The impact of DAA-mediated HCV eradication on CD4⁺ and CD8⁺ T lymphocyte trajectories in HIV/HCV coinfected patients: data from the ICONA Foundation Cohort

Running title: CD4⁺ and CD8⁺ trajectories in HIV/HCV

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Abstract

HCV infection has been hypothesized as a contributor of poor $CD4^+$ recovery in patients living with HIV (PLWHIV). Aim of this study was to evaluate $CD4^+$, $CD8^+$ cells and CD4/CD8 ratio trends before and after HCV treatment with direct acting agents (DAA) in PLWHIV. HIV/HCV patients enrolled in ICONA and HepaICONA cohorts with HIV-RNA $\leq$ 50 copies/mL who achieved a sustained viral response after DAA treatment were studied. A linear regression model was used to investigate $CD4^+$, $CD8^+$, and CD4/CD8 changes 12 months before and after DAA treatment. A total of 939 HIV/HCV patients were included, 225 (24.0%) female, median age: 53 years (IQR 50-56). At DAA initiation, $CD4^+$ T cell count was <350 cells/mm^3 in 164 patients (17.5%), and 246 patients (26.2%) had liver stiffness> 12.5 kPa. Trends of $CD4^+$ and CD4/CD8 ratio were similar before and after DAA in all study populations ($CD4^+$ change +17.6 cells/mm^3 (95%CI -33.5;69.4, p= 0.494); CD4/CD8 change 0.013 (95%CI -0.061; 0.036, p= 0.611). However, patients treated with ribavirin (RBV)-free DAA showed a significant decrease in $CD8^+$ cells (-204.3 cells/mm^3, 95%CI -375.0;-33.4, p=0.019), while patients treated with RBV experienced $CD8^+$ cell increase (+141.2 cells/mm^3, 95%CI 40.3;242.1, p=0.006). In conclusion, HCV eradication following DAA treatment does not seem to have an impact on $CD4^+$ T cell recovery in PLWHIV. However, a fast

decline of $CD8^+$T cells has been observed in patients treated without RBV, suggesting a favorable effect of HCV clearance on the general state of immune activation.

Keywords: HCV/HIV, DAA, CD4, CD8, immune activation.

Introduction

People living with HIV (PLWHIV) experience a state of chronic inflammation, established at the moment of HIV infection and, although reduced, persisting also during stable antiretroviral therapy (ART)-mediated HIV suppression [1]. Hepatitis C virus (HCV) co-infection can potentially enhance persistent immune activation, as demonstrated by higher levels of markers of inflammation, such as tissue factor activity, sCD14, IL-6, and IFNα [2,3], besides higher level of $CD4^+$ and $CD8^+$ T cell activation and accumulation of highly differentiated $CD8^+$ T cells [4–6] in HIV^+/HCV^+ patients as compared to HIV^+/HCV^- subjects. As inflammation and immune activation are currently considered the driving force for CD4 depletion in the course of HIV infection [7], HCV has been hypothesized as a possible contributor of poor $CD4^+$ cell recovery under ART in PLWHIV [8]. Few data are available so far on the impact that HCV eradication might have on immune activation and specifically on $CD4^+$ T lymphocyte recovery. Previous studies have shown that some markers of inflammation (IFNα, M-CSF, ICAM-1, VCAM-1, sFasL, TNFβ, sCD163) decreased in HIV/HCV patients after treatment with interferon-based regimens [9,10], but a possible influence of the immune modulating effect of interferon itself might have influenced these outcomes. Less is known on the possible effect of new interferon-free anti-HCV therapies with direct acting agents (DAA) that result in a high rate of HCV eradication in the absence of intrinsic immune modulating effects. Lopez-Cortes and colleagues recently demonstrated that, after successful DAA treatment, patients experienced a significant decrease of immune activation (i.e. HLA-DR and CD38 on $CD4^+$ and $CD8^+$ T cells), HIV-DNA, microbial translocation markers and D-dimer [11]. However, no changes on $CD4^+$ or $CD8^+$ T-cells counts were found, but, since the work was limited by relatively small sample size, definitive conclusions could not be drawn. To overcome this possible issue, and with the aim of identifying $CD4^+$, $CD8^+$ cells and CD4/CD8 ratio trends before and after DAA treatment in a cohort of HIV/HCV patients, we evaluated the data prospectively collected in the context of the ICONA and HEPA-ICONA observational cohorts.

