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*ABSTRACT BOOK OF*

## BIC INTERNATIONAL CONFERENCE

ADVANCES IN **HAEMOSTASIS** AND **BLEEDING DISORDERS**

**VENICE, ITALY** 17-19 SEPTEMBER 2021

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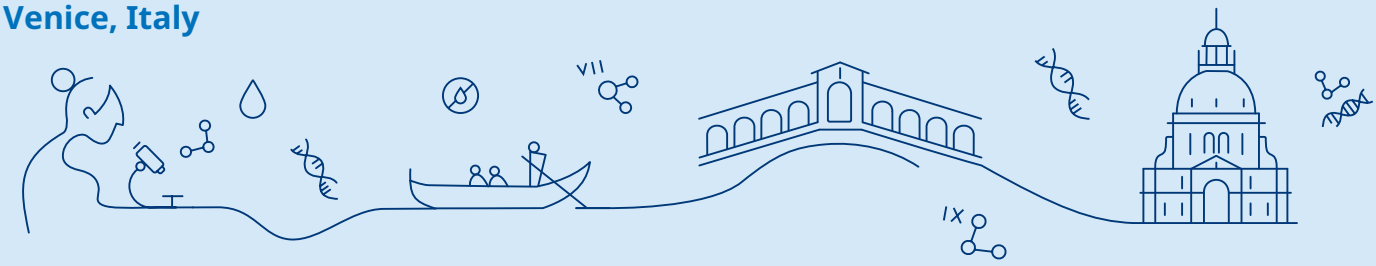
# **Continuing the commitment to patients with Haemophilia A:** Clinical evidence of N8-GP (turoctocog alfa pegol) in PTPs and PUPs

**BIC Symposium**
**18 September 2021**
**Venice, Italy**




# Agenda

**12:00 – 12:10** **Opening & Welcome**
*Prof. Pratima Chowdary*

**12:10 – 12:20** **Pathfinder Clinical Trial Programme: Efficacy and safety in adults PTPs**
*Prof . Pratima Chowdary*

**12:20 – 12:30** **Pathfinder Clinical Trial Programme: Efficacy and Safety in children PTPs**
*Prof. Gili Kenet*

**12:30 – 12:45** **Expanding experience into PUPs Efficacy and Safety results**
*Dr. Christoph Königs*

**12:45 – 13:00** **Q&A**



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# Abstract Book of
# 11th BIC International Conference *(Advances in Haemostasis and Bleeding Disorders)*
Venice, Italy, September 17-19 2021



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## ORAL COMMUNICATIONS



## POSTERS



## PUBLICATION ONLY

# ORAL COMMUNICATIONS

## SESSION 1

### OC 01

#### N-GLYCAN SHIELDED RHADAMTS13 FOR TREATMENT OF IMMUNE THROMBOTIC THROMBOCYTOPENIC PURPURA

Graça N.A.G.[1,2,*°], Ercig B.[1,3,4°], Kangro K.[2,5°], Arfman T.[1], Wichapong K.[4], Hrdinova J.[1,3,4], Kaijen P.[1], van Alphen F.[6], van den Biggelaar M.[1], Vanhoorelbeke K.[5*], Veyradier A.[7,8*], Coppo P.[8,9,10*], Reutelingsperger C.[3,4°], Nicolaes G.A.F.[3,4°], Männik A.[2°], Voorberg J.[1,11]

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*Background/Aims:* Immune Thrombotic Thrombocytopenic Purpura (iTTP) is a rare thrombotic microangiopathy which is caused by the development of autoantibodies directed towards ADAMTS13. The standard of care (SOC) relies on plasma exchange and immunosuppression. Recently, a nanobody preventing platelet binding to von Willebrand Factor (VWF) was introduced. High-dose wild-type recombinant human ADAMTS13 (rhADAMTS13) overrides inhibitory anti-ADAMTS13 antibodies in animal models of immune TTP. Currently the efficiency of this strategy for treatment of patients with iTTP is being assessed in the SOAR-HI trial (NCT03922308). Recently, we demonstrated that N-glycan shielding prevents the binding of pathogenic antibodies to ADAMTS13[1,2]. Here, we characterized the properties of N-glycan shielded ADAMTS13 in more detail and show its efficacy in an extended panel of patient's plasma.

*Methods:* Artificial N-glycans were introduced in the spacer domain of ADAMTS13. These variants were transiently expressed in CHO cell lines and tested for binding to autoantibodies of iTTP-patients (ELISA) and for activity employing fluorescent FRETS-VWF73 in the absence and presence of plasma of acute stage iTTP patients. We also assessed the activity of N-glycan modified ADAMTS13 on VWF multimers.

*Results:* One N-glycan inserted in position 608 of ADAMTS13 (K608N, NGLY3) enabled strong resistance to autoantibodies, and a higher residual activity when compared to the same concentration of wild-type-rhADAMTS13 (>25% difference; median difference = 23%; range 4–101%) for 14/21 patient samples tested (66% of samples). The NGLY3-ADAMTS13 variant was effective in resisting high inhibitory titer antibodies of patient plasmas, suggesting this variant is superior when compared to wild-type rhADAMTS13. We tested the ability of NGLY3-ADAMTS13 to process VWF using different ADAMTS13 activity assays. In all assays, NGLY3-ADAMTS13 demonstrated comparable activity to wild-type-ADAMTS13.

*Conclusions:* Our data suggests that the NGLY3-ADAMTS13 variant is more resistant to iTTP patients' autoantibodies than wild-type ADAMTS13 positioning NGLY3-ADAMTS13 as a superior treatment of iTTP.

References:

1. Graça NAG, Ercig B, Velásquez Pereira LC, Kangro K, Kaijen P, Nicolaes GAF, et al. Modifying ADAMTS13 to modulate binding of pathogenic autoantibodies of patients with acquired thrombotic thrombocytopenic purpura. Haematologica. 2020;105(11):2619–30.
2. Ercig B, Graça NAG, Kangro K, Arfman T, Wichapong K, Hrdinova J, et al. N-glycan mediated shielding of ADAMTS13 prevents binding of pathogenic autoantibodies in immune-mediated TTP N-glycan mediated shielding of ADAMTS13 prevents binding of pathogenic autoantibodies in immune-mediated TTP. Blood. 2021; First Edition:doi.: 10.1182/blood.2020007972.

### OC 02

#### TRANSPLACENTAL DELIVERY OF MATERNAL FVIII FOR INDUCTION OF FVIII-SPECIFIC IMMUNE TOLERANCE

Mimoun A.[1], Bou Jaoudeh M.[1], Peyron I.[2], Daventure V.[1], Delignat S.[1], Reyes-Ruiz A.[1], Christophe O.[2], Lenting P.[2], Denis C.[2], Lacroix-Désmazes S.[1*]

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*Background:* The main complication of FVIII replacement therapy in hemophilia A patients is the development of inhibitory anti-FVIII antibodies. Using FVIII-deficient mice, we have shown that the transplacental delivery of the Fcγ1-fused A2 and C2 FVIII domains during gestation induces partial immune tolerance to therapeutic FVIII in the progeny. Babies from A2-Fc/C2-Fc-treated mothers developed regulatory T cells and were partially protected, albeit not completely, from the development of FVIII inhibitors.

