (MRI) with gadolinium at the same time points and 12 months after initial presentation. RNA was extracted from purified CD3+ cell samples and processed for hybridization on to Affymetrix Human Exon GeneChips in accordance with the manufacturer's instructions. Analysis of differential expression was performed using Partek Genomics suite and lists of significantly changed genes were then analyzed using Pathway Studio in order to identify regulated pathways of interest. **Results:** A large number of genes (624, all $p < 0.01$) with differential expression between the control and patient samples were identified. Genes involved in T regulatory cell function comprised a distinct group. Interestingly, there was no difference in the GEP of patients comparing samples taken during first attack and remission. **Conclusions:** This gene expression study identified a panel of candidate genes and biological pathways expressed differently in patients with early MS. The differences were detectable during relapse and remission, and no relapse-specific GEP regulation could be identified.

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WITHDRAWN

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Confirmed association between multiple sclerosis and gene variations in interleukin-2 and interleukin-7 receptors in Danish multiple sclerosis patients

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Background: A genomewide association study from the International Multiple Sclerosis Genetic Consortium has recently identified an association between several common single nucleotide polymorphisms (SNPs) and multiple sclerosis (MS). In addition to the well documented, strong association between HLA-DRB1*1501 and MS, three SNPs located in the interleukin-7 receptor α (IL7RA)(rs6897932) and interleukin-2 receptor α (IL2RA)(rs12722489 and rs2104286) genes were also found to be highly associated with MS. The reason why these receptors confer MS susceptibility is not known but the gene variations have been suggested to affect the balance between soluble and cell-bound receptors. To confirm these results, we investigated the highly associated IL7RA and IL2RA gene variants in a Danish MS sample. **Objective:** To investigate the association between the three SNPs and MS in Danish individuals. **Methods:** The case-control study involved genotyping the polymorphisms in blood samples from 504 consecutive Danish Caucasian MS patients and 541 healthy donor controls. Of the MS patients, 462 had relapsing-remitting disease onset. All individuals were genotyped using TaqMan allelic discrimination. Predesigned primers and probes were obtained from Applied Biosystems. Polymerase chain reaction (PCR) and end-point scoring were performed with a 7500 real-time PCR system. Genotype frequencies were tested for Hardy-Weinberg equilibrium and comparison of allele frequencies was performed using SPSS statistical analysis. **Results:** All three SNPs, rs6897932, rs12722489 and rs2104286 were associated with MS. The risk allele frequencies in the controls were 71.2%, 80.1% and 69.5%, respectively. In all three SNPs, the more common allele seemed to confer MS susceptibility. Odds ratios for the rs6897932, rs12722489 and rs2104286 polymorphisms were 1.06 (95% confidence interval (CI) 0.77–1.47; $P = 0.001$), 1.74 (1.02–2.96; $P = 0.007$) and 1.56 (1.08–2.27; $P = 7 \times 10^{-5}$), respectively. **Conclusions:** In this study, we confirmed the previously found association between IL7RA and IL2RA variations and MS in Danish individuals.

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Increased release of ATP from erythrocytes of people with multiple sclerosis

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Background: Increased levels of nitric oxide have been reported in various fluids obtained from people with multiple sclerosis (MS). However, the origin of this nitric oxide is not known. The erythrocyte, via its ability to release ATP, is a recognized determinant of nitric oxide production in other cell types, for example, the endothelium. Recently, C-peptide, which is co-released with insulin in the beta cells, has been shown to stimulate erythrocyte-derived ATP release due to an increase in cellular glycolysis. Interestingly, our group has discovered that this increased cellular glycolysis requires that the C-peptide be activated by a metal such as zinc. Moreover, we have found that prolactin-releasing peptide (PRP) inhibits this C-peptide-induced release of ATP. **Objective:** To determine if the erythrocytes obtained from people with MS release abnormally high levels of ATP upon deformation. **Methods:** ATP release from erythrocytes was quantitatively determined using chemiluminescence generated from the luciferin/luciferase assay. Prior to the determination, the erythrocytes were either deformed via flow-induced shear or incubated with phys-

iological levels of zinc-activated C-peptide. The incubation with C-peptide was performed in the absence and presence of equimolar concentrations of PRP. **Results:** The average ATP release from erythrocytes obtained from healthy controls was 138 +/- 21 nM while the release from erythrocytes obtained from people with MS was 375 +/- 51 nM. The error bars are reported as the SEM for 11 controls and 18 MS samples. In the presence of C-peptide, ATP release increased by 80%; however, there was no increase when the C-peptide was incubated with the erythrocytes in the absence of zinc. Moreover, this increase was significantly decreased when the erythrocytes were incubated with PRP. **Conclusions:** The erythrocytes obtained from people with MS released more than twice the ATP in comparison to healthy controls. Moreover, PRP was found to inhibit ATP release from erythrocytes that were incubated with C-peptide.

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Brain manifestations in Japanese relapsing neuromyelitis optica

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Background: The revised criterion for neuromyelitis optica (NMO) was proposed in 2006 after the establishment of an NMO IgG assay. Aquaporin-4 was also expressed in the brain and we have shown on magnetic resonance imaging (MRI) that more than half of NMO patients previously had lesions in the brain. However, little information on brain symptoms in patients with relapsing NMO has been reported. **Objective:** To evaluate brain symptoms at relapse, we examined relapsing NMO patients with anti-aquaporin-4 antibody and centrally located long spinal cord lesion. **Methods:** We reviewed the medical records of 44 consecutive patients with relapsing NMO at the national hospital between 2005 and 2007. Clinical symptoms were evaluated by brain lesions, Expanded Disability Status Scale (EDSS) and brain MRI findings. **Results:** Twenty one (47%), 20 females and one male, out of 44 patients had brain symptoms. The mean age at onset was 35.7 +/- 14.2 years, the duration from onset was 8.6 +/- 6.1 years and the mean EDSS score was 7.5 +/- 1.9. The mean duration from onset to brain symptoms was 6.1 +/- 6.6 years. Brain symptoms were presented as disturbed consciousness in seven patients (33%), dysarthria in six (29%), hemiplegia in six (29%), aphasia in four (19%), convulsion in three (14%), hemi-facial nerve palsy in two (9.5%), dysphagia in one (4.8%), left visuo-spatial neglect in one (4.8%), ophthalmoplegia in (4.8%) and hypersomnia in one (4.8%). Brain MRI at relapse revealed that 19 patients (90%) had cerebral lesions, most of which were extended in subcortical lesions and some of which involved the cerebral cortex. Nine patients (43%) had brain-stem lesions, five (24%) had diencephalon lesions and three (14%) had cerebellar lesions. These lesions were enhanced by gadolinium in more than 90% of patients. **Conclusions:** In Japanese relapsing NMO, cerebral lesions are frequently involved clinically. Further investigations of these brain attacks may clarify the nature of NMO.

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A potentiation of the adverse effect of human leukocyte antigen-DR15 on the risk of multiple sclerosis by low infant sibling exposure: a population based case-control study

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Background: The risk of developing multiple sclerosis (MS) has been associated with human leukocyte antigen (HLA)-DRB1*1501-DQB1*0602 (HLA-DR15) genotype, low infant sibling exposure and high Epstein-Barr nuclear antigen (EBNA) IgG levels. **Objective:** To examine the interplay between these three factors. **Methods:** We conducted a population-based, case-control study in Tasmania (Australia)

based on 136 cases with definite MS and 272 community controls, matched on sex and year of birth. Measures included HLA-DR15 genotype, contact with younger siblings aged less than 2 years in the first 6 years of life, EBNA IgG levels and herpes virus simplex 1 (HSV-1) serology. **Results:** The association between HLA-DR15 and MS was 3.5-fold stronger among those who had low infant sibling exposure (less than 1 year cumulative exposure) (odds ratio (OR) 7.88 (3.43 - 18.11)) compared with those who had higher exposure (OR 2.21 (1.09 - 4.47)) (test for interaction $p=0.019$). A similar pattern of HLA potentiation was observed for HSV-1 IgG seronegativity ($p=0.038$). These interactions were observed irrespective of EBNA IgG levels. MS risk was increased 19-fold for those with all three risk factors compared with those without (OR 19.17 (6.49 - 56.60)). **Conclusions:** Exposure to infant siblings in early life may provide opportunities for additional infectious exposure that may be particularly beneficial for those with a 'high MS risk' HLA genotype for antigen recognition and response. Replication and understanding of how the adverse HLA-DR15 effect on MS may be down-regulated by higher infant contact in early life is now required.

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Prevalence of multiple sclerosis in New Zealand

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Background: Multiple sclerosis (MS) is thought to result from a complex interplay of genetic and environmental factors. Knowledge of MS prevalence across a whole country can provide valuable information on environmental and genetic factors that may contribute to the risk of developing MS. One such well described factor is the latitudinal gradient of MS prevalence. New Zealand is ideally suited to an observational study examining these factors, as it has a geographically well defined and manageable population size and extends over 13 degrees of latitude (34–47 degrees south). **Objective:** To undertake a nationwide survey of all persons with McDonald criteria definite MS resident in New Zealand on census day (March 2006). To determine whether there is a correlation between age-standardized prevalence of MS in New Zealand and latitude and to describe the level of disability associated with MS in New Zealand. **Methods:** A cross-sectional study was carried out utilizing multiple sources of notification, including MS society and hospital databases, direct advertising and private practice records. All cases were confirmed as definite MS. **Results:** Twelve thousand notifications for 6000 individuals were received, from which 2920 cases of definite MS were identified. In a population of approximately 4 million, this gives an overall prevalence of close to 75 per 100,000. There was a three-fold latitudinal gradient of MS from the most northern (50 per 100,000) to the most southern parts of the country (150 per 100,000). Analysis of disability data is currently underway. **Conclusions:** These results confirm that New Zealand has a high prevalence of MS and that there is a robust latitudinal gradient of MS prevalence. To our knowledge this is the first ever nationwide MS prevalence study (as opposed to registry) ever undertaken. **Supported by:** New Zealand HRC partnership funding grant with the National MS Society of New Zealand.

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Clinical and demographic features of pediatric multiple sclerosis: preliminary data from a Latin American multinational collaborative study group

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Background: Multiple sclerosis (MS) in children is increasingly recognized worldwide. Nevertheless, pediatric MS (Ped MS) remains poorly understood, particularly in our region. The current study sought to examine clinical and demographic characteristics of Ped MS in Latin America. **Objective:** To characterize the clinical and demographic features of Ped MS in Latin America. **Methods:** Eleven sites in six countries (Argentina, Brazil, Venezuela, Mexico, Colombia and Uruguay) were involved in this study. Children aged 17 years 11 months or younger by the time of clinical onset, and fulfilling consensus definitions proposed for Ped MS were included. Each site offered data of pediatric patients from a uniform study database. **Results:** Information was collected from 122 Ped MS patients: Argentina 57, Brazil 32, Venezuela 14, Mexico 12, Colombia 5, and 2 from Uruguay. Mean age at first event was 10.3 years (range 1.1 to 17.7 years). There were 71 girls (58%), and the female-to-male ratio was 1.4. Forty-seven percent of children were of mixed ethnicity (Mestizo), 40% were white/non-Caucasian, 11% were Caucasian and 2.5% were Amerindians. The clinical presentation was: acute disseminated encephalomyelitis (ADEM)-like phenotype 23%; optic neuritis 20%; brainstem syndrome 19%; hemimotor syndrome 15%; cerebellar ataxia 10%; polysymptomatic without encephalopathy 9%; spinal cord dysfunction 5%. Mean disease duration was 8 years (range 0.5 to 45 years). Currently, 74% of patients are classified as relapsing-remitting MS (RRMS), 20% secondary progressive with relapses, 5% secondary progressive without relapses and 1% as Marburg disease. No primary progressive courses were reported. Median Expanded Disability Status Scale (EDSS) score at last visit was 3.5 (range 0 to 10). **Conclusions:** An ADEM-like phenotype was the most frequent clinical syndrome at presentation. RRMS was the most frequent clinical course reported. The higher proportion of non-Caucasians in this Ped MS study group may reflect current Latin American demographics for that age range. The present study represents the first multinational collaborative study of Ped MS in Latin America.

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Parity, education and sun exposure may predict long-term disability in female patients with multiple sclerosis

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Background: Reproductive status, educational attainment and sun exposure are modifiable lifestyle traits that have been implicated in multiple sclerosis (MS) susceptibility. The roles of these factors in long-term disability progression in MS are not well established. **Objective:** To test whether parity, education level and sun exposure are associated with long-term disability (on the Expanded Disability Status Scale (EDSS)) in female patients with MS when controlling for leading predictors of disability in multivariable analysis. **Methods:** Analysis was based on longitudinal data from the New York State Multiple Sclerosis Consortium (NYSMSC) registry comprising more than 8000 registered patients from 16 MS centers in New York State organized to prospectively collect demographic and clinical data. Parity, educational attainment and sun exposure were patient-reported whereas disability and type of MS disease were physician-recorded. To analyze the effect of parity on MS disease progression, the dataset included women age 45 and older. **Results:** Among 2935 women with a mean age 54.2 (SD 7.3), mean disease duration 18 years (SD 10.8), 20.2% were nulliparous, 48% had progressive disease at enrolment, 39% had less than a 4-year/postgraduate degree and 92% reported less than an average of 1 hour/day of summer sun exposure. Results of adjusted logistic regression showed that nulliparity (odds ratio (OR)=1.6; 95% CI 1.2, 1.9), lower educational attainment (OR=1.5; 95% CI 1.2, 1.8), and less sun exposure (OR=1.8; 95% CI 1.2, 2.8) predict worse long-term disability (EDSS score greater than 6.0) independent of age, disease duration and type of MS disease. **Conclusions:** Our data support a protective effect of at least one live birth on a long-term disability milestone of EDSS score greater than 6.0. Higher education with socio-economic implications may afford MS patients sharper awareness of healthier behaviors, treatment compliance and access to better care. A potentially protective effect of environmental sun exposure warrants further investigation and correlation to vitamin D levels.

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Pregnancy and multiple sclerosis: a complete literature review
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Background: A large number of the people that receive the diagnosis multiple sclerosis (MS) are women in their peak reproductive years. Therefore, it is important to know how pregnancy affects MS.

Objective: To determine the short- and long-term effects of pregnancy on MS and the treatment possibilities. **Methods:** A complete literature survey was carried out based on the database PubMed and additional reading of the reference lists of the papers found in the database. Key words used in the search were multiple sclerosis, pregnancy and human. This search resulted in 535 hits. After reviewing all the abstracts, 66 studies remained and of these, 32 studies from 1959 to the present time were found to be of relevance to the subject and therefore, included in the survey. **Results:** The complete review revealed a pattern of the relapse rate being decreased during pregnancy and increased in the first trimester postpartum. Furthermore, the relapses postpartum were generally worse than those during pregnancy, but the overall disability progression did not increase compared with the predicted progression of MS. Many factors need to be taken into account when assessing whether pregnancy influences the progression of MS, but no studies showed significant worsening. The long-term effect of pregnancy does not seem to influence the course of MS negatively. During pregnancy, the treatment possibilities are relapse treatment with steroids. In some cases, long-term treatment with intravenous immunoglobulin (IVIG) is used for women with relapsing-remitting MS during and after pregnancy. **Conclusions:** Pregnancy does not influence the prognosis for women with multiple sclerosis. Most women will experience a decrease in attacks during pregnancy, but an increase in the first trimester postpartum. The disability during pregnancy and postpartum increases similarly to those patients without pregnancy. The treatment during pregnancy consists of relapse treatment with steroids and potentially long-term treatment with IVIG.

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Monthly ambient sunlight, vitamin D, infections and relapse rates in multiple sclerosis

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Background: Seasonal variation in multiple sclerosis (MS) relapses has been found in the northern hemisphere. The relationship between seasonal environmental factors, infections, serum vitamin D (25(OH)D) and MS relapses is undetermined. **Objective:** To examine the relationship between seasonal environmental factors, infections, serum 25(OH)D and MS relapses in the southern hemisphere. **Methods:** We prospectively followed a population-based cohort of relapsing-remitting MS (RRMS) patients in southern Tasmania (between January 2002 and April 2005). Correlations between monthly ambient environmental factors, predicted serum 25(OH)D, upper respiratory tract infections and relapse rates were examined using weighted Pearson's correlation and linear regression. **Results:** Of 199 definite MS patients, 142 had RRMS and were followed for a mean of 2.3 years. The relapse rate exhibited a moderate peak in winter with a nadir in summer (relapse rate =1.3(95% CI: 1.0–1.8) vs. 0.9 (95% CI: 0.7–1.4) per 1000 days of follow-up). Monthly relapse rates correlated with: (1) erythemal ultraviolet radiation when lagged 1.5 months prior, $r=-0.32$, $p=0.046$; (2) upper respiratory tract infection rate (no lag), $r=0.39$, $p=0.014$; and (3) predicted serum 25(OH)D (no lag), $r=-0.31$, $p=0.057$. The association between upper respiratory tract infection rate and relapses was reduced after adjustment for monthly erythemal ultraviolet radiation. None of the other ambient environmental factors examined (including ozone, particulate matter PM10, rainfall, temperature and sea level pressure) were significantly associated with relapses. **Conclusions:** Relapse rates were inversely associated with ambient ultraviolet radiation, vitamin D levels and positively associated with upper respiratory tract infections. The demonstrated lag between ultraviolet radiation but not serum 25(OH)D and relapse rates is consistent with a role for ultraviolet radiation-generated vitamin D in the alteration of relapse rates. Future work on the association between upper respiratory tract infections

and relapses should be considered in the context of ultraviolet radiation and vitamin D.

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P596

Clinical features and natural history of primary progressive multiple sclerosis

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Background: About 10 to 15% of patients with multiple sclerosis (MS) have a predominantly progressive course from onset, although a few relapses can occur during the progressive phase (PRMS). Also, in a few instances, a single relapse may occur before the progressive phase in patients with secondary progressive MS. In the past, all patients with a predominantly progressive phase and a single attack were termed transitional MS (TPMS), the prognosis of which is supposed to be similar to primary progressive multiple sclerosis (PPMS). **Objective:** To compare clinical features of PPMS and TPMS and to investigate possible factors related to a worse prognosis. **Methods:** In a retrospective cohort study, a convenience sample was identified from PRMS patients (they could have had a single relapse) who had visited our unit at least once in the last 15 years. **Results:** Up to now, 206 (95 men, 46%) have been included: 123 patients (59.4%) had no relapses and 83 (40.6%) had one before (45 patients) or during (38 patients) the progression. Patients with PPMS were older at clinical onset than TPMS (41.0 (IQR: 35.2 - 47.6) vs 37.9 (29.3 - 44.1) years, $p = 0.03$). Median times (months [IQR]) from clinical onset to EDSS of 4, 6 or 7 were significantly shorter in PPMS than in TPMS (62 [37.25 - 102.25] vs 123.5 [51.25 - 194.75], $p=0.001$; 95 [59 - 130] vs 156 [88 - 234], $p<0.001$; and 124 [83.75 - 187.25] vs 189 [115 - 284], $p<0.001$, respectively). Nevertheless we found no differences in median ages (years [IQR]) at Expanded Disability Status Scale (EDSS) of 4 (48.2 [41.4 - 55.0] vs 48.7 [44.2 - 53.6]), 6 (49.1 [41.3 - 56.9] vs 53.6 [45.3 - 58.2]) or 7 (49.7 [43.5 - 58.4] vs 59.2 [48.3 - 62.6]) between PPMS and TPMS. **Conclusions:** PPMS and TPMS patients had a similar behaviour in terms of age at a certain EDSS score. Older age at onset and faster early worsening implied a faster late progression.

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Apolipoprotein E4 genotype does not influence multiple sclerosis phenotype, severity, cognition or brain atrophy in a large Australian cohort

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Background: The associations of the apolipoprotein E (ApoE) polymorphism with multiple sclerosis (MS) disease severity have been studied with conflicting results. Several studies have reported an association of severe clinical disease course, cerebral atrophy and cognitive decline with ApoE e4 carrier status. Other studies, including a large meta analysis, have argued against this. Conflicting results are largely due to small sample sizes and variability of the progression measures used. **Objective:** To study the association of ApoE e4 allele status and MS severity, cognition and brain atrophy in a large cohort

to clarify the conflicting results recorded in smaller studies. **Methods:** The association between ApoE e4 carrier status and four measures of disease severity was tested in 1006 patients with relapsing-remitting MS (RRMS) and secondary progressive MS (SPMS). Severity measures included: MSSS score; progression index (Expanded Disability Status Scale (EDSS)/disease duration); age at first symptom and time between first and second symptoms. The symbol digit test (SDT) was used as a single cognitive marker in 869 patients. Brain volume was measured in 792 patients using the intercaudate ratio (ICR). Linear regression was used to test whether these measures were associated with number of ApoE e4 alleles. **Results:** Genotype frequency (in (%)) was: e2/e2 7 (1%); e2/e3 108 (11%); e2/e4 22 (2%); e3/e3 642 (64%); e3/e4 207 (21%) and e4/e4 20 (2%). There was no association between disease phenotype (RRMS or SPMS) and ApoE e4 carrier status. ApoE e4 status (homozygous or heterozygous) was not associated with any of the clinical disease severity markers. There was no association of ApoE e4 carrier status with the SDT score ($p=0.38$). There was no association of ICR with e4 carrier status ($p=0.64$). **Conclusions:** The ApoE e4 polymorphism does not influence MS progression or severity. Although limited cognitive testing and magnetic resonance imaging measures were used, results from this large cohort suggest that ApoE e4 carrier status has no influence on cognition or brain atrophy in MS patients. **Supported by:** Australian National Health and Medical Research Council Grant, National Multiple Sclerosis Society (USA) funding.

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Risk of developing multiple sclerosis is associated with variants in the ST8SIA1 gene

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Background: Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system of unknown etiology with both genetic and environmental factors playing a role in susceptibility. To date, the human leukocyte antigen (HLA) DR15/DQ6 haplotype within the major histocompatibility complex on chromosome 6p is the only genetic risk factor consistently associated with MS susceptibility. **Objective:** To find a gene on 12p12 which may contribute to MS susceptibility. Here we present two independent genetic studies supporting an allelic association of MS with polymorphisms in the ST8SIA1 gene, located on chromosome 12p12 and encoding ST8 alpha-N-acetyl-neuraminidase alpha-2,8-sialyltransferase 1. **Methods:** The initial association was made in a single three generation family where a single nucleotide polymorphism (SNP) rs4762896, was segregating together with HLA DR15/DQ6 in MS patients. This data was independently confirmed in a study of 274 family trios from Australia where the association was validated in individuals with sporadic MS, showing transmission disequilibrium of the paternal alleles for three additional SNPs, namely rs704219, rs2041906 and rs1558793, with $p=0.001$, $p=0.01$ and $p=0.01$, respectively. **Results:** The peripheral blood mononuclear cells of affected individuals in this family also displayed an alteration in the distribution of the ST8SIA1 protein on the cytoplasmic membrane with co-localization of the GD3 ganglioside. These collective findings strongly implicate ST8SIA1 as a novel susceptibility gene for MS. **Conclusions:** Cell surface gangliosides represent the major class of glycoconjugates on neurons and bear the majority of sialic acid within the central nervous system. Our data suggest that ST8SIA1 is an MS susceptibility gene transmitted primarily paternally, which may be regulated by genomic imprinting. We have evidence that ST8SIA1 enzyme is mainly expressed in peripheral blood on the B cell surface membrane, thus providing support for a potential role of B cells in this putative autoimmune disease.

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Low-contrast letter acuity and retinal nerve fiber layer thickness measured by optical coherence tomography in pediatric multiple sclerosis

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Background: Research in adult multiple sclerosis (MS) cohorts has shown that retinal nerve fiber layer (RNFL) thickness and macular volume measured by optical coherence tomography (OCT) correlate well with visual function, disease duration and magnetic resonance imaging measures of brain atrophy. These measures and their relation to visual function have not been explored in the pediatric population. **Objective:** To examine the feasibility and usefulness of low-contrast letter acuity testing and OCT scanning as potential clinical trial outcomes in pediatric MS. **Methods:** Children with relapsing-remitting MS, diagnosed by standard clinical and neuroimaging criteria, were enrolled as part of an ongoing study of visual and neurologic outcomes in MS at the Children's Hospital of Philadelphia. Patients and disease-free controls, category matched for age, underwent OCT-3 scanning for each eye as well as low- and high-contrast acuity testing. **Results:** Six children with MS (12 eyes), aged 16 (6 to 17 years), and 10 controls (20 eyes) have enrolled to date. Participants tolerated vision testing and OCT well without the use of mydriatic drops. RNFL thickness is reduced among the eyes of children with MS (96.4±17.9), particularly among eyes with a history of optic neuritis (n=4 eyes, 80.6±15.4), compared with control eyes (107.2±10.4). Total macula volume was similarly lower in MS (6.7±0.27) vs. control eyes (7.02±0.25). Among visual measures, low-contrast acuity is thus far the best discriminator of MS vs. control eyes, accounting for age and within-patient, inter-eye correlations (p=0.03 for low-contrast vs. p=0.49 for visual acuity, p=0.18 for RNFL thickness, GEE models). **Conclusions:** These preliminary data indicate that visual function testing and OCT are feasibly administered in children with MS aged 5 years and older. Even at this early stage in the study, low-contrast acuity and RNFL thickness differ between MS and control eyes, suggesting that these measures have promise for future consideration as visual outcomes in pediatric MS trials.

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P600

Multiple sclerosis in United States veterans of the Vietnam era and later service: multiple sclerosis in Alaska

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Background: To our knowledge, there has been no formal survey of the frequency of multiple sclerosis (MS) in Alaska. **Objective:** To investigate the frequency of MS in Alaska among the most recent series of US veterans. **Methods:** This analysis was conducted on 4951 service-connected MS cases and their 9378 controls. All veterans entered military service between 1960 and 1994. **Results:** Among MS cases and their controls, there were 11 MS and 32 controls resident in Alaska at service entry, but only one MS and three controls had also been born there. Included were seven MS and 28 controls among white males, who provided an adjusted case-control ratio of 0.47 (95% confidence interval (CI): 0.21–1.08). Against a reported rate of 45.23 for all US white males in 1976, this would provide an MS prevalence rate of 22 per 100,000 population (CI: 9.0–46.0), but six of these seven MS patients had been born outside Alaska. Prevalence for the one native-born MS would be 3.2 per 100,000 (CI: 0.08–17.08). Twenty-one migrant veterans (14 MS, 7 controls) born in Alaska had entered service from another state; the white males (9 MS, 7 controls) provided an adjusted case-control ratio of 2.44 (CI: 0.91–6.54) and an estimated prevalence rate of 115 (CI: 56.4–218.1) per 100,000. **Conclusions:** These data provide little evidence to refute inclusion of

Alaska as a low risk area, and indicate that Alaskan migrants to high-risk coterminous US increase their risk of MS well beyond that of their new neighbors.

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P601

Six-year follow-up of familial multiple sclerosis in Germany: a population-based survey

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Background: The prevalence of multiple sclerosis (MS) in Germany is about 60 in 100,000. Data from other countries found 15% of relatives to be affected by MS. So far, neither the population-based prevalence rate of familial MS, nor its clinical long-term outcome are known for Germany. **Objective:** To conduct a population-based epidemiological study on the prevalence of familial multiple sclerosis in Germany and to obtain 6-year clinical follow-up data compared with patients with sporadic disease. **Methods:** One hundred and fifty six patients with familial MS were identified using systematic telephone surveys and published announcements in one federal state of Germany (Hessen). As controls, 284 patients with non-familial sporadic MS were randomly and blindly selected from a database of 803 MS patients. To obtain homogenous data, all 96 siblings among the 156 familial MS patients were matched 1:1 for age, gender, clinical course and disease duration with 96 sporadic MS patients. All patients were followed for a 6-year period using a validated Expanded Disability Status Scale (EDSS) telephone version to assess clinical severity. To compare both groups, the 6-year follow-up progression index (PI=Delta EDSS/disease duration in years) was used as the primary end-point. SPSS 15.0 was used for statistical analysis. The study was approved by the local ethics committee. **Results:** One hundred and fifty six patients (with 96 siblings among them) with familial MS were identified among the 6,056,000 habitants of Hessen. The prevalence rate was 1.19×10^{-5} . The mean disease duration was 17.2±7.6 years (28% relapsing-remitting MS; 69% secondary progressive MS, 3% primary progressive MS). The 6-year PI in familial MS was 0.060±0.127 and 0.054 ±0.089 in sporadic controls (p>0.05). However, time to EDSS 7.0 (mandatory use of wheelchair) showed a trend to be shorter in familial MS, than in sporadic cases (18.3±6.9 vs. 20.4±9.3 years, p=0.09). **Conclusions:** Familial MS in Germany is rare, but the clinical course seem to be similar to that in sporadic cases. Phenotypically well characterized familial cases may therefore, be a good basis for genetic susceptibility studies in MS.

P602

The paraoxonases 2 gene polymorphisms and clinical course of multiple sclerosis

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Background: According to recent studies, the altered paraoxonases (PON) activity and concomitant increase in markers of lipids peroxidation were observed in multiple sclerosis (MS) patients. Sidoti et al. (2007) found that individuals with the PON55/LM-MM genotypes had a significantly higher risk of MS. Our previous study did not revealed any correlation between PON C311S polymorphism and the risk of MS in Polish patients. **Objective:** To test the hypothesis that the polymorphism in PON2 genes may be related to the clinical characteristics of MS. **Methods:** We studied the significance of PON2:

C311S, Q192R and L55M gene polymorphisms in 266 patients (174 females) with MS and in 456 healthy controls. In the MS population, mean Expanded Stability Status Scale (EDSS) score was 2.92, mean age was 37.1 years, mean disease duration was 7.8 years. PON2 genotyping was determined by using polymerase chain reaction and restriction enzyme digestion. **Results:** The distribution of PON2 genotypes in MS was SS - 59.6%, SC - 33.1% and CC - 7.3%, whereas in the controls it was SS - 58.9%, SC - 34.3% and CC - 6.8%. For PON2 Q192R in MS patients QQ - 51.3%, QR - 41.4%, RR - 7.3% and in the controls 55.4%, 37.6% and 7.0%, respectively. For PON2 L55M in MS patients LL - 42.8%, LM - 45.2% and MM - 11.9% (controls 38.9%, 50.3% and 10.8%). According to our results, the PON2 genotypes distribution did not differ between MS patients and controls. The LL and CC genotypes in MS patients were more frequently related to the relapsing-remitting course, but without reaching statistical significance. The patients with PON55/LM-MM genotypes were characterized by later onset of the disease ($p < 0.05$) and a slightly higher relapse index and shorter disease duration. **Conclusions:** The present study does not provide a strong support for the association between PON2 polymorphisms and clinical presentation of the disease.

P603

Mortality rate and cause of death in patients with multiple sclerosis in Isfahan, Iran

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Background: Mortality studies in patients with multiple sclerosis (MS) have suggested an association with latitude, female gender, race, suicide or skin cancer. Approximately 60 to 70% of deaths can be attributed to the disease itself or its complications. There is a relative paucity of data relating to mortality and cause of death. We attempted to determine survival from disease onset, causes of death, whether deaths were related to MS or its complications and the relative mortality of male and female MS patients compared with the general population. **Objective:** To determine the mortality rate and cause of death in MS patients in Isfahan, Iran. **Methods:** Data were obtained from the Isfahan Research Center of Multiple Sclerosis, ten neurologists who looked after almost all MS patients, patients' files and death certificates from 1995 to March 2008. **Results:** There were 53 deaths among 3560 MS patients known to have MS. MS was the underlying cause of death in 47.16%. Around 52.83% deaths were related to causes other than MS. The sex ratio was 1.03 to 1, men to women. The mean age at death for women was 49.46 years (55.46 years with MS as the related cause of death and 43.46 years with death unrelated to MS) and 56.1 years for men (55.33 years for death due to MS-related causes and 56.8 years with death unrelated to MS). The most common cause of death was respiratory disease in 33.9% patients following MS, sepsis and other infectious diseases in 9.43%, cardiovascular disease in 5.66% and renal failure in 3.77%. No accidental deaths, suicides or cancers were recorded. Median survival time from onset of disease was 15.2 years for women and 24.6 years for men. **Conclusions:** Multiple sclerosis was listed as the underlying cause of death in less than a half of deceased cases, which implies that surveys that rely only on the primary cause of death may underestimate the true mortality rate. The sex ratio of incident cases was similar to that in the general population in Isfahan (1.27 to 1, men to women). The mean age at death in patients who died from MS and its complications was an average of 5.67 years lower than those who died from unrelated causes. MS was the most common cause of death followed by respiratory infection, cardiovascular disease and renal failure.

Imaging – Part2

P604

Apparent diffusion coefficient imaging characteristics of biopsy-proven acute inflammatory demyelinating brain lesions

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Background: Diffusion imaging detects pathologic processes that alter water movement. Acute, often large, demyelinating lesions are diagnostically challenging and their diffusion characteristics are poorly defined. **Objective:** To describe diffusion imaging characteristics in a cohort of biopsy-confirmed acute central nervous system (CNS) inflammatory demyelinating disease (IDD). **Methods:** Pre-biopsy brain magnetic resonance imaging (MRI) scans ($n=40$) from 30 patients with biopsy-confirmed CNS IDD (excluding acute disseminated encephalomyelitis and neuromyelitis optica) were retrospectively reviewed. Biopsy and other enhancing lesions were analyzed for size, presence of T2W hypointense rim, T1W hypointensity, enhancement pattern and qualitative apparent diffusion coefficient (ADC) patterns. Clinical/radiographic variables were correlated to ADC patterns. Longitudinal radiographic/clinical changes were characterized among seven cases with serial studies. **Results:** A broad spectrum of biopsy lesion ADC patterns was seen at a single time point: facilitated, 43% (bright with or without isointense regions); peripherally restricted, 40% (dark ring/arc with bright center); mixed, 7% (heterogeneously bright and dark), restricted, 3% (homogeneously dark) and normal, 7% (isointense). Lesions of more than 2 cm were more likely to have a region of restricted diffusion (65% vs. 0%; $p < 0.01$). Enhancing rings/arcs and hypointense T2W rims were associated with rings/arcs on ADC (intra-class correlation coefficient [ICC] 0.53; $p < 0.002$, ICC 0.55; $p < 0.001$, respectively). Other enhancing lesions were present in 52% (median 4; range 1–40). Restricted diffusion of the biopsied lesion was associated with restricted diffusion of other enhancing lesions within a given patient (ICC 0.62; $p < 0.01$). Serial MRI scans demonstrated a change in ADC intensity pattern in four out of seven patients, over a median of 18 days (range 3–44). **Conclusions:** A variety of ADC patterns can be associated with acute demyelinating brain lesions, with larger lesions more likely to demonstrate areas of restricted diffusion. Although a single time point, ADC does not reliably discriminate demyelination from other pathologies. Rapid ADC intensity changes on serial studies may help differentiate demyelinating lesions from other pathologies, including neoplasm.

P605

Diffusion tensor imaging detects abnormality of the optic tracts following unilateral optic neuritis

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Background: Afferent visual pathways show promise as a model system to monitor tissue integrity in multiple sclerosis (MS), for testing potential neuroprotective and repair strategies. Diffusion tensor imaging (DTI) of the optic tracts (OT) is one pathway-specific method which correlates with visual outcome. However, the relationship of DTI values between MS patients and controls is unknown. **Objective:** To evaluate DTI of the OT in patients who have experienced acute unilateral optic neuritis (ON) versus normal controls. **Methods:** Nineteen patients underwent DTI at least 6 months after documented unilateral ON (when ultimate level of recovery was likely to be reached). Five normal volunteers (matched for age and gender to the patient group) also underwent DTI. Scans were acquired at 3T with 2 mm isotropic voxels. Regions of interest were manually placed in the OT near the lateral geniculate nucleus, where the OT was best visualized. The differences in between-group means were evaluated using bootstrap-derived confidence intervals (due to small sample size

and non-normality of the distribution). **Results:** Left and right OT DTI values were averaged due to symmetry of the values within patients. Fractional anisotropy (FA) values (mean +/- SD) were lower and more broadly distributed in the patient group (419.4 +/- 107.9) than in normal controls (493.3 +/- 59.6). The difference in group mean OT FA was significant (mean difference = 73.9, 95% CI=7, 0-137). Mean diffusivity, transverse diffusivity and longitudinal diffusivity did not differ significantly between patients and controls. **Conclusions:** FA appears to be a sensitive DTI measure of OT damage following unilateral ON, and may be useful as a pathway-specific outcome measure. **Supported by:** National Multiple Sclerosis Society (USA).

P606

Cortical lesion load predicts atrophy in the cortex of primary progressive multiple sclerosis: a longitudinal magnetic resonance imaging study

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Background: Inasmuch as cortical atrophy is an early phenomenon in multiple sclerosis (MS) and, to a certain extent, occurs independently of white matter (WM) pathology, its pathological substrate remains to be clarified. Thanks to the double inversion recovery (DIR) sequence, focal cortical inflammation (cortical lesions (CLs)) may be disclosed in vivo in MS, even in the early phase of the disease. **Objective:** To assess whether CLs and cortical atrophy are pathologically related. **Methods:** One hundred and ninety eight patients with a diagnosis of MS, 48 with primary progressive MS (PPMS), 105 with relapsing-remitting MS (RRMS) and 45 with secondary progressive MS (SPMS), were included in the study and underwent clinical (Expanded Disability Status Scale(EDSS)) and magnetic resonance imaging (MRI) examination. All patients were seen regularly every 6 months for 2 years. At baseline and at the end of the second year, the following MRI parameters were analyzed: CL number and volume, grey matter fraction (GMf), percentage grey matter volume change (PGVC), T2-weighted WM lesion load and number of contrast enhancing lesions. **Results:** CLs were frequently observed not only in RRMS and SPMS, but also in PPMS. The correlation between CL volume and GMf at baseline was stronger in PPMS ($r=-0.631$, $p<0.001$) compared with RRMS ($r=-0.383$, $p<0.001$) and SPMS ($r=-0.486$, $p<0.001$). Moreover, the correlation between PGVC and CL volume was much higher in PPMS ($r=0.682$, $p<0.001$) compared with RRMS ($r=0.212$, $p=0.003$) and SPMS ($r=0.297$, $p=0.021$). Finally, in PPMS, a multivariate analysis confirmed that both CL volume and patients' age were independent predictors of cortical atrophy and PGVC. **Conclusions:** Our results indicated that cortical atrophy is strictly related to the development of local inflammatory lesions in PPMS. Whether CL load may play a role in predicting the development of cortical atrophy needs to be confirmed in a larger patient series.

P607

Initial brain magnetic resonance imaging presentation in pediatric multiple sclerosis patients from white non-Hispanic and other ethnic backgrounds

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Background: Pediatric-onset multiple sclerosis (POMS) may affect non-Whites more often than adult-onset MS. Non-White POMS patients may have a worse clinical phenotype from onset of the disease. The influence of ethnicity on POMS brain magnetic resonance imaging (MRI) presentation is unknown. **Objective:** To compare MRI features of initial brain scans between White non-Hispanic and other ethnic background POMS patients. **Methods:** We queried the University of California, San Francisco pediatric MS data-

base for POMS patients with a brain MRI performed within 3 months of disease onset. Initial and second brain MRI scans were reviewed for lesions that were T2 bright, ovoid and well-defined, large (greater than 1 cm), confluent, and/or gadolinium enhancing, and for lesion resolution between the first and second scans. **Results:** Eight White non-Hispanic and 19 non-White POMS patients were identified (mean age = 13.2 +/- 2.7 years vs. 10.9 +/- 4.5 years, $p=0.1$; 62% vs. 47% females, $p=0.7$). On the initial scan, White and non-White patients had similar mean numbers of distinct T2 bright (24.7 +/- 14.6 vs. 23.6 +/- 16.5, $p=0.8$), well-defined, ovoid (21.7 +/- 13.6 vs. 18.2 +/- 16.6, $p=0.6$), large (5.5 +/- 6.1 vs. 6.2 +/- 6.9, $p=0.8$) and enhancing lesions (8.7 +/- 8.9 vs. 7.9 +/- 15.1, $p=0.9$). A similar proportion of patients in each group had subcortical (87% vs. 84%, $p=1$), deep gray matter (25% vs. 37%, $p=0.4$), brainstem (50% vs. 63%, $p=0.7$) and cerebellum involvement (50% vs. 37%, $p=0.7$) and confluent lesions (0% vs. 16%, $p=0.53$). Reduction of lesion burden between the first and second MRI was present in 43% of White vs. 67% of non-White POMS ($p=0.4$). **Conclusions:** This preliminary analysis showed no difference on initial brain MRI presentation between White non-Hispanic patients and patients from other ethnic backgrounds. Larger scale brain and spinal cord MRI studies are warranted to confirm these findings.

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Cortical adaptation in patients with clinically isolated syndrome; perspectives for predicting multiple sclerosis

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Background: Potentially adaptive cortical reorganization in multiple sclerosis (MS) has been reported in various studies. **Objective:** To depict the earliest steps of brain struggle to compensate for impairments in patients with clinically isolated syndrome (CIS). **Methods:** Eighteen right-handed patients with proven CIS and disease duration of less than a month were included. Neither of the cases had motor complaints. Ten healthy right-handed volunteers with matched demographic data served as controls. Each subject was conducted to four functional magnetic resonance imaging (MRI) trials in which they were asked to perform hand flexion-extension and ankle plantar and dorsiflexion on two sides of the body. A 1.5 T magnet was utilized and image analysis was carried out by means of FSL (version 4). **Results:** Within-group analysis showed larger clusters of activation in both stimuli (hand and ankle) in the CIS group compared with the healthy subjects. Most of the foci with maximum intensity were in the ipsilateral somatic sensory area, posterior parietal cortex and cingulate gyrus. Meanwhile patterns of activation when comparing different stimuli revealed more lateralization in hand activation in both control and CIS groups. In patients, most of activations were in the precentral gyrus for the ankle stimulus but hand movements showed a dominance in the postcentral gyrus. The latter finding was not obtained when analyzing the controls. In the case of between-group analysis, CIS vs. controls showed significant differences ($p=0.013$), either in areas related to motor tasks or novel regions. The most prominent regions of differences were the supplementary motor area, premotor cortex and inferior frontal gyrus on both sides and ipsilateral precuneus cortex. **Conclusions:** These sets of results indicate early functional changes in novel areas of the brain, which were not dealing with motor duties originally as well as increasing activations of areas dedicated to performing motor tasks, implying early adaptive change in the initial steps of the disease. From the clinical standpoint, a promising pre-diagnosis of MS would be possible before overt structural lesions in MRI.

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Six-year prospective multi-voxel brain magnetic resonance spectroscopy study of two cohorts of relapsing-remitting multiple sclerosis to examine the effect of glatiramer acetate on neuronal/axonal metabolic injury

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Background: Brain 1H-magnetic resonance spectroscopy (MRS) allows in vivo examination of neuronal/axonal integrity by quantifying the neuronal mitochondrial marker N-acetylaspartate (NAA). **Objective:** To investigate the effect of glatiramer acetate (GA) on neuronal metabolic injury by performing 1H-MRS in relapsing-remitting multiple sclerosis (RRMS) treated with GA for 6 years. **Methods:** Two cohorts of treatment-naïve, RRMS-initiated GA therapy were followed with serial brain 1H-MRS using an identical multi-voxel technique within a large central white matter volume of interest. Group 1 underwent MRS scans at baseline and annually for 6 years, whereas Group 2 underwent MRS scans at baseline and then at years 3 and 6. Data acquisition and post-processing were carried out in a blinded fashion. Nine healthy volunteers were also scanned annually. **Results:** Fifty three patients underwent serial brain 1H-MRS scanning in two groups. Group 1 (n=22) had patients who started GA therapy at enrolment (n=18) and during the course of the study (n=4). Mean age, disease duration and Expanded Disability Status Scale (EDSS) score were 43.5 years, 8.2 years and 2.77, respectively. Mean NAA/Cr at baseline was 1.97 (+0.24) and 2.20 + 0.16 (+11.6%) at year 6 (p<0.05). Group 2 (n=31) comprised patients who started GA therapy at enrolment. Mean age, disease duration and EDSS score were 35.1 years, 5.8 years and 2.53, respectively. Mean NAA/Cr at baseline was 1.99 + 0.1 and 2.12 + .08 (+6.53%) at year 6 (p<0.05). Combined results from the two cohorts treated showed improvement in the mean NAA/Cr from baseline at 1.99 to 2.15 at year 6 (+8.04%, p<0.05). Further analysis will be presented. **Conclusions:** This study represented the largest cohort of RRMS patients studied with serial MRS for 6 years. The study also supported the use of NAA as a reliable marker for assessing long-term disease progression and therapeutic response. It also confirmed our previously published findings suggesting a beneficial effect of GA on neuronal metabolic function in RRMS.

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P610

Optic nerve volume and diffusivity jointly predict multifocal visual evoked potential amplitude after optic neuritis

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Background: Acute inflammatory demyelination of the optic nerve, known as optic neuritis, is a common symptom of multiple sclerosis (MS). In extant optic neuritis, there is often non-recoverable visual dysfunction. Multifocal visual evoked potentials (mfVEP) can sensitively reveal changes in visual function across the visual field. However, it is still unclear how the extent of damage within the optic nerve, measured using magnetic resonance imaging (MRI), relates to changes in mfVEP. **Objective:** To determine the relationship between macroscopic volumetric and microscopic diffusion changes to the optic nerve, and changes to visual function after optic neuritis. **Methods:** Sixteen patients with optic neuritis and ten controls underwent optic nerve MRI using diffusion tensor imaging (DTI), high resolution T1 and T2 fluid-attenuated inversion recovery (FLAIR) imaging. For each optic nerve, fractional anisotropy (FA) and volume were measured. The amplitude and latency of mfVEP and logMAR

visual acuity was recorded for each eye. The affected side was compared with both the unaffected and control, and asymmetry coefficients ($[\text{Unaffected} - \text{Affected}] / \text{Unaffected}$) were used to study the relationship between structural and functional measures. **Results:** Significant abnormalities in MRI and mfVEP measures were detected in the affected side compared with the unaffected or control. There was no relationship between optic nerve FA and volume asymmetries in patients. However, variance in mfVEP amplitude asymmetry was jointly explained by both FA and volume ($\text{AMP}_{\text{asymm}} = (0.60) \cdot \text{VOL}_{\text{asymm}} + (0.50) \cdot \text{FA}_{\text{asymm}}$; $R = 0.80$; $P = 0.001$). Reduced mfVEP amplitude was associated with reduced visual acuity ($R = -0.80$; $P < 0.001$). **Conclusions:** In extant optic neuritis, there are microscopic changes in the optic nerve, which can be detected as reduced diffusion anisotropy, and macroscopic changes detected as optic nerve atrophy. Both diffusivity and volumetric changes are important predictors of visual dysfunction.

P611

Reduced diffusion anisotropy in optic radiation correlates with optic nerve atrophy after optic neuritis

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Background: Optic neuritis (ON) results from acute inflammatory demyelination within the optic nerve. It has been shown that in extant ON, degeneration within the optic nerve causes atrophy of cells within the lateral geniculate nuclei (LGN) of the thalamus. We hypothesized that atrophy of LGN cell bodies could also result in changes in the efferent white matter tract, optic radiation and could be measured using diffusion tensor imaging (DTI) as abnormal water diffusivity. **Objective:** To study diffusivity changes in the optic radiations of patients with extant ON and relate these changes to degeneration of the optic nerve, measured as optic nerve atrophy. **Methods:** Twenty-five control subjects and 15 patients underwent whole brain DTI. All DTI images were normalized to MNI152 space. Control subjects' optic radiata were mapped using a probabilistic tractography algorithm (FSL, FMRIB, Oxford). Individual control optic radiation tract maps were thresholded, binarized and a control optic radiation probability map created. The probability map was used to sample mean diffusivity (MD), fractional anisotropy (FA) and perpendicular diffusivity within the optic radiata bilaterally from controls and patients. In addition, patients' optic nerve volumes were measured from high resolution T1-weighted images. For optic nerve volume, an asymmetry coefficient was used ($[\text{Unaffected} - \text{Affected}] / \text{Unaffected}$) for correlation analysis. **Results:** Patient optic radiation showed increased MD ($P < 0.001$) and perpendicular diffusivity ($P < 0.001$) and reduced FA ($P < 0.001$) compared with the control. Optic radiation FA was negatively correlated with optic nerve volume asymmetry ($R = -0.74$; $P = 0.002$). **Conclusions:** In patients with extant ON, there are detectable diffusivity changes in the optic radiation, which are associated with optic nerve pathology.

P612

Brain volume evaluation in neuromyelitis optica

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Background: Neuromyelitis optica (NMO) involves spinal cord and optic nerves with a relative respect of brain. However, recent work argues for a possible implication of the brain in NMO, particularly in the region with a high concentration of aquaporin 4 (AQP4). **Objective:** To evaluate possible brain atrophy in NMO. **Methods:** Twenty four patients with NMO and 24 healthy volunteers (matched

for sex and age) were studied. All patients had T1-weighted magnetic resonance imaging (MRI) with three-dimensional acquisition. We then applied SIENAX post-treatment software. We evaluated the total brain parenchymal fraction (BPF) and both white (WM) and grey (GM) matter volume. **Results:** The BPF was significantly reduced in NMO compared with the controls: 1601.6 cm³ vs. 1666.1 cm³ ($p < 0.05$). This difference was due only to WM volume reduction: 755.6 cm³ vs. 834.9 cm³ ($p < 0.05$) and not to GM volume, which showed no difference in the two subgroups: 846 cm³ vs. 831.1 cm³ (ns). **Conclusions:** Our results suggested a mild but significant brain parenchymal atrophy in NMO compared with controls. This atrophy is due only to WM atrophy, in good correlation with AQP4 localizations.

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Unexpected multiple sclerosis: a 5-year follow-up of 65 patients with magnetic resonance imaging and clinical conversion profile
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Background: Subclinical demyelinating lesions may occur in the brain of asymptomatic individuals. The concept of pre-multiple sclerosis (MS) is now well recognized with incidental diagnosis. **Objective:** To report a descriptive study of clinical and a 5-year magnetic resonance imaging (MRI) follow-up in patients with subclinical demyelinating lesions. **Methods:** All patients underwent brain MRI for various medical problems not suggestive of MS and general physicians asked for medical advice from the neurologist. When demyelinating lesions fulfilled the Barkhof/Tintoré criteria and had normal neurological examination, patients were proposed for paraclinical studies (including blood samples, cerebrospinal fluid (CSF) and visual evoked potential) and MRI follow-up. **Results:** Sixty five patients were identified: 49 women and 16 men with a mean age of 35 years (16–49). The first brain MRI was performed for various medical events. Mean time between consultation and the first MRI was 4.6 months (1–48). All patients had normal biological screening and among CSF characteristics, 26 patients had oligoclonal bands and 23 had an elevated IgG index. Mean time for the second brain MRI was 6 months (3–30). On this second MRI, 80% had temporospatial dissemination. Twenty one patients had clinical conversion: optic neuritis 6, brainstem 4, sensitive symptoms 4, transverse myelitis 5, cerebellar 1, cognitive deterioration 1. Mean time between the first brain MRI and clinically isolated syndrome (CIS) was 3.6 years (0.8–5). Ten patients were treated with disease-modifying therapies (beta interferon 5, glatiramer acetate 3, other 2). **Conclusions:** Our study confirmed the burden of the preclinical phase with an increased ratio of MRI activity to clinical relapse. MRI with hypersignals and/or gadolinium enhancement fulfilling Barkhof/Tintoré criteria and evidence of dissemination in time and space before the clinical event is more rare. To our knowledge, this is the first CIS cohort with preclinical follow-up. Discussion about early treatment can take place considering the predictive value on conversion of the MRI burden of the disease.

P614

Catastrophic onset of central nervous system demyelinating disease: acute disseminated encephalomyelitis or multiple sclerosis?

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Background: Multiple sclerosis (MS) and acute disseminated encephalomyelitis (ADEM) are demyelinating diseases with certain common clinical and radiological features. Distinguishing ADEM from a first episode of MS is often difficult. **Objective:** To describe a case of catastrophic onset of demyelinating disease (ADEM or MS), which was treated with steroids and plasma exchange (PE). **Methods:** Description of clinical features, magnetic resonance imaging (MRI), blood and cerebrospinal fluid (CSF) studies, visual evoked potentials (VEPs), treatment and evolution. **Results:** A 51-year-old man was admitted with a subacute onset of diplopia, ataxia and right sensitive disturbances. An initial MRI showed multiple white matter enhancing lesions affecting both deep and superficial regions of both cerebral hemispheres and the cerebellum. Complete blood and CSF analysis were normal including negative oligoclonal bands. He was treated with intravenous steroids but his condition worsened to aphasia, frontal syndrome, tetraparesis, spasticity, right cerebellar syndrome and complex partial seizures (Expanded Disability Status Scale (EDSS) score 8.5). A second MRI showed new enhancing lesions in the brainstem, bilateral frontal lobes and juxtacortical temporal lobe. He was treated again with intravenous steroids with neither clinical nor MRI improvement. Spinal MRI was normal and VEPs were bilaterally affected. The patient was then treated with PE and his neurological condition improved. A further MRI showed no new lesions and reduction in the enhancement and lesion load. Seventeen months later his disability is moderate (cerebellar syndrome) without other relapses and the most recent MRI shows a reduction in the lesion load and no new lesions, nor any lesions revealing gadolinium enhancement. **Conclusions:** Demyelinating diseases may start with a catastrophic and multifocal onset, which can fail to respond to intravenous steroid therapy but may respond to PE. Differentiating between ADEM and MS can be difficult due to an overlap in clinical and radiological features. In these cases, the long-term evolution will help in establishing the definitive diagnosis.

P615

Magnetic resonance imaging with non-contrast T1-weighted fat-suppression sequence

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Background: Fat-suppression (FS) pulses may create an off-resonance magnetization transfer effect. The significance of hyperintensity on this sequence is unclear. **Objective:** We studied the frequency and change over time of hyperintense brain lesions in T1-FS imaging of relapsing-remitting multiple sclerosis (RRMS) patients and their correlation with clinical and other magnetic resonance imaging (MRI) measures of disease, including hyperintense lesions on non-FS-T1 imaging. **Methods:** Sixty one patients with early RRMS (median Expanded Disability Status Scale (EDSS) score at baseline=2) enrolled in the BECOME study (BETaseron COPaxone in MS with triple-dose gadolinium and 3-Tesla MRI end-points) were imaged repeatedly from baseline (T0) to 24 months (T24). A masked examiner (SLL) determined the presence of hyperintensities in T1-FS and non-FS-T1 sequences at T0 and T24. Only hyperintensities also seen in fluid-attenuated inversion recovery (FLAIR) and T2 sequences were studied. The correlation with the number of enhancing lesions (EL), combined active lesions (CAL), new enhancing lesions (NEL), new T2 lesions (NT2), EDSS progression and clinical relapses was analyzed by Spearman's coefficient, and the association with gadolinium enhancement over time by the Mann-Whitney U test. **Results:** T1-FS hyperintensities were observed in 96% of patients at T0 and 90% at T24, and were much more frequent than

non-FS-T1 lesions ($p < 0.01$). There was a decrease in T1-FS hyperintensities from T0 to T24 [median (IQR) 4 (5) versus 3 (4), $p = 0.02$]. T1-FS lesions at T0 were significantly correlated with CAL ($r = 0.47$), NEL ($r = 0.36$) and EL ($r = 0.45$) at T0, and with CAL ($r = 0.39$) at T24. The non-remission MRI pattern was associated with more T1-FS lesions at T0 ($p = 0.042$) and T24 ($p = 0.030$). We did find a significant correlation between T1-FS lesions and annualized relapse rates ($r = 0.256$), but not with EDSS progression. **Conclusions:** T1-FS hyperintensities are much more common than T1 hyperintensities on non-FS imaging and significantly correlate with disease activity clinically and by gadolinium enhancement.

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P616

Diffusion tensor imaging measures of forniceal damage correlate with episodic memory dysfunction in multiple sclerosis patients

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Background: An estimated 50% of patients with multiple sclerosis (MS) show cognitive impairment with 30 to 40% of MS patients demonstrating specific deficits in episodic memory. Recent pathologic data demonstrates significant hippocampal demyelination in a subset of patients with MS however, hippocampal white matter changes are difficult to evaluate using DTI due to the structure of the hippocampus. The fornix is the primary efferent of the hippocampus and well suited for evaluation with diffusion tensor imaging (DTI). The fornix has been strongly linked to memory function in a variety of disease processes. **Objective:** We hypothesize episodic memory dysfunction in MS patients dysfunction will be correlated with forniceal damage demonstrated by DTI changes, specifically, increased in axial diffusivity (λ_2) and reduced fractional anisotropy (FA). **Methods:** Fourteen MS patients with relapsing-remitting MS were studied using DTI and a battery of neuropsychological tests, including the California Verbal Learning Test (CVLT) and Brief Visual Memory Test (BVMT). Diffusion-weighted imaging used 71 non-collinear diffusion-weighting gradients (2.5x2.5x2.5 mm voxels, $b = 2000$ sec/mm², 8 $b = 0$ acquisitions). Regions of interest were drawn in the crus of the fornix and average values for FA, mean diffusivity, λ_2 and longitudinal diffusivity (λ_1) were measured. **Results:** Verbal episodic memory dysfunction measured by the CVLT demonstrated a strong correlation with reduced FA ($r = 0.652$, $p < 0.01$) and increased λ_2 ($r = 0.630$, $p < 0.008$) within the left fornix. Interestingly, in measures of spatial memory dysfunction, the BVLT demonstrated strong correlation with FA in the right fornix ($r = 0.766$, $p < 0.001$). **Conclusions:** Findings demonstrated strong correlations between decreased verbal episodic memory and diffusion abnormalities within the left hippocampus. Potential drawbacks to the study include confounding factors related to hippocampal atrophy and possible partial volume averaging of adjacent structures during DTI measurements. Further study using high-resolution DTI is required to confirm these results. Overall findings suggest deficits in episodic memory in MS subjects are strongly correlated with DTI measures of forniceal damage.

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P617

Patients with multiple sclerosis and trigeminal neuropathy: magnetic resonance imaging findings

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Background: Trigeminal neuropathy and multiple sclerosis (MS) are frequently associated. MS is the most frequent cause of brainstem lesions causing trigeminal neuralgia. Trigeminal neuralgia occurs in 1

to 2% of patients with MS. Only a few case reports and small series have described lesion topography on magnetic resonance imaging (MRI) in patients with MS and trigeminal neuropathy. **Objective:** To study the MRI characteristics of trigeminal lesions in MS. **Methods:** A retrospective analysis of 456 consecutive patients who underwent MRI for trigeminal neuropathy was performed. Patients with brainstem lesions suggestive for demyelination were selected. **Results:** Thirty one patients were identified. Seventeen patients (54%) had a linear plaque over the course of the fascicular part of the trigeminal nerve, 16 showed involvement of the spinal nucleus and tract (51%), 10 (32%) had a lesion of the principal sensory nucleus and the mesencephalic nucleus was involved in 7 patients (22%). One patient (3%) had contrast enhancement of the cisternal part of the trigeminal nerves. Eleven patients (35%) showed multiple lesion localizations. Twenty two out of 31 selected patients (71%) had trigeminal neuralgia, 6 (19%) had hypoesthesia and 2 (6%) had paresthesias. Twenty two had McDonald definite MS, 3 had clinically isolated syndrome with MRI dissemination in space and 7 patients had an isolated lesion. Twelve out of 17 patients (71%) with linear T2 hyperintensity of the fascicular part of the trigeminal nerve had McDonald definite MS. Eighteen out of 23 patients (78%) with T2 hyperintensity of one or several of the trigeminal nuclei had McDonald definite MS. **Conclusions:** A linear T2 hyperintensity along the fascicular part of the trigeminal nerve, with or without the involvement of the trigeminal nuclei, is highly indicative of MS.

P618

Diffusion-weighted imaging in non-compressive myelopathies: a 32-patient study

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Background: DWI (diffusion-weighted imaging) is frequently used for differentiation between cerebral lesions. However, the use of this sequence remains confidential in spinal cord pathology. **Objective:** To evaluate the diagnostic value of DWI and the measurement of apparent diffusion coefficient (ADC) in explorations of non-compressive myelopathies. **Methods:** Thirty two patients presenting a medullary syndrome due to a non-compressive myelopathy underwent spinal cord magnetic resonance imaging (MRI) between September 2005 and November 2007. For each patient, the ADC was calculated in pathological spinal cord. ADC values were also measured in healthy spinal cord of ten control patients. Statistical analysis was based on Student's t-test. **Results:** Fifteen patients presented an inflammatory myelopathy. Nine of these 15 patients presented a multiple sclerosis (MS), two patients an acute disseminated encephalomyelitis, one patient a neuromyelitis optica, one patient a systemic lupus erythematosus and two patients a myelopathy of unknown etiology. Five patients presented a spinal cord infarction. Six patients presented a parainfectious myelopathy. The remaining six patients had other etiologies. ADC values were significantly higher in inflammatory (mean ADC = $1.32 \pm 0.18 \times 10^{-3}$ mm²/sec) and parainfectious (mean ADC = $1.47 \pm 0.28 \times 10^{-3}$ mm²/sec) spinal cord lesions than in control healthy spinal cord (mean ADC = $0.93 \pm 0.07 \times 10^{-3}$ mm²/sec). ADC values were significantly lower in spinal cord infarction (mean ADC = $0.79 \pm 0.04 \times 10^{-3}$ mm²/sec). However, ADC measurements results did not show a significant difference between inflammatory and parainfectious myelopathies. **Conclusions:** These results are important to differentiate ischemic from inflammatory myelopathies when clinical presentation and extensive work-up are not able to make an etiologic diagnosis. Moreover, inflammatory and parainfectious myelopathies have the same presentation on DWI suggesting a similar physiopathological mechanism. If these results are similar to those described in cerebral explorations, ADC measurements remain for the moment limited because of technical reasons.

P619

Cognitive impairment in early multiple sclerosis: role of conventional and multimodal quantitative magnetic resonance imaging
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Background: Conventional and contrast-enhanced magnetic resonance imaging (MRI) cannot fully explain the commonly observed cognitive impairment in multiple sclerosis (MS) patients. So far, little is known about the stage at which tissue abnormalities occur. Cognitive studies with quantitative MRI measurements in early MS revealed inconsistent results. **Objective:** To evaluate the relationship between cognitive deficits in early MS, conventional and multimodal quantitative MRI. **Methods:** MRI brain scans were acquired from 47 patients within 3 months of the first MS-related clinical event. All patients underwent a neuropsychological assessment and were allocated into groups according to their cognitive performance. T2-hyper-intense lesion volume (LV), T1 LV and gadolinium-enhancing LV measurements were performed. Magnetization transfer ratio (MTR) histogram analysis, mean diffusivity (MD) and fractional anisotropy (FA) quantities and histograms of the normal-appearing white matter (NAWM) and grey matter (GM) were calculated. Additionally, single-voxel proton magnetic resonance spectroscopy (MRS) of the corpus callosum was performed with measurements of relative concentrations of N-acetylaspartate (NAA), creatine (Cr) and calculation of the NAA/Cr ratio. **Results:** Around 47% of MS patients had cognitive impairment. In this group, we found a significantly reduced NAWM-MTR compared with cognitively unimpaired MS patients ($p=0.03$). This was substantiated by MRS that differentiated significantly NAA concentration in the corpus callosum in patients with mild compared with more severe cognitive impairment ($p = 0.04$). Lesion quantification of T2 and T1 lesions did not significantly separate cognitively impaired from unimpaired patients. **Conclusions:** Cognitive deficits in patients after the first clinical attack are frequent and may be underestimated. While conventional MRI scan of the brain does not provide supportive evidence for cognitive impairment, MTR and MRS separated the cognitively impaired from the non-affected group early in the disease course. Our data depict the role of diffuse microscopic brain damage in the early stage of disease.

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P620

Apparent diffusion coefficient demonstrates greater sensitivity to multiple sclerosis lesions than fractional anisotropy and conventional magnetic resonance imaging
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Background: Multiple sclerosis (MS) is the most common cause of neurological problems in young adults. Up to one-third develop inter-nuclear ophthalmoplegia (INO) at some point. Lesions causing INO are located within the medial longitudinal fasciculus (MLF). The relative efficacy of conventional and more sophisticated magnetic resonance (MR) techniques to demonstrate these lesions is unknown. **Objective:** To investigate the relative specificity of diffusion tensor imaging (DTI) in the detection and quantification of MLF disruption in INO subjects. **Methods:** We compared the ability of DTI techniques to display MLF lesions with that of T2-weighted, proton density and fluid-attenuated inversion recovery (FLAIR) MR sequences. Twelve patients displaying clinical signs of INO and 12 control subjects underwent all procedures. Apparent diffusion coefficient (ADC) and

fractional anisotropy (FA) values were recorded in regions of interest extending from the medulla to the mid-brain. Reconstructed fiber tracts were validated by a neuroanatomist. Identification of lesions within the MLF region on conventional magnetic resonance imaging (MRI) was performed by a neuroradiologist. **Results:** DTI identified areas of abnormality in the MLF region for all INO subjects, while the MLF in 50% of INO subjects was reported as normal following conventional imaging. Subjects demonstrated significantly reduced ADC values compared with healthy controls on both sides: (right INO = 1.0166; right control = 0.9318; $p = 0.019$) (left INO = 0.9771; left control = 0.9286; $p = 0.029$). Lower FA values between subjects compared with healthy controls were not found to be significant: (right INO = 0.3772; right control = 0.3996) (left INO = 0.3907; left control = 0.3982). **Conclusions:** Compared with conventional MRI, ADC values identify areas of abnormality with greater sensitivity, allowing confirmation of lesions for a range of clinical signs. We propose that DTI could enable more accurate monitoring of disease progression, evaluate response to therapy and guide treatment choices.

P621

Voxel-based analysis of diffusion tensor magnetic resonance imaging-metrics of the corpus callosum in benign multiple sclerosis: relation to cognitive impairment

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Background: Cognitive impairment (CI), which has been reported in benign multiple sclerosis (BMS) patients, can be one of the major factors in determining their quality of life. Although numerous magnetic resonance-based studies have assessed the relationship between brain damage and CI in multiple sclerosis (MS), the mechanisms of such a dysfunction are still unclear. **Objective:** To investigate the relationship between the cognitive profile of BMS patients and the extent of tissue damage in the corpus callosum (CC) using voxel-based (VB) analysis of metrics derived from diffusion tensor (DT) magnetic resonance imaging (MRI). **Methods:** Conventional and DT MRI scans were acquired from 54 BMS patients (disease duration more than 15 years and EDSS score more than 3.0) and 21 healthy controls (HC). Neuropsychological tests (NPT) exploring memory, attention and frontal lobe cognitive domains were administered in patients. Mean diffusivity (MD), fractional anisotropy (FA) and T2-visible lesion maps were transformed into the standard space. In addition, a map of fiber bundle atrophy was calculated from the transformation. These maps were smoothed before VB statistical analysis limited to the CC. **Results:** Nine BMS patients (17%) had an abnormal performance in three or more NPT, fulfilling criteria for CI. VB analysis of CC changes between HC and BMS patients displayed widespread significant increased MD and decreased FA in BMS patients, while atrophy was restricted to the splenium. Compared with cognitively preserved patients, CI had increased MD in the right body, left splenium and left genu of the CC and higher lesion occurrence bilaterally in the splenium and body of the CC, which were close but not overlapped to MD clusters of abnormalities. PASAT performance correlated with FA changes in the majority of CC portions, while for the MD map this correlation was found only in a few clusters. **Conclusions:** Cognitive dysfunction in BMS is associated with an increased MD and higher lesion occurrence inside the CC.

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Magnetic resonance disease severity scale for patients with multiple sclerosis: a longitudinal study

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Background: We previously described a composite cerebral magnetic resonance imaging (MRI) scale combining T1 lesions, T2 lesions and whole brain atrophy in multiple sclerosis (MS) patients: the magnetic resonance disease severity scale (MRDSS). **Objective:** To test the strength of the MRDSS vs. individual MRI measures for sensitivity to longitudinal change and predicting clinical progression. **Methods:** In the comprehensive longitudinal investigation of MS at Brigham, we studied 103 patients (age (mean \pm SD) 42.7 \pm 9.1 years, EDSS score 3.3 \pm 2.2, 60% (n=62) relapsing-remitting, 32% (n=33) secondary progressive, and 8% (n=8) primary progressive). During follow-up of 3.2 \pm 0.3 years, 24 patients developed sustained disability progression. Baseline and follow-up brain MRI derived T2 hyper-intense lesion volume (T2LV), T1 hypo-intense lesion volume (T1LV), and brain parenchymal fraction (BPF). The ratio of T1LV to T2LV assessed lesion severity. The MRDSS for each patient was the combination of T2LV, BPF and T1/T2 ratio. Wilcoxon signed rank test assessed change in each measure over follow-up in 31 patients in whom analysis has been completed. **Results:** Patients had higher (worse) MRDSS at follow-up (5.75 \pm 2.3) vs. baseline (5.05 \pm 2.4) ($p < 0.00001$). For individual MRI standardized components of MRDSS, BPF decreased ($p = 0.010$), T1/T2 increased ($p = 0.0014$), but T2LV was unchanged ($p = 0.66$). Similar results were obtained for unstandardized MRI components. Change in MRDSS was larger than change in individual MRI components (i.e. 95% confidence interval for MRDSS did not overlap with confidence interval for individual MRI measures). Ongoing analysis will assess the full cohort allowing comparisons between change in MRI vs. disability over 3 years. **Conclusions:** These results suggest the improved sensitivity to longitudinal change of the MRDSS vs. individual MRI measures of lesions and atrophy. MRDSS shows potential as a tool to monitor disease evolution in MS.

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Statistical modelling of magnetic resonance imaging T2 parameters to determine precise cut-off points predictive for a second clinical event in clinically isolated syndrome patients at high risk

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Background: It is generally accepted that cut-off points (COP) for T2 lesion numbers (T2N) in clinically isolated syndrome (CIS) patients at high risk is nine, and this figure has been introduced into the selection criteria of a number of trials in CIS patients. Today, however, there are no robust data that mathematically address this figure with adequate statistical modeling, and no validation of its predictive value has been performed. **Objective:** To evaluate the COPs for a categorization of the number of T2N and T2 lesion volume (TLV) for the prediction of the occurrence of a second clinical event in CIS patients. **Methods:** We performed meta-analyses with 125 placebo patients of two CIS clinical trials with T2N and TLV baseline information. In iterative procedures (IP), categorizations for these magnetic resonance (MR) parameters were tested with Cox PH models and survival curves to find optimal COPs. In a second step, the predictive power of the identified COPs was tested in a multivariate Cox PH model that additionally included demographic, clinical baseline parameters and degree of recovery from the first attack. **Results:** A second clinical event was observed in 62 (49.6%) of the CIS patients, median time to event was 2.4 years. The median T2N at baseline was 17 (mean 22.8) with a corresponding volume of 2.83 cm³ (mean 5.68). A moderate

correlation between the T2N and TLV at baseline was found ($r=0.68$, $p < 0.0001$). The optimal COP for T2N was nine (HR=2.62 for >9 lesions, $p=0.007$) and 2 cm for the cube root TLV (HR=1.67 for ≥ 2 cm, $p=0.099$). Both MR parameters were not significant when demographic and clinical variables were added in a multivariate model. Strongest predictor was the degree of recovery from the first attack. **Conclusions:** Iterative procedures confirmed the figure of nine as the optimal COP for T2N. In multivariate analyses, neither T2N nor TLV had a significant impact on the prediction of a second clinical event in CIS patients, when the degree of recovery from the first attack was considered. After performing a complete validation process with a second 'closed' dataset however, the degree of clinical recovery showed only a strong trend as an outcome predictor.

P624

Migration of transplanted neural precursors during the chronic phase of experimental allergic encephalomyelitis is reduced compared with the acute phase

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Background: During the acute inflammatory phase of experimental allergic encephalomyelitis (EAE), intracerebroventricular (ICV)-transplanted neural precursor cells (NPCs) migrate in response to signals present within the inflamed central nervous system (CNS) (ref. 1) and contribute to the attenuation of inflammatory markers and EAE symptoms (ref. 2). (1) Ben-Hur T, van Heeswijk RB, Einstein O et al., Magn. Reson. Med. 2007; 57: 164–171. (2) Einstein O et al. Exp. Neurol. 2006; 198, 275–294. **Objective:** To investigate the migration and therapeutic value of NPC transplantation during the chronic phase of disease. **Methods:** To compare the biodistribution of NPCs delivered ICV during either the acute inflammatory (n=7) or the chronic (n=6) phase of EAE, we transplanted Feridex-labeled NPCs and applied serial (day 1, 3 and 7), non-invasive magnetic resonance (MR) cell tracking. Control animals without disease (n=5) were included. **Results:** Starting at day 3, in vivo and ex vivo magnetic resonance imaging (MRI) revealed that NPCs migrated extensively along the corpus callosum in acute EAE whereas, in chronic EAE, cell migration was less extensive and limited to regions of the corpus callosum contralateral to the site of cell injection. In some acute EAE brains, NPCs were found to exit from caudal regions of the corpus callosum and enter the brain parenchyma by radial migration into regions of the somatosensory cortex. **Conclusions:** Cell migration and distribution were determined by the phase of EAE as mediated by inflammatory factors within the micro-environment of the CNS. These findings have important ramifications for clinical translation of NPC therapy in that the cellular distribution and potential disease attenuation will depend on the clinical presentation and disease status of MS.

