since 1956

# BLOOD TRANSFUSION

OFFICIAL JOURNAL OF

**SIMTI** Società Italiana di Medicina Trasfusionale e Immunoematologia

**AICE** Associazione Italiana dei Centri Emofilia

**HDTM** Hrvatsko Društvo za Transfuzijsku Medicinu

**SETS** Sociedad Española de Transfusión Sanguínea y Terapia Celular

**SISET** Società Italiana per lo Studio dell'Emostasi e della Trombosi

**Blood Transfus 18, Supplement no. 4, November 2020**

-ISSN 1723-2007-

## ABSTRACT BOOK

**XXVI Congresso Nazionale della Società Italiana per lo Studio dell'Emostasi e della Trombosi - SISET**

Baveno (VB), 5 - 7 novembre 2020

**Guest Editors:** Armando Tripodi, Anna Falanga, Valerio De Stefano

**www.bloodtransfusion.it**

Edizioni SIMTI



Blood Transfusion - Bimestrale spedizione in abbonamento postale 70% - Poste Italiane SpA LO/MI

the specific role played by FVIII, von Willebrand factor (VWF) and thrombin on osteoclasts and osteoblasts obtained from a haemophilic patient.

**Methods.** In vitro assays assessed the osteoclastogenic potential of PBMC (Peripheral Blood Mononuclear Cells) isolated from a haemophilic patient. Osteoclastogenesis of healthy donors-PBMC were analysed in the presence of FVIII (2U/ml), VWF (20 μg/ml), FVIII/VWF (2U/ml) with or without osteoprotegerin (OPG 25 ng/ml), and thrombin (Thb 100 nmol/l). Moreover, the effects of these treatments on osteoblast differentiation and activity were also studied.

**Results.** PBMC from HA patient showed increased ability to form mature osteoclasts compared to those obtained from healthy controls. Moreover, RNA expression analysis performed on patient's osteoclasts revealed higher levels of RANK, TRAF6, CATHEPSIN K and TCIRG1 genes expression compared to control osteoclasts. VWF appears to play a major role, showing by itself ~45% inhibition of osteoclastogenesis comparable to OPG (the physiologic inhibitor), and even more if is complexed with FVIII (53% inhibition). Thrombin reduces osteoclast differentiation with variable effects (30-50% inhibition).

No alteration of alkaline phosphatase positivity was observed in control osteoblasts treated with Thb and VWF, whereas incubation with FVIII leads to a statistically significant reduction, also revealed in osteoblasts treated with FVIII/VWF.

**Conclusion.** All these data support that bone loss observed in haemophilic patients could be related to increased osteoclast formation and activity and that coagulation factors directly impact on bone cells.

## OC078 - Contribution of asialoglycoprotein receptor ASGR2 5' UTR polymorphisms to full-length FVIII concentrate pharmacokinetics

Barbara Lunghi [1] - Massimo Morfini [2] - Nicola Martinelli [3] - Sabrina Frusconi [4] - Dario Balestra [1] - Alessio Branchini [1] - Silvia Linari [5] - Giovanna Marchetti [6] - Giancarlo Castaman [5] - Francesco Bernardi [1]

*University of Ferrara, Dept of Life Sciences and Biotechnology, Ferrara [1] - Italian Association Hemophilia Centers, (AICE), Milan [2] - University of Verona, Dept of Medicine, Verona [3] - Careggi University, Genetic Diagnostics Unit, Laboratory Dept,, Florence [4] - Careggi University Hospital, Center for Bleeding Disorders, Dept of Oncology,, Florence [5] - University of Ferrara, Dept of Biomedical and Specialty Surgical Sciences, Ferrara [6]*

**Background.** The asialoglycoprotein receptor mediates endocytosis of galactose- and N-acetylgalactosamine-terminating glycoproteins, and binds with high affinity the FVIII B domain, particularly through its N-linked oligosaccharide structures. Evidences in mouse models support a role for this receptor in the VWF and FVIII clearance. The human oligomeric receptor is composed of major (ASGR1) and minor (ASGR2) subunits. Alternative splicing of the *ASGR2* mRNA originates multiple RNA transcripts, potentially encoding transmembrane and soluble isoforms. The transcript variants differs among individuals, and several polymorphisms have been detected in the 5'UTR.

To investigate the relation between the *ASGR2* 5'UTR polymorphisms and patient variability in FVIII pharmacokinetic (PK) outcomes.

**Methods.** Twenty-eight HA patients with FVIII:C ≤ 2 IU/dL underwent 55 FVIII single dose (21.4-51.8 IU/kg) PKs using pd-FVIII and/or FL r-FVIII concentrates. FVIII:C was measured up to 72 hours and analyzed by two-compartment PK model. PK parameters were evaluated in relation to *F8* mutations, *ABO* blood-group and eight polymorphisms in the *ASGR2* 5'UTR, investigated by sequencing.