*Aim:* To develop molecules that mediate the transplacental delivery of FVIII.

*Materials and Methods:* We engineered three monovalent anti-FVIII IgG1 (FabFc) specific for the A2 (BOIIB2), C1 (KM33) or C2 (BO2C11) domains of FVIII. The IHH and N297 residues in the Fc fragment of KM33 FabFc were independently mutated to Alanine residues to abrogate binding to the neonatal Fc receptor (FcRn) or Fcγ receptors (FcγR), respectively. FabFc-mediated transplacental delivery of human B domain-deleted FVIII was tested FVIII-KO mice. FVIII was quantified in fetuses' plasma by ELISA.

*Results:* FVIII injected to pregnant mice following pre-incubation with KM33 or BO2C11 FabFc reached fetuses' circulation with concentrations of 0.12±0.02 and 0.07±0.03 nM, respectively.

In contrast, FVIII injected alone or following pre-incubation with BOIIB2 FabFc or with KM33 FabFc IHH was not delivered to fetuses. Removal of the N-glycosylation at N297 did not affect the levels of transplacentally delivered FVIII.

*Conclusions:* FabFc allowed transplacental delivery of FVIII to levels equivalent to 10-20% normal FVIII plasma levels. The transplacental delivery of FVIII was mediated by the Fc fragment of the FabFc and implicated binding to the FcRn. Binding of FabFc to the FcgR did not modulate the amounts of transplacentally delivered FVIII, suggesting that FcR-mediated catabolism of FVIII/FabFc immune complexes does not affect materno-fetal transfer of FVIII. KM33 and BO2C11 FabFcs that compete with VWF binding to FVIII were more proficient at transplacental deliver than the A2-domain specific BOIIB2 FabFc.

### OC 03

#### DEVELOPING A MODEL FOR STUDYING VON WILLEBRAND DISEASE WITH HIPSC-DERIVED ENDOTHELIAL CELLS

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*Background:* Several recognized protocols exist to differentiate human induced pluripotent stem cells (hiPSCs) into endothelial cells (hiPSC-ECs). Even though these hiPSC-ECs mimic primary ECs relatively accurate, both low levels of von Willebrand factor (VWF) and round Weibel-Palade bodies (WPBs), instead of characteristic tubular shaped WPBs, indicate an immature EC phenotype. To be used as a proper model, these cells require a mature EC phenotype. Exposure to a histone deacetylase inhibitor (HDACi) during differentiation may cause remodeling of the gene expression profile and may improve *in vitro* maturation of hiPSC-ECs.

*Aim:* To improve the maturity of the EC phenotype of hiPSC-ECs by the addition of a HDACi during the differentiation process.

*Methods:* Peripheral blood mononuclear cells from three healthy donors were reprogrammed into hiPSCs and subsequently differentiated into ECs. The HDACi sodium butyrate was added at different time-points and concentrations during differentiation. VWF

production and secretion was measured, together with the expression of VWF related transcription factors.

*Results:* The addition of sodium butyrate did not have an effect on VWF secretion from the hiPSC-ECs, neither at basal levels nor after histamine stimulation. However, in the cell lysates a small increase in VWF production was seen, which was confirmed by confocal microscopy. Even though an increase in the number of WPBs was observed, these organelles still lacked the tubular shape and remained visible as round immature structures as shown previously. qPCR analysis did show an increase in the expression levels of several VWF related transcription factors after the addition of sodium butyrate, leading to an increase in VWF expression and production.

*Conclusion:* Even though the addition of sodium butyrate increases VWF expression and production, possibly through increased expression of VWF related transcription factors, the hiPSC-EC phenotype remains immature. We are currently investigating other markers and adjusting the differentiation factors to improve the maturation process of hiPSC-ECs.

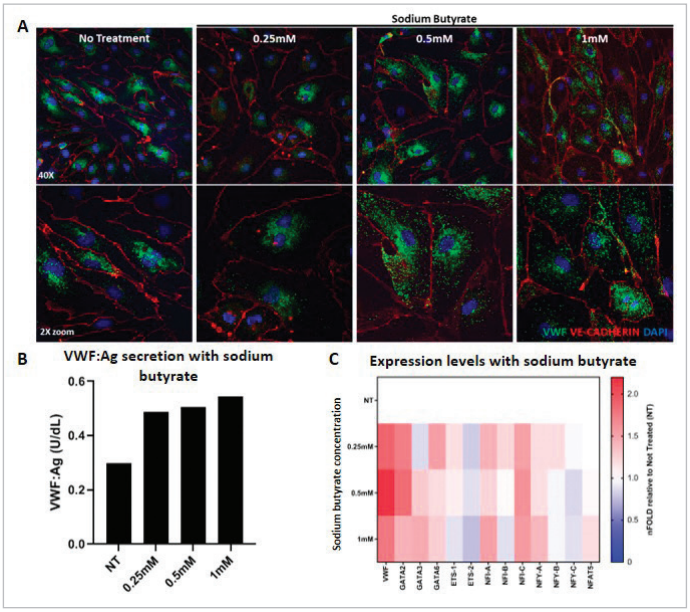


Figure. VWF levels of hiPSC-ECs during differentiation with sodium butyrate. When hiPSC-ECs are incubated with sodium butyrate at different concentrations for 16 hours, there is an increase seen in (A) VWF production, (B) basal VWF secretion, VWF:Ag measured over 24 hours) and (C) the expression levels of VWF and several VWF related transcription factors.

## OC 04

### ANTI-CYSTEINE/SPACER AUTOANTIBODIES THAT OPEN THE CONFORMATION OF ADAMTS13 ARE A COMMON FEATURE OF THE AUTOIMMUNE RESPONSE IN IMMUNE-MEDIATED THROMBOTIC THROMBOCYTOPENIC PURPURA

De Waele L.[1*], Curie A.[1,2], Kangro K.[1], Tellier E.[4], Kaplanski G.[4,5], Männik A.[3], Tersteeg C.[1], Joly B.S.[6], Coppo P.[7], Veyradier A.[6], De Meyer S.F.[1], Roose E.[1], Vanhoorelbeke K.[1]

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*Background/Aims:* Immune-mediated thrombotic thrombocytopenic purpura (iTTP) is caused by an autoantibody-mediated deficiency of ADAMTS13 (A Disintegrin And Metalloprotease with ThromboSpondin type 1 repeats member 13). Patient autoantibodies have epitopes in all ADAMTS13 domains: Metalloprotease (M) and Disintegrin-like (D) domain, a first Thrombospondin type-1 repeat (T1), a Cysteine-rich (C) and Spacer (S) domain, 7 additional Thrombospondin repeats (T2-T8) and 2 CUB (CUB) domains, and almost all patients have autoantibodies against the CS domains. In healthy individuals, ADAMTS13 circulates in a folded conformation where the central S domain interacts with the C-terminal CUB domains. We recently showed that ADAMTS13 adopts an open conformation in iTTP patients and that patient immunoglobulin G's (IgGs) can open ADAMTS13. We now hypothesize that the anti-CS and anti-CUB autoantibodies can disrupt the S-CUB interaction in folded ADAMTS13 thereby inducing an open conformation.