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P625

Longitudinal cortical thickness measurement in patients with multiple sclerosis

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Background: Cortical thinning has been reported to occur in multiple sclerosis (MS) patients, but precise, validated measurement methods are needed for quantitative studies. **Objective:** To report the sensitivity and precision of a new algorithm for detection of cortical thinning in MS patients; to compare the degree of cortical thinning between different MS groups and healthy controls (HC); and to determine the clinical and magnetic resonance imaging (MRI) correlates of cortical thinning. **Methods:** MS patients were selected based on their atrophy rates from a cohort of 87 patients participating in a 4-year

atrophy study. Group A included the five patients with the highest rates of whole brain atrophy; Group B, the five patients with the highest rates of gray matter atrophy; Group C, the five patients with the lowest rates of whole brain atrophy. Age-matched HC were selected for comparison. A new cortical longitudinal atrophy detection algorithm (CLADA) was used to measure cortical thickness. CLADA uses T1-weighted images from all time-points to create a subject-specific cortical model, which is deformed to fit the image of each time-point. CLADA's scan-rescan reproducibility is 0.55%. **Results:** Cortical thinning was observed in all groups. Mean annual rates of cortical thinning (SD) were -0.629% (0.57) in MS patients and -0.305% (0.28) in HC. Mean annual rates of cortical thinning for each MS subgroup were -0.897% (0.61) for those with highest rate of whole brain atrophy, 0.749% (0.58) in those with highest rate of gray matter atrophy, and -0.215% (0.30) for those with the lowest rate of whole brain atrophy. **Conclusions:** The new method was sensitive enough to detect cortical thinning in MS patients compared with the controls, and results correlate with the severity of brain atrophy. These results provide important evidence to validate the method. MRI and clinical correlates of cortical thinning will be presented.

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P626

3T magnetic resonance imaging relaxometry detects T2 prolongation in the global and regional brain normal-appearing white matter in multiple sclerosis

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Background: 3T magnetic resonance imaging (MRI) boosts the sensitivity in detection of overt multiple sclerosis (MS) brain lesions, but its role in detecting damage in the normal-appearing white matter (NAWM) has not been established. **Objective:** To assess the ability of 3T MRI-based relaxometry to detect T2 prolongation in NAWM in MS. **Methods:** We tested if 3T MRI R2 relaxometry detected damage in NAWM of MS patients (n=13) vs. age-matched normal controls (NL) (n=11). Baseline characteristics of the MS group were: age (mean SD) 42.5±5.4 (range 33–51 years), disease duration 9.0±6.3 (range 1–22 years), expanded disability status scale (EDSS) score 2.5±1.7 (range 1–6.5), 11 relapsing-remitting, one primary progressive and one secondary progressive. R2 (1/T2) brain maps, created from 3T axial images at two spin echo times, were segmented to derive global and regional (region-of-interest-derived) cerebral R2 histograms of the NAWM. The regional NAWM areas were frontal lobe, parietal lobe, pons and the callosal splenium and genu. **Results:** Mean NAWM R2 relaxation rate was lower (indicating T2 prolongation) in MS than NL in the whole brain (p=0.00047), frontal lobe (p=0.00023), parietal lobe (p=0.0088) and callosal genu (p=0.0026). Similarly, R2 histogram peak position was lower in NAWM in MS than NL in the whole brain (p=0.019), frontal lobe (p=0.0011), callosal genu (p=0.0034) and splenium (p=0.034). No significant correlation with clinical characteristics was found with any of the R2 histogram metrics in this small sample. We continue to accumulate additional subjects to confirm and extend these findings. **Conclusions:** 3T MRI R2 relaxometry can detect tissue damage in the global and regional cerebral NAWM of MS patients that is missed by conventional lesion measures. Such findings may represent demyelination, inflammation and/or axonal loss.

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P627

Choice of echo time in the study of metabolic alterations in normal appearing white matter

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Background: 1H-magnetic resonance spectroscopy (MRS) was proven to be useful in the study and quantification of microscopic disease in the normal appearing white matter (NAWM) of patients with multiple sclerosis (MS). The result obtained by 1H-MRS depends strongly on the echo time (TE) used. With a short TE (20–35 ms), we are able to detect a larger number of metabolites; a longer TE (≥ 135 ms) presents fewer overlapping metabolites facilitating its quantification. What is of more advantage for the study of NAWM still needs to be elucidated.

Objective: To compare 1H-MRS results in the NAWM of MS patients for short and long TE, and to determine which is more sensible to detect metabolic alterations. **Methods:** Sixteen patients with MS and 15 controls of matched age performed single voxel 1H-MRS 1.5 T examination of the NAWM using the PRESS technique with two different TEs (30 and 135 ms). The 2 x 2 x 2 cm³ 1H-MRS voxel was placed either in the region of frontal or parietal white matter using a TR=1500 ms. 1H-MRS raw data were processed and analyzed using the LCModel software and for each voxel absolute values of N-acetyl aspartate (NAA), Cr, Cho, ml and Glx were obtained. Results were compared for controls and patients for each TE. **Results:** Only with a long TE was it possible to observe a decrease of NAA in the NAWM of patients (p ≤ 0.05). Other metabolites were not different. For short TE, no statistically significant differences between controls and patients were observed. **Conclusions:** In order to observe a NAA decrease in the NAWM of MS patients, long TE 1H-MRS was more sensible. However, it still needs to be elucidated if this decrease really reflects neuronal damage or if it is a T2 relaxation effect.

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N-acetyl aspartate decrease in normal appearing white matter: neuronal damage or T2 effect?

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Background: 1H-magnetic resonance spectroscopy (MRS) showed that N-acetyl aspartate (NAA) is decreased in the normal appearing white matter (NAWM) of multiple sclerosis (MS) patients, reflecting neuronal loss or dysfunction. Most 1H-MRS studies in MS are performed with a long echo time (TE ≥ 135 ms). The disadvantage of a long TE is that metabolite intensities in the spectrum are strongly affected by T2 relaxation effects and knowledge of T2 is needed to estimate concentrations. Individual T2 measurement is very time-consuming, so generally a common T2 value is assumed for all subjects. Therefore, it should be tested whether T2 is not different in MS disease. **Objective:** To estimate T2 metabolite relaxation times in the NAWM of MS patients and to determine whether they are different from controls. **Methods:** Sixteen patients with MS and 15 controls of matched age performed single voxel 1H-MRS 1.5 T examination of the NAWM using the PRESS technique with two different TEs (30 and 135 ms). The 2x2x2 cm³ 1H-MRS voxel was placed either in the region of the frontal or parietal white matter using a TR=1500 ms. 1H-MRS raw data were processed and analyzed using the LCModel software and absolute values of NAA, Cr and Cho were obtained. T2 calculation was performed assuming a mono-exponential T2 decay of the metabolite signal. Statistical analysis was applied in order to detect differences between patients and controls. **Results:** All metabolites studied presented shorter T2 relaxation times in patients when compared with controls. This decrease was statistically significant (p ≤ 0.05) for NAA

and Cho in the frontal region and for NAA and Cr in the parietal region. **Conclusions:** Metabolite T2 relaxation times are reduced in the NAWM of MS patients and this can lead to underestimation of metabolite concentrations when interpreting long TE 1H-MRS results. **Supported by:** FAPESP grant #99/03597-9.

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Conventional and non-conventional quantitative magnetic resonance imaging outcomes differ when comparing multiple definitions of benign multiple sclerosis

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Background: Although benign multiple sclerosis (BMS) has been recognized for more than 50 years, a precise and reliable definition has yet to be elucidated. **Objective:** To investigate differences in quantitative magnetic resonance imaging (MRI) outcomes using four different definitions of BMS. **Methods:** A cohort of 730 consecutive multiple sclerosis (MS) patients enrolled at one site in the New York State Multiple Sclerosis Consortium (NYSMSC) were classified as BMS based on their Expanded Disability Status Scale (EDSS) score and disease duration in years (ddy) using four common literature definitions (number of BMS cases ranged from 184 to 78): BMS-1 (EDSS score ≤ 2 , ddy ≥ 10), BMS-2 (EDSS score ≤ 3 , ddy ≥ 10), BMS-3 (EDSS score ≤ 3 , ddy ≥ 15) and BMS-4 (EDSS score ≤ 2 , ddy ≥ 15). Primary MRI outcomes consisted of: T2-lesion volume (LV), T1-LV, gadolinium-LV, whole brain atrophy (NBV) and whole brain magnetization transfer ratio (MTR). Secondary MRI outcomes comprised a number of non-conventional MRI measures in gray matter (GM) and white matter (WM) tissue compartments. Differences in BMS vs. non-BMS groups for each definition were assessed using multivariate analysis of covariance procedure corrected for age, sex and total months of disease-modifying treatment. **Results:** NBV was significantly higher only in the BMS-2 definition. BMS-1, -2 and -4 definitions had significantly lower T2- and T1-LVs in comparison to non-BMS groups. BMS-3 was the only definition that did not reflect any significant differences for primary MRI outcomes. More robust differentiation of BMS vs. non-BMS groups was possible using non-conventional MRI measures. In particular, GM-based measures were significantly higher in all BMS definitions, except for BMS-4. **Conclusions:** Differences in primary and secondary MRI outcomes of BMS vs. non-BMS are affected by the particular definition used. Non-conventional MRI measures can contribute to better differentiation between BMS and non-BMS.

P630

Protective clinical, demographic and conventional and non-conventional magnetic resonance imaging predictors of benign multiple sclerosis: a large cohort study

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Background: Benign multiple sclerosis (BMS) is the mildest form of multiple sclerosis (MS) that is clinically apparent. Due to lack of general consensus regarding what constitutes the BMS phenotype, specific diagnostic criteria and definitive protective predictive factors have not been identified. **Objective:** To determine protective clinical, demographic and conventional and non-conventional magnetic resonance imaging (MRI) predictors of BMS vs. non-BMS in a large cohort of patients. **Methods:** Analysis of clinical, demographic and MRI characteristics of BMS (n=78) vs. non-BMS (n=444) groups in a consecutive cohort of 730 clinically definite MS patients enrolled at one site in the New York State Multiple Sclerosis Consortium (NYSMSC)

was conducted. Clinical and MRI characteristics of BMS (Expanded Disability Status Scale (EDSS) score ≤ 2 , disease duration ≥ 15 years) vs. non-BMS were compared using two steps. In the first step, univariate and multivariate analyses were conducted. The variables that were found to be significant in the first step were entered into a binary logistic regression model to identify the best clinical and MRI protective predictors of BMS vs. non-BMS. Odds ratio (OR) and 95% confidence intervals were calculated. **Results:** Lower T1-lesion volume (p = 0.012), white matter volume (p = 0.017), age at onset (p < 0.0005) and having a relapsing-remitting MS (RRMS) course (p < 0.0005) were significant protective predictors of BMS vs non-BMS. When two groups were matched for disease duration (≥ 15 years), higher cortical volume (p = 0.004), lower age at onset (p < 0.0005), and having RRMS (p < 0.0005) were protective predictors. **Conclusions:** Non-conventional MRI measures may contribute to identifying protective factors of BMS vs. non-BMS. Lower rate of cortical atrophy in disease duration matched analysis model was the most protective MRI outcome for the BMS group.

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SepINRIA v1.7.2: multiple sclerosis brain magnetic resonance imaging visualization, comparison and analysis software

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Background: SepINRIA is a software offering visualizations, comparisons and analysis of multiple sclerosis (MS) brain magnetic resonance imaging (MRI). **Objective:** To provide clinicians with a tool to quantify lesion burden and atrophy. **Methods:** SepINRIA works on a convenient database in which new DICOM files can be added. Images can be visualized in 2D or 3D. Two images (either of the same or different sequences) can be aligned and visualized in the same window (side by side or by image fusion). Lesion segmentation can be obtained manually, semi-automatically or fully automatically from T1, T2, PD and T2-fluid-attenuated inversion recovery (FLAIR) sequences. Quantitative values (number of lesions, volume, etc.) are then computed. Manual and semi-automatic modes can be used to perform a segmentation of reference. In this case, a quantitative comparison of the segmentations can be realized. Manual and automatic brain atrophy evaluations are also available. In the manual method, specific points have to be identified. Then the distances between these points are computed and give linear measures (width of brain, lateral ventricles and third ventricle). In the automatic method, the brain parenchymal fraction (BPF) is computed from an automatic segmentation of the brain based on T1, T2 and DP sequences taking into consideration partial volume effects. This is done simultaneously for each date of examination. Evolution of the BPF reflects the atrophy. The use of SepINRIA has been simplified and optimized. Data can also be exported into DICOM files. **Results:** SepINRIA is available on Linux, MacOSX, Windows and can be downloaded at: <http://www-sop.inria.fr/asclepios/software/SepINRIA/> **Conclusions:** We developed a software to analyze MS brain MRI. The images alignment function is already useful in a clinical context. Automatic lesion segmentation and evaluation of brain atrophy are still undergoing research. Neurologists can perform lesion segmentation or atrophy measurements of reference due to the manual lesion segmentation and linear measurement functions.

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Comparison between T2-weighted fast spin echo and fast short time inversion recovery pulse sequences in the depiction of cervical cord multiple sclerosis plaque

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Background: Improving the sensitivity of magnetic resonance imaging (MRI) in the detection of multiple sclerosis (MS) plaques in the cervical cord is important in the early diagnosis and follow-up of MS. **Objective:** To compare the sensitivity of T2-W fast spin echo (FSE) and fast short time inversion recovery (fast-STIR) sequences for the

better depiction of plaques in the cervical spinal cord MRI of MS patients. **Methods:** Eighteen proved cases of relapsing-remitting MS (RRMS) (3 men and 15 women) were examined on a 1.5 T MRI system and sagittal images in T2 W FSE and fast-STIR sequences were obtained. Acquired images were compared side by side in a PACS workstation, then images were scored for the presence and number of plaques and their detectability as well as myelographic effects and overall image quality. **Results:** Cervical cord plaques were seen better on fast-STIR images, which showed the highest lesion contrast. A mean of 2.44 cord lesions per patient were seen on T2-FSE images and 2.94 cord lesions per patients on fast-STIR images (20% more than on T2-FSE). Two or more cervical cord lesions were found on nine patients with T2-FSE images (50%) and 11 patients on fast-STIR images (61%). Although T2-FSE provided better myelographic effect and overall better image quality, lesion visibility was improved by fast-STIR. **Conclusions:** We found the fast-STIR sequence a sensitive technique for the assessment of spinal cord involvement in MS. In respect to the growing number of MS patients in Iran, it may have an important role in the diagnosis of the disease and in confirming MacDonald's criteria. Fast-STIR sequence not only provides high lesion contrast but could also be easily implemented on many local MRI machines.

P633

Symptomatic demyelinating lesions located at the middle cerebellar peduncles are associated with good prognosis?

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Background: Brainstem inflammatory-demyelinating syndrome (BIDS) is a frequent manifestation of multiple sclerosis (MS) onset. Three or four Barkhof magnetic resonance imaging (MRI) criteria and the presence of oligoclonal bands in the cerebrospinal fluid (CSF) are associated with a higher risk of conversion to MS in these patients. However, this condition can also be a monophasic disease with a good prognosis. **Objective:** To describe nine patients affected by BIDS with the symptomatic lesion located at the middle cerebellar peduncles and less than three Barkhof MRI criteria with a good clinical and radiological follow-up. **Methods:** Description of clinical, MRI, CSF, neurophysiological features and evolution of these patients. **Results:** Seven women and two men were studied. Mean age at assessment was 35 years and the time of follow-up was 19 months (range 3 to 36 months). All subjects had symptoms indicating affection of the brainstem (facial sensory symptoms, gait disturbances and vertigo) and the symptomatic lesion was located at the middle cerebellar peduncles. Four patients had demyelinating lesions in other regions of the brain but did not fulfil more than two Barkhof MRI criteria. Five of the patients had positive oligoclonal bands in the CSF. All had normal visual evoked potentials. None of the patients had a second relapse and their control spinal cord and cranial MRI did not show dissemination in time for MS diagnosis at the follow-up. **Conclusions:** Symptomatic demyelinating lesions located at the middle cerebellar peduncles, which fulfil less than three Barkhof MRI criteria can be associated with good clinical prognosis.

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Regional diffusion tensor imaging differences in multiple sclerosis patients with little focal damage and the relation to neurocognition

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Background: Focal damage, reflected as T2 hyperintense and T1 hypointense lesions, cannot fully account for cognitive impairment in multiple sclerosis (MS), which is a frequent symptom occurring early in the disease. The investigation and localization of damage in the total white matter (WM) could provide more knowledge on the roots of cognitive deficits in MS. Diffusion tensor imaging (DTI) measures have shown to be sensitive for WM damage, not only inside lesions but also for damage in the so-called normal-appearing white

matter (NAWM). **Objective:** To localize WM damage in MS patients using a new hypothesis-free, post-processing technique, tract-based spatial statistics (TBSS), and relate it to neurocognitive deficits. **Methods:** DTI data was investigated in 30 MS patients (median Expanded Disability Status Scale (EDSS) 3.0, range 0–6.5, mean disease duration 3.6 years, SD 3.5), which were selected to have little focal WM damage (mean 5.6 mL, SD 7.7) on standard T2-weighted images, and 31 age-matched healthy controls. The letter digit substitution test (LDST) was used to assess processing speed, Stroop test to evaluate inhibition and attention, and the location learning test (LLT) for visuospatial memory. Cognitive tests were controlled for fatigue, depression and premorbid IQ where appropriate. **Results:** Patients were found to have a lower fractional anisotropy (FA) compared with controls in the fornices, left corona radiata, inferior longitudinal fascicles of both hemispheres, both optic radiations and parts of the forceps major and the genu of the corpus callosum. Patients showed just-normal inhibition and attention and impaired processing speed compared with controls. In patients, processing speed correlated with abnormal FA in the corpus callosum (Pearson's $r = 0.26$; $P = 0.05$). Despite the damage in the fornices, known to be involved in memory function, patients exhibited normal visuospatial memory, which may indicate (partial) reorganization of memory function. **Conclusions:** We found 12 areas of FA decrease using a hypothesis-free method of analyzing DTI images of MS patients with little focal WM damage. Impaired processing speed was associated with reduced FA in the corpus callosum.

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Clinical and conventional magnetic resonance imaging features of non-disabling multiple sclerosis: a large-scale, multicenter, multinational, cross-sectional study

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Background: Although it is well known that multiple sclerosis (MS) may have a benign course (BMS), the structural features of non-disabling MS remains poorly defined. Conventional magnetic resonance imaging (MRI) studies of BMS have failed to find consistent correlates to be used for a reliable identification of these patients during therapeutic counselling. **Objective:** To evaluate the clinical and conventional MRI features of a large and heterogeneous population of patients with non-disabling MS. **Methods:** We studied 167 patients with BMS (i.e. having an Expanded Disability Status Scale (EDSS) score of 3.0 or less and a disease duration of at least 15 years) and 173 patients with non-disabling relapsing-remitting MS (NDRRMS) (i.e. having an EDSS score of 3.0 or less and a disease duration of between 5 and 14 years), who were retrospectively identified from the MS clinics in six centers. For all patients, demographic and clinical data had to have been collected within 2 weeks from a brain MRI scan, including high resolution, T2-weighted sequences. Centralized image analysis consisted of T2-hyperintense lesion volume (LV) measurement, using a semi-automated local thresholding technique. Data analysis was adjusted for patients' age and enrolment center. **Results:** Patients with BMS were older than those with NDRRMS ($p < 0.001$). The mean disease duration was 21.6 years for BMS and 8.2 years for NDRRMS. Median EDSS did not differ between the two groups of patients. The mean T2 LV was higher in BMS than in NDRRMS patients (12.99 vs. 8.48 ml, $p < 0.001$). T2 LV did not differ between those 61 BMS patients with low disability (EDSS < 2.0)/long disease duration (> 20 years) and the remaining 106 with higher disability/shorter disease duration. T2 LV was correlated with MS duration ($r = 0.29$, $p < 0.001$). **Conclusions:** The burden of T2-visible

lesions does not seem to be a distinctive feature of non-disabling MS, but rather a mere reflection of disease duration.

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Optimizing diffusion measurements for large-scale, multicenter multiple sclerosis trials: a pan-European study

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Background: Diffusion tensor imaging (DTI) is increasingly being used in the study of multiple sclerosis (MS), but little is known about the issues related to its application in multicenter studies. **Objective:** To apply a standardized acquisition scheme for DTI of MS patients using different scanners; to investigate which is the inter-scanner variability of DTI-derived metrics and whether the sensitivity to disease-related changes varies accordingly. **Methods:** Twenty-nine healthy subjects and 25 relapsing-remitting MS patients with low disability were studied in five centers. Four 1.5 T scanners and three 3.0 T scanners from two different manufacturers were used. A pulsed gradient spin echo single shot echo planar DTI sequence was used with the following scheme: TR (ms):5000–9000, TE (ms): 90–125, pixel size: 2.5x2.5 mm, slices: 50, slice thickness (mm): 2.5; diffusion-encoding gradients directions: 30, b-value (s/mm²): 900; number of acquisitions with b=0: 4–6. A high-resolution dual echo sequence was also acquired. The DTI was estimated by linear regression and fractional anisotropy (FA) and mean diffusivity (MD) maps calculated. After removing lesions and masking, normalized FA and MD histograms from the normal-appearing brain tissue were created. **Results:** The subjects' age was not significantly heterogeneous among centers. There was a significant inter-scanner heterogeneity for average MD/FA and histogram peak height values in both controls and patients. After correcting for scanner, pooled data analysis showed that average MD was significantly higher, average FA significantly lower and MD peak height significantly lower in patients than in controls (p<0.001, p=0.038 and p<0.001). There was no significant inter-scanner heterogeneity for the observed differences between patients and controls in any variable. **Conclusions:** A careful standardization of the acquisition scheme can make DTI data collection in multicenter MS studies feasible and reliable for detecting disease-related changes without major inter-center heterogeneities, which would affect the data pooling for centralized analysis.

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Comparative value of different criteria for dissemination in space and time in patients with a clinically isolated syndrome affecting the spinal cord

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Background: Different criteria have been proposed to establish dissemination in space (DIS) and time (DIT) for patients presenting with a clinically isolated syndrome (CIS). Their value in patients with a CIS affecting the spinal cord has not been studied. **Objective:** To compare different set of criteria for DIS and DIT in patients with a cord CIS followed-up for a mean duration of 4.4 +/- 1.6 years. **Methods:** We compared four sets of DIS criteria and three sets of DIT criteria in 114 patients with a diagnosis of acute myelitis. Inclusion criteria were sensory and/or a motor clinical episode lasting less than

30 days compatible with a cord lesion localization and evidence of a cord lesion on magnetic resonance imaging (MRI). Other causes of myelopathy (compression, infarction, trauma or tumor) and myelitis (infections, neuromyelitis optica or systemic diseases) were excluded. Clinical, MRI and cerebrospinal fluid (CSF) data were retrieved from patients' files. Follow-up data were collected retrospectively or prospectively. Studied DIS criteria were three positive out of four Barkhof/Tintore criteria without considering the cord lesion or with the cord criteria, two brain lesions and a positive CSF (raised IgG index and/or oligoclonal bands) and new criteria from Swanton (at least one lesion in two out of four areas: infratentorial, periventricular and juxtacortical, without cord). DIT criteria were from McDonald 2001, Polman 2005 and Swanton 2006. Ages, sex and sets of DIS and DIT criteria were included in a logistic regression model. **Results:** During follow-up, 58% of patients were diagnosed for multiple sclerosis (MS) according to Polman et al. criteria, 86% of which had had a second episode and had been diagnosed for clinical-defined MS (CDMS), according to Poser et al. criteria. The presence of three out of four Barkhof/Tintore criteria and/or two lesions on brain MRI and positive CSF (whether or not the cord lesion was considered) was more sensitive and accurate for prediction to CDMS than the new criteria from Swanton. Bayesian statistics of all DIT criteria were similar. **Conclusions:** CSF data were useful for DIS. Whether or not the cord lesion is considered in DIS criteria does not matter. The new criteria are not better for spinal cord CIS.

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Predictive features of conversion to multiple sclerosis in clinically demyelinating isolated syndromes affecting the spinal cord

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Background: Multiple sclerosis (MS) frequently begins by a clinically isolated syndrome (CIS) affecting the spinal cord. However, little is known about the clinical characteristics differentiating CISs which will evolve to a diagnosis of MS in the following years from those which remains as CIS. **Objective:** To compare the clinical, spinal cord magnetic resonance imaging (MRI), brain MRI and cerebrospinal fluid (CSF) characteristics of patients with a spinal cord CIS evolving to diagnosed MS during a 2-year follow-up and patients with isolated cord CIS. **Methods:** We identified 135 patients with a diagnosis of isolated myelitis in medical files from the Department of Neurology of the University Hospital, Bordeaux, France from 2000 to 2007. Acute myelitis was diagnosed on the basis of a sensory and/or motor clinical episode lasting less than 30 days compatible with a cord lesion localization. Evidence of a cord lesion on MRI was requested. Other causes of myelopathy (compression, infarction, trauma or tumor) and myelitis (infectious, neuromyelitis optica, systemic diseases) were excluded. Clinical, MRI and CSF data were retrieved from patients files. Follow-up data were collected retrospectively or prospectively. **Results:** Eight patients were excluded because of an alternative cause of disease. Around 60% of patients were followed for more than 2 years. During follow-up 58.5% of patients were diagnosed for MS according to Polman et al. criteria, 86% of which had a second episode and were diagnosed for clinical-defined MS (CDMS) according to Poser et al. criteria. The diagnosis of isolated myelitis (I-CIS) was maintained at the end of the follow-up for 34% of patients, 63% of which had been followed for at least 2 years. Patients with MS were younger and had fewer cardio-vascular risk factors. The length of the initial stage was higher in the MS patients. Raised CSF IgG index and oligoclonal bands were more common in the MS group. Cord lesion on MRI was less extended on axial slices (less than the half of the spinal cord section) in MS patients. MS patients more frequently fulfilled at least one of the Barkhof-Tintore criteria on brain MRI. **Conclusions:** Clinical, MRI and CSF features differentiate isolated myelitis from CDMS myelitis.

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Cortical thinning in patients with multiple sclerosis: a follow-up study after 8 years

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Background: In patients with multiple sclerosis (MS), the focus of attention has recently shifted considerably towards the cortical involvement of the disease, detecting focal cortical thinning with disease progression alongside an overall reduction of the cortical thickness. So far, little is known about the evolution of cortical thickness and the regional changes of the cortex over time. We formerly evaluated cross-sectional, in vivo cortical thickness and its relationship to disability and disease duration in 20 patients with definite MS. **Objective:** To evaluate longitudinally the degree and pattern of cortical thinning. **Methods:** Eighteen patients from the former cohort (20 patients) were rescanned at a median follow-up time of 8 years. High-resolution 3D T1-w brain scans were acquired with the same 1.5 Tesla GE scanner. All scans (time points 1 and 2) were re-evaluated in a blinded fashion. Statistical thickness difference maps were generated by performing t-tests between patient and control groups. **Results:** Mean age and disease duration at the second time point was 44 and 13.5 years, respectively. Median Expanded Disability Status Scale (EDSS) score was 5.3. At time point 2, a significant overall reduction of the mean cortical thickness in relation to time point 1 was observed. In all patients groups that were formed at time point 1 according to disease duration and disability, we found at time point 2 an increase in the number of patchy areas of highly significant focal cortical thinning. **Conclusions:** This study provided evidence that a continuous, focal thinning of the cerebral cortex takes place as the duration of disease increases.

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Ultra-high field phase contrast imaging in multiple sclerosis patients

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Background: Ultra-high field magnetic resonance imaging (MRI) (7 Tesla) investigations in humans may open a diagnostic window for in vivo studies of pathologies on a macromolecular level. Phase contrast imaging at 7 Tesla has recently gained attention due to increased contrast compared with imaging at conventional magnitudes and to potentially novel and complementary information content. Possible susceptibility related sources of phase differences between gray matter (GM) and white matter (WM), such as blood deoxy-hemoglobin tissue myelin and tissue iron content have been discussed. However, none of these factors can fully explain the observed in vivo phase difference. **Objective:** To demonstrate for the first time that the proton resonance frequency is shifted due to the microscopic exchange between free water and mobile macromolecules (Zhong et al. 2008). The phase difference can be expected to depend on macromolecule content and type in different pathological states during multiple sclerosis (MS) lesion evolution. **Methods:** We investigated the direct magnetic resonance phase images acquired at 7 Tesla in patients with definitive MS. A gradient echo sequence with TR/TE of 750/18 ms with 200 μ m in plane resolution and 2 mm slice thickness was used in addition to standard T2 imaging for lesion detection. **Results:** GM and WM contrast was up to 10-fold higher at 7 Tesla than that for conventional magnitude images. MS lesions detected as T2 hyperintensities showed varying contrast in phase contrast suggesting different underlying macromolecular content. **Conclusions:** Since MS lesions have been shown to contain different macromolecule content compared with normal WM, and different macromolecule content during lesion evolution, this high sensitivity towards pathological alterations in combination with the good spatial resolution makes ultra-high field phase mapping a promising tool for the differentiation of MS pathology.

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Cortical lesions in patients with multiple sclerosis: their relation with cortical atrophy and impact on physical and cognitive impairment

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Background: The incidence of cortical lesions (CLs), their relation with other imaging metrics of disease, and their impact on physical and cognitive impairment remain unknown in multiple sclerosis (MS) patients. **Objective:** To assess the incidence of CLs and their impact on clinical and imaging metrics of MS disease using high resolution, high signal-to-noise ratio images. **Methods:** Twenty-one MS patients underwent a clinical examination (Expanded Disability Status Scale (EDSS) and the minimal assessment of Cognitive Function in MS (MACFIMS)) and a 3.0 Tesla magnetic resonance imaging (MRI) using a multichannel receiver coil. Isotropic 1.0 mm³ 3D spoiled gradient-recalled-echo inversion prepared (IR-SPGR) and a 3D fluid-attenuated inversion recovery (FLAIR) sequence, conventional T1 and T2-weighted images were acquired. 3D-IRSPGR and 3D-FLAIR were registered and inspected for CLs. Hypointensities on 3D-IRSPGR and hyperintensities/hypointensities on 3D-FLAIR either entirely lying in the cortex or touching it to some extent were counted. Measurements of cortical thickness, brain and white matter lesion volumes were obtained. Unpaired t-tests determined the significance of differences in clinical and MRI metrics between CLs+ and CLs- patients. Spearman correlations established the associations between quantity of CLs and other MS measures. **Results:** Results obtained thus far on the 3D-IRSPGR showed a total of 90 CLs (median 4.0, range 1–39) in 13 patients (61.9%). CLs+ patients had a greater volume of T2 lesions than CLs- patients ($p=0.037$), but no group differences in cortical thickness were present. Of the examined clinical variables, only the California Verbal Learning Test, Long Delay (CVLTLD) is lower in CLs+ patients ($p=0.0097$). Significant correlations are found only between the number of CLs and T2 ($p=0.0081$) and T1 ($p=0.0399$) lesion volume, and CVLTLD scores ($p=0.0088$, $r=-0.601$). **Conclusions:** The association between hypointense CLs on 3D-IRSPGR and measures of cognitive and physical impairments is not straightforward. Also, no correlation exists between cortical focal pathology and diffuse atrophy in MS patients.

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Predicting conversion to secondary- progressive multiple sclerosis using magnetic resonance imaging-based regional atrophy measures

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Background: Multiple sclerosis (MS) is associated with progressive atrophy of the brain and spinal cord. Association between spinal cord atrophy and disability has been shown. In an earlier study, we showed that medulla oblongata volume (MOV) is a reliable biomarker of spinal cord atrophy in MS. We have also shown that it can be used to differentiate between relapsing-remitting MS (RRMS) and secondary progressive (SPMS) patients. **Objective:** To investigate the potential of MOV as an indicator of transition from RRMS to SPMS. **Methods:** Thirty patients were selected from the Comprehensive Longitudinal Investigation of Multiple Sclerosis at the Brigham and Women's Hospital (CLIMB). Magnetic resonance imaging (MRI) scans of 15 patients who converted from RRMS to SPMS ('converters') were compared with those of 15 RRMS patients with matched disease duration (first symptom to last MRI), who did not convert to SPMS ('non-converters'). There were a total of 61 MRIs for the converters (4.1 MRIs per patient) and 58 MRIs for the non-converters (3.9 MRIs per patient). The patients underwent axial conventional spin-echo dual-echo head MRI and clinical evaluation including Expanded Disability Status Scale

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(EDSS). For each patient, for each time point, the caudal MOV was manually outlined on three consecutive MRI slices. A rule, derived from a decision-tree based classifier, was applied to each time point's MOV measure to assess disease progression status by MRI (MRI-RR or MRI-SP). Clinical stratification of each time-point used a threshold of EDSS score higher than 4 to distinguish clinical-RR from clinical-SP. **Results:** For the converters, 80% (49/61) of time points were correctly classified as RRMS or SPMS. For the non-converters, 93% (54/58) of time points were correctly distinguished. The classification algorithm can make two types of errors. In the converters, 8 time points were MRI-SP while still clinical-RR. In the non-converters, only four such cases were found. In the converters, four time points were classified as MRI-RR although clinical-SP. **Conclusions:** We showed that in a longitudinal setting, MOV can discriminate between RRMS and SPMS patients and might presage clinical transition to SPMS. Further experiments are needed to validate these intriguing findings.

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Statistical parametric mapping of 99mTc-ethyl cysteinate dimer SPECT in neuromyelitis optica: first results

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Background: One study reported perfusion abnormalities in three cases of neuromyelitis optica (NMO) using SPECT by visual analysis with normal brain magnetic resonance imaging (MRI). **Objective:** To investigate differences in regional cerebral blood flow between healthy subjects and NMO patients using quantitative voxel-based analysis (SPM) and to examine differences between patients with mild and severe visual dysfunction. **Methods:** Fourteen relapsing-NMO patients (Wingerchuck et al.), 12 females and 2 males, mean age 43.5 ± 10 years, age of disease 10.2 ± 9.5 years, Expanded Disability Status Scale (EDSS) score 4.4 ± 3, visual FS 2.9 ± 2.3, pyramidal 3 ± 2 and 12 healthy subjects (10 females, 2 males, age 54 ± 3 years). The NMO patients were: Group I EDSS-visual FS from 0 to 2, six cases; Group II EDSS-visual FS from 3 to 6, eight cases. SPECT imaging 99m Tc-ethyl cysteinate dimer (ECD) with double-head GC, high resolution fan-beam collimators was performed. The group comparisons were: healthy subjects vs. whole group NMO; healthy subjects vs. Group I (normal to mild dysfunction) and healthy subjects vs. Group II (more severe dysfunction). **Results:** No significant differences were found between the whole group of NMO patients and the healthy subjects. However, for Group I (normal/mild dysfunction), a pattern 1 with a hypoperfusion in the right middle temporal gyrus Brodmann A. 21 MT region (or V5) - 'high level' visual area for motion perception was observed. For Group II (more severe visual dysfunction), a pattern 2 was observed with an hypoperfusions in the right calcarine cortex, Brodmann A BA17, V1 - 'low level' visual area to detect basic features of the object. **Conclusions:** The hypoperfusions observed in both groups could be a remote effect from optical nerve lesions or a ECD hypofixation as aquaporin 4 regulates blood vessel permeability or it could be a functional neuroplasticity phenomenon.

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Tissue-specific atrophy changes on disease modifying drug therapy

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Background: Disease modifying drugs (DMD), beta-interferons and copaxone, have shown partial efficacy in multiple sclerosis (MS). Early identification of responders is essential. **Objective:** To investigate the magnetic resonance imaging (MRI) counterparts of clinical response to DMD with special interrogation of tissue-specific atrophy para-

eters. **Methods:** Relapsing-remitting MS (RRMS) patients starting on DMD were recruited. Expanded Disability Status Scale (EDSS) and relapses were recorded every 3 to 6 months. MRI scans were performed at DMD onset and after 1 year. T1, T2 and Gd+ lesion volumes were calculated and gray matter (GM), white matter (WM) and brain parenchymal fractions (BPF) were obtained with SPM99. Patients with relapses or EDSS progression during follow-up were considered clinically active. **Results:** One hundred and six patients were analyzed (mean (sd) age and disease duration: 29.7 (8.1) years and 4.4 (4.1) years, respectively). Median EDSS: 1.5 (interquartile range: 1.0–2.0). Mean (sd) number of relapses in the previous 2 years was 2.05 (0.76). At baseline, significant correlations were observed for GM with disease duration ($r=-0.28$, $p=0.004$) and EDSS ($\rho=-0.21$, $p=0.031$) and for WM with number of relapses in the previous 2 years ($r=-0.22$, $p=0.026$). At 1 year, decreases in BPF (-0.67%) and GM(-1.34%) were observed ($p<0.001$), but not in WM (0.6%, $p=0.5$). Patients with more than two Gd+ lesions at baseline had larger decreases in WM than patients with fewer(-1.08% vs. 1.20%, $p=0.012$). Larger T2, T1 and Gd+ T1 lesion volumes at baseline were significantly correlated with larger BPF and WM decreases on therapy, but not with GM changes. No significant differences in mean atrophy rates were observed between clinically active and inactive patients at 2 (n=100) and 4 (n=29) years. **Conclusions:** No relationship was observed between clinical response to DMD and atrophy development in this short follow-up period. WM changes relate to changes in inflammatory activity, while GM changes do not, and correlate with disability.

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Multiple sclerosis patients reveal neural compensation during information processing with no additional performance decline: a longitudinal functional magnetic resonance imaging study

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Background: Cognitive impairment is present in approximately 50% of individuals with multiple sclerosis (MS) however, its neural manifestations have yet to be determined. Even with in-depth neuropsychological assessments, it may be difficult to detect subtle cognitive deficits. **Objective:** To investigate disease progression and related cognitive impairment using functional magnetic resonance imaging (fMRI). One example of impairment reported in MS is information processing. **Methods:** The current fMRI study examined six MS patients and matched healthy controls at baseline (time1), and the same patients again after 12 months (time2). The Computerized Tests of Information Processing (CTIP) was administered to uncover neural activity related to increased cognitive load (semantic processing). Imaging and performance (error rate and reaction time) data were compared. **Results:** Fixed-effects analyses revealed that during high-load cognitive tasks, controls show large right frontal (inferior to middle) activity while patients show none. Left occipital cortex activity was observed in all participants, although was more apparent in patients. Patients also revealed bilateral cerebellar and temporal pole activations that were absent in the controls. Overall, compared with controls, patients had slower reaction times, but only patients at time2 showed higher error rates. However, between patient comparisons did not reveal significant performance changes over time, regardless of apparent neural changes. Patients at time1 revealed bilateral postcentral and left precentral gyrus activations, yet this was not apparent at time2. In contrast, patients at time2 showed more activity in the bilateral temporal poles and introduced supramarginal gyrus activation. **Conclusions:** Our fMRI results suggested that MS patients utilized different neural mechanisms for increased information processing. Specifically, patients did not recruit the expected prefrontal cortex. There is a suggestion of progression of disease over time, given that at time2 patients recruited the right supramarginal gyrus and increasingly the temporal pole in order to limit additional performance decline.

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Neural compensation in cognitively impaired patients with multiple sclerosis during a response inhibition taskCarole S. Scherling¹, Andra Smith¹, Lisa Walker³, Matthew Hogan², Mark S. Freedman⁴¹University of Ottawa, School of Psychology, Ottawa, Ontario, Canada;²University of Ottawa, Faculty of Medicine (Division of Neurology), Ottawa,Ontario, Canada; ³Ottawa Hospital, Psychology Department, Ottawa,Ontario, Canada; ⁴Ottawa Hospital, Multiple Sclerosis Clinic, Ottawa, Ontario, Canada

Background: Multiple sclerosis (MS) produces cognitive sequelae in approximately half of affected individuals. Cognitive deficits can occur early in the disease course and often contribute significantly to diminished quality of life. Neuropsychological tests may lack the sensitivity to detect subtle cognitive changes. Whereas, structural neuro-imaging studies have provided insight into pathology, functional magnetic resonance imaging (fMRI) has highlighted the concomitant functional changes. **Objective:** To undertake longitudinal investigations to document the progression of these functional changes over time. **Methods:** The current study examined six MS patients across two fMRI scans, separated by 12 months (time 1 and time 2). Matched healthy controls were scanned at time 1. A go/no go task was administered to uncover neural activity related to response inhibition. Imaging and behavioral data (accuracy and reaction time) were compared. **Results:** Fixed-effects analysis showed that compared with controls, patients revealed more temporal lobe activity and less parietal lobe and cerebellar activity. Concurrently, patients had higher error rates than controls. Patients did not demonstrate any change in behavioral data over time. Patients at time 1 and time 2 showed orbital frontal lobe activity, but time 2 frontal activations also included middle and superior gyri. Furthermore, patients at time 1 revealed limbic and posterior cerebellar activity, unlike time 2. Yet, overall activation clusters in the frontal and temporal lobes were larger in time 2. Finally, a closer look at time 2 neural activity showed that along with a lack of cerebellar activity, the supramarginal gyrus and the frontal lobes had increased activity. This indicated heightened self-monitoring, yielding behavioral control, thereby suggesting the use of greater neural compensation at time 2. **Conclusions:** Results in our sample suggested that individuals with MS recruited different neural mechanisms than controls for response inhibition. As the disease progresses in the short term, maintaining the same performance level is accomplished via compensatory changes in the recruited neural network.

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Multiple sclerosis lesion characterization with 7 Tesla magnetic resonance imaging

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Background: Conventional magnetic resonance imaging (MRI) shows limited correlation with disease burden, thus improved contrast/resolution features may be useful for structural lesion differentiation. **Objective:** To evaluate 7 Tesla MRI for visualizing and characterizing multiple sclerosis (MS) lesion substructure with the long-term goal of bringing 7 Tesla MRI into clinical practice, thus expanding capabilities for early diagnosis and treatment assessment. **Methods:** Ten MS patients (33–53 years) were studied at 7 Tesla using: 2D-susceptibility weighted imaging (SWI): TR/TE/flip=1600/12/500; 2D-white matter attenuated inversion recovery TSE (WHAT): TR/TI/TE=8000/500/14; and T2-weighted gradient spin echo (GraSE): TR/TE 4000/70. Phase images were reconstructed from SWI data, all images were visually compared and the numbers of lesions seen with each sequence were counted. **Results:** Overall, lesions had highest contrast on WHAT. High spatial resolution and excellent gray matter (GM)/white matter contrast allowed the depiction of several juxtacortical lesions and a few GM lesions. WHAT images also provided excellent depiction of perivascular spaces. SWI magnitude images depicted only 93% of the lesions seen on WHAT. This is due to the decreased

contrast in the weakly PD-weighted SWI magnitude images compared with WHAT. SWI phase images showed several interesting features. Some lesions were seen on the magnitude images, but not on the phase images indicating that contrast in these lesions is due to free water increase. Other lesions were seen only on phase but not magnitude images. These lesions must have a significant presence of paramagnetic material, for example, iron. Furthermore, many lesions showed a dark outer ring on the SWI phase images that was not seen on magnitude or WHAT images. In addition, both magnitude and phase SWI sequences showed that most lesions are associated with venous structure. **Conclusions:** This ongoing study indicates that 7 Tesla MRI provides several novel contrast mechanisms for detailed characterization of MS lesions and depiction of their internal structure. Future studies are needed to correlate 7 Tesla MRI with clinical findings.

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T2 in normal-appearing gray matter in multiple sclerosis measured at 3 Tesla and 7 Tesla magnetic resonance imaging

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Background: In standard field-strength, T2-weighted magnetic resonance imaging (MRI), decreased signal was reported in the deep gray matter of multiple sclerosis (MS) patients and attributed to increased brain iron, which was also observed in the histopathology of the MS brain. **Objective:** To assess if brain iron content can be evaluated in vivo through quantitative T2 measurements in MS patients compared with healthy controls at 3 Tesla and 7 Tesla. **Methods:** T2 measurements were obtained in 41 MS patients (20–60 years) and 15 healthy controls (18–56 years). Clinical evaluation of the MS patients included Expanded Disability Status Scale (EDSS) scores, timed 25 foot walk and 9-hole peg test. T2 measurements at 3 Tesla and 7 Tesla (Philips) were acquired with a dual-spin echo sequence (TE=10/60 ms) and a 8 echo Gradient Spin Echo (GraSE) sequence (TE=9–72 ms) and T2 values were computed from manually traced regions of interest (ROIs) of the thalamus, globus pallidus, putamen, caudate and normal-appearing frontal gray and white matter. Initial quantitative comparison included patients with relapsing-remitting MS and healthy controls aged over 30 years, since normal brain iron levels are near constant for that age group. **Results:** Average T2 in MS patients for the measured regions was similar or larger than average T2 in control subjects. Inter-observer T2 analysis showed no differences. Initial analysis did not indicate any correlation between T2 and clinical measures (EDSS, 25 foot walk and 9-hole peg test). **Conclusions:** To the best of our knowledge, these are the first reported 3 Tesla and 7 Tesla T2 measurements in MS patients. Our finding that MS patient T2s are longer than control T2s is contrary to prior studies reporting signal decrease in T2 scaled to the cerebrospinal fluid signal, which was attributed to brain iron increase in MS. Larger T2 values reflect increased free water due to demyelination. Thus, our study indicated that advanced T2 measurement methods are needed that allow the separation of T2 components from brain iron (decreased T2) and free water (increased T2).

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Magnetization transfer ratio: a predictor of neuronal loss in multiple sclerosis cortical gray matter?Klaus Schmierer¹, Harold Parkes¹, Po-Wah So², Shu F. An¹, Sebastian Brandner¹, David H. Miller¹, Tarek A. Yousry¹¹Neuroinflammation, NMR Research Unit, UCL - Institute of Neurology, London, United Kingdom; ²Imperial College London, Biological Imaging Centre, Imaging Sciences Department, Hammersmith Hospital, London, United Kingdom

Background: In multiple sclerosis (MS) brain white matter (WM), magnetization transfer ratio (MTR) is associated with myelin content and, to lesser degree, axonal count. The substrate of MTR changes in MS cortical grey matter (CGM) is less clear. **Objective:** To probe the

association between myelin content (MyC) and neuronal density (ND) with T1, T2 and MTR in MS CGM using high-field magnetic resonance imaging (MRI) and histology. **Methods:** Thirteen blocks of postmortem MS brain were selected based on the presence of CGM lesions (GML), and scanned at 9.4 T to acquire the following datasets (slice thickness 1 mm, FOV 30x30 mm, matrix size 256x192): (i) spin echo (SE) T1, (ii) SE T2 and (iii) gradient-echo with-/out saturation pulse for MTR maps. Tissue blocks were then processed for embedding in paraffin and sections immuno-stained for myelin-basic protein (MBP) and cresyl-violet (CV). Regions of interest (ROI) were identified on histological sections and registered to MRI maps. Transmittance of MBP-stained sections was used to estimate MyC. ND was estimated in ROI using uniform random sampling of CV sections. Student's paired t-test and regression models were applied. **Results:** Sixteen GML (nine juxtacortical (GM only), five subpial, two affecting all layers) were analyzed. Non-lesional GM (nlGM) and GML differed in T2 ($p<0.01$), MTR ($p=0.04$) and MyC ($p<0.01$); trend difference was detected for T1 ($p=0.09$), and no difference for ND ($p=0.8$). Correlations were detected between (i) MTR and ND ($r=0.67$, $p=0.02$) and (ii) T1 and ND ($r=-0.52$, $p=0.05$). These correlations remained robust when MyC was included in the regression model. Neither MTR nor T1 were predictors of MyC. No association was detected between MyC and ND ($r=0.09$, $p=0.7$). **Conclusions:** Whereas in MS WM, MTR mainly depends on myelin content, MTR (and T1) in the cortex seems largely dependent on ND. Lack of association between MyC and ND suggests that neuronal damage in CGM is partially independent of MyC. **Supported by:** Wellcome Trust, grant #075941.

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Multiple sclerosis cortical grey matter changes following fixation detected by multimodal magnetic resonance imaging

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Background: In formalin-fixed multiple sclerosis (MS) brain magnetic resonance (MR) indices may be affected by disease and fixation. The latter may affect the inference of likely in vivo changes from MR and/or histology studies. Effects of fixation on MR indices in MS white matter (WM) have been described. **Objective:** To assess changes following fixation of MR indices in MS cortical grey matter (CGM). **Methods:** Fifteen MS brain samples were studied unfixed at 51 hours (SD 28) post-mortem, and after 64 days (40) of fixation. Using a 1.5 T scanner, 2D datasets were acquired to calculate: (i) T1, (ii) T2, (iii) magnetization transfer ratio (MTR), and (iv) fraction of macromolecular protons (fB) (ten cases only). CGM maps were produced to investigate post-fixation changes of MR indices in CGM, contrast between CGM and normal-appearing white matter (NAWM) and correlation between CGM and mean WM (NAWM and WM lesions) using the Student's t test and regression analysis. **Results:** Differences between unfixed and fixed CGM were detected for T1 (1114 ms (SD 216) vs. 617 ms (114), $p<0.01$), T2 (4 ms (28) vs. 42 ms (30), $p=0.04$), MTR (29.1 pu (2.5) vs. 24.1 pu (3.3), $p<0.01$). No difference was detected for fB. Contrast of T2 between CGM and NAWM was reduced following fixation ($p<0.01$); no such changes were observed for other MR indices. T1 (fixed, $r=0.87$, $p<0.01$) and MTR (unfixed, $r=0.58$, $p=0.03$ and fixed, $r=0.81$, $p<0.01$) in CGM correlated with respective WM values, whereas the remaining MR indices did not. **Conclusions:** In CGM, formalin-fixation affected all MR indices except fB. The lack of association of CGM and WM in fB and T2 suggests partially independent pathological processes in the two tissue compartments, as further evidenced by the change in T2 contrast between the tissues. Histological examination of the samples will probe the relative contribution to MR indices of CGM lesions and NA cortex. **Supported by:** Wellcome Trust, grant #075941.

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Three dimensional surface mapping of subregional hippocampal atrophy in multiple sclerosis: correlation with clinical variables

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Background: Hippocampal atrophy preferentially affects the CA1 region in early multiple sclerosis (MS). Volume loss, especially in the left hippocampus, is correlated with deficits in verbal learning. The spatial localization and extent of hippocampal volume change in MS in association with clinical variables has not been characterized. **Objective:** To localize hippocampal atrophy in MS patients compared with controls using a three-dimensional surface displacement mapping technique, and to determine if these changes are correlated with clinical measures. **Methods:** The right and left hippocampi (CA1-3, dentate gyrus and subiculum) were manually segmented from a standard T1-weighted scan obtained at 1.5 T (resolution 1 mm³) by a single trained researcher. A total of 23 relapsing-remitting MS (RRMS) patients and 18 controls were studied. Using a previously described high resolution surface mesh algorithm, average surface maps were generated for controls and RRMS patients. Differences in surface landmarks between the groups were determined over the entire left and right hippocampus. Significance of detected displacements was determined using the Wilcoxon ranked-sum test adjusted for multiple comparisons with the permutation test. The relationship of clinical variables (performance on an unrelated word list learning task, PASAT performance and T2 lesion volume measures) was assessed using linear regression. **Results:** Surface displacements in the RRMS patients were greater in the anterior hippocampal region and statistically significant displacement was detectable in the left anterior subiculum. Surface displacement was greater for the left versus the right hippocampus. Worsening verbal learning performance was correlated with greater displacement in the left anterior CA regions, while PASAT performance and T2 lesion volumes were not correlated with regional hippocampal changes. **Conclusions:** Changes in hippocampal volumes in RRMS are localized to anterior regions as revealed by three-dimensional surface mapping. Left anterior hippocampal changes are associated with deficits in verbal learning but not T2 lesion volumes. The hippocampus is vulnerable to clinically relevant changes in MS.

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Combined diffusion tensor and magnetization transfer magnetic resonance imaging of the human optic nerve in multiple sclerosis

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Background: Quantitative magnetic resonance imaging (MRI) of the optic nerve (ON) is technically challenging, but critically important for detecting the onset/progression of multiple sclerosis (MS). Brain and spinal cord magnetization transfer (MT) and diffusion tensor imaging (DTI) are sensitive to MS pathology and correlate with disability, but have not been combined to assess the optic nerve. **Objective:** To develop combined MT/DTI of the ON in MS patients and assess the derived, quantitative metrics. **Methods:** DTI and MT were performed on six MS patients coronal to the ON as it exits the optic globe. DTI: 15 gradient directions, $b=400$ s/mm², 25 slices (1.2x1.2x2.5 mm resolution), 4 minutes. MT: 2 volumes with/without 10.5 μ T MT-prepulse (1.5 kHz off-resonance), 25 slices (0.67x0.88x2.5 mm resolution), 7 minutes. Fractional anisotropy (FA), perpendicular (λ_{perp}), parallel (λ_{para}) and mean diffusivity (MD) were calculated from DTI data and MT ratio (MTR) from the MT data. Regions of interest (ROIs) were placed bilaterally in the center voxels of the optic nerve slice-by-slice from the orbit to the chiasm. **Results:** The mean (\pm SD) diffusion parameters

over both optic nerves were: FA = 0.60 ± 0.14 (range = 0.35–0.8) MD = 0.0014 ± 0.0003 mm²/sec (range = 0.001–0.002), λ_{perp} = 0.0011 ± 0.0003 mm²/sec (range = 0.0009–0.0015), λ_{para} = 0.0021 ± 0.0004 mm²/sec (range = 0.0014–0.0027) and MTR = 0.50 ± 0.03 (range = 0.3–0.54). The mean and range of these measures agree with literature values for dense white matter tracts of the brain and spinal cord. Comparison with controls and optical coherence tomography-based measures of the retinal nerve fiber layer thickness will be presented on a larger sample. **Conclusions:** We showed that combined MT and DTI of the optic nerve is possible, and the absolute values and ranges are in the expected range for dense fiber tracts. The variability in these patients indicates that with higher sample size it may be possible to detect that can be related to visual dysfunction and non-MRI based imaging of the anterior visual pathway. This may provide an early marker for disease progression.

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Whole spinal cord lesion detection with 1.5T and 3T MRI and clinical correlation in patients with multiple sclerosis

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Background: There is growing interest in using higher field MRI for assessing brain pathology in MS while few studies have examined its role in assessing spinal cord damage. **Objective:** Assess the relationship between spinal cord T2 lesions and clinical status in multiple sclerosis (MS) using both 1.5 and 3T MRI. **Methods:** Whole spine T2-weighted fast spin-echo MRI was performed at 1.5T and 3T in 32 MS patients [1 clinically isolated; 26 relapsing-remitting, 5 primary or secondary progressive; Expanded Disability Status Scale (EDSS) score (mean±SD) 2+/-1.9 (range 0–6.5), disease duration 8.18±7.4 (range 0.2–39) years]. Protocols were optimized and matched on voxel size. T2 hyperintense lesions were quantified using a semi-automated edge finding tool with scans randomized anonymously and intermixed with normal subjects. **Results:** At 1.5T, moderate correlations were found between whole spinal cord lesion number (Spearman $r_s = 0.46$, $p = 0.01$) or volume ($r_s = 0.36$, $p = 0.04$) and EDSS score. However, 3T lesion-EDSS correlations were weak (lesion number $r_s = 0.06$, $p = 0.76$; lesion volume $r_s = 0.13$, $p = 0.46$). In contrast, correlation between lesion volume and timed 25-foot walk were similar at 1.5T (Pearson $r = 0.38$, $p = 0.04$) and 3T ($r = 0.45$, $p = 0.01$). A higher whole cord lesion volume was present in progressive vs. relapsing patients at 1.5T (520±514 vs. 180±290 mm³, $p = 0.03$), which showed a non-significant trend at 3T (485±495 vs. 139±157 mm³, $p = 0.09$). Whole cord lesion number and volume did not differ between MRI platforms in the MS group ($p > 0.05$). No lesions were found in normal controls. **Conclusions:** This study shows significant relationships between whole spinal cord T2 hyperintense lesion load and clinical status in patients with MS. The 1.5T and 3T MRI scanning protocols yielded relatively similar results for many but not all comparisons.

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Development of positron emission tomography imaging techniques to visualize central nervous system myelin

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Background: As therapeutic strategies aimed at promoting remyelination are likely to emerge in the next few years, there will be a need to develop imaging techniques measuring the putative remyelination enhancement. **Objective:** To identify compounds that could be used to image central nervous system (CNS) myelin by positron emission tomography (PET), we evaluated several amyloid markers for their ability to stain myelin. **Methods:** We took advantage of the autofluorescence properties of several Congo red and thioflavinT derivatives, conferred by their chemical structure, to investigate their binding on CNS myelin. The most promising compounds were radiolabeled with carbon-11 and their ability to image CNS myelin by PET was evaluated in adult healthy monkeys, and in monkeys with a chemically induced demyelinated lesion. **Results:** We have shown that several Congo red derivatives could be used as myelin markers, and that BMB (1,4-bis(p-aminostyryl)-2-methoxy benzene), once radiolabeled with carbon-11, allowed the visualization of myelin by PET. Since thioflavinT derivatives bind to the same molecular target as Congo red in senile plaques (the multiple beta-sheet structures), we hypothesized that they may also stain myelinated fibers. We found that the thioflavin derivative N-[11C]methyl]-2-(4'-methylaminophenyl)-6-hydroxybenzothiazole ([11C]PIB), might represent a suitable myelin biomarker for PET imaging. We further investigated whether this compound could image chemically induced demyelinated lesions in the monkey CNS, and we are planning a preliminary clinical trial in patients with multiple sclerosis. The most recent results will be presented. **Conclusions:** Congo red or thioflavinT derivatives could represent promising agents to image CNS myelin by PET.

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Quantification of neuronal loss in progressive multiple sclerosis: a positron emission tomography study with [11C]-flumazenil

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Background: It is now well admitted that neuronal injury plays a crucial role in the appearance and progression of neurological disability in multiple sclerosis (MS). However, the extent of neuronal loss in the gray matter of MS patients remains poorly known. **Objective:** To assess whether positron emission tomography (PET) using [11C]flumazenil, a specific central benzodiazepin receptor (BZR) antagonist, allows the detection and quantification of neuronal loss in MS. **Methods:** Ten patients with progressive MS were compared with ten healthy volunteers. PET was performed on a high resolution research tomography dedicated to brain imaging (HRRT, Siemens Medical Solution, spatial resolution of 2.5 mm) using [11C]-flumazenil. The PET images were acquired following co-injection of [11C]-flumazenil (285 ± 59 MBq) and unlabeled flumazenil (10 µg/kg). Quantification of the receptor density (in pmol/mL) was calculated voxel by voxel using the partial saturation method, allowing the acquisition of parametric images. Brain regions of interest were drawn on T1-weighted magnetic resonance images using semi-automated methods and were co-registered with PET images. As BZR are expressed by virtually all cortical, striatal and

thalamic neurons, the determination of the total number of BZR in each segmented brain structure reflects the neuronal viability. **Results:** Only mild modifications in the density of BZR were observed in the cortical and subcortical structures analyzed. However, when the density of BZR was adjusted for volume decrease in each structure, allowing a more accurate measurement of neuronal loss, clear differences were found between groups. The magnitude of the neuronal loss was between 0 to -30% depending on the region. Interestingly, the level of neuronal loss in the thalami and gyrus precentral was in accordance with previous neuropathological studies. **Conclusions:** Our preliminary results suggest that PET using [¹¹C]flumazenil and the partial saturation method allows the quantification of neuronal loss in MS. **Supported by:** ARSEP grant. Biogen-Idex subvention.

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Phosphorus spectroscopy of normal-appearing white matter in multiple sclerosis

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Background: Studies using proton (1H) magnetic resonance spectroscopy (MRS) in patients with multiple sclerosis (MS) have shown a reduction of N-acetyl aspartate (NAA) throughout the normal-appearing white matter (NAWM). As NAA is a neuro-axonal-specific metabolite synthesized by mitochondria and its reduction in the NAWM is partially reversible, its decrease in NAWM not only indicates axonal loss but probably also impaired axonal mitochondrial metabolism. It is, however, unclear whether reduced axonal mitochondrial activity is a primary event or secondary to axonal degeneration. **Objective:** To study the high-energy phosphorus compound phosphocreatine (PCr) in the NAWM of MS patients. **Methods:** Twelve patients with MS (six men and six women; age range 39 to 58 years, mean 50.6 years; four with relapsing-remitting MS (RRMS) and eight with progressive MS) and five healthy controls (four men and one woman; age range 44 to 57 years, mean 51.4 years) underwent phosphorus (31P) MRS using a 3T MRI system and a surface coil to position a volume of interest (45 cc) in the right centrum semiovale. The processed spectra were curve-fitted to provide metabolite peak areas. **Results:** We found a significantly increased ratio PCr/total 31P in patients compared with controls (0.263 ± 0.039 vs. 0.223 ± 0.018 ; $p = 0.012$, unpaired t-test). In addition, the metabolite ratio PCr/total ATP was significantly increased (0.605 ± 0.129 vs. 0.510 ± 0.034 ; $p = 0.034$, unpaired t-test), mainly caused by an increase in PCr. **Conclusions:** This preliminary study showed an increase in the relative amount of PCr, compared with the total phosphorus spectrum and the total ATP peak areas in NAWM. PCr acts as an energy source to maintain a constant ATP level dependent on variable energy demands. Its increase in NAWM could indicate that this energy reservoir is not properly metabolized, lending further support to the hypothesis of a primary-impaired energy metabolism in the NAWM of patients with MS.

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Does multiple sclerosis become an ischemic disease over time? A 3-year follow-up study of patients positive for the presence of anti-phospholipid antibodies

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Background: We recently showed that multiple sclerosis (MS) anti-phospholipid antibodies (APLA)-positive (+) patients present higher

macroscopic and microscopic brain damage compared with MS APLA-negative (-) patients. The mechanisms by which APLA can contribute to ischemic damage in the brain of MS patients include prothrombotic mechanism or vasospasm. **Objective:** To investigate the evolution of clinical and magnetic resonance imaging (MRI) measures in MS patients, according to their APLA status. **Methods:** Forty-seven (47) relapsing-remitting MS patients were enrolled and followed for 3 years. Twenty six MS patients were APLA+ and 21 were APLA- at baseline. There were no differences at baseline between the two groups for age, sex, disease duration MRI characteristics, disability or time on disease modifying treatment. All patients were on interferon beta-1 IM at baseline and remained on the same treatment over follow-up. A number of MRI measures were calculated at baseline and 3 years, including perfusion-weighted MRI. **Results:** APLA+ patients developed more damage in several MRI measures compared with APLA- patients: diffusion entropy (+4% vs. -2.5%, $p=0.019$), magnetization transfer ratio (MTR) of T1-LV (-3.3% vs. +4.7%, $p=0.04$), T2-LV (-2.4% vs. +1.1, $p=0.064$), normal-appearing gray matter (NAGM) (-2.6% vs. +0.8, $p=0.067$) and normal-appearing brain tissue (NABT) (-2.1 vs. +0.01, $p=0.075$) and gray matter (GM) volume (-2.5% vs. -1.7%, $p=0.071$). APLA+ patients showed strong correlations between decline in whole brain and GM volumes, deterioration in MTR and diffusivity measures, and prolonged mean transit time (MTT) of GM and lesions ($r=0.5$ to 0.7 , $p<0.01$). This was not seen in APLA- patients. There was a tendency for more severe clinical deterioration in the MS APLA+ group over 3 years. **Conclusions:** The relationship of APLA and MS remains intriguing. Discerning demyelinating from ischemic types of lesions in MS APLA+ patients is mandatory. Strong correlations between prolonged MTT and deterioration in MRI measures over 3 years suggest that APLA-related microvascular damage is an important component of the MS disease process.

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Differentiating multiple sclerosis from other causes of demyelination using diffusion weighted imaging of the normal appearing corpus callosum

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Background: Differentiating between multiple sclerosis (MS) and other diseases affecting central nervous system (CNS) white matter can be diagnostically challenging on the basis of clinical presentation and conventional magnetic resonance imaging (MRI). Abnormalities in diffusion weighted imaging (DWI) have been described in MS, with higher apparent diffusion coefficients (ADCs) described in normal-appearing gray and white matter. **Objective:** The purpose of this study is to evaluate whether ADC values in gray and white matter brain regions differ in patients diagnosed with MS compared with patients diagnosed with diseases such as neurosarcoidosis (NS) and acute disseminated encephalomyelitis (ADEM). **Methods:** Thirty MS patients, 21 NS patients and four ADEM patients underwent DWI in a 1.5T GE scanner. MS patients included 13 males and 17 females with a mean age of 44 years; non-MS patients included 10 males and 11 females with a mean age of 45 years. Quantitative analysis of data was performed on the Advantage Workstation using GE Functool software. Regions of Interest (ROIs) were placed on axial images in the following locations: right, left and center in the genu of the corpus callosum, right, left and center in the splenium of the corpus callosum, right and left frontal and occipital periventricular white matter regions and both thalami. Care was taken to avoid placing ROIs in areas of visible T2 lesions whenever possible. ADC values for each ROI were determined. A Student's t-test was applied to compare MS with non-MS patients. **Results:** Mean ADC values were significantly higher in MS patients than in non-MS patients in the right ($p=.0007$), center ($p=.0219$) and left ($p=.0112$) of the genu of the corpus callosum and in the right ($p=1.7 \times 10^{-6}$), center ($p=5.4 \times 10^{-4}$) and left ($p=1.3 \times 10^{-5}$) of the splenium of the corpus callosum. In non-callosal white matter regions and gray matter regions, there were no statistically significant differences between MS and non-MS groups. **Conclusions:** Elevated ADC levels within the corpus callosum on DWI may potentially help to differentiate between patients with MS and patients with other diseases affecting the CNS white matter.

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Diffusion tensor imaging of the corpus callosum and cognitive deficits in multiple sclerosis

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Background: Cognitive symptoms of multiple sclerosis (MS), including deficits in attention, memory, information processing and executive functioning, correlate only modestly with disease burden as detected on standard brain magnetic resonance imaging (MRI). Diffusion tensor imaging (DTI), an imaging method used to quantify the degree and direction of water flow within tissue, has been shown to have the ability to detect differences between brain with and without MS. **Objective:** To determine whether the differences detected by DTI are correlated with the extent or pattern of cognitive dysfunction in MS. To determine whether there is a correlation between DTI metrics of fractional anisotropy (FA) and mean diffusivity (MD) in regions of interest (ROIs) within the brain and performance on subtests of the Minimal Assessment of Cognitive Function in MS (MACFIMS), a measure of cognitive function in multiple domains. **Methods:** Eighteen MS patients underwent MACFIMS testing and DTI in six directions in a 1.5T GE scanner. Using GE Funtool software, ROIs were placed on axial images in the genu, body and splenium of the corpus callosum (CC) and various gray and white matter brain regions. MD and FA of the regions were measured. Pearson correlation coefficients were used to describe the strength of the association between cognitive scores and DTI metrics. **Results:** Elevated MD within regions of the CC significantly correlated with poor performance on multiple cognitive subtests. MD of the genu and left body of the CC each correlated with eight out of nine cognitive measures assessed; MD of the splenium and right body of the CC with six out of nine measures. **Conclusions:** Disruption of the integrity of the CC may preferentially contribute to cognitive dysfunction in MS. Alternatively, the substrates for cognitive dysfunction may be more diffuse, but DTI may be best suited for detecting disruption in the CC, a highly organized white matter tract.

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Magnetic resonance imaging findings in patients with clinically isolated syndrome suggestive of multiple sclerosis

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Background: Clinically isolated syndrome (CIS) is an event due to a single central nervous system (CNS) lesion that lasts longer than 24 hours and resolves within several weeks of onset and is typically the first manifestation of multiple sclerosis (MS). A relationship between the number of T2 hyperintense magnetic resonance imaging (MRI) abnormalities during the CIS episode and the risk of developing MS has been found. MRI abnormalities may also lead to consideration for early therapy. Identifying asymptomatic lesions will help to determine the risk of developing MS and possible treatment. **Objective:** To assess the frequency of asymptomatic brain lesions in subjects with spinal cord syndrome or spinal cord lesions in patients with a syndrome above the spinal cord. **Methods:** Retrospective study of consecutive patients with CIS that presented to a specialized MS clinic. **Results:** Thirty eight subjects were identified, 76% female and 24% male. Age of CIS onset was 25 years or younger in 18%, 26–35 years in 40%, 36–45 years in 16%, 46–55 years in 16% and older than 55 years in 10%. Ten (26%) reported family history of MS. Optic neuritis (26%) and transverse myelitis (38%) were the most frequent clinical manifestations. The initial study was a brain MRI in 26 (68%) of the patients; 23 (88%) were abnormal while 4 (15%) showed dissemination in space according to Barkhof criteria. Initial studies were cervical and thoracic MRIs in nine and three patients, respectively; all were abnormal. Twenty two patients (86%) with initial brain MRI underwent cervical and thoracic MRIs; 14 (63%) had abnormalities suggestive of MS. All the patients with initial spine MRIs underwent brain MRIs; five (41%) showed abnormalities suggestive of demyelination. **Conclusions:** Around 63% of patients with initial brain and

41% with initial spinal cord MRIs showed evidence of asymptomatic demyelination in the clinically unaffected area of the CNS. Results suggest that imaging the entire CNS is valuable in determining the risk for developing MS after CIS.

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Predicting disability in patients presenting with optic neuritis: the role of early magnetic resonance imaging parameters

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Background: There is limited information on the relationship between early magnetic resonance imaging (MRI) findings and future disability in patients presenting with optic neuritis (ON). **Objective:** To investigate ON in patients, the influence of number, location and activity of lesions and non-lesion MRI measures obtained on early MRI scans on disability at a median 6-year follow-up. **Methods:** Of 143 ON patients who were prospectively recruited for a serial MRI and clinical follow-up study starting within 3 months of symptom onset, 106 had reached a scheduled 5-year follow-up time point, and of these, 100 were evaluated clinically. Baseline MRI scans were obtained within 3 months of ON onset and repeated 3 months later. Lesion number, location and activity measures were analyzed on the baseline scan and lesion activity measures were studied at follow-up. Brain atrophy and magnetization transfer ratio (MTR) and magnetic resonance spectroscopy measures from normal appearing brain tissues were also analyzed. Patients were grouped by Expanded Disability Status Scale (EDSS) at 5 years (0, 1, 1.5–2, ≥2.5) and ordinal logistic regression was performed to assess the association between early MRI findings and subsequent disability. **Results:** At median 6-year follow up, 48% had converted to CDMS (median EDSS 2) and 52% remained a clinically isolated syndrome (median EDSS 1). In the final models, both the presence and the number of spinal cord lesions at baseline (odds ratios (OR) 3.30 and 1.94) and new T2 lesions at follow up (OR 7.12, 2.06) were significant independent predictors of higher disability. Disability was also predicted by the presence of Gd-enhancing lesions (OR 2.78) and the number of infratentorial lesions (OR 1.82) at baseline. Spinal cord lesions were the sole predictor of disability in patients who converted to CDMS. **Conclusions:** In ON patients, Gd-enhancing lesions, infratentorial and spinal cord lesions and new T2 lesions are predictors of subsequent disability.

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Diffusion tensor imaging of giant lesions in multiple sclerosis

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Background: Giant brain lesions (GL) are uncommon in multiple sclerosis (MS) and can represent a diagnostic dilemma. Conventional brain magnetic resonance imaging (MRI) often fails to provide the etiology. Diffusion tensor imaging (DTI) is a novel imaging technique that enables evaluation of the micro-structure of lesions, thus may serve as a powerful tool to appraise GL. **Objective:** To evaluate and characterize GL microstructure in comparison with acute and chronic MS lesions. **Methods:** MRI was obtained on a 3T system from 42 MS patients. Region of interest measurements on DTI maps were obtained for 7 GL, 15 acute and 20 chronic MS lesions. DTI was acquired using a single shot EPI sequence: TE=75, TR=14000, B value=1000 s/mm², FOV 240x240 mm, matrix 128x128, 31 independent orientations, slice thickness of 2.6 mm, no gap. Post-processing was performed by DTI-studio software and DTI metrics were calculated for ADC, FA, eigen value 1 (E1) for parallel diffusivity, and eigen value 2–3 (E2, E3) for perpendicular diffusivity. **Results:**

Mean lesion volume was significantly higher for the GL group: GL 4.49 ± 2.0 cm³, acute lesions 0.59 ± 0.21 cm³, and chronic lesions 0.73 ± 0.33 cm³, $p < 0.001$. The E1 values were similar between all lesion groups. Significant differences were demonstrated for the ADC, FA, E2 and E3 between the GL group vs. acute or chronic lesions. Mean ADC values (s/mm²) were 1.8×10^{-3} , 1.5×10^{-3} , 1.4×10^{-3} for the GL group, acute and chronic MS lesions, respectively, $p < 0.001$. E2 values (1.8×10^{-3} , 1.53×10^{-3} , 1.51×10^{-3} , $p < 0.001$), and E3 values (1.6×10^{-3} , 1.39×10^{-3} , 1.23×10^{-3} , $p < 0.05$) were higher in GL compared with acute or chronic lesions. Mean FA values were significantly lower in the GL group compared with the acute and chronic lesions (0.10, 0.13, 0.25, respectively, $p < 0.001$). **Conclusions:** The higher ADC and lower FA values in GL indicate an area with a more severe axonal loss and microstructural damage. The higher values of E2 and E3 implicate severe demyelination and microscopic edema. Altogether our results demonstrated that GL represent a separate lesion entity characterized by a very large volume, acute inflammation, severe axonal loss and destructive demyelination.