Materials and methods

The Italian Cohort Naive Antiretrovirals Foundation Study (ICONA) is a multi-centre, prospective and observational cohort study, recruiting ART-naïve PLWHIV from 1997. Since 2013, the ICONA Network has been enlarged to include a new cohort of PLWHIV co-infected with HCV, who are

chronically infected with HCV and naïve to DAA treatment at enrolment: the HepaICONA study. The ICONA and HepaICONA studies have been approved by Institutional Review Boards of all the participating centres. Patients enrolled in HepaICONA cohort may be both ART-naive or experienced, provided that they have never received DAA treatment before enrolment. Data are collected prospectively from the date of entry in the cohort till last available follow-up for all patients who agree to participate and sign consent forms, in accordance with the ethical standards of the committee on human experimentation and the Helsinki Declaration. Demographic, clinical, laboratory data and information on therapies are prospectively collected and recorded in anonymous form. CD4 and CD8 counts are collected at every visit, on average, every 6 months. For the present study, we selected HIV/HCV coinfected patients enrolled in ICONA and HepaICONA, who received DAA treatment between April 2013 and September 2018 and who achieved a sustained viral response 12 weeks after the completion of DAA treatment (SVR12). Patients were included in the analysis if they were on ART for >12 months, with HIV-RNA ≤50 copies/mL at DAA start, and with at least one available $CD4^+$ and $CD8^+$ count at DAA initiation (baseline) and at least one further measurement 12 months before and/or 12 months after baseline. The change in biomarkers before treatment was evaluated as the difference between baseline value (at DAA start) and the value measured 12 months before (the closest value in the interval [-18; -6] months). The change in biomarkers after treatment was evaluated as the difference between the value at baseline (at DAA start) and 12 months later and after SVR was achieved (the closest value in the interval [+6; +18] months). Each patient contributed to the analysis with the change in $CD4^+$, $CD8^+$ and CD4/CD8 i) before treatment or ii) after treatment or iii) both. A linear regression model was used to compare the change of $CD4^+$, $CD8^+$, and CD4/CD8 before and after DAA treatment. Because we selected people who eradicated HCV with DAA and we used the markers variation observed in the pre-DAA period as the not exposed group, this selection essentially operates as an instrumental variable for the association between DAA and variation in the marker post SVR. Furthermore, about half of patients were included both as exposed and not exposed so that for these individuals partial exchangeability was automatically established. Therefore, the unadjusted analysis should provide the causal effect of DAA driven eradication on marker changes.

A formal interaction test between the biomarker changes post eradication and main confounders at baseline (RBV use, CD4 count, stiffness, risk factor for HIV acquisition and age) was performed. Multivariable models were also adjusted for HCV genotype and exact time between measurements in the time windows.

Results

A total of 939 HCV/HIV patients were included in the study, 225 (24.0%) female, with median age of 53 years (IQR 50-56). Median follow up was 13.7 months (IQR 8.6-17.5). The risk factor for HIV acquisition was intravenous drug use in the majority of patients (N=687, 73.2%), while 190 reported unprotected intercourse (N=118, 12.6% heterosexual and N=72, 7.7% were MSM). At the time of DAA initiation, they all had HIV-RNA≤50 copies/mL and $CD4^+$ T cell count was >500 cells/mm^3 in 620 (66.0%) patients, 351-500 cells/mm^3 in 155 (16.5%) and ≤350 cells/mm^3 in 164 (17.5%), while $CD4^+$ T cell nadir was ≤200 cells/mm^3 in 165 (17.6%) patients. Median time of ART use at DAA start was 7.1 years (IQR: 4.0-11.3), and the combination of nucleoside reverse transcriptase inhibitors (NRTI) + integrase strand transfer inhibitors (INSTI) was the most represented ART regimen (37.0%), followed by NRTI+ non-nucleoside reverse transcriptase inhibitors (NNRTI), (18.9%), and NRTI+ protease inhibitors (PI), (17.0%).