*Materials and Methods:* To investigate our hypothesis, anti-CS and anti-CUB autoantibodies were first purified from the total IgGs of 13 acute iTTP patients by CS- or CUB-coupled affinity chromatography and their specificity was evaluated in ELISA. The ability of the affinity purified anti-CS or anti-CUB autoantibodies to open ADAMTS13 was tested in our in-house developed ADAMTS13 conformation ELISA.

*Results:* Affinity purified anti-CS (10/13 patients, Figure 1A) and anti-CUB (4/13 patients, Figure 1B) autoantibody fractions showed binding to respectively CS or CUB while little to no binding to other ADAMTS13 fragments was observed. Interestingly, all purified anti-CS autoantibody fractions (10/10 patients) were able to induce an open ADAMTS13 conformation (Figure 1C, conformation index > 0.5). In contrast, only half of the purified anti-CUB autoantibody fractions (2/4 patients) opened the conformation of ADAMTS13 (Figure 1D).

*Conclusion:* Our data shows that the presence of anti-CS autoantibodies inducing an open ADAMTS13 conformation is a common feature of the autoantibody response in iTTP patients.

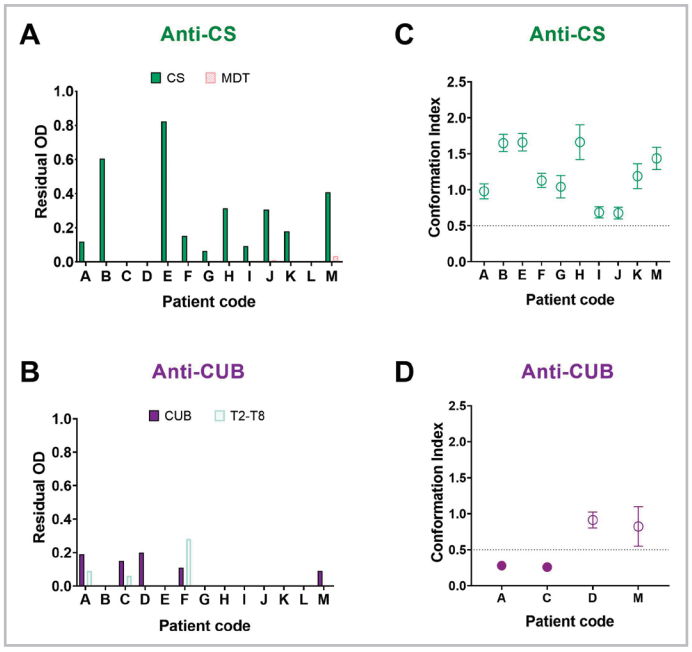


Figure 1.

## OC 05

### SYNTAXIN 5 IS ESSENTIAL FOR GOLGI INTEGRITY, WEIBEL-PALADE BODY BIOGENESIS AND STIMULUS-INDUCED VON WILLEBRAND FACTOR SECRETION FROM ENDOTHELIAL CELLS

Kat M.[1*°], Karampini E.[1°], Olins J.[1], Mulder A.A.[2], van Alphen F.[1], Jost C.R.[2], Koning R.I.[2], Geerts D.[3], van den Biggelaar M.[1], Margadant C.[4], Voorberg J.[1,5], Bierings R.[1,6]

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*Background:* Von Willebrand factor (VWF) is a multimeric hemostatic protein primarily synthesized in endothelial cells (ECs) that facilitates platelet arrest from the circulation at the site of vascular damage and initiates plug formation. VWF is temporarily stored in endothelial storage organelles, the Weibel-Palade bodies (WPBs), whose biogenesis and morphology are strongly related to VWF anterograde trafficking and Golgi architecture. We have previously identified ER-to-Golgi SNARE SEC22B as a novel regulator of WPB elongation, through its involvement in Golgi integrity and VWF anterograde transport.

*Aim:* In this study we aimed to further elucidate the determinants of WPB morphology by looking into the role of endothelial SEC22B interaction partners.

*Materials and Methods:* Using a mass spectrometry-based approach we identified a vast array of proteins within the SEC22B interactome that potentially regulate WPB biogenesis and morphology, including Golgi Qa SNARE STX5, which we confirmed by immunoblotting (Fig.1A). To investigate the role of STX5 in WPB biogenesis we depleted STX5 in endothelial cells using shRNA silencing and analyzed WPB biogenesis and ER and Golgi morphology using confocal and electron microscopy.

*Results:* STX5-depleted ECs exhibited ER dilation, extensive Golgi fragmentation (Fig.1B,C) and decreased WPB length (Fig.1B,D). This subsequently led to reduced intracellular VWF levels (Fig.1E), stimulus-induced VWF secretion (Fig.1F), and VWF multimerization (Fig.1G). Finally, we examined the localization of well-known WPB markers and found that the shorter, stimulus-unresponsive WPBs in shSTX5 ECs still contained Ang-2, P-selectin, Rab27A, and CD63.

*Conclusions:* Taken together, our study has identified SNARE protein STX5 as a novel regulator of WPB biogenesis.

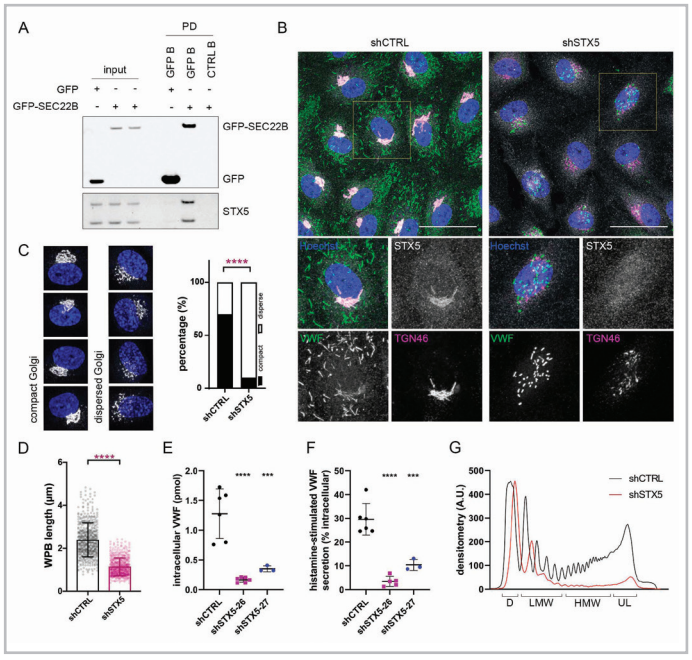


Figure 1.