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The demographic, clinical and magnetic resonance imaging features of transverse myelitis in children

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Background: Transverse myelitis (TM) is an acute inflammatory process affecting the spinal cord. **Objective:** This study characterizes the demographic, clinical and neuroimaging features of TM in children, of which little is known. **Methods:** Analysis of all children with TM at the Hospital for Sick Children, Toronto, Canada, from 1999 to 2006 was performed using standardized data collection forms, a pre-determined magnetic resonance imaging (MRI) scoring tool and the WeeFIM scale. **Results:** Forty children met inclusion criteria, 38 were followed for a mean of 3.2 ± 2.0 years, one died during the illness, and one child was lost to follow-up. The female:male ratio was 1:0.86 for children under 10 years age and 1:2.38 in patients aged 10 to 18 years. Twenty four (60%) patients were White Canadian and 12 (30%) were Asian; 22 (55%) were first or second generation immigrants to Canada. Twice as many patients presented in the colder months of the year, with 29 (72.5%) reporting a prodromal illness. Focal lesions on spinal MRI were seen in nine (23.1%) patients and 28 patients (71.8%) had longitudinally extensive TM (LETM). None of the 12 patients tested for neuromyelitis optica IgG were positive (seven with LETM). Twenty-one out of 34 (61.8%) had brain MRI lesions; eight (23.5%) fulfilled MacDonald criteria for dissemination in space. At maximal deficit, 37 out of 39 patients (92.5%) had impairments in ≥ 2 WeeFIM skill domains. At last follow-up, six (20%) children remain wheelchair-dependent, and eight (21%) have persisting bladder or bowel difficulties. Children of Asian heritage were more likely to have LETM and a poor outcome. Six of 38 children (15.7%) were later diagnosed with multiple sclerosis. **Conclusions:** TM in children has a propensity for extensive spinal cord involvement and a significant risk for permanent disability. The heightened incidence in winter months and the common report of prodromal illness implicates an infectious trigger, the discovery of which may provide an opportunity to prevent this serious illness.

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Magnetic resonance imaging markers of brainstem neuronal injury or inflammation are not significantly associated with multiple sclerosis fatigue

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Background: The etiology of fatigue in multiple sclerosis (MS) is unclear, but may be due to injury of the brainstem reticular activating system. **Objective:** To determine whether MS fatigue is associated with magnetic resonance (MR) markers of brainstem axonal injury or inflammation and/or demyelination. **Methods:** Fifty three MS patients and 28 normal controls were recruited for a cross-sectional study. Fatigue was assessed with the fatigue severity scale (FSS) and the multidimensional fatigue inventory (MFI). Subjects underwent magnetic resonance imaging (MRI) and magnetic resonance spectroscopic imaging (MRSI) (repetition time 1500 ms, echo time 135 ms) using a 1.5T Siemens Sonata. Metabolite ratios of total N-acetyl-groups (NA)/Creatine (Cr), a marker of axonal integrity, and Choline (Cho)/(Cr), a marker of inflammation and/or demyelination, were measured in the midbrain and pons. Focal T2-weighted lesions in the brainstem were segmented manually, and the volumes were calculated in the 10 most and 10 least fatigued patients (FSS). Technically adequate MRSI data were available for 33 patients and 21 controls. **Results:** Brainstem NA/Cr and Cho/Cr did not differ between MS patients and controls, although Cho/Cr tended to be higher in MS (age-adjusted p value = 0.13). The FSS and MFI general, physical and mental fatigue were not correlated with markers of brainstem axonal injury (reduced NA/Cr) or inflammation/demyelination (increased Cho/Cr). Brainstem T2W lesion volume did not differ between the 10 most and 10 least fatigued MS patients. Four out of 10 most and 4/10 least fatigued MS patients had brainstem lesions. **Conclusions:** Fatigue in MS was not associated with MRSI markers of brainstem neuro-axonal injury or inflammation and/or demyelination, nor with focal brainstem lesions. These results support our previous findings that other parameters such as behavioral and psychosocial factors are important in explaining a part of MS fatigue. Additional potential causes include diffuse nervous system dysfunction and/or the effects of immune mediators.

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Neurobrucellosis mimicking demyelinating disease

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Background: Brucellosis is still common in developing countries. Acute, subacute or chronic meningitis, meningoencephalitis, polyradiculoneuritis, myelitis and involvement of cranial nerves are the most common features of neurobrucellosis. Bacterial infection might trigger an immune mechanism leading to demyelination in a portion of chronic neurobrucellosis cases. **Objective:** Neurobrucellosis should be considered in the differential diagnosis of demyelinating disease in Turkey because of its endemic nature. **Methods:** This prospective study included 14 consecutive patients with neuro-

brucellosis diagnosed and treated between 2002 and 2006 at the Haydarpaşa Numune Education and Research Hospital. Seven patients were male and seven were female. Seven were followed in infectious disease clinics and the other seven patients were followed in neurology clinics. All patients had diagnostic criteria for neurobrucellosis. In all patients, neuroimaging techniques, such as computed tomography and magnetic resonance imaging were performed at the onset of illness and during the following period. **Results:** The cases were classified into two different clinical categories. The first group had meningovascular complications as prominent signs. The patients had presented with some symptoms such as fever, stiff neck and cranial nerve palsies. The second clinical group had implied diffuse central nervous system involvement. In this group, brain or spinal cord involvement were prominent signs. The patients presented with symptoms such as cerebellar dysfunctions, transverse myelitis, neurosensory deafness, hemiplegia and aphasia. In seven patients, serum cultures were positive for *Brucella* species. Oligoclonal bands were negative in all these patients. The pathologies that were observed in neuroradiological examinations consisted of meningeal contrast enhancement, white matter changes and vascular changes that correlated with the clinical manifestations. **Conclusions:** Neurobrucellosis may appear with different clinical manifestations and mimicking multiple sclerosis. The diagnosis may be difficult. Early diagnosis and early treatment is effective on the progress of neurobrucellosis.

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Cerebral atrophy is an independent and detectable outcome measure within a 6-month period in patients with relapsing-remitting multiple sclerosis

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Background: Cerebral atrophy is considered to partly reflect the neurodegenerative component of multiple sclerosis (MS), and is more closely related to clinical disability compared with conventional magnetic resonance imaging (MRI) parameters. The rate of atrophy therefore, is a conceivable outcome measure for clinical trials monitoring the effect of new neuroprotective agents. As such, better knowledge of MRI factors predisposing to the development of atrophy is important in order to adopt the rate of atrophy as an outcome measure in future short-term clinical trials. **Objective:** To evaluate the rate of cerebral atrophy in a 6-month period, and to investigate the predictive and explanatory value of MRI outcome measures in relation to cerebral atrophy. **Methods:** In a natural history cohort of 154 relapsing-remitting multiple sclerosis (RRMS) patients, gadolinium (Gd)-enhanced brain MRI scans were performed monthly for 6 months. Primary outcome was the percentage brain volume change (PBVC) over 6 months. Using multiple linear regression analysis, the following possible predictive or explanatory variables in the relationship with PBVC were considered; baseline normalized brain volume (NBV), baseline number and volume of Gd-enhancing lesions, baseline number of T2 lesions, baseline T2 lesion load, number and volume of on-study Gd-enhancing lesions, on-study T2 lesion load, on-study number of new persisting black holes (PBH). All models were corrected for age, sex and disease duration. **Results:** There was a significant atrophy over 6 months (PBVC -0.33%; $p < 0.0001$), which is consistent with previous reports (annualized PBVC -0.67%/year). The number of T2 lesions at baseline ($p=0.024$), the on-study Gd-enhancing lesion volume ($p=0.044$) and the number of on-study PBHs ($p=0.003$) were associated with an increased rate of atrophy. **Conclusions:** Within a 6-month period, a significant atrophy rate could be detected, relatively unaffected by baseline MRI variables. On-study associations of PBVC and PBH suggest axonal loss to be an important driving mechanism. Our finding suggests that atrophy measurement is an independent and meaningful outcome measure in short-term clinical trials in RRMS patients.

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Magnetization transfer ratio histogram parameter best reflects the differences between normal and relapsing-remitting multiple sclerosis patients and the course of disease

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Background: Advanced magnetic resonance imaging (MRI) techniques in the diagnosis and treatment of patients with multiple sclerosis (MS) is still an area of research. Methodologies and parameters based on the use of magnetization transfer imaging that will have a relationship with the course of disease may be defined and validated in every institution according to international protocols. **Objective:** To establish the parameter derived from the histogram of magnetization transfer ratio (MTR) images best characterizes the differences between the MS patients group and control group and the parameter that reflects the relationship with the Expanded Disability Status Scale (EDSS) and duration of symptoms. **Methods:** The study group consisted of 33 patients (22 women and 11 men), with clinically diagnosed relapsing-remitting MS. The control group consisted of 14 volunteers. MRI was performed in a 1.5T scanner equipped with phased array 12-element head coil. Magnetization transfer imaging (MTI) was performed with a 2D GRE (TR 700 ms, TE 12 ms, Flip 20°, 256x128, 26x5.0 mm slices) with and without a magnetization transfer pulse. The images were processed using ImageJ software and plugins to remove the scalp and calculate MTR maps and histogram of the whole brain. The following parameters were derived from the resulting histogram: mean MTR, MTR mode, peak height, MTR25%, MTR50% and MTR75%. **Results:** All MTR parameters, except MTR mode, from the control group were significantly different when compared with the patients group. No significant correlation was found between EDSS and any of the MTR parameters. Significant negative correlations existed between the duration of symptoms and MTR parameters, especially MTR25% and peak height. **Conclusions:** Our methodology could differentiate MS patients from normal subjects and permitted a good relationship with duration of the symptoms. We would like to obtain separated histograms for white and gray matter to correlate better with the EDSS.

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Lesion probability maps: clinical correlations of brain lesion distributions in multiple sclerosis

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Background: In multiple sclerosis (MS), the presence of asymptomatic brain lesions challenges the prediction of disability based on conventional brain magnetic resonance imaging (MRI). As lesion location is crucial in determining symptomatology, previous studies have aimed at correlating pre-defined lesion locations, as well as voxel-wise lesion probability, to disability. Until now, infratentorial and spinal cord lesions have not been taken into account in these analyses. **Objective:** To explore relations between lesion location and disability using standard-space voxel-wise analyses. **Methods:** Non-parametric, permutation-based statistics were used to voxel-wise correlate lesion presence (using binary T2 brain lesion masks, including infratentorial lesions) to the following parameters: demographic patient characteristics; timed walk test (TWT); paced auditory serial addition test (PASAT); nine-hole peg test (NHPT), total T2 lesion

volume on brain MRI (TLV); presence and number of diffuse and focal spinal cord lesions. To identify statistically significant ($p < 0.05$) clusters, a cluster-forming threshold of $t=3.1$ was used. **Results:** We analyzed 327 patients (62% female; 84% relapse-onset; mean disease duration 7.8 years; mean age 39 years, median Expanded Disability Status Scale (EDSS) 3.0). Voxel-wise analyses showed that periventricular lesion presence was significantly correlated to worse disability scores, the secondary progressive disease phase and longer disease duration. Multivariate analyses revealed that disease phase correlations were determined by disease duration, and that all correlations with age or disease duration depended on TLV correlations. In cluster-wise analyses, the relation between worse disability scores and periventricular lesion presence remained significant, but when corrected for TLV, no significant clusters remained. Spinal cord lesions did not correlate with lesion probability in any location and did not influence TWT results when included in its analyses. **Conclusions:** In this large cross-sectional study, TLV proved more important in determining disability than lesion location. This may be explained by brain plasticity and the cumulative influence on disability by a higher TLV, irrespective of lesion location. **Supported by:** Dutch MS Research Foundation, Voorschoten, Netherlands, grant 05-566-MS.

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Diffusely abnormal white matter in multiple sclerosis: relationship to disease progression in an 8-year long-term follow-up

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Background: Diffusely abnormal or 'dirty'-appearing white matter (DAWM) refer to areas of increased T2 signal intensity in white matter, distinct from typical multiple sclerosis (MS) lesions. **Objective:** To determine the relationship of DAWM to clinical and magnetic resonance imaging (MRI) markers of MS disease progression in patients with relapsing-remitting MS (RRMS) followed for up to 8 years. **Methods:** Three hundred and forty eight patients with RRMS originally enrolled in a randomized, 2-year, placebo-controlled clinical trial of subcutaneous interferon beta-1a (the PRISMS study) were studied at baseline and 8 years later for those who participated in the long-term follow-up (LTF). The presence of, and change in DAWM over time (unchanged/decreased/increased) were determined by two radiologists. T2 burden of disease (BOD) and brain parenchymal volume (BPV) were determined using a semi-automated algorithm. Patients were clinically evaluated using the Expanded Disability Status Scale (EDSS). **Results:** DAWM was seen in 88 out of 348 (25.3%) patients at baseline. At LTF, DAWM was unchanged in 61/88 patients (69.3%), decreased in 25/88 (28.4%), increased in 2/88 (2.3%) and new in only 3/348 patients (0.9% of those enrolled). Patients with DAWM had lower baseline T2 BOD ($p=0.0014$) and larger BPV ($p < 0.001$). At LTF, DAWM patients had the greatest reduction in BPV ($p=0.008$) and those patients with changes in DAWM (decreased, increased, new) had the greatest increase in T2 BOD ($p=0.03$). There were no differences in baseline or 8-year EDSS score, or EDSS progression, in patients with and without DAWM, or with changes in DAWM. **Conclusions:** DAWM is present in 25.3% of patients with RRMS. Patients with DAWM or changes in DAWM demonstrated more rapid progression of MRI markers of disease, including T2 BOD and BPV, but did not demonstrate corresponding differences in disability as estimated by the EDSS, which emphasizes physical disability. Given the primarily posterior periventricular location of DAWM, it may be of greater relevance to cognitive processing or visual spatial processing not accounted for by the EDSS.

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A measure of region of interest reproducibility dependence with lesion load

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Background: Regions of interest (ROIs) are commonly used to delineate lesions on magnetic resonance imaging (MRI) scans in multiple sclerosis (MS) patients. Reproducibility metrics such as number, size, volume, Kappa and concordance (the fraction of mask overlap) are used to validate a new classification method or operator. As a result of the size and shape of lesions, these metrics have an undesirable dependence on the underlying lesion load (LL). In practice, measured agreement between independently formed lesion masks improves when formed on images with high LL. **Objective:** To determine the importance of metric selection and the extent to which total LL influences reproducibility measures. **Methods:** Forty image masks were formed using an automated lesion classifier and compared with a set of 40 gold standard masks. Correlations were calculated between three mask reproducibility metrics: Kappa; the fraction of lesions from the two masks that intersect at one or more voxels, which we term the 'overlapping lesion fraction' (OLF); and concordance. We also measured each metric's dependence on the logarithm of the number of voxels in the gold standard mask as a measure of LL. **Results:** All of the reproducibility metrics were strongly correlated. The weakest correlation was between OLF and concordance (Spearman Rho = 0.8888, $p < .000001$). Spearman Rho values comparing LL with Kappa, OLF and concordance were 0.7513, 0.7214, and 0.7801 ($p < .000001$ for all), respectively. Minimum, median and maximum LL of the gold standard masks was 2.8, 10.7, and 44.9 ml, respectively. **Conclusions:** Despite large differences in what the reproducibility metrics calculated, all were strongly correlated. This indicates that metric selection for comparing masks does not greatly impact final outcome. However, all mask comparison metrics were also strongly correlated with the underlying LL. Therefore, the underlying LL should be reported along with any mask comparison metric.

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Lesion outlining using minimum area contour change

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Background: A common approach of defining hyperintense lesions on dual-echo/fluid-attenuated inversion recovery (FLAIR) magnetic resonance imaging (MRI) scans in patients with multiple sclerosis (MS) uses the intensity value at the maximum gradient near where an operator clicks to contour and define a lesion's region of interest (ROI). Hence, a single contour value uniquely defines the ROI and reduces variability compared with a freehand approach. **Objective:** To eliminate user variability in outlining lesions and automatically track lesion size and intensity across scans using the minimum area contour change (MACC) concept. **Methods:** MACC uniquely selects a contour value and hence, the outline of the lesion. Initial ROIs are produced using a contouring-thresholding, semi-automated operator assisted approach. The MACC method creates a rectangular boundary with length and width twice that of the lesion. Within the rectangle, an indexed family of contours is created starting from the average intensity value (indexed 1) and ending with the maximum value of the original lesion (indexed N). The MACC value for index n (greater than 1) is the area ratio of the contours indexed n and n-1. Given that contours for progressively higher contours are fully contained within contours of lower values (for image topology near lesions), the MACC value is always less than or equal to 1.0. The MACC value approaches 1.0 when two neighboring contours are close, correlating with a high gradient along the contour's edge. The contour with the highest MACC value is selected and is subsequently editable in JIM4. **Results:** Testing on scans from seven subjects showed 87.5% of the ROIs from the MACC method had near perfect agreement (within 15% or

4 sq. mm) with ROIs drawn in JIM4. Using ROIs from the first time point, the method correctly outlined 85.1% of the ROIs on a second time point. **Conclusions:** The MACC method improves reproducibility while reducing operator processing time.

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Visualization and quantitative assessment of normal-appearing white matter changes in multiple sclerosis patients: Q-space analysis of the slow diffusion component at 3 Tesla

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Background: Histopathologic studies and several quantitative magnetic resonance (MR) markers have indicated pathology in the normal-appearing white matter (NAWM) in multiple sclerosis (MS) patients. However, it has been challenging to visualize this. **Objective:** To employ Q-space imaging of the slow diffusion component to detect NAWM changes in a cohort of well-characterized MS patients. **Methods:** Seventy one MS patients: 48 women, 23 men; clinically isolated syndrome (CIS) 1 patient, relapsing-remitting MS (RRMS) 53 patients, secondary progressive MS (SPMS) 12 patients, primary progressive MS (PPMS) 5 patients; mean duration of disease 15.6 years (3–38 years); mean Expanded Disability Status Scale (EDSS) 3.2(0–6.5) and 10 normal controls (NC) (5 women, 5 men). 3T-Siemens-ALLEGRA: structural magnetic resonance imaging (MRI) and including high b-value diffusion-weighted imaging (DWI) for q-space analysis. DWI: six directions, 16 b-values ranging: b=0 to b=9021 sec/mm² by linearly increasing the diffusion gradient amplitude, delta/DELTA=43/48 ms; TE/TR=125/1450 ms. Structural imaging was used for segmentation of cerebrospinal fluid (CSF), gray matter and white matter. Lesions were marked on fluid-attenuated inversion recovery (FLAIR) images. Finally, the NAWM masks of nine supratentorial probability-of-zero-displacement (PZD) maps were analyzed quantitatively and qualitatively. **Results:** The mean white matter PZD values were 1617±43 for NC and 1545±63 for patients, which were different (two-tailed t-test (p <0.005). A cluster analysis (allowing for three clusters, 1456±50, 1547±21, 1610±24) demonstrated differences of 49/71 patients to NC, which closely matched the visual analysis, that identified the same individuals as showing pathology. There was no correlation of EDSS with mean NAWM PZD, T1 or T2 lesion volumes. There was an inverse correlation of PZD with T1 or T2 lesion volumes (cc=-0.61;-0.66). **Conclusions:** In this 3T study, we used an adapted protocol for clinical use of color-coded PZD maps that were highly sensitive and could detect reductions of the slow diffusion component in the NAWM of MS patients. This was both visually and quantitatively evident and underlines the usefulness of this imaging and analysis approach to detect otherwise covert pathology. Clinical characteristics were not predictive of the NAWM changes, and particularly in patients with slight functional compromise (EDSS 0–2), additional MRI information on NAWM may be useful.

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Electrophysiological changes in the fellow eye of optic neuritis patients: evidence for early subclinical inflammation in multiple sclerosis

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Background: There is now evidence that diffuse inflammatory damage to the central nervous system (CNS) occurs very early in multiple sclerosis (MS). **Objective:** To study the evolution of diffuse CNS inflammation in early MS by examining the fellow (unaffected) eye of patients with acute optic neuritis (ON) using multifocal visual evoked potentials (mfVEP). **Methods:** Forty-eight patients with a first episode of unilateral typical optic neuritis were enrolled. At enrolment, 33 patients had brain or spinal cord demyelinating magnetic reso-

nance imaging (MRI) lesions. During the course of the study, 14 patients progressed to clinically definite MS (MS group) while 19 patients remained in the high-risk group for the development of MS (HR group). Fifteen patients had ON as a clinically isolated syndrome without MRI lesions elsewhere in the CNS and were classified as low risk for MS (LR group). Twenty-five age-matched healthy volunteers were also enrolled. mfVEP were performed at 1, 3, 6 and 12 months after ON. Latency of mfVEP was used as a marker of demyelination, while amplitude was used to assess the combined effect of inflammation and neurodegeneration. **Results:** Average amplitude and latency of the unaffected eyes were compared between the four groups. At each visit, the MS group demonstrated the largest (and highly significant) divergence of amplitude and latency from normal group, while the LR group did not show any significant difference from the controls. The HR group occupied an intermediate position, while still statistically different from the controls. None of the groups demonstrated any significant longitudinal changes in amplitude or latency of the fellow eyes during the follow-up period. **Conclusions:** Our results suggested the presence of early, subtle inflammation and demyelination in the fellow eye of ON patients with MS/high-risk characteristics, which possibly reflect diffuse sub-clinical MS-related changes in the CNS. This change was already present at 1 month after onset of ON and was proportional to the risk of development of MS.

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Column-specific spinal cord magnetization transfer, not T2*w, imaging relates to sensorimotor function in multiple sclerosis

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Background: Magnetic resonance imaging (MRI), such as T1 and T2*w, assists in diagnosing multiple sclerosis (MS) and identifying new lesions. However, the correlation with neurological deficits is imperfect, perhaps because it poorly characterizes underlying microstructural damage. Magnetization transfer (MT) MRI allows quantification of white matter columns of the spinal cord. **Objective:** To demonstrate that column-specific MT MRI correlates better with sensorimotor dysfunction in MS than T2*w MRI. **Methods:** Forty MS subjects underwent 3 Tesla MRI and quantitative measurements of sensorimotor function. From axial MTw cervical spinal cord (C2–C6) images, we calculated the cerebrospinal fluid (CSF)-normalized MT (MTCFSF) signal intensity as well as the T2*w signal in the dorsal column (DC) and lateral column (LC) white matter. We evaluated sensorimotor function using walking, ankle strength, standing balance and vibration sensation tests. **Results:** The MTCFSF in the LC was significantly correlated with strength (R=-.34, p=0.04) and walking speed (R=-.43, p=.004); MTCFSF in the DC was significantly correlated with sensation (R=.48, p=0.002) and balance (R=.39, p=.02). By contrast, T2*w in the LC and DCs were far less correlated with these impairments (LC: R=.03, p=.86, R=-.08, p=.65; DC: R=.26, p=.11, R=-.27, p=.08). Progressive MS subtype was a strong predictor of sensorimotor impairment. When subtypes were pooled, a stepwise multiple regression showed MTCFSF of the LC to be the significant predictor of strength (R²=0.13), and the DC to be the significant contributor of sensation (R²=0.23). **Conclusions:** Column-specific spinal cord MTCSE, but not T2*w, imaging results were significantly related to sensorimotor impairments in individuals with MS. We suspect this is because MTCSE is sensitive to myelin health, whereas T2*w MRI reports on inflammation. This suggests that it is the myelin damage that correlates with neurological deficits. Since the spinal cord is somatotopically organized, the correlations presented here further indicate that these sensorimotor measures may be used as surrogate markers of spinal cord damage.

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Increased coarseness of MRI texture in acute multiple sclerosis lesions affects their subsequent recoveryYunyan Zhang¹, Hongmei Zhu², J. R. Mitchell¹, Luanne M. Metz¹¹University of Calgary, Calgary, Alberta, Canada; ²York University, Toronto, Ontario, Canada

Background: T2 hyperintensity in multiple sclerosis (MS) is pathologically non-specific and dominant pathological processes within individual T2 lesions cannot be determined either using conventional magnetic resonance imaging (MRI). Texture analysis using the polar Stockwell transform (PST) demonstrates promise in detecting subtle signal alterations on MRI. PST low frequency energy (LFE) is increased during inflammation and demyelination in mice when texture irregularity increases. **Objective:** To measure LFE image texture in relation to lesion activity before, during, and after enhancement on 1.5T T2-weighted MRI in MS. **Methods:** Twenty relapsing-remitting MS (RRMS) patients were scanned bimonthly for twelve months. Gd-enhancing lesions that evolved from a region of NAWM were evaluated at month -2, during enhancement, and at month 8. Lesions were classified as resolved or persisting at month 8. Ten regions of interest (ROIs) in both the contralateral NAWM and in other regions of NAWM were also evaluated (controls). PST spectra were computed for each ROI. Texture difference was assessed using ANOVA ($P \leq 0.05$ as significant). **Results:** Twelve lesions from 10 patients were included. Five resolved and seven persisted at month 8. The average sum of LFE (sumLFE) increased during Gd-enhancement then improved by month 8. The sumLFE was also higher ($P < 0.05$) in persisting than in resolving lesions in pre-lesional NAWM (by 60.4%), during the acute phase (by 70.4%), and during the chronic phase (by 65.8%) and was consistently higher than in control NAWM ($P < 0.05$). **Conclusions:** LFE increased during tissue disruption (Gd-enhancement) then decreased during recovery. Higher sumLFE in persisting lesions suggests more severe tissue injury and predicted less recovery. Persistently high LFE in lesions that recover on T2 weighted MRI suggests that this texture analysis approach is a more sensitive measure of tissue abnormality which may facilitate evaluation of neuroprotective or reparative therapies using conventional MRI in MS.

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Diffusion tensor imaging detects early axonal injury in a rat spinal cord axotomy modelJiangyang Zhang¹, Melina V. Jones², Cynthia A. Deboy², Daniel S. Reich², Paul N. Hoffman², Kazim A. Sheikh², Susumu Mori¹, Peter A. Calabresi²¹Department of Radiology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA; ²Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Background: Axonal injury is common in patients with multiple sclerosis. Diffusion tensor imaging (DTI) is sensitive to changes in white matter microstructures related to axonal injury. Recent studies demonstrated that DTI derived axial diffusivity (Da) and radial diffusivity (Dr) measurements are potential markers of axonal and myelin injuries, respectively (1, 2). **Objective:** To investigate the sensitivity and specificity of these markers to axonal and myelin injuries in the spinal cord. **Methods:** We used ex vivo DTI and immunohistology to examine axonal injury in the rat spinal cord after unilateral L2-L4 dorsal root axotomy at multiple time points (from 16 hours to 30 days). **Results:** Three days after axotomy, DTI revealed a longitudinal lesion in the ipsilateral dorsal column extending from lumbar to cervical cord. The lesion showed significantly reduced Da and increased Dr at day 3 as compared to the contralateral unlesioned dorsal column tracts. These findings coincided with loss of phosphorylated neurofilaments, accumulation of non-phosphorylated neurofilaments, and no clear loss of myelin (stained by luxol fast blue). At day 30, DTI of the lesion continued to show significantly decreased Da. There was a slow increase in Dr, which correlated with gradual clearance of myelin without further significant change in neurofilament levels. **Conclusions:** These results show that Da can detect

axonal degeneration within 3 days after injury. The clearance of myelin in later stages may contribute to late Dr increase, whereas the cause of its early increase at day 3 remains unclear.

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Short-term change of new contrast enhancing lesion activity in secondary progressive multiple sclerosis without treatmentYinshan Zhao¹, Anthony Traboulsee¹, John Petkau², William Hunter³, David K. Li⁴¹Department of Medicine, University of British Columbia, Vancouver, British Columbia, Canada; ²Department of Statistics, University of British Columbia, Vancouver, British Columbia, Canada; ³Angiotech Pharmaceuticals, Inc., Vancouver, British Columbia, Canada; ⁴Department of Radiology, University of British Columbia, Vancouver, British Columbia, Canada

Background: Previous studies showed new contrast enhancing lesions (CEL) on monthly MRI scans decreased over a 9-month period in placebo-treated relapsing-remitting multiple sclerosis (RRMS) patients. **Objective:** We now examine the changes of new CEL activity in secondary progressive (SP) MS patients, who generally have less CEL activity. **Methods:** A post-hoc analysis was performed on 62 placebo patients from a clinical trial of Micellar Paclitaxel in SPMS (Angiotech). Patients were not pre-selected for MRI activity. Monthly MRI scans were taken at screening, baseline and months 1-6. Patients were considered as a single group and by screening CEL count level subgroups: "no", "low" and "high" (0, 1-3, >3 CEL). A random effects Poisson model was used to examine the pattern of the monthly activity rates. **Results:** 36, 17 and 9 patients had "no", "low" and "high" CEL counts at screening. The monthly new CEL rates (95% CI) of all patients at baseline, months 1-3 and 4-6 were 1.3 (0.7, 2.3), 1.3 (0.8, 2.1), 1.0 (0.6, 1.7). The changes were not significant. There was little change in the monthly rates of the "no" subgroup: 0.3 (0.1, 0.9), 0.2 (0.1, 0.4) and 0.3 (0.1, 0.7) at the three time periods, respectively. The corresponding rates were 1.3 (0.7, 2.5), 1.4 (0.8, 2.5), 1.0 (0.5, 1.7) for the "low" subgroup and 5.2 (2.3, 12.0), 5.8 (2.8, 12.3), 3.9 (1.8, 8.3) for the "high" subgroup. When assessed under a linear time trend, the rates for both "low" and "high" subgroups decreased by 9.5% (2.1%, 16.3%) per month or 45% (12%, 66%) from baseline to month 6. **Conclusions:** These SP patients with CEL at screening experienced a decline of new CEL activity in the absence of an active treatment. The rate of decrease was similar to that observed in placebo RRMS patients with CEL activity at screening.

Neuroimmunology

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Plasma chitotriosidase activity in patients with multiple sclerosis
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Background: Human chitotriosidase (Chit) is a member of the chitinase family synthesized by activated macrophages. Chit should be viewed as a component of the innate immunity that may play an important role in defence against chitin-containing pathogens. The plasma levels of this phagocyte derived enzyme are markedly elevated in some pathological conditions such as Gaucher and Niemann-Pick diseases. A recent study has shown that Chit may be involved in the pathogenesis of multiple sclerosis (MS). **Objective:** To evaluate Chit activity in healthy controls (HC) and patients with different clinical forms of MS, and the effect of treatment with interferon-beta (IFN β). **Methods:** Chit activity was determined in plasma samples from 175 untreated MS patients and 42 HC by means

of an enzyme assay as described by Hollak et al. (1994). The MS group comprised of 50 patients with primary progressive MS (PPMS), 39 patients with secondary progressive MS (SPMS), and 86 patients with relapsing-remitting MS (RRMS) (50 patients during clinical remission and 36 patients during relapse). Chit activity was also determined in patients from the RRMS group after treatment with IFN β . Plasma Chit activity was expressed as nmol/ml/h. **Results:** Mean plasma Chit activity was significantly increased in patients with SPMS compared to patients with RRMS in remission ($p < 0.05$). Comparisons of mean plasma Chit activity between RRMS patients during clinical remission and relapse did not reveal significant differences. Finally, IFN β treatment was associated with a significant increase in plasma Chit activity compared to untreated patients. **Conclusions:** These findings suggest that Chit may play a role in the pathogenesis of MS and underscore the importance of innate immunity in the progressive phases of MS and the response to IFN β treatment.

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Effect of interferon β and the development of neutralising antibodies, on reactivation of latent EBV, in patients with multiple sclerosis

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Background: EBV is associated with several putative autoimmune diseases, including multiple sclerosis (MS). Raised anti-EBNA1 IgG titres are a risk factor for developing MS and we have previously shown them to be associated with Gd-enhancing lesions on MRI. Dysregulated EBV infection has been described in MS postmortem brain tissue. Interferon- β , a disease-modifying therapy, has known anti-viral and immunomodulatory effects. **Objective:** We sought to study the effect of interferon- β on EBV reactivation, peripheral B cell viral load and immunological markers in people with MS. **Methods:** 42 treatment naive subjects and 20 healthy controls had blood, saliva and urine samples obtained at regular intervals: pre treatment and months 1, 2, 3, 6, 9, 12, 18 and 24 and at any time of relapse. Plasma, whole blood and PBMC fraction were tested for EBV DNA by real-time PCR. Serological markers anti-EBNA1 IgG, VCA IgM/G and EA IgG and CMV, measles and VZV IgG were measured. Nabs to IFN β were measured using the Luciferase Reporter Gene assay. Preliminary 12-month data was analysed to determine within subject changes in EBV activity and between groups (Nab positive and Nab negative). **Results:** 100% of MS subjects were seropositive for EBV infection, 85% healthy controls. EBV DNA was detected in whole blood, PBMC and saliva but not in the plasma fraction of both patients and controls. Nabs were detected in 6 subjects. EBV load (copy number/ 10^6 PBMCs) in PBMC fraction were significantly higher in Nab+ve (598, 95%CI 236–960) subjects than Nab-ve (354, 95%CI 181–526) and healthy controls (201, 95%CI 143–259), $p = 0.007$. EBNA1 IgG titre was significantly different between the MS (1596 95% CI 1371–1820U/ml) and healthy control cohorts (493 95% CI 454–531U/ml), $p < 0.005$. **Conclusions:** These results support the hypothesis that latent rather than lytic EBV infection plays a role in the pathogenesis of MS. High EBNA1 IgG titres, which correlate with the frequency of anti-EBV cytotoxic T cells, suggest a greater immune response to a higher EBV+ve B cell load in subjects with MS. This was higher in the Nab+ve than Nab-ve cohort which may suggest a treatment effect of IFN β . **Supported by:** EU Framework 6 grant to the NABINMS Cortium. UK & Northern Ireland MS Society.

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Reactivity to myelin peptides in a selected group of patients with relapsing-remitting multiple sclerosis

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Background: Previous studies using low doses of antigen have allowed highly discriminatory selection of a group of immunodominant epitopes of myelin between patients and controls **Objective:** To evaluate, in vitro, cellular reactivity to a mix of 7 immunodominant myelin peptides and its evolution with time in patients with relapsing-remitting MS as previous step for a protocol of antigenspecific immunological tolerance. **Methods:** We studied 18 controls and 40 patients without immunomodulatory or corticosteroid therapy in 3 months previous to blood extraction. We performed proliferation assays with peripheral blood mononuclear cells to a definite mix of myelin peptides: MBP13-32, MBP111-129, MBP 146-170, MBP83-99, MOG1-20, MOG35-55, PLP139-154 at concentrations of 2 and 10 μ M of every peptide. Lymphocyte proliferation was measured by incorporation of 3H thymidine. Individual wells were considered positive if their cpm was $\geq 3SD$ above the negative control wells. Those individuals with more than 50% positive wells were considered positive. In 20 patients, a second proliferation was performed after 3 months. **Results:** A positive reaction to mix of peptides was detected in 29 of 40 patients (73%) and 3 out of 18 controls (16%). The cellular reactivity was dose-dependent (39 % to 2 μ M and 73% to 10 μ M). Those patients with positive reactivity ($n = 29$) had higher disability (EDSS, 2.5[2–4] vs 1.0[1–2], $p = 0.02$), more relapses (8.23 \pm 3.4 vs 4.75 \pm 1.9, $p < 0.001$), and shorter time of evolution from the last relapse (9 \pm 2.6 vs 14 \pm 5.3 months, $p = 0.036$). The cellular reactivity after 3 months was maintained in 16 of the 20 patients analysed. Three patients, previously positive, presented a negative reaction after initiating treatment with interferon. One patient, previously negative, became positive after relapse. **Conclusions:** Patients with MS proliferate significantly to a mix of immunodominant myelin peptides. The reactivity was maintained with time but either relapses or immunomodulatory therapy changed it. These results suggest that this selected mix of myelin peptides may be potentially useful in protocols of induction of antigen specific tolerance in MS.

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Neuroprotective and immunomodulatory effects of leukemia inhibitory factor during neuroinflammatory responses in multiple sclerosis

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Background: Leukemia inhibitory factor (LIF) is produced by infiltrating immune cells and glial cells during auto-immune and injury responses in the central nervous system (CNS). During these responses, LIF promotes survival of glial cells and neurons. While studies indicate that LIF has immunomodulatory potential, data on underlying events are lacking. **Objective:** (1) Study mechanisms of LIF-mediated protection of mature oligodendrocytes (OLG). (2) Determine the effect of LIF on macrophage function. **Methods:** LIF mediated effects were studied in primary rat OLG and macrophage cultures. Cell viability was measured by AnnexinV/PI. Activation of signalling pathways was analyzed by western blotting and RPA. Myelin phagocytosis was determined using Dil-labelled myelin and flow cytometry. **Results:** We show that LIF protects OLG selectively against the synergistic insult of the proinflammatory cytokines IFN- γ and TNF- α , while it does not protect against oxidative stress nor against staurosporine induced apoptosis. We further demonstrate that LIF protection is independent of suppressors of cytokine signalling and Bcl-2 mRNA expression levels. Our results indicate that LIF induces a shift in the cellular machinery towards a pro-survival execution program, illustrated by an enhanced expression of isoforms of the anti-apoptotic molecule 14-3-3 and an

activation of the Jak/STAT3 and the phosphatidylinositol 3 kinase/Akt pathways. We further demonstrate that LIF modulates macrophage function by inhibiting the production of oxygen radicals and TNF- α , both mediators of CNS injury in neuroinflammatory diseases such as multiple sclerosis (MS). We show that LIF plays a role in myelin phagocytosis as it stimulates myelin uptake by macrophages. These effects of LIF on macrophage function are accompanied by activation of the JAK/STAT3 signalling pathway, without affecting AKT and ERK activation. **Conclusions:** These results demonstrate that LIF has both neuroprotective and anti-inflammatory properties and enhances myelin clearance, implicating it as an important factor in lesion modulation in immune mediated demyelinating diseases such as MS.

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TRAIL and BAFF serial serum levels during interferon β therapy in MS patients

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Background: Tumor necrosis factor(TNF) related apoptosis-inducing ligand(TRAIL) is a member of the TNF family with immunomodulatory capacity having a protective role against autoimmunity. Critical for B cell survival, B cell activating factor (BAFF) affects humoral and cell-mediated responses. **Objective:** This study focuses on expression of TRAIL levels on lymphocytes of multiple sclerosis (MS) patients and evaluates effect of interferon β therapy on soluble (s)TRAIL and BAFF levels. **Methods:** 35 MS patients and 18 controls were included. Their initial, 3rd, 6th, 9th and 12th month serum samples under interferon β treatment were collected. TRAIL expression and sTRAIL and sBAFF levels were analysed by flow cytometry and enzyme-linked immunosorbent assay respectively. **Results:** MS patients' mean basal flow-TRAIL expression rates (11.11 \pm 15) were significantly higher than control group (2.16 \pm 1.64). After treatment for 3 months, mean f-TRAIL levels of patient group were found to be 3.14 \pm 5.30(p<0.025) whereas after 6 months it was 2,00 \pm 3,21(p<0.025). MS patients' mean basal sTRAIL levels were not different from controls (p>0.05). Mean sTRAIL level was 634.75 \pm 195.44ng/ml and these values significantly increased during the sixth month of therapy. Mean basal BAFF level in MS patients (2.11 \pm 0.2pg/ml) was significantly higher than controls (p<0.05). No statistical significance of sBAFF levels has been found during 9 months of treatment. **Conclusions:** In pre-treatment phase the sTRAIL levels of patients were not significantly different than controls. Basal f-TRAIL expression rates were found to be higher in patients. After 6 months of treatment, f-TRAIL expression rates were lower where sTRAIL levels increased significantly. Detected decrease of f-TRAIL rates after therapy may depend on the cleavage of membranous TRAIL by specific metalloproteases into sTRAIL. In the pre-treatment period BAFF levels have been found significantly higher in the MS group; however, treatment had no significant effect. These findings cast doubt on whether TRAIL and BAFF have a role in this complex autoimmune process or their effect is merely a secondary compensatory mechanism that downregulates the inflammatory response.

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Assessment of CD4+CD25+ T regulatory (Treg) cells and Foxp3-expressing cells in patients with multiple sclerosis

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Background: CD4+CD25+ Treg cells play a key role in active suppression of B and T effector cells and maintenance of peripheral tolerance. Foxp3 is specifically expressed in Treg cells and it is one of the critical regulators of their development and function. **Objective:** To evaluate

the proportions of Treg that express the markers CD4, CD25 and Foxp3 and related cytokines in multiple sclerosis (MS) patients and investigate the effect of immunomodulatory drugs on these cells. **Methods:** Peripheral blood lymphocytes (PBL) were obtained from MS patients and healthy controls and stained for FACS analysis with anti CD4, anti CD25, anti CD11c, anti CD56 and anti Foxp3 abs. Patients' serum was evaluated for cytokine levels by enzyme-linked immunosorbent assay. An Excel correlation test between the scores on the Functional Independence Measure (FIM) scale and the percentage of Treg cells was performed. **Results:** A decrease in CD4+CD25+Treg cells was observed in MS patients (1.276%). The proportion of these cells increased following immunomodulatory treatments (5.18%). The expression of Foxp3 was low in CD4+T lymphocytes of MS patients (0.933%) and was upregulated following immunomodulatory treatment (5.061%). Foxp3 was also expressed in other immune populations, such as CD11c+ and CD56+ cells. The pattern of FoxP3 expression in CD11c+ cells was similar to that in CD4+T lymphocytes, while the highest expression of Foxp3 in total PBLs and in CD56+NK cells was found in untreated MS patients (76.93% and 6.84% respectively). The level of IFN- γ was increased, paralleled by a decrease in IL-4 (24.616 pg/ml and 26.91pg/ml respectively), which were partially corrected following treatment. A 30% correlation was found between the level of Treg cells and the function of MS patients in ADL tasks measured by FIM total score. **Conclusions:** Our results demonstrate that CD4+CD25+Treg cells are reduced in MS and that treatment with immunomodulating medications upregulates Treg and Foxp3+T lymphocytes and CD11c+ cells. Our findings also contradict the popular acceptance of Foxp3 as an exclusive Treg marker. An increase in IFN γ /IL-4 cytokine ratio was observed in MS patients and a correlation between Treg level and FIM scores of disability.

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Measuring the cellular immune response against Epstein-Barr virus in multiple sclerosis

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Background: Several different lines of evidence demonstrate an association between Epstein-Barr virus (EBV) and multiple sclerosis (MS), but whether EBV infection contributes to the pathologic process in MS is unclear. One possibility is that the cellular immune response against EBV is altered in MS. A deficient anti-EBV cytotoxic response would result in an excessive inflammatory response that could contribute to central nervous system damage. The available literature is limited, but suggests that the protective cytotoxic response against EBV is indeed decreased in MS, while the proliferative response is increased. **Objective:** To measure the cellular immune response against EBV-infected cells in MS patients and controls. **Methods:** First, an EBV-infected lymphoblastoid cell line (LCL) was generated for each subject using standard methods. Then a second blood sample was drawn, and peripheral blood leukocytes (PBL) were isolated using Ficoll. CD4 and CD8 enriched cells were prepared from PBL using negative selection with magnetic beads. The PBL, CD4, and CD8 cells were stimulated with autologous LCL in assays for cytotoxicity, proliferation, and number of interferon- γ secreting cells. **Results:** To date, we have tested four MS patients and three controls. The two subjects with the most effective cytotoxic response were controls. Two of the MS patients and one control had a negligible cytotoxic response. The proliferative responses were similar in MS and controls. The two controls with high cytotoxic activity also had the highest number of interferon- γ secreting cells. Cytotoxic activity and interferon- γ secretion were found in both the CD4 and CD8 subsets in MS and controls. **Conclusions:** Our preliminary results support the idea that the cytotoxic response against EBV may be decreased in MS. This might result in an increased inflammatory response at sites of EBV infection, and contribute to the disease process in MS. We are currently testing more subjects.

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P685**Effects of cytokine mixtures typical of Th1 and Th2 cells and monocyte/macrophages on early gene expression in CNS neurons: differential modulation of signaling pathways**

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Background: Axonal damage and neuronal loss lead to much of the permanent disability in multiple sclerosis (MS). Damage to demyelinated axons has been postulated as a major cause of neuronal damage and death. However, cytokines (Cyt) may also have deleterious direct or indirect effects on neuronal function. **Objective:** To investigate how Cyt mixtures typical of Th1 and Th2 cells and monocyte/macrophages (M/M) differentially regulate neuronal gene expression. **Methods:** Cultures of neonatal rat central nervous system neurons consisting of 88–90% neurons, identified by anti-neuronal antibodies, and 10–12% astrocytes, were treated with the different Cyt mixtures for 6 hours. No cytotoxicity was observed compared to controls. RNA was extracted and analyzed using Affymetrix REA230 2.0 microarrays. To screen for genes and pathways of interest, we accepted changes in expression of >1.2 fold, $p < 0.05$ compared to control as significant. We determined the number of genes modulated in the different but somewhat overlapping KEGG pathways. **Results:** All three Cyt mixtures regulated Cyt-Cyt receptor interactions, inositol phosphate metabolism, and signaling via toll-like receptors, notch, and MAPK, with Th1 and M/M affecting more genes than Th2. Both Th1 and M/M regulated pathways related to antigen processing/presentation, cell adhesion, apoptosis, complement/coagulation cascades, hematopoietic cell lineage, actin cytoskeleton, and signaling through adipocytokines, wnt and JAK/STAT. Th1 Cyt uniquely regulated genes related to glycine/serine and carbohydrate metabolism, and M/M Cyt, extracellular matrix, and arachidonic acid, glycosphingolipid, arginine/proline and histidine metabolism. Th2 Cyt uniquely regulated calcium signaling, neuroactive ligand-receptor interactions, long term potentiation and Fc epsilon signaling. Interestingly, M/M and Th2 Cyt regulated B cell receptor signaling, while Th1 Cyt had no effect. **Conclusions:** The data suggest that Th1, M/M and Th2 Cyt have different effects on early gene expression in neurons, which may prove to be important in both damage and protection of neurons and axons in MS.

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P686**Ectopic germinal centres in the brain of people with multiple sclerosis**Cosimo Maggiore¹, Gusta Trillo-Pazos², Geoff Keir², Gilberto Pizzolato³, Paolo Edomi³, Ahuva Nissim⁴, Gavin Giovannoni¹

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Background: In order to ascertain the mechanisms of damage and to develop new therapies in multiple sclerosis (MS), it is essential to understand the neuropathology of the disease. The differences seen in MS lesions in the earliest stages seem to converge into a homogeneous pattern in the active demyelinating phase. The aetiology of the immune response and the mechanisms underlying the chronicity and the neurodegenerative aspect of the disease are still unknown. In a significant proportion of people with MS (PwMS) affected by a secondary progressive disease, ectopic B-cells follicles with germinal centers (EGCs) can be detected in the meninges, and recent studies have implicated EBV as a possible trigger of disease activity. **Objective:** To establish the presence of EGCs and EBV infection in MS. **Methods:** Immunohistochemically lesions and lymphoid infiltrates were identified on snap frozen brain blocks from PwMS. Markers for lymphoid tissues and for lesions (GFAP, MAP2, PLP and CNPase) were used. We stained for EBV in the lesions and in the ectopic lymphoid organs using antibodies targeting

EBNA-1, LMP-1 and LMP-2a antigens of latent EBV infection. **Results:** The presence of EGCs (CD20+, CD21+, CD3+, CD68+ markers for B-cells, T-cells and macrophages) and the expression of latent EBV antigens (EBNA1, LMP-1 LMP-2) in brains of PwMS have been confirmed. These germinal centres were microdissected using laser capture microdissection to test for the presence of viral gene expression using different approaches. **Conclusions:** In contrast with classic germinal centres, ectopic ones are found in non-lymphoid tissue and are associated with chronic inflammation, the local production of immunoglobulin, and the chronic upregulation of chemokines that are normally absent in these tissues. Our preliminary data suggest that in MS the same pathogenic mechanisms underlying some chronic infectious and inflammatory diseases and that EBV could be the trigger agent in EGCs in secondary progressive MS. Currently other viruses, potentially associated with MS, are being investigated using a microgenomics approach.

P687**Tight junction proteins in immune cells: new players in multiple sclerosis?**Ilana Mandel¹, Tamar Paperna¹, Lea Glass-Marmor¹, Sara Dishon², Ariel Miller¹

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Background: Multiple sclerosis (MS) is associated with enhanced immune cell migratory capacity. Migration of immune cells, predominantly lymphocytes and macrophages, into the central nervous system through the blood brain barrier (BBB) elicits inflammation, culminating in demyelinating lesions. Tight junctions (TJ) protein complexes expressed by endothelial cells are major contributors to the cell-cell barrier that comprises the BBB. **Objective:** To compare TJ expression in peripheral blood lymphocytes (PBLs) of healthy individuals versus MS patients at various disease states (relapse versus remission) and to examine the effect of MS immunosuppressive and immunomodulatory drugs on TJ expression. **Methods:** PBL samples were collected from healthy individuals and MS patients, prior to and following treatment with interferon-beta or glucocorticoids (methylprednisone; GC). Expression levels of TJs in RNA from PBLs were assessed by real-time polymerase chain reaction; protein levels were analyzed by Western blots. Immunofluorescence studies performed on peripheral blood mononuclear cells from healthy individuals were visualized by confocal microscopy. **Results:** We observed that TJ proteins, known to be expressed mainly by endothelial and epithelial cells, were consistently expressed by PBLs, in both MS and control samples. GC treatment led to a significant reduction in RNA levels for the TJ genes encoding JAM3 and claudin 5. A decrease in claudin 5 protein levels was detected as well. Immunofluorescence studies revealed that TJ were expressed predominantly by T and B lymphocytes and not by other PBLs, such as monocytes and granulocytes. Interferon beta treatment did not seem to affect TJ expression levels in PBLs. **Conclusions:** Our results demonstrate that TJ are expressed in B and T cells, and that their levels may be affected by MS disease state and treatment. These findings indicate a novel role for TJ within immune cells to be considered, suggesting new possibilities for interference with immune cell extravasation in MS.

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P688**NMO-IgG antibodies targets astrocytes in contact with oligodendrocytes in the optic nerves and the spinal cord**Romain Marignier¹, Pascale Giraudon², Chantal Watrin², Gaelle Cavillon², Veronique Rogemond², Sandra Vukusic¹, Jérôme Honnorat², Christian Confavreux¹

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Background: NMO-IgG is a specific autoantibody of Devic's neuromyelitis optica (DNMO), an inflammatory demyelinating disorder restricted to the optic nerves and the spinal cord. NMO-IgG binds

selectively to aquaporin-4 (AQP4), a water channel highly expressed in the astrocytic foot processes at the blood brain barrier. Recent findings support a direct pathogenic role for this antibody. However, AQP4 involvement has not yet clearly found either the specific location of DNMO tissue lesions or demyelination mechanisms. **Objective:** To investigate the specific cellular staining of NMO-IgG antibody in the target tissues in DNMO. **Methods:** Serum of 29 patients (transverse myelitis group n=10, DNMO group n=11 and multiple sclerosis (MS) group n=8) was examined for immunoreactivity on rat spinal cord, optic nerves and whole brain. Tissues were fixed in 4% paraformaldehyde (PFA). All the sera were previously tested for NMO-IgG detection using classic 10% formol fixation. **Results:** For both fixation methods, a specific staining was observed in 4/10 sera in the transverse myelitis group, 6/11 in the DNMO group and 0/8 in the MS group. When present, the staining was homogeneous for all the sera, whatever the clinical presentation. In the formol fixed tissues, NMO-IgG immunoreactivity concentrated in astrocytic foot processes around blood vessels, as expected. In contrast, using PFA 4% fixation, in the brain, immunoreactivity for NMO-IgG was present in the processes and soma of astrocytes only in specific areas (glia limitans externa, subependyma, cerebellum and hippocampus). In the spinal cord, a diffuse staining for astrocytes, both in the white and grey matter, was observed. Moreover, in the spinal cord and the optic nerves, all the AQP4+ cells had the ability to bind NMO-IgG. Finally, in these tissues, some of the NMO-IgG+ astrocytic processes encircled oligodendrocytes. **Conclusions:** NMO-IgG staining is diffuse in the optic nerves and the spinal cord. This antibody has the ability to target astrocytic endfeet around blood vessels but also astrocytes in close contact with myelinating cells, especially in the optic nerves and the spinal cord.

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Differential gene and protein expression profiles in patients with CIS

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Background: Additional biomarkers are needed to more accurately identify clinically isolated demyelinating syndrome (CIS) patients eligible for early immunomodulatory therapy. **Objective:** To identify a profile of differentially expressed genes (DEGs) in the peripheral blood mononuclear cells (PBMCs) and proteins in the cerebrospinal fluid (CSF) derived from CIS patients. **Methods:** Gene expression profile was detected using Affymetrix (HG-U133) arrays (~45,000 probes) in PBMCs derived from 15 untreated CIS patients and 7 matched healthy controls (HCs). CSF protein profile was detected by RayBio® Human Cytokine Antibody Array (120 proteins) in 10 CIS patients and 14 matched HCs. Differential proteomic CSF profile was established by comparing CSF profiles with the corresponding sera. **Results:** Genomic profiling of PBMCs revealed 277 DEGs in CIS patients when compared to HCs, including 21 up-regulated and 8 down-regulated immune response (IR) genes. Among the significantly up-regulated IR genes were IL1R1, IL21R, and CCR6, which are identified as markers of Th17 cells. A differential analysis of the CSF and corresponding sera from CIS patients revealed selectively up-regulated 29 proteins, including TGF- β 1, IL-6, and its receptor sgp130, which all play a role in the Th17 cells differentiation. Proteins with the most significant increase in the CSF in comparison to the sera were chemokines fractalkine/CX3CL1, eotaxin-3/CCL26, SDF-1/CXCL12, MIP-3 α /CCL20 and I-309/CCL1, which constitute chemokine gradient that mediates inflammatory cell migration into the central nervous system (CNS). Proteomic profiling of CSF from CIS patients in comparison to HCs revealed up-regulated fibroblast growth factor (FGF)-6 and GDNF, neuroprotective neurotrophins secreted in response to the inflammatory damage within the CNS. Up-regulated fractalkine, a chemokine secreted by neurons, reflects the neural damage. MCP-1, a Th2-related chemokine, was decreased in CSF samples from CIS patients. **Conclusions:** Our results identified gene expression changes in PBMCs consistent with a critical role of Th17 cells in the development of autoimmune response. CSF pro-

teomic profiles identified higher expression of neuroprotective factors in CIS patients.

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Interferon- β modulates STAT6-activated inflammatory gene expression through the induction of SHP-1 in multiple sclerosis PBMCs

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Background: The protein tyrosine phosphatase SHP-1 is a crucial negative regulator of proinflammatory cytokine signaling both in the immune and central nervous systems (CNS). Mice lacking SHP-1 display pronounced virus-induced inflammatory demyelinating disease in the CNS. We have recently reported that SHP-1 activity is induced by interferon- β (IFN- β) both in the CNS and immune system of mice (Christophi et al., J Neurochem. 2008). Also, we have shown that peripheral blood mononuclear cells (PBMCs) of multiple sclerosis (MS) patients display a stable deficiency of SHP-1 expression that leads to heightened STAT6 phosphorylation (pSTAT6) and increased STAT6-activated gene expression relevant to the mechanisms of inflammatory demyelination (Christophi et al., Lab Invest. 2008). **Objective:** To examine whether SHP-1 expression and activity is modulated by IFN- β . **Methods:** Cultured PBMCs of normal subjects and MS patients were treated with IFN- β , and SHP-1 expression, pSTAT6 and pSTAT1 activation, and inflammatory gene expression were analyzed. **Results:** Treatment of cultured PBMCs from MS patients with IFN- β increased the expression of SHP-1 to levels seen in PBMCs of normal control subjects. Moreover, IFN- β treatment significantly lowered levels of pSTAT6 in PBMCs of MS patients, consistent with heightened SHP-1 activity. Conversely, depleting SHP-1 using siRNA effectively increased the levels of pSTAT6 in PBMCs of normal subjects to levels equal to MS patients and abolished the suppression of pSTAT6 by IFN- β . Finally, multiple STAT6-responsive genes were increased in MS PBMCs relative to PBMCs of normal subjects and these levels were reduced to normal values with IFN- β . To the contrary, STAT1 phosphorylation and STAT1-responsive genes were increased following IFN- β treatment. **Conclusions:** Thus, cultured PBMCs of MS patients display a stable deficiency of SHP-1 expression, which affects SHP-1 activity on STAT6 and the ability to regulate STAT6-responsive genes that may be relevant to MS inflammatory disease processes.

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Specific inhibition of peptidylarginine deiminase attenuates demyelination

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Background: Multiple sclerosis (MS) is the most common demyelinating disease in humans. The mechanism by which myelin is destabilized and degraded is obscure. In studying this process, we have found that myelin basic protein (MBP) isolated from white matter from MS patients was modified by the conversion of arginine \rightarrow citrulline. This change is mediated by the enzyme peptidylarginine deiminase2 (PAD2) and results in a reduction of positive charge on MBP, thereby compromising its ability to interact with negatively

charged lipids, resulting in myelin destabilization. **Objective:** PAD2, which is up-regulated in MS due to hypomethylation of its promoter, is localized to the myelin sheath and the axon and represents a therapeutic target. **Methods:** We used the amidino compound 2-chloroacetamide (2CA), an irreversible active site inhibitor of PAD2, to treat mouse models of demyelination. These included a transgenic mouse model of demyelination and an autoimmune model of demyelination. **Results:** We have shown it to inhibit PAD2 in vitro and to bind to cysteine-656 in the active site of the enzyme by mass spectrometry. We have treated two animal models of demyelination with 2CA and showed disease was attenuated in both. In one, a transgenic mouse expressing supernumerary copies of DM20, a myelin proteolipid protein, was treated three times per week after signs of disease were evident. Attenuation of clinical signs and morphological evidence of remyelination were observed. When vitamin B12 was included in the treatments, further attenuation was observed and PAD activity was reduced by 50%. In a second animal model, myelin/oligodendrocyte glycoprotein induced experimental autoimmune encephalitis with peptide 35–55, were treated on alternate days with 2CA, beginning after onset of clinical signs, disease was attenuated. In histological brain sections, a reduction of lymphocyte infiltration was observed, consistent with a reduction in splenocytes isolated from the spleens of treated animals. These studies are in progress. **Conclusions:** We concluded that inhibition of enzyme activity in combination with promoter methylation (which decreased PAD transcription) represents a novel therapeutic strategy. **Supported by:** This project was supported by an operating grant from MS Society of Canada awarded to MAM and FGM.

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Multiple sclerosis patients resisted to glucocorticoids therapy: abnormal expression of hsp90 in the GR complex

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Background: The majority of multiple sclerosis (MS) patients respond favourably to glucocorticoids (GS) for their relapse treatment (SS MS). Unfortunately, a small subset of MS patients fail to adequately respond even to high dose of GS (SR MS). The mechanism of GS therapeutic unresponsiveness is not resolved. GS signalling depends on ligation of glucocorticoid receptors (GRs) with hsp90 in cytoplasm. **Objective:** In this study we have examined molecular mechanisms of unresponsiveness to GS in MS patients. **Methods:** GR transcripts were assessed in peripheral blood mononuclear cells (PBMCs) by real-time polymerase chain reaction in SS and SR MS patients. GR expression was assessed by Western blotting. The amount of heat shock protein 90 (hsp90) in GR cytoplasmic complex was assessed by immunoprecipitation. Hsp90 was shown to stabilize the GR complex, prevent its translocation to nucleus and inhibit GR transcription. **Results:** In PBMC of SR MS, transcripts for all three isoforms of GR, alpha, beta and gamma, were reduced by about half compared to SS MS patients. We have not found an increase in the beta and gamma transcripts of GR, which might serve as dominant negative mutants, over GR alpha in SR MS. The amount of hsp90 in the GR complex in cytoplasm was significantly higher in SR MS compared to SS MS. **Conclusions:** Molecular mechanism of GS unresponsiveness in MS patients might be related to increased presence of hsp90 in the GR cytoplasmic complex leading to inhibition of GR translocation to nucleus. There results might elucidate the mechanism of unresponsiveness to GS in MS patients.

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Stem cells ameliorate EAE via an indoleamine 2,3-dioxygenase (IDO) mechanism

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Background: Stem cells (SC) have been shown to have beneficial effects during autoimmune demyelination but underlying mechanisms remain unknown. In mice, lineage negative cells expressing the Sca1 molecule (Lin-Sca1+) represent a pluripotent population of bone marrow SC (BMSC) depleted of mature hematopoietic precursors and enriched in mesenchymal SC. **Objective:** In this study we have assessed the immunoregulatory role of pluripotent Lin-Sca1+ bone marrow stem cells (Lin-Sca1+BMSC) in experimental autoimmune encephalomyelitis (EAE), a model of multiple sclerosis. **Methods:** Syngeneic pluripotent Lin-Sca1+BMSC were transferred at peak of disease to mice with PLP139-151-induced EAE. At different time points after transplantation proliferation of T cells and IFN-gamma secretion were assessed. Accumulation of PKH26 stained SC in mouse organs was analyzed by flow cytometry. The role of indoleamine 2,3-dioxygenase (IDO) was established using Western blot analysis and specific competitive inhibition with 1-methyl-DL-tryptophan (1-MT). **Results:** Lin-Sca1+BMSC transfer enhanced recovery (p=0.000950), prevented relapses (p=0.0076), reduced central nervous system damage and enhanced remyelination. T cells from treated mice showed decreased proliferation to PLP139-151 (p=0.0019) and elevated interferon-gamma production. In dendritic cells (DC) increased induction of IDO was observed. Specificity of IDO involvement was confirmed by demonstration that in the presence of CD11c+ DC, with high IDO expression, PLP-induced proliferation was inhibited and the IDO-inhibitor, 1-MT, abrogated the immunoregulatory effect of Lin-Sca1+BMSC (p=0.000912). Relapse prevention correlated with inhibition of antigen spreading, the latter evidenced by loss of T cell responsiveness to PLP178-191 and MBP85-99 during chronic disease. **Conclusions:** Thus, pluripotent SC induce IDO in DC, leading to inhibition of antigen reactivity and spreading in EAE.

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A molecular approach for determining the CD8+ T cell epitopes in a murine model of multiple sclerosis

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Background: It is generally accepted that non-chronic progressive multiple sclerosis (MS) is an immune mediated disease. Growing evidence has demonstrated a potential role for CD8+ T cells in this disease. CD8+ T cells are the most numerous lymphocyte within active MS lesions, are clonally expanded in the blood and cerebrospinal fluid of MS patients and have been associated with central nervous system damage. However, the antigen specificity of CD8+ T cells within MS patients has not been elucidated. Our lab has demonstrated the ability to inhibit antigen specific CD8+ T cells within the Theiler's murine encephalomyelitis virus (TMEV) animal model of MS resulting in reduced pathological and clinical symptoms. **Objective:** To create a molecular approach to elucidating the antigen specificity of CD8+ T cells within the TMEV model of MS. **Methods:** The TMEV virus genome was dissected into 16 overlapping 550 base-pair segments and placed into expression vectors. Likewise, mouse strain specific MHC class I molecule genes were placed into plasmids. Separate experimental groups of cells from the COS-7 cell line were then transfected with a segment of the TMEV virus and an MHC class I molecule. TMEV virus specific CD8+ T cells were then isolated and co-cultured with transfected COS-7 cells. An enzyme-linked immunosorbent assay was then performed on the media of transfected cells in order to detect IFN- γ expression, an indicator of antigen recognition by CD8+ T cells. **Results:** MHC class I molecules were successfully expressed on transfected cells as detected by flow cytometry. Additionally, virus specific CD8+ T cells expressed IFN- γ in response to a known TMEV

antigen, indicating the MHC class I molecules successfully presented viral peptides. **Conclusions:** This system of antigen detection was proven successful for the TMEV model of MS and may be directly translated to human MS in the future through the use of library expansion.

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NMO-IgG immunofluorescence sensitivity exceeds aquaporin-4 IgG immunoprecipitation: combined assays improve sensitivity

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Background: Multiple laboratories have confirmed the high sensitivity and specificity of AQP4-specific IgG for neuromyelitis optica (NMO) spectrum disorders. Limited reports suggest that immunoprecipitation and cell-binding assays (AQP4-IgG) are more sensitive than immunohistochemical (NMO-IgG). **Objective:** We compare sensitivity and specificity of our standardized quantitative GFP-linked AQP4 immunoprecipitation and immunofluorescence assays for two groups in whom service NMO-IgG testing was requested (total = 6335). We evaluate their utility, independently and combined, in a clinical setting. **Methods:** Two groups were evaluated serologically for NMO-IgG and AQP4-IgG (normal value ≤ 10 nmol/L). Group 1 consisted of Mayo Clinic patients (n=100) and controls (n=735), 2004–2007. Patient diagnoses: NMO, 40; longitudinally extensive transverse myelitis (LETM, ≥ 3 vertebral segments), 43 (relapsing 17); relapsing optic neuritis (ON), 17. Control diagnoses: MS, 249; TM (<3 segments), 64; monophasic ON, 40; ADEM, 6; other, 376. In Group 2 were non-Mayo Clinic patients (n=5500), October 2007–March 2008. **Results:** Group 1 - Sensitivities for NMO: NMO-IgG 58%; AQP4-IgG 33%; combined assays 63%. Sensitivities for relapsing LETM: NMO-IgG 29%; AQP4-IgG 6%; combined 29%. Specificities for NMO and relapsing LETM: NMO-IgG 99.7%; AQP4-IgG 99.5%; combined 99.5%. Group 2 - 574 patients were positive in one or more assays (10%). The seropositives were: NMO-IgG alone, 167 patients (29%); AQP4-IgG alone, 76 (13%); both, 331 (58%). Clinical diagnoses were available for 124 seropositive patients (22%). All NMO-IgG positive patients (n=108; with and without AQP4-IgG) had an NMO spectrum disorder (100%). Of 16 patients positive only for AQP4-IgG (median value, 32.6 nmol/L; range, 10.2–867 nmol/L) 15 had an NMO spectrum disorder (95%) and 1 was at risk for NMO (monophasic ON). **Conclusions:** In a large clinically-based study, seropositivity in immunofluorescence or immunoprecipitation assays was specific for NMO spectrum disorders. AQP4 immunoprecipitation was less sensitive; combined testing improved sensitivity. Supported by a grant from the Ralph Wilson Medical Research Foundation.

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Opioid growth factor (OGF) and low dose naltrexone (LDN) inhibit immunological responses associated with EAE

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Background: The first phase of experimental autoimmune encephalomyelitis (EAE) is characterized by the production of pro-inflammatory cytokines, inflammation, and the recruitment of activated T lymphocytes and antibodies to the central nervous system (CNS). Somatic hypermutation prior to lymphocyte entry into the CNS, along with epitope spreading, molecular mimicry, and bystander

activation once T and B lymphocytes have infiltrated into the CNS, contribute to disease progression. Both the opioid growth factor (OGF) and low dose naltrexone (LDN) repress the progression of EAE in mice. **Objective:** To determine the mechanism of OGF and LDN action on EAE. **Methods:** Immediately after induction by myelin/oligodendrocyte glycoprotein injection, mice were treated intraperitoneally once daily with 0.1 mg/kg naltrexone (MOG-LDN), 10 mg/kg OGF (MOG-OGF) or saline (MOG-vehicle). Spleens were collected at both 5 and 8 days following MOG injection, from which lymphocytes were isolated and incubated for 72 hr in the presence of either MOG35-55 peptide or phytohemagglutinin (PHA; a T cell mitogen). T lymphocyte proliferation was measured using a standard thymidine incorporation assay. **Results:** MOG35-55-stimulated lymphocytes isolated at 5 days from MOG-LDN and MOG-OGF mice had significantly ($p<0.05$) reduced thymidine uptake (1508 ± 583 and 945 ± 384 cpm, respectively) relative to MOG-vehicle mice (3267 ± 778 cpm). Lymphocytes isolated at 8 days from MOG-OGF and MOG-LDN mice treated with PHA had significantly decreased thymidine incorporation relative to controls. **Conclusions:** These data demonstrate that early in the induction of EAE, treatment with OGF or LDN reduces MOG-primed lymphocytes and suggests a mechanistic pathway in the understanding of EAE pathogenesis and opioid action. These data suggest that therapeutic treatment with either OGF or LDN suppress T cell proliferation.

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Oral fingolimod (FTY720) treatment reduces peripheral IL-17-producing TH17 cells in patients with multiple sclerosis

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Background: Fingolimod (FTY720) is an oral sphingosine-1-phosphate receptor modulator, which showed efficacy in a phase II clinical trial in patients with multiple sclerosis (MS). Therapeutic dosing of fingolimod retains lymphocytes in the secondary lymphoid organs, thereby reducing naive T cells and central memory T cells (TCM), but not effector memory T cells (TEM) in blood. Little is known about effects of a long-term mono-therapy with fingolimod on encephalitogenic IL-17 producing TH17 cells, which possibly contribute to inflammatory processes in autoimmunity. **Objective:** To determine in patients with MS the effect of fingolimod on peripheral IL-17 producing TH17 cells. **Methods:** T cell subsets were phenotypically characterized by flow cytometry in peripheral blood mononuclear cells from patients with MS treated with fingolimod (n=5), untreated patients with MS (MSUT, n=5) and healthy donors (HD, n=6). IL-17 production was determined in purified CD3+ T cells after stimulation with anti-CD3/CD28 monoclonal antibodies. **Results:** In HD and MSUT, the majority of IL-17 producing CD4+TH17 cells express the chemokine receptor CCR6 (HD: 83%; MSUT: 81%). CCR6 is mainly expressed in TCM (HD: 36%, MSUT:39%) and to a lesser degree in TEM (HD: 13%, MSUT: 12%) and naive T cells (HD: 3%, MSUT: 5%). CCR6 is expressed in 22% and 24% of CD4+ T cells in HD and MSUT, whereas it can be found only in 4% of CD4+ T cells in fingolimod-treated patients. Stimulation of equal amounts of CD3+ T cells revealed reduced IL-17 production in fingolimod-treated patients by $>80\%$ when compared to HD and MSUT. **Conclusions:** Oral fingolimod strikingly reduces the numbers of CCR6+CD4+ central memory TH17 cells in blood circulation, most likely by retaining these cells in the secondary lymphoid organs. This would also reduce the recirculation of proinflammatory TH17 cells to the central nervous system (CNS), and the inflammatory activity in CNS lesions of patients with MS.

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WIN 55,212-2 downregulates VCAM-1 in a viral model of multiple sclerosis

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Background: Brain endothelial cell infection represents one of the first events in the pathogenesis of Theiler's virus-induced demyelinating disease (TMEV-IDD), a model of multiple sclerosis (MS). Vascular adhesion molecule-1 (VCAM-1) represents one of the most important adhesion molecules involved in the transmigration of blood leukocytes across the blood-brain barrier (BBB) that is an essential step in the pathogenesis of MS. There is considerable evidence to suggest the potential therapeutic value of cannabinoids (CBs) in the treatment of MS and their experimental models. However, the mechanisms by which CBs mediate their effects are only partially known. **Objective:** We investigated whether VCAM-1 could be target of CBs in neurovascular endothelium by *in vivo* and *in vitro* approach. **Methods:** *In vivo:* At the time of TMEV infection, SJL/J mice were treated with the CB1/CB2 agonist, WIN55,212-2 (1.5mg/kg) for 3 days. Motor function was evaluated by rotarod and activity cage test. VCAM-1 expression, CD4 lymphocytes infiltration and microglial activity was analyzed by immunohistochemistry in brain sections of these mice. *In vitro:* VCAM-1 production was measured by enzyme-linked immunoabsorbent assay. **Results:** We showed that WIN at the time of virus infection downregulated VCAM-1 expression, reduced perivascular CD4+ T lymphocyte infiltration and limited microglial responses to areas close to the injection site. This early treatment interferes with the later development of TMEV-IDD since WIN-treated mice reduced disease severity. To gain insight into the signaling pathways involved in VCAM-1 regulation, we used brain endothelial cell cultures. Here we report that WIN downregulates VCAM-1 through a novel mechanism that involves peroxisome proliferator-activated receptors gamma (PPAR γ). **Conclusions:** Overall, our data suggest that downregulation of adhesion molecules in brain endothelium is one of the mechanisms by which CBs diminish central nervous system inflammation in MS.

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Visualization and characterization of cytotoxic T cell-neuron interactions: antigen-presentation determines specific CD8 T cell locomotion and separates an immediate and a delayed pathway of acute neuronal injury

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Background: Cytotoxic T lymphocytes are important effector cells in the immune response and they are involved in the pathophysiology of autoimmune diseases such as multiple sclerosis (MS). The cells release the content of their cytotoxic granules into the immunological synapse to trigger apoptosis of the target cell. **Objective:** Here we question the underlying mechanisms of antigen-specific killing of neurons by CD8+ T cells with a focus on early electrophysiological changes. **Methods:** We use immunohistochemical and electrophysiological assays in combination with time-lapse video-microscopy and two-photon microscopy in an ovalbumin specific co-culture system of hippocampal neurons and cytotoxic T lymphocytes (OT1 cells). **Results:** CTL killed cultured, MHC class I-induced central nervous system (CNS) neurons quickly and depending on cell-cell contact. Migration pattern of CD8 T cells, characteristics and number of immune-neuronal contacts are determined by the presentation of the TCR-specific antigen on neurons. Real-time electrophysiological recordings revealed that CD8 T cells can induce immediate changes in membrane capacitance and resistance

(appr. 60% decrease; \leq 10 minutes). With a similar kinetic and MHC restriction, neurons showed a rapid influx of Ca²⁺. Single-cell recordings using TCR-transgenic T cells (OT-I) deficient for granzyme B or perforin or neutralizing Fas-FasL interactions showed that immediate effects crucially depended on perforin. In contrast, the amount of neuronal injury in cocultures over 6 hours showed no differences between granzyme B-deficient, perforin-deficient or WT-CTL. **Conclusions:** Interaction of CD8 T cells with CNS neurons and its functional consequences are determined by neural antigen presentation, TCR-specificity and direct cell-cell contact. Immediate changes of basal cellular parameters upon neuronal contact are caused by perforin alone. However, a second pathway of inducing delayed neuronal injury seems to involve perforin and granzyme B.