Main characteristics of the study population are shown in Table 1. Two hundred forty-six (26.2%) patients had an advanced stage of liver fibrosis, with liver stiffness exceeding 12.5 kPa, and median HCV-RNA at DAA start was 6 log_{10} UI/mL (IQR: 5.5-6.5). DAA combinations that were more frequently used in the cohort were sofosbuvir+velpatasvir ± ribavirin (N=247, 26.3%) sofosbuvir+ledipasvir ± ribavirin (N=239, 25.5%), sofosbuvir+daclatasvir ± ribavirin (N=156, 16.6%), ombitasvir/paritaprevir/ritonavir+dasabuvir ± ribavirin (N= 91, 6.7%) and glecaprevir/pibrentasvir (N=60, 6.4%). Globally, ribavirin was used in 332 (35.4%) patients. The duration of DAA treatment was 8 weeks in 57 patients (6.2%), 12 weeks in 635 (68.6%) and 24 weeks in 234 (25.3%).

About half (44%) of the study population contributed to immunological values before and after baseline, 17% and 39% contributed with values only before or only after DAA initiation respectively. Mean $CD4^+$ and $CD8^+$ T cell changes one year before and one year after DAA treatment are showen in Table 2. $CD4^+$ and $CD8^+$ changes were similar before and after DAA

treatment, with a mean difference in $CD4^+$ change of +17.6 cells/mm^3 (95%CI -33.5;+69.4, p= 0.494) and in $CD8^+$ of -68.1 cells (95%CI -170.2;+34.0, p= 0.191). The difference in CD4/CD8 ratio change was -0.013 (95%CI -0.061; +0.036, p= 0.611).

The analysis was subsequently repeated to take into account if patients received also ribavirin (RBV) in association with DAA treatment or not. Patients who were treated with RBV-free DAA regimens showed stable $CD4^+$T cell counts, with an adjusted difference in change of -3.9 cells/mm^3 (95%CI -91.0; +83.2, p= 0.930), but they showed a significant change in the $CD8^+$T cell trajectory, that was raised before DAA treatment and declined thereafter, with adjusted difference in change of -204.3 cells/mm^3 (95%CI -375.0;-33.4, p=0.019) (Table 3). The CD4/CD8 ratio did not change significantly after treatment, with an adjusted difference in change of -0.033 (95%CI -0.112;+0.047, p= 0.418). Furthermore, in patients treated with RBV, $CD4^+$T cells did not change significantly, with an adjusted difference in change pre- and post-DAA of +33.9 cells/mm^3 (95%CI -13.6;+81.5, p=0.161). In contrast, $CD8^+$ T cells had different trend pre- and post-DAA treatment, with a stable count before and a rise after DAA treatment and an adjusted difference in change of +141.2 cells/mm^3 (95%CI +40.3;+242.1, p=0.006). In particular, the formal interaction test between RBV use and pre/post change of $CD8^+$ was found significant (p=0.002). The adjusted difference in CD4/CD8 ratio change was -0.022 (95%CI -0.083; +0.038, p= 0.470).

The exploration of a possible interaction between biomarker change post eradication and the confounder measured at baseline: CD4 count (≤200, >200), stiffness (F1-F3, F4), risk factor for HIV acquisition (MSM, IDU) and age (≤50 years, >50 years) did not show significant results (Tables S1, S2, S3, S4).

Discussion

The present analysis describes $CD4^+$ and $CD8^+$ changes in HIV/HCV coinfected patients before and after being successfully treated with DAA, and with almost 1,000 people included, is, to our knowledge, the largest study available to date on this issue. According to our data, DAA treatment in HCV/HIV patients does not show a beneficial effect on $CD4^+$ T cell recovery in the months following treatment, regardless of whether RBV was included in the combination. Indeed, although a $CD4^+$ increase has been noticed after treatment, the adjusted difference in $CD4^+$ T cells from the pre-DAA period was not significant. This result confirms similar previous

studies performed in smaller groups of patients [11,12]. The reason why a direct effect on $CD4^+$ was not found remains to be ascertained. This could be simply due to short follow-up post HCV eradication. However, although the enhanced immune activation in HCV/HIV coinfection might play a major role in HIV infected patients who fail to recover their $CD4^+$ T cells [13], results are conflicting, with most studies reporting a negative effect of HCV on $CD4^+$ T cell recovery and others reporting no effect [8]. Moreover, the same number of circulating $CD4^+$ T cells can reflect diverging functional immune characteristics in different patients [14], and studies focusing on the immune activation following HCV eradiation showed a significant decline of activated $CD4^+$ and $CD8^+$ T cells, despite their stable absolute counts [11,12]. In our work, only the absolute number of $CD4^+$ was available, and thus it is not assessable if their post-DAA stability could be associated to a reduction of immune activated or exhausted cells, rather than to low replication of naive T-cells.