## OC 06

### FROM COAGULATION TO ANGIOGENESIS: POSSIBLE ROLE OF FVIII IN ENDOTHELIAL FUNCTIONALITY

Olgasi C.[1*], Cucci A.[1], Borsotti C.[1], Walker G.[1], Molineris I.[2], Anselmi. F.[2], Assanelli S.[1], Sgromo C.[1], Oliviero S.[2], Follenzi A.[1*]

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*Background:* Hemophilia A (HA) is a rare bleeding disorder caused by the absence or dysfunction of Factor FVIII (FVIII). Clinical manifestations are spontaneous bleeding episodes that primarily consist of hemarthroses and intracranial hemorrhages. Standard therapies are ineffective in preventing the bleeding episodes and they can occur without any clear cause. To date, the impairment of vessel stability in HA patients and a correlation between FVIII and endothelial functionality has never been explained.

*Aim:* To elucidate the potential role of FVIII in endothelial stability and investigate the significant differences between HA and healthy endothelial cells (ECs).

*Methods:* iPSCs-derived ECs differentiated from HA patients and healthy donors were used as the model system. HA-ECs were transduced with a lentiviral vector (LV) carrying the B-domain deleted form of FVIII under the control of an endothelial promoter (LV-VEC.FVIII). Differences in healthy, HA and LV-VEC.FVIII-transduced HA ECs were evaluated by performing RNA-Seq and proteomic analyses. Finally, to investigate EC functions, *in vitro* and *in vivo* assays were conducted.

*Results:* RNA-Seq and proteomic analyses, revealed a differential expression profile, with the down regulation of several genes and protein profiles in HA vs healthy cells, suggesting an impairment in HA ECs stability. The impaired phenotype was partially attenuated in LV-VEC.FVIII transduced HA ECs. These data were validated *in vitro*, showing a weakening in vessel-formation capability, migration potential and permeability for HA ECs. The transduction with LV-VEC.FVIII improved ECs functionality, suggesting the potential involvement of FVIII. Finally, in a mouse model of severe HA, it was demonstrated an altered permeability and tubulogenesis potential of HA vessels when compared to wild type mice.

*Conclusions:* These preliminary results, if confirmed in primary ECs, will provide new insights into unexplored roles for FVIII, offering new therapeutic gene and cell therapy strategies in the management of HA patients.

# POSTERS

## COVID-19

### PO 01

#### PROTHROMBOTIC ALTERATIONS OF VON WILLEBRAND FACTOR LEVEL AND ADAMTS13 ACTIVITY IN HOSPITALIZED COVID-19 PATIENTS

Sinkovits G.[1*], Mező B.[1,2], Réti M.[3], Müller V.[4], Iványi Z.[5], Gál J.[5], Gopcsa L.[3], Reményi P.[3], Szathmáry B.[6], Lakatos B.[6], Szlávik J.[6], Bobek I.[7], Prohászka Z.Z.[1], Förhécz Z.[1], Csuka D.[1], Hurler L.[1], Kajdácsi E.[1], Cervenak L.[1], Kiszel P.[2], Masszi T.[1], Vályi-Nagy I.[3°], Prohászka Z.[1°]
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**Corresponding author; email sinkovits.gyorgy@med.semmelweis-univ.hu*

*Background:* Severity of the COVID-19 disease is associated with the dysregulation of inflammation and haemostasis. Endothelial cells play a central role in regulating both processes.

We aimed to determine VWF antigen (VWF:Ag) level, ADAMTS13 activity (ADAMTS13:Ac) and their ratio in samples of COVID-19 patients, and to analyse their associations with disease severity and thromboembolic complications.

*Methods:* Between April 20 and June 2, 2020, 128 PCR-positive COVID-19 patients were included in our observational study. Patients were stratified according to severity at the time of sampling. The following groups were defined: (1) convalescent, outpatient (n=26), (2) hospitalized, not requiring oxygen support (n=31), (3) hospitalized, receiving oxygen therapy (n=36), (4) critical, requiring intensive care (n=35). VWF:Ag level was determined by ELISA; ADAMTS13:Ac was determined by FRET. Non-parametric statistical tests were used.

*Results:* VWF:Ag levels were significantly elevated in all groups of hospitalized patients (median values: 196%, 270% and 383% in groups 2, 3 and 4, respectively). ADAMTS13:Ac was decreased in patients requiring oxygen support or intensive therapy (median 75% and 50% in groups 3 and 4, respectively). Consequently, the VWF:Ag/ADAMTS13:Ac ratio was increased in all hospitalized patients, the rate of increase correlated with disease severity (median 1.97, 3.71 and 10.73 in groups 2, 3 and 4, respectively). The VWF:Ag level and VWF:Ag/ADAMTS13:Ac ratio were higher in patients with thromboembolic complications (median 390% vs. 213% and 6.0 vs. 2.3, respectively), however, these differences were not present in subgroups stratified by disease severity. We found several significant correlations between the above parameters and those related to the pathophysiology or severity of the COVID-19 disease.

*Conclusion:* Our results show that VWF:Ag, ADAMTS13:Ac and their ratio correlate strongly with disease severity and with several markers of inflammation, coagulation and complement activation, which may indicate a central role of endothelial cells in the pathogenesis of immunothrombosis in COVID-19.

## FACTOR VIII INHIBITORS

### PO 02

#### SELECTION OF IMMUNO-DOMINANT T CELL EPITOPES FROM THE REPERTOIRE OF FVIII DERIVED PEPTIDES PRESENTED ON MHC CLASS II.

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*Background/Aims:* The main complication of haemophilia A treatment is the development of neutralizing antibodies (inhibitors) against factor VIII (FVIII). The eradication of FVIII inhibitory antibodies relies on the immune tolerance induction (ITI). Since ITI is efficient in only 60-80% of the cases, novel strategies are needed to more efficiently induce tolerance in haemophilia A patients with inhibitors. Within the EDUC8-consortium we are exploring novel approaches for ITI which includes tolerogenic targeting of FVIII derived CD4+ T cell epitopes coupled to different classes of nanoparticles. Here we employed bioinformatics and advanced peptide presentation assays to identify promiscuously presented FVIII derived peptides with the ultimate goal of selecting suitable candidate-peptides for tolerogenic nanoparticle-mediated targeting.

*Materials and Methods:* A database of FVIII derived peptides presented on different MHC class II alleles was assembled and compared to predicted MHC class II presented epitopes using the 'Immune Epitope Database (IEDB)' website. An additional dataset of naturally processed FVIII peptides was generated by incubating human FVIII with immature monocytes-derived DCs from HLA-typed healthy donors. Specific attention was directed towards the identification of FVIII peptides presented on HLA-DP4 since this MHC class II allele is highly prevalent in the Caucasian population.

*Results:* The 'Immune Epitope Database' website-based analysis of FVIII presented peptides revealed a large number of FVIII core peptides. Detailed inspection of the data set revealed that several FVIII derived peptides were presented by multiple HLA-DR and HLA-DQ alleles. To supplement the current data set we successfully developed a protocol to study peptide presentation on HLA-DP utilizing a monoclonal antibody that specifically bound to HLA-DP4. This provides a basis for studying the HLA-DP4 presented peptide repertoire of FVIII.

Conclusions: Taken together, our data provide an inventory of promiscuously presented FVIII-derived peptides which will guide novel approaches of nanoparticle mediated induction of tolerance in haemophilia A.