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Glatiramer acetate induces interleukin-1 receptor antagonist and inhibits the induced production of interleukin-1 β in human monocytes

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Background: Mechanism(s) of action of glatiramer acetate (GA) in multiple sclerosis (MS) remain elusive. It is likely that the pro-inflammatory cytokine interleukin-1 β (IL-1 β) plays an important part in MS. IL-1 β is a mediator of inflammation in experimental autoimmune encephalitis (EAE), and is detected in central nervous system-infiltrating macrophages and microglial cells. The secreted form of IL-1 receptor antagonist (sIL-1Ra) is a naturally occurring inhibitor of IL-1 β that ameliorates the course of EAE. IL-1 β may therefore represent a suitable therapeutic target in MS. Cellular contact of human monocytes with stimulated T cells up-regulates IL-1 β and sIL-1Ra production. **Objective:** To study the effects of GA on the production of IL-1 β and sIL-1Ra in human monocytes and to extend these observations to the effects of GA on cytokine levels in EAE and in MS patients. **Methods:** *In vitro:* We activated human monocytes either with GA, or by direct contact with stimulated T cells, using soluble extracts of isolated membranes (CesT), in the presence or in absence of GA. *In vivo:* Cytokine levels were assessed by enzyme-linked immunoabsorbent assay in human and mouse serum. **Results:** *In vitro,* GA induced transcription and production of sIL-1Ra in human monocytes through signaling pathways involving PI3K δ , GSK3 β and MEK2. In T cell contact-activated monocytes, GA strongly diminished the production of IL-1 β at the transcriptional level and enhanced sIL-1Ra transcript expression. In contrast, in lipopolysaccharide (LPS)-activated monocytes, GA enhanced both IL-1 β and sIL-1Ra production. Thus, GA differentially affected cytokine production following conditions related to chronic (CesT) and acute (LPS) inflammation, respectively. sIL-1Ra serum levels were increased in EAE mice treated with GA compared to untreated animals. Similarly, in patients with relapsing-remitting MS, GA treatment enhanced levels of sIL-1Ra as compared with untreated patients and healthy controls. **Conclusions:** GA directly affects human and murine monocytes by triggering a bias toward a less inflammatory profile. This new mechanism might participate in the therapeutic effects of GA in MS.

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P701**The relationship between oxidative burst and severity of multiple sclerosis**

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Background: Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase reduces oxygen in phagocytic cells during elimination of pathogens to form a number of reactive oxygen species. This process, known as the respiratory burst, is an important effector function of the innate immune system. We have recently shown that the respiratory burst is significantly lower in patients with severe Guillain-Barré syndrome. **Objective:** To determine if the level of respiratory burst in patients with multiple sclerosis (MS) influences disease severity. **Methods:** We here examined the respiratory burst in leukocytes isolated from 61 patients with relapsing-remitting MS (RRMS) and secondary-progressive MS (SPMS) and 61 age-matched healthy controls. The severity was also evaluated by a Multiple Sclerosis Severity Score (MSSS) and by onset characteristics predicting the subsequent course of MS. Leukocytes were activated by formyl-Met-Leu-Phe (fMLF), Trp-Lys-Tyr-Met-Val-Met-NH₂ (WKYMVM) or phorbol myristate acetate (PMA), and superoxide anion production was measured with isoliminol/luminol-enhanced chemiluminescence (CL). Myeloperoxidase (MPO) activity in isolated leukocytes was measured spectrophotometrically. **Results:** A lower superoxide anion production was found after stimulation with fMLF (p=0.025), PMA (p=0.026) and WKYMVM (p=0.047) in patients with more severe course, evaluated with the MSSS. There was no difference in superoxide anion production between patients with RRMS and SPMS, or between cases with different predictors at onset. No significant differences in superoxide radical production were found between patients and controls after stimulation with fMLF, WKYMVM and PMA. There were no differences in MPO activity between patients and controls, nor between subgroups of patients. **Conclusions:** The present study indicated a relationship between the respiratory burst, which is a part of the innate immune response, and the severity of MS. This suggests that the individual capacity to generate oxygen radicals, in analogy with findings in the Guillain-Barré syndrome, may determine the severity of MS.

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P702**Heat shock protein 70 is critically involved in myelin antigen specific CD4 T cell autoreactive responses**

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Background: Heat shock proteins (Hsp), a highly conserved molecular chaperone of a protective capability, have also been implicated in the generation of immune responses. The gain of function experiments showed that overexpression of inducible form of hsp70 enhanced in vitro autoimmune response to myelin basic protein (MBP). **Objective:** To assess the role in vivo of hsp70 in myelin antigen recognition we used mice deficient in one of the major genes encoding inducible hsp70, hsp70.1. **Methods:** In this study we used experimental autoimmune encephalitis (EAE) induction, enzyme-linked immunosorbent assay, and antigen presentation assay. **Results:** Immunization of hsp70.1KO mice with myelin/oligodendrocyte glycoprotein (MOG) peptide35-55 showed their almost complete resistance to EAE induction. The EAE resistance correlated with loss of MOG35-55 proliferative T cell responses and interferon gamma production as well as downregulation of IL17 production. Interestingly hsp70 deficiency resulted in CD4 T cell activation dysfunction demonstrated by antigen presentation assays which showed that

MOG35-55 reactivity vanished when T cells from hsp70.1KO mice were co-cultured with wild type APC. The mechanism of CD4 T cell failure in hsp70.1KO mice in responding to MOG35-55 was TCR dependent and involved increased susceptibility to activation-induced apoptosis. **Conclusions:** These results provide direct proof of the in vivo role in hsp70 in recognition of self myelin antigen MOG and strengthen the hypothesis that stress condition associated with hsp70 induction enhances autoimmune reactions.

P703**Prognostic value of intrathecal IgG synthesis in multiple sclerosis**

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Background: Despite an increased understanding of the underlying patho mechanisms in multiple sclerosis (MS), the immunological parameters that faithfully mirror disease activity are only emerging. However, the ability to predict the subsequent clinical course of MS on the basis of clinical and other supportive data at presentation would be invaluable. **Objective:** We examine the oligoclonal band (OCB) pattern and intrathecal IgG production (IgG index) to ascertain if these are useful in predicting the subsequent clinical course of MS. We also assess the influence of some clinical features (such as course of the disease) in the laboratory results, in order to identify which factors might be related with the disease progression. **Methods:** IgG index was measured in 54 MS patients. Progression index (PI), type of disease course and the presence of OCBs were compared in patients with normal, high and very high IgG Index. **Results:** Patients with very high IgG index had a higher PI (0.1 ± 0.13) in comparison with the two other groups (0.06 ± 0.05 in high IgG index group and 0.05 ± 0.07 in normal IgG index group, $P > 0.05$). Secondary progressive (SP) patients had a higher IgG index than relapsing-remitting (RR) patients (2.04 ± 1.24 for SP vs. 1.78 ± 1.45 for RR, $P > 0.05$). PI was higher in OCB positive MS patients (0.08 ± 0.10) vs. OCB negative ones (0.05 ± 0.04). **Conclusions:** Our results did not reveal significant prognostic value for IgG index in MS patients. However, they indicate a trend towards better prognosis of the disease in patients with lower values of IgG index.

P704**Effects of glial cell-derived cytokines on human B cells: implication for multiple sclerosis pathogenesis**

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Background: B cells are increasingly implicated in multiple sclerosis (MS) pathogenesis. Persistence of B cells in the central nervous system (CNS) compartment may reflect a permissive environment provided by local glial factors, but whether this requires presence of specific B cell antigens, and/or T cell help, has not been established. **Objective:** To test whether glial-derived factors expressed in the inflamed MS CNS could influence B cell responses including immunoglobulin class switching and production of oligoclonal IgG bands (OCB). **Methods:** B cells isolated from the peripheral blood of normal subjects were cultured with or without activation or addition of selected factors including IL-6, IL-15, BAFF and IL-10 - all known to be abnormally expressed by glial cells in the CNS of MS patients. B cell survival and expression of CD80 and CD86 co-stimulatory molecules, and of CD27 and CD38, were measured serially by flow cytometry. Total IgG concentration and IgG subclasses were quantified by standard enzyme-linked immunosorbent assay. Culture supernatants were further studied by isoelectric focusing electrophoresis with IgG immunofixation to detect OCB. **Results:** IL-6, IL-15 and B cell activating factor (BAFF) synergistically supported: (i) greater than

2-fold increase in B cell survival; (ii) 3-fold increase in the frequency of CD80-positive B cells; (iii) 2-fold increase in CD86 expression; and (iv) significant ($p < 0.05$) increase in lymphotoxin production from activated B cells. Addition of IL-10 induced significant increases in (i) the percentage of plasmablasts ($p < 0.05$), (ii) the total IgG production ($p < 0.01$), (iii) the proportion of IgG1 subclass, and (iv) the presence of OCB. Thus, even in the absence of specific antigen, or the presence of T cell help, glial products expressed in the inflamed MS CNS can promote B cell survival, upregulate their antigen-presenting cell machinery and induce high levels of class-switched IgG.

Conclusions: At least a part of increased IgG found in the MS CNS may be generated in situ without specific antigen and T cell help.

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P705

Aquaporin-4-antibody seroconversion occurs before the onset of neuromyelitis optica

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Background: Neuromyelitis optica (NMO) is characterized by severe optic neuritis and longitudinally extended myelitis (LEM). Recurrent optic neuritis or LEM alone is considered a high-risk syndrome of NMO. A serum antibody against aquaporin-4 (AQP4) water channel protein has been detected exclusively in patients with NMO and the high-risk syndrome. However, there has been no report of a case in which AQP4-antibody was detected before the onset of the disease. We herein report a case of NMO in which AQP4-antibody was detected prior to the onset. **Results:** A 34-year-old woman with no history of neurologic diseases developed severe paraparesis and numbness in both legs. Brain magnetic resonance imaging (MRI) was normal, but spinal cord MRI depicted LEM with diffuse swelling of the cord and a T2-hyperintensity lesion extending from C6 to T7, mainly involving the central gray matter on the axial view. AQP4-antibody titer at that point was 128x in the assay previously reported (Takahashi et al, 2006, 2007). She was treated with high-dose intravenous methylprednisolone and plasma exchange, which resulted in the clinical improvement and a decline of AQP4-antibody titer (8x). Later a stored blood sample of the patient that was taken 3 months before the onset of LEM for blood donation, when she was in good health, was found, and it was also positive for AQP4-antibody (32x). **Conclusions:** To our knowledge, this is the first case in which AQP4-antibody was detected before the onset of NMO. Our data suggest that AQP4-antibody seroconversion occurred before the clinical development of NMO or the high-risk syndrome, and it virtually ruled out the possibility that AQP4-antibody was produced secondarily as a result of the tissue destruction by NMO.

P706

Multiple sclerosis. predictive value of cerebrospinal fluid findings

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Background: Cerebrospinal fluid (CSF) study of immunoglobulins (Ig) and proteins is an essential parameter in the diagnosis of MS complementing data obtained by magnetic resonance imaging (MRI) **Objective:** To detect immunobiological parameters that show different stages in the course of the disease and could help us establish progressive degrees of demyelination. **Methods:** 148 patients were studied, men and female, aged between 11 and 53, with diagnosis of Clinically Definite MS (McDonald criteria), remitting relapsing forms, with EDSS ranging between 0 and 6. We performed simulta-

neous analysis of native CSF and serum by electrophoresis searching Ig oligoclonal banding (OB). Kappa and Lambda Light chains were studied by polyacrylamide gel in sodium dodecyl sulfate. Specific Neuronal Enolase (SNE) and Heavy Neuro Filaments (HNF) were studied by Enzyme linked immunosorbent Assay (ELISA). IgG Index and serum albumin were studied by nephelometry. Immune electrophoresis (IEF) was used to characterize IgG precipitation arch, and ELISA to analyze soluble Immunocomplexes. In all cases we studied the post-translational modifications of IgG in citrullination and glycosylation. All patients were studied when in clinical relapse, previous to treatment, with brain and spinal chord MRI. **Results:** Correlation between CSF findings and EDSS enabled us to differentiate 5 intrathecal demyelinating profiles of progressive aggressiveness: Type I: increased daily IgG synthesis rate. Type II: IgG OB is added. Type III: IgG alteration of the precipitation arch by IEF is added. Type IV: Free light chains -mostly Kappa ones- are found in plus. Type V: Detection of soluble Antigen-Antibody Immunocomplexes, SNE and HNF, are added. In Type V, we found post-translational modifications of the newly formed Immunoglobulin, mostly cytrulline aggregates. **Conclusions:** The existence of the different profiles mentioned contributes to individualize the immunopathological reaction of each patient, and if aggressive progression is found, it opens the possibility of trying a more early and rational therapeutic intervention.

P707

A qualitative comparison of anti-IFN β antibodies in patients with and without neutralizing antibodies using Biacore™ technology

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Background: Traditionally, anti-IFN β antibodies that develop in patients treated with IFN β are categorized as NAb- or NAb+, based on their ability to neutralize an in vivo effect of IFN β . A comprehensive profiling of these antibodies should also include an assessment of affinity because besides titre and specificity, the biological effectiveness of an antibody molecule is highly dependent on its affinity. **Objective:** To assess the qualitative differences of anti-IFN β antibodies in NAb- and NAb+ patients over a period of time. **Methods:** We assessed serial samples of 6 NAb- and 12 NAb+ patients, with IFN β treatment durations of between 66 to 198 months, to characterize the affinity maturation of these antibodies using a biosensor-based approach (Biacore™). Biacore™ utilizes the principles of surface plasmon resonance with data from the dissociation phase of the antigen-antibody reaction being inversely proportional to relative antibody affinity. **Results:** In NAb- patients, mean antibody dissociation rates decreased only very slightly from 0.00130 ± 0.00025 s⁻¹ to 0.00105 ± 0.00020 s⁻¹ at mo 18, followed by a gradual increase to 0.00243 ± 0.00099 s⁻¹ at month 60. In patients with neutralizing antibodies (NAb+), mean antibody dissociation rates decreased from 0.00118 ± 0.00030 s⁻¹ at month 6 to 0.00021 ± 0.00008 s⁻¹ at month 36, followed by a slight increase to 0.00027 ± 0.00003 s⁻¹ at mo 60. Relative dissociation rates were significantly correlated with NAb titres (Spearman's correlation, $R_2 = 0.537$, $p < 0.001$). **Conclusions:** Our study shows that there is little improvement in antibody affinity over time in NAb+ patients. This suggests that it is the normal maturation process of an antibody response in the presence of continuous antigen challenge.

P708

NABs to IFN in Vancouver multiple sclerosis clinic: by straightening-up some parameters, the field would be less controversial

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Background: NABs can arise in multiple sclerosis (MS), bioavailability may be compromised and clinical effect reduced. Controversies exist and technical clarification is needed. **Objective:** To summarize our experience and demonstrate that minor changes in approach will help. **Methods:** From January 2003 to December 2007 we followed yearly (12 to 204 months) DMD-treated MS patients at the UBC MS clinic clinically, with enzyme-linked immunoabsorbent assay (ELISA), CPE, and since 2007 with the Luciferase assay and Biacore™ assay. **Results:** We show that clinical response to IFN (RR) is blunted in NAB+. This appears for years 3 and 4 and is more evident in Beta-1a than Beta-1b patients. CPE was done on 1372 samples using antigen homologous to treatment received. Sera + by ELISA were sent for CPE. 693 ELISA+ sera were sent for CPE. CPE was + in 119/553 Betaseron®, 154/673 Rebif® and 11/166 Avonex® treated patients. Titers (TRUs/mL: mean±sem) were significantly lower for Betaseron® (1045±152), than for Rebif® (7736±1682) and Avonex® (4783±1836) treated patients. Luciferase sensitivity and specificity were slightly better than CPEs. Titers by both techniques correlated well: 0.92 for Beta-1b and 0.98 for Beta-1a. Using as antigen in the assay the same antigen than used for treatment had consequences on titers reporting: Using beta-1a as an antigen to assay beta-1b injected patients results in an overestimation of titers by a factor of 1.5 to 6 (e.g. beta-1b patient; titer against Beta-1b=290TRU/mL; titer against Beta-1b=1147TRUs/mL). There was a predominant IgG4 response in NAB+ patients (pred. beta-1a), IgG4 was minimal in BAB+ NAB- patients (pred. beta-1b). Patients with IgG4 Babs had less chance of reverting to negative. We used Biacore™ on selected samples and found that Ab binding was higher in NAB+ than in BAB+ NAB- patients. Further, affinity correlated with NAB titres $R^2=0.374$ ($p < 0.001$). **Conclusions:** We prefer to use homologous antigen to assay NABs levels and avoid overestimation of titers in Beta-1b treated patients. Clinical correlations should be done independently among the different products. NAB+ appear to have higher affinity antibodies than Bab+Nab-. These observations can help our understanding of clinical/biological correlations.

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P709

CD8low frequency: a marker for disease prognosis and progression in multiple sclerosis?

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Background: We have recently reported a reduction in frequency of CD8lowCD4neg cells in untreated relapsing-remitting multiple sclerosis (RRMS) subjects compared to healthy subjects (5.2% vs 7.6%, $p=0.0006$). The same deficit is seen in individuals with a clinically isolated demyelinating syndrome (CIS). This cell population consists largely of CD3-CD56+ NK cells and has been reported to be increased in frequency after Daclizumab treatment (Bielekova, PNAS 2006). **Objective:** To assess the utility of this biomarker in prognosticating disease course and to characterize changes in this parameter with MS treatments. **Methods:** The study uses data acquired in the MS Registry, a project that includes the prospective collection of flow cytometric data on peripheral blood from healthy control subjects (n=31), and different subtypes of subjects with MS (n=349). A t-test was used to explore differences in CD8low frequency between subjects categories.

To assess the correlation of CD8low frequency with disease course, we used logistic regression and the MS Severity Score (MSSS), and random effect modeling to assess longitudinal magnetic resonance imaging volumetric data. **Results:** While a reduced CD8low frequency is associated with untreated CIS or RRMS, this reduction is not observed in untreated subjects in the progressive phase of the disease (primary or secondary). In terms of treatment, glatiramer acetate does not significantly alter the distribution of this biomarker; however, IFNβ significantly reduces further the frequency of CD8low cells. Within treatment categories, there was no association of this biomarker with responder/non-responder status. Finally, CD8low frequency did not correlate with our clinical outcomes (MSSS, rate of brain atrophy, rate of T2 lesion accumulation). **Conclusions:** Although, CD8low frequency may have a role as a susceptibility biomarker, particularly in the inflammatory phase of MS, it does not appear to be useful as a prognostic marker in MS. This measurement appears to be dynamic during treatment, with opposing effects noted with IFNβ here, relative to published reports of increased frequency with Daclizumab treatment.

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P710

Recombinant antibodies derived from plasma cell clones in multiple sclerosis cerebrospinal fluid do not react with myelin proteins

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Background: Intrathecal IgG synthesis, persistence of oligoclonal IgG bands (OCBs) and memory B cell clonal expansion are well-characterized features of the humoral response in the cerebrospinal fluid (CSF) of multiple sclerosis (MS) patients. However, the role of B cells and antibodies in disease remains enigmatic. Because OCBs are directed against disease-relevant antigens in central nervous system infectious and inflammatory diseases, the targets of CSF OCBs in MS may provide important clues to the cause of disease. **Objective:** To characterize binding of recombinant antibodies (rAbs) derived from MS CSF plasma cell clones to myelin proteins. **Methods:** We produced more than 50 different human IgG1 monoclonal rAbs by co-expressing the paired heavy- and light-chain V region sequences of MS CSF plasma cell clones in tissue culture cells. Humanized control rAbs were also generated from anti-myelin hybridomas in which murine V region sequences were fused to human constant region sequences for expression. Purified rAbs were assayed extensively for binding to myelin basic protein, proteolipid protein, myelin oligodendrocyte protein, other myelin proteins and anti-nuclear antigens (ANA) by an array of immunoassays that included immunostaining, protein immunoblotting, binding of rAbs to myelin chip arrays and enzyme-linked immunoabsorbent assays (ELISAs). **Results:** Whereas rAbs derived from anti-myelin hybridomas and positive control anti-myelin mAbs reacted strongly with myelin antigens in multiple immunoassays, none of the MS CSF rAbs displayed immunoreactivity to major myelin proteins. In immunocytochemistry, only a few rAbs reacted weakly with nuclei or cytoplasmic granules in neurons, glial cells and inflammatory cells in MS and control brain. ELISAs for ANA immunoreactivity were also negative. **Conclusions:** Our findings indicate that well-characterized myelin antigens are not the major target of the humoral immune response in MS CSF. The identification of novel MS-specific antigens is being pursued.

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P711

Molecular mechanism involved in the recombinant alpha IFN treatment in patients with multiple sclerosis

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Background: Type I interferons (IFN-alpha/beta) were the first agents to show clinical efficacy for multiple sclerosis (MS) treatment, as a disease modifying therapy. Human IFNs (alpha, beta) interact with the alpha IFN receptor. The interaction with the receptor components results in the activation of a signaling pathway leading to the regulation of specific genes and proteins which contributes to their numerous biological effects. **Objective:** We hypothesize similar molecular mechanisms of action for both IFNs, since alpha and beta IFNs are type I IFNs and they bind to the same receptor, therefore a common signaling pathway is to be expected. In our study, we evaluated the possible molecular mechanisms involved in the in vivo effects of the recombinant alpha-2b IFN (r-alpha-2b IFN) treatment during 6 months in relapsing-remitting MS patients. We compared the modulation of genes involved at different steps of MS pathogenesis: antigen presentation, Th1/Th2 cytokines expression, brain-blood barrier (BBB) integrity and natural and adaptive regulatory T cells. **Methods:** Total RNA from peripheral blood mononuclear cells of nine rIFN-a2b treated and five untreated MS patients, was extracted and amplified for CD86, CD28, CTLA-4, TNF- α , IFN- γ , CCL2, CCR5, IL-13, MMP-9, TIMP-1, CD25, TGF- β , IL-10, and the transcriptional factor Foxp3 by reverse transcription-polymerase chain reaction. **Results:** We found a significant down-regulation of the CD28 costimulatory signal ($p=0.043$) and MMP-9 metalloproteinase ($p=0.043$) in the group of patients treated with r-alpha-2b IFN. Furthermore, the chemokine CCL2 ($p=0.011$) and the regulatory cytokine TGF-beta were up-regulated with the treatment as well ($p=0.036$). There were no differences concerning the modulation of the genes studied, in untreated patients. **Conclusions:** The modulation of key points of the pathogenesis of this disease such as the activation mechanisms, BBB integrity and the immunoregulatory and immunosuppressive cytokines, demonstrated in our study, could explain the beneficial effects of r-alpha 2b IFN treatment in MS patients.

P712

Neurologic manifestations of Behçet disease: description of 15 patients in Valencia (Spain)

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Background: Behçet's disease (BD) is a multisystemic, relapsing and inflammatory process that can involve the central nervous system (CNS). The most widespread diagnostic criteria are those of the International Study Group of Behçet's Disease (ISGBD) and those of the Japanese Committee (JC). Neurological symptoms occur in 10–50% of the cases with a relapsing-remitting (RR) or progressive course. **Objective:** To describe a series of patients diagnosed with BD with neurological manifestations, and to illustrate their evolution and treatment used. **Methods:** We report 15 BD patients with neurological manifestations. The systemic symptoms/signs, the pattern of neurological involvement, the complementary explorations and the evolution were recorded. **Results:** There were three clinically different patterns of neurological involvement: parenchymal (50%), meningeal (46.2%) and intracranial hypertension (IH) due to venous sinus thrombosis in one patient (3.8%). Up to 43% had psychiatric manifestations. 66.7% fulfilled the ISGB criteria, whereas 93.3% were diagnosed according to the JC criteria (20% of complete form and 73.3% of incomplete form of BD). 60% of the patients had magnetic resonance imaging lesions in CNS. Cerebro-spinal fluid (CSF) analysis was performed in 11 patients, with abnormal results in 10 and presence of oligoclonal bands in one. Five patients were HLA-B51

positive. Most patients were treated with corticosteroids, and azathioprine was added in 80% of them. 50% were treated with cyclophosphamide and plasmapheresis was performed in one with good results. 80% had a RR evolution, 66.6% of them with sequels. 20% developed a secondarily progressive course in spite of treatment. **Conclusions:** BD does not have a pathognomonic clinical symptomatology or laboratory findings. In our series, the ISGBD criteria were not sufficiently sensitive compared with those of the JC. Disability related with the neurological involvement is important, with 53.3% of patients developing some sort of sequelae and 20% with a progressive course.

P713

Loss of therapeutic effects of glatiramer acetate in CX3CR1-/- mice
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Background: Glatiramer acetate (GA) is a random myelin polypeptide-based therapy that has been approved for the treatment of relapsing-remitting multiple sclerosis (MS) for over a decade. Its mechanism of action remains elusive, although recent studies have suggested that multiple cellular and/or soluble factors might be involved in mediating GA's effects in MS and in experimental autoimmune encephalomyelitis (EAE). NK cells are potentially regulatory cells in MS and EAE. The fractalkine receptor, CX3CR1, is expressed on glial cells in the central nervous system (CNS). Soluble fractalkine and CX3CR1 dictate the migration of NK cells to the CNS under pathological circumstances. Consequently, NK cells homing to the CNS are significantly impaired in CX3CR1 deficient mice. **Objective:** To investigate whether the impaired homing of NK cells to CNS influences the therapeutic effects of GA during EAE. **Methods:** EAE was induced in CX3CR1-/- mice by immunization of MOG35-55 peptide with adjuvant CFA. Groups of wild type mice and CX3CR1-/- mice were treated with GA or PBS upon EAE induction. Clinical parameters were monitored and compared among the groups. **Results:** GA conferred moderate protection against EAE in wild type mice (mean clinical score 2.43 ± 0.24 vs. 1.56 ± 0.19 , $p < 0.05$; maximum clinical score 4.0 ± 0.50 vs. 3.12 ± 0.21 , $p < 0.05$, respectively). In contrast, GA treatment did not alter clinical course of EAE in CX3CR1-/- mice (mean clinical score 2.78 ± 0.28 vs. 2.84 ± 0.29 , $p < 0.05$; maximum clinical score 4.13 ± 0.51 vs. 4.12 ± 0.62 , $p > 0.05$, respectively). **Conclusions:** The therapeutic effects of GA in EAE are completely lost in CX3CR1-/- mice. Our current work is focusing on dissecting how NK cells might contribute to the therapeutic effects of GA using combination of cell transfer and monoclonal antibodies.

P714

Myelin modulates adhesion and migration of immune cells

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Background: A hallmark of multiple sclerosis (MS) is the infiltration of activated immune cells into sites of myelin destruction. Myelin contains several neurite outgrowth inhibitory molecules, Nogo, MAG and OMpg, that bind to a common receptor, Nogo receptor 1 (NgR1). In the experimental autoimmune encephalomyelitis model of MS, recent studies demonstrated that Nogo neutralization not only improved axonal recovery, but also reduced inflammatory infiltrates. This suggests that myelin-associated signals may alter immune cell trafficking in the central nervous system (CNS). **Objective:** To determine whether myelin signals influence immune cell adhesion and migration. **Methods:** Western blotting and RT-PCR were used to assess the expression of NgR1 and its co-receptors in immune cell subsets isolated by FicolI density gradient centrifugation followed by magnet-assisted cell sorting. Adhesion and migration assays were performed to test the ability of immune cells to respond to myelin substrates, a source of NgR1 agonists. **Results:** We demonstrate that human T cells, B cells and monocytes express NgR1 and its co-receptors, with highest NgR1 expression in T cells. Expression of the NgR1 complex may

enable immune cells to respond to myelin, altering their adhesion and migration. Myelin substrates resulted in a dose-dependent inhibition of the adhesion of T cells with a maximum decrease of approximately 50%. Migration of T cells through myelin-coated transwells was enhanced approximately 2-fold. To determine whether these effects were mediated through NgR1, we performed the assays in the presence of PiPLC (cleaves NgR1 from the cell surface), and Y27632 (inhibits ROCK, a kinase in the NgR1 signaling cascade in neurons). Both agents attenuated the myelin-mediated inhibition of adhesion and enhancement of migration of T cells. **Conclusions:** We propose that myelin signals, at least in part mediated through the NgR1 complex, modulate adhesion and migration properties of T cells, which may influence their ability to traffic within myelinated areas of the CNS. NgR1 may therefore be a therapeutic target for both immuno-modulatory and regenerative strategies that may prevent damage and support repair in the CNS of MS patients.

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P715

Diagnostic value of IgG and free light chain quantification in CSF of patients with multiple sclerosis

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Background: Quantitative measurements of multiple sclerosis (MS) markers by automated nephelometry deliver rapid and reproducible results. As the widely used IgG index generally shows low sensitivity and specificity, new quantitative markers are needed to assist MS diagnostics. Recent studies demonstrate elevated levels of free kappa and lambda light chains in the cerebrospinal fluid (CSF) of MS patients. **Objective:** Our purpose was to determine the diagnostic performance of free light chains and IgG and whether different index calculations incorporating these markers increase their diagnostic value in our cohort. **Methods:** CSF and serum samples from 438 unselected patients, including an MS group of 70 patients (41 MS, 29 CISSMS), were analysed using nephelometry (Behring ProSpec; free light chain immunoassay of Freelite™, The Binding Site, Birmingham, UK). We then retrospectively correlated results with the patients' diagnoses. **Results:** Best diagnostic performance is achieved using the free kappa light chain index: sensitivity 0.96; specificity 0.86. The sensitivity (0.83) and the specificity (0.81) of the free lambda light chain index are distinctly lower compared to the free kappa light chain index. However, these values still outperform the results for the IgG index (sensitivity 0.8 and specificity 0.77). Combined indices incorporating two or three of these markers cannot exceed a sensitivity of 0.96 but show significantly lowered specificities. **Conclusions:** In this study an elevated free kappa light chain index represents the most sensitive and specific quantitative diagnostic parameter for MS. As it is measured by automated, routinely available methods the quantification of free kappa light chains can provide a rapid and reproducible indication of intrathecal immunological process supporting current MS diagnostic criteria.

P716

The inhibitory function of CD4CD25+ T regulatory cells is impaired in relapsing-remitting multiple sclerosis but not in primary progressive multiple sclerosis

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Background: Multiple sclerosis (MS) is an autoimmune disorder directed against self antigens of the central nervous system. CD4(+)CD25(+)FoxP3(+) regulatory T cell (Treg) mediated suppression is an essential mechanism of self-tolerance. Treg function was found to be impaired in relapsing-remitting MS (RRMS) patients. Recent findings suggest that the pathophysiology of primary progressive MS (PPMS) is substantially different from RRMS, raising the possibility

that neurodegenerative processes independent of inflammatory processes are of particular importance in PPMS. **Objective:** To study the suppressive function of a mixture of CD25(high) and CD25(intermediate) expressing T(reg) cells in myelin basic protein (MBP)- and PWM-induced proliferation in PPMS patients, RRMS patients and healthy controls. **Methods:** 35 untreated RRMS patients (13 males and 22 females, 40±10 years, disease duration 7.4±5.9 years), 10 age matched untreated patients with PPMS (disease duration 12±5.5 years, range 3–25 years), and 34 age matched healthy controls were studied. **Results:** Suppression of MBP-induced proliferation was observed in 89% of healthy controls. Patients with PPMS did not differ significantly from healthy controls, with 70% of PPMS patients showing suppression. Suppression was more frequently found in PPMS patients and healthy controls than in RRMS patients (45% of RRMS individuals showed suppression of MBP-induced proliferation, p=0.029). Suppression of PWM-induced proliferation did not differ between controls and PPMS patients, while suppression was less frequently found in RRMS patients (91% in controls, 80% in PPMS vs. 69% of RRMS patients, p=0.022). Relative T reg counts did not differ significantly between both RRMS and PPMS compared to healthy individuals. **Conclusions:** Our findings suggest that impaired Treg function is involved in the pathogenesis of RRMS but not in primary MS.

P717

Cerebrospinal fluid Baff levels in multiple sclerosis and other neurological diseases

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Background: Myeloid cells produce and secrete Baff (B-cell activating factor of the tumor necrosis factor superfamily). Three receptors for Baff have been identified. Their expression is highly restricted to B-cells. Baff is a potent survival factor for B-cells. Activated T-cells may also express some receptors for Baff. **Objective:** To measure Baff levels in the cerebrospinal fluid (CSF) of patients with multiple sclerosis (MS) compared to patients with other neurological diseases. **Methods:** MS patients (n = 45) were compared to patients with amyotrophic lateral sclerosis (ALS, n = 6), patients with other inflammatory neurological disease (OIND, n = 16), and patients with non-inflammatory neurological disease (NIND, n = 7). Baff levels were measured by an enzyme-linked immunosorbent assay. **Results:** The mean Baff level ± SEM for patients with: MS = 95.8 ± 13.6, ALS = 184.8 ± 54.2, OIND = 453.4 ± 180.2, and NIND = 124.3 ± 25.5 pg/ml. For MS patients, the mean Baff level for patients with primary progressive disease (PPMS) = 68.0 ± 19.5, and for patients with relapsing-remitting disease (RRMS) = 110.1 ± 18.6 pg/ml. When patients with RRMS were divided into those with relapse (n = 5) and those with stable disease (n = 25), the mean Baff level was 186.0 ± 55.6 and 94.9 ± 18.4 pg/ml, respectively. **Conclusions:** CSF Baff levels in MS are not different from those in NIND and are significantly lower than those in patients with OIND. Baff levels are higher in patients with RRMS compared to those with PPMS, although the difference is not significant. However, CSF Baff levels are higher during relapse, and are comparable to the levels in patients with OIND. These data show that Baff is present in the CSF, and suggest that Baff may play a role in MS relapses.

P718

Signaling mechanisms that regulate oligodendrocyte precursor migration

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Background: During development oligodendrocyte precursors (OPCs) migrate to target regions of the central nervous system (CNS) where they eventually differentiate into mature, myelinating oligodendrocytes. The secreted guidance cue Netrin-1, expressed by the floor plate cells of the embryonic spinal cord, acts as a chemorepellent for OPCs, promoting their dispersal to dorsal regions of the spinal cord. **Objective:** We investigated the intracellular mechanisms by which netrin-1 induces OPC repulsion, and examined the interaction

between the netrin-1 receptor deleted in colorectal cancer (DCC) with components of the actin cytoskeletal machinery. In particular, we focused on the Rho family of small GTPases, RhoA, Rac1 and Cdc42. **Methods:** OPCs were purified from newborn rat cortices using a mixed glial culture shake-off method. OPC migration was investigated using a Boyden chamber assay. Pharmacological inhibitors of ROCK (Y27632 and H1152), an effector of RhoA, were used to test the requirement for RhoA activity in netrin-1-mediated repulsion of OPCs. Biochemical pulldown methods were used to investigate changes in GTPase activity in OPCs stimulated with netrin-1. **Results:** Inhibition of ROCK disrupted netrin-1-induced chemorepulsion of OPCs. In addition, treatment of OPCs with these inhibitors also disrupted netrin-1 induced process retraction, a characteristic morphological change accompanying repulsion. **Conclusions:** These findings provide evidence that RhoA is a critical downstream component of the signalling pathway activated by netrin-1 in OPCs. To obtain a more complete picture of the signals influencing OPC migration, we are currently investigating how Rac1 and Cdc42 activity complements the activation of RhoA. **Supported by:** Multiple Sclerosis Society of Canada Fonds de la Recherche en Sante du Quebec.

P719

The activating zeta chain of the T cell receptor is increased on multiple sclerosis lymphocytes

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Background: The T cell receptor (TCR) complex is comprised of [alpha+beta] antigen-recognition chains, plus [epsilon+zeta] chains. The TCR recognizes antigens presented by major histocompatibility proteins. Activation of the TCR complex transmits a stimulatory signal through the zeta chain. Chronic antigenic stimulation and cytokines may alter levels of TCR components on T cells. **Objective:** Connective tissue disease, tumors, and pregnancy reduce zeta chain levels on T cells, but TCR-zeta chain levels in multiple sclerosis (MS) are unknown. **Methods:** Expression of the intracellular portion of the T cell zeta chain was quantitated with flow cytometry on MNC from healthy controls (NL), therapy-naïve relapsing-remitting (RRMS) patients, and RRMS and secondary progressive MS (SPMS) patients on glatiramer acetate therapy. **Results:** TCR-zeta was increased on CD3+ and CD4+ T cells in MS compared to controls. The frequency of CD3+,TCR-zeta-high cells was 24% in NL; 40% in MS; and 46% in glatiramer-treated MS. CD3 (TCR-epsilon) levels were comparable in all three groups. Patients on glatiramer therapy had reduced TCR-zeta on CD4+ lymphocytes (ratio of zeta-high/zeta-low was 1 in NL; 20 in MS; and 6 in glatiramer-treated). Glatiramer-treated, however, had an increase in TCR-zeta expression on CD8+ lymphocytes (ratio = 1 in NL; 2 in MS; 14 in glatiramer-treated). **Conclusions:** Over-expression of TCR-zeta could increase immune reactivity in MS to antigens acting through the TCR-Ag-MHC complex. The increase in TCR-zeta on lymphocytes suggests a correlation with increased CD4 activity in MS, and possibly with less T cell apoptosis in therapy-naïve MS. The increase of TCR-zeta on CD8 cells is possibly linked to increased CD8 regulatory cell function during glatiramer therapy.

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P720

Beneficial effects of pregnancy in multiple sclerosis reflected by cytokine pattern and prolactin

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Background: Multiple sclerosis (MS) relapse frequency is reduced during pregnancy. In experimental studies pregnancy-associated

hormones were found to be involved in myelination. **Objective:** To define biomarkers concurring with measures of disease activity in MS patients during pregnancy and post-partum. **Methods:** Thirty-four women were followed during pregnancy at 3 month intervals until month 6 post-partum. Expanded disability status scale (EDSS), paced auditory serial addition test (PASAT), 50 m timed walk and nine-hole peg test were performed in the first and third trimester and at month 6 after delivery. Quantitative cytokine mRNA expression for IL-4, IL-10, IL-17, IFN- γ and TNF- α , as well as serum levels of estradiol and prolactin, were determined. In 20 patients longitudinal visual evoked potentials (VEP) were obtained. **Results:** The annualized relapse rate dropped from 0.91+/-0.94 before pregnancy to 0.22+/-0.38 (p=0.004) and reversed to 0.45+/-0.69 (p=0.02) in the first 6 months after delivery. Measures of clinical disease activity significantly improved during pregnancy and no rebound was observed at month 6 post-partum. IFN- γ and IL-17 decreased and Th-2 cytokines increased from the first to third trimester. High prolactin levels at month 6 after delivery correlated with improved latencies on VEP. **Conclusions:** This study confirms the beneficial effect of pregnancy in MS. The observed higher levels of prolactin at month 6 after delivery in patients with improved VEP is compatible with a positive effect of this hormone on regenerative processes in the central nervous system (CNS) and is in accordance with recent data that prolactin can induce remyelination. Prolactin may be a potential candidate for treatment development in MS.

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P721

Toll-like receptor modulation of multiple sclerosis relevant B cell responses

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Background: Toll-like receptors (TLRs) are important innate receptors involved in activation of adaptive immune responses. Infections have been implicated in triggering or exacerbating the course of multiple sclerosis (MS). It is likely that this is partially due to TLR stimulation in the periphery and/or in the central nervous system. B cells have emerged as important cells at the innate-adaptive immune interface and are increasingly implicated in MS pathophysiology. **Objective:** The purpose of this project is to determine the effects that TLR-mediated signals have on B cell effector responses. **Methods:** We studied the effects that TLR8 and TLR9 signaling had on B cell survival, proliferation and effector cytokine production using our current paradigm of ex vivo B cell activation involving (1) CD40 ligation alone to mimic T cell help without antigen stimulation (bystander activation of B cells) or (2) BCR cross-linking followed by CD40 ligation to mimic activation by antigen followed by T cell help. **Results:** TLR8 signaling significantly enhanced B cell survival (n=4, p=0.002), while TLR9 signaling did not. Both TLR8 (n=7, p<0.0001) and TLR9 (p=0.02) signaling enhanced B cell proliferation, but only TLR9 signaling also enhanced B cell cytokine production. In fact, TLR8 stimulation significantly suppressed B cell production of IL-6 (n=7, p<0.0001), IL-10 (p=0.02), and lymphotoxin (p=0.0004) under both modes of activation, in spite of the enhanced proliferation. This reciprocal effect of TLR8 signaling on B cell proliferation and cytokine production did not appear to reflect plasma cell differentiation and IgG production (n=4). **Conclusions:** TLR8 and 9 activation differentially affect B cell activation, which will impact activation of other immune responses. Determining the mechanism by which TLR8 signaling increases survival and other B cell responses may prove important in future treatment strategies, as TLR activation is being increasingly implicated in many autoimmune diseases, including MS.

P722

Increased CD5⁺ B cell number and IgM levels in cerebrospinal fluid of early multiple sclerosis patients correlate with cortical inflammation

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Background: The pathologic process underlying the development of cortical lesions (CLs) in multiple sclerosis (MS) is poorly understood. **Objective:** Since follicle-like B cell aggregates have been demonstrated in MS meninges, we investigated whether CLs are associated with a peculiar intrathecal B cell response. **Methods:** Paired cerebrospinal fluid (CSF) and serum were sampled from 15 patients with clinically isolated syndrome (CIS)/possible MS and 21 with relapsing-remitting multiple sclerosis (RRMS) [22F, 14M; F/M: 1.6, mean age: 36 (17–71)]. CD5⁺ B cells were analyzed in serum and CSF by flow-cytometry. IgM concentration was determined by specific nephelometric assay. IgM index was considered elevated when ≥ 0.08 . CLs were detected by double inversion recovery (DIR) sequences, using a 1.5T MR scanner. **Results:** IgM index was elevated in 16 patients (44%) and normal in 20 (56%). CIS, pMS and RRMS patients were equally distributed in the two groups based on IgM index. Eleven patients with increased IgM index had CLs (69%), as against only 3 patients with normal IgM index (15%). A significant association and correlation between IgM index and CLs was found ($p=0.0017$, Fischer's Exact Test; $p=0.0095$, Spearman Rank Test). IgM index poorly correlated with pleocytosis and IgGOCB, unlike IgG index, which was not associated with CLs ($p=0.18$). CLs were not associated with IgGOCB ($p=0.083$), as well. The percentage of CD5⁺ B cell in the CSF was significantly increased in patients with elevated IgM index, regardless of their IgG index or the presence of IgGOCB. **Conclusions:** We observed a correlation between elevated IgM index and the presence of CLs in MS. The association of higher IgM index with increased percentage of CSF CD5⁺ B cells, together with the lack of association with other inflammatory parameters, may support the hypothesis that this peculiar IgM-producing B cell subclass may be involved in early gray matter pathology.

P723

Vitamins D and A levels and regulatory T cells in patients with multiple sclerosis

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Background: Vitamin D (vitD) and vitamin A (vitA) can promote the development of regulatory T cells (Treg cells), which can suppress multiple sclerosis (MS) disease activity. 1, 25 dihydroxyvitamin D (dH-vitD) is the active form of vitD and is produced from 25-hydroxyvitamin D (H-vitD). dH-vitD activity is also mediated by interactions between the vitD receptor and retinoid receptor. **Objective:** To examine the relationship between vitD, vitA and Treg cell percentages and Treg precursors in patients with MS. **Methods:** Blood samples were obtained from 21 patients with relapsing-remitting MS not on treatment with disease modifying therapy. Serum H-vitD and dH-vitD levels were analyzed by immunoextraction followed by enzyme immunoassay. Retinol (vitA alcohol) levels were measured by reverse phase HPLC. Mononuclear cells in the samples were examined for percentages of Treg (CD3+CD4+CD25+FoxP3+) cells by flow cytometry. Also examined were total naïve T cells (CD3+CD4+CD45RA+ cells), from which develop Treg cells, and CXCR3+ naïve T cells, which include Treg precursors that can traffic into areas of inflammation, including the central nervous system, in response to proinflammatory chemokine. **Results:** The patient group was comprised of 18 females; 13 patients were Caucasian and eight were African American. H-vitD and dH-vitD levels were in the normal range and Treg cell percentages were low in this patient group. H-vitD levels correlated inversely with Treg percentages ($R=-0.54$; $p=0.01$). However, Treg cell percentages correlated directly with dH-vitD/

H-vitD and retinol/H-vitD ratios ($R=0.61$; $p=0.0035$ and $R=0.52$; $p=0.015$, respectively). Also, the calculated vitD ratio correlated inversely with percentages of CXCR3+ naïve T cells, ($R=-0.47$; $p=0.036$). **Conclusions:** An appropriate balance between vitD and vitA may be required to maintain adequate Treg cell numbers in patients with MS, and associations between such measures may not be restricted to individuals of a specific race or ethnicity. Further studies are required to better understand the mechanisms that underlie these effects and their specific consequences on regulatory T cell development and function.

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P724

Diminished myelin-specific T cell activation associated with increase in CTLA4 and Fas molecules in multiple sclerosis patients treated with interferon beta

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Background: Multiple sclerosis (MS) is a chronic inflammatory disease of the white matter of the central nervous system characterized by focal areas of demyelination. Interferon- β (IFN β) provides an effective treatment which lessens the frequency and severity of exacerbations in relapsing-remitting MS, but the mechanisms by which IFN β is efficient remain uncertain. **Objective:** The hypothesis is that the increase in Fas and CTLA4 molecules in MS patients may lead to lymphocyte apoptosis, which suggests possible mechanisms underlying the therapeutic response to IFN β . **Methods:** A total of 47 patients with stable relapsing-remitting MS, secondary progressive MS and primary progressive MS; 55 patients with relapsing-remitting MS in treatment with IFN β 1b and 30 normal subjects were studied and the treated patients had been receiving IFN β treatment for 18–24 months. Were evaluated proliferative response, quantification of CTLA4 molecules, quantification of surface Fas molecule, quantification of surface Fas on CD4+ and CD8+ cells, and quantification of soluble Fas in sera. **Results:** Our data demonstrate that IFN β impairs the proliferative response to myelin basic protein (MBP) and myelin, as well as increasing the expression of the CTLA4 intracellular molecule. Moreover, this treatment increases the expression of surface Fas molecules, as well as of the soluble form of these molecules. **Conclusions:** IFN β treatment reduces the proliferative response of lymphocytes to myelin antigens, as well as inducing the expression of intracellular CTLA4 molecules and surface Fas molecule on CD4 T lymphocytes. This increase in surface Fas molecules should be favorable to the induction of apoptosis; although soluble Fas molecules released during the treatment may result in the survival of some of the T cells. Thus, the beneficial effects of IFN β treatment, i.e., the reduction of myelin-specific T cell activation and the reduction in clinical signs, may be the result of the fact that the mechanisms of apoptosis have prevailed, thus reducing the inflammatory response despite the presence of the soluble Fas.

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P725

Imatinib mesylate reduced production of extracellular matrix and connective tissue growth factor by astrocytes

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Background: Imatinib mesylate is an inhibitor of the transforming growth factor β (TGF β) and platelet-derived growth factor (PDGF) signaling pathways. **Objective:** This study was undertaken to test the potential use of imatinib mesylate as an antifibrotic drug for the treatment of gliosis in multiple sclerosis (MS). **Methods:** The expression of extracellular matrix (ECM) proteins (procollagen 1, procollagen 2 and fibronectin) and connective tissue growth factor (CTGF) in astrocytes

was analyzed after treatment with TGF β or PDGF by real-time polymerase chain reaction, Sircol collagen assay and Western blot. **Results:** The mRNA expression of ECM proteins and CTGF induced by these growth factors was also strongly and dose-dependently inhibited by imatinib mesylate. Imatinib mesylate efficiently reduced basal mRNA expression of procollagen 1, procollagen 2 and fibronectin in astrocytes. These results were confirmed at the protein level. We also found that imatinib mesylate significantly reduced the collagen released from astrocytes in culture as measured by Sircol assay. In addition, imatinib mesylate did not alter astrocytes proliferation or induce apoptosis and necrosis. **Conclusions:** Our data show that biologically relevant concentrations of imatinib mesylate have potent antifibrotic effects on astrocytes in vitro. Considering its favorable side effects profile, the oral administration and the experience with its use in other diseases, imatinib mesylate might be a candidate for the treatment of gliosis in MS.

P726

Absence of Epstein-Barr virus RNA and Epstein-Barr virus-specific intrathecal antibody production in multiple sclerosis cerebrospinal fluid

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Background: Numerous studies have implicated Epstein-Barr virus (EBV) infection with multiple sclerosis (MS). Latent- and lytically-infected B cells have been found in MS plaques, including perivascular infiltrates, and MS central nervous system (CNS) germinal centers. **Objective:** To analyze MS plaques and cerebrospinal fluid (CSF) for evidence of EBV infection. **Methods:** Nested real-time polymerase chain reaction (PCR) for EBV-encoded non-translated RNA (EBER)-1, as well as control GAPDH- and IgG-specific transcripts was performed on cDNA prepared from single B95-8 EBV-infected marmoset cells, single ARH-77 EBV-infected human B cells, single plasma cells in MS CSF and MS plaques. ELISA was used to detect anti-EBV antibodies in CSF and serum of MS and inflammatory CNS controls (IC), after which the anti-EBV antibody index was calculated. Recombinant antibodies generated from clonally-expanded MS CSF plasma cells were tested for binding to EBV-infected B95-8 cells by immunocytochemistry. **Results:** IgG and GAPDH were detected in positive control cells, in all MS plaques and in single plasma cells from MS CSF. EBER-1 transcripts were detected in single B95-8 cells, single ARH-77 cells, and lytic or latent infection confirmed by detection of EBV-generated BFRF-1, LMP-1 and EBNA-2 transcripts. In contrast, EBER-1 transcripts were not found in seven plaques from five MS patients or in cDNA from 155 single CSF plasma cells from four MS patients with relapsing-remitting and secondary-progressive disease. Although anti-EBV IgG antibodies were found in serum and CSF of both MS and IC patients, analysis of the anti-EBV antibody index revealed no evidence of intrathecal synthesis. A panel of recombinant antibodies derived from MS CSF plasma cell clones also failed to stain the B95-8 cells. **Conclusions:** EBER-1 transcripts were not found in MS CSF plasma cells or MS plaques. Furthermore, there was no evidence of intrathecal synthesis of anti-EBV antibodies in MS patients. Overall, our findings argue against a pathogenic role for EBV infection in MS. **Supported by:** NIH PO1 NS32623.

P727

Roles of Interleukin-15 in multiple sclerosis: enhanced peripheral expression and greater impact on CD8 T cell cytotoxicity

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Background: Cytokines play crucial roles in shaping T cell responses in numerous autoimmune diseases. Increasing evidence suggests that CD8 T cells partake in multiple sclerosis (MS) pathogenesis. Activated CD8 T cells are detected in the central nervous system (CNS) of MS patients but contribution of cytokines to their activation has not been

elucidated. Interleukin-15 (IL-15) is pivotal in the generation and maintenance of memory CD8 T cells. Antigen presenting cells, e.g. monocytes, are the main sources of this cytokine, expressing a biologically active IL-15/IL-15R α complex on their surface, which can be up-regulated by IFN- γ . **Objective:** We compared the levels of IL-15/IL-15R α on human peripheral blood monocytes in healthy controls and MS patients. We assessed whether IL-15 has more impact on CD8 T cell effector functions in MS patients compared to healthy controls. **Methods:** Peripheral blood mononuclear cells from human donors were stained directly ex-vivo for IL-15, IL-15R α and cell specific markers. Purified CD8 T cells were shortly stimulated in vitro with anti-CD3 in the presence or absence of IL-15 and then analyzed using flow cytometry-based assays for proliferation (CFSE) and effector functions (lytic enzyme, i.e. Granzyme B). **Results:** Most monocytes (over 90%) were IL-15R α positive and a subset of these cells expressed surface bound IL-15 (10–62%). More monocytes expressed the IL-15/IL-15R α complex in MS than in control samples. Addition of IL-15 to anti-CD3 activated CD8 T cells led to a significant dose-dependent increase of proliferation. IL-15 also boosted IFN- γ and Granzyme B production by the same cells. Moreover, the impact of IL-15 on Granzyme B production by CD8 T cells was greater in MS patients compared to healthy controls. **Conclusions:** Our results confirmed the reported increased amounts of IL-15 in MS patients' blood, but also underlined that enhanced IL-15 is provided as biologically active IL-15/IL-15R α complex on the monocyte's surface. We showed that CD8 T cells from MS patients displayed augmented cytotoxic capacity (Granzyme B) compared to healthy controls in response to IL-15, thus underscoring the pro-inflammatory impact of IL-15 on CD8 T cells in the context of MS.

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P728

Expression of brain-derived neurotrophic factor is regulated by fingolimod (FTY720) in cultured neurons

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Background: Oral fingolimod (FTY720) is a sphingosine-1-phosphate (S1P) receptor modulator, which exerts a beneficial effect in multiple sclerosis (MS) by directing auto-aggressive lymphocytes to remain in the lymph nodes and away from the central nervous system (CNS). Oral fingolimod also readily crosses the blood-brain barrier allowing for potential additional effects within the CNS. Therefore, we explored the effect of fingolimod on brain-derived neurotrophic factor (BDNF) biosynthesis, as BDNF may protect neurons and may indirectly affect myelination by increasing neuronal activity. **Objective:** To determine if fingolimod increases the levels of BDNF in neuronal cell cultures and to characterize the signalling pathways involved in the regulation of BDNF expression. **Methods:** After 14–16 days of in vitro culture, primary cortical neuron cultures and embryonic stem cell-derived (ESCD) neurons were treated on alternate days for up to 6 days with fingolimod-phosphate (fingolimod-P). Protein extracts were processed for Western Blots, using anti-BDNF, anti-P-CREB and anti-ERK1/2 antibodies and were normalized for β III-Tubulin expression. Total mRNA was reverse transcribed and analyzed by qRT-PCR using specific TaqMan probes for BDNF and GAPH (internal control). **Results:** There was a dose-dependent increase in BDNF mRNA in both primary cortical and ESCD neurons following fingolimod-P treatment. This increase was blocked by tetrodotoxin and the MAP kinase inhibitor U0126. BDNF protein levels were up-regulated two-fold by fingolimod-P in both cell cultures; the up-regulation was preceded by an increase in the phosphorylation of ERK1/2 and the transcription factor CREB. **Conclusions:** Fingolimod phosphate can increase levels of BDNF in cultured neurons indicating that neuronal activity, and possibly myelination, is also increased. These findings suggest that fingolimod may have CNS as well as peripheral effects, which may be beneficial in the treatment of MS.

P729

Gene expression analysis identifies Th1, Th17 and IL10-related immune activation in multiple sclerosis

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Background: DNA array studies have shown abnormal gene expression in multiple sclerosis (MS), but the relationship between gene expression and disease activity is uncertain. **Objective:** To assess the relationship between gene expression in blood and cerebrospinal fluid cells (CSF) and disease activity in relapsing-remitting (RRMS). **Methods:** We used Affymetrix Human Genome Focus chips and real-time reverse transcriptase polymerase chain reaction (RT-PCR) to study gene expression in blood mononuclear cells (MNCs) from 36 patients with RRMS in clinical remission and 12 healthy controls. In addition, gene expression was studied by real-time PCR in blood and CSF MNCs from 13 patients with a clinically isolated syndrome (CIS), 17 MS patients in relapse, and 10 neurological control subjects. **Results:** Using a 5% false discovery rate (FDR), we found higher expression of 775 (12.6%) and lower expression of 26 (0.4%) out of 6,147 genes in RRMS patients than in healthy controls. Analysis of transcription factor binding sites within upregulated genes suggested increased activity of several transcription factors, including STAT1 and STAT6. Increased expression of mRNA encoding STAT1, STAT3, STAT4 and STAT6 in MS was demonstrated by RT-PCR. Furthermore, patients with RRMS in clinical remission had increased expression of cytokines utilizing these STAT molecules for signaling. Interleukin (IL)-10 expression was increased in blood in RRMS patients in clinical remission, and correlated negatively with disease activity on Gd-enhanced magnetic resonance imaging (MRI). In patients with CIS or MS relapse gene expression changes were more pronounced in CSF than in blood cells, and CSF cells had lower expression of IL-10 and higher expression of the T helper type 1 (Th1) cytokine interferon-gamma and the Th17 transcription factors RORA and RORC than controls. **Conclusions:** Using an unbiased gene expression analysis, we have demonstrated increased activity of signaling pathways and increased expression of cytokines and transcription factors related with Th1, Th2, Th17, and IL-10-related immune activation in MS. Patients without disease activity have lower IL-10 expression and patients with active disease have increased Th1 and Th17 immune activation in CSF.

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P730

Peroxisome proliferator-activated receptors (PPARs) in relapsing-remitting multiple sclerosis patients and the influence of immunomodulatory therapy

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Background: Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors that regulate metabolic and immune processes. PPAR agonists have been shown to improve clinical and inflammatory activity of experimental autoimmune encephalomyelitis. In these animal models of multiple sclerosis (MS), a gender-dependent expression of PPAR α was suggested to be associated with the susceptibility for this disease. However, the implications

of PPARs for the pathogenesis and therapeutic strategies of MS remain uncertain. **Objective:** The aim of this work is to analyse the expression of PPAR α , PPAR γ , and PPAR δ mRNA in peripheral blood mononuclear cells (PBMCs) of MS patients and normal controls and to study the influence of immunomodulatory treatments. **Methods:** 15 female and seven male patients with relapsing-remitting MS in stable clinical conditions and 14 female and eight male controls were studied. In 13 patients, analysis of PPAR expression was performed before and 6 months after the beginning of interferon beta (n= 9) or glatiramer acetate (n= 4) therapy. Expression levels of PPARs mRNA were measured by quantitative RT-PCR in PBMCs. The Student's t-test was used for parametric and the Wilcoxon rank sum for non-parametric analysis. **Results:** Before treatment, expression levels of PPAR mRNA in female MS patients were unchanged in comparison to controls. In males, PPAR α and PPAR δ expressions were increased (26% in patients in comparison to controls (p=0.028 and p=0.036, respectively). After immunomodulatory therapy, PPAR γ expression decreased by 40% in female (p=0.0002) and PPAR δ decreased by 24% in male patients, (p=0.055), in comparison to pre-treatment levels. **Conclusions:** These results suggest that PPARs may have a gender-related influence on MS pathogenesis and response to immunomodulatory therapy. Further studies are warranted to determine the roles of PPARs in the disease and as potential targets for new therapeutic approaches.

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The primary analysis of signal nerve ending proteins as possible markers of axonal damage

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Background: The presynaptic signal proteins is of interest as possible markers of axonal damage, primarily in patients with multiple sclerosis (MS). An analysis of six major presynaptic proteins in cerebrospinal fluid in patients with MS and other central nervous system (CNS) pathology was fulfilled: brain abundant, membrane attached signal protein 1 (BASP1), myristoylated alanine-rich C kinase substrate (MARCKS), Growth Associated Protein 43 (GAP-43), phosphorylated GAP-43 (pGAP-43), tau-protein, Amyloid beta 1-42 immunoreactivity. **Objective:** The quantitative evaluation in cerebrospinal fluid (CSF) of six presynaptic proteins for early diagnostics of MS and neurodegenerative diseases. **Methods:** The analysis of a six presynaptic proteins in CSF in 14 patients with MS and two patients with clinically isolated syndrome (CIS) was examined. The control group consist of seven patients with vascular dystonia. The age of patients with MS was from 19 to 48 years, male - 68,7%, female - 31,3%. The patients have relapsing-remitting, secondary-progressive MS. Expanded disability status scale (EDSS) score was from 2 to 6.5. The content of the proteins in CSF was estimated immunochemically by standard protocol ELISA. **Results:** The significant increase of BASP1, MARCKS, tau-protein level in CSF patients with MS in 35,7% is revealed as compared with patients of the control group. It is necessary to note that CIS patients showed a doubtful increase of all testing proteins level in CSF. The not significant increase of GAP-43, pGAP-43 level in CSF in patients with MS was shown. The significant increase of A β 1-42 in CSF patients with MS in 25% is revealed. More thorough selection of patients and further study is required. **Conclusions:** The patients with rapidly progressive (malignant) MS have significant increase of BASP1, MARCKS, tau-protein, A β 1-42 levels in CSF. The patients with MS do not have a pattern of change of GAP-43, pGAP-43 levels in CSF.

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From experimental autoimmune encephalomyelitis to multiple sclerosis: IL-16 regulates inflammation and axonal damage in autoimmune diseases of the central nervous systemDusanka S. Skundric¹, William W. Cruikshank², Djordje Gveric³¹Wayne State University, Detroit, Michigan, USA; ²Boston University, Boston, Massachusetts, USA; ³University College London, London, United Kingdom

Background: Multiple sclerosis (MS) is an autoimmune inflammatory, demyelinating, neurodegenerative disease. Experimental autoimmune encephalomyelitis (EAE) serves as a model for MS. We originally developed a relapsing-remitting EAE model in (B6 x SJL) F1 mice, which has a great deal of resemblance with human pathology. In both diseases, infiltration by autoaggressive myelin-specific CD4+ T cells correlates with disease activity and central nervous system (CNS) pathology. The cytokine IL-16 recruits exclusively CD4+ cells. IL-16 is produced by activated T cells, B cells, dendritic cells and microglia. **Objective:** We sought to find out if intrathecal regulation of IL-16 correlated with disease activity, demyelination and axonal dysfunction in (B6 x SJL) F1 EAE mice and human MS tissue. In addition we compared how well our EAE model compares to the human situation regarding regulation of IL-16. **Methods:** We examined brain and spinal cord samples from 24 mice with relapsing-remitting EAE, and from 39 MS autopsies, taken from patients with relapsing-remitting clinical disease. Relative levels of IL-16, active caspase-3, T-bet, Stat-1 (Thy 701), and NF(M+H)P were measured by Western blot. We employed two-color immunostaining and confocal microscopy to identify phenotypes of IL-16 containing cells in frozen tissue sections from MS lesions. **Results:** In EAE and MS lesions, we found markedly increased levels of pro- and bioactive IL-16 (80 kD and 22 kD, respectively). Levels of IL-16 corresponded with an increase in active-caspase-3, T-bet and Phosphorylated Stat-1. Increase in bioactive IL-16 in CNS corresponded to increased phosphorylation of axonal medium and heavy chain neurofilaments, suggesting axonal damage. We readily observed IL-16 immunoreactivity confined to infiltrating CD4+, CD8+ T cells, B20+ B cells, CD83+ dendritic cells, and to a lesser extent to Mac-1+ microglia. **Conclusions:** Data show production of IL-16 in EAE and MS lesions. Increased levels of IL-16 correlate to Th1 CD4+ cell infiltration and axonal damage. Our results suggest an important role of IL-16 in regulation of acute and chronic inflammation and subsequent axonal damage in EAE and MS.

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sRAGE: A potential biomarker of multiple sclerosis disease severity
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Background: RAGE, the receptor for advanced glycation end products (AGEs), has been implicated in diabetes, vascular disease and in neurodegeneration. Inhibition of RAGE reduces the infiltration of inflammatory immune cells into the central nervous system (CNS) and suppresses experimental autoimmune encephalomyelitis (EAE). The soluble isoform of RAGE (sRAGE) is reduced in patients with coronary heart disease and Alzheimer's Disease compared to the level in healthy control subjects. We hypothesized that sRAGE may represent an effective biological marker of multiple sclerosis (MS) disease severity. **Objective:** To determine the differences in the serum levels of sRAGE between MS patients and healthy control subjects and to investigate whether serum sRAGE levels correlate with MS disease severity as indicated by the expanded disability status scale (EDSS). **Methods:** Serum levels of sRAGE were measured in 37 MS patients, and 22 age- and gender-matched healthy control subjects by ELISA. MS patients were clinically stable and naïve to MS disease modifying drugs including interferon beta, natalizumab, glatiramer acetate for the previous 6 months. **Results:** Serum levels of sRAGE were

significantly lower in MS patients (998 ± 52.6 pg/ml) compared to levels in healthy controls (1292.2 ± 77.1 pg/ml) ($p=0.005$). Serum sRAGE levels tended to be lower in female patients compared to male patients ($p=0.05$). An inverse relation between sRAGE and EDSS, and between sRAGE and rate of clinical relapse in the previous two years ($R^2=0.166$, $P=0.012$) were observed. No significant correlations were found between serum sRAGE levels and MS disease duration, and between serum sRAGE levels and age at disease onset. **Conclusions:** The reduced serum sRAGE in MS patients relative to controls supports the role for RAGE axis in MS clinical pathology, as lower levels of sRAGE may be associated with enhanced inflammatory responses. Based on this data, further investigations are warranted to assess the clinical relevance of serum sRAGE in a large and diverse MS cohort and its association with magnetic resonance imaging (MRI) indicators of disease severity/progression.

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P734

Characterization of HHV-6 U24 protein and its possible implication in multiple sclerosis

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Background: Human Herpesvirus Type 6 (HHV-6) infection has been tentatively associated with at least a subset of multiple sclerosis (MS) patients. An identical sequence was found between HHV-6 U24 protein and myelin basic protein, a candidate autoantigen in MS. It was shown that greater than 50% of T cells recognizing residues 93 to 105 of myelin basic protein (MBP) cross-reacted with and could be activated by a synthetic peptide fragment corresponding to 1 to 13 of HHV-6 U24 in MS patients. This domain within MBP contains a major site of phosphorylation, Thr97. It has been shown that the phosphorylation state of MBP determines its subcellular location, and its ability to bind negatively charged lipids. In MS patients and in an experimental animal model of MS, Thr97 is significantly less phosphorylated. The in vivo impact of MBP hypophosphorylation is yet unknown, but phosphorylation at this site may, for example, protect against proteolytic degradation and subsequent presentation of MBP epitopes to the immune system. **Objective:** Our goal was to determine whether the region of U24 that is identical to MBP was also a target of MAPK (Erk2) kinase. We also wanted to see if U24 could directly induce experimental autoimmune encephalitis (EAE), an animal model of MS. **Methods:** A recombinant form of U24 from E.coli was expressed and purified. Its secondary structure was probed with circular dichroism. Erk2 was used to 32P-label U24, which was then analyzed by SDS-PAGE and autoradiography. Thin Layer Chromatography (TLC) and MALDI-TOF mass spectrometry were used to determine the position of the phospho-site in U24. To find whether U24 protein could be used to induce EAE, we directly injected U24 into female Lewis rats. **Results:** We have shown that both U24 protein is likely to be membrane-bound, and that it is recognized by Erk2 kinase. U24 can be phosphorylated at the Thr position in the stretch of sequence shared with MBP. Preliminary results on the Lewis rats indicate that U24 does not induce direct EAE responses. **Conclusions:** By demonstrating that U24 can be phosphorylated at a position equivalent in MBP, we have identified a molecular target for kinases which may interfere with normal phosphorylation of MBP and in turn play a role in the pathogenesis of MS.

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NMO-IgG and anti-aquaporin 4 antibody simultaneously examined in a large series of Japanese neuromyelitis opticaKeiko Tanaka¹, Takashi Tani², Masami Tanaka³, Masatoyo Nishizawa⁴, Kenji Sakimura⁵, Makoto Matsui⁶¹Neurology, Kanazawa Medical University, Kahoku-Gun, Ishikawa, Japan; ²Niigata University, Niigata, Niigata, Japan; ³Utano National Hospital, Kyoto, Kyoto, Japan; ⁴Niigata University, Niigata, Niigata, Japan; ⁵Niigata University, Niigata, Niigata, Japan; ⁶Kanazawa Medical University, Uchinada, Ishikawa, Japan

Background: Neuromyelitis optica (NMO)-IgG, whose target molecule is an aquaporin 4 (AQP4), is reported to be a specific marker for NMO. NMO is thought to be prevalent in Asia and it is important to diagnose them for proper treatment. We established the immunofluorescence detection system of NMO-IgG and AQP4 antibody (AQP4-Ab) and compared the sensitivity of each method in a large series of Japanese NMO together with IgG subclass and antibody titers. We also examined the reactivity of AQP4-Ab negative sera with AQP1. **Objective:** Seventy AQP4-Ab positive sera were examined for immunohistochemical staining of rat cerebral sections. IgG subclass were examined with 20 AQP4-Ab positive sera. Disease activities and antibody titers were compared in 20 patients. AQP4-Ab negative sera were examined for the reactivity with AQP1. **Methods:** Antibodies for AQP4 or AQP1 were detected using AQP4 or 1-transfected HEK 293 cells. IgG subclass were examined using anti-human IgG1, IgG2, IgG3, IgG4 and IgM for the second antibody. Antibody titer was checked with serial dilution of each sera to find the staining end points. **Results:** Seventy AQP4-positive sera were all bound to rat cerebral pia/subpia/Virchow Robin lining; however, perivascular staining was not seen in 26% of them. Among AQP4-Ab negative sera, some showed fibrous and linear staining around choroid plexus, and those were all negative for AQP-1 antibody. We also examined IgG subclass with these 70 sera; 95% showed IgG1, 20% were IgG2 or IgG3, 25% were IgG4 and 15% were IgM (overlapping results). We also examined the relevance of antibody-titers and disease activity and the high titer tended to be seen in those with active and early stages of NMO, even if they did not show long spinal cord lesions. **Conclusions:** All of these results confirm that the NMO-IgG/AQP4-Ab are closely related to the pathogenesis of NMO.

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Ninjurin-1 as a putative adhesion molecule of the blood-brain barrierSimone Terouz¹, Hania Kebir¹, Aurore Dodelet-Devillers¹, Igal Ifergan¹, Monique Bernard¹, Romain Cayrol¹, Danica Stanimirovic², Alexandre Prat¹¹University of Montreal, Montreal, Quebec, Canada; ²Institute for Biological Sciences, Ottawa, Ontario, Canada

Background: In multiple sclerosis (MS), inflammatory demyelinating lesions are associated with a compromised blood-brain barrier (BBB) and with perivascular infiltration of immune cells into the central nervous system (CNS). The movement of leukocytes from the blood to the CNS is orchestrated by many factors including cell adhesion molecules that enable immune cells to adhere and cross the BBB. Nerve injury-induced protein (Ninjurin)-1 was described as an adhesion molecule that promotes nerve regeneration, in a homotypic Ninjurin-1-Ninjurin-1 fashion. We recently identified Ninjurin-1 in a proteomic screen for BBB-associated proteins using human BBB-derived endothelial cells (ECs). **Objective:** To study the role of Ninjurin-1 in the trans-BBB migration of immune cells. **Methods:** Expression, regulation and localization of Ninjurin-1 on human BBB-ECs was determined by Western blot (WB), flow cytometry and immunocyto- and immunohisto-fluorescence. The functional role of Ninjurin-1 has been assessed using an in vitro model of the human BBB. **Results:** We confirmed expression of Ninjurin-1 in vitro on BBB-ECs by WB and flow cytometry, in situ by confocal microscopy and we show regulation of Ninjurin-1 upon treatment with pro-inflammatory cytokines TNF, IFN-gamma and IL-17. Furthermore, Ninjurin-1 immunoreactivity was detected in vitro on astrocytes and in situ in MS lesion and in CNS material obtained from its animal

model, experimental autoimmune encephalomyelitis (EAE). We demonstrate that Ninjurin-1 is present on peripheral blood immune cells, with a preferential expression on CD14 monocytes, as compared to CD4, CD8 or CD19 lymphocytes. Finally, using a modified Boyden chamber assay, we show that migration of ex vivo CD14 monocytes across the human endothelium is significantly reduced following treatment of BBB-ECs with a competing Ninjurin-1 peptide (PARWGWWRHGPIN) and that Ninjurin-1 blockade increases BBB permeability. **Conclusions:** Our findings suggest that Ninjurin-1 is a novel adhesion molecule of the CNS endothelium that acts as a regulator of monocyte migration into the brain.