Nevertheless, we observed a notable change in $CD8^+$ T cells, with different trends in patients treated with or without ribavirin. The results seem to be biologically plausible. After the removal of the HCV antigenic trigger, a $CD8^+$ T cell reduction would be expected, as a consequence of reduced systemic inflammation, in accordance with the reduction of HLA-DR and CD38 expression that have been previously reported after DAA treatment [11,12,15]. Moreover, the reduction of intrahepatic $CD8^+$ T cell density is the predominant change that has been detected in liver biopsies of DAA treated patients [16]. Nonetheless, we found a reduction of $CD8^+$ T cells only in patients who were treated with RBV-free DAA regimens, and, on the contrary, an increase in others. The reasons for these discrepant results remain unclear, and are probably multifactorial. A possible direct effect of ribavirin on immune response stimulation has been previously hypothesized, with possible T-cell proliferation induction at low dosages, consequent to IL-2 production and polarization of the T cell responses towards a type 1 cytokine profile [17], although an inhibition of T cell proliferation is expected at higher dosages [18]. On the other side, in our study, ribavirin was mainly used in combination with DAA for treating patients with more advanced stages of liver fibrosis [19]. It is thus possible that the reduction of inflammation and of $CD8^+$ T cells could be seen only in patients with lower fibrosis, while cirrhotic patients might show a paradoxical increase in $CD8^+$ T cells due to recover from a previous state of cytopenia, that could be induced by cirrhosis, but also by ribavirin [4,20]. However, neither in patients treated with

ribavirin nor in others the variation of $CD8^+$ T cells led to a variation in the CD4/CD8 ratio one year after treatment, thus, the relevance of the data remains to be ascertained.

The findings of our study are mainly limited by its observational nature and by the fact that $CD4^+$ and $CD8^+$ T cell subsets and their proportion in the different study time-points could not be determined. Moreover, the study lacks a comparison group of HIV/HCV coinfected patients who did not clear HCV-RNA so that we can be sure that the changes observed can be attributed to DAA-driven eradication alone. However, the very high success rate of DAA treatment even in the co-infected population strongly limits the sample size of non-responders. Furthermore, the main analysis relies on unconditional exchangeability achieved using biomarker changes prior to DAA initiation as control and, for the effect of RBV, on the assumption that our model of the confounding pattern is correctly specified and there are no unmeasured confounding factors.

In conclusion, HCV eradication following DAA treatment does not seem to have an impact on $CD4^+$ T cell recovery in PLWHIV, at least in the first months. However, a fast decline of $CD8^+$ T cells has been observed after treatment in the subset of patients treated without RBV, suggesting a favorable effect of HCV clearance on the general state of immune activation. A longer follow-up, and a control group that do not eradicate HCV might be required to evidence more significant changes in the lymphocyte population after DAA treatment.

Data Availability Statement: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Significance Statement: Inflammation and immune activation are currently considered the driving force for $CD4^+$ T cell depletion in the course of HIV infection. As a higher expression of inflammation markers has been found in HIV^+/HCV^+ compared to HIV^+/HCV^- patients, we investigated if HCV eradication with new direct acting agents (DAA) had an impact on $CD4^+$ T cell recovery in people living with HIV. We did not find a significant change in $CD4^+$ T cells after DAA, but the fast decline of $CD8^+$ T cells observed in patients treated without ribavirin, suggested a favorable effect of HCV clearance on the general state of immune activation in the course of HIV infection.