### PO 03

#### THE IGG-DEGRADING ENZYME FROM *STREPTOCOCCUS PYOGENES* AS A NEW TOOL FOR THE ELIMINATION OF FACTOR VIII INHIBITORS

Bou Jaoudeh M.[1*], Mimoun A.[1], Delignat S.[1], Daventure V.[1], Astermark J.[2], Lacroix-Désmazes S.[1]
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*Background:* The development of neutralizing anti-factor VIII (FVIII) IgG, known as "FVIII inhibitors", complicates the treatment of hemophilia A (HA) patients and renders the administration of exogenous therapeutic FVIII obsolete. Bypassing agents such as activated factor VII or bispecific antibodies are not as effective as FVIII in correcting major bleeds caused by injuries or surgery. IdeS, an enzyme from *Streptococcus pyogenes* that hydrolyzes human IgG, has been successfully used in kidney transplantation to prevent antibody-mediated allograft rejection. The aim of this study is to see whether IdeS may eliminate FVIII inhibitors from the plasma of inhibitor-positive patients.

*Methods:* IdeS was incubated with i) plasma from 119 patients with severe or mild/moderate HA, with or without FVIII inhibitors

or ii) human monoclonal anti-FVIII $IgG_{1k}$ or $IgG_{4k}$ specific for the A2 (BOIIB2), A3 (KM41), C1 (LE2E9), or C2 (BO2C11) domains of FVIII. SDS-PAGE and Western blot analyses confirmed IgG hydrolysis and release of the Fc fragment. ELISA was used to measure residual FVIII recognition using a secondary anti-Fc detection antibody.

*Results:* IdeS hydrolyzed recombinant monoclonal IgG as well as polyclonal IgG present in patient's plasma. In ELISA, IdeS treatment reduced FVIII recognition by 10 folds, confirming the dissociation of the Fc from the $F(ab')_2$ fragments. Since IdeS preserves the integrity of the $F(ab')_2$ fragments, treatment had no significant effect on the inhibitory activity against FVIII *in vitro*.

*Conclusions: In vitro*, IdeS efficiently hydrolyzes anti-FVIII IgG. The proof of concept for the elimination of FVIII inhibitors by IdeS *in vivo* will be demonstrated in FVIII-deficient mice reconstituted with exogenous human anti-FVIII IgG. It will also show whether IdeS-mediated transient removal of FVIII inhibitors can be applied to all inhibitor-positive HA patients or should be limited to those with low inhibitor titres.

## PO 04

### MIGHT PROTHROMBIN-543R>L, A MAJOR DETERMINANT OF MEXICAN-AMERICAN COAGULATION POTENTIAL, CONTRIBUTE TO THE DISPARATELY ELEVATED FVIII-INHIBITOR-RISK IN HISPANIC HEMOPHILIA-A PATIENTS?

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*Background/Aims:* Hemophilia A (HA) is caused by heterogeneous factor (F)VIII-gene (*F8*) mutations and plasma FVIII deficiencies that variably impair intrinsic pathway coagulation amplification. To prevent hemarthrosis-induced crippling, prophylactic FVIII infusions begin when severe HA patients (HAPs) are toddlers but ~30% develop FVIII inhibitors. Gain-of-function mutations in common pathway coagulation factor genes (e.g., FII-G20210A) - which increase coagulation potential and venous thromboembolic risk significantly-decrease bleeding and FVIII utilization by severe HAPs. Since decreased bleeding and FVIII utilization should decrease FVIII inhibitor risk, we sought to identify loss-of-function mutations in common-pathway coagulation-factor-genes that decrease coagulation-potential as we hypothesize they should increase FVIII inhibitor-risk in severe HAPs by increased bleeding and FVIII utilization.

*Materials/Methods:* We screened for protein-altering variants decreasing coagulation potential in large Mexican-American pedigrees of the South Texas Family Study (STFS). We focused initially on FII coagulant activity (FII:C) and both the PT and aPTT global tests of coagulation potential. These subjects were genotyped using the Illumina Exome-24 chip. All protein altering variants were analyzed for associations with FII:C, PT, and aPTT. We performed linear mixed model analyses to estimate trait heritabilities and interrogate single-nucleotide variations (SNVs) for evidence of genetic-association. To control for multiple-testing, associations were considered significant if their p-value falls below the Bonferroni-adjusted significance level.

*Results:* Heritability-estimates for FII:C, aPTT, and PT were highly significant at 0.49, 0.49, and 0.54 (for all, p<1.0E-10) (Table 1). All three hemostasis traits were significantly associated with the same Chromosome-11 SNV (rs143064939) 1628G>T in the FII-gene (F2) which encodes 543R>L and has a large effect-size on each trait (for all, p<9.0E-07) although only that for FII:C is shown (Figure 1). The "signs" of their effect are physiologically consistent in that individuals with 1628G>T have lower FII:C levels but correspondingly prolonged aPTT and PT times.

*Conclusions:* The consistent effects of this variant in non-HAPs of the STFS suggest that severe-HAPs who are heterozygous or homozygous for FII-543L will compared to severe HAPs with the same causative *F8* mutation but homozygous for FII-543R would experience more frequent/severe bleeding and greater tFVIII-utilization. Thus, we hypothesize that FII- 543R>L likely contributes to the disparately high-incidence of FVIII-inhibitor-development in HA patients of Mexican ancestry.

| Heritability | | | Association with rs143064939 | |
|---|---|---|---|---|
| Trait | H2 | p-value | beta | p-value |
| FII | 0.49 | 1.57E-13 | -0.80 | 5.53E-09 |
| aPTT | 0.49 | 1.46E-12 | 0.70 | 8.08E-07 |
| PT | 0.54 | 5.34E-19 | 0.77 | 1.74E-08 |

Table 1. Linear mixed model estimates for FII, aPTT, and PT heritabilities and associations with rs143064939.

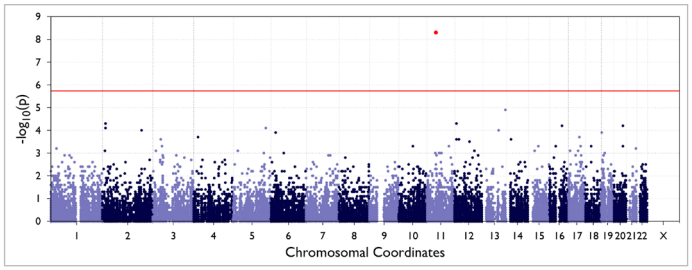


Figure 1. Manhattan plot for associations of FII activity with exome-wide nonsynonymous-SNPs on theIllumina-(human)-exome-24 chip.

## HAEMOPHILIA

### PO 05

#### PRECLINICAL EFFICACY AND SAFETY ASSESSMENT OF A RECOMBINANT FACTOR IX VARIANT THAT FUNCTIONS INDEPENDENTLY OF FACTOR VIII IN COMBINATION WITH CURRENT HEMOPHILIA A THERAPEUTICS

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*Background/Aims:* Factor (F)IX-FIAV functions independently of the cofactor VIIIa and ameliorates the hemophilia A (HA) phenotype *in vitro/vivo* [Quade-Lyssy et al. J.Thromb.Haemost. 2014]. Here we assessed the efficacy and safety of purified recombinant FIX-FIAV in combination with conventional HA therapeutics in mild, moderate, and severe HA patient plasma.