**Results.** Patients grouping by the *ASGR2* g.5173T/C (c.-95T/C) and *ABO* genotypes displayed several significant differences in PK parameters. The c.-95TT homozygotes (n=9) differed from homozygotes and carriers of the C allele (n=19) for the K 1-2 (P=0.048), K 2-1 (P=0.021), Alpha (P=0.022), Alpha HL (P=0.01) and CLD2 (P=0.046) parameters. Homozygotes (n=5) for the common TT haplotype (H1), including the most frequent 5'UTR alleles conserved in primates, showed significantly lower K 1-2, Alpha and CLD2 values, and higher Alpha HL values than the CC homozygous genotypes.

In linear regression models including the *ASGR2* c.-95T/C and *ABO* genotypes, with PK parameters as dependent variables, the K 2-1, Alpha and Alpha HL parameters were significantly predicted by the *ASGR2* c.-95T/C (P=0.032, β coefficient 0.373; P=0.033, β coefficient 0.401 and P=0.016, β coefficient -0.426, respectively). In the non-O patients (n=19) the *ASGR2* c.-95T/C genotypes were associated with a significant gradient of K 1-2 (P=0.032), K 2-1 (P=0.042), Alpha (P=0.020), Alpha HL (P=0.011) and Cl (P=0.045) values.

**Conclusions.** Frequent *ASGR2* c.-95T/C genotypes showed in HA patients a significant and coherent association with several parameters of full-length FVIII PK. The association was detectable after correction for *ABO* genotypes, and produced clear PK parameter differences in non-O blood group patients. The influence on specific RNA transcripts and FVIII PK parameters of the homozygous *ASGR2* H1 haplotype deserves further investigation.

## OC079 - Identification of novel genetic risk factors in the conserved haplotype region surrounding the LCT locus on chromosome 2q21

Andrea Cairo [1] - Silvia Spena [2] - Emanuela Pappalardo [2] - Flora Peyvandi [3]
*Fondazione I.R.C.C.S. Ca' Granda, Ospedale Maggiore Policlinico, Fondazione Villa, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milano [1] - University of Milan, Department of Pathophysiology and Transplantation, Milano [2] - Fondazione I.R.C.C.S. Ca' Granda, Ospedale Maggiore Policlinico, Fondazione Villa and University of Milan, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center and Department of Pathophysiology and Transplantation, Milano [3]*

**Background.** Inhibitors development affects about 30% of patients with severe hemophilia A (HA). Different environmental and genetic risk factors are involved in this process. Recently, we identified a SNV (rs3754689) in the *LCT* gene potentially linked with this predisposition. Since this variant is benign and is located in a conserved haplotype block, we hypothesized that the association signal captured by this variant is probably located in coinherited, neighboring genes like *R3HDM1, UBXN4* and *miRNA-128-1*, previously linked with autoimmune disorders and therefore potential clinically relevant targets. The aim of this study is to identify novel genetic risk factors associated with inhibitor development in *R3HDM1, UBXN4, CXCR4, MCM6, miRNA-128-1* genes in patients with severe HA

**Methods.** A cohort of 246 Italian patients with severe HA with (72) or without (174) inhibitor was subjected to target sequencing by TruSeq Custom Amplicon (Illumina). Data were analyzed according to the guidelines reported by the Broadinstitute (https://software.broadinstitute.org/gatk/best-practices/). Statistical analysis was performed by Plink, PlikSeq. ENCODE project and Enhancer Atlas were consulted to evaluate the role of the variants in the gene expression.

**Results.** 228 variants passed the quality controls: 56 common (MAF≥1%) and 172 rare (MAF<1%). Logistic regression of common variants confirmed the protective role of the rs3754689 missense variant previously identified. Moreover, other 4 variants resulted significantly associated. Two of these (rs3213892; rs3816155) are localized in the intron 13 of the LCT; a genomic region involved in the regulation of the UBXN4 expression. Rare variants resulted not associated with the development of inhibitor.

**Conclusions.** This study confirmed the association of the chromosomal region around the LCT locus with inhibitor development in patients with severe HA. Further investigations are necessary to evaluate the expression of target genes and miR-128-1.

## OC080 - Recombinant FVIII in pharmacokinetic studies: a comparison among assays

Enrico Dosio [1] - Alessandra Valpreda [1] - Jacopo Agnelli Giacchello [1] - Cristina Dainese [1] - Federica Valeri [1] - Alessandra Borchiellini [1]
*University Hospital City of Health and Science, Regional center Hemorrhagic and Thrombotic diseases, Hematology Unit and Lab, Torino [1]*

**Background.** Personalized factor VIII (FVIII) prophylaxis is the best treatment for Hemophilia A. Recombinant FVIII (rFVIII) therapy with modified molecules is a challenge for laboratories. Literature reports discrepancies between rFVIII levels detected with one stage clotting assay (OSA) with different reagents and chromogenic substate assay (CSA), with a high risk of over/underestimating FVIII levels. Here we report pharmacokinetic (PK) data from personalized prophylaxis with different rFVIII.

**Methods.** From 2017 to 2019 we performed 41 PK studies with 10 different rFVIII, chosen on product and patient characteristics. For each PK point, factor levels were measured on two replicates with coagulometer ACL TOP