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		All population (n=939)	No RBV (n=607)	RBV (N=332)	p
Gender, n(%)	M	714 (76.0%)	454 (74.8%)	260 (78.3%)	0.227
	F	225 (24.0%)	153 (25.2%)	72 (21.7%)	
Italian nationality, n(%)	No	103 (11.0%)	94 (15.5%)	9 (2.7%)	<0.001
	yes	836 (89.0%)	513 (84.5%)	323 (97.3%)	
Age, yrs, median IQR		53 (50-56)	53 (50-56)	53 (50-55)	0.292
Mode of HIV transmission, n(%)	heterosexual	118 (12.6%)	80 (13.2%)	38 (11.5%)	0.012
	IDU	687 (73.2%)	437 (72.0%)	250 (75.3%)	
	MSM	72 (7.7%)	57 (9.4%)	15 (4.5%)	
	other/unknown	62 (6.6%)	33 (5.4%)	29 (8.7%)	
AIDS diagnosis, n(%)	No	784 (83.5%)	512 (84.3%)	272 (81.9%)	0.339
	Yes	155 (16.5%)	95 (15.7%)	60 (18.1%)	
CD4 nadir, cell/mm^3, n(%)	<=200	165 (17.6%)	97 (16.0%)	68 (20.5%)	0.083
	200+	774 (82.4%)	510 (84.0%)	264 (79.5%)	
CD4 at BL, cell/mm^3, n(%)	0-350	164 (17.5%)	92 (15.2%)	72 (21.7%)	0.003
	351-500	155 (16.5%)	91 (15.0%)	64 (19.3%)	
	500+	620 (66.0%)	424 (69.8%)	196 (59.0%)	
cART change for DAA use, n(%)	no	671 (71.5%)	440 (72.5%)	231 (69.6%)	0.345
	yes	268 (28.5%)	167 (27.5%)	101 (30.4%)	
Type of cART regimen, n(%)	NRTI+NNRTI	177 (18.9%)	131 (21.6%)	46 (13.9%)	0.001
	NRTI+PIB	160 (17.0%)	91 (15.0%)	69 (20.8%)	
	NRTI+INSTI	347 (37.0%)	215 (35.4%)	132 (39.8%)	
	PI monotherapy	39 (4.2%)	33 (5.4%)	6 (1.8%)	
	NUCS-sparing	152 (16.2%)	102 (16.8%)	50 (15.1%)	
	other	64 (6.8%)	35 (5.8%)	29 (8.6%)	
Stiffness at BL, n(%)	<12.5	557 (59.3%)	418 (68.9%)	139 (41.9%)	<0.001
	12.5+	246 (26.2%)	109 (18.0%)	137 (41.2%)	
	not available	136 (14.5%)	80 (13.1%)	56 (16.9%)	
HCV-RNA at BL, log_{10}, median (IQR)		6 (5.5-6.5)	6 (5.5-6.5)	6 (5.4-6.4)	0.076

Table 1. Main characteristics of study population at DAA initiation of 939 patients who contributed at least one pair between T1-T2 and T2-T3.

Length of HCV therapy, n(%)	8w	57 (6.2%)	55 (9.2%)	2 (0.6%)	<0.001
	12w	635 (68.6%)	442 (73.8%)	193 (59.0%)	
	24w	234 (25.3%)	102 (17.0%)	132 (40.4%)	
HCV genotype, n(%)	1	528 (57.1%)	345 (57.7%)	183 (56.0%)	0.191
	2	22 (2.4%)	18 (3.0%)	4 (1.2%)	
	3	221 (23.9%)	132 (22.1%)	89 (27.2%)	
	4	153 (16.5%)	102 (17.0%)	51 (15.6%)	
	other	1 (0.1%)	1 (0.2%)	0	
Type of DAA, n(%)	sof+dac (+/-rbv)	156 (16.6%)	80 (13.2%)	76 (22.9%)	<0.001
	sof+vel (+/-rbv)	247 (26.3%)	237 (39.0%)	10 (3.0%)	
	sof+lep (+/-rbv)	239 (25.5%)	138 (22.7%)	101 (30.4%)	
	ombit/parit + das (+/-rbv)	91 (6.7%)	23 (3.8%)	68 (20.5%)	
	glecaprevir	60 (6.4%)	60 (9.9%)	0	
	grazoprevir (+/- rbv)	48 (5.1%)	38 (6.3%)	10 (3.0%)	
	sof/sim (+/- rbv)	46 (4.9%)	26 (4.3%)	20 (6.0%)	
	other	52 (5.5%)	5 (0.8%)	47 (14.2%)	

Table 2. Mean and standard deviation (SD) of CD4, CD8, ratio and white cells at 3 time points (T1=12 months before DAA initiation, T2= DAA initiation, T3= 12 months after DAA initiation).