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NMO-IgG and AQP-4 antibodies in different demyelinating disease phenotypes: correlations with clinical and paraclinical parametersCarla Tortorella¹, Maddalena Ruggieri¹, Giovanni Bosco Zimatore¹, Antonio Frigeri², Anna Lia¹, Elisabetta Di Monte¹, Angelo Ghezzi³, Angela Rizzo³, Maria Pia Amato⁴, Bahia Hakiki⁴, Francesco Patti⁵, Alessandra Lugaresi⁶, Elisa Merelli⁷, Patrizia Sola⁷, Vita Di Renzo¹, Paolo Livrea¹, Maria Svelto², Maria Trojano¹¹Dept. of Neurological and Psychiatric Sciences, University of Bari, Bari, Italy; ²Department of General and Environmental Physiology, Bari, Italy; ³Az.Osp.S.Antonio Abate, Gallarate (MI), Italy; ⁴Department of Neurological Science, University of Florence, Firenze, Italy; ⁵Department of Neurology, University of Catania, Catania, Italy; ⁶Department of Neuroscience, University of Chieti, Chieti, Italy; ⁷Department of Neurology, University of Modena, Modena, Italy

Background: NMO-IgG is an autoantibody selectively binding the AQP-4 water channel. Even if it has been considered a specific marker of neuromyelitis optica (NMO) its pathogenetic role in demyelinating disease is still not completely clarified. **Objective:** The aims of this study were to confirm sensitivity and specificity of NMO-IgG and AQP-4 Abs in the diagnosis of NMO and to correlate NMO-IgG seropositivity with clinical, cerebrospinal fluid (CSF) and magnetic resonance imaging (MRI) metrics in different phenotypes of demyelinating disease. **Methods:** We examined 150 patients: 57 multiple sclerosis (MS), 19 optic neuritis (ON), 31 isolated myelitis, 16 recurrent forms of ON or myelitis and 27 recurrent forms of ON and myelitis. Serum NMO-IgG was tested by indirect immunofluorescence whereas AQP-4 by HeLa cell line transfected with mouse AQP-4 c-DNA. **Results:** One MS, two ON, seven myelitis and four recurrent forms of ON or myelitis were NMO-IgG seropositive. By using Wingerchuck '99 criteria all patients with recurrent forms of ON and myelitis were classified as NMO (11 NMO-IgG positive, sensitivity 40%) whereas only 14 patients of this group fulfill Wingerchuck '06 criteria for NMO (eight NMO-IgG positive, sensitivity 57%). The specificity of the serum NMO-IgG test for NMO was 98% vs MS (p=0.0001) and 88% vs other demyelinating diseases (p=0.0001). 103 serum samples were tested for AQP-4. The sensitivity of serum AQP-4 Abs was 40% and 70% when the older or newer diagnostic criteria were used respectively, whereas its specificity was 100% vs MS (p=0.0001) and 90% vs other demyelinating diseases (p=0.0001). NMO-IgG seropositive patients not fulfilling the 2006 criteria were characterized by higher CSF/serum albumin ratio (p=0.01) and higher frequency of negative or "aspecific" brain MRI in comparison to the seronegative ones (p=0.02). **Conclusions:** Our data confirm the specificity of NMO-IgG and AQP-4 Abs in the diagnosis of NMO, but suggest that sensitivity may be related to the diagnostic criteria used. NMO-IgG seropositivity seems to also identify a subgroup of demyelinating diseases mainly characterized by blood-brain barrier (BBB) damage and negative or "aspecific" brain MRI. A pathogenetic role of NMO-IgG production in BBB impairment might be speculated. **Supported by:** Progetto Strategico Regionale, Regione Puglia, 2007.

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Phase I safety study of RTL1000, a recombinant T-cell receptor ligand specific for MOG peptide, in multiple sclerosisArthur A. Vandenberg¹, Gregory G. Burrows², Edward P. Ravey², Halina Offner²¹Neuroimmunology Research, Portland VA Medical Center, Portland, Oregon, USA; ²Oregon Health & Science University, Portland, Oregon, USA

Background: A Recombinant T-Cell Receptor Ligand (RTL1000) - covalently linked HLA-DR2 alpha-1 and beta-1 domains with attached myelin oligodendrocyte glycoprotein (MOG-35-55 peptide) was developed as a T-cell specific therapy for multiple sclerosis (MS). **Objective:** The primary objective of this study is to evaluate the safety profile of a single intravenous (IV) dose of RTL1000, as assessed by clinical examinations and magnetic resonance imaging (MRI). This study is a first-in-human double-blind, placebo-controlled, Phase 1, dose escalation study of RTL1000 in relapsing and progressive MS subjects. **Methods:** Five cohorts (receiving escalating doses of 2 mg, 6 mg, 20 mg, 60 mg and 200 mg), with six subjects per cohort (four receiving drug, two receiving placebo) are included, with all subjects followed over 90 days for clinical and MRI changes. Blood samples collected over 28 days are evaluated for RTL1000 pharmacokinetics and immunogenicity, and for changes in T cell responses to MOG peptide and control antigens. Safety data from each cohort will be reviewed by an independent Data Safety Monitoring Board (DSMB) prior to enrollment of the next cohort. **Results:** The first two cohorts have been completed as of January 2008, with the third being enrolled in February 2008. RTL1000 was detectable in plasma in subjects receiving 6 mg of the drug. Immunological studies indicate sufficient reactivity in a subset of subjects to MOG peptide at entry to allow assessment of RTL1000 effects when the study is unblinded at its completion. **Conclusions:** An update of unblinded data on characteristics of enrolled subjects, and pharmacokinetics, immunogenicity and immunological changes induced by RTL1000 will be presented and discussed.

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Immunological changes in peripheral blood in two doses of methyprednisolone therapy of multiple sclerosis relapse and correlation with sex

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Background: In treatment of multiple sclerosis (MS) relapses high doses of intravenously (IV) given methyprednisolone (MP) are used, however its mechanism of action is not fully known. **Objective:** We have studied 34 patients (24 women and 10 men), who were randomized to receive MP IV in doses: 1000 mg and 500 mg for five consecutive days in double manner. The control group was 19 healthy volunteers. **Methods:** Patients were asked to give blood on days 0 and 7 from IV MP therapy, controls twice in 7-day intervals. Expanded disability status scale (EDSS) examination was performed on days 0 and 7 after starting treatment. We analyzed surface antigens expression on human leukocyte subsets: CD+CD19-, CD3-CD19+, CD3+CD4+, CD3+CD8+, CD3-CD56+CD16+, and interleukin-6(IL-6) and interleukin-8 (IL-8). **Results:** As data were found to be normally distributed, the Wilcoxon matched Paris Test was used ($p \geq 0.05$). 14 women and four men received 1000 mg IVMP, eight women and six men received 500 mg IVMP. Both doses of IVMP were efficient in promoting clinical recovery. In women treated with 1000 mg of MP increase of granulocytes producing IL-6 and decrease of CD14+ IL-8 cells was noted. In men the same changes gives 500 mg MP. IL-6 promote immune response by enhancing T-cell activation, but IL-6 was also known to promote survival, migration and differentiation of oligodendrocytes precursors into myelin basic protein positive cells. IL-8 is responsible for immune tissue damage and for attracting the immune cells to the area of inflammation. **Conclusions:** There is still a need for further investigation to elucidate the mechanism of action of MP.

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Interferon-beta induced restoration of regulatory T-cell function in multiple sclerosis is prompted by an increase of newly generated naïve Treg

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Background: Naturally occurring regulatory T-cells (Treg) are functionally impaired in patients with relapsing-remitting multiple sclerosis (RRMS). We recently showed that prevalences of newly generated CD31- coexpressing naïve Treg (recent thymic emigrant, "RTE"-Treg) are critical for suppressive function of circulating Treg and a shift in the homeostatic composition of Treg subsets related to a reduced de novo generation of RTE-Treg may contribute to the MS-related Treg dysfunction. Interferon beta (IFN-beta), an immunomodulatory agent with established efficacy in MS, reduces relapse rates and decreases disease progression. Emerging evidence suggests that Treg suppressive capacities may increase in MS patients undergoing treatment with IFN-beta although the mechanisms mediating this effect are uncertain. **Objective:** To evaluate the effect of IFN-beta on the suppressive activity and the homeostasis of circulating Treg in patients with MS. **Methods:** We determined frequencies, phenotypes, and suppression activities of Treg in peripheral blood samples obtained from 20 patients with RRMS before and serially after treatment institution with IFN-beta. **Results:** Suppressing capacities of Treg were consistently upregulated at three and six months of IFN-beta treatment. The restoration of Treg function was paralleled by increased proportions of naïve RTE-Treg and a coincidental reduction of memory Treg. Total naïve CD4+ T-cells also shifted towards higher numbers. **Conclusions:** These findings suggest that the increase of Treg inhibitory capacities mediated by IFN-beta can be explained by its impact on the homeostatic balance within the Treg compartment.

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Direct evidence of a thymic abnormality in relapsing-remitting multiple sclerosisJulia L. Williams¹, Helen Mason¹, Yves Lapierre², Jack P. Antel², David G. Haegert¹¹McGill University, Department of Pathology, Montreal, Quebec, Canada; ²McGill University, Department of Neurology, Montreal, Quebec, Canada

Background: Naïve CD4 T cells have a central role in initiating the autoimmune response in multiple sclerosis (MS). Naïve T cells occupy an ecological niche in the periphery that is regulated by homeostatic mechanisms. Some investigators claim a naïve T cell homeostatic abnormality occurs in MS but the basis of this abnormality could not be clearly established. **Objective:** Our objective was to test the hypothesis that relapsing-remitting multiple sclerosis (RRMS) patients have premature thymic involution leading to reduced generation of recent thymic emigrants (RTEs) and followed by compensatory homeostatic proliferation of naïve CD4 T cells. **Methods:** To test this hypothesis, we used a FACSAria cell sorter to isolate subsets of naïve CD4 T cells (10 patients and 10 age-matched healthy controls), based on expression levels of CD31. Specifically, we isolated subsets of RTEs (CD31-hi and CD31-lo) and non-RTEs (CD31-neg). Using real-time polymerase chain reaction (PCR), we quantified signal joint T cell receptor excision circles (sjTRECs) and β TRECs in DNA from these subsets as markers of thymic output. Additional homeostatic markers (Bcl-2, CD127 and Ki-67) were analyzed by flow cytometry within the different subsets. **Results:** In controls, true RTEs (CD31-hi) showed a significant decline in T cell receptor excision circles (TREC) levels with increasing age, whereas in RRMS comparable cells had consistently low TREC levels irrespective of age. Second, in RRMS, RTEs and non-RTEs showed significantly increased expression of the IL-7 receptor (CD127), whereas non-RTEs had increased Bcl-2 levels. **Conclusions:** We report the first definitive evidence of a thymic abnormality in RRMS that leads to reduced thymic output and compensatory homeostatic proliferation affecting both RTEs and non-RTEs. These naïve CD4 T cell changes may increase autoreactivity and predispose to RRMS.

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P742

Cytokine and chemokine profiles in the cerebrospinal fluid and serum of multiple sclerosis

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Background: Cytokines and chemokines are soluble mediators of the immune system involved in transmigration of immune cells to the central nervous system (CNS) and subsequent development of tissue damage in multiple sclerosis (MS). **Objective:** Our aim was to identify cytokines and chemokines that could be used as potential therapeutic targets. **Methods:** We analyzed serum and CSF cytokines and chemokines including granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage (GM)-CSF, interferon (IFN)- γ , tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, macrophage inflammatory peptide (MIP)-1 β , and monocyte chemoattractant protein (MCP)-1 in 176 patients with different subtypes of MS. Their levels were correlated with disease activity expressed by annual relapse rate and neurological disability expressed by the expanded disability status scale (EDSS). For statistical processing one-way ANOVA or its non-parametric parallel (Kruskal-Wallis analysis) as well as Spearman's correlation analysis were used. **Results:** In CSF, the level of MIP-1 β in relapsing-remitting (RR) MS was higher than in secondary progressive (SP) MS (39.5 pg/ml vs 14.8 pg/ml, $p < 0.01$) or primary progressive (PP) MS (39.5 pg/ml vs 12.2 pg/ml, $p < 0.01$). During relapse, CSF MIP-1 β increased (38.8 pg/ml vs 14.0 pg/ml, $p < 0.05$), and CSF MCP-1 decreased (122.1 pg/ml vs 200.3 pg/ml, $p < 0.05$) in comparison with those in the clinically stable phase. The serum cytokine and chemokine profiles in patients with different subtypes of MS, or between relapse and stable stages, were without marked differences. **Conclusions:** The higher level of CSF MIP-1 β in RRMS, as well as the upregulated MIP-1 β and downregulated MCP-1 in CSF during relapse, are consistent with increased inflammatory activity in the early phase of MS, especially during relapse. Our results suggest that MIP-1 β and MCP-1 are important players in the inflammatory demyelinating pathogenesis of MS, and they could be used as potential therapeutic targets or markers to predict the disease subtypes or disease activity.

P743

Peripheral T cells are the therapeutic targets of high dose glucocorticoids in experimental autoimmune encephalomyelitis

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Background: Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS). Presently there is no cure available for MS and therapy remains unsatisfactory. **Objective:** Acute relapses and optic neuritis are treated with high doses of glucocorticoids (GC) but the underlying mechanism is still debatable. Thus, we aimed at further elucidating the mode of GC action in the treatment of MS. **Methods:** We induced experimental autoimmune encephalomyelitis (EAE) in C57Bl/6 mice by immunization with Myelin Oligodendrocyte Glycoprotein using mutant mouse strains carrying different cell-type-specific mutations in the glucocorticoid receptor (GR) gene. Subsequently, the mice were analysed by immunohistochemistry and flow cytometry. **Results:** We could show that dexamethasone (Dex) ameliorates the disease course of EAE in a dose-dependent manner. The study of heterozygous GR knock-out mice and hematopoietic stem cell chimeras that lack the GR especially in leukocytes revealed that the cytosolic GR is a prerequisite for mediating therapeutic GC effects since no Dex effect could be

observed. Using cell-type specific GR-deficient mice we could further show, at the cellular level, that the expression of the GR in T cells is essential for mediating beneficial effects while it is dispensable in myeloid cells. Analysis of the molecular mechanisms showed that these effects were achieved through induction of apoptosis and down regulation of adhesion molecules in peripheral but not in CNS-residing T cells. Furthermore, we observed that Dex inhibited T cell migration into the CNS. **Conclusions:** We describe for the first time the underlying mode of GC action at the cellular and molecular level in the treatment of EAE and with these findings we anticipate that therapeutic approaches in the treatment of acute relapses in MS may be improved in the future.

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P744

EMMPRIN: A novel regulator of neuroinflammation in multiple sclerosis

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Background: Several members of the matrix metalloproteinase (MMP) family are upregulated in multiple sclerosis (MS) and its animal model, Experimental Autoimmune Encephalomyelitis (EAE), where the balance of their multiple roles appears to be detrimental. Thus, inhibiting MMP activity is a reasonable approach in MS, but pharmacological inhibitors of MMPs lack selectivity and it is unclear as to which MMP member is more critical for targeting. An alternate approach is to address whether there is a common inducer of the expression of MMP members in MS, and to target this molecule. The extracellular MMP inducer (EMMPRIN, CD147) is found on the surface of various cell types in an inactive state. In the tumor literature, EMMPRIN induces stromal and tumor cells to produce and activate various MMPs. **Objective:** Our specific objective was to determine whether EMMPRIN has a role in MS and EAE. **Methods:** EAE was induced in C57Bl/6 mice with MOG35-55 peptide. At different times thereafter, lymphoid (spleen and lymph nodes) and neural (spinal cord) tissues from EAE-afflicted mice were analyzed. Sections from patients who died with MS were also examined. **Results:** The activation of T-cells in culture led to the upregulation of active EMMPRIN. In EAE, we found increased numbers of EMMPRIN positive cells in the central nervous system (CNS) from the onset of EAE signs, and these continued to rise with disease progression. EMMPRIN immunoreactivity was detected in the CNS in both infiltrating and resident cellular populations including astrocytes. Interactions of T-cells with astrocytes resulted in the increased expression of MMPs through a process that appeared to be EMMPRIN-dependent. In MS tissues, EMMPRIN was significantly upregulated compared to controls, and elevated expression was in lesions rather than normal appearing tissue. Finally, EMMPRIN null mice, although susceptible to EAE, showed a slightly delayed onset and reduced severity in EAE disease versus wildtype controls. **Conclusions:** Targeting EMMPRIN could help influence the marked elevation of MMPs and disease process of EAE and MS.

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P745

Impaired hypothalamo-pituitary-adrenal axis activity in multiple sclerosis patients

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Background: Previous studies have shown that multiple sclerosis (MS) patients exhibit chronic hypothalamo-pituitary-adrenal (HPA) axis activation, the underlying mechanisms of which remain unclear. Most studies have been performed in limited patient series without clear separation between subgroups, therefore yielding conflicting data. **Objective:** To investigate HPA axis activity in well-defined MS patient subgroups. **Methods:** A total of 173 patients with clinically definite MS were studied, 40 with primary progressive (PP), 41 with secondary progressive, 58 with relapsing-remitting (RR) but in remission, and 34 with

RR in acute relapse. Sixty healthy subjects served as controls. No patients were under steroid or other immunomodulatory therapy. Plasma cortisol, adrenocorticotrophic hormone (ACTH), and Dehydroepiandrosterones (DHEAs), as well as urine cortisol levels were measured using commercial RIAs. Glucocorticoid receptor (GR) binding assay in peripheral blood mononuclear cells (PBMC) was performed using [3H]-dexamethasone (DEX). PBMC production of corticotrophin-releasing hormone (CRH), IL-1, IL-6 and TNF-alpha was evaluated using ELISPOT. **Results:** All four groups of patients displayed significantly higher cortisol, ACTH and DHEAs plasma concentrations and urine cortisol values than controls, with primary progressive multiple sclerosis (PPMS) patients and relapsing-remitting multiple sclerosis (RRMS) in acute relapses subjects showing the most prominent differences during exacerbations. GR binding assays did not differ between patients and controls regarding binding capacity, however all MS patient groups showed decreased GR affinity and sensitivity compared to controls. The number of IL-1, IL-6 and TNF-alpha secreting cells was significantly increased only in RRMS patients during exacerbations. Finally, MS patients showed a significant increase in PBMC CRH secreting cell numbers. **Conclusions:** Our analysis indicates that MS patients show HPA axis hyperactivity, and that although lymphocytes from MS patients express similar GR numbers to controls, their binding affinity and glucocorticoid sensitivity appears to be reduced.

WITHDRAWN

P747

A novel type of proinflammatory human dendritic cell (slan-DC) in neuroinflammatory central nervous system disease: first data on the presence and function in multiple sclerosis

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Background: To demonstrate histopathologically the presence of slan-DC in different inflammatory central nervous system (CNS) disorders and the effect of immunomodulatory treatment on slan-DCs in comparison to plasmacytoid dendritic cells (PDCs) and monocytes in patients with multiple sclerosis (MS). **Objective:** Slan-DCs are a subpopulation of proinflammatory myeloid human blood DCs which express selectively the epitope 6-sulfo-LacNAc. As already shown slan-DCs play an important role in proinflammatory processes and therefore we wanted to evaluate whether these cells are present in inflammatory CNS disorders and could be modulated by immunomodulatory therapies. **Methods:** By immunohistochemical analysis, we evaluated different inflammatory CNS disorders like MS lesion, meningitis, encephalitis and vasculitis. We used an antibody against the 6-sulfo-LacNAc epitope on the surface of slan-DCs (mAb DD2) in combination with an anti-CD68 antibody for macrophage/microglia staining. In addition, we investigated blood samples from untreated and treated MS patients with immunomodulatory therapy (IFN, GA) or relapse therapy (methylprednisolone): after isolation of peripheral blood mononuclear cells (PBMCs), sorted slan-DCs were cultured with LPS, R848 or without stimulation. Slan-DCs, PDCs and monocytes were characterized regarding their frequency, cytokine profile as well as surface activation markers by four-color fluorescence flow cytometry. **Results:** Slan-DCs are present in different inflammatory CNS disorders. Especially in areas with active inflammation, a remarkable number of CD68+ cells are slan-DC presenting in a typical perivascular localization. Slan-DCs are modulated differently by immunomodulatory therapies in comparison to PDCs or monocytes. During relapse therapy with methylprednisolone cortisone there is a decrease in slan-DC frequency whereas PDCs and monocytes increase in frequency. There is decreased expression of TNF- α , CD83, CD150 after LPS or R848 stimulation. **Conclusions:** We show for the first time that slan-DCs are present in the inflamed CNS in significant quantities and can be specifically modulated by immunomodulatory therapies and methylprednisolone treatment.

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P748

Epstein-Barr virus is associated with gray matter atrophy and lesion injury in multiple sclerosis

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Background: Because Epstein Barr virus (EBV) specifically affects immune cells and is capable of causing the dysimmunity that is a hallmark of multiple sclerosis (MS), it has long been considered a promising hypothesis as the environmental trigger or disease-modifying pathogen for MS. **Objective:** To determine whether the presence of anti-EBV antibodies is associated with magnetic resonance imaging (MRI) measures of brain injury and neurodegeneration in MS patients. **Methods:** A total of 135 MS patients (86 females; 49 males) underwent brain MRI and testing for antibodies against EBV. The MRI measurements included gadolinium-enhancing (Gd) lesion volume (LV), T1- and T2-LVs and fractions of whole brain parenchyma (BPF), white matter (WMF) and gray matter (GMF). The anti-EBV panel included measurements of the levels of anti-EBV early antigen (EA) IgG, anti-EBV nuclear antigen (EBNA) IgG and anti-EBV viral capsid antigen (VCA) IgG. The relationships between antibody levels and MRI parameters were assessed in regression analysis. Limited longitudinal analysis of the effects of anti-EBV VCA IgG antibodies on evolution of MRI measures was available for a subset of 50 patients after mean follow-up of 3.1 years. **Results:** The proportion of MS patients positive for anti-EBV-EA IgG, anti-EBV-EBNA IgG and anti-EBV-VCA IgG antibodies were 17.0%, 97.0% and 99.3%, respectively. The anti-EBV-VCA IgG levels were significantly associated with higher T2- and T1-LVs and lower GMF and BPF. The GMF ($R^2 = 0.24$ for overall model and Standardized $\beta = -0.26$, $p = 0.002$) and BPF ($R^2 = 0.39$ for overall model and Standardized $\beta = -0.28$, $p < 0.001$) showed the strongest negative associations with anti-EBV-VCA IgG levels. Higher decline in BPF was significantly associated with increased baseline anti-EBV VCA IgG levels ($R^2 = 0.61$, $p < 0.001$ for overall model and Standardized $\beta = -0.38$, $p = 0.008$). **Conclusions:** Our data suggest that the presence of anti-EBV antibodies is associated with MRI markers of GM atrophy and lesion injury in MS. Increased levels of anti-EBV VCA IgG at baseline were associated with increased loss of brain volume over 3-year follow-up.

P749

Identification of a new antigen candidate of multiple sclerosis using an open reading frame selected phage display library

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Background: To date the epitope specificity in multiple sclerosis (MS) is unknown. The study of autoimmune response in MS is complicated by epitope spreading, its heterogeneity and pathogenetic relevance of the autoantibodies. Antibodies to myelin components are routinely detected in MS patients, but their diagnostic and clinical value is still controversial. Until now peptide-based research has been important in attempts to identify autoantigens in MS, but random peptide libraries have been almost always used. Since pathogenic autoantibodies recognize conformational epitopes, the use of a cDNA phage display library seems more appropriate. **Objective:** To overcome the problem of traditional cDNA fragment libraries rich in non-functional clones, we set a novel phage display vector able to select real open reading frame (ORF) cDNA fragments. **Methods:** We produced a cDNA library from healthy human brain with a dimension of 2x10exp5 and about 90% of diversity; the length of epitopes encoded by cDNA fragments was of about

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30–130 aminoacids. Then we performed two biopanning of this library using a pool of purified IgG from 90 MS relapsing-remitting (RR) patient sera and 12 clinically isolated syndrome (CIS) patient liquor. **Results:** After three rounds of selection more than 25% (for RR sera) and 48% (for CIS liquor) of the clones showed an O.D. in Phage-ELISA screening higher than mean O.D. of 0,5 with a negative control of 0,2 O.D. After automated sequencing and databank characterization, 21 clones from RR and 17 from CIS selection were identified; the clones more reactive were also common in the two selections. 90% of all clones were real ORFs and had a mean size of 80 aminoacids. **Conclusions:** Currently we are confirming and validating these new candidate autoantigens of MS by classical immunoassays. This kind of serum and liquor profiling could assist in distinguishing between types of MS, monitoring its progression, and setting antigen-dependent therapeutic strategies.

Neuropsychology/Neuropsychiatry

P750

Efficacy of the cognitive stimulation in patients with multiple sclerosis
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Background: Cognitive dysfunction has been reported to be present in 45 to 65% of multiple sclerosis (MS) patients. Memory, attention and speed of information processing are the most frequent affected cognitive domains. Cognitive rehabilitation increases the capabilities for processing and using information, and also improves the quality of life of MS patients. **Objective:** To determine the efficacy of a process of cognitive stimulation in MS patients. **Methods:** Nineteen MS patients, 16 female and 3 male, mean age 31.8 years and 14 year mean education level were included in our study. Fourteen relapsing-remitting MS, two secondary progressive and three primary progressive MS patients regardless time of diagnosis, treatment or expanded disability status scale (EDSS) were evaluated with the Rao Neuropsychological Screening Battery (NSB) for MS and the Wechsler Adult Intelligence Scale III before and after the process of cognitive stimulation. The cognitive stimulation was performed during an eight month period with a weekly personalized session. The before and after scores were analyzed using the statistical 'T' test for related samples considering a significant statistical difference when $p < 0.05$ (SPSS.10th version) was used. **Results:** NSB final results showed significant cognitive improvement in long term storage, recall and delayed recall memories (0.006, 0.010, 0.003) respectively. Verbal fluency also improved ($p < 0.013$). The WAIS III showed improvement in the final total and Executive IQ performance and perceptual organization (0.008, 0.001 and 0.005) respectively. **Conclusions:** The results obtained from this population showed the efficacy of cognitive stimulation in seven of the 15 studied variables.

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P751

Cognitive profile of multiple sclerosis in the Mexican population
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Background: The earliest description of multiple sclerosis (MS) cognitive dysfunction was described by Charcot in 1877. The most commonly affected cognitive domains reported are memory, attention and speed of processing information. MS prevalence in Mexico has

been reported as low as 1 to 13 per hundred-thousand inhabitants. A clinical profile has been recently reported. There are no published papers reporting the neuropsychological or cognitive profile in the Mexican population. **Objective:** To define the cognitive profile in the Mexican population. **Methods:** One hundred and seventy-seven patients and 250 controls were included for study regardless of gender, time of diagnosis, treatment, type of MS or expanded disability status scale (EDSS) score. The Spanish validated version from Cáceres et al. of Rao Neuropsychological Screening Battery (NSB) was applied. **Results:** Demographic data did not show significant statistical differences between patients and controls. Sixty-four percent of patients showed cognitive dysfunction and the most affected cognitive domains were memory (60%), sustained attention (54%) and speed of information processing (51%). **Conclusions:** This is the first reported study of the cognitive dysfunction in the Mexican population with a significant sample and important number of controls. The frequency characteristics and affected domains of Mexican MS patients do not differ from that reported in other countries.

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P752

Neuropsychiatric symptoms in the early stage of multiple sclerosis
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Background: It is known that patients with multiple sclerosis (MS) can present neuropsychiatric symptoms. However it unclear how often they occur, especially in the early stage of the disease. **Objective:** The present study aims to investigate the prevalence and the range of neuropsychiatric symptoms in the early phases of MS. **Methods:** Twenty-seven MS patients with mean disease duration of 2.7 years (SD 0.27, range 1–6) attending the Neuroimmunology and Multiple Sclerosis Unit of the Doctor Josep Trueta Hospital in Girona (Spain) were studied. Our study protocol included an interview to collect demographical and clinical data, and the assessment of the Neuropsychiatric Inventory (NPI), the Expanded Disability Status Scale (EDSS) and the Multiple Sclerosis Functional Composite (MSFC). A descriptive analysis and a calculation of proportions were performed. Proportions between groups were compared using the chi-square test. Given that NPI scores were not normally distributed, comparisons between groups were made using the Mann-Whitney test. The correlations between NPI subscores and demographic and clinical variables were analyzed using the Spearman rank correlation. **Results:** Twenty-two (81.5%) patients exhibited some degree of psychiatric symptoms. Irritability was the most common symptom (74.1%) followed by anxiety (51.9%), depression (40.7%), apathy (29.6%), sleep disorders (29.6%), appetite disturbances (18.5%), euphoria (18.5%), disinhibition (7.4%) and delusions (3.4%). Median subscale scores revealed that the most severe symptom was depression. Participants with lower educational level (≤ 12 years) were more depressed (72.7% vs. 25%, $p = 0.022$), anxious (64.3% vs. 23.1%, $p = 0.031$), apathetic (87.5% vs. 26.3%, $p = 0.008$) and had more sleep disorders (75% vs. 31.6%, $p = 0.038$). A positive and significant correlation was found between the anxiety score and the PASAT test ($r = 0.542$, $p = 0.045$). No other significant correlation was found. **Conclusions:** Neuropsychiatric manifestations are very common among MS patients in the early stages of the disease and they are not related with disability.

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P753**Early cognitive impairment in patients with clinically isolated syndrome suggestive of multiple sclerosis: a 1-year follow-up**

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Background: Cognitive impairment has been traditionally reported to be associated with the later stages of multiple sclerosis (MS). However, a few studies have shown that it may be detectable during the early phase of MS, and even after a Clinically Isolated Syndrome (CIS). **Objective:** The aim of the study was to evaluate the presence of cognitive impairment in patients with CIS (pwCIS) and to assess the evolution of cognitive function in pwCIS after a 1-year interval. **Methods:** Fifty-three consecutive pwCIS (37 women, 16 men; mean age of 31.4 years) assessed between 3 and 5 months after the attack and 24 age-, sex-, and educational level-matched healthy control (HC) subjects were included in the study. At 1 year, 44 pwCIS were re-assessed. A comprehensive battery of neuropsychological tests was used to explore cognitive functions. Emotional tests were also administered. Three methods were used to estimate the early cognitive impairment at baseline and longitudinal analysis. Method 1 used comparisons test to test. Method 2 used normalized composite scores of the neuropsychological tests grouped in the following domains: speed of information processing, attention, verbal and visuospatial memory and executive functions. Method 3 used comparisons of number of tests failed. $p < 0.05$ was the level of statistical significance. **Results:** pwCIS and HC were comparable in emotional status. At baseline pwCIS did worse only in motor tasks, and vocabulary tests. No worsening was observed in pwCIS after one year of follow-up and in fact they did improve in most tests and domains. PwCIS did improve in emotional tests. **Conclusions:** The findings of the current study suggest that cognitive involvement is not so common in pwCIS and that there is no cognitive decline early in the course of the disease in a sample of consecutive patients carefully diagnosed with CIS.

P754**Psychiatric and cognitive onset in multiple sclerosis**

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Background: Psychiatric and cognitive symptoms are rarely reported as the first manifestation of multiple sclerosis (MS). **Objective:** To describe clinical and magnetic resonance imaging (MRI) characteristics of patients who presented with cognitive or atypical psychiatric symptoms as the first and predominant manifestation of MS. **Methods:** Among the MS outpatients followed in our neurological department we have identified 21 patients who presented either with isolated cognitive impairment (n=14) or with psychiatric symptoms (n=7), as the first manifestation of the disease. Brain atrophy was evaluated by measuring the width of the third ventricle and compared to healthy controls (n=9) and MS patients: primary progressive multiple sclerosis (PPMS) (n=10) and relapsing-remitting multiple sclerosis (RRMS) (n=10) with similar disease duration and without cognitive/psychiatric symptoms. **Results:** Mean age at presentation was 43 years for cognitive-onset MS patients and 29 years for psychiatric-onset MS patients. Mean disease duration was 7.4 years (2–14 years). Among patients with cognitive presentation, 8/14 had at least one relapse during the follow up. Neuropsychological symptoms were characterized by progressive memory decline and frontal subcortical dementia, which remained the predominant feature of the disease for all patients. Psychiatric presentation consisted in psychotic symptoms in 4/7 patients and in severe mood disorders in 3/7 patients. Relapses occurred during the follow-up for 4 patients. Three psychiatric-onset patients evolved towards a severe cognitive impairment during the first years of the disease. Initial brain MRI performed in a mean delay of 3 years (0–11 years) from onset showed

multiple focal non-confluent white matter lesions in 12 patients and diffuse or confluent lesions mostly located in the periventricular white matter in nine patients. Brain atrophy was more severe in patients who presented or evolved towards a cognitive impairment and progressed faster than in other MS patients. **Conclusions:** MS can rarely present with psychiatric or cognitive symptoms. Patients with psychiatric onset may further evolve towards a severe cognitive deterioration. Cerebral atrophy is more pronounced and progressed faster among patients who developed early dementia.

P755**Cortical activation changes are present in patients with clinically isolated syndrome during both unconscious and conscious processing**

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Background: The global neuronal workspace theory predicts a stochastic all-or-none dynamical transition between unconscious and conscious processing. This dynamical transition, that does not represent a proper functional state, can be characterized by the measure of the threshold for access to consciousness. We have recently demonstrated that this parameter was delayed in patients with early relapsing-remitting multiple sclerosis (RRMS) suggesting that unconscious and/or conscious functional states were altered. However, behavioural measures demonstrated normal unconscious and conscious processing in patients. **Objective:** To clarify this point, we aimed at comparing the functional activation and deactivation patterns related to unconscious and conscious processing between clinically isolated syndrome (CIS) patients and healthy controls. **Methods:** An event related functional magnetic resonance imaging (fMRI) study using a visual backward masking task (comparison of a number to 5) was performed in patients with CIS (n=8) and matched controls (n=7) on a 1.5T MR scanner (Magnetom Vision plus Siemens). Activation and deactivation maps were obtained for unconscious and conscious processing in each group (random effect, SPM2, $p < 0.005$, $k=10$). **Results:** Normal unconscious and conscious behavioural performances were observed in patients. During unconscious processing, healthy controls activated right amygdala and right BA40, and deactivated right BA8 and left BA46. Relative to controls, CIS patients showed lower activation in right amygdala and right BA40, but higher deactivation in BA 24, BA19, right BA39, left BA19 and right BA7. During conscious processing, controls activated bilateral BA24, right BA7, bilateral cerebellum and right BA39. Controls deactivated right BA2/4, right BA9, right BA10, left BA42, left BA46 and left BA6. Relative to controls, CIS patients showed higher activation in left BA6 and right cerebellum, higher deactivation in BA19 and lower deactivation in left BA6. **Conclusions:** Event-related fMRI demonstrates altered cortical activation patterns relative to unconscious and conscious processing in CIS patients in the absence of behavioural impairment.

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P756**Happiness and personal growth are preserved in interferon-beta-1a treated patients with multiple sclerosis**

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Background: Multiple sclerosis (MS) is a chronic debilitating disease that adversely affects happiness and may arrest personal growth. Happiness is defined as both a predominance of positive emotions and as a construct closely related to satisfaction with life. There is a growing interest amongst clinicians and consumers in measuring happiness and personal growth as indicators of patients' well-being. **Objective:** To evaluate happiness and personal growth in relapsing-remitting MS patients treated with interferon-beta-1a (Rebif) in comparison with age and gender matched healthy subjects. **Methods:** The

Oxford Happiness Inventory (OHI), the Satisfaction with Life Scale (SLS) and the Personal Growth Initiative Scale (PGIS) were endorsed by 105 relapsing-remitting MS patients (70 females, mean age 44.4±13.3 years, Expanded Disability Status Scale (EDSS) 3.6±2.3) and 87 healthy subjects (66 females, mean age 39.7±11.5 years). Clinical and demographic data were extracted from the patients' computerized medical files. All patients were treated for at least 6 months with interferon-beta-1a (Rebif). The study was approved by the local IRB committee. **Results:** Mean happiness scores in MS patients for the OHI (103.1 ±17.0) and the SLS (23.9± 6.8) as well as the PGIS (40.1 ±7.6) were not statistically different from those of healthy subjects. Categorical analysis of the SLS demonstrated a different distribution wherein more MS patients (24.8%) were found in the "unhappy" subgroup (SLS score<20) as compared to healthy subjects (12.6%, p=0.08), while rates were similar in the extremely happy subgroup (SLS>30). **Conclusions:** In treated relapsing-remitting MS patients, happiness was found to be rated similarly to that of healthy subjects despite a greater percentage of patients who reported being unhappy. This may reflect positive treatment effects, hedonic adaptation or their combination.

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P757

Memory impairment in multiple sclerosis: correlation with deep gray matter and mesial temporal atrophy

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Background: Magnetic resonance imaging (MRI) research in multiple sclerosis (MS) samples reveals pathology in both the cerebral cortex and deep gray matter (DGM). The classical subcortical dementia hypothesis has been ascribed to MS and is supported by studies highlighting the role of thalamic atrophy in neuropsychological outcomes. However, the importance of mesial temporal (MLT) atrophy in MS is untested and poorly understood. New structural imaging techniques permit volumetric measures of multiple regions within the MLT lobe and DGM. **Objective:** To determine the relative importance of MLT and DGM structures in predicting MS performance on memory tests presented in the auditory/verbal and visual/spatial spheres. **Methods:** Cross-sectional analysis of 50 MS patients undergoing structural brain MRI and neuropsychological testing was carried out. Using Freesurfer software, the volumes of MLT [hippocampus, amygdala] and DGM [thalamus, caudate] structures were calculated. Neuropsychological testing contributed measures of new learning, delayed recall and recognition memory, in the auditory/verbal and visual/spatial memory modalities. **Results:** Significant correlations between higher regional volume and better test performance were observed across all memory tests. For measures of free recall or new learning, DGM volumes were most strongly predictive of outcomes. In contrast, measures of recognition memory were predicted only by MLT volumetric measures. **Conclusions:** For the first time, the predictive validity of MLT and DGM atrophy are simultaneously compared in MS using reliable and validated neuropsychological measures. We find that both compartments play significant but different roles in the amnesia of MS.

P758

Does immunomodulating therapy have stabilizing effects on cognition in multiple sclerosis?

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Background: Cognitive deficits are common in multiple sclerosis (MS). They are only weakly associated with physical disability but substantially correlated with supratentorial lesion-load as well as with measures of general brain atrophy. **Objective:** The aim of our study was to evaluate possible cognition-related effects of immunomodulatory treatment in MS-patients over a 2-year-period by using atten-

tional and mnemonic indicators as outcome measures. **Methods:** We studied a clinical population of altogether 20 MS-patients. The group consisted of seven male and 13 female patients with relapsing-remitting MS (mean age: 38.9 yrs, mean expanded disability status scale (EDSS): 2.2 at baseline). During the study period all patients were on stable, immunomodulatory medication. Cognitive performance was evaluated at baseline (before starting medication) and subsequently after 6 months for a total period of 2 years. Group performance at baseline was compared to standardized norms derived from matched, healthy individuals on the basis of their scores on a variety of cognitive tests of general intellectual abilities, attention and memory. **Results:** Although there were no statistical differences between mean scores of general intellectual performance, different cognitive profiles were already present at the beginning of the study. Moreover, non-parametric intra-group-comparisons revealed a slight attenuation of alertness-related responses in the patient-group between baseline and week 104 although simple reaction-time latencies remained stable. **Conclusions:** Our study indicates that subtle cognitive dysfunctions may be present in early phases of MS, even when physical disability is not evident. Furthermore, our data speak in favour of a general stabilizing effect of immunomodulatory therapy on cognition. Nevertheless, we could also demonstrate that there are particular cognitive functions which are prone to decline over time even in medicated patients, leading to mental disturbances which might interfere with everyday functioning and quality of life. **Supported by:** Serono.

P759

Impact of the topographic lesion distribution on different cognitive deficit patterns

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Background: Multiple Sclerosis (MS) is often associated with cognitive dysfunction. A weak correlation between cerebral lesion burden and cognitive performance has been pointed out in the literature almost consistently. Recent studies however assume that the distribution of lesions is more critical for the pattern of cognitive dysfunction. **Objective:** The current study aimed to explore the relationship between the distribution of cerebral lesions and the pattern of cognitive decline both quantitatively and qualitatively. **Methods:** 37 MS patients underwent comprehensive neuropsychological assessment as well as magnetic resonance imaging. On the basis of lesion burden and topographic distribution the patients were assigned to three subgroups characterized either by few distinct lesions (1), large, confluent periventricular lesions (2) or a combination of both (3). Based on this subdivision it was hypothesized that a confluent lesion pattern would invariably result in memory deficits, whereas distinct lesions would result in specific neuropsychological impairment depending on lesion size and localization. Moreover, a combination of both periventricular and extraperiventricular located lesions would most probably be reflected by global cognitive decline. **Results:** Discriminant analysis yielded a significant discriminant function with Wilk's Lambda values of 0,02 ($\chi^2 = 81,82$, $p = 0.003$), indicating a dissociation between the three subgroups on the basis of their cognitive results. More specifically, and depending on the lesion pattern, the groups differed significantly in their attentional performance ($\chi^2 = 6,77$; $p = 0.034$), mental speed ($\chi^2 = 11,85$; $p = 0.003$) and memory function ($\chi^2 = 10,56$; $p = 0.005$; $\chi^2 = 7,18$; $p = 0.028$), with group number 1 consistently displaying the least cognitive impairment. **Conclusions:** Our data confirm the assumption of a crucial role of lesion distribution in relation to cognitive deficits.

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P760**Affective and cognitive aspects in relapsing-remitting multiple sclerosis patients 24-months follow-up of the ITACA Study**

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Background: Multiple Sclerosis (MS) is the leading cause of disability in young adults and its progression, involving cognitive and physical aspects, may influence both the Quality of Life (QoL) and the affective status of the patients. **Objective:** To evaluate the long-term effects of different first-line disease modifying therapies (DMTs) (GA or IFN-Beta) on cognitive functions, affective status, fatigue and QoL in patients with relapsing-remitting multiple sclerosis (RRMS) over a 24-month period. **Methods:** An observational, longitudinal, multi-centre, Italian study was undertaken. All patients underwent a comprehensive Neuropsychological (Nps) evaluation including Rao's Brief Repeatable Battery of Neuropsychological Test (BRB-N), Stroop Colour Word Test and Short Intelligence Test (TIB). The MS Quality of life/54 items (MSQoL/54) and the Montgomery Asberg Depression Rating Scale (MADRS) were used to assess QoL and the presence of depressive symptoms. The Fatigue Severity Scale (FSS) and physical disability (EDSS) were also evaluated. **Results:** 752 RRMS patients were enrolled by 79 centres participating in the ITACA Study. 397 patients were treated with GA and 355 patients with IFNs; at baseline evaluation the two groups did not show any significant difference in the different neuropsychological aspects. During the 24-month follow up 58 patients dropped out. In the whole group of patient a mild, but statistically significant increase ($p < 0.05$) of physical disability was observed over time, while FSS did not change. Although the Nps mean scores at baseline, 12 and 24 months were within the normal ranges, specific Nps tests showed a slight improvement in patients on DMTs. The multi-varied analysis showed a significant correlation among all the Nps evaluations. MADRS mean score did not detect significant depressive symptoms in both subgroups of patients, but a decreasing trend of the score was observed at two-year follow-up. Both depression and disability scores were inversely related with Nps scores. **Conclusions:** Finally, at two year evaluation there were no significant differences over time either in patient perception of mental or in physical health.

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P761**Foreign accent syndrome as first sign of multiple sclerosis**

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Background: Foreign accent syndrome (FAS) corresponds to a dysprosodia perceived as a foreign accent by listeners. It occurs after mild cerebral damages concerning the left hemisphere, usually from traumatic or vascular origin. **Objective:** We report the third case in biomedical literature of FAS induced by multiple sclerosis (MS) and the second in which it represents first sign of the disease. **Methods:** A 39-year-old, right-handed woman, only speaking the French and English languages, suffered from a sub-acute right facial palsy. It was followed by occurrence of a German foreign accent without aphasia or apraxia. **Results:** Blood and cerebrospinal fluid (CSF) exams were normal except for the presence of oligoclonal bands in CSF. Brain magnetic resonance imaging (MRI) revealed a lesion in the left pre-rolandic white matter with hypersignal on T2-weighted images which was enhanced after gadolinium injection, associated with small lesions of deep white matter on T2 weighted images. The most important lesion was located just near the pyramidal tract on tractography and near the facial motor area on functional MRI. Single photon emission computed tomography (SPECT) showed a diaschisis with hypoperfusion of the right pre-rolandic, temporal and thalamic areas. We concluded with the diagnosis of pseudotumoral MS. **Conclusions:** FAS consists of aspecific speech rhythm disorders, without dysarthria or language disorders. These disorders are attributed by the listener to a foreign accent. The abnormalities noted in this case with new func-

tional imaging, allows us to better understand the underlying subtle mechanisms of speech regulation. It confirms that MS may begin with this range of symptoms.

P762**Sensitivity of neuropsychological tests to cognitive impairment in multiple sclerosis**

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Background: Approximately half of persons with multiple sclerosis (MS) are cognitively impaired. Domains most commonly impacted include episodic learning/memory, information processing speed/efficiency, executive functions, and visuospatial processing. **Objective:** The objective of this study was to compare the sensitivity of neuropsychological tasks in identifying cognitive impairment in persons with MS. Neuropsychological tests from the Brief Repeatable Battery (BRB) were compared to other measures, including selected tasks from the Delis-Kaplan Executive Function System (D-KEFS). **Methods:** To date we have tested 86 persons with definite MS (median Expanded Disability Status Scale (EDSS) = 3.0, range 1-7) without major depression and 50 age and gender matched healthy controls. Participants completed the BRB (Selective Reminding Test, 10/36 Spatial Recall Test, Symbol Digit Modalities Test, Paced Auditory Serial Addition Test, Controlled Oral Word Association Test) and additional measures including Judgment of Line Orientation, and the following Delis-Kaplan Executive Function System (D-KEFS) tasks: Sorting Test, Color-Word Interference, and Verbal Fluency. Scores on all tests were converted to Z scores based on the performance of the healthy control group. Tasks were assessed as to the frequency with which persons displayed impaired performance, scoring 1.5 or more standard deviations below the mean of healthy controls. **Results:** Impaired performance in the MS group was most frequent (37%) for the D-KEFS Color-Word Interference Task, a modification of the Stroop, using a score that controls for word reading and color naming speed (mean Z score on this task was -1.87). Scores on the Symbol Digit Modalities Test were the next most frequently impaired (32.6%). **Conclusions:** In a battery of tasks sensitive to MS cognitive impairment, the D-KEFS Color Word Interference Task appeared particularly well suited to distinguishing between MS and healthy performance, and might prove useful as a screening measure of cognitive impairment in future clinical trials.

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WITHDRAWN**P764****Verbal fluency as a brief clinical screening tool for cognitive impairment in multiple sclerosis: an alternative to the Paced Auditory Serial Addition Test?**

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Background: Cognitive impairment affects approximately 40% of patients with multiple sclerosis (MS), with 1 in 2 patients not detected by neurological examination alone. The Paced Auditory Serial Addition Test (PASAT) is widely used as the sole cognitive test in evaluating patients and has a sensitivity of 74% and a specificity of 65% for cognitive impairment. However administration requires audio playback facilities and support materials. The verbal fluency test

is a component of the Addenbrooke's Cognitive Examination and can be administered with no support materials. **Objective:** To evaluate verbal fluency assessment as a clinical screening tool for cognitive impairment in MS compared to the PASAT. **Methods:** A prospective case series of 30 patients attending an MS research clinic in Cambridge, England were assessed using PASAT and verbal fluency tests. **Results:** 30 patients were recruited (14 male, 16 female) with mean age 47 years (range 21–64). Mean disease duration was 11.6 years (range 1–26). Seven patients (23%) had primary progressive multiple sclerosis (PPMS), five (17%) had relapsing-remitting multiple sclerosis (RRMS), and eighteen (60%) had secondary progressive multiple sclerosis (SPMS). Five patients (17%) had moderate or severe depression and a further five patients had mild depression using the BDI-II assessment scale. Fourteen patients (47%) reported subjective cognitive impairment that was not associated with age, gender, disease phenotype or duration but was associated with depression on the BDI-II scale (Mann Whitney U-test, $p=0.0023$). PASAT and verbal fluency scores were correlated ($r = 0.47$, $p = 0.01$). This correlation was maintained after exclusion of depressed patients ($r = 0.52$, $p = 0.01$). **Conclusions:** Verbal fluency scores are correlated with PASAT scores in MS patients. The verbal fluency score may therefore be a useful alternative brief clinical screening tool for contrast impairment when facilities and time are limited.

P765

Association of retinal nerve fibre layer thinning with cognitive impairment in multiple sclerosis

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Background: Patients with multiple sclerosis (MS) have retinal nerve fibre layer (RNFL) thinning by optical coherence tomography (OCT), even among eyes with no history of acute optic neuritis. RNFL thinning is associated with brain atrophy by magnetic resonance imaging (MRI), suggesting that visual pathway axonal loss reflects more global processes of degeneration in MS. **Objective:** To determine whether RNFL thinning in MS is associated with cognitive impairment as defined by a standard battery of tests. **Methods:** As part of an ongoing study, patients with MS ($n=34$) and disease-free controls ($n=7$) underwent monocular and binocular low contrast letter acuity testing. OCT-3 scans were performed for each eye to measure average RNFL thickness (360 degrees around disc) and total macula volume. Cognition was assessed using the Symbol Digits Modalities Test (SDMT) and Neuropsychological Screening Battery for MS (NSBMS). **Results:** Patients ($n=34$) were aged 48 ± 9 years with disease duration 9.4 (1.7 – 29.0) years; most had relapsing-remitting multiple sclerosis (RRMS) (30/34). Cognitive impairment was identified in eight patients (24%) using standards for the NSBMS (at least two subtests below the 5th percentile); none of the controls were cognitively impaired. Accounting for age and within-patient, inter-eye correlations, average RNFL thickness was lower in eyes of patients with cognitive impairment (78.1 ± 8.4 vs. 86.0 ± 14.8 microns, $p=0.04$, GEE models). Total macula volume was also reduced in patients with cognitive impairment (6.19 ± 0.53 vs. 6.57 ± 0.56 mm³, with a trend toward significance ($p=0.07$). SDMT raw scores were also lower among patients with RNFL thinning ($p=0.03$), and distinguished MS patients from controls, accounting for age ($p=0.02$). Cognitive impairment was associated with poorer performance on low-contrast acuity (monocular: $p=0.02$ – 0.03 ; binocular: $p=0.002$ – 0.005). **Conclusions:** Patients with cognitive impairment have greater degrees of RNFL thinning in MS. Anterior visual pathway axonal loss is therefore likely to reflect more global aspects of axonal degeneration that contribute to cognitive impairment and other forms of disability.

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P766

Attentional network efficiency in early multiple sclerosis

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Background: Cognitive impairment is already prevalent during early stages of multiple sclerosis (MS). However, although attention is amongst the most frequently impaired cognitive domains, it has been mostly studied in later stages of the disease, when patients often suffer severe physical disabilities. Recently developed attention models allow specific assessment of independent attentional networks including, alerting, orienting and executive control. In order to evaluate each of these networks independently in patients at early stages of the disease, we selected a test which clearly differentiates between them, the Attentional Network Test (ANT). **Objective:** 1) To study attentional network efficiency in patients with recently diagnosed MS. 2) To assess specific performance in the different attentional networks. **Methods:** Nineteen MS patients (defined using Poser's and McDonald's criteria with Expanded Disability Status Scale (EDSS) ≤ 2 diagnosed in ≤ 3 years) and nineteen age, sex and education level matched-controls were enrolled in this study. Patients were evaluated using a comprehensive neuropsychological battery and the ANT. **Results:** Group comparisons did not reveal significant differences in overall accuracy, nor differences in orienting or conflict attention network efficiency. In contrast, significant differences were detected in the alerting network. The control group showed significantly greater alerting effect ($p=0.006$) when compared to MS patients, who derived no benefit from an alerting cue. **Conclusions:** Patients with recently diagnosed MS show some dissociation in attentional network efficiency, particularly in the alerting network. Future studies should investigate this finding in greater depth, to assess whether noradrenergic system involvement is also part of the alerting network dysfunction observed here in patients with early MS.

P767

Decision-making under ambiguous and risky conditions in early multiple sclerosis

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Background: Previous studies have detected decision-making (DM) deficits in advanced stage multiple sclerosis (MS) patients under ambiguous conditions. However, DM has not been studied in recently diagnosed patients, nor been evaluated under different risk conditions. **Objective:** To study DM processes in recently diagnosed MS patients, differentiating DM under ambiguous conditions -tested using the Iowa Gambling Task (IGT)- and under predictable conditions -using the Game of Dice Task (GDT). **Methods:** Nineteen MS patients (defined following Poser's and McDonald's criteria with Expanded Disability Status Scale (EDSS) ≤ 2 diagnosed in ≤ 3 years) and 19 age, sex and education level matched-controls were enrolled. All patients were evaluated through extensive neuropsychological battery testing. DM abilities were assessed under ambiguity and under risk conditions using the IGT and GDT respectively. **Results:** Standard neuropsychological tests showed differences in verbal fluency ($p=0.04$), sustained attention (PASAT) ($p=0.02$) and naming ($p=0.05$). Depression was also detected in MS patients using Beck's Depression Inventory ($p=0.005$). MS patients obtained significantly lower scores than controls in Total IGT Score ($p=0.01$), in IGT Block 4 ($p=0.01$) and Block 5 ($p=0.03$). No significant differences were found between groups regarding number of safe or risky choices made during the GDT ($p=0.4$). **Conclusions:** Patients were sensitive to large rewards but did not acknowledge punishment when decisions were made under an implicit set of rules (IGT). However, when rules were clear and explicit (GDT), patients showed no preference for risky choices. These results suggest that recently diagnosed MS patients may present

dissociation in different DM processes. DM appears to be altered under ambiguous choice conditions and preserved under risky ones with explicit rules.

P768

The differential relationship between expanded disability status scale scores and neurocognitive impairment

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Background: Multiple Sclerosis (MS) is a debilitating demyelinating disorder which is linked to a variety of cognitive deficits. The research regarding the relationship between expanded disability status scale (EDSS) scores and neuropsychological functioning has been mixed. **Objective:** The purpose of the current study was to evaluate the relationship between different levels of EDSS scores and composite indexes of the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS, Randolph, 1998). **Methods:** This sample consisted of 49 individuals diagnosed with MS (mean age 45.39 years, standard deviation = 9.20 years). The average time since diagnosis was 7.71 years with a standard deviation of 9.00 years). The participants were divided by EDSS scores; 24 patients had EDSS scores of 2.0 or less and 25 had scores of 2.5 and greater. **Results:** Linear regression analysis indicated that EDSS scores were an excellent predictor of overall neurocognitive impairment, as measured by the RBANS, for the patients with EDSS scores of 2.5 and above ($R^2=.537$; $p=.000$) and a very poor predictor for patients with EDSS scores under 2.5 ($R^2=.018$; $p=.535$). Significant correlations were found at the 0.05 level for the patients with high EDSS scores between EDSS scores and all five composites of the RBANS (Immediate Memory, Visual-Spatial, Language, Attention, and Total Scale). There were no significant correlations for the patients with low EDSS scores. **Conclusions:** In this study, patients with more severe physical symptoms of MS demonstrated a strong relationship between EDSS scores and neurocognitive impairment. Indeed, EDSS scores accounted for 53.7% of the variability in the Total Scale of the RBANS. Conversely, EDSS scores were very poor predictors of neurocognitive impairment for patients with lower EDSS scores. This study reinforces the notion that EDSS scores should not be used to extrapolate neurocognitive functions for patients with few functional problems. The implications will be discussed in regards to practitioners and researchers.

P769

Cognitive evolution over 5 years can be predicted by magnetic resonance imaging in early relapsing-remitting multiple sclerosis

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Background: Little is known about the predictive value of early magnetic resonance imaging (MRI) abnormalities on change of cognitive performances. **Objective:** To determine MRI predictors on cognitive outcome in early relapsing-remitting multiple sclerosis (RRMS) patients. **Methods:** Forty-six patients, recruited at the time of their diagnosis and followed-up for 5 years, were tested for clinical and cognitive parameters at baseline and after one, two and five years and underwent brain MRI at baseline and two years. Cognitive evaluation of these patients, and 56 matched healthy subjects, included the Brief Repeatable Battery (BRB), Wais-R similarities subtest, Go no go and Stroop test. MR parameters included lesion load (LL), brain parenchyma fraction (BPF), ventricular fraction (VF), mean magnetisation transfer ratio (MTR) on lesion and normal appearing brain tissue (NABT) masks. **Results:** At baseline, patients presented deficits for verbal and visuo-spatial memory, attention, information processing speed (IPS), inhibition and conceptualization. At five years, SDMT and PASAT2s scores remained significantly different from controls. Seventy-two percent of patients deteriorated over five years for inhi-

bition task (go no go/Stroop), 28.3% for PASAT 2s and 21.7% for PASAT 3s. PASAT 2s scores deteriorated significantly from two to five years ($p=0.02$). Over five years, Expanded Disability Status Scale (EDSS) deteriorated significantly ($p<0.01$). After linear regression analysis, independent and significant correlation between some baseline MR parameters and some cognitive score changes over five years: BPF with SDMT change ($R^2=0.24$, $p<0.001$), LL with PASAT3 change ($R^2=0.15$, $p<0.01$), mean NABT MTR with PASAT2 change ($R^2=0.27$, $p=0.001$), mean lesion MTR with WLG change ($R^2=0.16$, $p<0.01$). Attention performance changes (SDMT: $R^2=0.19$, $p=0.01$, PASAT 3: $R^2=0.11$, $p=0.02$, and PASAT 2: $R^2=0.38$, $p<0.0001$) were associated with VF change over two years. **Conclusions:** Attention and IPS deficits remained consistent over five years. Working memory (PASAT2) deteriorated. The main predictor of IPS/attention deterioration over five years is progressive central brain atrophy (VF change) over two years after MS diagnosis.

P770

Cognitive aging in patients with multiple sclerosis: an analysis of processing speed

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Background: Although researchers have often pointed to similarities between the cognitive changes that occur in conjunction with multiple sclerosis (MS) and those occurring over the course of normal aging, no studies of the combined impact of aging and disease have been reported. **Objective:** To provide a cross-sectional analysis of age-related cognitive slowing in MS patients compared to healthy controls. **Methods:** Samples of 245 MS patients and 177 healthy controls ranging in age from 18 to 74 were divided among five age cohorts. Processing speed was assessed using the preliminary word-reading and color-naming trials of the Stroop Test. Previous research has shown performance on these two trials to constitute excellent measures of simple information processing speed. **Results:** A significant slowing on each trial was evident for patients compared to controls within each age cohort, and processing speed declined across the age cohorts for both patients and controls. However, the plots depicting the age-related declines for patients and controls were unequivocally parallel. **Conclusions:** Once their diagnosis is clinically established, individuals with MS patients are already processing information at a slower rate than their healthy peers. Thereafter, patients appear to experience the same course of cognitive slowing with age as their healthy peers. We found no evidence that the disease process of MS interacts with the effects of aging to compel a more precipitous decline in processing speed.

P771

The Symbol-Digit Modalities Test more accurately reflects multiple sclerosis patient-perceived cognitive impairment compared to the Paced Auditory Serial Addition Test

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Background: The impact of multiple sclerosis (MS) on cognition can be evaluated using quantitative measures such as the Paced Auditory Serial Addition Test (PASAT) and the Symbol-Digit Modalities Test (SDMT). The PASAT is currently the cognitive component of the Multiple Sclerosis Functional Composite (MSFC); however, the SDMT has been recognized as a promising alternative. The Multiple Sclerosis Neuropsychological Screening Questionnaire (MSNQ) is a reliable, self-report measure of patient-perceived cognitive impairment correlated with depression. **Objective:** To evaluate the utility of the SDMT, versus the PASAT, in reflecting patient-perceived cognitive impairment detected by the MSNQ. **Methods:** SDMT, PASAT and MSNQ data from 72 patients with MS (mean age 49 years) were examined at baseline and at follow-up (mean 585 days). T-tests were used to assess changes in cognitive function. Correlations between subjective

(MSNQ) and quantitative (SDMT, PASAT) measures were calculated. **Results:** Mean baseline SDMT, PASAT and MSNQ scores were 51.3, 38.3 and 27.2, respectively. Mean score changes from baseline to follow-up revealed significant improvements in neuropsychological performance (SDMT: 2.7, PASAT: 2.5) and patient-perceived cognitive impairment (MSNQ: -1.9). Significant correlations between MSNQ and SDMT scores were found at baseline ($r=-0.39$, $p<0.01$) and at follow-up ($r=-0.34$, $p<0.01$). PASAT scores correlated significantly with MSNQ scores at baseline ($r=-0.33$, $p<0.01$), but not at follow-up ($r=-0.20$, $p=0.1$). **Conclusions:** Compared to the PASAT, the SDMT appears to better reflect patient-perceived cognitive impairment detected by the MSNQ, particularly at follow-up. These results support the potential role of the SDMT as a cognitive component of the MSFC; however, further investigation is warranted.

P772

Conservative behavior in multiple sclerosis, a reflection of prefrontal cortex dysfunction

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Background: Different studies have shown the relationship between cognitive impairment in multiple sclerosis patients and prefrontal cortex function based on neuro-imaging and neuropsychological assessment. Thus few investigations have been done to complete the image of prefrontal cortex dysfunction in these patients. **Objective:** To illustrate a complete image of cognitive impairment in MS patients considering the assessment of Dorsal-Lateral and Ventro-Medial prefrontal cortex function. **Methods:** 43 relapsing-remitting MS patients (27 females)(Expanded Disability Status Scale (EDSS) mean=3.46±1.79) and 40 healthy age-gender-education-matched controls included the study. We used neuropsychological assessment tasks specific for Dorsal-Lateral prefrontal cortex: WCST (Wisconsin Card Sorting Task) and TPT (Time Perception Task) and for Ventro-Medial prefrontal cortex: Iowa Gambling Test (IGT), Delayed Discounting Task (DDT), Balloon Analogue Risk Task (BART). **Results:** MS patients performed poor results in WCST which assesses cognitive flexibility and executive functions (preservative-error: $M\pm SD=15.49\pm 9.94$ vs. $8.778.6\pm 2$, $P\text{-value}=0.007$). They also showed over-estimation and over-reproduction of time intervals doing TPT which evaluates working memory and chronological speed of cognition. IGT results showed that MS patients have more delay in making more risky choices (required time for choosing from risky cards mean=4292±2552 millisecond vs. 2814±1465, $p\text{-value}=0.015$). DDT, which assesses the process of reward values over delays, showed that MS patients have lower discounting amounts over delays. MS patients also had lower levels of risky behavior tendency according to BART results (saved balloons=20.75±4.17 vs. 18.55±4.39, $p\text{-value}=0.034$). **Conclusions:** The result of this study strengthened the hypothesis of a conservative behavior in decision making both logically and emotionally in MS patients. Some possible explanations for such result could be "Multiple Disconnection Syndrome" particularly seen in frontal lobe lesions in MS patients, lower processing speed and the social effect of disease stigma on patients' behavior. Thus more investigation must be done to clarify the nature of such behavior in MS patients.

P773

Diffusion tensor imaging abnormalities in depressed multiple sclerosis patients

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Background: Depression is common in multiple sclerosis (MS) and has been linked to the presence of brain lesions and atrophy in anterior frontal and temporal regions. However, lesions only account for a minority of the depression variance. To date, no study has explored the etiological role of subtle abnormalities in normal appearing brain tissue (NABT) in depressed MS patients. **Objective:** To investigate the importance of NABT in the pathogenesis of depression. **Methods:** Forty nine depressed MS patients (40 female, mean age= 45(SD = 9.0) years, mean Expanded Disability Status Scale (EDSS) = 2.5, mean duration of MS= 129.7(SD = 97.4) months) meeting the McDonald criteria completed the Beck Depression Inventory Revised (BDI-II) before undergoing magnetic resonance imaging (MRI) (1.5T GE) that included diffusion tensor imaging (DTI) sequences. Lesion volume was defined using semi-automated software. All brains were segmented into gray matter, white matter, and cerebrospinal fluid (CSF) according to in-house software. Thirteen discrete brain regions per hemisphere were defined using a Semi-Automated Brain Region Extraction analysis (SABRE). Fractional Anisotropy (FA) and Mean Diffusivity (MD) values were obtained for NABT in anterior fronto-temporal regions known to subserve mood regulation. The BDI-II was divided according to an established factor analysis into cognitive-affective and somatic subgroups. Correlations were sought between the imaging and psychometric data. Significance was set as $p<0.01$ to control for multiple comparisons. **Results:** Modest albeit significant inverse correlations were found between the scores on the cognitive-affective subscale of the BDI-II and FA values in bilateral superior frontal NABT regions ($r=-0.45$; $p<0.01$ for both). A positive correlation was also found between depression scores on the full BDI-II and the MD value derived from the dominant inferior frontal region. **Conclusions:** The data provide evidence that depression is linked to abnormalities in frontal NABT. Furthermore, an association emerged between the magnitude of depression and the severity of the brain changes. This study extends the findings derived from brain lesion and atrophy analyses and underlies the importance of brain pathology in the pathogenesis of depression in MS patients. **Supported by:** Multiple Sclerosis Society of Canada.

P774

Emotional perception, memory and reaction in multiple sclerosis patients

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Background: Multiple sclerosis (MS) lesions may result in cognitive deficits, mainly in the explicit episodic memory. On the other hand, a high prevalence of emotional disorder in MS has been reported. It is known that the emotional information processing involves the functioning of many regions of the central nervous system (CNS). However, few studies have investigated the emotional processing in MS. **Objective:** The aim of this study was to evaluate the perception, the memory and the reaction of MS patients in the presence of affective charge and neutral stimuli. **Methods:** Twenty high arousal unpleasant figures and 20 neutral figures were presented to 21 MS patients and to 21 healthy individuals. A subjective perception task of the pictures was performed and the physiologic measures of skin conductance and heart rate were recorded, which were followed by the free recall task. **Results:** MS patients present an appropriate emotional perception. Concerning memory, they recalled a lower number of figures than the control group and did not present an increment in recalling the unpleasant figures. Nevertheless, they recalled better the

high arousal figures than the neutral ones. In terms of emotional reaction, a similar average variation in the skin conductance between those two groups was perceived. Referring to the heart rate, the patients did not distinguish between unpleasant and neutral figures and presented a similar bradycardia in the presence of both types of figures. Moreover, an habituation failure in the physiological responses was noticed in comparison with the responses to the initial and final emotional figures. **Conclusions:** Automatic mechanisms, activated by the arousal and mediated by the functioning of the amygdala, are preserved in MS, while there is a damage in the processing of the affective valence of the stimuli, involving the cognitive and cortical processes. The impairment in the emotional processing may make the MS patients more susceptible to both affective and mood disturbance.

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Mitoxantrone does not worsen cognitive function in patients with multiple sclerosis

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Background: Mitoxantrone (MTX) is an antineoplastic agent that has been shown to reduce disability and relapse frequency in patients with secondary progressive (SPMS), progressive relapsing (PRMS) and worsening relapsing-remitting (RRMS) multiple sclerosis. It is well known that cognitive decline may occur in some patients after chemotherapy. The effect of MTX treatment on cognitive functions has been scarcely studied. **Objective:** The aim of our study was to assess whether cognitive decline occurs after treatment with MTX (12 mg/ m2 every month for the first 3 months and then 12 mg/ m2 every 3 months until completing one year of treatment) in patients with MS. **Methods:** We assessed twenty-three consecutive patients who received MTX with a neuropsychological battery focused on attention, executive functions, memory and visuospatial and visuo-constructive functions. The cognitive battery was administered at baseline and after 1 year of treatment with MTX. Other covariables were analyzed as The Hospital Anxiety and Depression Scale (HAD), Expanded Disability Status Scale (EDSS), relapses and years of education. **Results:** The mean age was 33.13 (SD= 6.49) and the average years of education was 13.18 (SD= 3.96). The average disease duration was 9.8 years (SD= 5.43) and the median EDSS was 6.0 (3.0–7.0); the number of relapses in the year previous to treatment was 1.34 (SD= 1.26). After 1 year, improvement was seen in the verbal fluency test (FAS) while no changes were obtained in all the other tests. Clinical variables were not related with cognitive measures. Anxiety and depression scores were not related with the improvement in FAS test. **Conclusions:** Mitoxantrone seems not to produce cognitive decline in patients with MS during the first year of treatment.

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Brain magnetic resonance imaging lesion volume correlates with physical disability and cognitive impairment in multiple sclerosis: comparison of 1.5T and 3T

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Background: There is growing interest in using higher field magnetic resonance imaging (MRI) to assess brain pathology in multiple sclerosis (MS). It is unknown if 3T improves clinical correlations compared to 1.5T MRI. **Objective:** To compare the relationship between

brain lesions and clinical status in MS at 1.5T and 3T. **Methods:** 2D FLAIR was performed at 1.5T and 3T in 32 MS patients [1 clinically isolated syndrome; 26 relapsing-remitting, 5 primary or secondary progressive; Expanded Disability Status Scale (EDSS) (mean±SD) 2±1.9 (range 0–6.5), disease duration 8.18±7.4 (range 0.2–39) years]. MRI protocols were optimized and matched on voxel size. FLAIR lesions were quantified using a semi-automated edge finding tool with anonymous scan randomization. Cognitive testing was based on consensus panel standards emphasizing domains usually affected in MS. **Results:** Correlation between lesion volume and EDSS score was weak at 1.5T (Spearman $r=0.195$, $p>0.05$) but significant at 3T ($r=0.388$, $p=0.03$). After accounting for age, sex, and depressive symptoms, associations were observed between 1.5T lesion volume and Brief Visuospatial Memory Test-Revised-Total Recall (BVRT-R-TR) ($R^2=0.417$, $p=0.01$) and Symbol Digit Modalities Test (SDMT) ($R^2=0.549$, $p=0.001$) and between 3T lesion volume and BVRT-R-TR ($R^2=0.383$, $p=0.03$), SDMT ($R^2=0.699$, $p<0.00001$), and Judgment of Line Orientation ($R^2=0.356$, $p=0.01$). Other cognitive measures including Paced Auditory Serial Addition Test-3, Controlled Oral Word Association Test, and California Verbal Learning Test-II were not associated with lesion volume at 1.5T or 3T ($p>0.05$). Whole brain lesion volume was 22% higher at 3T on average (10,800±14,799mm³) than 1.5T (8,834±13,210mm³, $p=0.01$). **Conclusions:** 3T can demonstrate a higher brain lesion load and generally stronger associations with clinical status than 1.5T in MS. Higher field brain MRI may help improve clinical-MRI correlations in the monitoring of MS.

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Usefulness of mismatch negativity for simple cognitive dysfunction assessment in multiple sclerosis patients

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Background: Cognitive disturbances are an important and frequent complain of multiple sclerosis (MS) patients and must be evaluated for different reasons (impact on quality of life, socio-professional activities...). Classical cognitive evaluations are relatively time consuming and not easily repeatable and would be suggested to patients after a first short and simple screening. The usefulness of Event-related potential (ERPs) especially the P300 component was often reported in the literature but to our knowledge only one study (Jung et al. 2006) explored correlations between pre-attention and mismatch negativity (MMN) component. **Objective:** To estimate the usefulness of MMN for detecting early cognitive dysfunction in MS. **Methods:** This work allows the inclusion of 30 MS patients and 30 healthy subjects with normal auditory evoked potentials. All of them will be assessed for attention and memory using computerized attention tests, Paced Auditory Serial Attention Test (PASAT), Digit Spans and California Verbal Learning Task (CVLT) and ERPs elicited using a passive oddball paradigm in auditory modality. N1 and P2 components will be recorded and MMN component will be calculated as the potential difference between deviant and standard stimuli. Amplitudes, latencies and areas in both groups will be compared using Kruskal-Wallis and Chi2 tests with a significant level at $p<0.05$. **Results:** Today 24 MS patients and 30 healthy subjects were included. Preliminary analysis of ERPs on 18 patients (age: 47±12,1, disease duration: 9,5±7,29, Expanded Disability Status Scale (EDSS): 3,31±2,87) and 29 healthy subjects (age: 35,9±9,95) showed that MMN were detected in 96,6% of healthy subjects against 77,8% of patients ($p<0.05$). The comparison of N1 and P2 components in both groups didn't show any significant difference. In addition, MS patients showed significant reduced MMN amplitudes whatever age, EDSS or disease durations. **Conclusions:** MMN amplitude reduction suggests pre-attention dysfunctions and could represent an objective index of cognitive dysfunction. Complete data for the whole group of patients and correlations between cognitive scores and ERPs components will be presented during the meeting.

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Complex pathological pathways of altered social cognition in multiple sclerosis

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Background: Cognitive changes have been reported in multiple sclerosis (MS), but data about altered social cognition are scarce. **Objective:** Here, we established a database to examine complex cognitive and affective changes in 50 patients with ambulatory relapsing-remitting MS and 50 healthy controls (HS). **Methods:** To investigate, Theory of Mind (ToM), Baron Cohen's Adult Eyes Test, Faces Test and a test for detecting social Faux Pas were applied. In addition, Baron Cohen's Empathy Test, Weschler Adult Intelligence Scale (WAIS), Beck's Depression Inventory (BDI) and Spielberger's Trait Anxiety Inventory (STAI) scores were also used. Nonparametric Mann Whitney U-test was used for statistical analysis. Patients were divided into two subgroups based on duration of the disease (short-term MS, disease duration: 3.27±1.3 years, n=25; long term MS, disease duration: 15.1±6.83 years, n=25). **Results:** Patients with MS had lower PQ compared to healthy subjects (p<0.01) and were more depressed (p<0.01). A significant defect in social cognition was detected in all measures as well (Faces Test p<0.03, Eyes test p<0.01, Faux Pas p<0.01). Empathy was significantly worse in patients with long-term compared to short-term disease (p<0.03). Depressed patients had higher Expanded Disability Status Scale (EDSS) (p<0.01), worse empathy (p<0.03), lower IQ, PQ, VQ (p<0.01 in all), poor performance in Faces Test (p<0.01) and more anxiety (p<0.01) compared to non-depressed patients. Next, all data of patients with MS were correlated. A number of correlations were found, which were used to test hypothetical pathways by structural equation modelling (SEM). Higher EDSS contributed to lower PQ and worse performance in the Faces Test, which influenced empathy. Depression could contribute to lower PQ and decreased performance in the Faces Test directly, but could influence them indirectly through anxiety and decreased empathy as well. **Conclusions:** Our data indicate that even in the absence of marked cognitive deficits social interactions can be hindered by a number of altered pathways creating pathological vicious circles.