*Methods:* Plasma was obtained from 21 HA patients (>18yrs; seven/phenotype) and analyzed for FVIII/FIX levels, FVIII mutation, Bethesda Units, and aPTT. Plasma was spiked with 100% (5µg/mL) FIX-FIAV with or without FVIII, emicizumab, aPCC, or FVIIa. Following FXIa-triggered thrombin generation (TG) analysis, the TG lag-time was quantified in terms of FVIII-like activity using a FVIII calibration for each patient plasma (n=3/phenotype). This study was approved by the local medical ethics committee, and patients provided written informed consent.

*Results:* Efficacy assessment using the TG lag-time revealed that FIX-FIAV mitigated the HA phenotype from severe to moderate (<0.01% → 5±4% FVIII-like activity), from moderate to mild (4±2% → 13±5% FVIII-like activity), and from mild to normal (17±13% → 34±12% FVIII-like activity). The combination of FIX-FIAV with FVIII replacement therapy enhanced the FVIII-like activity on average by 2-fold with 10%, 27%, or 100% FVIII added to HA plasma. This confirms that the FIX-FIAV cofactor-dependent activity is regulated by FVIII. Emicizumab (7, 26, or 55µg/mL) spiking revealed a dose-dependent increase in FVIII-like activity up to 164±38% in severe HA plasma and higher for moderate/mild HA. The combination with FIX-FIAV minimally enhanced the FVIII-like activity (~1.3-fold). Combination of FIX-FIAV with the bypassing agents (1U/mL) aPCC or rFVIIa indicated no substantial synergistic effects in this system.

*Conclusions:* FIX-FIAV could serve as a potential treatment for HA as it mitigates the HA phenotype in patient plasma. While further safety assessment is warranted, no severe procoagulant effects were observed for the combination of FIX-FIAV with conventional HA therapeutics.

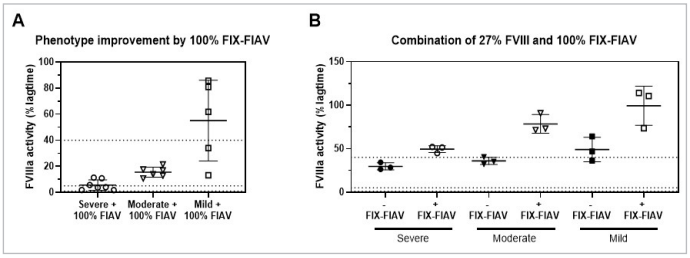


Figure. Effect of FIX-FIAV. Panel A: phenotype improvement determines as FVIII activity at 100% (5µg/mL) FIX-FIAV addition to severe, moderate or mild HA patient plasma. Panel B: FVIII activity with 27% FVIII in the absence (-FIX-FIAV) or presence (+ FIX-FIAV) of 5% (5µg/mL). Dotted lines represent 1, 5 or 40% FVIII activity. The FVIII activity was quantified using a FVIII calibrator of the TG lag-time for each patient plasma.

### PO 06

#### THE SAFETY AND EFFICACY OF N8-GP IN PREVIOUSLY UNTREATED PATIENTS (PUPS) WITH SEVERE HAEMOPHILIA A: INTERIM RESULTS FROM THE MAIN AND EXTENSION PHASES OF PATHFINDER6

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*Background/Aims:* N8-GP is a recombinant, glycoPEGylated, extended half-life factor VIII (FVIII) replacement product approved for the treatment of patients with haemophilia A. This clinical trial evaluates safety and efficacy of N8-GP in previously untreated patients (PUPs) with severe haemophilia A.

*Methods and Materials:* Pathfinder™ 6 (NCT02137850) is an ongoing, open-label, single-arm, phase 3a trial investigating N8-GP treatment in severe haemophilia A PUPs, enrolling male children, <6 years old, with no exposure to FVIII concentrates and no FVIII inhibitors. The primary endpoint was the FVIII inhibitor incidence (two consecutive tests ≥0.6 Bethesda units (BU); high-titre was ≥5 BU). For this analysis, ≥50 patients had ≥50 exposure days (EDs) to N8-GP. Patients with ≥10 EDs or inhibitors were included in inhibitor incidence analysis. Patients <24 months old were treated with pre-prophylaxis or prophylaxis. Patients ≥24 months old were treated with prophylaxis.

*Results:* 80 patients received ≥1 N8-GP dose and were included in this analysis. Of the 67 patients eligible for FVIII inhibitor analysis, 20 developed inhibitors (10 high-titre), resulting in an incidence rate of 29.9% (14.9% high-titre). Seven patients began immune tolerance induction therapy; 5 were successful, 1 withdrew and 1 has ongoing therapy. Sixty-five patients received N8-GP prophylaxis for an average 2.17 years. In these patients, the median ABR (interquartile range) was 1.42 (0.76; 3.13), 91.3% of bleeds were treated with ≤2 injections and the haemostatic success rate was 90.5% (Table). No unexpected AEs were observed. Temporarily decreased incremental recovery was observed in 17 non-inhibitor patients (examined in a separate abstract).

*Conclusions:* Compared with previous studies, patients in pathfinder6 had an inhibitor incidence within the expected range and a high-titre incidence within the lower range. Patients on prophylaxis had a median 1.42 ABR and a 90.5% success rate for treatment of bleeding episodes, indicating that PUPs can be successfully treated with N8-GP.

| | Pre-prophylaxis | Prophylaxis |
|---|---|---|
| Number of patients | 54 | 65 |
| Number of patients with bleeds, N (%) | 46 (85.2) | 54 (83.1) |
| Number of bleeds | 159 | 266 |
| Mean treatment period (years) | 0.470 | 2.168 |
| Estimated ABR* (95% CI) | 6.62 (5.34; 8.21) | 2.37 (1.79; 3.15) |
| Median ABR (IQR) | 6.63 (2.61; 13.04) | 1.42 (0.76; 3.13) |
| Bleeds treated with ≤2 injections, % | 87.2 | 91.3 |
| Success rate for treatment of bleeds**, % (95% CI) | 82.7 (73.1; 89.4) | 90.5 (84.9; 94.1) |

Table. Annualised bleeding rate (ABR) and haemostatic success of patients in Pathfinder6