All study population

	T1-T2 (12 months pre DAA initiation and BL)							T2-T3 (BL and 12 months post DAA initiation)						
Biomarker	**N**	**Mean 1**	**SD1**	**Mean 2**	**SD2**	**Difference**	**p-value**	**N**	**Mean 2**	**SD2**	**Mean 3**	**SD3**	**Difference**	**p-value**
CD4 count	577	669	336	666	310	-2.9	0.741	777	685	672	700	341	+15	0.487
CD8 count	577	881	430	951	943	+71	0.057	777	978	967	980	613	+2.5	0.944
CD4/CD8 ratio	577	0.87	0.47	0.87	0.46	-0.007	0.963	777	0.86	0.67	0.85	0.48	-0.012	0.539
White blood cells	522	6360	2184	6263	2078	+470	0.152	761	6169	2097	6659	2305	+490	<0.001

Patients treated with RBV

Biomarker	T1-T2 (12 months pre DAA initiation and BL) N	Mean 1	SD1	Mean 2	SD2	Difference	p-value	T2-T3 (BL and 12 months post DAA initiation) N	Mean 2	SD2	Mean 3	SD3	Difference	p-value
CD4 count	190	614	348	605	296	-8	0.641	300	615	315	642	315	+28	0.029
CD8 count	190	888	519	905	505	-17	0.413	300	884	556	986	704	+102	0.003
CD4/CD8 ratio	190	0.82	0.53	0.84	0.48	+0.017	0.442	300	0.85	0.50	0.84	0.55	-0.003	0.844
White blood cells	142	6103	2199	6085	2276	+272	0.871	239	5899	2126	6287	2287	+389	<0.001

Patients NOT treated with RBV

Biomarker	T1-T2 (12 months pre DAA initiation and BL) N	Mean 1	SD1	Mean 2	SD2	Difference	p-value	T2-T3 (BL and 12 months post DAA initiation) N	Mean 2	SD2	Mean 3	SD3	Difference	p-value
CD4 count	387	696	326	696	312	-0.3	0.978	477	729	818	736	352	+7	0.839
CD8 count	387	869	388	982	1093	+113	0.037	477	1037	1150	977	549	-60	0.262
CD4/CD8 ratio	387	0.89	0.44	0.88	0.45	-0.02	0.521	477	0.87	0.75	0.85	0.43	-0.018	0.561
White blood cells	357	6478	2170	6345	1977	+585	0.115	384	6328	2194	6930	2231	+602	<0.001

Table 3. Unadjusted and adjusted difference in the change of CD4, CD8, ratio and white cells by means of linear regression in the two strata of patients: a) treated with RBV and b) not treated.

a) Patients treated with RBV, N=332				
	Unadjusted difference in change		Adjusted* difference in change	
Biomarker	**Variation Pre-Post DAA (95% CI)**	**p-value**	**Variation Pre-Post DAA (95% CI)**	**p-value**
CD4				
Post-DAA vs. pre-DAA	36.2 (-5.7, 78.0)	0.090	33.9 (-13.6, 81.5)	0.161
CD8^				
Post-DAA vs. pre-DAA	119.0 (30.3, 207.7)	0.009	141.2 (40.3, 242.1)	0.006
ratio				
Post-DAA vs. pre-DAA	-0.021 (-0.074, 0.033)	0.095	-0.022 (-0.083, 0.038)	0.470
white blood cells				
Post-DAA vs. pre-DAA	407 (99, 714)	0.010	279 (-162, 720)	0.214
b) Patients NOT treated with RBV, N=607				
CD4				
Post-DAA vs. pre-DAA	7.2 (-69.5, 84.0)	0.853	-3.9 (-91.0, 83.2)	0.930
CD8^				
Post-DAA vs. pre-DAA	-173.7 (-324.8, -22.6)	0.024	-204.3 (-375.0, -33.4)	0.019
ratio				
Post-DAA vs. pre-DAA	-0.009 (-0.080, 0.060)	0.783	-0.033 (-0.112, 0.047)	0.418
white blood cells				
Post-DAA vs. pre-DAA	734 (487, 982)	<0.001	710 (359, 1061)	<0.001
***adjusted for time between measurements, age, stiffness and HCV genotype; ^the difference in change of CD8 is not adjusted for age**				