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Pathological laughing and crying in five patients with multiple sclerosis

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Background: Pathological laughing and crying (PLC) has an approximately prevalence of 10%-20% among patients with stroke, 40% with Alzheimer's disease, 19%-49% with amyotrophic lateral sclerosis, and 7%-10% among patients with multiple sclerosis (MS). Various damages of prefrontal, temporal cortex, internal capsule, hypothalamus, thalamus, brainstem and the cerebellar pathways may cause pathological laughing or crying. **Objective:** We interviewed 160 clinically or laboratory definite MS patients for any complains of PLC. In only five patients we have found PLC such as sudden, involuntary displays of laughing or crying or both, without associated subjective feelings of depression or euphoria. **Methods:** They are documented on age, gender, physical disability (Expanded Disability Status Scale), duration of disease. All of the patients' magnetic resonance imaging (MRI) results with gadolinium are examined. **Results:** The patient group, consisting two women and three men, had a mean age of 41 (31-50), a mean Expanded Disability Status Scale (EDSS) score of 5.0 (3.0-6.0), and disease duration of 12.8 years (6-21). Four of the patients were in secondary progressive and one patient was in the relapsing-remitting phase of the disease. Patient PLC was not associated with disease exacerbations. All patients had numerous brainstem and cerebellar relapses, which caused obvious disabilities including gait ataxia, intention tremor, dysmetria, dysarthria, nystagmus and other cerebellar dysfunctions. **Conclusions:** PLC is especially found in the chronic progressive phase of the disease mostly associated with

cognitive impairment and brainstem lesions. Patients may not inform us about their complaints of PLC. Either they are not taking it seriously or they hesitate to inform about complains of sudden involuntary laughing or crying because of the social factors. Another reason might be they have not connected their PLC to MS.

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Learning and memory in pediatric multiple sclerosis

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Background: In the pediatric setting understanding patterns of cognitive impairment will allow for increased appreciation of the potential impact of cognitive functioning on school performance. Extant studies in pediatric onset multiple sclerosis (MS) observe cognitive dysfunction across a range of cognitive domains. **Objective:** To describe verbal learning and memory function in a well-characterized population of children with MS. **Methods:** Twenty-one consecutive individuals with pediatric MS/clinically isolated syndrome (CIS) attending the UCSF Regional Pediatric MS Center were administered the California Verbal Learning Test (CVLT) - Child version or Adult version (if age >= 16 years) in the context of a larger neuropsychological and clinical evaluation. CVLT scores were compared to available age-adjusted normative data. Participants were classified as impaired if performance fell at or below 1.5 SD below population mean. **Results:** The mean age of disease onset was 14±3.3 years, mean age at evaluation was 15.4±3.5. The sample represented 76% girls, and a range of race/ethnicity groups (38% Hispanic/Latino, 10% White non-Hispanic, 14% Asian, 14% African-American, and 24% mixed). Impairment rates on CVLT indices of learning and memory are as follows: verbal learning - 19% impaired, learning slope - 24% impaired, short delay and long delayed recall - 24% impaired, recognition - 10% impaired. **Conclusions:** Deficits in memory and learning were present in nearly one-quarter of patients with child-onset MS after approximately one year of disease evolution. The pattern of impairment reflects difficulties both with respect to decreased initial acquisition of verbal information and retrieval of this information over time. Recognition and identification of learned information was only slightly poorer than expected normative performance for the group. A greater understanding of difficulties in learning and memory for children with MS is warranted due to the potential for a detrimental impact on school performance and overall functioning.

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Executive dysfunction represents an underestimated component of cognitive impairment in multiple sclerosis

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Background: Subtle deficits in various cognitive domains exist in approximately 30 to 70% of multiple sclerosis (MS) patients. Converging evidence suggests that cognitive deficits can be found even in early stages of the disease. It is more controversial what type of deficits are the earliest to appear and how this is affected by the scrutiny of the evaluation. **Objective:** The aim of this ongoing study is to more fully explore the spectrum of cognitive impairment in consecutive MS outpatients with a focus on early MS. **Methods:** To the present, 87 MS patients (29 clinically isolated syndrome (CIS); mean Expanded Disability Status Scale (EDSS) 1.2 (SD 1.0), mean disease duration 0.7 (SD 2.6) years and 58 relapsed-remitting multiple sclerosis (RRMS); mean EDSS 2.1 (SD 1.3), mean disease duration 8.8 (SD 8.4) years) underwent the Brief Repeatable Battery of Neuropsychological Tests (BRB-N) and the Wisconsin Card Sorting Test (WCST) to specifically search for executive dysfunction. **Results:** While the global index score of the BRB-N was normal for both subgroups, the subtests showed deficits in the domains of memory in 6.9% (CIS 0.0%, RRMS 10.3%), long-term memory in 8.0% (CIS 3.4%, RRMS 10.3%), concentration in 17.4% (CIS 10.7%, RRMS 20.7%), and

for executive functions in 6.9% (CIS 3.4%, RRMS 8.6%). Using the WCST, however, more than doubled the proportion of subjects scoring abnormal results (16.5%; CIS 14.3%, RRMS 17.5%). The differences between the subgroups were statistically not significant. **Conclusions:** These results confirm that cognitive impairment occurs at the earliest stages of MS and increases with ongoing disease. The frequency of domain specific deficits may be underestimated by using a global test battery such as seen for executive dysfunction.

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Evaluation and correlation of cognitive event-related potentials (P300) and neuropsychological tests in patients with clinically isolated syndrome

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Background: Numerous studies have described the cognitive event-related potentials (ERPs) latency and amplitude as neurophysiological markers of cognitive function in multiple sclerosis (MS), although some authors have found no correlation between ERPs and cognitive skills. **Objective:** This study determined the specific patterns of cognitive dysfunction and the profile of abnormalities of cognitive ERPs. Correlations between clinical, neuropsychological, and P300 data were analyzed. **Methods:** The latencies and amplitudes of N100, N200, P200 and P300 were evaluated in 20 patients with clinically isolated syndrome (CIS) and in 20 healthy controls. Cognitive dysfunction was assessed, using a comprehensive neuropsychological test battery. **Results:** Patients had impairments in verbal learning and long-term memory (65%), evaluating attention (55%), executive function (25%), and visuospatial skills (20%). The latencies and amplitudes of ERPs recorded from the Fz, Cz, and Pz electrode positions were not related to age, gender, disease type, or disease duration in both groups. The N200 and P200 latencies were prolonged and the N100, P200, and N200 amplitudes were reduced in all recording positions in the patients relative to those in the controls ($p < 0.05$). However, the N100 latencies, P300 latencies and amplitudes recorded from all electrode positions in the CIS patients did not differ from those of the normal subjects ($p > 0.05$). There were no statistical differences in the latencies or amplitudes of ERPs between patients with and without cognitive deficits. Verbal learning, verbal fluency, semantic retrieval, and executive function were negatively correlated with the P300 latencies at all recording positions. **Conclusions:** The P300 potential latency and amplitude do not appear to be affected in the CIS. Cognitive tests appear to be more sensitive for detecting abnormalities that may occur before the P300 becomes abnormal.

WITHDRAWN

WITHDRAWN

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Cognitive functioning in pediatric patients with acute inflammatory disorders of the central nervous system

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Background: A proportion of individuals with pediatric multiple sclerosis (MS) have cognitive deficits, however little is known about cognitive functioning in patients with acute disseminated

encephalomyelitis (ADEM) or those with ADEM-like presentations some of whom are later classified as having MS. Understanding the cognitive functioning of these individuals is critical for assisting in academic and other school related activities. **Objective:** To assess and compare cognitive functioning in pediatric patients with ADEM, ADEM-like presentations, and MS. **Methods:** Neuropsychological testing was performed on individuals with diagnoses using International Pediatric MS Study Group consensus criteria of ADEM (n=10), ADEM-like presentations (n = 12) of whom half were ultimately reclassified as MS, and MS with non-encephalopathic onset (n=58). All patients were referred to the National Pediatric MS Center between 2003 and 2008. Exclusion criteria were blindness, under age 5, and history of learning disability or attention deficit disorder. Neuropsychological testing was supervised by a licensed neuropsychologist. All patients underwent evaluation after stabilization of the acute syndrome and at least 1 month after discontinuation of steroids. Cognitive impairment was defined as performance 1.5 standard deviations below age matched norms on two or more of six cognitive domains (executive, visual spatial, memory, attention, psychomotor speed and behavior). Cognitive impairment scores were compared to the EDSS score at the time of testing. **Results:** Cognitive deficits were present in 50% (5/10) of ADEM cases, 58% (7/12) of the patients with ADEM-like presentations, and 43% (25/58) of MS cases. Even in monophasic ADEM, impairments were present as far as 2 years following the acute event. There was no association between EDSS and impairment level. **Conclusions:** Cognitive impairment is as frequent in ADEM as in MS and can persist years after the acute event. The impairment is not explained by EDSS.

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Magnetic resonance imaging indices of brain pathology in multiple sclerosis predict executive control deficits

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Background: Cognitive deficits are common in persons with multiple sclerosis (MS), particularly on time-dependent tasks. However, relations between neuropsychological tests and Magnetic resonance imaging (MRI) indices of brain pathology are moderate at best. **Objective:** We examined associations between performance on a computerized test that measures efficiency of three neuroanatomically-distinct attentional networks and MR indices of brain pathology in MS. **Methods:** Twelve female MS patients (Expanded Disability Status Scale (EDSS): 0–6) and 12 matched controls completed the Attentional Networks Test (ANT-I). In the ANT-I, an arrow is presented above or below a central fixation point and subjects must indicate its direction. Alerting is manipulated by presenting or omitting a warning tone prior to the target. Orienting is manipulated exogenously by presenting an uninformative visual cue prior to the target at one of the two possible target locations. Executive control is assessed by surrounding the target arrow with flanking arrows that point in the same or opposite direction. Participants also underwent MRI scans at 1.5 T with the following sequences: T1 SPGR, T1 Hi-Res SPGR (with focus on the thalamus), T2 FRFSE and FLAIR. Images were processed using AFNI and FSL to yield measures of white matter hyper-intensities (WMHIs), cortical and subcortical volumes, and thalamic volumes, specifically. **Results:** MS patients had greater WMHI volumes ($p < 0.0001$), more central atrophy ($p = 0.03$), and a trend toward reduced thalamic volumes ($p < 0.10$). Patients were slower on all ANT-I conditions ($p < 0.05$) but were most impaired in the executive network, particularly on alerted trials ($p = 0.04$). When MS subjects were divided into two subgroups based on severity of MRI pathology, an association between the extent of pathology and the degree of executive impairments was evident. **Conclusions:** The ANT-I is sensitive to attentional control deficits in MS. The degree of impairment in control of attention is associated with the extent of subcortical atrophy and the disruption of the white matter fiber tracks subserving cortical-subcortical circuits. **Supported by:** Research grant from the Multiple Sclerosis Society of Canada, Studentship from the Multiple Sclerosis Society of Canada, Capital Health research Fund.

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Cognition, fatigue and health-related quality of life in clinically isolated syndrome suggestive of multiple sclerosis in an international study - baseline data from CogniCIS

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Background: No large longitudinal studies on cognition in patients with clinically isolated syndrome (CIS) have been conducted. The pattern of cognitive deficits associated with CIS, and its interaction with fatigue, depression and health-related quality of life (HRQoL) in different patient populations worldwide requires investigation. **Objective:** To globally assess cognition, fatigue, depression and HRQoL in CIS patients and the interrelations of these parameters. **Methods:** CIS patients receiving no disease-modifying treatment undergo 6-monthly assessments over 2 years. For cognitive testing, all patients completed the Paced Auditory Serial Addition Task (PASAT) and the Faces Symbol Test (FST). A subset of 119 patients completed the Brief Repeatable Battery of Neuropsychological Tests (BRB-N). Fatigue was measured by the Fatigue Severity Scale (FSS), depression by the Center for Epidemiologic Studies Depression Scale (CES-D), HRQoL by the European Quality of Life 5-dimensional questionnaire (EQ-5D) and the Functional Assessment of MS (FAMS). **Results:** Baseline data are presented from 394 CIS patients in 16 countries (Europe 289, Asia 30, New World 75), with median age of 34.0 years and a median of 5.72 months since first symptoms. The median Expanded Disability Status Scale score was 1.0 (Europe 1.0, Asia 2.0, New World 1.0). Cognitive scores are presented in detail from the PASAT, the complete BRB-N and from the Faces Symbol Test (FST). 31.2% of patients had scores likely indicative of fatigue. 28.0% of CES-D scores were likely indicative of depression. Median EQ-5D index score was 0.81, VAS 80.0, median FAMS total score was 148.00. Correlations of the above parameters are presented. **Conclusions:** This large exploratory study provides new information on the pattern of cognitive dysfunction in CIS patients with defined demographics and neurological status. Patients' self-reported outcomes are similar, with regional variations. Future longitudinal data will define patterns of change over time and possible links to disease progression. **Supported by:** Bayer Schering Pharma AG.

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Evolution of performance in the Paced Auditory Serial Addition Test with "Dyad score" in different subtypes of multiple sclerosis

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Background: Commonly, score used in the Paced Auditory Serial Addition Test (PASAT) is the number of correct responses (CR). Multiple sclerosis (MS) patients give fewer series of two or more consecutive CR. This strategy of skipping items intermittently decreases the difficulty and can mask changes in performance of cognitively deteriorated patients. "Dyad score" (DY), a modified scoring method to the PASAT, consists of counting the total number of two CR given consecutively. This score reflects performance according to the intended task demands. **Objective:** To compare patterns of responses and performance's evolution during the PASAT of cognitively deteriorated and intact MS patients with remittent-relapsing (RRMS), primary (PPMS) and secondary progressive (SPMS) form. **Methods:** 228 patients (115 RRMS, 90 SPMS and 23 PPMS) were assessed by a neuropsychological battery (French adaptation of the Brief Repeatable Battery of Neuropsychological Tests (BRB-N)). PASAT was analyzed by the number of CR and DY for each condition (three and two seconds), each half part and each series of ten items ("decade"). **Results:** A significant relationship between global cognitive and PASAT's deteriorations in each MS form ($p < 0.03$), each PASAT condition and each score were found. DY and CR by "decades" decreased significantly for each condition and for each form ($p < 0.000$), with or without global

cognitive deterioration (ANOVA, $p < 0.027$; $p < 0.004$; $p < 0.000$), without interaction's effect. SPMS patients gave significantly fewer CR and had lower DY only in PASAT three seconds ($p < 0.04$ – $p < 0.0000$), compared to RRMS and PPMS, but not in PASAT two seconds. PPMS patients had fewer CR but not lower DY compared to RRMS. All scores of PASAT were inter-correlated, except in PPMS. **Conclusions:** This study underlines a decrease of the performance of the PASAT during the task in each form. However, we did not find different patterns of response of the PASAT between cognitively deteriorated and intact patients. However, SPMS patients seem to have different profiles of responses than RRMS and PPMS patients only in PASAT three seconds.

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The combined use of CNS-Vital Signs™ computerized test battery and California verbal learning test yields complementary findings in the assessment of cognitive impairment in relapsing-remitting multiple sclerosis

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Background: Cognitive functions are impaired in about 30% of patients with relapsing-remitting multiple sclerosis (RRMS). We have already demonstrated the value of the CNS-VS™ battery in the screening for cognitive impairment in RRMS patients. However, computerized test batteries do not allow a satisfactory assessment of verbal memory functions. We tried to overcome such limitations by testing a group of RRMS patients also with the California verbal learning test (CVLT) for a finer evaluation of verbal learning strategy. **Objective:** To define the potential of the CNS-VS™ computerized test battery and CVLT for the evaluation of cognitive impairment in RRMS. **Methods:** Twenty-eight (10 males) RRMS patients (aged 30.9 ± 8.7 yrs; Expanded Disability Status Scale (EDSS) 1.5 ± 0.6 ; disease duration 5.7 ± 4.6 yrs) and 12 healthy controls matched for age, sex, and education underwent combined neuropsychological testing by the CNS-VS™ computerized battery and CVLT. Cognitive domains explored included recognition memory, psychomotor speed, reaction time, complex attention, cognitive flexibility, verbal learning/recall, and encoding strategy. Scores were defined abnormal when below 1.5 SD from the mean of controls and patients were considered as cognitively impaired when dysfunctional in at least one cognitive domain. **Results:** The selective use of the CNS-VS™ battery or CVLT allowed the detection of cognitive impairment in 12 (43%) or in seven (25%) RRMS patients respectively. The combined evaluation with both test procedures demonstrated cognitive dysfunction in 15 RRMS patients (54%). Only four RRMS (14%) patients exhibited impairment on both assessment measures. **Conclusions:** The additional use of the CVLT to the CNS-VS™ battery results in a higher proportion of cognitively impaired RRMS patients. Neuropsychological dysfunctions as detected by the two procedures are only partially overlapping, suggesting that the two testing measures explore different, but complementary, cognitive domains. The CVLT provides critical information on verbal memory and learning strategies, leading to a more thorough assessment of cognitive decline in RRMS patients.

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A new score of cerebral functional system: proposition established among the LORSEP cohort

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Background: Estimate of the prevalence of cognitive impairment among patients with multiple sclerosis (MS) ranges between 40 and 70%. Today, the cerebral functional system (CFS) of the Expanded Disability Status Scale (EDSS) is subjective including several clinical signs such as cognitive dysfunction and depression. **Objective:** To define a new cognitive functional system (NCFS) based on a neuropsychological evaluation (NE). To assess change of the EDSS with this new score. **Methods:** Prospectively, we have included 215 MS patients

of the LORSEP cohort between March 2004 and June 2007. Patients' data have been entered in the EDMUS (European Database of Multiple Sclerosis) system. NE evaluated cognitive functions (information processing speed, attention, executive functions, memories, language, visuo-spatial abilities and arithmetic), fatigue with the validated translation of FIS and depression. The NCFS was devised with grades from 0 (normal) to 5 (severe dementia), depression excluded and fatigue included. Grade 1 of the NCFS was integrated in the EDSS as another FS. The NCFS and new EDSS including NCFS were established with the data of the NE and compared with the EDSS. **Results:** Patients (median EDSS =3.0, current age: 43.5 (SD=10,8) years, disease duration: 11.2 (SD=8,6) years), included 149 women (69,3%). 98,1% of these patients presented cognitive impairment with the NCFS compared to 62,3% with the CFS. NCFS was higher than CFS ($p<0,001$; CFS: 1.0 (SD=1,0) ; NCFS: 1.5 (SD=0,8)) and EDSS has been changed for 31% of the 113 patients with a EDSS<3.5 even though EDSS for all patients changed slightly (from 3.3 to 3.4). Change of EDSS was not associated with current age and age at onset of MS. **Conclusions:** We propose a new cotation of CFS based on NE, including fatigue, excluding depression and integrating the grade 1 at EDSS. This changes the EDSS for the low level of EDSS and integrates better cognitive deficit in MS.

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Duloxetine in multiple sclerosis patients with depression, pain and bladder dysfunction

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Background: Psychiatric symptoms are frequent in multiple sclerosis (MS) patients; nearly one in two patients with MS will experience depression during their lifetime. Pain, fatigue, bladder dysfunction and cognitive impairment are common symptoms of MS as well. **Objective:** The first aim of this study is to evaluate efficacy of Duloxetine, a dual reuptake inhibitor of serotonin and norepinephrine, in MS patients with depression, pain, fatigue and bladder dysfunction. The second aim is to evaluate duloxetine efficacy in improving cognitive functions in a subgroup of these patients with mild cognitive impairment. **Methods:** Twenty-five MS patients with mood depression, pain and bladder dysfunction were treated with Duloxetine 60 mg daily for at least 3 months. Several scales including Beck Depression Inventory, Chicago Multiscale Depression Inventory, Multiple Sclerosis Quality Of Life 54, Visual Analogue Scale for pain and bladder function, Modified Fatigue Impact Scale and a battery of neuropsychological tests were administered at baseline and after 1 and 3 months of therapy in order to assess depression, quality of life, fatigue, pain, bladder function and cognitive function. **Results:** Five out of 25 MS patients discontinued duloxetine because of gastrointestinal side effects. The remaining 20 patients reported a significant improvement ($p < 0.05$) in depression, quality of life, fatigue and pain. A slight improvement was reported in cognitive and bladder functions. **Conclusions:** Several antidepressant drugs can be successfully used for the treatment of depression in MS. Preliminary data of this study showed a positive effect of duloxetine on depression in MS; furthermore in these patients duloxetine seems to be useful in improving pain and fatigue.

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Factors influencing depression in the multiple sclerosis population in Vojvodina

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Background: Depression in multiple sclerosis (MS) is a complex phenomenon, influenced by several biological and psychological as well

as local social and cultural factors, with an impact on different aspects of disease and life of the patients. **Objective:** To evaluate the interrelation of depression in multiple sclerosis and its relation with neurological deficit, quality of life, fatigue and other factors in the MS population. **Methods:** We applied the following measuring instruments: Expanded Disability Status Scale (EDSS), Paced Auditory Serial Addition Task (PASAT), SF-36, MSQOL-54, Beck Depression Inventory (BDI) and Fatigue Severity Scale (FSS) in 120 relapsing-remitting multiple sclerosis (RRMS) patients, EDSS 0–6,5, age 18–55. **Results:** Incidence of depression in our population is 63.35% (mild 31.7, moderate 21.7 and severe 10%), in female 84.8% (mild 42.4, moderate 27.2 and severe 15.1%), and in male 36.4% (mild 18.5, moderate 14.8 and severe 3.7%). Depression is correlating with EDSS score ($r=3,18$) and even more with some EDSS subsystems (EDSSAMB and EDSSPYR) based on multiple regression analyses. Depression is most frequent in patients with EDSS 0–1.5 and more frequently severe in group of patients with EDSS 2–3.5 than in patients with either lower or higher EDSS score. Analyzing the prevalence and severity of depression in different age groups (20–29, 30–44 and 45–55 years), in younger patients depression is more severe (severe depressed 20, 5 and 5% in each age group respectively) and more frequent (70, 62 and 58% respectively). Depression is correlating with quality of life ($p=0,05$). Other factors associated with depression in this study are: fatigue, unemployment, two or more children, not married. **Conclusions:** Depression is more frequent than in other studies, associated with younger age, EDSS, quality of life and fatigue.

P793

Mental health co-morbidities in pediatric multiple sclerosis

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Background: Depression and other psychiatric disorders can occur in over half of adults with multiple sclerosis (MS). The prevalence of psychiatric difficulties in pediatric MS is unknown. **Objective:** To determine the prevalence of depression and other psychiatric disorders in a pediatric MS cohort. **Methods:** Pediatric MS patients ($n=28$) referred to a Southeastern US Pediatric MS Center from September 2007 to April 2008 were evaluated by a child psychiatrist using DSM-IV-TR criteria. All patients met the International Pediatric MS Study Group definition for MS, including five patients (18%) with CIS who fulfilled magnetic resonance imaging (MRI) criteria for dissemination in time. Psychiatric assessment was performed while patients were relapse-free and off steroids. **Results:** This cohort was 50% female, 46% African-American with a mean age of 15.3 ± 0.6 years at the time of initial psychiatric evaluation. Most patients were recently diagnosed with MS, with 1.3 ± 0.3 years since onset of symptoms and 2.9 ± 0.4 relapses. Over 70% ($n = 20$) were diagnosed with a psychiatric co-morbidity, including depression ($n=17$), anxiety ($n=5$) and attention deficit hyperactivity disorder ($n=5$), with seven patients receiving two or more diagnoses. Five patients had been treated for mental health conditions prior to onset of demyelinating symptoms. Treatment included selective serotonin reuptake inhibitors ($n=11$), stimulant therapy ($n=2$) or other psychotropic medications ($n=2$). All MS patients with depression or anxiety were also referred for counseling in their communities, but less than half ($n=7$) received psychological services. All nine patients with followup psychiatric evaluation reported improved symptoms whether treated medically or behaviorally. No correlation with psychiatric diagnoses was observed with respect to age, sex, race, disease duration or number of relapses. **Conclusions:** Psychiatric disorders are common in pediatric MS, affecting >70% of this pediatric MS cohort with depression being the most common diagnosis. Many of these patients had difficulty accessing local mental health services.

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P794

Sexual dysfunction in multiple sclerosis

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Background: Sexual dysfunction is frequent and important symptom among patients suffering from multiple sclerosis (MS); however there's no clear definitions about some aspects of its clinical presentations and etiology. **Objective:** The aim of this study was to assess the nature of sexual dysfunction in unselected sample of MS patients. **Methods:** 72 relapsing-remitting (RR), nine secondary progressive (SP), two primary progressive (PP) MS patients and six clinical isolated syndrome (CIS) patients were included in this study. Patients were submitted to MSISQ-19 (Multiple Sclerosis Intimacy and Sexuality Questionnaire-19) and ASEX (Arizona Sexual Experiences Scale) to evaluate sexual dysfunction in MS. They also filled out a Beck Depression Inventory, STAI-1 and STAI-2 to assess depression and anxiety that could be related with sexual dysfunction. **Results:** In 44 female and 45 male patients with mean age of 37 (range 21 to 56) and mean EDSS score of 2.2 (range 0 to 7.50), 34 patients reported primary sexual dysfunction (38.2%); 39 patients reported secondary sexual dysfunction (46.1%) and 22 patients reported tertiary sexual dysfunction (25.8%). There was no significant difference between male and female patients in total sexual dysfunction score, but secondary sexual dysfunction was found significantly more frequent in female patients ($p < 0.05$). The most frequent complaints among female patients were less intense orgasm (22.2%) and inadequate vaginal lubrication (22.7%). Difficulty getting/keeping satisfactory erection (20%) and loss of confidence about sexuality (17%) were common complaints in male patients. We also assessed sexual dysfunction by comparing MS types. **Conclusions:** We found RRMS patients and progressive MS patients (SP and PP) were significantly more dysfunctional when compared to CIS patients.

P795

Relationship between magnetic resonance imaging measures and presence or absence of depressive symptoms and cognitive impairment in relapsing-remitting multiple sclerosis: the COGIMUS (COGNITION IMPAIRMENT IN MULTIPLE SCLEROSIS) study

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Background: Cognitive impairment (CI) and depression are common in MS, affecting as many as 65 and 50% of patients, respectively. **Objective:** To evaluate the difference in baseline magnetic resonance imaging (MRI) measures of patients with or without CI and/or depressive symptoms. **Methods:** Patients aged 18–50 years with relapsing-remitting multiple sclerosis (RRMS) (McDonald criteria) and Expanded Disability Status Scale (EDSS) score ≤ 4.0 were recruited to the prospective, multicenter, observational, dose-controlled COGIMUS study and received IFN beta-1a, 22 or 44mcg subcutaneously three times weekly. MRI assessment, a complete neurologic examination, the Hamilton Rating Scale for Depression (HRSD) and Rao's battery of neuropsychologic tests for CI were performed at baseline and regular intervals for 3 years by psychologists or evaluating neurologists blinded to patients' treatment. Depression was defined as HRSD score ≥ 11 . CI was defined as having ≥ 3 positive tests for CI. A cognitive-impaired index was constructed using mean and SD from a sample of normal Rao's battery scores. **Results:** Baseline depression and cognition data for 548/550 (99.6%) enrolled patients are reported. Patients were divided into four categories: neither depressed nor CI, ($n=319$; 58.0%); not depressed, CI, ($n=90$; 16.4%); depressed, not CI,

($n=108$; 19.6%); both depressed and CI, ($n=31$; 5.6%). T2 lesion data were available for 287 patients. Mean (SD) volume at baseline in each category was: neither depressed nor CI, 4,560 (5,130); not depressed, CI, 6,892 (8,187); depressed, not CI, 6,284 (6,423); both depressed and CI, 12,644 (10,982) ($p=0.002$; Kruskal-Wallis ANOVA). T1 hypointense lesion and Gd+ lesion volumes were greatest in patients with both depression and CI. Patients neither depressed nor CI had mean (SD) cognitive-impaired index measurements lower than patients who were depressed and CI (6.7 [4.2] vs 18.0 [5.1]; global comparison between groups, $p < 0.001$; Kruskal-Wallis ANOVA). **Conclusions:** These findings suggest a direct association between MS brain lesions, cognitive function and mood in patients with RRMS with low levels of disability.

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P796

The role of education and depression on different aspects of multiple sclerosis related fatigue

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Background: Fatigue is one of the most disabling symptoms in multiple sclerosis (MS), consisting in a subjectively perceived lack of physical and/or mental energy interfering with intended activities of daily life. An association of depression, educational level and fatigue has been described but little is known about the specific correlations of these factors with mental and physical fatigue. **Objective:** Taking advantage of the ability of the Fatigue Scale for Motor and Cognitive Functions (FSMC) to separately assess mental and physical fatigue we investigated the relation between education, depression and these two main components of fatigue. **Methods:** In 309 MS patients (mean age: 43.4, SD: 9.95; mean Expanded Disability Status Scale (EDSS): 3.4, SD: 1.63; 206 female, 103 male) we assessed physical and cognitive fatigue by the FSMC and depression by the Beck Depression Inventory (BDI). Education was categorized according to highest achieved degree. **Results:** Bivariate correlations revealed a significant correlation between depression and both, physical and cognitive fatigue ($p < 0.01$). Educational level was negatively correlated to physical and cognitive fatigue, with a significant association only to cognitive fatigue ($p < 0.01$). This relation remained stable when depression was controlled for by partial correlation analyses. **Conclusions:** The results of the present study indicate that depression has a strong impact on both fatigue aspects but, against previous assumptions, does in itself not preferentially influence cognitive fatigue. Educational level showed only a low correlation to fatigue with a tendency for low educated patients reporting more cognitive and high educated patients reporting more physical fatigue. This finding might reflect better coping with cognitive fatigue potentially by means of a superior cognitive capacity in higher educated patients as compared to lower educated individuals.

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P797

Multi-parametric magnetic resonance imaging correlates of cognitive impairment in early multiple sclerosis

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Background: Lesion metrics are modest correlates of cognitive dysfunction in early multiple sclerosis. Additional magnetic resonance imaging parameters indicative of pathology in the normal-appearing brain tissue and of axonal damage are of interest in explaining the disease mechanisms leading to cognitive impairment and can potentially explain additional variance in cognitive function. **Objective:** To identify and compare correlates of cognitive impairment in early MS using multi-parametric MRI. **Methods:** Design: cross-sectional. Subjects: 61 patients (19 CIS, 42 MS, EDSS mean(sd)=1.48(1.15)). MRI parameters: T1 and T2 lesion metrics, grey

matter, white matter and brain parenchymal fractions, magnetization transfer ratio (MTR) histogram metrics of normal-appearing grey and white matter and proton spectroscopy metabolite concentrations. Neuropsychological domains: IQ decline, attention/speed of information processing, recall memory and executive functions. Statistical analysis: Multiple linear regressions controlling for age, gender, pre-morbid IQ, level of education, Expanded Disability Status Scale (EDSS), anxiety and depression scores. **Results:** 48% of patients failed at least one test (\leq 5th percentile of published norms). Volumes of T1 and T2 lesions predicted performance on the Paced Auditory Serial Addition Test (PASAT3) ($p < 0.001$, $p = 0.019$ respectively), Symbol Digit Modalities Test (SDMT) ($p = 0.004$, $p < 0.001$) and immediate ($p = 0.037$, $p = 0.034$) and delayed ($p = 0.038$, $p = 0.005$) Story Recall subtests of the Adult Memory and Information Processing Battery. Number of T2 lesions predicted decline in verbal IQ ($p = 0.038$) and PASAT3 ($p = 0.035$) and SDMT ($p < 0.001$) scores. Of the volumetric parameters, a lower gray matter ($p = 0.007$) and brain parenchymal fraction ($p < 0.001$) predicted SDMT scores. Only T2 lesion volume tended to remain significant when modeled together with other significant MRI parameters. No MTR or spectroscopic parameters were associated with cognitive performance. **Conclusions:** Attention/speed of information processing and memory scores were correlated with lesion metrics and measures of brain atrophy, but T2 lesion volume emerged as the best predictor. This suggests that both inflammatory activity and axonal damage contribute to cognitive impairment in early MS.

P798

Alexia without agraphia followed by thunderclap headache as the presenting feature of rapidly evolving severe multiple sclerosis: case report

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Background: A 41 year old male noticed an inability to read the written word whilst on holiday in Canada in August 2007. He had no other symptoms at all and was able to write. He had no problems with numeracy or spoken language. **Objective:** To describe the clinical story of an unusual presentation of multiple sclerosis (MS). **Methods:** Case report. **Results:** On return to the UK he sought advice from his GP and had a brain magnetic resonance image (MRI). Within hours of the MRI scan he developed a sudden, severe brief global headache which returned several times and he was admitted to the medical admissions unit. CT Brain scan and Lumbar puncture were negative for blood products and the rest of the cerebrospinal fluid (CSF) test was normal other than for marginally raised protein. CT Angiogram was normal. The MRI scan surprisingly showed multiple deep, periventricular and subcortical white matter lesions consistent with an inflammatory process. Investigations for vasculitis, infection and sarcoid were negative. The diagnosis of MS could not be made on clinical or MRI grounds in light of the atypical presentation and single MRI. Six weeks later he was asymptomatic and had no neurological signs.

After a further five weeks he re-presented with a brainstem syndrome consisting of ataxia, vertigo and diplopia. He had a 6th nerve palsy and ipsilateral facial weakness and had an EDSS of 6.0. Symptoms partly resolved with oral methyl prednisolone.

Within a further six weeks his condition had deteriorated such that he had virtually lost independent mobility and was depressed with suicidal ideation. He was admitted to the Neurology Unit for steroids, rehabilitation and psychological intervention. Repeat MRI scan showed multiple new lesions, some with Gadolinium enhancement. He was subsequently treated with the monoclonal antibody Alemtuzumab. **Conclusions:** Alexia without agraphia is an unusual syndrome first described by Dejerine in 1892. It is thought to be caused by interhemispheric disconnections in the posterior circulation territory, perhaps in the splenium of the corpus callosum. We believe this is the first description of this syndrome presenting as an MS clinically isolated syndrome.

P799

"I am not the only person with this problem": exploring the experiences of persons with multiple sclerosis in a cognitive rehabilitation group

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Background: 40–60% of persons with MS (PwMS) develop cognitive impairments over the course of the disease. It is recommended that PwMS experiencing cognitive difficulties undergo neuropsychological assessment to identify their cognitive strengths and weaknesses, exploring how these impairments impact on their life. PwMS should be then be offered cognitive rehabilitation to help minimize their problems and/or compensate for their impairments. It is suggested such rehabilitation should begin when the person's cognitive difficulties are mild to help enhance uptake. In order to attempt to provide better support/guidance to PwMS with cognitive difficulties known to a MS Psychology Service, a cognitive rehabilitation group was designed. **Objective:** The objective of the intervention was to enhance understanding of how MS affects cognition and to encourage the use of compensatory strategies in a peer supported environment. **Methods:** The intervention was delivered over seven sessions and for the purpose of this short study two groups were delivered. In total 13 PwMS attended. After completion, participants completed evaluation forms providing quantitative and qualitative data about their experience. **Results:** 92% of participants were pleased with delivery, format and content of the groups. Twelve of the participants reported they would recommend the group to someone else. Overall participants reported the group provided the opportunity to meet other PwMS with similar difficulties, share experiences and learn new skills/strategies to compensate for cognitive difficulties. One participant was dissatisfied with the group. **Conclusions:** The findings of this study suggest cognitive groups may be an effective way of delivering rehabilitation to PwMS. Due to limited resources in the MS Psychology Service, it can be considered as an economic way of delivering good quality support. However the delivery of the group may need further consideration to ensure it is an enjoyable experience for all participants.

P800

How appropriate is cognitive behavioural therapy computer software for the treatment of depression in people with multiple sclerosis?

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Background: People with multiple sclerosis (PwMS) are at elevated risk of depression which can adversely affect their associated health outcomes. The UK National Health Service (NHS) acknowledges a shortage of therapists and recommends cognitive behavioural therapy (CBT) software for people with mild to moderate depression. However, the applicability of generic CBT software for the treatment of depression in PwMS is unclear. **Objective:** An assessment of the appropriateness of computerized CBT for the treatment of depression in PwMS. **Methods:** A qualitative study, using in-depth interviews, evaluation sheets, and framework analysis was undertaken. Seventeen participants were recruited with relapsing-remitting or secondary progressive MS, with self-reported mild/moderate depression, and who had not received psychotherapeutic intervention within the last year. Level of disability ranged from Expanded Disability Status Scale (EDSS) 1.5 - 7.0. Nine participants were diagnosed, by the study psychologist, with major depressive disorder, using the Mini-International Neuropsychiatric Interview. Participants used one of two CBT programmes, 'Beating the Blues' or 'MoodGym'. **Results:** All

participants identified at least one useful CBT technique. Almost all found both CBT programmes inappropriate for PwMS. Those using 'MoodGym' found it wordy and felt they were being encouraged to do activities they were no longer physically capable to do as an answer to their depression; they also felt it was aimed at teenagers. PwMS using 'Beating the Blues' saw the value of the theoretical approaches and techniques but had problems applying it to their experiences of living with MS. Both programmes contained depression symptom inventories which users found difficult to answer honestly or consistently, believing that they should not be scoring symptoms which they felt were caused by their MS rather than depression. **Conclusions:** CBT techniques have the potential to support PwMS. CBT programmes need to be more explicit about the applicability of techniques to PwMS. Guidance is also needed on how to answer depression symptom inventories.

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P801

Insomnia, fatigue and somatic symptoms overestimate the frequency of depression in the Beck inventory

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Background: Depression occurs in above 60% of multiple sclerosis (MS) patients. Its frequency must be interpreted regarding the kind of tests applied. Some authors consider that the evaluation of somatic symptoms in the Beck inventory (BI) overestimate the actual frequency of depression in MS patients. **Objective:** Determine the influence of somatic complaints over the frequency and severity of depression. **Methods:** We analyzed 138 MS patients, regardless of clinical variety, severity or time of diagnosis. The current BI was applied and evaluated as usual. After that, insomnia, fatigue and somatic complaint items were excluded from the inventory and the MBI (modified BI) was re-evaluated. **Results:** Eight patients (6%) were excluded because they did not complete the inventory, 94 female (72%) and 36 male (28%) patients were included. Mean age was 35±11. All of them completed the test. The BI showed 55% of MS patients with normal scores, mild depression was present in 23% while severe depression was present in 22%. When somatic items were excluded the results were 65% with normal scores, 21% mild and 14% severe depression. Statistical analysis with SPSS (13.0 version) showed significant differences ($p < 0.001$). **Conclusions:** MS includes a wide range of symptoms including fatigue, insomnia and somatic complaints. Even more, fatigue is considered a pivotal symptom for MS diagnosis. Excluding these common findings from the Beck inventory slowed down the probability of diagnosing depression in our patients. We obtained a 10% difference in normal scores and an 8% difference in severe depression. We conclude that BI scores should be carefully interpreted in diagnosing depression in MS patients and that the frequency of diagnosis of depression in MS patients is overestimated.

P802

Information processing in multiple sclerosis: accuracy versus speed

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Background: Findings from several studies support the conclusion that the primary cognitive deficit in multiple sclerosis (MS) patients entails a slowing in the speed of information processing and that this deficit is not an artifact of ancillary motor impairments. Furthermore, a few studies have shown that, when patients are permitted sufficient time to respond, their accuracy of performance is comparable to that of healthy controls. The present study was designed to offer further support for this perspective by examining patients' performance on a

set of tests that provided both explicitly-timed measurement and covertly-timed measurement of processing speed. The covertly-timed measures also allowed assessment of patients' accuracy of response. **Objective:** To use multiple measures to assess speed of information processing in MS patients. **Methods:** Forty MS patients and 40 healthy controls completed computerized versions of the Rotated Figures Test (RFT), Remote Associates Test (RAT), Tower of London (TOL), Picture Naming Test (PNT) and Stroop Color-Word Interference Test (Stroop). In the RFT, RAT, and TOL, subjects' attention was drawn toward performing the various tasks correctly, and the speed with which they arrived at problem solutions was covertly timed by the computer "behind the scenes." The PNT and Stroop were administered afterwards; these tests assessed rapid serial processing and were explicitly timed. **Results:** Results indicated that MS patients answered with significantly greater latencies than controls across the full array of explicitly- and covertly-timed measures. Accuracy of performance was similar between the groups on two of three covert measures (RAT, TOL), but did differ on the Rotated Figures Test. **Conclusions:** Overall, slowing in MS patients' speed of information processing was found across a range of cognitive measures, adding further evidence that this deficit plays a principal role in cognitive dysfunction in MS.

P803

Multiple sclerosis: cognitive dysfunction study in Brazilian patients

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Background: Cognitive dysfunctions (CD) often occur in multiple sclerosis (MS). Brain injuries and disconnections in neural ways can result in cognitive damage, commonly found in MS. In relation to neuropsychologic aspects, several authors suggest that only a cognitive study test can demonstrate who has intellect functions committed independently of clinical type evolution. Low or no association between physical impairment and CD seems to exist. **Objective:** To evaluate cognitive aspects as memory and attention in a sample of MS Brazilian patients and to verify eventual relationships among CD and depression, anxiety and fatigue. **Methods:** We studied 30 adult patients, assisted in public and private units of health, with MS diagnosis confirmed. Neuropsychologic test - RAVLT (Rey Verbal Auditory Learning Test) and Beck depression inventory and fatigue severity scale were applied. All the patients signed the Term of Free and Clarified Consent. The study was approved by the Committee of Ethics in Research of the Brasilia Health Department. **Results:** The mean age of patients was 35.5 varying from 18 to 63 years. There were 23 (76.7%) female patients. Sixty percent (18 patients) had between 13 to 16 years of study. CD was 52% among female and 71% among male patients. The highest percentage of CD was found among patients between 28 to 37 years old (73%). CD was found to be indirectly proportional to the number of years of study. Anxiety degrees were associated with CD ($p = 0.03$). Depression degrees were also associated with CD but it was not statistically significant ($p = 0.2$). Any association between fatigue and CD was not found. **Conclusions:** Studies of evaluation of the cognitive function in MS are few and controversial. The influence of the anxiety level in CD as well as probable involvement of the depression in CD was verified. On the other hand, it was verified that the fatigue was very frequent in patients with MS as has already been demonstrated in several studies. However in this study we didn't observe an association between fatigue and cognition. This conclusion is similar to that reported by Rao et al. and Krupp et al. and different to that one reported by Pia Amato et al.

P804

Corpus callosum volume and fiber integrity as correlates of processing speed in children and adolescents with multiple sclerosis
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Background: The corpus callosum (CC) plays an important role in attentional control and transmission of information through inter-hemispheric interaction. Processing speed has been associated with integrity of callosal fibers in adults with multiple sclerosis (MS) and in healthy control children. **Objective:** To explore the relationship between CC volume loss and processing speed in children and adolescents with MS. **Methods:** Twenty-three children (18 females) with MS ranging from 11 to 19 years old (mean=16.51, SD=2.23) participated, 10 of whom have had magnetic resonance imaging (MRI) data analyzed. MRI segmentation algorithms were used to quantify CC volume. Diffusion tensor imaging was used to calculate fractional anisotropy (FA) values for four CC regions: genu, anterior and posterior body, and splenium. Performance on age-normed measures of visual searching speed, visuo-motor scanning, and fine-motor dexterity were associated with CC measures using correlational analyses. **Results:** Overall group performance on measures of visual search speed (mean=-2.11, SD=2.24), visuo-motor scanning (mean=-0.84, SD=0.16), and hand dexterity (mean=-1.78, SD=3.24) was mildly to moderately impaired relative to normative data. Reduced volume in the genu, but not other CC segments, was correlated with slower speed on a visual search ($r=0.80$, $p=0.01$) and visuo-motor scanning task ($r=0.82$, $p=0.003$), and approached significance on a measure of fine-motor dexterity ($r=0.52$, $p=0.10$). Lower FA within the splenium ($r=0.79$, $p=0.007$) and posterior body ($r=0.70$, $p=0.02$), but not the genu or anterior body of the CC, was associated with slower visual searching speed. FA values were not associated with performance on visuo-motor scanning and dexterity. **Conclusions:** Findings suggest that MRI measures of the CC, particularly in the posterior structures (splenium, posterior body), are associated with speed of visual information processing. The moderate associations between lower FA values in the CC and reduced processing speed in children with MS provide impetus for our ongoing research exploring diffusion tensor imaging (DTI) measures of global white matter integrity and neurocognitive functioning.

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P805

The effect of memory training on the quality of life in patients with multiple sclerosis

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Background: Cognitive impairment is a common problem in multiple sclerosis (MS) and is characterized by difficulties in recent memory, sustained attention, executive functions and information processing speed. These deficits are associated with poorer health-related quality of life (QoL). Specific training may improve cognitive functioning, but it is unclear whether it also results in better QoL. **Objective:** In this open label pilot study, we examined the impact of a specific memory training on health-related QoL in nine patients with MS who reported having problems with their memory. **Methods:**

Patients had to be relapse-free for at least one month and of age between 18 and 55 years. They used no medication influencing memory and had no moderate or severe psychiatric problems. Three tests were taken at baseline and immediately after the training: MSQoL-54 for measuring the QoL, the Memory Questionnaire for the subjective and the Rivermead Behavioural Memory Test (RBMT) for the objective memory. The memory training program was based on a newly developed training by Berg et al., that is individually tailored to the patients' particular complaints and needs. After identification of the main memory problems patients were individually taught several mnemonic strategies over 9 weeks. **Results:** The MSQoL physical health composite score improved in seven out of nine patients (mean score before training 40.8 and after training 46.9) and the MSQoL mental health composite score improved in five out of nine patients (mean score 61.1 before and 66.8 after training). The RBMT profile and screening test showed an improvement for four out of eight patients (mean score 9.4 before and 11.2 after training) and the memory questionnaire improved in seven out of nine patients (mean score before 93.7 and 72.2 after training). **Conclusions:** The findings in this pilot study suggest that individualized cognitive training may improve mental and physical QoL in MS patients, in combination with improvement in objective and subjective memory. More studies, preferably randomized controlled trials, are needed to confirm these findings.

P806

Cognitive impairment, social functioning and fatigue in patients with relapsing-remitting multiple sclerosis: the COGIMUS (COGNITION IMPAIRMENT IN MULTIPLE SCLEROSIS) study

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Background: Cognitive impairment (CI) develops in 40–65% of patients with multiple sclerosis (MS), sometimes appearing before the onset of physical disability. Altered social functioning is also common, as is fatigue, which affects as many as 90% of patients with MS. All these factors contribute to impaired quality of life. **Objective:** To determine the impact of CI on measures of social functioning and fatigue in patients with MS. **Methods:** The prospective, multicenter, observational, dose-controlled COGIMUS study recruited patients aged 18–50 years with relapsing-remitting (RR) MS (McDonald criteria) and Expanded Disability Status Scale (EDSS) score ≤ 4.0 , who received interferon beta-1a, 22 mcg or 44 mcg subcutaneously three times weekly. Patients underwent a complete neurological examination at baseline and regular intervals thereafter for 3 years. The Fatigue Impact Scale (FIS), Environmental Status Scale (ESS) and Rao's battery of neuropsychological tests were administered regularly, by psychologists or evaluating neurologists blinded to patients' treatment, to assess fatigue, social functioning and cognition, respectively. Fatigue and social function in patients with and without CI (defined as ≥ 3 positive tests for CI) were compared. **Results:** A total of 550 patients were recruited. Data on cognition and social functioning were available for 548 (99.6%) patients and on fatigue for 547 (99.5%) patients. The mean (SD) ESS total score was numerically higher in patients with CI than in those without (2.0 [3.5] versus 1.4 [2.4], respectively; $p=0.357$). Mean scores on three of the seven ESS components were significantly higher in patients with CI than those without: 'Actual work status', $p=0.045$; 'Personal assistance required', $p=0.040$; 'Transport', $p=0.011$. The mean (SD) total FIS scores were 25.4 (25.1) in patients with CI and 27.5 (24.0) in those without ($p=0.20$). Scores in the FIS subscales were higher in patients with CI than in those without, but the differences did not reach significance. **Conclusions:** CI affects aspects of social functioning and may influence fatigue in patients with RRMS.

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P807

Information processing in multiple sclerosis patients

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Background: Cognitive impairment (CI) affects 46% of patients in Argentina. Speed of information processing deficit is often seen in patients with multiple sclerosis (MS). It refers to the rate at which cognitive processes can be executed. When two tasks are presented simultaneously or at a short stimulus onset asynchrony (SOA), a systematic delay in the execution of the second task is observed while response times (RT) to the first task are unaffected. This phenomenon, referred as Psychological Refractory Period (PRP), has been widely used in normal subjects to understand the temporal organization of different stages of information processing. **Objective:** Here we investigate PRP performance in patients with MS. Since a primary aspect of the pathophysiology of MS is demyelination of long distance fibers, we hypothesized that the dynamics of information processing may be affected, even at early stages of the disease when no other CI are evident. **Methods:** 10 MS patients with relapsing-remitting pattern and 10 healthy controls were evaluated. Age: 36.92 ±10.97; Education: 14.98 ±2.60; Expanded Disability Status Scale (EDSS): 1.18 ±0.64; Disease evolution: 6.36 ±4.99. In order to evaluate the PRP a computerized test was used. Cognitive Battery: Brief Repeatable Battery-MS, Symbol Digits Test, Trail Making A and B and Conners Test. **Results:** The performance in the dual-task is significantly impaired in MS patients. First, MS patients perform slower than controls in PRP tasks. This is particularly accentuated in the response time to the second task, which is affected by dual-task interference. Second, we observed a strong effect of notation in the patient group: the difference between the RT in words-written numbers vs digits-written numbers trials was significantly higher than in the control group. In addition, patients manifested a systematic difficulty in executing both tasks in close succession.

Conclusions: We can conclude that MS patients with no evident signs of CI have already compromised some particular aspects of information processing.

P808

Neuropsychology of primary progressive multiple sclerosis a study of Brazilian patients from Rio de Janeiro

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Background: The majority of patients with primary progressive multiple sclerosis (PPMS) develop spinal cord involvement. Brain lesions and cognitive impairments are less described compared to relapsing-remitting multiple sclerosis (RRMS). Data about the prevalence of cognitive alterations in PPMS are contradictory (Comi et al. -7% vs. Solari et al. -50%). The results of a former study in RRMS Brazilian patients from Rio de Janeiro (Negreiros et al. 2008) showed that 50% presented cognitive abnormalities detected by neuropsychological tests. **Objective:** To estimate the frequency of the cognitive alterations in PPMS Brazilian patients, and to evaluate possible similarities or differences with RRMS. **Methods:** A Battery of Neuropsychological tests was applied in 26 PPMS patients assisted in a reference service for MS treatment in Rio de Janeiro (Brazil), and classified according to the criteria of Thompson (2000). 26 controls were enrolled, matched to sex, age and level of education. The battery applied was the same used to evaluate RRMS patients, and assessed the following cognitive functions: tracking for dementia, attention/concentration, processing of information, fluency verbal, memory and abstract thinking and the Beck Scales for mood disturbances. The statistical analysis was done according to the study of Rao (1991). **Results:** In comparison to the controls, 50% of the PPMS patients showed cognitive alterations in neuropsychological tests. The recent memory (60%), verbal fluency (40%) and processing of information were the cognitive functions (40%) more frequently impaired. Depression was more frequent in patients than controls, but no association was found between

cognitive deficit and depression. **Conclusions:** The rate of cognitive impairment in our PPMS patients was similar to the results of Solari et al. The prevalence of alterations in neuropsychological tests was similar in Brazilian PPMS and RRMS patients.

P809

Cognitive decline predicts disability progression in relapsing-remitting multiple sclerosis

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Background: Cognitive deficits occur in approximately 50% of patients with multiple sclerosis (MS) and are considered poorly correlated with the physical disability or disease duration. **Objective:** To compare the evolution of cognitive functions and physical disabilities in newly diagnosed MS patients and to examine the influence of cognitive deterioration on further physical disability. **Methods:** Relapsing-remitting MS patients with Expanded Disability Status Scale (EDSS) equal to or below 3 were prospectively included. Neuropsychological functions were assessed using the BCogSEP, which is a French language validated battery. Physical functions scored by the EDSS and neuropsychological functions were evaluated at baseline and after one and two years. The disability was reassessed a few months or years later. **Results:** Seventy-five patients were included with a mean (SD) age of 35.7 (8.2) years. The mean EDSS scores were 1.4 (0.7), 1.8 (0.8) and 2.1 (0.8) at baseline, after 2 years and after a mean follow-up of 3.8 years (2.9) respectively. Twenty-nine patients (38%) were considered cognitively impaired at baseline. After two years, cognitive functions deteriorated in 24 patients (31%) (9 and 15 cases with and without disability progression respectively). Disability progression was noted in 18 cases (24%)(9 and 9 cases with and without cognitive deterioration respectively). The cognitive and the physical deteriorations were not significantly associated. At the end of the follow-up, among the 24 patients with a cognitive decline during the first two years, 16 patients had disability progression. Among the 51 patients without cognitive decline during the first two years, 12 patients only had a disability progression at the end of the follow-up ($p < 0.01$). **Conclusions:** During the first two years of follow-up in a relapsing-remitting MS group, a cognitive decline was not associated with a deterioration in physical disability. However, this early cognitive decline is highly predictive of a disability progression during a longer follow-up.

P810

How does a single neuropsychological session lasting different times modify cognitive performance and fatigue in multiple sclerosis patients?

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Background: Cognitive fatigue can be measured as an inability to maintain initial levels of attentional performance. Researchers do not agree as to whether a longer and continuous effort in a cognitive task increases mental fatigue and changes neuropsychological performance. Furthermore different initial levels of cognitive impairment may complicate the cognitive fatigue performance and ratings. **Objective:** The objectives of this study were: to examine the cognitive performances and ratings of subjective cognitive fatigue using cognitive fatiguing tasks lasting different times and to evaluate the correlation between subject cognitive fatigue and the objective performances. **Methods:** Of 70 individuals with MS, only 30 patients presented significant fatigue (measured with vigilance test of Test for Attentional Performance) and initial cognitive deficit (Symbol Digit Modalities Test) and these were randomized in three groups, matched for different initial levels of cognitive deficit. Each group completed a neuropsychological training in a single session lasting different times (15, 30, 45 minutes). Subjective measure of fatigue (cognitive Modified Fatigue Impact Scale (MFIS) subscale) and attentional test (Paced

Auditory Serial Addition Task (PASAT)) were rated before and after the session. **Results:** There were no significant differences between the three groups on PASAT. Although cognitive MFIS subscale did not significantly differ for the three groups, the value is close to significance ($F = 3,191, p = 0.058$). There was no significant correlation between change in subjective fatigue and cognitive performance. **Conclusions:** Changes in performance over time showed improvement rather than deterioration. These results confirm that patients' subjective ratings of their fatigue are not valid indicators of their actual performance on cognitive tests and that the cognitive fatigue does not decline more rapidly over the time engaged in mental activity.

P811

Cognitive dysfunction in patients with early relapsing-remitting multiple sclerosis, Expanded Disability Status Scale ≤ 3.0 and high educational level

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Background: Cognitive deficits at early stages of multiple sclerosis (MS) can have a severe impact on patients' quality of life, affecting everyday tasks as well as professional and social activities. Yet studies of cognitive impairments in the early phase of MS have been rare, with often heterogeneous patient samples in terms of other parameters such as educational level, Expanded Disability Status Scale (EDSS) or disease duration. **Objective:** The present study aimed to evaluate and characterize cognitive functions in patients with EDSS ≤ 3.0 and high educational background at an early stage of relapsing-remitting MS (RRMS). **Methods:** 16 RRMS patients allocated from our outpatient clinic with EDSS ≤ 3.0 (mean:1.9), disease duration ≤ 5 years (mean: 2.94 years) and educational background GCSE-level or higher were compared to 16 healthy participants matched for age, sex and educational level. A comprehensive neuropsychological test battery was used to evaluate MS-related cognitive deficits. In addition self report measures for depression, fatigue and quality of life were applied. **Results:** Compared to healthy participants, MS patients performed significantly worse in tests for verbal and working memory, mental speed, and executive function while performance in visuo-spatial memory was unaffected. Compared to normative data 13 of 16 MS patients were classified as cognitively impaired in at least one of the applied tests. Variability in individual test scores was also higher in the MS patient group compared to the healthy control group. In addition patients scored significantly higher on self report measures for fatigue (clinically relevant) and depression (not clinically relevant) and lower on quality of life than healthy participants. **Conclusions:** Cognitive decline and fatigue are already apparent at an early stage of MS when physical status is not yet severely affected. These results emphasize the importance of early neuropsychological assessment since cognitive decline and fatigue significantly influence patients' professional life and everyday activities. **Supported by:** Swiss MS Society.

P812

Cognitive impairment in multiple sclerosis correlates highly with a delayed P3 component in event-related potential testing

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Background: Cognitive impairment (CI) occurs in up to 70% of multiple sclerosis (MS) patients. Subtle CI is difficult to detect clinically and correlates poorly with MRI measures. The Paced Auditory Serial Addition Test (PASAT) is useful in assessing CI in MS but may be influenced by anxiety and years of education. Event-related potentials (ERPs: time-locked electroencephalograms (EEGs)) are a non-invasive method of detecting cognitive activity. Low-probability, unexpected

stimuli elicit a large positive wave at approximately 300 ms (the P3 component) in the averaged ERP. Delayed latencies of the P3 are a reflection of impaired cognitive functioning. **Objective:** The aim of the present study was to assess the relationship between P3 latency and PASAT performance in MS patients. **Methods:** Fourteen MS patients (eight men; seven RRMS, five SPMS, two PPMS) were examined; mean age was 41 yrs, median Expanded Disability Status Scale (EDSS) 2.5 (range 0–8), mean duration of illness 14.8 yrs (range 1.5–44). Each subject completed the PASAT prior to ERP recording. ERPs were recorded using a 128-channel high-density EEG array. The P3 paradigm consisted of tones, separated by an inter-stimulus interval of 2 s, presented binaurally for 205 trials in a pseudorandom order. Frequent non-target (80%) and infrequent target (20%) tones were presented at 500 Hz and 1000 Hz, respectively. Subjects were instructed to press a button as quickly as possible following a target tone. **Results:** The mean P3 latency was 405.2 ms (standard deviation 75.6). The mean PASAT score was 75% (range 42–98). The correlation between P3 latency and PASAT score was significant (Spearman's $\rho = -0.71, p < 0.01$). **Conclusions:** Impaired P3 latency correlated highly with the PASAT and may be a reliable, objective test of CI in MS. The P3 latency is independent of motor disability measured by the EDSS. We propose that ERPs are useful in the assessment of CI in MS.

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P813

COMT Val158Met genotype and pain perception in patients with multiple sclerosis

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Background: The Val158Met polymorphism of the catechol O-methyltransferase (COMT) gene has been linked to pain perception in healthy adults. Research suggests that the met allele is associated with increased synaptic dopamine, which is in turn associated with decreased endogenous opioid activity. **Objective:** To determine whether COMT genotype predicts pain perception in patients with multiple sclerosis (MS). We hypothesized that patients would show higher pain self-ratings than controls and that within the patient group, COMT met allele homozygotes would report higher levels of pain than val carriers. **Methods:** Participants included patients with mild to moderate relapsing-remitting or secondary progressive MS (n=25) and demographically matched healthy controls (n=12). Genotyping was completed using custom TaqMan assays. Current pain severity was rated on a 10-point Likert Scale. The extent to which pain interfered with everyday activities ("pain interference") was measured using the Pain Effects Scale. Participants also completed depression and fatigue self-ratings and underwent brain magnetic resonance imaging (MRI). **Results:** Patients' subjective pain severity and pain interference ratings exceeded those of the healthy adults (both $p < 0.001$). Patients homozygous for the met allele (n=7) reported higher pain interference than val carriers (n=18; $p = 0.02$). COMT genotype showed no effect on pain severity ($p > 0.05$). Separate covariance analyses indicated that the relationship between COMT genotype and pain interference remained significant even after accounting for age, sex, disease subtype and duration, disability, mood, fatigue, lesion volume, brain parenchymal fraction, and medications (analgesics, antidepressants, disease-modifying medications). Further analyses revealed no evidence of a dose effect of the COMT met allele. **Conclusions:** This preliminary study suggests that COMT Val158Met genotype predicts pain perception in patients with MS. We are now conducting a larger independent replication study in MS assessing additional candidate polymorphisms implicated in pain perception. Further research on the genetic and molecular basis of pain in MS may lead to novel therapeutic approaches. **Supported by:** National Institute of Neurological Disorders and Stroke (K23NS045830, R03 NS056228), National Multiple Sclerosis Society (USA) (RG3248, PP1214), Hitchcock Foundation.

Pathology

P814

Tumor-like lesion in neuromyelitis optica

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Background: Neuromyelitis optica (NMO) is an idiopathic relapsing inflammatory disease with severe attacks of optic nerves, spinal cord or both. Other brain areas are rarely affected. It is still discussed whether NMO is a separate disease or a subtype of classical multiple sclerosis (MS). Clinical, cerebrospinal fluid (CSF) and magnetic resonance imaging (MRI) parameters suggest that at least in the initial stages of NMO antibody-driven inflammation mainly affects components of the blood-brain barrier and the surrounding perivascular tissue. Serum auto-antibodies directed against aquaporin-4 (AQP4) water channel protein seem to play a pathogenetic role. This assumption may also be supported by autopsy and serum studies. **Objective:** We correlated serum findings with neuropathological findings of a brain biopsy. **Methods:** (1) neuropathological examination (including immunohistochemistry) of brain biopsy and (2) serum levels of anti-NMO-IgG (AQP4) autoantibodies. **Results:** A 64 year old female with NMO for 5 years experienced an exacerbation under immunosuppressive therapy with azathioprine. A large cystic tumor-like lesion infiltrated the corpus callosum. Biopsy revealed a semi-necrotizing demyelinating lesion with macrophages and loss of myelin, axons, astrocytes and AQP4. Loss of AQP4 was accompanied by deposition of activated complement complex. In serum AQP4 autoantibodies were detectable. Perivascular infiltrates consisted of CD4+ T-Lymphocytes, CD20+ B-lymphocytes, CD79a+ plasma cells and eosinophilic granulocytes. **Conclusions:** (1) Our observation is in accordance with an AQP4 autoantibody-driven immune pathogenesis of lesions in NMO. (2) NMO may give rise to large focal lesions in the forebrain, which in aged patients may be difficult to differentiate from brain tumors.

P815

Diffuse inflammation in the spinal cord of progressive multiple sclerosis patients

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Background: Recent neuropathological studies of brain samples revealed the presence of a diffuse inflammatory reaction in the normal appearing white matter (NAWM) of progressive multiple sclerosis (MS) patients, which is associated with diffuse axonal injury. However, the nature of the inflammatory response is still poorly studied in the spinal cord of MS patients, although it constitutes a common area of involvement which atrophy correlates with irreversible disability. **Objective:** The aim of our study was (i) to characterize the inflammatory reaction in the cervical spinal cord of patients within the progressive phase of MS; (ii) to correlate T-cells and macrophages/microglia infiltration with axonal loss. **Methods:** Qualitative and quantitative analysis of inflammation, demyelination and axonal loss was performed on autopsy cervical spinal cord tissue from 14 primary and secondary progressive MS patients and from five control subjects, using immunohistochemical techniques. **Results:** Whatever the number of active demyelinating lesions, a diffuse inflammatory reaction took place in the NAWM, the gray matter and the meninges of MS spinal cord. In the NAWM, macrophages/microglia massively accumulated whereas T-cell infiltration is mild and sparse. Moreover, we confirmed the presence of a diffuse axonal loss in the cervical spinal cord, which reaches a mean level of 30% compared to control subjects. We are currently determining to

which extent diffuse axonal loss correlates with the level of T-cell and/or macrophages/microglia infiltration. **Conclusions:** To our knowledge, this is the first quantitative study of both inflammatory reaction and axonal loss in the spinal cord of MS patients. As was reported to occur in the brain, the progressive phase of MS is associated with a diffuse inflammation throughout the whole spinal cord, which is dominated by macrophages/microglia accumulation.

P816

Influence of factors present in the cerebrospinal fluid of patients with multiple sclerosis on the axonal damage

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Background: The permanent neurologic disability that appears in multiple sclerosis (MS) depends on the degree of neuroaxonal affection. Neurofilaments are composed of three protein subunits: heavy (NFH), middle (NFM) and lightweight (NFL). NFL, the largest component of the axonal cytoskeleton, whose presence is diminished in MS lesions, is essential for the correct assembly of neurofilaments and maintenance of axonal calibre. The correct phosphorylation of the subunits NFM and NFH form a stable network that determines the speed of the nervous impulse and regulates several aspect of NF dynamics, rendering subunits more resistant to proteolysis, increasing axonal calibre, and promoting NF-NF interactions. Cdk5, a serine/threonine kinase that phosphorylates C-terminal regions of NFM and NFH, plays an important role. **Objective:** The objective of this study was to determine how the presence of factors in the cerebrospinal fluid (CSF) are capable of affecting the axon: structure, function, and pathophysiology. **Methods:** CSF samples from healthy volunteers and MS patients with clinically definite, relapsing-remitting (RRMS) and secondary progressive (SPMS) multiple sclerosis, were collected at the Neurological Departments of Hospitals of Valencia, Spain. The study was approved by the local ethics committees. Primary cultures of cerebellar neurons from rats were incubated with CSF from both MS patients and healthy matched controls. We analyzed: 1) cellular levels of NFL and mRNA expression of NFL, using immunocytochemistry and quantitative Real-time reverse transcription polymerase chain reaction (RT-PCR) respectively; 2) degree of phosphorylation of NFM and NFH; 3) Cdk5 kinase activity using a fluorescence-based assay. **Results:** Results show: 1) a significant decrease of axonal NFL and NFL mRNA expression, induced by CSF from all the MS patients compared with CSF from controls; 2) Cdk5 neuronal activity decreased; 3) a correlation of Cdk5 activity with the reduction of both degree of phosphorylation of NFM and NFH. CSF of some MS patients with acute relapse induced a clear increase of Cdk5 activity with respect to that of both MS and controls. **Conclusions:** Thus, we speculate the presence of factors in the CSF of MS patients capable of producing axonal damage.

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P817

Increased expression of HERV-H Env and HERV-W Env epitopes on the surface of peripheral blood mononuclear cells from patients with active multiple sclerosis

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Background: The leading hypothesis assumes that multiple sclerosis (MS) occurs as a result of exposure of genetically susceptible individuals to an unknown environmental agent(s). Several herpesviruses together with human endogenous retroviruses (HERV-H/-W) have been associated with development of MS for years, but none of the associations are conclusive. Both virus groups are known to interact

with each other, inducing synergistic immune responses. Recently, we have shown that inactivated herpesvirus preparations are capable of inducing reverse transcriptase (RT) activity, a hallmark of retrovirus activation. This RT activity was significantly higher in MS patients vs. healthy controls, and the reactivation is initiated early in the patients. **Objective:** To further substantiate the presence of activated HERVs in MS. **Methods:** A flow cytometric evaluation of cell membrane expression of HERV-H Env and HERV-W Env epitopes on peripheral blood mononuclear cells (PBMCs) from 20 patients with active MS, 20 patients with stable MS, and from 20 healthy individuals was carried out. We have also analyzed serological reactivity to the expressed HERV-H and HERV-W Env epitopes using Time-Resolved Immunofluorometric Assay (TRIFMA). **Results:** The statistical analysis showed significantly increased expression of HERV-H/ -W Env together with an increased number of B cells in patients with active MS. B cells and monocytes are the only PBMCs expressing the proteins. Moreover, patients with active MS, display increased antibody reactivities towards HERV-H and -W epitopes. The higher antibody reactivities in sera from patients with active MS correlate with the higher levels of HERV-H Env and HERV-W Env expression on B cells and monocytes. We did not find such correlations for stable MS patients or for healthy controls. **Conclusions:** These findings indicate that both HERV-H and -W Envs are expressed in higher quantities on the surface of B cells and monocytes in patients with active MS, and the expression of the proteins may be associated with exacerbations of the disease.

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P818

Influence of cerebrospinal fluid from multiple sclerosis patients on voltage sodium channels in cerebellar granular cell culture of rats

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Background: The permanent disability present in multiple Sclerosis (MS) depends on the degree of neuroaxonal affection, and it is in this sense where the neuroprotection concept acquires more relevance. At the present time, only the capacity of remyelination of the MS plaque has been established as a neuroprotector factor. The electrical conduction through the axon is an essential condition for remyelination, and it depends on the voltage activated sodium channels (Nav). **Objective:** To search cerebrospinal fluid (CSF) of MS patients and CSF of corresponding control subjects (CO) in respect to their ability to influence the expression of distinct isoforms of Na⁺ channels in rat cerebellar granular cell cultures (RCGC). **Methods:** We studied CSF of 27 MS patients (16 relapsing-remitting, 11 secondary-progressive) and 10 non-neurological controls. Primary cultures prepared from newborn rat cerebellum were treated with 5% concentrations of CSF on the 7th day in vitro for 2 hours. We assessed whether RCGC express 1.8 channels. The expression of Nav 1.2, Nav 1.6 and Nav 1.8 isoforms in the CSF-treated or untreated RCGC were studied by immunofluorescence staining and confocal microscopy analysis, and reverse transcription polymerase chain reaction (RT-PCR) using primers specific for several Nav alpha subunits. Changes in the intracellular sodium concentration ([Na⁺]_i) in RCGC were measured using the sodium ion fluorescence indicator Sodium Green by confocal laser-scanning microscopy. **Results:** RCGC were shown to express the Nav 1.8 isoform. The CSF of MS patients reduced the cellular expression of 1.6 and 1.8 Nav channels revealed by immunocytochemical staining. mRNA analysis revealed a reduced

transcription of the mRNA for the 1.2 and 1.8 Nav channel alpha subunits. Supporting these results the [Na⁺]_i increase induced by the CSF-CO is inhibited by the CSF-MS. **Conclusions:** Obtained data indicate that the constitution of compounds and/or factors present in the CSF-MS is changed, compared to CSF-CO. The CSF-MS modify the expression of some Nav channel isoforms and the Na⁺ homeostasis. A change of Nav channel expression might play a role in the altered axonal conduction in MS.

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P819

Myelinating oligodendrocytes in the adult human cortex and cortical lesions of multiple sclerosis patients

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Background: Demyelination of the cerebral cortex in patients with multiple sclerosis (MS) is well documented. We, and others (Albert et al., Brain Pathol, 17 (2007), 129–138), have observed areas within cortical MS lesions that are highly suggestive of remyelination. To date, however, a systematic study of oligodendrocyte lineage cells in control and MS cerebral cortex has not been reported. **Objective:** To characterize the density and distribution of PLP-positive oligodendrocytes and NG2-positive oligodendrocyte progenitor cells in control and MS cerebral cortex. **Methods:** One-centimeter slices of control and MS postmortem brains were fixed, sectioned and immunostained for NG2, PLP, MHC Class II, neurofilament and Hu. Eighty-one cortical lesions from seven MS patients and 19 cortical regions from five controls were examined. **Results:** In control brains, PLP-positive myelin was dense immediately adjacent to the pial surface. Myelin sheaths were sparse in the deep aspect of Layer I and Layer II but increased in density in deeper layers. PLP-positive cell bodies and processes extending to myelin sheaths were frequent. This was interpreted as evidence for active myelination. Surprisingly, many control sections showed patches of decreased myelin sheath density in Layers I and II with PLP-positive cell bodies and processes extending to myelin sheaths. Sixty-six percent of cortical MS lesions showed evidence of remyelination based on the presence of individual or clusters of PLP-positive cell bodies with processes extending to myelin sheaths. The density, distribution, and morphology of NG2-positive cells in cortical demyelinated lesions were similar to that seen in the non-demyelinated cortex. **Conclusions:** The cerebral cortex of patients without neurological disease frequently show evidence of active myelination. These results raise the possibility that myelin is continuously regenerated or remodeled in the normal mature adult brain. Similar foci in cortical MS lesions are interpreted as evidence of remyelination.

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P820

Coenzyme Q10 in the cerebrospinal fluid of multiple sclerosis patients

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Background: Recently published data suggest that oxidative stress may play a role in the pathogenesis of multiple sclerosis (MS). **Objective:** Since Coenzyme Q10 (CoQ10) is an essential electron carrier in the mitochondrial respiratory chain and a powerful antioxidant, we measured this antioxidant in the blood and cerebrospinal fluid (CSF) of patients with MS and sought possible relations between CoQ10 levels and clinical and magnetic resonance imaging (MRI) features. **Methods:** CoQ10 concentration in blood and CSF from 37 patients with MS and 39 control patients with various

neurological diseases other than MS (NC) was measured by high-performance liquid chromatography with an electrochemical detection system. We measured albumin levels by nephelometric analysis and oligoclonal bands (OCBs) by standard immunoblotting. We also evaluated intrathecal CoQ10 synthesis as CoQ10 index: [CoQ10 CSF/CoQ10 serum]/ [albumin CSF/albumin serum]. We used the Mann-Whitney-U test for the statistical analysis of differences between MS and NC, and between MS groups with or without clinical (age at onset, course, Expanded Disability Status Scale (EDSS) score, relapse) and MRI (enhancing, T1, spinal cord lesions) variables when the spinal tap was performed. **Results:** The plasma concentrations of CoQ10 were similar in MS and in controls (NC), whereas CSF levels were significantly ($p < 0.001$) lower in MS patients (median values, 150 pM) than in controls (450 pM). Both CSF CoQ10 levels and CoQ indices were significantly higher during clinical relapses than in remission phases ($p < 0.025$ and $p < 0.005$, respectively). However, the CoQ10 index, when calculated and compared independently of disease stage, did not differ between the two groups. **Conclusions:** These preliminary data indicate that: (1) the CoQ10 level is substantially lower (~ three orders of magnitude) in CSF than that in blood; and (2) the CSF CoQ10 level is lower in MS patients than in NC. The lower CSF CoQ level in MS patients cannot be explained by decreased CoQ10 biosynthesis because CoQ indices were similar in MS and NC. Notably, CoQ10 was higher during clinical relapses, which may indicate a protective effect against oxidative stress damage in MS. In conclusion, our results suggest a role for CoQ10 in MS, but further studies are required to confirm this hypothesis.