Disclosures: GK: Grant and research support from Alnylam, Bayer, BPL, Opko Biologics, Pfizer, Roche, Takeda. Ad boards, honoraria for consultancy/lectures from Alnylam, Bayer, Biomarine, CSL, NovoNordisk, Opko Biologics, Pfizer, Takeda, ROCHE, Sanofi, Uniquore. CK: Speaker or advisory boards for Bayer, Biotest, CSL Behring, Novo Nordisk, Roche/Chugai, Sanofi/Sobi, Takeda. Institutional research support from Bayer, Biotest, CSL Behring, Novo Nordisk, Pfizer, Sanofi/Sobi. TM: Advisory boards for Baxalta/Shire/Takeda, Bayer, Novo Nordisk, Chugai and Pfizer and received educational and investigational support from Chugai and Novo Nordisk. Received honoraria from Shire/Takeda, Bayer, Bioverative/Sanofi, Chugai, CSL, JB, KMB, Kirin, Nichiyaku, NOVO, Octapharm, Sysmex. GY: Personal fees from Novo Nordisk during the conduct of the study. CM: Consultancy or speaker for Bayer, Biotest, CSL Behring, Grifols, Novo Nordisk, Roche, Takeda; travel support from Bayer, Biotest, CSL Behring, Novo Nordisk; institutional research support from Bayer, Baxalta/Shire/Takeda, Biotest, CSL Behring, Novo Nordisk, SOBI. SD, AHM and HS: Employees of Novo Nordisk

## PO 07

### DOES DIFFERENCE BETWEEN LABEL AND ACTUAL POTENCY OF FACTOR VIII CONCENTRATE AFFECT PHARMACOKINETIC-GUIDED DOSING OF REPLACEMENT THERAPY IN HEMOPHILIA A?

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*Background:* To account for inter-individual variability in the pharmacokinetics (PK) of factor concentrates, PK-guided dosing is increasingly implemented in hemophilia patients. Although this is performed on label potency, European Pharmacopoeia legislation allows a potency difference of ±20% between label and actual potency. It is unknown if these differences affect PK guidance.

*Aims:* This study explores the effects of potency difference on individual factor VIII (FVIII) PK parameters and the prediction of FVIII trough levels of dosing regimen.

*Methods:* We analyzed individual preoperative PK profiling data from severe and moderate hemophilia A patients included in the OPTI-CLOT randomized controlled perioperative trial. Actual potency of administered (batches of) standard half-life FVIII concentrates was provided by pharmaceutical companies to determine potency difference. Two individual PK parameter estimations and concentration-time curves were constructed by nonlinear mixed-effects modelling using both label and actual potency. Finally, we explored the effect of both the identified and the maximum legislated potency difference on predicted FVIII trough levels when infused in a low and high dose regimen.

*Results:* In 44 of 50 included patients, actual potency was higher than label potency. The median potency difference was 6.0% (range -9.2% to 18.4%) and resulted in varying PK estimations. This difference, however, resulted in almost identical FVIII concentration-time curves. Importantly, predicted FVIII trough levels were linearly correlated to the actual dose, when calculating dosing regimens (Figure).

Conclusion: Potency differences linearly affect predicted FVIII trough levels of dosing regimens. Therefore, our study indicates that it is not necessary for clinicians and pharmacologists to take potency into account when applying PK guidance of FVIII concentrates in hemophilia A patients.

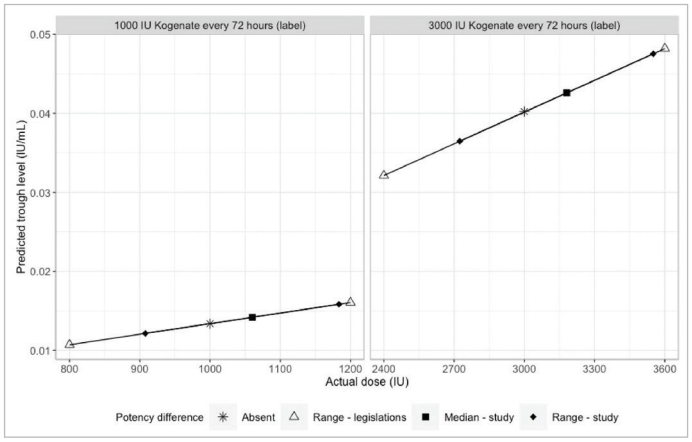


Figure. Predicted FVIII trough levels corresponding to both a low (left panel) and high dose regimen (right panel). The exact actual FVIII concentrate doses on the left panel are as follows: 800 IU (minimum legislated variation of -20%), 908 IU (minimum identified variation of -9.2%), 1000IU (variation absent), 1060.3 IU (median identified variation of +6.3%), 1183.6 IU (maximum identified variation of +18.36%) and 1200 IU (maximum legislated variation +20%). The exact actual FVIII concentrate doses on the right panel are as follows: 2400 IU (minimum legislated variation of -20%), 2724.0 IU (minimum identified variation of -9.2%), 3000 IU (variation absent), 3180.9 IU (median identified variation +6.3%), 3550.80 IU (maximum identified variation +18.36%), 3600 IU (maximum legislated variation of +20%). The linearity of the predicted data points demonstrates the correlation between label and actual FVIII concentrate dose on predicted FVIII trough levels.

## PO 08

### CONFIRMED SAFETY PROFILE OF BAY 94-9027 PROPHYLAXIS FOR ≥5 YEARS: OUTCOMES FROM THE PROTECT VIII AND PROTECT VIII KIDS EXTENSION STUDIES

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*Background/Aims:* Simulations using preclinical toxicology and pharmacokinetics/pharmacodynamics data predicted no long-term safety concerns in humans for the extended-half-life, site specifically PEGylated (polyethylene glycosylated), recombinant factor VIII (FVIII) product, BAY 94-9027. The long-term clinical safety of BAY 94-9027 was assessed in previously treated patients (PTPs) with severe haemophilia A (FVIII<1%), in the extension studies of the PROTECT VIII (NCT01580293;12–65 years old) and PROTECT VIII Kids (NCT01775618;<12 years old) trials. We report the long-term safety of BAY 94-9027 prophylaxis in PTPs for ≥5 years from their respective extension studies.

*Materials and Methods:* Throughout the PROTECT VIII and PROTECT VIII Kids extension studies, safety outcomes were assessed every 6 months and at last study visit, including adverse events (AEs), inhibitor development (titre, ≥0.6 Bethesda units/mL), anti-PEG antibodies, renal biomarkers and quantitative plasma levels of free PEG.

*Results:* At extension completion, 75 patients completed ≥5 years of BAY 94-9027 prophylaxis (median [range], years: PROTECT VIII [n=36], 6.2

[5.0–7.0]; PROTECT VIII Kids [n=39], 6.1 [5.2–6.6], respectively). Median FVIII consumption (IU/kg/year) was 3332 in PROTECT VIII and 4160 in PROTECT VIII Kids using infusion schedules of twice-weekly, every-5-days or every-7-days. Nine patients experienced non-serious study-drug-related AEs; two patients experienced study-drug-related serious AEs (Table). No renal AEs were reported; renal biomarkers remained within normal levels at the last study visit (Table). Three patients had low, unconfirmed anti-FVIII antibody titre (range: 0.6–1.5 BU/mL), negative at the second test; none developed anti-PEG antibodies. No deaths or thrombotic events were reported. Free PEG was detectable in 14 (18.7%) patients (maximum value:0.152 mg/L), confirming the predicted range based on simulations from preclinical studies.