P821

Butyrylcholinesterase activity in multiple sclerosis

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Background: Butyrylcholinesterase (BuChE) belongs to a family of proteins that include hydrolases and adhesion molecules. BuChE is involved in hydrolysis of the neurotransmitter acetylcholine, metabolism of lipids, esters and amides and in nervous system development. BuChE is expressed in white matter, glia and neurons. BuChE has been implicated in inflammatory and neurodegenerative processes. **Objective:** To compare BuChE activity in brain tissues from multiple sclerosis (MS), Alzheimer's disease (AD) and normal controls. **Methods:** Post-mortem brain tissues from MS, AD and matched controls (two each) were obtained from the Maritime Brain Tissue Bank. MS tissue containing plaques and surrounding normal-appearing tissues were identified and blocked, as were homologous regions in AD and control brains. Tissues were fixed, cryoprotected and cut in 50µ thick sections. Adjacent sections were stained for BuChE, AChE, astrocytes, microglia, macrophages, and with luxol fast blue/cresyl violet for myelin and cells. Sections were analyzed using light microscopy. **Results:** BuChE activity was present in glia, neurons and white matter in control, AD and MS brains. In AD, BuChE was associated with neuritic plaques and neurofibrillary tangles, as observed previously. In MS, within plaques, there was generalized loss of BuChE activity but the remaining activity was associated with cells and processes that lacked characteristics of glia. In peri-plaque regions, there was increased BuChE activity and many cells stained for markers for microglia/macrophages. In the normal-appearing white matter there were patches showing loss of BuChE activity but a prominence of BuChE-stained cells with glial morphology. **Conclusions:** BuChE has been implicated in neurotransmission, metabolism, and nervous system development as well as neurodegenerative lesions in AD. In inflammation, cholinesterases are upregulated in myeloid cells. We find different patterns of changes in BuChE activity between MS, AD and normal brains that suggest BuChE may play a role in inflammatory and neurodegenerative processes in MS.

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P822

Gene expression profiling of grey matter lesions in secondary progressive multiple sclerosis

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Background: Multiple sclerosis (MS) is an inflammatory demyelinating disease with widespread cortical lesions as one of the pathological hallmarks. However, our understanding of pathogenetic mechanisms involved in grey matter lesion formation is limited. **Objective:** Here, we aim to provide a transcriptome analysis of cortical grey matter lesions, hence advancing our understanding of lesion pathogenesis and identifying potential novel biomarkers and therapeutic targets. **Methods:** Grey matter lesions (type 3 - subpial lesions) were identified in the superior frontal gyrus (SFG) from 10 patients with secondary progressive MS and age-matched with SFG grey matter from controls with no neurological disorders. Gene expression analysis was performed on RNA extracted from grey matter lesions dissected from snap frozen tissues with the Illumina whole genome HumanRef8 v2 BeadChip. **Results:** A total of 2,058 genes were differentially expressed ($p < 0.01$) with a greater than a 1.4-fold change in 688 genes. Levels of mRNA from selected genes were confirmed using quantitative real-time polymerase chain reaction. The most highly expressed gene transcripts found in grey matter lesions were in response to oxidative stress (metallothioneins and S100A10), cellular stress (heat shock proteins), inflammation (ALOX5AP), cell motility (CYP2J2, osteopontin/CD44, palladin), blood-brain-barrier disruption (PECAM1 and tissue inhibitor of metalloproteinase 1), cell cycle control (BCL-6, PML and RUNX3) and interferon (IFITM1, 2 & 3). Most under expressed genes were associated with myelin loss (MOBP), neuronal loss (parvalbumin and VGF nerve growth factor inducible) and lipid/cholesterol metabolism (coenzyme A reductase). **Conclusions:** Cell cycle control was the most overrepresented functional category placing an emphasis on apoptosis and attempts at apoptotic rescue. A detailed assessment of the cell populations in which this is occurring may prove useful in advancing our knowledge in lesion pathology.

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P823

Hippocampal demyelination and memory deficit in multiple sclerosis patients

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Background: Multiple sclerosis (MS) is an inflammatory-mediated demyelinating disease of the central nervous system. Greater than 50% of MS patients become cognitively impaired and 30–40% have memory dysfunction. Some MS patients present with memory deficits and show relatively little physical disability and few white matter lesions. This raises the possibility that hippocampal pathology, unrelated to white matter lesion load, may cause memory impairment in MS patients. **Objective:** As hippocampus plays a crucial role in memory function, we investigated whether hippocampal demyelination is a cause of memory dysfunction in MS patients. **Methods:** We used a combination of immunohistochemistry, genome wide expression profiling, real time polymerase chain reaction (PCR) and magnetic resonance imaging (MRI) studies to ascertain the role of hippocampal demyelination in memory dysfunction in MS patients.

Results: Approximately 60% (13/24) of hippocampi from postmortem MS brains were demyelinated. Compared to control hippocampi, neuronal densities did not appear significantly altered in demyelinated or myelinated MS hippocampi. Pathological changes characteristic of Alzheimer's disease (AD) brain or ischemic injury were not detected in MS hippocampi or adjoining entorhinal cortex. We compared gene transcript profiles in myelinated and demyelinated MS hippocampi with control hippocampi and hippocampi from AD brains. Altered transcripts in demyelinated hippocampi reflect reduced synaptic plasticity while transcript profiles in AD hippocampi reflect neuronal degeneration. Gene families significantly altered in demyelinated MS hippocampi included those related to synaptic plasticity, axonal transport, neurotransmitters function and memory/learning. These genes were not decreased in RNA profiles from myelinated MS hippocampus. **Conclusions:** These data support the hypothesis that hippocampal demyelination reduces synaptic plasticity and contributes to cognitive dysfunction in MS patient.

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P824

Peripheral arterial compliance is compromised in young multiple sclerosis patients

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Background: A reduction of arterial elasticity in patients with autoimmune and inflammatory diseases such as rheumatoid arthritis and systemic lupus erythematosus has been previously reported and believed to be secondary to systemic inflammation. Similar involvement of peripheral arteries is not expected in a disease that affects exclusively the central nervous system, such as multiple sclerosis (MS). **Objective:** The primary purpose of this study was to examine large arterial compliance and small arterial compliance (C1 and C2 respectively) in patients with MS and compare them to healthy age-matched controls. Our hypothesis is that healthy controls will have more compliant arteries compared to individuals with MS. **Methods:** Men and women between the ages of 20 to 39 years, nine with relapsing-remitting MS ($\mu = 34.4 \pm 6.3$ yrs), and nine healthy, age-matched controls ($\mu = 30.2 \pm 4.4$ yrs) volunteered for this study. Arterial compliance was measured by using Pulse Contour Analysis (PCA), which records and analyzes the blood pressure waveform data from the Arterial PulseWave Sensors. **Results:** There were no significant differences between the two groups for age, height or weight ($p > 0.05$); however, there were significant differences in C1 and C2 between the groups ($p < 0.05$). The MS group had less arterial compliance of both large (C1) (14.8 vs 19.9 ml/mmHg), and small (C2) (6.1 vs 10.5 ml/mmHg) arteries compared to healthy controls. Absolute C2 recordings for the young MS group corresponded to values seen in people in the sixth decade of life. **Conclusions:** Arterial compliance is significantly compromised in young individuals with MS, compared to age-matched controls, suggesting a systemic effect of an inflammatory process that is believed to be confined to the central nervous system.

P825

Association of multiple sclerosis and amyotrophic lateral sclerosis

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Background: Multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS) are two different neurological diseases. Their targets are different central and/or peripheral nervous system with mechanisms partly divided. **Objective:** To report two cases of rare concurrence of MS and ALS. **Methods:** Case report **Results:** A twenty five year old woman developed a progressive right hemiparesis. Magnetic resonance imaging (MRI) of the brain showed several hypersignal T2

in white matter, oligoclonal bands were found in cerebro spinal fluid (CSF). The diagnosis was a first relapse of MS with total recovery. After 10 years without symptoms, she developed weakness of the legs, her reflex were exaggerated. Fasciculations and amyotrophy in limbs and tongue appeared. Progressively, she presented bulbar signs with nasal voice, and deglutition disorders. The electromyogram confirmed the diffuse anterior horn involvement. El Escorial ALS Criteria were fulfilled. She died nine months later. The autopsy confirmed both diagnoses: MS and ALS. A forty nine year old woman presented yearly neurological attacks with paresthesia in the limbs, visual loss of one eye or weakness of limbs. The diagnosis was a relapsing-remitting MS according to the McDonald criteria with MRI of the brain and CSF positive. She received several treatments (azathioprine, methotrexate, mitoxantrone) and became secondary progressive at fifty seven years of age. Six years later, she presented a worsening with weakness in upper limbs, loss of reflex, fasciculations, and bulbar signs (deglutition disorders, hypophonia). The electromyogram confirmed the diffuse anterior horn involvement. El Escorial ALS Criteria were fulfilled. She died four months later. **Conclusions:** Only five cases of such association have been reported in the literature. Although exceptional, this association of ALS and MS leads to a discussion of a common etiological immunological dysregulation, the role of immunoactive treatments or an unusual coincident combination. For this last hypothesis, the risk would be less than one case per year for one million inhabitants.

P826

The link between quaking proteins and P53 in myelination

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Background: The study of multiple sclerosis (MS) has been greatly advanced with the use of mouse models such as the quaking viable mouse (qkv). Homozygous qkv/qkv mice display rapid tremors, tonic-clonic seizures and severe hypomyelination of the central nervous system (CNS). The qkl gene encodes for the alternatively spliced KH domain RNA binding proteins QKI-5, -6, and -7. The qkv mutation results in the loss of isoforms 6 and 7, which have been shown to regulate myelin basic protein mRNA export as well as oligodendrocyte (OL) maturation. Although populations of oligodendrocyte precursor cells (OPCs) are observed in MS lesions, remyelination does not occur, suggesting OPC differentiation pathway impairment. The terminal differentiation of OPCs into mature OLs has been shown to be dependent on p53 in vitro. The *GLD-1* C. elegans homolog of QKI is known to associate with the p53 cep-1 mRNA, influencing its activity. It is likely that QKI proteins also serve the same function in mammalian cells. The Richard lab has identified the consensus sequence for the QKI Response Element (QRE), being NACUAAY-N(1-20)-UAAY. The p53 mRNA harbors a similar QRE within its 3'-UTR (UAYUAAYnnnUAAY) that we have shown does indeed interact with the QKI isoforms in vitro. **Objective:** To investigate QKI and p53 interactions during CNS myelination **Methods:** p53^{-/-} mice were bred to qkv/qkv mice to generate p53^{-/-} mice within the qkv/qkv background. Progeny were monitored closely for behavioral changes. CNS morphology of compound mutant mice was compared to that of the qkv/qkv mice and p53^{-/-} mice using immunocytochemistry. **Results:** Compound mutant qkv/qkv;p53^{-/-} mice have an earlier onset of seizures compared to qkv/qkv mice and exhibit an ataxic gait. Immunocytochemistry revealed no difference in corpus callosum myelin levels, however degeneration in the cerebellum was observed. **Conclusions:** These studies demonstrate that QKI and p53 are genetically linked to influence neurological defects including seizures and ataxia.

P827**Expression of chemokine-like receptor-1 (CMKLR1) and chemerin within the central nervous system: implications for regulation of experimental autoimmune encephalomyelitis**

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Background: Chemoattractant receptors, adhesion molecules, and their ligands have been implicated in recruitment of inflammatory cells to the central nervous system (CNS) during multiple sclerosis (MS). Chemokine-like receptor-1 (CMKLR1; also known as ChemR23 or Dez) is a G protein-coupled receptor that shares phylogenetic homology with a subfamily of chemoattractant receptors, including C5a-R and C3a-R. CMKLR1 is primarily expressed by monocytes, macrophages, and plasmacytoid dendritic cells. We have previously shown that CMKLR1-deficient mice are resistant to progressive experimental autoimmune encephalomyelitis (EAE), a model for human MS. It also has been shown that mRNA for chemerin, a natural ligand for CMKLR1 and a proteolytically regulated chemoattractant, is expressed at relatively low levels in the CNS. **Objective:** We sought to determine sources of chemerin within the CNS, as well as to identify CMKLR1-expressing cells in the CNS of mice with clinical EAE. **Methods:** We used a novel anti-CMKLR1 monoclonal antibody to examine CMKLR1 expression on primary mouse CNS mononuclear cells and a microglial cell line. We developed an enzyme-linked immunosorbent assay (ELISA) to measure chemerin levels in vitro. **Results:** CMKLR1 and chemerin are expressed in the spinal cord of mice with EAE. Flow cytometric analysis indicates that a subset of CNS-infiltrating macrophages express CMKLR1 during acute EAE, while mouse microglia constitutively express CMKLR1. Mouse brain extract contains detectable levels of chemerin protein, and microglia produce chemerin in response to stimulation with tumor growth factor beta in vitro. **Conclusions:** These data implicate CNS-resident microglia as a potential source of active chemerin, which may serve to recruit CMKLR1-expressing cells to inflammatory lesions during EAE. **Supported by:** National Multiple Sclerosis Society (USA) Postdoctoral Fellowship FG 1703-A-1.

P828**The UK Multiple Sclerosis Tissue Bank: resource for research**

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Background: The Multiple Sclerosis Tissue Bank (MSTB) at Imperial College London houses a large collection of human brain and spinal cord samples that are available to researchers investigating causes of central nervous system (CNS) inflammatory disorders, in particular multiple sclerosis (MS). The MSTB operates an easy and open access policy to tissue and welcomes applications from academic, public and private sector researchers. **Objective:** A major objective of the MSTB is to help understand what causes multiple sclerosis and assist in the development of better drug treatments by providing high quality brain tissue. The MSTB also aims to enhance the public's awareness of MS, promote the work of the tissue bank and increase the numbers of volunteers who are willing to sign up to the donor scheme. **Methods:** In the MSTB we use coronal sectioning of the fresh brain and alternate freezing and fixation of the slices for tissue preparation. The collection mainly contains snap-frozen and fixed-frozen and cryo-protected blocks of tissue but also thick frozen brain slices and whole formalin-fixed brains. Other tissue preparation methods can be implemented on user request. Furthermore, when possible, we also supply fresh tissue for cell isolation and culture. The tissue quality is suitable for applications ranging from histochemistry to genetic/genomic procedures and all samples are linked to a detailed clinical and neuropathological report. **Results:** The MSTB has been successful in actively supporting over 160 projects, many of which are ongoing. Through careful screening and reporting we ensure that tissue is suitable for a particular research project and when possible or necessary we also engage in collaborative studies, provide advice and

quality control. **Conclusions:** The MSTB is therefore a valuable resource for MS research with easy access to high-quality human CNS tissue which is available to research projects from academic institutions and the biotechnology/pharmaceutical industry.

Supported by: The UK MS Society.**P829****The role of Fas in toxin-induced demyelination**Amke R. Hesse¹, Jasmin Held², Ari Waisman³, Tanja Kuhlmann¹*¹Institute of Neuropathology, University Hospital Muenster, Muenster, NRW, Germany; ²University Medical Center, Goettingen, Niedersachsen, Germany; ³Johannes Gutenberg-University, Mainz, Rheinland-Pfalz, Germany*

Background: Multiple sclerosis (MS) is the most frequent demyelinating disease of the central nervous system of young adults. In early MS lesions oligodendrocytes with the morphological criteria of apoptosis are found. Additionally, oligodendrocytes expressing apoptosis related proteins such as Fas (CD95/ APO-1) have been described in MS lesions. **Objective:** Fas is a cell surface receptor belonging to the TNF-receptor superfamily transducing cell death signals when ligated by the Fas ligand. Mice lacking Fas expression exclusively in oligodendrocytes show a delayed disease onset as well as decreased demyelination and reduction of oligodendroglial cell death in experimental autoimmune encephalomyelitis (EAE), an animal model of MS. The aim of our study is to determine whether the Fas-mediated cell death of oligodendrocytes is also crucial for oligodendroglial cell death in toxin-induced demyelinating animal models. **Methods:** We examined the upregulation of Fas by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) in the corpus callosum of mice fed with cuprizone, a copper chelator, that induces oligodendroglial cell death and demyelination. Additionally, we compared the extent of oligodendroglial cell death and demyelination in the corpus callosum of wild type (WT) mice and mice lacking Fas especially in oligodendrocytes (FAS-KO) in the cuprizone model. **Results:** We found an upregulation of Fas-mRNA in the corpus callosum of mice fed with cuprizone which correlated with the occurrence of activated caspase 3-positive oligodendrocytes. Preliminary results show similar numbers of apoptotic oligodendrocytes in WT and FAS-KO mice fed with cuprizone for 6 or 10 days. **Conclusions:** Preliminary results indicate that Fas is dispensable for oligodendroglial cell death in the cuprizone model.

P830**Substantial pyramidal cell loss in the motor cortex of secondary progressive multiple sclerosis cases with meningeal B-cell follicles**
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Background: We have previously described a subset (45%) of secondary progressive multiple sclerosis (SPMS) cases with meningeal aggregates of B cells, plasma cells and follicular dendritic cells, resembling ectopic B-cell follicles. SPMS cases with ectopic follicles (F+) had an earlier disease onset, a more aggressive disease course and died younger than SPMS F- cases. SPMS F+ had a greater number of subpial demyelinated lesions. **Objective:** As part of our studies into the mechanisms of neurodegeneration in progressive MS we describe the extensive loss of pyramidal neurons and increased microgliosis in the SPMS F+ motor cortex. **Methods:** Quantitative analysis of SPMS post mortem tissue of precentral gyrus from 10 F- and 10 F+ SPMS cases in comparison to matched controls using immunofluorescence revealed a gradient of neuronal loss (55% reduction layer II, 18% reduction layer VI). To examine the fate of individual neuronal populations, pyramidal and non-pyramidal cells of layers III and V were identified morphologically by NeuN/ Smi32 expression. **Results:** F+ SPMS had greater losses within normal grey matter (NGM) and lesion grey matter (LGM) than F- compared to control. Strikingly, the density of pyramidal neurons was reduced by 30% in F+ NGM (p< 0.05) and by 65% in LGM (p< 0.05 and 0.001; layer III and V, respectively). Activated Caspase 3+ or TUNEL+ neurons were restricted to superficial

layers. Pyramidal neuronal abnormalities included expression of PCNA/cyclinD, and condensed/fragmented nuclear structures. A hallmark of F+ GM was an increased density of HLA-DR+, CD68+ and iNOS+ ramified microglia, and occasional parenchymal CD8+ T cells. **Conclusions:** Cortical grey matter of follicle positive SPMS is characterized by overlying inflamed meninges, a gradient of cell damage, activated microglia and a substantial reduction in the density of pyramidal neurons that may give rise to the increased disability and younger age at death in this population. **Supported by:** MRC.

P831

Immune phenotype and aquaporin staining in inflammatory infiltrates in minor salivary glands of patients with neuromyelitis optica, peripheral Sjögren's disease, and multiple sclerosis

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Background: Sjögren's disease can cause both central nervous system (CNS) and peripheral nervous system (PNS) disease. CNS disease has diverse manifestations, such as focal lesions, multifocal brain and spine lesions, and neuromyelitis optica (NMO). PNS disease is largely a sensory-motor axonal neuropathy. Both in the CNS and PNS, most patients have intense inflammation in minor salivary glands. There is no information whether similar immune cells cause damage in salivary glands taken from patients with CNS versus PNS Sjögren's disease. **Objective:** To determine the identity of immune cells and aquaporin staining pattern in labial salivary glands taken from patients with NMO, peripheral Sjögren's disease, and multiple sclerosis (MS). **Methods:** Immunohistochemistry was used to stain for aquaporin-5 and immune cells in labial salivary glands. **Results:** Immunohistochemistry for aquaporin-5 showed diffuse staining in all cases. The staining was most intense in the abluminal wall of the acini and less intense in the basolateral surface and ductal cells. In all cases, areas of inflammation had qualitatively less intense staining pattern in the abluminal surface of the acini, implying damage to the aquaporin-5 channels. The inflammatory infiltrates, when seen, were organized in a follicle-like structures, with majority of CD19+ B cells forming central clusters surrounded by CD3+ T cells. The regulatory T cells (Fox3+) were also organized in the periphery, where CD3+ cells were abundant. The CD138+ plasma cells however were diffusely seen throughout the whole tissue, without any discrete aggregation. Qualitatively, no differences were observed in the amount of staining for immune markers between NMO and peripheral Sjögren's cases. **Conclusions:** Minor salivary gland inflammation in both NMO and peripheral Sjögren's disease is mediated by follicle-like structures. Both NMO and Sjögren's disease have qualitatively similar immune mechanisms causing peripheral tissue damage. It remains to be seen whether similar humoral responses are generated by inflammatory processes seen in CNS and PNS Sjögren's disease. **Supported by:** Brain Research Foundation.

P832

T cell- and macrophage-associated pathology correlates with response to corticosteroids

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Background: Four different immunopathological patterns of demyelination in early active lesions of multiple sclerosis (MS) have been identified, suggesting pathogenic heterogeneity. Individual treatment regimens based on the immunopathological subtype seem promising. Corticosteroid pulse therapy is the standard treatment for acute relapses in MS patients. However, a subgroup of patients seems to be steroid-unresponsive. Recently, selective response to therapeutic plasma exchange in steroid-unresponsive MS patients has been associated with antibody/complement mediated MS pathology (Pattern II). **Objective:** We hypothesized that Pattern I patients would

more likely improve with a high dosage of corticosteroids compared to other MS patterns since their lesions are characterized by a prominent macrophage and T-cell infiltration. **Methods:** 84 patients with a pathologically confirmed fulminant central nervous system (CNS) inflammatory demyelinating disease were retrospectively studied. Immunopathological pattern classifications and clinical outcomes were assessed independently, based on medical records. **Results:** 46 patients were treated with a high dosage of corticosteroids during the acute attack. 16 out of 19 Pattern I patients were considered as treatment successes to a high dosage of corticosteroids, showing complete or marked functional neurological improvement. In contrast, only four out of 19 patients with Pattern II pathology revealed marked neurological improvement, while the remaining 15 patients did not show treatment success. All investigated patients with Pattern III pathology (n=8) did not respond to high dose corticosteroids. **Conclusions:** We conclude that MS patients with T cell/macrophage-mediated demyelination (Pattern I) are more likely to respond favorably to a high dosage of corticosteroids than patients with other patterns of myelin destruction.

P833

Extensive myelin deposition in the meninges of patients with multiple sclerosis

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Background: Multiple sclerosis (MS) is a chronic inflammatory disease characterized by myelin breakdown throughout the central nervous system. Myelin debris has been observed within MS lesions, in cerebrospinal fluid and in cervical lymph node, but the route of myelin transport is unknown. Drainage of interstitial fluid from the brain parenchyma occurs via perivascular spaces and leptomeninges, but the presence of myelin debris in these tissues has never been described. **Objective:** To describe whether myelin products are present in leptomeningeal tissue from MS patients. **Methods:** Formalin-fixed brain tissue containing meninges from a total of 28 MS patients, nine patients without neurological disease and six Alzheimer's disease (AD), and five stroke patients was included. Immunohistochemistry and double-labelling was performed for myelin antigens (PLP, MBP, MOG, CNPase), dendritic cells (DCs; DC-SIGN) and macrophages (CD68). Immunostainings were scored semi-quantitatively. **Results:** Results of this study show extensive extracellular deposition of myelin antigens (PLP, MBP, MOG and CNPase) in the leptomeninges of MS patients. We show that this is MS-specific, as myelin proteins were infrequently or not at all observed in meningeal tissue of non-neurological controls, AD and stroke patients. Although the vast majority of the myelin products were found extracellularly, myelin antigens were occasionally also observed within DC-SIGN+ dendritic cells and CD68+ macrophages. **Conclusions:** We postulate that, following myelin or oligodendrocyte damage in the brain parenchyma, myelin proteins are drained from MS lesions to the perivascular spaces, and from there to the meninges. Chronic availability of myelin antigens in the meninges may modulate the disease process, by either enhancing chronic inflammation or suppressing immune responses inducing tolerogenic effects. **Supported by:** Dutch MS Research Foundation (grant numbers 02-498MS and 06-587MS).

P834

Meningeal inflammation in multiple sclerosis

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Background: Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system. Recently,

immunohistochemical studies demonstrated the occurrence of extensive grey matter demyelination in MS. Mechanisms underlying the development of grey matter lesions are unknown and it has been speculated that meningeal inflammation might be involved in (sub-pial) cortical demyelination. **Objective:** Here, we describe the occurrence of meningeal inflammation in a large post-mortem MS dataset and correlate these data with the presence and extent of cortical demyelination. **Methods:** A total of 103 paraffin-embedded tissue blocks from 28 MS patients and 17 tissue blocks from nine non-neurological controls were labelled for proteolipid protein (PLP), CD3 (T-cells), CD20 (B-cells), CD68 (macrophages), Granzyme B (cytotoxic T-cells), and DC-SIGN (mature/immature dendritic cells). Cell density counts of (cytotoxic) T-cells, B-cells, macrophages and dendritic cells were performed systemically, in randomly chosen areas in the meninges not directly adjacent to cortical lesions. Separate cell counts were performed for meninges directly adjoining cortical lesions. MS tissue sections stained for PLP were measured morphometrically for the percentage of demyelinated cortex. Subsequently, the extent of meningeal inflammation was (spatially) correlated to the extent of cortical demyelination. **Results:** So far, analysis of a subset of our dataset revealed significantly more T-cell infiltrates in the meninges of MS patients compared with controls. However, no relation was found between the extent of global T-cell infiltration in the meninges of MS patients and the extent of cortical demyelination. Additionally, and more specifically, no differences between the extent of T-cell infiltration in the meninges directly adjacent to cortical lesions and normally myelinated cortex were found. **Conclusions:** The infiltration of T-cells in the meninges of MS patients does not seem to be correlated with cortical demyelination. Additional immunostainings for B-cells, cytotoxic T-cells, macrophages and dendritic cells are currently being processed. These results will be presented at the meeting. **Supported by:** Dutch MS Research Foundation (grant numbers 02-498MS and 06-587MS).

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Does the anatomical localization of multiple sclerosis lesions influence the extent of remyelination?

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Background: Multiple sclerosis (MS) lesions are characterized by demyelination, inflammation, astrogliosis and a relative loss of axons. Simultaneously to these destructive processes, repair mechanisms, such as remyelination, occur in MS lesions. Remyelination is a well known phenomenon in early MS. In chronic MS lesions remyelination is limited in the majority of lesions. The underlying cause for limited remyelination in chronic MS lesions is not known. **Objective:** The aim of this study was to analyze and compare the extent of remyelination in early and chronic MS lesions. Additionally, we examined whether the anatomical localization of the lesions influences the extent of remyelination. **Methods:** We analyzed the extent of remyelination in 53 biopsies from 52 patients (early MS) and in 174 lesions from 36 autopsies (chronic MS). **Results:** Out of 53 early MS lesions only 22.6% (n = 12) were completely demyelinated. Out of the remaining lesions 3.8% (n = 2) showed few myelinated fibres, while 30.2% (n = 16) and 41.5% of the lesions (n = 22) displayed patchy remyelination or remyelination throughout the sampled lesion area. Complete remyelination resembling shadow plaques was found in 1 lesion (1.8%). In chronic MS lesions out of the 174 lesions 40% (69 lesions) were completely demyelinated, 34% (n = 59) showed less than 50% remyelination while 12.6% (n = 22) were characterized by remyelination of more than 50% of the lesion area. Only 13.8% lesions (n = 24) showed complete remyelination. Among chronic MS lesions subcortical lesions displayed a higher fraction of lesions with marked remyelination than periventricular lesions (subcortical 44.4%; periventricular 22.9%). The majority of cerebellar lesions (72.2%) was characterized by complete demyelination. **Conclusions:** Remyelination is a frequent event in early MS lesions. In contrast, in chronic MS remyelination is less frequently observed.

Furthermore, our findings indicate that the anatomical localization of a lesion might influence the extent of remyelination.

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P836

Mitochondrial dysfunction within chronically demyelinated axons in multiple sclerosis

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Background: The mechanisms of 'slow burning' axonal degeneration in multiple sclerosis (MS) are not well understood. One potential cause is mitochondrial dysfunction. Mitochondria are the main site of ATP production in axons and are particularly important in demyelinated axons to maintain membrane potential for nerve conduction and calcium homeostasis. We propose that mitochondrial defects in chronically demyelinated axons lead to cytoskeletal changes and ultimately axonal loss in MS. **Objective:** Identify adaptive changes in relation to mitochondrial mass and cytochrome c oxidase (COX) activity and explore mitochondrial defects within chronically demyelinated axons in MS lesions. **Methods:** COX activity and mitochondrial mass (porin immunoreactivity) were determined within morphologically intact large (>2 µm diameter) axons in chronic active and inactive MS lesions (n=32), normal white matter (NWM) in spinal cord and brain from MS cases (n=14) and controls using COX histochemistry, immunohistochemistry and confocal microscopy. Axons were identified using antibodies against [phosphorylated (SMI31) and non-phosphorylated (SMI32) neurofilaments] and amyloid precursor protein (APP). The phosphorylation status of the neurofilaments, which affects their interaction with mitochondria, was determined by Western blotting. **Results:** The majority of demyelinated SMI31 reactive large axons contain increased COX activity and mitochondrial mass compared with myelinated axons in NWM and controls. The neurofilaments within SMI31 reactive demyelinated axons appear to be hyperphosphorylated. The chronically demyelinated axons with APP or strong SMI32 reactivity contain significantly less COX activity and mitochondrial mass compared with SMI31 reactive demyelinated axons. In lesions, COX activity inversely correlates with the density of HLA reactive microglia/macrophages. **Conclusions:** The adaptive changes within chronically demyelinated axons include hyperphosphorylation of neurofilaments, increase in COX activity and mitochondrial mass. These axons are vulnerable to COX dysfunction and mitochondrial depletion, with APP accumulation, non-phosphorylated neurofilaments, impaired nerve conduction and presumably axonal loss.

P837

Central nervous system vasculitis with selective involvement of the left cerebral and infratentorial structures

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Background: Because of the lack of systemic features and since the clinical involvement is limited to central nervous system (CNS), CNS vasculitis represents a puzzling differential diagnosis with multiple sclerosis (MS). **Objective:** To report on two unusual cases of persistent hemi-lateralized brain vasculitis. **Methods:** Observational case report. **Results:** The first patient presented partial seizures 20 years ago, when she was 25 years old. A brain magnetic resonance image (MRI) showed cortical-subcortical lesions of the left cerebral hemisphere, all with enhancement after gadolinium (Gd) infusion. The cerebrospinal fluid (CSF) exam was normal. After 6 years the patient had gait imbalance and diplopia, which improved after iv steroid therapy. MRI showed multiple enhancing lesions on the left pons and cerebellum. The

patient was treated with Azathioprina and low dose oral prednisolone, followed by high-dose iv Cyclophosphamide, which she is still taking with clinical benefit. Numerous brain MRI performed during the follow-up revealed only a mild increase of the number of the lesions with occasional persistence of Gd-enhancement. The second patient is a 33-year-old female who presented language and mnesic deficits 4 years ago. A brain MRI showed a mild atrophy of the left cerebral hemisphere, with multiple lesions in the left cerebral and cerebellar hemisphere, a few of them with Gd-enhancement. Oligoclonal bands were detected in the CSF. The cognitive impairment worsened and after 2 years the patient developed behavioral changes. A new brain MRI showed an increased number of white matter lesions, most of them with enhancement, and a left hemi-atrophy of the brain. Because of the rapid progression of the disease, the patient was treated with Alemtuzumab. Both patients underwent a brain biopsy, which revealed perivascular lymphomononuclear infiltrates, mostly T lymphocytes and macrophages; the second patient had also CD20-positive B cells. The histopathological features confirmed the clinical diagnosis of CNS vasculitis. **Conclusions:** These have been the first cases described of CNS vasculitis, with a selective involvement of the left cerebral and infratentorial structures over a long time.

P838

Diffuse mitochondrial and neuronal damage in the temporal cortex of multiple sclerosis patients is associated with increased occurrence of seizures

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Background: Studying the clinical histories and the neuropathology of 263 multiple sclerosis (MS) subjects in the UK MS Tissue Bank we have previously shown a remarkably higher (14%) prevalence of seizures in MS cases compared to the general population (4%), but the basis of this selectivity is unknown. **Objective:** Our preliminary data indicates a possible association between mitochondrial respiratory chain complex IV defects and epilepsy in MS cases. The following increased oxidative stress could be a crucial initiating event that affects respiratory chain function leading to neuronal death, but the potential correlation with seizures in patients with MS is still unclear. **Methods:** Using post-mortem tissue and corresponding clinical summaries from the UK MS Tissue Bank cases we have performed quantitative and qualitative analysis of the mitochondrial dysfunction and neurodegeneration in the temporal cortex of MS cases with and without seizures and of control cases. Possible correlations with neuropathology and inflammatory conditions in cortical lesions and normal grey matter have also been evaluated. **Results:** Occurrence of seizures in the MS population was mainly detected in the latter phase of the disease and strong correlation was found between seizures and earlier disease and disability onset in examined MS cases. Moreover we found that the presence of seizures in MS patients is strongly associated with increased grey matter demyelination and atrophy, largely affecting the temporal gyrus. Neuronal loss/damage was detected in the temporal cortex of seizure-positive MS cases. Furthermore, mitochondrial respiratory chain complex deficient cells were detected in the cerebral cortex of seizure-positive MS cases. **Conclusions:** Mitochondrial, neuronal and myelin damage in the temporal cortex of MS cases could be directly involved in the increased incidence of epileptic events in the MS population, affecting the disease progression and clinical course.

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P839

Natalizumab decreases the numbers of dendritic cells and CD4+ T cells in cerebral perivascular spaces

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Background: Natalizumab therapy reduces the number of CD4+ T cells within the cerebrospinal fluid (CSF) ten-fold more than the number of CD8+ T lymphocytes. In addition, the effect on cell numbers persists at least 6 months after cessation of treatment. **Objective:** To extend our studies on the prolonged, and differential, effect of natalizumab on T lymphocyte numbers in the CSF, we investigated the number and phenotypes of leukocytes, and the expression of major histocompatibility complex (MHC) I and II in cerebral perivascular spaces (CPVS). We hypothesized that natalizumab reduces the number of antigen presenting cells (APCs) in CPVS. **Methods:** Inflammatory cell numbers in the CPVS of cerebral tissue were assessed by immunohistochemical staining from a patient with multiple sclerosis (MS) who developed progressive multifocal leukoencephalopathy (PML) during natalizumab therapy. Controls included location-matched cerebral autopsy material of patients without central nervous system (CNS) disease, MS patients not treated with natalizumab, and patients with PML not associated with natalizumab therapy. **Results:** The absolute number of CPVS in the patient with MS treated with natalizumab was significantly lower than in all control groups due to extensive destruction of the tissue architecture. The expression of MHC class II molecules, and the numbers of CD209+ dendritic cells (DC) were significantly decreased in CPVS in the patient with MS treated with natalizumab. No CD4+ T cells were detectable. **Conclusions:** Our observations may explain the differential and prolonged effects of natalizumab therapy on leukocyte numbers in CSF.

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P840

Acute demyelination of corpus callosum axons leads to severe axonal conduction deficit and cortical neuronal cell death

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Background: The corpus callosum (CC), the main transverse tract of nerve fibers and callosal projection neurons (II/III, V and VII cortical layers) important for cognitive processes and for motor coordination, show cortical pathology, severe atrophy and axonal loss in long-standing multiple sclerosis (MS) patients. **Objective:** To evaluate i) the degree and physical pattern of CC axonal conduction dysfunction following demyelination; and ii) the potential demyelination-induced callosal pathology as a cause of widespread callosal projection neuronal apoptosis. **Methods:** Preferential demyelination of the CC without primary and minimal secondary inflammation was achieved by 0.2% cuprizone diet (CPZ) fed mice. To analyze consequences of potential remyelination similar to during MS remission some animals were put on normal diet and allowed to remyelinate. The experiments concentrate on the axon tracts of the CC. CPZ mice were analyzed prior to maximal, during maximal, and after maximal demyelination onset. Live brain slices were assayed for CC conduction abnormalities

by compound action potential (CAP), then were post-fixed and analyzed by immunohistochemistry. **Results:** Acute demyelination of CPZ CC was evident between 3 and 6 weeks. CAP amplitude was reduced ~80% in CPZ compared to control CC. Recovery to control level CAP amplitude was observed in remyelinating 3 week CPZ mice. Only 20% of normal CAP recovery was evident in the 6 week CPZ group. Demyelination, focal axonal swelling, amyloid precursor protein accumulation and global nodal protein disorganization (decreased Caspr, re-organized Nav1.6 and Kv1.2 protein) were observed in CPZ mice. Demyelinating and remyelinating 6 week CPZ mice had intense nodal pathology and many Caspase-3+ callosal projection neurons. **Conclusions:** Acute demyelination in the absence of primary inflammation is sufficient to induce callosal dysfunction that in turn causes conduction deficits and potential retrograde callosal neuron cell death. Initial MS therapy should include neuroprotective drugs, along with anti-inflammatory drugs to reduce demyelination-induced axon pathology and neuronal loss.

P841

Patterns of demyelination, inflammation and neurodegeneration in deep grey matter

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Background: Grey matter (GM) demyelination is now recognized as an important feature of multiple sclerosis (MS). While most studies have focused on demyelination, inflammation and neurodegeneration in cortical GM, less data are available on such features in deep GM nuclei. **Objective:** The aims of this study were 1) to characterize the distribution and the extent of deep GM lesions (DGML) in MS brains, 2) to evaluate neurodegenerative changes in DGML, 3) to characterize the mechanisms of inflammation in DGML, in comparison to white matter (WM) and cortical lesions. **Methods:** This study was performed on coronal sections of 14 brains of MS patients, and on three control brains, using immunohistochemistry to detect significant antigens (MBP, HLA-DR, CD68, GFAP, synaptophysin, fibrinogen, IgG, VCAM-1, neurofilaments, beta-APP, CD3, CD20, CD138, ICAM-1). **Results:** DGML were frequently detected in MS brains, more often in the periventricular regions of the thalamus and in the caudate nucleus, but also in putamen, globus pallidus, claustrum, amygdala, hypothalamus and substantia nigra. DGML more often appeared as mixed WM/GM lesions, but also some pure GM lesions were observed, frequently stopping sharply at the border with the WM, similarly to what is often observed in cortical lesions. Active DGML presented features (presence of macrophages, blood-brain barrier damage, perivascular lymphocyte infiltration), more similar to WM lesions than to intracortical lesions. Gliosis and axonal loss were far more severe in the WM part than in the GM part of mixed deep GM/WM lesions. A reduction of neuronal density was observed in DGML, if compared to adjacent NAGM and to controls. No reduction of synaptophysin immunostaining was detected in DGML. **Conclusions:** Demyelinating lesions are frequent in deep GM in MS, with features reminiscent of cortical lesions. Neurodegenerative changes are commonly observed in DGML. The mechanisms of active demyelination in DGML might resemble more those of WM lesions than those of intracortical lesions.

P842

Laquinimod given before and after disease onset reduces inflammatory cell infiltration and demyelination in experimental autoimmune encephalomyelitis

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Background: Laquinimod is a new orally active, well-tolerated substance that suppresses contrast-enhancing lesions in multiple

sclerosis (MS) patients. In animal models of MS, laquinimod inhibits the development of acute and chronic experimental autoimmune encephalomyelitis (EAE). Laquinimod modulates the cytokine balance in favor of Th2/Th3 cytokines. **Objective:** The aim of this study was to assess the effect of laquinimod on demyelination and inflammation in MOG-induced EAE in mice which received laquinimod from the day of immunization or after disease onset. **Methods:** C57BL/6 mice with MOG-induced EAE were treated daily with either DDW or laquinimod (25mg/kg). Laquinimod treatment was started either from the day of immunization (day 0–28) or after disease onset (day 13–28). The spinal cords were processed and evaluated by standard histological analysis. Inflammation, macrophage infiltration, T cell density and demyelination were quantified. **Results:** Mice treated with laquinimod from the day of immunization showed a significantly reduced inflammatory index (0.9±1.6) compared to controls (3.6±0.9) and mice treated with laquinimod after disease onset (3.2±1.1). In total white matter, the animals treated with laquinimod from days 13–28 displayed a marked reduction of macrophages (65.6±15.9/mm² vs. 181.9±136.2/mm², p<0.01) and T cells (48.4±19.6/mm² vs. 94.0±58.9/mm², p=0.056) compared to vehicle-treated mice. However, when this evaluation was related to lesional areas only, these treated animals showed similar densities of macrophages and T cells in lesions as controls, partly due to reduced lesional areas after treatment. Compared with control mice (9.3%±4.3%), the extent of demyelination was significantly reduced in both treatment groups (treated on days 0–28: 2.1%±4.8%; p<0.001; treated on days 13–28: 3.8%±1.9%; p=0.001). **Conclusions:** Treatment with laquinimod is effective in ameliorating the extent of demyelination as well as macrophage and T cell infiltration in white matter, even when treatment is applied after disease onset. These results indicate that laquinimod may play a role in future treatment of MS.

P843

Caveat mTOR: mTOR and LC3 as markers of axonal pathology in multiple sclerosis

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Background: Axonal degeneration, a well-established consequence of chronic axonal demyelination, is a major contributor to the progressive neurological decline experienced by multiple sclerosis (MS) patients. The mechanism by which demyelinated axons degenerate, however, is not fully understood. Recent studies have suggested that certain neurodegenerative diseases may be caused by either a defect in, or an over-exuberance of, basal autophagic mechanisms. The relationship between demyelination, axonal pathology and autophagy has not yet been elucidated. mTOR and LC3, proteins in the autophagy pathway, may have a role in axonal pathology in MS. **Objective:** We investigated whether autophagy markers are expressed by axons in MS brains. **Methods:** We studied white matter (WM) from adult human MS brains and age-matched controls. Using immunohistochemistry, protein analysis and magnetic resonance imaging (MRI), we studied markers for autophagy along with other markers of axonal degeneration. **Results:** We observed that axons in demyelinated WM lesions selectively and robustly express two major molecules in the autophagy pathway, mTOR and LC3, and punctate immunoreactivity was noted throughout axoplasm. Further, a subset of axons in putative Normal-Appearing White Matter (NAWM), identifiable by mTOR and LC3 immunoreactivity, were swollen and tortuous. These axons may contribute to a pathological correlate of Dirty-Appearing White Matter (DAWM), which has been described as subtle changes in T2 by MRI. **Conclusions:** The presence of these molecules may signal either up-regulation of basal autophagy or a failure of autophagosome turnover; both may be attempts to rescue the cell from unfavorable energy conditions. Additionally, it is important to consider the role of LC3 under conditions of axonal pathology. It is possible that LC3 is accumulating in these axons in the wake of catastrophic microtubule depolymerization and is not a harbinger of autophagy. While suggestive of both autophagy and microtubule dysfunction, LC3 and mTOR persist as novel markers of axonal pathology in MS brains. It remains to be determined if these

markers represent a classic autophagy response or a new, axon-specific degradation pathway.

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P844

Alternatively and classically activated macrophages migrate differently in organotypic central nervous system cultures

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Background: Macrophages play an important role in the pathogenesis of multiple sclerosis (MS). Different subtypes of macrophages exist with different functions in immune response and tissue repair. Two extremes are classically activated (CA) macrophages, which can be cytotoxic, and alternatively activated (AA) macrophages, which can be growth promoting. Both types of macrophages have been found in MS lesions, with CA being located mostly at the active border and AA more in the center of the lesion and perivascular. **Objective:** The aim of the study is to discover whether the presence of differently activated macrophages in distinct locations could be caused by differences in migration. **Methods:** The migration of differently activated macrophages was studied in organotypic central nervous system (CNS) cultures and the effect of either demyelination or neuronal damage on migration. Finally, the intrinsic migratory capacity of macrophages was studied by directed migration under the influence of chemokines. **Results:** A higher number of AA macrophages migrated into untreated spheroids compared to CA macrophages. This difference in migration was also seen after the induction of demyelination or neuronal damage. Comparing demyelination and neuronal damage no difference in the number of migrating macrophages was observed. Both CA and AA macrophages could migrate deep into the spheroids and reach neuronal cell bodies and axons; however this was observed more frequently for AA macrophages. To study whether the differences in migration were due to intrinsic migratory capacity of the macrophages, we studied the migration across a filter in a blind well chamber. A higher number of AA macrophages migrated in response to CXCL12 and MCP-1 compared to CA macrophages. Macrophages migrated very well towards a neuronally conditioned medium, indicating the presence of a soluble chemokine in the medium. **Conclusions:** The intrinsic migratory capacity of AA macrophages is greater compared to CA macrophages, even in the CNS environment. In spheroids AA macrophages migrate deeper, possibly due to neuronal attraction, which might also play a role in the localization of AA in MS lesions.

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Surrogate Markers (non-MRI)

P845

Development of a multiplex assay for simultaneous measurement of neurofilament subunits in cerebrospinal fluid of patients with multiple sclerosis

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Background: Neurofilaments (NF) are the principal component of the axoskeleton and are released during axonal damage. Body fluid levels of NF are important candidate markers for axonal degeneration in neurological disorders such as multiple sclerosis (MS). At present, enzyme-linked immunosorbent assays (ELISAs) are available for the quantification of any of the three NF subunits. Immunoassays such as ELISAs provide sensitive, specific and precise quantification of single

biomarkers. However, when applied to the expanding number of biomarkers that will be identified by proteomics, they become expensive and time-consuming to perform and large sample volumes are needed. Thus, multiplex-immunoassays for simultaneously measurement of different proteins in small volumes of precious body fluids (e.g. cerebrospinal fluid (CSF)) are needed. **Objective:** To develop a multiplex assay for NF heavy and light chain subunits, capable of discriminating both subunits in CSF of neurologic patients and negative controls. **Methods:** In Luminex, biomarkers are captured on an array of fluorescently-coded microspheres and detected by the binding of fluorochrome-labeled detection antibodies. The multiplexed assays are analyzed on a Luminex that is equipped with two lasers; one excites the microspheres and one the reporter dye. **Results:** We showed that the multiplex-NF-immunoassay is a reproducible technique with a sensitivity that is comparable to that of solid-phase ELISAs. Further, the stability of the neurofilament subunits under different pre-analytical conditions (different storage temperatures, variation in time until processing, effects if freezing-thawing) was shown. Studies in selected MS patient groups validated the use of this assay to identify axonal damage and for prognosis of MS. **Conclusions:** Multiparameter assays provide a powerful tool for measuring complex protein patterns in body fluids of patients with neurodegenerative diseases.

P846

Glial fibrillary acidic protein in cerebrospinal fluid is a marker for progression in multiple sclerosis

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Background: Glial fibrillary acidic protein (GFAP) is a neuro-specific protein and the major intermediate filament of the cytoskeleton of astrocytes. It is considered to be the morphological basis of astrogliosis and the main protein constituent of chronic lesions in multiple sclerosis (MS). Increased levels of GFAP have been demonstrated in cerebrospinal fluid (CSF) after severe head injury, during dementia and also in MS. **Objective:** To determine whether GFAP is valuable as a biomarker of progression in MS. **Methods:** Twenty-five patients, encompassing 15 relapsing-remitting (RRMS) and 10 secondary progressive (SPMS), and 28 healthy controls were examined twice with a median time of 9 (range 8–10) years apart. Neurological deficits were scored with the expanded disability status scale (EDSS) and CSF was obtained by lumbar puncture. GFAP was analyzed with enzyme-linked immunosorbent assay (ELISA). **Results:** MS patients had increased GFAP levels compared with healthy controls ($p < 0.05$), with the highest levels in the SPMS group ($p < 0.01$). The GFAP levels increased in patients and also controls over time ($p < 0.01$). The GFAP-level correlated with increased EDSS in MS patients at both examinations ($r = 0.47$ $p < 0.05$, $r = 0.51$ $p < 0.01$) respectively, and was more pronounced for SPMS ($r = 0.863$ $p < 0.01$, $r = 0.668$ $p < 0.05$). Although, the GFAP level correlated with age in the control group at the second examination ($r = 0.503$, $p < 0.01$) the relationship between GFAP and EDSS in MS was independent, when controlled for age, at both examinations ($r = 0.44$ $p < 0.05$ and $r = 0.47$ $p < 0.05$). We found that GFAP levels could have a predictive value. There was a significant correlation between GFAP measured at the first time-point and EDSS at the second time-point ($r = 0.41$ $p < 0.05$), with the most pronounced relationship in the SPMS group ($r = 0.67$ $p < 0.05$). **Conclusions:** Increased GFAP levels are associated with MS progression. The GFAP level in CSF might be useful as a predictor for progression. GFAP might be a biomarker for evaluating therapeutic efficacy in MS.

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Viral seroprevalence among Canadian children with clinically isolated syndromes

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Background: Environmental factors, such as viral infection, have been implicated in the pathobiology of multiple sclerosis (MS). Children experiencing their first clinically isolated syndromes (CIS), and thus at risk for MS, are diagnosed in close proximity to putative exposures, with limited time for acquisition of irrelevant viral infections. **Objective:** To evaluate the seroprevalence of common viruses among Canadian children presenting with CIS. **Methods:** Serum samples at presentation were analyzed with standardized assays for IgG antibodies against Epstein Barr virus (EBV), cytomegalovirus (CMV), parvovirus B19, varicella zoster (VZV) and herpes simplex viruses (HSV). **Results:** Of 90 children (mean 9.97 years; female:male ratio 1.19: 1), 51 (57%) were seropositive for remote EBV exposure, 32 (36%) for CMV, 30 (33%) for parvovirus B19, 20 (22%) for HSV, and 77 (86%) for VZV. To date, 17 (19%) meet criteria for diagnosis of MS, 75% of whom were seropositive for remote EBV infection, which did not differ from the remaining 73 children (52%, $p=0.5$, corrected for age). As expected, EBV seropositivity increased with age (OR=1.17, $p<0.001$). EBV nuclear antigen titres were higher in children with confirmed MS (mean=212.9 (s.d.=83.3) vs. mean=186.0 (s.d.=69.8; $p=0.3$). The likelihood of remote EBV infection did not differ by clinical presentation when adjusted for age. However, among the EBV-positive children, monolesional presentations were more common than polylesional ($p=0.03$) or acute disseminated encephalomyelitis (ADEM) ($p<0.001$). 31 children were seronegative for EBV, including four children diagnosed with MS (23.5%). Seropositivity for CMV, parvovirus B19, VZV, HSV did not differ as a function of confirmed diagnosis of MS. **Conclusions:** Of the five common viruses studied, only remote EBV infection and higher EBV titres were implicated as possible associations with an early diagnosis of MS in children at risk. Analysis of the remaining 135 children enrolled in our study ('validation cohort') will be performed to confirm these findings. EBV exposure is not a requisite inciting feature of MS, as approximately 25% of children sampled prior to confirmed MS diagnosis are EBV negative. Further studies are ongoing to identify other putative viral triggers.

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P848

The timed 100-meter walk test: an easy-to-use sensitive tool to detect and evaluate restricted walking capacities in multiple sclerosis

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Background: The timed 25-foot walk (T25FW) weakly correlates with overall walking capacities of multiple sclerosis (MS) patients. We developed the timed 100-meter walk test (T100T), which besides reflecting speed may be more sensitive to other walking parameters such as gait and spasticity-related fatigue. **Objective:** The primary aim of this study was to develop a quantitative ambulation test that correlates with the maximal walking distance in MS patients. **Methods:** In the T100T, the patient is instructed to walk as fast as possible on a distance of 100 meters. Eighty-eight MS patients with an Expanded Disability Status Scale (EDSS) score from 0 to 5.5 and 60 normal controls performed the T100T and the T25FW. In addition, 88 normal controls and 30 patients performed the tests twice. **Results:** T25FW ($R^2 = 0.79$) and T100T ($R^2 = 0.89$) correlated with the nonlinear distribution of EDSS scores. The correlation between T100T and T25FW values was high ($r_2 = 0.81$) for the low (0 to 3.0) and high (3.5–5.5)

scores of EDSS. The intra-class correlation coefficient was better for T100T ($r = 0.921$) than that of T25FW ($r = 0.836$). The range of T100T values in MS patients (40.4 to 114.7 seconds) was 10-fold wider than that of the T25FW (3.0 to 9.1 seconds). The univariate distribution analyses demonstrated that abnormal T100T values appear to be more sensitive than T25FW to predict walking limitations. Finally, the correlation with the reported and/or actual maximal walking distance without aid and rest was significantly better for T100T. **Conclusions:** The T100T proves to be superior to the T25FW in terms of discriminatory power for the detection and evaluation of restricted walking capacities in MS. The T100T should be of interest for clinical trials studying disability worsening and improvement across the spectrum of EDSS. It may provide more sensitive measure for ambulation change in quantifying progressive MS pathology.

P849

Intrathecal IgM response against S-nitrosocysteine in multiple sclerosis

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Background: There is currently no biomarker in multiple sclerosis (MS) to help monitor the clinical activity. Here we present a potential surrogate marker, IgM to S-nitrosocysteine epitope (SNO-cys), that we detected in MS cerebrospinal fluid (CSF). **Objective:** Anti-SNO-cys IgM were previously found in serum in correlation with MS clinical activity. Our aim was to detect them in CSF and determine whether they were produced in the brain. Evaluation of intrathecal synthesis requires the serum to be tested simultaneously with the CSF to rule out a passive transfer of immunoglobulins from blood. **Methods:** Pairs of serum and CSF obtained from 17 MS patients and 19 neurological controls were tested for SNO-cys IgM, MOG IgM, and total IgM content with in-house enzyme-Linked immunosorbent assays (ELISAs). **Results:** Anti-MOG IgM were negative in all CSF. A significant increase of SNO-cys IgM was found in the CSF of 41% of MS patients when compared to the neurological control group ($P=0.012$), as well as a significant increase in total IgM in MS CSF versus the control CSF ($P=0.0003$). In contrast, there was no significant difference in serum between the two groups. The high correlation between SNO-cys IgM and total IgM in MS CSF ($r=0.81$, $P<0.0001$) prompted calculation of intrathecal synthesis using an approach developed by Blennow et al. (1993). In brief, this method is based on a linear function with the IgM index acquired from numerous healthy volunteers as a constant, and calculates the IgM intrathecal production using individual albumin quotient and serum IgM content. In this manner, we found that whenever SNO-cys IgM were above a cut-off threshold this coincided with positive intrathecal IgM production. **Conclusions:** These data lend support to brain production of a novel class of antibodies SNO-cys IgM, and suggest the IgM intrathecal synthesis in MS is underestimated by commonly used formulas. Further analysis is under way to correlate MS clinical activity to SNO-cys IgM levels.

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P850

Neuromyelitis optica (NMO) positive antibodies confer a worse course in relapsing-NMO in Cuba and French West Indies

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Background: Neuromyelitis optica IgG (NMO IgG) antibodies are a useful tool for the diagnostic criteria of NMO. Japanese studies suggest a worse course in cases with positive antibodies. **Objective:** To compare the NMO IgG antibodies (+/-) with demographic, clinical, disability and magnetic resonance imaging (MRI) data. **Methods:** We studied 48 relapsing-NMO (R-NMO) patients (Wingerchuck et al. 2006), from Cuba (24) and the French West Indies (24). Demographic, clinical, disability, laboratory and fatality index data were obtained from the cases. NMO-IgG antibodies were tested on sera by an indirect immunofluorescence (Lennon et al. 2004). Cuban patients were tested at Barcelona and West Indian cases at Lyon. Brain and spinal cord MRI was done in all cases but for spinal cord in acute relapses and at its remission. **Results:** Sixteen cases were NMO IgG (+) and 32 NMO IgG (-). NMO IgG (+) have more attacks (9.3 +/-6.7) than NMO IgG (-) (5.0 +/-3.1) (p = 0.0003), with more spinal attacks (5.9 +/-5.4 vs 2.5 +/- 2.2; p = 0.003), higher Expanded Disability Status Scale (EDSS) (6.6 +/-2.3 vs 4.4 +/-2.0; p = 0.003), with FS motor (3.6 +/-1.6 vs 2.3 +/-1.5; p = 0.02) and FS sensorial (2.9 +/-1.8 vs 1.7 +/-1.5; p = 0.02). Brain MRI showed that NMO IgG (+) had three or more periventricular lesions (60% vs NMO IgG (-)15.6%; p = 0.002) and more deep white matter lesions (73.3% vs 34.4%; p = 0.02). Spinal cord MRI at acute relapses did not differ between NMO IgG (+) or (-). Spinal cord MRI lesions at remission demonstrated that NMO IgG (+) had more involvement of: number of vertebral segments (6.0 vs 3.9; p=0.04); more grey matter (88.9% vs 47.8%; p=0.04); white matter (77.8% vs 30.4%; p=0.02) and holocord involvement (55.6% vs 13.06%; p=0.02). **Conclusions:** NMO IgG positive antibodies confer a worse course with more attacks and more disability in R-NMO.

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P851

Lachrymal isofocusing: an adapted method for diagnosis of multiple sclerosis

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Background: In clinically isolated syndrome (CIS), cerebrospinal fluid (CSF) immunoglobulin G oligoclonal bands detection (OCB) is a well known predictive factor of conversion to multiple sclerosis (MS), but CSF examination is considered to be invasive. Few studies have

shown the usefulness of tears-OCB detection for the diagnosis of MS. **Objective:** The aim of this study was to assess the predictability of tears-OCB detection in patients with CIS, for the presence of CSF-OCB. **Methods:** We included prospectively patients with CIS and compared the results of CSF-OCB and tears-OCB collected by a Schirmer strip. We used classic isoelectrofocusing on agarose gel followed by an immunoblotting process method. A alternative dilution technical is used for this analysis. **Results:** Fifty-three patients were included. CSF-OCB and tears-OCB were found in respectively 65.9% and 36.4% of patients with a concordance rate between CSF and tears of 70.5%. All patients with tears-OCB had CSF-OCB. **Conclusions:** According to our results, tears-OCB detection, if positive, may replace CSF-OCB detection in patients with CIS. Many lumbar punctures currently realized in this objective could thus be avoided.

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P852

Measuring upper limb functioning in multiple sclerosis: is the Disabilities of Arm, Shoulder and Hand (DASH) fit for purpose?

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Background: Multiple sclerosis (MS) frequently, and often progressively, affects people's upper limb function. However, there are currently no MS-specific rating scales to measure this important outcome. Despite this, many site-specific measures of upper limb functioning exist. Probably the best known is the Disabilities of the Arm, Shoulder, and Hand (DASH) which has been used in over 250 studies, mostly in musculoskeletal conditions. **Objective:** As the DASH's measurement performance in MS is unknown, our objective was to determine whether it is fit for purpose as a measure in MS research. **Methods:** Two phases of psychometric analyses were conducted on DASH data from n=300 persons with multiple sclerosis. First, traditional psychometric methods were used including tests of: data quality, acceptability, scaling assumptions, score reliability and validity. Second modern psychometric methods (Rasch analysis) were used including tests of: ordering of response categories, statistical and graphical tests of fit, and item dependency. **Results:** Traditional psychometric analyses implied that the DASH is a reliable and valid measure of upper limb function in MS. However, Rasch analysis diagnosed problems with the DASH (response category disordering [9/30 items], item misfit [13/30 items], and item dependency [six pairs of items]) and identified how they might be fixed. **Conclusions:** This study raises two key implications for measuring upper limb functioning in MS. First, the DASH could be a useful measure if its measurement problems are solved. Specifically, reduce the number of response categories, select the most clinically conformable sub-sets of items, and expand these to form more clinically meaningful sub-scales. Second, this study further illustrates the value of Rasch analysis for diagnosing and solving measurement problems, and maximizing outcome measurement in MS.

P853

Use of a customized TaqMan low density array to investigate the effects of interferon beta neutralizing antibodies demonstrates a differential inhibition of gene expression

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Background: The clinical effects of neutralizing antibodies (NAbs) to interferon beta (IFNβ) during treatment of relapsing-remitting multiple sclerosis (RRMS) are not clearly understood. IFNβ induces a large number of biological responses making it difficult to identify a single mechanism of action responsible for its efficacy in MS. Although cellular responses to IFNβ can be considered wide-ranging, only two basic approaches are used to measure activity: the antiviral protection and MxA assays. Neither of these provide information directly linked

to the immunomodulatory activities of IFNB. **Objective:** Based on different gene expression patterns observed in peripheral blood mononuclear cells of RRMS patients naive to treatment and those treated with IFNB-1b we have defined a regulated set of IFNB and RRMS associated genes. **Methods:** This unique gene set includes counter regulated genes, those associated with Th1-Th2 response, adhesion, chemotaxis, IFN signalling, and cell cycle responses. This 'fingerprint' of genes was formatted onto a customized microfluidic gene card (IFN Response in clinical Samples; IRIS) with relevant housekeeping and cell lineage genes as controls. Sera from RRMS patients were assayed using IRIS to measure Nab dependent inhibition of gene expression; neutralizing titres were calculated using the Kawada formula. **Results:** Neutralization of gene expression was not the same for all genes analyzed and a wide gradient of inhibition was observed that was not dependent on the magnitude of gene expression. Effects of NABs generated against IFNB-1b, IFNB-1a and the WHO standard will be presented. **Conclusions:** Our results demonstrate that measuring the effects of NABs using IRIS offers a major advantage over other assays by providing a more comprehensive way to monitor IFNB neutralizing activity. Current efforts to make use of this approach to clarify the effects of NABs on IFNB treatment and determine if IRIS can predict response to treatment will be discussed.

P854

Influence of time of day on Expanded Disability Status Scale, motor, and psychological fatigability in multiple sclerosis patients

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Background: Multiple sclerosis (MS) patients often experience substantial changes of neurological function during the daytime, specifically after physical exercise. **Objective:** To investigate Expanded Disability Status Scale (EDSS) as well as functional scores (FS) and cognitive changes during one single day in MS patients. **Methods:** 24 MS patients with baseline EDSS scores between 3.0 and 6.0 were examined in the morning, after physical exercise (home trainer; average power: 50 watts), and in the late afternoon on one single day. At all three time points EDSS (including all FS), walking distance and time, timed 50 meter walk and the Paced Auditory Serial Addition Task (PASAT) were evaluated. **Results:** The median EDSS scores and single FS remained unchanged at all time points. The mean walking distance was significantly reduced after exercise but recovered before the third examination (319 vs. 260 vs. 326 meters) and the median 50 meter walking time was significantly longer after exercise and returned to baseline at the evening examination (38 vs. 44 vs. 39 seconds). As expected, there was a learning effect of the PASAT with significantly improved mean values at the second examination which persisted until the last testing (39 vs. 43 vs. 44 correct answers). The EDSS scores were most strongly influenced by walking distance and the 50 meter timed walk. **Conclusions:** Single FS and overall EDSS scores were independent of the time of day although motor fatigability occurred. The time of day of the clinical examination does not influence robust but relatively insensitive outcome measures as the EDSS but might have an influence on more sensitive measures as timed walking which contributes to the Multiple Sclerosis Functional Composite (MSFC).

P855

Search of new biological markers of interferon-beta therapy

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Background: Myxovirus A (MxA) is a protein encoded by the MX1 gene with potent antiviral activity. MxA has proven to be a sensitive measure of the biological response to interferon-beta (IFNB) treatment and its activity is significantly reduced during the development of neutralizing antibodies (NABs). Nevertheless, the use of MxA as a biological marker for IFNB treatment in multiple sclerosis (MS) has been criticized for the lack of evidence of its role on disease pathogenesis

and clinical response to IFNB. **Objective:** To identify new biomarkers of IFNB treatment that are specifically induced by type I IFNs and may have immunomodulatory effects on MS. **Methods:** Gene expression microarrays (Affymetrix Human Genome U133 Plus 2.0) were performed in peripheral blood mononuclear cells from eight MS patients at baseline and after 3, 12 and 24 months of IFNB treatment. Four patients were negative for NABs and four patients developed NABs at 12 and/or 24 months. Changes in gene expression induced by IFNB over time in NAB positive and NAB negative patients were analyzed. **Results:** Nine genes followed a pattern in gene expression over time similar to the MX1 and were selected for further experiments. These genes were significantly induced by IFNB treatment and their expression was greatly reduced by the presence of NABs. Changes in gene expression of the selected genes were validated by real time PCR. In vitro experiments to characterize their specific induction by type I (IFNB and IFN- α) but not type II (IFN- γ) IFNs are currently in progress. **Conclusions:** Selected genes are potential new biomarkers of IFNB bioactivity that may have functional roles in MS and in the clinical response to IFNB.

P856

Serum levels of the B cell chemokine attractant CXCL13 are predictive of the presence and number of enhancing lesions on brain magnetic resonance images in patients with multiple sclerosis

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Background: There is a need for biomarkers of disease activity in multiple sclerosis (MS). The highly variable course of MS marked by relapses and/or progression makes it imperative to identify useful biomarkers to help optimize therapy in individual patients over time. **Objective:** To determine if serum levels of the B cell attractant chemokine CXCL13 are related to the presence of inflammatory lesions on matched gadolinium-enhanced brain magnetic resonance images. **Methods:** Seventy-five subjects with clinically isolated syndrome (CIS) or relapsing-remitting multiple sclerosis (RRMS) were prospectively followed with monthly brain MRI for up to two years. Participants were also seen every 3–4 months and at time of suspected relapses for clinical assessments. Blood was drawn every six months right before MRI and frozen for later analyses. We measured serum levels of CXCL13 by Enzyme-Linked Immunosorbent Assay (ELISA) (R&D Systems). **Results:** A total of 229 serum samples with matched MRIs from 75 patients were evaluated for CXCL13 levels. In patients with two or more serum samples, there were 14 patients who always had serum levels ≥ 40 pg/ml, 39 patients who intermittently had serum levels ≥ 40 pg/ml, and only seven patients whose serum levels were consistently < 40 pg/ml. Serum samples corresponding to MRI with gadolinium enhancement had significantly higher levels of CXCL13 than samples obtained at times of non-enhancing MRIs (44.2 pg/ml (n=111) versus 60.6 pg/ml (n=118), $p < 0.01$). Serum CXCL13 ≥ 40 pg/ml was highly predictive of having ≥ 5 enhancing lesions on MRI (sensitivity 76%). **Conclusions:** We found a significant and important correlation between elevated serum levels of the B cell chemokine CXCL13 and the presence and number of enhancing lesions on brain MRI. Serum CXCL13 was strongly associated not only with an active scan, but more importantly with having a pattern of consistently active brain MRI over time. The potential clinical utility of this serum marker is supported by the findings that a serum CXCL13 level ≥ 40 pg/ml had high sensitivity for a very active brain MRI (≥ 5 enhancing lesions), and good sensitivity (66%) to the presence of at least one enhancing lesion.

P857

Neural cell adhesion molecule as a biomarker of neuro-regeneration in experimental autoimmune encephalomyelitis
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Background: Neural cell adhesion molecule (NCAM), also known as CD 56, is expressed mainly in the nervous system (neurons, astrocytes, oligodendrocytes), but also in skeletal muscle and by natural killer cells. It mediates cell-cell interactions in both the developing and mature nervous system and is critical to axonal growth, guidance, synaptogenesis and interactions of motor axons and muscle. **Objective:** To objectively assess NCAM levels in control, remission, and chronic experimental autoimmune encephalomyelitis (EAE) mice and to compare them with their disease course. **Methods:** Spinal cords were removed from ABH mice with EAE at remission (following and acute attack and one relapse) and end stage of the disease (chronic) as determined by clinical score, and from control animals. Tissues were homogenized in T-PER with protease inhibitors and stored at -80C until use. NCAM protein levels were calculated using a sandwich enzyme-linked immunosorbent assay (ELISA) and controlled for total protein. **Results:** NCAM values were significantly elevated in remission compared to either control or chronic tissues. Mean NCAM values were as follows: control 134.9 ± 18.3 pg/mg, chronic 189.2 ± 24.3 pg/mg and remission 595.1 ± 39.9 pg/mg; p=0.007. The rise in NCAM values was associated with an improvement in the clinical score of the mice. **Conclusions:** We report that NCAM values are elevated during the remission stage in EAE, which goes in hand with clinical improvement. However, this rise is not maintained in the chronic stages of EAE. NCAM measurements can therefore be utilized in measuring neuroregeneration in EAE, and assessing therapeutic efficacy of neuroprotective agents.

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P858

Increase of serum uric acid levels in multiple sclerosis during treatment with natalizumab

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Background: Uric acid (UA), a product of purine metabolism, is an endogenous peroxynitrite scavenger. Several pieces of evidence have demonstrated that the mean serum UA level in clinically active multiple sclerosis (MS) patients was significantly lower than in clinically inactive MS patients or controls. **Objective:** To propose UA as a marker of disease activity. **Methods:** UA serum level were measured in 12 relapsing-remitting MS patients, who underwent Expanded Disability Status Scale (EDSS) evaluation and brain magnetic resonance imaging (MRI), and in nine healthy age and sex-matched controls. In all subjects the blood samples were taken after 5 days of the same diet. The Student t test was used to compare the groups. The linear regression analysis was used to determine the correlation between UA levels, EDSS (mean: 3; SD: 1.1), annualized relapse rate (ARR: mean: 1; SD: 0.4) and number of MRI enhancing lesions (mean: 1.8; SD: 1.7). Eight of the MS patients underwent natalizumab treatment (300 mg endovenously, every month). The T-paired test was used to compare UA levels before and after 6 months of therapy. **Results:** There was a significant difference between mean UA serum level in MS patients (mean: 3.6 mg/dl; SD: 0.8) and controls (mean: 4.6 mg/dl; SD: 0.9) (p=0.01). A significantly inverse correlation between UA levels and EDSS were found (r=-0.57; p=0.05), while the statistical analysis showed no considerable correlation with ARR (r=-0.1, p=0.64) and MRI activity (r=-0.08; p=0.8). Finally mean serum UA level was significantly increased after 6 months therapy with natalizumab (p=0.003). **Conclusions:** Although the role of UA in MS pathogenesis is unknown, it might reflect the disability degree and the response to the treatment.

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P859

Biomarkers of disease activity in multiple sclerosis patients treated with immunomodulatory agents

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Background: Disease activity is highly variable in multiple sclerosis (MS) patients both before and under immunomodulatory treatment. Several effects on the expression of immunologically relevant molecules are known to occur in blood during treatment with IFN-β and glatiramer acetate (GA). However, the extent to which biomarkers of immune activation can identify disease activity during immunomodulatory treatment is uncertain. **Objective:** We aimed at identifying blood biomarkers for disease activity under immunomodulatory treatment. **Methods:** In 39 patients with relapsing-remitting MS scheduled for IFN-β (n=28) or GA (n=11) treatment, we collected blood samples before and after 3 and 6 months of treatment. IFN-β treated patients had blood samples drawn 9–12 hours post injection; in GA treated patients blood samples were not timed. Expression of TNF-related Apoptosis-Inducing Ligand (TRAIL), Interleukin(IL)10, IL12A, IL23, IL27, IL1B, IL1RN, IL15, FOXP3, Matrix Metalloproteinase-9, and the cyclin inhibitors p22 and p57 was determined by real-time reverse transcriptase polymerase chain reaction (RT-PCR) with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as reference molecule. Magnetic resonance imaging (MRI) with Gadolinium (Gd) contrast was performed before treatment and after 3 and 6 months of treatment. **Results:** The number of Gd+ lesions before treatment correlated with the number of Gd+ lesions after both 3 and 6 months of treatment. Expression of IL10, TRAIL and IL27 correlated negatively with MRI disease activity before treatment. None of the markers correlated with MRI disease activity at any time during treatment. **Conclusions:** Differences in disease activity shown by MRI during the first 6 months of immunomodulatory treatment are not reflected by differences in the expression of several biological response-markers in blood, whereas Gd+ lesions before therapy predict MRI activity on therapy. This may suggest that the variation in the observed MRI disease activity is not associated with a biological non-responder phenomenon. Rather, it may be a matter of variation in underlying disease activity, as reflected by the association of MRI activity before and during treatment.

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P860

Lessons from the Cannabinoids in Multiple Sclerosis (CAMS) Study: part 2 - what happens when the Ashworth scale makes the grade?

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Background: In the sister abstract (Lessons from the Cannabinoids in Multiple Sclerosis (CAMS) Study Part 1) we demonstrated that the Ashworth scale total body spasticity score did not meet requirements for reliable or valid measurement. **Objective:** Here we attempt to solve some of the problems identified, optimize the Ashworth's performance, and re-analyze the results of the CAMS study. **Methods:** In Stage 1, we Rasch analyzed data from a sub-sample of patients (n=98) to use a dataset uncontaminated by inter-rater variability. This identified two key problems: too many response categories; upper limb muscles did not "fit" coherently with lower limb muscles. In Stage 2, we attempted to solve these problems by constructing an instrument from the Ashworth data that satisfied both clinical and psychometric requirements. This involved creating an eight-item lower limb spasticity scale with less response categories. In Stage 3, we re-analyzed the CAMS dataset (n=667) post hoc. **Results:** When compared with the

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original 20-item scale, the new eight-item scale: - generated significantly different spasticity estimates for 89% of people; - generated group level results more consistent with patient-reported improvements (effect sizes: placebo = 0.19; marinol = 0.30; Cannador = 0.29); - detected more individuals with significant improvements (n=109 vs 74). Nevertheless, the targeting of both 20- and eight-item scales was suboptimal, thus underestimating changes in spasticity in all groups. **Conclusions:** Poor measurement undermines the inferences from clinical trials. Here, improving the Ashworth scale altered the results of the CAMS study. Although the treatment effect was still not statistically significant the poor targeting of both the original and new Ashworth scales compromised their ability to detect change and, as such, underestimates the potential treatment effect. Further development could produce a clinician scale suitable for clinical trials. However, for now, we are still left uncertain as to the role of cannabis as a symptomatic treatment for people with MS.

P861

Optic coherence tomography in cases with optic neuritis

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Background: Optic neuritis (ON) refers in general to any inflammatory optic neuropathy and is often an early sign of MS. The evaluation of optic nerve has a great importance for clinical practice with respect to both differential diagnosis and demyelination or axonal damage of optic nerves. Optic Coherence Tomography (OCT) is seen as a new method that could be beneficial in this issue, and moreover there are few papers on this subject in the related literature. **Objective:** The aim of the study is to determine demyelination or axonal damage and establish the prognosis of ON during acute and chronic stages. **Methods:** In the present study 20 patients with ON were evaluated. Retinal fiber layer thickness (RFLT) in the patients were measured and compared with the demographic and clinical features. Relation visual evoked potentials (VEPs) were also measured and compared with RFLT. **Results:** Eleven of the patients were in acute/subacute periods, nine were in chronic periods. The average RFLT of the involved eyes was 98.8, and that in non-involved eyes was 105.41 micrometer. There was an average relation between average RFLT and VEP latencies (R=0.41). The strongest relation was in the quarter temporal area (r=0.51). There was not any significant relation with orbital and brain magnetic resonance imaging (MRI) findings. An average relation between RFLT and VEP was established. The lack of definite difference of RFLT between patients with or without ON indicated subclinical involvement. **Conclusions:** In conclusion, the relationship with MRI could be appreciated in a healthier manner if the number of cases is to be increased. The findings indicate that OCT, being a non-invasive and sensitive method, can be used as a primary ending point in the evaluation of ON.

P862

Performance of an anti-GAGA4 IgM assay in distinguishing multiple sclerosis (MS) patients from control groups in a US cohort: a cross sectional retrospective study

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Background: Previous studies have shown that anti-Glc(α-1,4)Glc(α) (GAGA4) IgM antibody enzyme immunoassay (EIA) can distinguish multiple sclerosis (MS) patients from other neurological diseases (OND) and healthy controls (HC). **Objective:** To confirm the ability of anti-GAGA4 IgM EIA to differentiate diagnosed MS patients from

HC and OND patients positive for other neurological related antibodies in a US cohort. **Methods:** Cross-sectional retrospective analysis of frozen sera from a US cohort consisting of 640 MS patients (48.5 ± 11.8yrs), 100 HC (39.9 ± 12.3yrs) and 31 OND controls (61.2 ± 16.4yrs) was carried out. Sera with masked identity were diluted 1:1200 and EIA units (EU) of anti-GAGA4 IgM were measured by gMS® Dx Immunoassay (Glycominds, Lod, Israel) and normalized by dividing by the square root of total IgM levels (mg IgM/mL serum). Anti-GAGA4 IgM cutoff level for determining antibody status (positive/negative) was determined at the upper 15 percentile of non-MS controls. **Results:** A correction for age was performed since anti-GAGA4 IgM EU/(mg/mL)0.5 levels decreased with age (slope coefficient -0.455(EU/(mg/mL)0.5/year), p<0.0001, linear regression). MS patients had significantly higher (p<0.0001, Mann-Whitney U test) levels of anti-GAGA4 IgM (median, 49.0 EU/ (mg/mL)0.5 than both HC (median, 35.5 EU/(mg/mL)0.5) and OND controls (median, 31.8 EU/(mg/mL)0.5. ROC curve analyses showed that anti-GAGA4 IgM has the ability to discern MS from HC (AUC: 0.70) and MS from OND controls (AUC: 0.83). The cutoff for antibody positivity was set at 57 EU/ (mg/mL)0.5, leading to a sensitivity of 38.8% (95% CI: 35.0–42.7%) for detecting MS patients with a specificity of 84% (95% CI: 75.3–94.6%) for HC and 100% (95% CI: 86.7–100%) for OND controls. Interestingly, anti-GAGA4 IgM levels were slightly increased with longer duration of MS (Spearman's rho=0.08, p=0.04). **Conclusions:** We confirmed that the anti-GAGA4 IgM EIA assay can differentiate MS patients from healthy controls and patients positive for other neurological related antibodies.

P863

Quantification of the retinal nerve fiber layer during acute unilateral optic neuritis and association with tests of visual function

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Background: Optic neuritis (ON) is the initial presentation in 20% of cases of multiple sclerosis (MS). ON can lead to swelling of the optic nerve. Optical coherence tomography (OCT) is a new non-invasive way to quantify the thickness of the retinal nerve fiber layer (RNFL) and macular volume (MV). **Objective:** To quantify the thickness of the RNFL and the MV in acute unilateral ON. To investigate the association of the RNFL and MV with tests of visual function. **Methods:** Forty patients with acute unilateral ON and no prior history of demyelinating disease were examined with OCT to measure the RNFL and MV of both eyes within 28 days after onset. Visual function was tested with a Low-Contrast Sloan Letter Chart (LCLC) (100%), Velhagen colour plates, visual field and visual evoked potential latency. **Results:** The RNFL was significantly increased in the affected eye acute phase compared to the fellow eye (131.3 μm SD 55.5 vs 98.1 μm SD 11.6 p=0.0005). There was no significant difference between the macular volume (7.26 mm³ SD 0.49 vs 7.15 mm³ SD 0.40 p=0.0859). The RNFL of the affected eye showed no significant change associated with the visual tests. The fellow eye showed a significant association with the LCLC 100% yielding a regression coefficient of -0.55 μm (p=0.0103). The MV of the affected eye showed no significant change associated with the visual tests. The fellow eye showed a significant increase of 0.09 mm³ per 1 dB increase in the mean deviation of the visual field (p=0.0027). **Conclusions:** Acute unilateral ON causes significant thickening of the RNFL compared to the fellow eye but no associations with visual tests could be found in the affected eye. More data will be analyzed.

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P864

Does soluble CD40L represent a marker for disability in multiple sclerosis?

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Background: A challenging area in multiple sclerosis (MS) care is finding a biomarker that may be an indicator of disease severity or prognosis. Many of these biomarkers center on the T-cell and cytokines surrounding their activation. Recently, there has been a renewed interest in the B-cell's role in disease progression. B-cells can be activated with the help of T-cells via co-stimulation of receptor pairs CD40 (B-cell) and CD40L (T-cell). It has been suggested that elevation of CD40L may be an indicator of progression. **Objective:** To determine plasma beta-amyloid (AB), soluble CD40 and CD40L in persons with MS. **Methods:** A group (n=15) of MS patients and healthy individuals (n = 31) were recruited as part of a biomarker project. During the visit, blood was collected, and neurologic exam was performed (Expanded Disability Status Scale (EDSS) was extracted) and memory testing was administered (included Mini Mental State Examination (MMSE)). The study was approved by the Western IRB. A Mann-Whitney test was performed due to small sample size and the two-sided alpha was set at 0.05. **Results:** The MS group consisted of 15 females with mean age of 51.5±9.0 years and EDSS score of 2.7. Thirty-one healthy individuals were used as controls (mean age=64.55±3.3). Soluble CD40 was elevated in those with MS compared to controls (median=87.5 [46.8–127.5] pg/ml vs. 31.6 [18.0–57.2] pg/ml, p=0.002). CD40L was not found to be significantly different in MS compared to controls but it was found to positively correlated with EDSS (rho=0.612, p=0.015). Interestingly, AB40 was also found to be elevated in persons with MS (median=83[67–93] pg/ml) compared to controls (median=62[52–77] pg/ml). **Conclusions:** Although it is not a surprise to see changes in inflammatory parameters in MS, it is interesting to see elevated soluble CD40 receptor given the recent evidence of B-cells importance in MS treatment (e.g. rituximab). In addition, the correlation between CD40L values and EDSS supports prior studies where CD40L was suggested to be linked to disease progression. In the future, we plan to expand our database, follow these patients longitudinally, and explore our finding of elevated plasma AB40 in MS.

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P865

Low sensitivity and specificity of AQP4 IgG in multiple sclerosis and neuromyelitis optica in Australians

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Background: The spectrum of demyelinating disease in Western countries is characterized by a predominance of conventional multiple sclerosis (MS) and a small proportion of neuromyelitis optica (NMO). It has been argued that relapsing NMO and optic-spinal MS is different from conventional MS, and that a recently identified serum autoantibody AQP4 IgG can distinguish NMO from conventional MS. **Objective:** Our study aimed to investigate the prevalence of the anti-AQP4 antibody in conventional MS, NMO and longitudinally extensive spinal cord lesion (LESCL) patients from a Western Australian MS cohort. **Methods:** A subset of 94 patients from a population based Western Australian MS cohort (1) of 842 cases was examined and AQP4 IgG testing performed using a validated assay at Kyushu University. **Results:** There were 74 classic MS (CMS) of whom 23 were primary progressive (PPMS), 19 NMO, two longitudinally extensive transverse myelitis and one tumefactive demyelinating case. There were only two cases found to be AQP4 IgG seropositive. One positive case was found among 74 CMS patients. The 23 PPMS were all seronegative. Only one of the 19 NMO patients was seropositive. In our WA MS cohort there were three NMO patients previously NMO-IgG negative (Mayo Clinic), but only one was included in this pilot

study and was also AQP4 IgG negative. **Conclusions:** Our study showed a very low rate of anti-AQP4 antibody in Caucasian MS patients in comparison with other studies using the same laboratory. These results emphasize the need for further study on the clinical utility of AQP4 serology (2). We are preparing a comprehensive serological and immunogenetic examination of the total cohort of 842 patients.

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P866

Clinical and magnetic resonance imaging features of patients with anti-aquaporin-4 antibody

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Background: A serum IgG autoantibody that selectively binds to aquaporin-4(AQP4) water channel is considered a specific biomarker of neuromyelitis optica(NMO). **Objective:** To identify the characteristics of patients with anti-AQP4. **Methods:** We analyzed the demographic, clinical and magnetic resonance imaging (MRI) findings of 41 patients with anti-AQP4 antibody or NMO-IgG. **Results:** There was a female predominance (90%) with mean age of onset of 29.6 (8–52) years. Over the 6.1±4.8 years of average disease duration, optic neuritis (ON) occurred in 68%, myelitis in 98%, and brain involvement in 44% of patients. Only 66% of patients had both ON and myelitis. The mean annual relapse rate was 2.1±1.6. Most patients had severe relapses in their clinical courses; 82% with ON had visual acuity less than 0.1 in at least one eye and 75% of patients with myelitis were unable to ambulate without assistance. Longitudinally extensive cord lesions preferentially affected the central gray matter were found in 98% with myelitis and 40% of them were extended into the brainstem. Cord atrophy was also common (58%). On brain MRI, 73% showed abnormal lesions in their disease courses. 60% literally fulfilled the Barkhof MRI criteria, however their MRI features were atypical for prototypic MS in terms of commonly involved locations and configuration. Most lesions were typically located in the cerebral peduncle(63%), medulla(53%), posterior limb of internal capsule(50%), cerebellar peduncle(40%), and adjacent to the third and fourth ventricles (33%). Extensive confluent hemispheric lesions were found in 40% and elongated curvilinear lesions of subcortical white matters in 27%. Some brain lesions were also longitudinally extensive from the internal capsule to the cerebral peduncle which appears to be synonymous of longitudinally extensive myelitis. The prognosis was poor. Eighteen patients (44%) were functionally blinded in at least one eye and 15 patients(37%) needed assistance on gait due to poor recovery. **Conclusions:** Our results show that the clinical and MRI features of patients with anti-AQP4 antibody are distinct from prototypic MS. In addition, brain involvements were common and their MRI findings were characteristic enough to distinguish from MS.

P867

Electrophysiologically-eloquent pontine inflammatory lesion causes reversible loss of wave V on brainstem auditory evoked response

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Background: There is disagreement in the literature as to the precise location of generators of wave V of brainstem auditory evoked response (BAER). We have found no reported cases where an isolated

inflammatory brainstem lesion would cause selective loss of wave V with preservation of other parts of the waveform. **Objective:** To describe a case where a small, isolated demyelinating/ inflammatory lesion in the area of lateral lemniscus caused a reversible loss of wave V. **Methods:** Case report. **Results:** A 45 year-old woman presented with acute-onset numbness over the right side of the face, mouth, tongue and fullness in the right ear, and was found to have a 10x8x8 mm enhancing lesion in right mid-pontine tegmentum. BAERs showed no recordable wave V, while latencies of waves I and II on both sides, and all the responses on the left side, were within normal limits. Work-up for systemic inflammatory disorders and infectious etiologies was unremarkable. She was given a diagnosis of Idiopathic Inflammatory Demyelinating Disease. No treatment was pursued. At 16-month follow-up, she had near-resolution of clinical symptoms and radiographic findings, and complete re-normalization of BAER waveform. **Conclusions:** The lesion in our patient allows for exquisite localization of wave V generators on the BAER to an area less than a cubic centimeter in volume in mid-pontine tegmentum. The lesion would be expected to disrupt the auditory pathway at the level of lateral lemniscus and our report, therefore, supports the hypothesis that generators of wave V (or critical inputs thereto) are located in the lateral lemniscus. The case also demonstrates that even complete absence of one of the evoked responses due to an inflammatory lesion may be fully reversible following the resolution of the lesion.

WITHDRAWN

P869

Composite methylation profiles of blood DNA as biomarkers for multiple sclerosis

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Background: Lack of an objective molecular biomarker for detection and monitoring of multiple sclerosis (MS) precludes disease detection and delays objective evaluation of drug efficacy. **Objective:** To establish if consistent alterations in DNA methylation can be detected in plasma of MS patients, we evaluated methylation patterns in patients with relapsing-remitting MS (RRMS). These patterns were compared with healthy controls in order to assess whether differences in methylation can be used for disease detection. **Methods:** Our assay for methylation in 56 genes (MethDet-56) utilizes digestion of DNA with methylation-sensitive restriction enzymes and then amplification of surviving fragments with gene-specific primers. Signals from undigested and digested parts of the same sample are compared via microarray-mediated methylation analysis (M3A). Data are presented as the signal ratio between undigested and digested samples. Circulating cell-free plasma DNA from untreated RRMS patients in remission was used for analysis. The Fisher's Exact test, naïve Bayes algorithm, and k-fold cross-validation were used for statistical evaluation. **Results:** CG-rich regions of two genes were most frequently (over 75% of samples) differentially methylated in RRMS and control samples. k-fold cross-validation indicated that the training set produced 80% sensitivity and 66% specificity of MS detection, while the testing set achieved 72% sensitivity and 57% specificity. **Conclusions:** Our data establish that detection of circulating free DNA in RRMS plasma via MethDet assay is feasible and can achieve clinically significant accuracy in discriminating MS from healthy controls.

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P870

Prediction of development of neutralizing antibodies to interferon-beta in patients with multiple sclerosis

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Background: The efficacy of interferon beta (IFNβ) is well established in patients with relapsing-remitting multiple sclerosis (RRMS). Nevertheless, IFNβ induces the formation of neutralizing antibodies (NABs) in a certain proportion of patients. Development of NABs is associated with loss of clinical, magnetic resonance imaging (MRI), and biological effects of IFNβ. **Objective:** To characterize the gene expression signature present at baseline in patients that initiate treatment with IFNβ and predict development of NABs after therapy. **Methods:** NABs were tested in serum samples from 52 RRMS patients at baseline and after 12 and 24 months of IFNβ treatment. The gene expression signatures were determined with microarrays (Affymetrix Human Genome U133 Plus 2.0) in peripheral blood mononuclear cells at the same time points as before. Differentially expressed genes at baseline between NAB positive and NAB negative patients were analyzed with the statistical platform R and Bioconductor. A prediction algorithm comparing several predictors in a cross-validated process was developed in order to identify the best set of genes that discriminate between NAB positive and NAB negative patients. **Results:** NAB positive and NAB negative patients were distinguished by a gene expression signature that was already present at baseline. Prediction studies identified a group of genes that predicted development of NABs to IFNβ at 12 or 24 months with high accuracy. **Conclusions:** These results suggest that development of NABs may indeed be predicted early and will help to identify those patients who will show a lack of response to treatment due to NABs.

P871

Evidence of axonal loss in clinically isolated syndromes by optical coherence tomography?

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Background: Optical coherence tomography (OCT) is a new biomarker of neurodegeneration in multiple sclerosis (MS). Previous studies showed that retinal nerve fiber layer (RNFL) is decreased in MS eyes with and without a history of optic neuritis (ON). In MS, RNFL atrophy is correlated with brain atrophy and disability assessed by Expanded Disability Status Scale (EDSS). In clinically isolated syndrome (CIS), axonal loss has already been demonstrated by proton magnetic resonance imaging (MRI) spectroscopy. No data is known about axonal loss demonstrated by OCT in CIS. **Objective:** To determine whether CIS patients have an early retinal axonal loss by measuring RNFL and macular thickness with OCT. **Methods:** Fifty-six CIS patients underwent retinal evaluation with OCT Stratus 3000 (4.0) within the year from the onset of the clinical symptoms. Fast RNFL thickness (3.4) and Fast macular thickness map protocols were practiced by the same trained ophthalmic engineer. ON eyes were excluded from the study. Thirty two healthy controls were recruited. We compared average, temporal, superior, nasal, inferior RNFL thickness and macular volume between CIS and controls. Comparisons were performed by Student t-test. **Results:** Among 56 CIS patients, there were 11 ON, 11 brainstem syndromes, six hemispheric brain syndromes, 18 spinal cord syndromes and 10 multifocal syndromes. Thirty-five patients (62.5%) presented dissemination in space according to Barkhof's criteria. Average, temporal, superior, nasal, inferior RNFL thickness (mean±SD μm) and macular volume (mean±SD μm³) of the CIS population were respectively 98.98±10.26 μm, 66.44±11.95 μm, 124.5±15.36 μm, 75.46±14.71 μm,

125.54±16.82 μm and 6,86±0,32 μm³. There was no significant difference between CIS and controls (p>0,05) in all RFNL and macula parameters. **Conclusions:** At the CIS time, OCT evaluation does not show retinal axonal loss. Clinical and MRI follow-up is necessary in order to compare OCT measurements of CIS that converse in MS and those that do not.

P872

Lessons from the Cannabinoids in Multiple Sclerosis (CAMS) Study: part 1 - did Ashworth's scale make the grade?

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Background: The Cannabinoids in Multiple Sclerosis (CAMS) study concluded that cannabinoids do not reduce muscle spasticity in people with MS. This finding contradicts substantial anecdotal clinical evidence and animal studies. **Objective:** We explored this discrepancy by examining the CAMS primary outcome measure, the Ashworth scale, to determine if any measurement limitations might have influenced the study's interpretations. **Methods:** We re-analyzed the CAMS Ashworth data (n=667) in which ten limb muscles on either side of the body were scored and summed to give a total spasticity score. Although this summation process appears clinically reasonable (conceptually sound), the numbers generated by it must also satisfy requirements for measurement (be psychometrically sound) for valid inferences to be made from the analysis of Ashworth total spasticity scores. We used Rasch analysis to test the extent to which summed Ashworth scales satisfied psychometric requirements for measurement. **Results:** The Ashworth scale total body muscle spasticity score did not satisfy requirements for reliable and valid measurement. Empirical evidence did not support the six-response categories for 17 of the 20 muscles selected. Scores for many individual muscles had substantial floor effects indicating suboptimal scale-to-sample targeting, and therefore limited ability to detect clinical change. The majority of upper limb muscle scores did not fit as components of an overall total body scale score, indicating that combining upper and lower limb muscles compromises measurement performance. **Conclusions:** Summing Ashworth scale grades across multiple muscles to generate an estimate of a person's total spasticity is clinically appealing. However, our analysis indicates the scores generated by this method did not satisfy requirements of measurements for statistical analysis. Could this finding explain, in part, the discrepancy between patients' positive perceptions of cannabis benefit and the negative results of CAMS? The questions raised by this study underscore why state of the art clinical trials must use rating scales proven to be fit for purpose.

P873

Serum neurofilament levels suggest axonal damage is more extensive in neuromyelitis optica than in neuromyelitis optica or multiple sclerosis optic neuritis

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Background: Axonal loss is considered to be responsible for sustained disability. The quantification of serum neurofilament levels allows the estimation of the degree of axonal damage. **Objective:** We hypothesized that the serum neurofilament levels would reflect the clinical impression that axonal damage may be more severe in patients with neuromyelitis optica (NMO) and chronic relapsing inflammatory optic neuropathy (CRION) than in patients with optic neuritis in the context of multiple sclerosis (MSON), or healthy controls (CTRL). **Methods:** Seventy-six patients were recruited into this prospective study (20 MSON, 19 CRION, 9 NMO, 20 healthy volunteers). Serum neurofilament levels (NfH-SMI35) were measured using a standard enzyme-linked immunosorbent assay (ELISA). Serum aquaporin

4 antibodies (AQP-4) were tested at the Mayo Clinic laboratories by indirect immunofluorescence. **Results:** Serum NfH-SMI35 levels were significantly different between the groups (F3,56=4.05, p=0.011). Serum NfH-SMI35 levels were significantly higher in patients with NMO (mean 0.79 +/- standard deviation 1.51 ng/mL) compared to patients with CRION (0.13+/-0.16 ng/mL, p=0.007), MSON (0.09+/-0.09, p=0.008) and healthy controls (0.01+/-0.02 ng/mL, p=0.001). **Conclusions:** This study suggests that axonal loss following ON is more extensive in patients with NMO compared to CRION or MSON. This is probably due to concomitant myelitis in NMO.

P874

Early diagnosis and biomarker discovery from mass spectral profiles: application to multiple sclerosis

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Background: Mass spectral characterization of body fluids followed by multivariate analysis represents a useful approach to reveal biomarkers in proteomic research. Cerebrospinal fluid (CSF) is a perfect source to search for new biomarkers to improve early diagnosis of neurological diseases like multiple sclerosis (MS). **Objective:** A common objective for spectral profiling is to reveal differences in composition between fractions of body fluids from healthy controls and persons with a specific disease. The aim of the present study is to identify MS specific disease markers in CSF. **Methods:** Matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry and multivariate statistical techniques were used to examine the low molecular weight CSF proteome for potential biomarkers. We applied the technique for three groups of patients: patients with multiple sclerosis, other neurological diseases and orthopedic patients (controls). CSF was drawn from patients undergoing lumbar puncture as part of routine diagnostic evaluation or operation. Samples were prepared and whole mass spectral profiles are collected using MALDI-TOF. The obtained data were pretreated and analyzed using the Sirius software (PRS AS). **Results:** Analysis provided information about similarities and differences between the three patient groups and within these groups. Spectral regions that influenced separation between the groups were detected and biomarker candidates were identified by graphical display. This technique was also shown to reduce the risk of finding false biomarker candidates. **Conclusions:** When comparing CSF from MS patients with healthy controls (limited study) by MALDI-TOF mass spectrometry, we were able to find some potential biomarkers.

P875

Optical coherence tomography after acute optic neuritis: a potential outcome measure for neuroprotection trials

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Background: Optical coherence tomography (OCT) can quantify axonal loss in the retinal nerve fiber layer following acute optic neuritis (ON). Short-term outcome measures are needed to test potential neuroprotective drugs. OCT may provide a useful measure of neuroprotection in this context. **Objective:** To evaluate the change in retinal nerve fiber layer thickness (RNFLT) following acute optic neuritis (ON) and to estimate the sample size needed to use OCT as an outcome measure in neuroprotection trials. **Methods:** We are performing a prospective cohort study in patients with acute ON. Patients have OCT, visual evoked potentials, visual fields, and visual acuity tested within 45 days of onset of ON and are re-evaluated at 3, 6, and 12 months. Preliminary results are reported for 16 patients, 10 of whom have 6 month data. These data are used to perform preliminary sample size calculations. **Results:** Acutely, in ON the mean RNFLT is greater in affected than unaffected eyes due to swelling (108 vs. 101 μm, SD 20.1 vs. 15.9, p=0.3). In the ON eyes the mean RNFLT drops to 89 μm (SD 16.9) between 45 and 120 days, a decrease of

-19 μm ($p=0.045$ vs. baseline). This decrease in RNFLT is presumably due to a combination of axonal loss and the resolution of swelling. In unaffected eyes there is no significant change in RNFLT. It is estimated that of the 19 μm change, -8 μm is attributable to resolution of swelling and -11 μm is due to axonal loss. Based on preliminary results, a placebo-controlled trial of a putative neuroprotectant in acute ON would be estimated to require a sample size of 102 to see a 50% reduction of axonal loss with a power of 0.8 and $\alpha=0.05$ using baseline and 4 month measurements. **Conclusions:** Preliminary results from this cohort study provide an estimate of the change in RNFLT after acute OCT. These results will be useful for sample size estimations for clinical trials using OCT as an outcome measure.

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P876

Anti-thyroid antibodies identify a subgroup of multiple sclerosis patients

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Background: Multiple sclerosis (MS) is a complex autoimmune disease which may be associated with other autoimmune diseases, most frequently autoimmune thyroid disorders. Thyroid dysfunction is commonly reported in the clinic, consisting of a reported incidence of hypo or hyperthyroidism. Often diagnostic testing supporting an autoimmune pathogenesis for these thyroid conditions is absent.

Objective: To understand if autoimmunity is the basis for thyroid disease in the MS patients and to determine the incidence of anti-thyroid antibodies. **Methods:** A total of 640 clinically definite multiple sclerosis (CDMS) patients were tested for the presence of anti-thyropoetin (anti-TPO) and anti-thyroglobulin (anti-TG) antibodies. Antibodies to the thyroid antigens were measured by chemiluminescent microparticle immunoassay. The levels of > 100 IU for anti-TPO and > 50 IU for anti-TG antibodies were considered positive as described in the literature. **Results:** The criteria effectively delineated patients with significant levels of antibody ($p < 0.0001$). In this cohort of 640 CDMS patients, 72 (64 female and eight male) patients were positive for either or both antibodies. The presence of the antibodies was associated with a 43% incidence of clinical thyroid disease. The female to male ratio for the patient population as a whole was 3.15 to 1; however, the ratio increased to 8 to 1 in the antibody positive patients. The anti-TPO antibodies were more likely to be found in ambulatory patients than those with more advanced disease ($p < 0.05$). The presence of antibodies was not substantially influenced by disease duration or patient age. **Conclusions:** Anti-thyroid antibodies in MS patients are associated with a distinct gender bias and a substantial incidence of clinically significant thyroid disease. The presence of thyroid specific autoantibodies in MS patients may be an indicator of a more generalized autoimmunity and may identify an important subgroup of MS patients.

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P877

Serum N-acetyl-aspartate in relapsing-remitting multiple sclerosis Maddalena Ruggieri, Carla Tortorella, Rosaria R. Leante, Edmondo Ceci, Elisabetta Di Monte, Pietro Iaffaldano, Vita Di Renzo, Paolo Livrea, Maria Trojano

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Background: Lower N-acetyl-aspartate (NAA) levels were recently demonstrated in cerebrospinal fluid (CSF) of multiple sclerosis (MS) patients with higher disability and lower brain volume. So far no studies are known that measured NAA in serum of MS patients. **Objective:** To evaluate serum NAA concentrations in relapsing-remitting (RR) MS patients and correlate them with CSF levels, clinical phenotype and

measures of disease activity and severity. **Methods:** Fourteen clinically isolated syndromes (CIS) suggestive of MS, 45 RRMS and 117 healthy controls (HCs) were included into the study. Serum and CSF NAA levels were evaluated by using a stable isotope dilution gas-chromatography-mass-spectrometry method. All MS patients underwent clinical examination including the Expanded Disability Status Scale (EDSS) score evaluation at the time of serum and CSF collection.

Results: Significant higher ($p < 0.0001$) NAA serum levels (1.68 ± 0.6 micromoli/L) were found in MS patients than in HCs (0.24 ± 0.24 micromoli/L). Significant inverse correlations were found between NAA serum levels and age ($p < 0.05$) in HCs, and disease duration ($p < 0.05$) and number of relapses ($p < 0.01$) in MS. The levels tended ($p = 0.07$) to be higher in CIS than in RRMS. A significant positive ($p < 0.0001$) correlation was found between serum and CSF (0.76 ± 0.3 micromoli/L) concentrations. No correlations were found between serum and CSF NAA values and EDSS scores. **Conclusions:** The results suggest that the increase in serum NAA concentration in MS might indicate a leakage in peripheral blood, through a defective blood-brain barrier, of an enhanced NAA production from reversibly affected neurons, early in the course of the disease. NAA could function as an efflux metabolic water pump for the removal of neuronal metabolic water that has accumulated during inflammatory neuronal damage.

P878

Motor cortex excitability alterations in patients with clinically isolated syndrome and early relapsing-remitting multiple sclerosis Sven Schippling¹, Suzan Grossmann¹, Maxim Bester², Roland Martin¹

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Background: Due to the progress in imaging techniques for diagnostic purposes, the role of evoked potentials (EP) in the assessment of multiple sclerosis (MS) has changed in the recent past. The current diagnostic criteria for MS are largely based on magnetic resonance imaging (MRI) findings. However, whereas MRI has greater sensitivity as compared to EP in the diagnosis of MS, the correlation between MRI parameters and clinical disability is poor. Among the EP, transcranial magnetic stimulation (TMS) has recently been shown to be a sensitive surrogate marker of disability and disease progression even in early stages of the disease. However, little is known about longitudinal changes in motor cortex excitability in early MS patients not presenting with overt clinical motor signs and symptoms. **Objective:** It is the aim of this prospective study, to evaluate TMS as a functional measure of impaired motor cortex excitability in CIS and early relapsing-remitting MS (RRMS) patients, and to test TMS outcomes as a predictor for the progression to clinically definite MS. **Methods:** 15 patients with clinically isolated syndrome (CIS) without clinical motor signs, 15 early RRMS patients (Expanded Disability Status Scale (EDSS) 0-3.5; disease duration ≤ 3 years) and 15 control subjects are prospectively followed by clinical, TMS and MRI examinations at baseline and months 9 and 18. With TMS, measures of transcallosal inhibition (TI) are assessed. Apparent diffusion coefficient (ADC) and fractional anisotropy (FA) maps are derived from DTI. **Results:** At baseline, patients with CIS as well as RRMS show significant alterations in motor cortex excitability as compared to controls. The ipsilateral silent period as one measure of TI is prolonged both in CIS and RRMS, irrespective of a history of motor symptoms. MRI data is currently being analyzed and correlation analysis will be performed on TI and FA maps of transcallosal fibers connecting primary motor cortices. **Conclusions:** Our data so far provides evidence that, by TMS, motor impairment is detectable in patients without clinical motor signs in disease stages as early as CIS.

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P879

Effect of interferon- β alone or in combination with atorvastatin on active MMP-9 and TIMP-1: results from the Swiss Atorvastatin and Betaferon in Multiple Sclerosis (SWABIMS) TrialJohann Sellner¹, Isabell Greeve², Stephen L. Leib³, Heinrich P. Mattle²¹Department of Neurology, Klinikum rechts der Isar, Technische Universität München, München, Germany; ²Department of Neurology, Inselspital, Bern University Hospital, and University of Bern, Switzerland, Bern, Switzerland; ³Institute of Infectious Diseases, University of Bern, Bern, Switzerland

Background: Potential immunomodulatory effects of statins have attracted considerable interest in the treatment of multiple sclerosis (MS). However, statins may abolish the presumably beneficial effects of interferon- β on proteolysis in MS, that is, matrix-metalloproteinase-9 (MMP-9) and tissue inhibitor of MMP (TIMP)-1. **Objective:** To determine the treatment effect of interferon- β alone and in combination with atorvastatin on serum levels of active MMP-9, TIMP-1 and active MMP-9/TIMP-1 ratio in patients with relapsing-remitting (RR) MS. **Methods:** Sequential serum samples were obtained from 28 patients participating in the Swiss Atorvastatin and Betaferon in Multiple Sclerosis Trial. All patients were treated *de novo* with interferon- β 1b (250 μ g subcutaneously) for 3 months and then randomized to a treatment of either a monotherapy with interferon- β 1b (n=12) or a combination of interferon- β 1b and atorvastatin (40 mg orally, n=16) for further 6 months. Samples from 10 age-matched healthy subjects served as controls. Active MMP-9 and TIMP-1 levels were determined at baseline, 3, 6 and 9 months with commercial enzyme-linked immunoabsorbent assay (ELISA) kits. **Results:** In patients with MS, significantly higher levels of active MMP-9 (P<0.001) and active MMP-9/TIMP-1 ratio (P<0.01) were detected prior to initiation of treatment, compared with healthy controls. Serum levels of TIMP-1 were significantly decreased in patients with MS (P<0.05) but were raised following a 3 month treatment with interferon- β 1b. Serum levels of active MMP-9, and TIMP-1 and the active MMP-9/TIMP-1 ratio were not influenced by atorvastatin when given in combination with interferon- β 1b during the study period. **Conclusions:** A proteolytic dysregulation on the basis of increased active MMP-9 and lower TIMP-1 serum levels was detected in patients with MS. Treatment with interferon- β 1b corrected this imbalance by raising TIMP-1 serum levels. Our study did not detect a detrimental neutralisation or a synergistic effect on the proteolytic balance by adding atorvastatin to interferon- β 1b in patients with RRMS. **Supported by:** Swiss Multiple Sclerosis Society.

P880

Comparison of optical coherence tomography and scanning laser polarimetry in subgroups of multiple sclerosisDorine Siepman¹, Marijke Wefers Bettink-Remeijer², Rogier Q. Hintzen¹¹MS Centre ErasMS, Erasmus MC, Rotterdam, Netherlands; ²Rotterdam Eye Hospital, Rotterdam, Netherlands

Background: Optical coherence tomography (OCT) and scanning laser polarimetry (GDx ECC) are non-invasive methods used to assess retinal nerve fiber layer (RNFL) thickness, which may be a reliable tool to monitor axonal loss in multiple sclerosis (MS). **Objective:** To compare the ability of OCT and GDx ECC in discriminating between eyes with and without previous optic neuritis (ON) and between eyes of patients with primary progressive MS (PPMS) and relapse-onset MS (relapsing-remitting MS (RRMS)/secondary progressive MS (SPMS)). We also examined correlations between OCT and GDx ECC measurements and disability (using the Expanded Disability Status Scale (EDSS)) and impact of MS (using the Multiple Sclerosis Impact Scale (MSIS-29)). **Methods:** Ophthalmologic examination (visual acuity, visual field mean deviation (MD), visual field pattern standard deviation (PSD)), OCT 2.27, OCT 3.4 and GDx ECC were performed in 65 MS patients (26 RRMS, 10 SPMS, 29 PPMS). 28 patients (43%) had a history of ON. Adjustments were made for age and disease duration. **Results:** The average and each quadrant RNFL thickness was lower for eyes with previous ON (p<0.01 for all parameters). OCT and GDx ECC did not differ in the capacity to distinguish between eyes with and without previous ON. No differences were found between PPMS and

RRMS/SPMS. OCT and GDx ECC measurements were highly correlated ($\rho=0.72$, p<0.01). Visual field-MD values correlated with OCT means ($r=0.36-0.39$, p<0.01) and GDx ECC TSNIT average ($r=0.46$, p<0.01). In patients without previous ON, EDSS correlated with MD ($r=-0.36$, p<0.01), OCT nasal quadrant ($r=-0.33$, p<0.05) and macular volume ($r=-0.33$, p<0.05). For MSIS-29 physical impact score, significant correlations were found with MD ($r=-0.47$, p<0.01) and PSD ($r=0.45$, p<0.01). **Conclusions:** RNFL is significantly decreased in eyes of MS patients with previous ON. No differences between PP, RR and SP subgroups were found, after adjustment for age and disease duration. OCT and GDx findings were highly correlated and showed good correlations with visual field (MD). In patients without previous ON, only OCT parameters showed significant correlations with EDSS. **Supported by:** Dutch MS Research Foundation Rotterdam Eye Hospital.

P881

A comparison of multiple sclerosis patients who have negative cerebrospinal fluid oligoclonal bands to those with positive testing: baseline characteristics and prognosis

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Background: It is suggested that multiple sclerosis (MS) patients with negative cerebrospinal fluid (CSF) oligoclonal bands (OCB-) have a more benign disease. **Objective:** To review the baseline characteristics and clinical outcomes in a group of MS patients who are OCB- compared with those with OCB+ results during the same period. **Methods:** A retrospective study of 451 patients who had CSF analysis performed after 1 January 1993 (using the FDA approved test of isoelectric focusing and immunoblotting) and who were followed through to 1 November 2007. **Results:** All patients met current diagnostic criteria (revised McDonald) for MS. There were only 48/451 (10.6%) OCB- versus 403/451 (89.4%) OCB+ patients. Mean age at onset for OCB- was 37.1 \pm 8.8 versus 34.5 \pm 10 years OCB+ (p=ns); female:male OCB-:OCB+ was 2.7 versus 2.0 (p=ns); 7/48 (14.6%) OCB- had a positive family history with at least one first-degree relative with MS versus 77/403 (19.1%) for OCB+ (p=ns). The central nervous system (CNS) location for the first attack for OCB-:OCB+ was: brainstem-cerebellum 22.9% versus 22.6% (p=ns); spinal cord 31.3% versus 40.7% (p=ns); optic nerve 12.5% versus 16.1% (p=ns); supratentorial 29.2% versus 13.2% (p=0.003); multifocal involvement 4.2% versus 7.4% (p=ns). Clinical course for OCB- versus OCB+ was 22.9% versus 15.1% primary progressive MS (p=ns); 18.8% versus 12.4% secondary progressive MS (p=ns); 56.3% versus 71% relapsing-remitting (RR) MS (p=0.036); and 2.1% versus 0.5% (p=ns) progressive relapsing MS. CSF findings outside of OCB were similar between groups with the exception of significantly more WBC in OCB+ versus OCB- 7.8 \pm 10.9 versus 2.4 \pm 2.5 (p=0.002). More OCB+ compared to OCB- patients' MRI met McDonald criteria around the time of CSF sampling (37.7% versus 27.1%; p=0.034). There were no differences in the number of relapses or the overall amount or speed of Expanded Disability Status Scale (EDSS) progression between the groups. **Conclusions:** Fewer OCB- patients have RRMS, but more tend to present without a distinct CNS location (that is, supratentorial) for their first event. They also tend to have a less inflammatory CSF and fewer MRI lesions at the time of sampling. Overall, however, their prognosis seems no different from that of OCB+ patients although the trend was for more to have a progressive versus relapsing course of disease.

P882

Comparison of the degenerative and inflammatory markers in cerebrospinal fluid in multiple sclerosis patients with relapsing-remitting course of disease and after clinically isolated syndrome
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Background: Multiple sclerosis (MS) is an autoimmune inflammatory disease of the central nervous system (CNS) leading to

demyelination and axonal loss. Cerebrospinal fluid (CSF) examination is the basic examination to evaluate inflammation activity and the degree of degeneration in the CNS. **Objective:** To assess whether there are any differences in inflammatory and degenerative marker levels in CSF in relapsing-remitting MS (RRMS) and after clinically isolated syndrome (CIS). **Methods:** We examined 149 patients: 66 MS patients (46 RRMS and 20 CIS) and 83 controls with other non-inflammatory diseases. We evaluated markers of BBB integrity: albumin quotient and prealbumin, inflammatory markers: CRP, C3, C4, transferrin, haptoglobin, beta-2-microglobulin, orosomucoid, markers of tissue destruction: ApoA-I, Apo-B, cystatin C, neuron-specific enolase, tau-protein and beta-amyloid. The intrathecal synthesis was assessed according to the number of oligoclonal IgG bands in alkaline fraction in IEF and IgG quotient. We also evaluated alfa-1-antitrypsin, which is considered as protective factor in the CNS. **Results:** We found increases in some inflammatory and degenerative CSF markers even after CIS. Intrathecal synthesis was higher in RRMS, but the number of oligoIgG bands was higher after CIS. As inflammatory markers, values of transferrin, beta-2-microglobulin and neuron-specific enolase were higher after CIS; haptoglobin and transferrin were lower. As degenerative markers, values of ApoA-I, tau-protein were higher after CIS; the value of Apo-B was higher in RRMS. The values of beta-amyloid and alfa-1-antitrypsin were normal. **Conclusions:** There were no statistically significant differences between particular degenerative and inflammatory markers in patients after CIS and RRMS, except for cystatin. We found statistically significantly lower values of cystatin in CSF in RRMS patients compared with patients after CIS. It is an indicator of high disease activity even after the first attack of MS.

P883

Systemic mitochondrial membrane hyperpolarisation in primary progressive multiple sclerosis

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Background: Mitochondrial dysfunction may relate to neuronal and axonal failure in multiple sclerosis (MS) and provides a putative link between the inflammatory and neurodegenerative aspects of the disease. Mitochondrial mechanisms include immune cell, neuronal and glial apoptosis. Work in other T-cell mediated autoimmune disease (systemic lupus erythematosus (SLE)) has found systemic mitochondrial hyperpolarization is a key event in T-cell activation and apoptosis. A systemic mitochondrial functional or genetic abnormality, especially of respiratory chain complex I, has been suggested previously. **Objective:** Controls and patients with primary and secondary progressive multiple sclerosis were studied (1) to determine whether any difference exists in systemic mitochondrial membrane potential and (2) to search for the presence of a systemic abnormality of mitochondrial respiratory chain enzyme function. **Methods:** Following venesection, platelets were extracted by differential centrifugation from age and sex matched controls (N=17), persons with primary progressive (PPMS, N=16) and secondary progressive multiple sclerosis (SPMS, N=9). Platelets were then: (1) prepared and loaded with the mitochondrial membrane potential sensitive fluorescent dye JC-1, with or without 10 μ M rotenone (selective Complex I inhibitor) for flow cytometric analysis; or (2) disrupted via nitrogen cavitation to yield a crude mitochondrial pellet used for enzymatic assay of respiratory complexes I, II/III, IV and citrate synthase by spectrophotometry. **Results:** Patients were matched for disability (mean Expanded Disability Status Scale (EDSS) score 6.1, SPMS/PPMS) and disease duration (SPMS/PPMS 16 years). No difference in complex I, II/III or IV enzymatic activity was found across groups ($p=0.56$, $p=0.22$, $p=0.23$, respectively). Platelets of patients with PPMS were relatively hyperpolarized at rest and exhibited marked attenuation of rotenone induced hyperpolarization ($p=0.005$). **Conclusions:** No difference in systemic mitochondrial respiratory complex enzyme activity was demonstrated. In PPMS evidence for relative systemic mitochondrial hyperpolarization was found and supported by

attenuation of the rotenone effect. Further work characterizing this finding in the context of the pathophysiology of PPMS is planned. **Supported by:** Multiple Sclerosis Research Australia.

P884

Autoantibody profiling in multiple sclerosis: characterization of novel antigenic candidates

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Background: Autoantibodies play an important role in multiple sclerosis (MS) but may also be of interest as diagnostic or prognostic biomarkers for MS. **Objective:** To identify antigenic targets specifically interacting with antibodies present in the cerebrospinal fluid (CSF) of MS patients. **Methods:** Serological antigen selection was applied using a cDNA phage display library derived from MS brain plaques. **Results:** A panel of eight antigenic targets was identified that showed 86% specificity and 45% sensitivity in discriminating between MS patients and controls. Autoantibody reactivity was demonstrated in different MS subtypes and was found at an early stage in a subset of MS patients. An increased reactivity towards the antigenic targets (size ranging from 11 to 121 amino acids) was observed in patients with higher clinical disability. Bioinformatic analysis revealed a novel antigen SPAG16, while the remaining antigens constitute novel epitopes encoded by transcript variants of known genes. Several clones represented epitopes from cerebral proteins, or epitopes from novel cDNA sequences originating from brain libraries. Three out of eight antigenic targets represented epitopes similar to viral proteins such as Epstein-Barr virus (EBV). Recombinant proteins were expressed for four of the antigenic targets and synthetic peptides were obtained for the remaining candidates. Immunoreactivity for each purified recombinant protein and synthetic peptide was confirmed. Inhibition assays using synthetic peptides representing cDNA inserts confirmed specific antibody reactivity of MS CSF to the MS brain plaque-derived peptides. We generated monoclonal antibodies and currently study the expression of these genes in healthy and diseased central nervous system (CNS). **Conclusions:** In conclusion, we identified a panel of eight new brain-derived antigenic epitopes that show increased immunoreactivity in MS patients. Autoantibody profiles against epitopes derived from MS brain tissue could serve as diagnostic markers or form the basis for the identification of a subgroup of MS patients who may benefit from specific therapies.

P885

Quantitative cerebrospinal fluid peptide profiling of multiple sclerosis patients by mass spectrometry

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Background: We have previously reported our findings on differential peptide expression in cerebrospinal fluid (CSF) of multiple sclerosis (MS) patients, using matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry to identify 11 differentially expressed proteins. **Objective:** Using more advanced mass spectrometry techniques we examined differential peptide expressions quantitatively. Secondly, these techniques were used to examine differences between relapsing-remitting (RR) and primary progressive (PP) MS. **Methods:** MALDI Fourier transform mass spectrometry was used to quantitatively measure 163 CSF samples of MS patients and controls (four groups: MS, clinically isolated syndrome (CIS), inflammatory controls and non-inflammatory controls), using peak height to determine the amount of peptide present ($R^2=0.99$) in each sample. Samples were subsequently analyzed using specialized software to pinpoint differences between MS patients and controls. An Orbitrap mass spectrometer was employed to measure 12 RRMS and 12 PPMS CSF samples to detail differences in CSF peptide profile between these two

MS types. **Results:** A total of over 600 peptide peaks were found to be quantitatively differentially expressed in the comparisons between the four groups. Of these peptide peaks we were able to identify 89. Among the identified proteins were previously reported proteins chromogranin A, complement C3 and clusterin, which were expressed at higher levels in MS CSF compared with non-inflammatory controls. Other interesting peptides were two Ig Kappa fragments. In the comparison between the two different MS types small differences were observed in CSF peptide profile. Three peptides that were upregulated in PPMS were identified. So far, the identified differences appear to be related to the innate immune response. **Conclusions:** Using these more advanced techniques, we were able to confirm our previous findings and add a large number of other differentially expressed proteins in CSF when comparing MS patients with controls.

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P886

Marked increase of cerebrospinal fluid glial fibrillary acidic protein in neuromyelitis optica: an astrocytic damage marker

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Background: Neuromyelitis optica (NMO) is a neurologic inflammatory disease characterized by severe optic neuritis, longitudinally extended transverse myelitis and autoimmunity to aquaporin-4 (AQP4) predominantly localized in astrocytic foot processes. It was recently demonstrated that loss of AQP4 and glial fibrillary acidic protein (GFAP) were the prominent features of NMO lesions, suggesting astrocytic impairment in the pathogenesis of NMO. **Objective:** To reveal a useful clinical biomarker of NMO, we measured the cerebrospinal fluid (CSF) concentrations of astrocytic markers, GFAP and S100B, in acute exacerbations of NMO and other diseases. **Methods:** We performed enzyme-linked immunosorbent assays of GFAP and S100B in CSF, obtained from the patients with NMO (n=10), multiple sclerosis (MS) (n=10), acute disseminated encephalomyelitis (ADEM) (n=3), spinal infarction (n=3), and other neurologic diseases (OND) (n=5). **Results:** The CSF GFAP levels during relapse in NMO ($7666.0 \pm 15\,266.5$ ng/ml) were significantly over several thousand times higher than those in MS (0.7 ± 1.5) or OND (0.6 ± 0.3), and much beyond those in spinal infarction (354.7 ± 459.0) and ADEM (0.4 ± 0.2). They returned to a close to normal level along with clinical improvement soon after corticosteroid therapy in NMO. There were strong correlations between the CSF GFAP or S100B levels and expanded disability status scales or spinal lesion length in NMO ($r > 0.9$). **Conclusions:** CSF GFAP and S100B may be useful biomarkers in NMO, and astrocytic damage is strongly suggested in the acute phase of NMO.

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Proteomic analysis in cerebrospinal fluid of patients with clinically isolated syndromes suggestive of multiple sclerosis

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Background: Clinical and radiological variables assessed at the time of clinically isolated syndromes (CIS) are strong predictors of conversion to multiple sclerosis (MS) and long-term disability. However, the clinical course is unknown and time to conversion and response to treatment highly variable. Alternative prognostic factors of conversion and disability are needed. **Objective:** To identify biomarkers that predict conversion to MS and disability by means of proteomic analy-

sis in cerebrospinal fluid (CSF) samples from CIS patients. **Methods:** The present study is based on longitudinal clinical, CSF and magnetic resonance imaging (MRI) data prospectively acquired from a cohort of patients with CIS recruited since 1995. Patients were classified into converters and non-converters to MS based on clinical and radiological findings, and presence of oligoclonal bands in CSF. Four different pools of CSF were created, two pools from CIS patients that will convert to MS, and two pools from non-converters. Each pool contained CSF samples from five patients. After quantification, proteins were labeled with iTRAQ isobaric tags, and pools were analyzed using two-dimensional liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS). MS/MS spectra were searched in the human protein database using the Paragon algorithm and data were analyzed with ProteinPilot software. This technology allows, on the one hand, the identification of the proteins present in the sample and, on the other hand, the relative quantitation of these proteins between converters and non-converters. **Results:** A total of 166 proteins were identified in CSF samples. Eighteen proteins were found to be differentially expressed between converters and nonconverters to MS (p-value of the ratio between the two conditions was less than 0.05). Of these proteins, 11 were overexpressed in converters to MS and 7 overexpressed in non-converters. **Conclusions:** Although additional experiments with new pools are in progress, differentially expressed proteins represent potential biomarkers of conversion to MS in CIS patients.

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Serum uric acid levels are decreased in patients with relapsing-remitting multiple sclerosis, in particular during relapses

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Background: Previous studies indicate that patients suffering from relapsing-remitting multiple sclerosis (RRMS) have low serum levels of the endogenous antioxidant uric acid (UA). **Objective:** The aim of this study was to examine whether patients with RRMS have decreased serum levels of UA and if the value of UA changed during relapses. We correlate the value of UA to Expanded Disability Status Scale (EDSS) score and the disease duration. **Methods:** The mean levels of UA were compared in the following groups: (1) optic neuritis (ON), (2) early multiple sclerosis (EMS) patients with ON as the first attack, who meet the McDonald criteria for magnetic resonance imaging (MRI) of the cerebrum and have positive IgG index and oligoclonal bands in cerebrospinal fluid (CSF); (3) healthy control group (HC); (4) RRMS during stable disease; and (5) RRMS during a relapse (ATTACK). Furthermore to correlate the UA values in the RRMS group to EDSS scores and the disease duration. The serum reference interval was 150–350 μ M for women and 200–450 μ M for men. A Student's *t* parametric test was used to evaluate the statistical significance. **Results:** The mean UA value for RRMS was statistically significantly lower in comparison with the HC and EMS groups ($P=0.0055$ and $P=0.0124$) and for the ATTACK group in comparison with HC, ON, EMS and RRMS ($P=0.0006$, $P=0.0041$, $P=0.0025$ and $P=0.0148$). **Conclusions:** These results support the hypothesis that serum levels of UA are decreased in patients with RRMS and even more decreased during the relapse. However, the UA values are not lowered early in the development of multiple sclerosis during the first attack. This suggests that disease duration and progression might have an influence on the UA values.

P889

Cerebrospinal fluid oligoclonal bands and/or an elevated IgG index does not increase the risk of clinically definite multiple sclerosis in clinically isolated syndrome patients with brain magnetic resonance imaging lesionsJennifer Smrtka¹, Claire Riley¹, Shuang Wang², Mark J. Tullman¹¹Columbia University Medical Center, New York, New York, USA; ²Columbia University Mailman School of Public Health, New York, New York, USA

Background: Cerebrospinal fluid (CSF) oligoclonal bands (OCBs) and/or an elevated IgG index are present in more than 90% of clinically definite multiple sclerosis (CDMS) patients. However, studies examining whether OCBs increase the risk of CDMS in patients with symptoms suggestive of a first attack of multiple sclerosis (MS) and brain magnetic resonance imaging (MRI) lesions have yielded conflicting results. The CSF was examined in some of the patients who were subsequently enrolled in the IFN β or glatiramer acetate (GA) placebo-controlled clinically isolated syndrome (CIS) trials, which required a minimum of two or three brain MRI lesions consistent with demyelinating disease for entry. This provided an opportunity to determine whether CSF OCBs and/or an elevated IgG index increase the risk of CDMS in these patients. **Objective:** To determine whether CSF OCBs and/or an elevated IgG index increase the risk of CDMS in CIS patients with brain MRI lesions consistent with demyelinating disease. **Methods:** We attempted to analyze CSF data from subjects randomized to placebo in the GA and the IFN CIS trials. We tried to obtain data that were not published from the study sponsors. The CSF was examined in 32 of the placebo patients in the IFN β -1a CHAMPS trial. However, data regarding IgG index were not collected, so these patients were excluded from our analysis. The results of the GA PreCISe trial were presented recently, but the CSF data are not yet available. The IFN β -1b BENEFIT and IFN β -1a ETOMS trials are included in this analysis. A chi-squared test was used to compare the proportion of patients with positive and negative CSF who developed CDMS. **Results:** CSF was examined in 226 of the 331 placebo patients (68%). OCBs and/or an elevated IgG index (positive CSF) were present in 83% (187/226) of patients; 47% of patients (87/187) with positive CSF and 38% of patients (15/39) with negative CSF developed CDMS, respectively (odds ratio, 1.39; 95% confidence interval, 0.69–2.82; $p=0.36$). **Conclusions:** CSF OCBs and/or an elevated IgG index are present in most CIS patients with MRI brain lesions consistent with demyelinating disease, but does not increase the risk of conversion to CDMS over a 2-year period. Additional analyses with baseline demographic, clinical, and MRI characteristics, as well as the PreCISe data are planned and will be presented.

P890

A successful validation of the ability of anti-GAGA4 IgM antibody assay to differentiate multiple sclerosis patients from other neurological diseases patients in a German cohort: a cross-sectional retrospective analysisJohannes Brettschneider¹, Sana Abdul¹, Ella Fire², Jennifer Yarden², Larissa Spector², Nir Dotan², Hayretin Tumani¹¹Neurology, University of Ulm, Ulm, Germany; ²Glycominds Ltd., Lod, Israel

Background: Previous studies (Israeli, Canadian-Belgian cohorts) demonstrated that anti-Glc(α 1,4)Glc(α) (anti-GAGA4) IgM antibody levels are higher in diagnosed and first presentation multiple sclerosis (MS) patients than other neurological disease (OND) patients, thus enabling the identification of a subgroup of MS patients. Corrections for total IgM and age were shown to improve the performance of this assay. **Objective:** To validate the ability of anti-GAGA4 IgM EIA assay to differentiate between relapsing-remitting MS (RRMS) and OND patients in a German cohort. **Methods:** Retrospective analysis of frozen sera from a German cohort of 126 patients (age between 18 and 65 years): 83 RRMS patients (age: mean \pm SD, 37.4 \pm 10.0 years; 65% female), 8 primary progressive MS (PPMS) patients (age: mean \pm SD, 42.5 \pm 12.2 years; 50% female) and 35 OND patients (15 other inflammatory neurological disorders (OIND), 20 other non-

inflammatory neurological disease (ONIND), age: mean \pm SD, 53.5 \pm 10.2 years; 37% female). Sera were diluted 1:1200 and levels of anti-GAGA4 IgM antibodies were measured by gMS[®] Dx immunoassay, normalized by dividing by the square root of total IgM levels (mg IgM/ml serum). Based on results from a former study, correction for patients' age was done by adding 0.455 (EU/(mg/ml)^{0.5}) per year from the age of 20 years. Anti-GAGA4 cutoff level for determining antibody status (positive or negative) was set at 57 EU/(mg IgM/ml serum)^{0.5}. **Results:** Significantly higher levels of anti-GAGA4 IgM antibodies were observed in RRMS patients (median: 48.0 EU/(mg/ml)^{0.5}), $p=0.0001$, Mann-Whitney U test) compared with OND patients (median: 33.3 EU/(mg/ml)^{0.5}). Receiver operating characteristic (ROC) curve analysis demonstrated that anti-GAGA4 can differentiate between RRMS and OND patients (area under the curve (AUC): 0.812). Anti-GAGA4 was positive in 29/87 RRMS patients (sensitivity: 33.3%, 95% confidence interval: 23.6–44.3%); negative in 33/35 OND patients, (specificity: 94.3%, 95% confidence interval: 80.8–99.1%). PPMS patients had relatively low levels anti-GAGA4 IgM antibodies (median: 39.1 EU/(mg/ml)^{0.5}) and only one of the eight patients had a value above the cutoff. **Conclusions:** The ability of anti-GAGA4 to differentiate between RRMS and OND patients was further validated. **Supported by:** Glycominds Ltd.

P891

Cerebrospinal fluid proteome profile in patients with clinically isolated syndrome and relapsing-remitting multiple sclerosis

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Background: Proteome analysis of cerebrospinal fluid (CSF) could be a useful diagnostic tool to identify candidate biomarkers in patients with clinically isolated syndrome (CIS) and relapsing-remitting multiple sclerosis (RRMS). **Objective:** To compare the CSF proteome profile of patients with (a) RRMS, (b) CIS who will remain with CIS, and (c) CIS who will develop a second relapse within a follow-up time of 2 years (CIS-RRMS). **Methods:** Pooled CSF samples of $n=8$ patients with RRMS, $n=8$ patients with CIS and $n=8$ patients CIS-RRMS were studied using 2-dimensional difference in gel electrophoresis (2-D-DIGE) and matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry. Spots with significantly different volumes were selected for protein identification and further validation studies using immunoassays. **Results:** In the first set of analysis 23 spots corresponding to 13 distinct proteins could be identified to be significantly downregulated in patients with CIS when compared with patients with RRMS. Five proteins were found to be significantly upregulated. In the second set of analyses comparing patients with CIS and CIS-RRMS, 22 spots corresponding to 13 distinct proteins were found to be significantly different, 2 of which were downregulated, whereas 11 proteins were upregulated in patients remaining with CIS after 2 years. 2-D-DIGE results of two of the identified proteins (alpha-2-HS-glycoprotein, proapolipoprotein) were confirmed by Western blot analysis. **Conclusions:** 2-D-DIGE is a promising semi-quantitative tool to compare the CSF proteome profile of patients with CIS and RRMS. The diagnostic relevance of the candidate biomarker proteins identified so far are currently validated by quantitative immunoassays in conjunction with blood-CSF barrier related evaluation.

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Characterization of the peripheral milieu in children with acquired inflammatory central nervous system demyelination

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Background: Although studies in adult patients with multiple sclerosis (MS) have implicated various inflammatory mediators in disease pathogenesis, serological markers of initiating or early disease mechanisms have not yet been identified. Pediatric patients with clinically isolated syndromes (PD-CIS) at risk for MS provide a unique opportunity to study the early inflammatory biology of central nervous system (CNS) demyelination. **Objective:** To: (i) compare levels of selected peripheral inflammatory molecules in children with CIS with those in control subjects; and (ii) examine whether particular measures at disease presentation (CIS) are associated with the tendency to develop chronic relapsing illness (MS). **Methods:** Prospectively collected serum samples from 65 children under the age of 16 with the diagnosis of CIS were stratified into high-risk (HR) and low-risk (LR) categories for MS, based on presenting-phenotype or magnetic resonance imaging (MRI) lesion burden. Samples were similarly obtained from age- and gender-matched healthy children (n=50), and neurological disease controls (n=22). Serum was blindly analyzed by multiplexed bead based arrays (Luminex Corp.) using an initial "test cohort" and subsequent "validation cohort" for levels of particular cytokines, chemokines, adhesion molecules and matrix proteases, selected based on previous implications in adult MS studies. **Results:** Analysis of cytokine levels demonstrated significant differences between in IL-1b (p=0.03), IL-4 (p=0.026), IL-8 (p=0.006), IL-10 (p=0.043) and TNF α (p=0.026), between the first 29 CIS patients (LR=14, HR=15) and 34 controls ("test cohort"). These cytokine levels were not different between the HR and LR CIS groups. Validation of these findings is being conducted in the remaining patients (n=36) and controls (n=38), as well as complete analysis of the 22 selected chemokines, adhesion molecules and matrix proteases. **Conclusions:** Initial results suggest that levels of selected cytokines (IL-1b, IL-4, IL-8, IL-10, TNF α) differ in the periphery of children with CIS compared with controls. Our study should provide additional insights into the early mechanisms of acquired CNS demyelination.

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Genetic variation in IFN signaling is associated with responsiveness to IFN-b in multiple sclerosis

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Background: Previously, we showed that baseline status of the IFN type-I activity negatively correlates with pharmacological effects of IFN-b treatment. **Objective:** It is hypothesized that inter-individual differences in the IFN type-I response program at baseline, and concomitant variation in IFN-b responsiveness may be the result of inter-individual variation in IFN-b biology. In this study we investigated the role of genetic variation in IFN signaling components in relation to heterogeneous pharmacological response to IFN-b. **Methods:** To determine biological response, peripheral blood was collected from 30 relapsing-remitting multiple sclerosis (RRMS) patients before and during IFN-b therapy. From 20 untreated RRMS patients peripheral blood was collected at two time points over a 3 to 12 month time period to analyze stability of baseline values over time. Baseline stability and biological response-rate, were analyzed by Taqman low-density arrays using the mean gene-expression level of a set of 10 IFN type-I response genes. Genetic variation was determined in several

components of the IFN signaling cascade using Taqman genotyping assays and conventional genomic polymerase chain reaction (PCR). The ratios of gene-expression levels before and during therapy were compared with occurrence of genetic variation in components of the IFN signaling pathway. **Results:** The extent of biological response correlated negatively with baseline expression of the type-I IFN response gene-set (R=-0.3891; p=0.0336). Baseline stability over time was reflected by a correlation coefficient of r=0.54; P=0.029 (n=20). Next we determined the association of genetic variation in IFN signalling components with IFN type-I response gene activity at baseline and after pharmacological intervention with IFN-b. This analysis revealed a significant association between genetic variation in one of the IFN signaling components and baseline IFN type-I response gene expression (P=0.0198). Accordingly, a significant reduced biological response was observed for patients who contained the genetic variant (P=0.0057). **Conclusions:** Variation in IFN signaling may determine (part) of the heterogeneity in pharmacological and clinical response to IFN-b in RRMS.

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Evaluation of modified Montreal cognitive assessment in multiple sclerosis: a pilot study

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Background: Multiple sclerosis (MS) is associated with cognitive impairment in 40-70% of patients, including slowness of information processing. Mild cognitive dysfunction (MCD) in MS may occur prior to physical disability but is difficult to assess using current bedside cognitive tools. The Montreal Cognitive Assessment (MoCA) is a 10 minute bedside test, which is useful in patients with Mild cognitive impairment (MCI). We propose the Modified MoCA (MoMoCA), which includes time taken to complete the MoCA as a measure of speed of information-processing. **Objective:** To obtain preliminary evidence that the timed MoMoCA can help identify MCD in the MS population, as it will take longer for these patients to complete the test. **Methods:** In this pilot study 30 MS subjects with Expanded Disability Status Scale (EDSS) scores under 6.0 and 30 subjects in a non-MS group were compared using the following tests: EDSS, MoMoCA (including score and the time taken to complete the test), Mini Mental State Examination (MMSE), Multiple Sclerosis Screening Questionnaire (MSNQ), Modified Fatigue Impact Scale (MFIS) and Paced Auditory Serial Additions Test (PASAT). **Results:** Interim analysis in 20 MS subjects age 25-55 with mean EDSS score of 2.7 indicates that the length of time to complete the MoMoCA was 7.2 minutes. MMSE and MoMoCA score alone were not sensitive enough to identifying MCD in MS. In our study 90% of subjects had fatigue and this affected PASAT scores. The final analysis will be presented at the meeting. **Conclusions:** MoMoCA may be a sensitive test to measure speed of information-processing and a good indicator of MCD in patients with MS. PASAT scores are useful in subjects who do not experience fatigue. Further comparisons in a larger MS population versus non-MS subjects would be beneficial.

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P895**NMO-IgG diagnostic and prognostic performance**

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Background: NMO-IgG, a serum IgG which reacts with aquaporin-4 (AQP4), is a specific biomarker for neuromyelitis optica (NMO) and related disorders. It was reported in 2004 by Mayo Clinic investigators and several independent teams have studied the performance characteristics of new assays for AQP4 antibodies. The studies have varied in the selection of cases (NMO, opticospinal multiple sclerosis (OSMS), limited/inaugural forms-longitudinally extensive transverse myelitis (LETM) and recurrent optic neuritis(ON)); controls (multiple sclerosis (MS), other neurological or autoimmune disease) and in immunoassay technique. **Objective:** To summarize data on assays that detect AQP4-specific antibody according to case and control selection and immunoassay technique. **Methods:** We carried out a systematic review of the indexed literature from 2004 to 2008. **Results:** Eleven case-controls and two longitudinal studies of seropositive inaugural syndromes were reviewed. In total 1475 individuals were tested for NMO-IgG combining the case-control studies: 305 NMO cases, 140 limited syndromes suggestive of NMO and 1030 controls. When compared with MS controls, the specificity ranged between 85% and 100%. The sensitivity range was 47% to 91%. The sensitivity was lower when OSMS (LETM not required) was used as the case definition or when studying children. Sensitivity and specificity for isolated or recurrent LETM was only slightly lower than for NMO. Immunoassays included indirect immunofluorescence (IF) with rodent central nervous system (CNS) substrate, indirect IF on cells stably transfected with AQP4-cDNA and radio-immunoprecipitation assay using recombinant AQP4. The sensitivity and specificity did not differ importantly between assay techniques, although conflicting results by two groups were reported when the reference IF assay on mouse brain at Mayo Clinic was compared directly with IF on transfected cell lines. For patients with a single episode of LETM and with ON, seropositivity for NMO-IgG predicted recurrence of LETM and ON. **Conclusions:** NMO-IgG is highly specific for the diagnosis of NMO and related disorders, and predicts the risk of recurrence of myelitis and/or ON in patients with limited or inaugural presentations of NMO. Superiority of one immunoassay technique has not been demonstrated, so further study is required.

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P896**Effect of heat exposure on cortical excitability and fatigue in multiple sclerosis patients**Andrea T. White¹, Scott L. Davis², Luke M. Wendt¹¹University of Utah, Salt Lake City, Utah, USA; ²University of Texas Southwestern, Dallas, Texas, USA

Background: Heat exposure produces fatigue and a worsening of symptoms in many multiple sclerosis (MS) patients and is thought to be due to the effects of demyelination in the central nervous system. **Objective:** The purpose of this study was to determine the effect of heat stress and fatigue on cortical excitability in MS patients. **Methods:** Nine relapsing-remitting MS patients (aged 40 ± 7 years; diagnosed for 5 ± 1.3 years; Expanded Disability Status Scale (EDSS) range of 1–3) who experience heat-related fatigue underwent transcranial magnetic stimulation (TMS). Recruitment curves were generated at 100%, 110%, 120%, and 130% of resting motor threshold (RMT). Motor-evoked potential (MEP) amplitudes were recorded before and after 3 minutes of sustained, maximal thumb abduction. Fatigue and subjective ratings of thermal sensation (TS) and discomfort (TD) were assessed throughout the protocol. All testing occurred under both thermoneutral (TN) and heat-stressed (HS) conditions. **Results:** Significant treatment differences were observed for core and mean skin temperatures and heart rate (37.65 ± 0.13 versus 37.01 ± 0.37 °C; 37.44 ± 0.55 versus 33.73 ± 1.48 °C; and 91 ± 8.5 versus 73 ± 8.5 bpm, for HS and TN, respectively). Subjective ratings for TS (7.3 ± 0.6 versus 4.4 ± 0.7), TD (3.8 ± 1.1 versus 1.3 ± 0.5), and fatigue (45 ± 28 versus 19 ± 18) were significantly higher ($p < 0.001$) and peak force was

significantly lower during HS versus TN (3831 ± 1585 versus 4498 ± 1253, $p < 0.05$). TMS recruitment curve slopes were less steep during HS, with significant differences at 120% and 130% RMT ($p < 0.05$). MEP amplitudes were lower at baseline during HS and remained lower throughout 10 minutes postexercise recovery. **Conclusions:** HS increased fatigue and decreased peak force and resulted in decreased cortical excitability, evidenced by changes in recruitment curves and MEP amplitudes before and after exercise. Slowed or blocked conduction in demyelinated cortical pathways secondary to heat stress may account for these changes.

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P897**Fetuin-A is a biomarker for disease activity and treatment efficacy in multiple sclerosis**

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Background: Fetuin-A was identified in a cerebrospinal fluid (CSF) proteomics study that revealed an elevation of this protein in patients with multiple sclerosis (MS) compared with controls. Fetuin-A is known to be involved in a variety of biological functions that may affect blood-brain barrier integrity and immune dysregulation including its activity as a TGFβ1 antagonist. **Objective:** We investigated whether Fetuin-A levels in the CSF and brain correlated with disease activity in MS. We also examined whether this protein could be used as a biomarker for treatment response to natalizumab. **Methods:** Fetuin-A levels in CSF and plasma were determined by enzyme-linked immunosorbent assay (ELISA) and disease activity was determined by clinical and magnetic resonance imaging (MRI) findings. Fetuin-A protein and mRNA expression were analyzed by immunohistochemistry and quantitative polymerase chain reaction (PCR). The pathological studies were extended to experimental autoimmune encephalomyelitis (EAE). The *in vitro* effects of Fetuin-A on TGFβ1 mediated inhibition of T-cell proliferation was also analyzed. CSF Fetuin-A levels were also determined in natalizumab-treated patients at baseline and after six months of therapy. All patient samples were obtained with IRB informed consent. **Results:** CSF levels of Fetuin-A in patients with active disease (mean=1600 µg/ml) were significantly elevated ($p=0.0003$) in comparison with patients with stable disease (mean=1177 µg/ml), while no differences were found in plasma Fetuin-A levels. In brain tissue Fetuin-A levels were markedly elevated in all areas of demyelination in comparison with other regions in the same brain and also in comparison with control brains. Fetuin-A mRNA expression levels were significantly higher in MS brain tissue compared with levels in normal brain (~40 000 fold increase; $p<0.0001$). Fetuin-A treatment resulted in a significant decrease in T-cell proliferation *in vitro* ($p=0.0007$). In natalizumab treatment responders CSF Fetuin-A levels were significantly lowered post-treatment. In EAE, Fetuin-A was specifically increased in acute inflammatory lesions. **Conclusions:** These findings suggest that Fetuin-A is a biomarker of disease activity in MS and can be used as an indicator of therapeutic efficacy. The EAE findings provide an experimental model for further investigation.

P898**Retinal nerve fiber thickness in inflammatory demyelinating diseases of childhood onset**

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Background: Acquired central nervous system (CNS) demyelination has been reported to occur in approximately 0.4/100 000 children. Although visual problems may be found in up to two-thirds of this

cohort, standardized tools to monitor the anterior optic pathway (AOP) in this cohort are currently unavailable. **Objective:** To evaluate the use of a standardized visual battery in children with acquired demyelinating diseases, focusing on average retinal nerve fiber layer thickness (ARNFLT) as measured by optical coherence tomography (OCT). **Methods:** Case-control study including a consecutive patient cohort followed at the Pediatric Multiple Sclerosis (MS) Center at The Jacobs Neurological Institute. Consensus definitions for pediatric demyelinating disease were followed. Controls were healthy children. All patients and controls received visual acuity (VA)/low-contrast letter acuity testing and optical coherence tomography (OCT). **Results:** Data from 31 children and 15 controls was included. Average age of the patient/control cohort was 13.2/9.8 years (range 4.3–17/4–17). The female to male ratio was 1:1.6/1:1.5. A history of optic neuritis (ON) was present in 18/31 patients (58%). Diagnoses

included MS (12), chronic relapsing inflammatory optic neuropathy (2), ON (7), transverse myelitis (TM) (2), and acute disseminated Eencephalomyelitis (ADEM) (8). ARNFLT in patients was $89.8 \pm 19.5 \mu\text{m}$ ($100 \pm 12.8 \mu\text{m}$ in unaffected eyes versus $76.2 \pm 18.5 \mu\text{m}$ in affected eyes) and $107 \pm 12.4 \mu\text{m}$ in controls. The ARNFLT in MS patients was decreased, even in eyes without a history of ON: $93.9 \pm 13 \mu\text{m}$ versus $81.7 \pm 13.2 \mu\text{m}$ in affected eyes, while in ADEM/TM it was $109 \pm 7.2 \mu\text{m}$ in unaffected eyes versus $67.2 \pm 17.3 \mu\text{m}$ in affected eyes. ARNFLT in ON was $97.3 \pm 12.6 \mu\text{m}$ in unaffected eyes versus $76.9 \pm 21.2 \mu\text{m}$ in affected eyes. **Conclusions:** Patients with a history of demyelinating disease show evidence of decreased RNFLT, often bilateral, even in the absence of a history of ON. OCT may be a valuable tool to assess and monitor AOP dysfunction in children with demyelinating diseases.

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