*Conclusion:* No patients presented unexpected AEs or renal function abnormalities. Long-term safety of BAY 94-9027 was demonstrated over ≥5 years' prophylaxis across all age groups, confirming the preclinical long-term safety predictions for BAY 94-9027.

| | PROTECT VIII n = 36 | PROTECT VIII Kids n = 39 | Total N = 75 |
|---|---|---|---|
| **AEs** | | | |
| Any AE, n (%)<br>Mild<br>Moderate<br>Severe | 35 (97.2)<br>3 (8.3)<br>22 (61.1)<br>10 (27.8) | 37 (94.9)<br>2 (5.1)<br>30 (76.9)<br>5 (12.8) | 72 (96.0)<br>5 (6.7)<br>52 (69.3)<br>15 (20.0) |
| Any study-drug-related AE*<br>Mild<br>Moderate<br>Severe | 5 (13.9)<br>3 (8.3)<br>2 (5.6)<br>0 (0) | 4 (10.3)<br>2 (5.1)<br>1 (2.6)<br>1† (2.6) | 9 (12.0)<br>5 (6.7)<br>3 (4.0)<br>1† (1.3) |
| AE-related deaths | 0 (0) | 0 (0) | 0 (0) |
| Any study-drug-related SAE* | 0 (0) | 2‡ (5.1) | 2‡ (2.7) |
| **Renal function biomarker** mean (±SD) at final study visit | | | |
| Albumin (mg/L) in urine<br>*(Random sample)* | n = 32<br>20.5 (39.4) | n = 25<br>9.6 (6.9) | ND |
| β-2 Microglobulin (µg/L) in urine<br>*Normal range adults: <300[1]* | n = 33<br>127.9 (130.9) | n = 36<br>103.7 (81.2) | ND |
| Cystatin C (µg/L) in blood<br>*Normal range adults and children: 530–950[2]* | n = 35<br>708.6 (95.8) | n = 39<br>685.9 (92.0) | ND |
| Kidney Injury Molecule-1 (µg/L) in urine<br>*Normal range adults: <1[3,4]* | n = 28<br>0.8 (0.6) | n = 33<br>0.8 (0.6) | ND |
| Lipocalin-2 (µg/L) in serum<br>*Normal range adults: 42–177[#]* | n = 30<br>94.8 (43.5) | n = 39<br>64.9 (16.7) | ND |
| Lipocalin-2 (µg/L) in urine<br>*Normal range adults: 0.4–72[#]* | n = 34<br>6.9 (4.4) | n = 36<br>6.0 (5.4) | ND |
| Creatinine (mg/dL) in serum<br>*Normal range: 0.5–1.3[#]*<br>Mean change from baseline | n = 36<br>0.8 (0.1)<br>–0.04 | ND | ND |
| Calculated Creatinine clearance (mL/min) in serum<br>*Normal range: 77–160[5]*<br>Mean change from baseline | n = 36<br>139.6 (31.4)<br>6.3 | ND | ND |
| **Detectable PEG measurement** (> LLOQ 0.1 mg/L) | | | |
| Free PEG in plasma, n (%) | 3§ (8.3) | 11¶ (28.2) | 14 (18.7) |

*As judged by the investigator. †One patient experienced severe muscle spasms. ‡Both study-drug-related SAEs were suspected (unconfirmed) FVIII inhibitors (≥0.6 Bethesda units/mL). §In 2 patients, PEG was detected at a single visit and negative during the rest of the study; in 1 patient PEG was detected at the last visit only. ¶Six patients had detectable PEG only once in the study: 3 during extension, with no PEG detected during the rest of the study, and another 3 patients at the last visit only; 5 patients had detectable PEG in plasma at repeated time points (≤4; including last visit); #provided by the central laboratory, Covance.
AE, adverse event; FVIII, factor VIII; ND, no data available; PEG, polyethylene glycol; SAE, serious AE; SD, standard deviation.

Table. Safety outcomes during the PROTECT VIII and PROTECT VIII Kids extension studies

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## LABORATORY METHODS

### PO 09

#### THE SPECIFICITY OF THE ASSAY DETERMINES THE MEASUREMENT OF FVIII ANTIGEN LEVELS IN PATIENTS WITH SEVERE HAEMOPHILIA A

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*Background/Aims:* Recently, Rosendaal et al. (Blood 2017;130:1757-1759) established an association between non-null mutations and inhibitor protection in severe haemophilia A patients depending on the type of FVIII concentrate used. In the same cohort, Spena et al. (J Thromb Haem 2018;16:778) established an association between circulating FVIII and inhibitor protection. Interestingly, in this study, the FVIII:Ag was performed with monoclonal antibodies recognising FVIII light chain, which may have influenced FVIII:Ag measurement in patients carrying light chain mutations. Our objective was to compare the measurement of FVIII:Ag with assays specific for different FVIII regions.

*Materials and Methods:* Plasma was collected from patients with severe haemophilia A treated with emicizumab. FVIII:Ag levels were measured with two commercial assays, one using monoclonal antibodies recognising the FVIII light chain (Asserachrom®), one using polyclonal antibodies (Vizulize®), and with an in-house assay using monoclonal antibodies recognising the FVIII B-domain associated to the heavy chain.

*Results:* No FVIII:Ag was detected with any of the 3 methods in plasma of subjects with gene inversion (n = 9), stop codons (Q305X and L462X; n = 2) or deletions (one partial deletion of exon 26 and one large deletion from exon 7 to 22). Interestingly, our in-house assay did not detect any FVIII:Ag in a patient with mutation V234F whereas the Asserachrom® assay did not detect any antigen in the samples from the patients with the mutations Y2256D and A2061D (Table). By contrast, FVIII:Ag plasma levels of ≥1% were detected with Vizulize® in these 3 patients.

*Conclusions:* Measured FVIII:Ag levels vary according to the specificities of the various assays used. Thus, the type of FVIII:Ag assay may influence the prediction of the risk of inhibitor development. This observation should be considered in studies evaluating the predictive value of FVIII:Ag measurement on the risk of inhibitor formation.

| Mutation | FVIII:Ag (%) | | |
|---|---|---|---|
| | In house | Vizulize® | Asserachrom® |
| **V234F** | <0,5 | 2,0 | 2,5 |
| **Y2256D** | 2,4 | 1,0 | < 0,5 |
| **A2061D** | 2,4 | 2,2 | < 0,5 |

Table. Samples from patients with severe haemophilia A due to a missense point mutation in the FVIII gene

### PO 10

#### EVALUATION OF THE DIFFERENT PLATELET-DEPENDENT VON WILLEBRAND FACTOR ACTIVITY ASSAYS CAPACITY TO ASSESS THE IN VIVO INHIBITORY EFFECT OF CAPLACIZUMAB ON THE VWF-PLATELET INTERACTION.

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*Background:* Caplacizumab is an anti-von Willebrand factor (VWF) humanized Nanobody® for the treatment of acquired thrombotic thrombocytopenic purpura (aTTP). This nanobody binds to the VWF A1-domain and sterically prevents its interaction with the platelet glycoprotein Ibα (GPIbα). The measurement of the platelet-dependent VWF activity may be used for therapeutic drug monitoring of caplacizumab. This activity, usually measured with the VWF ristocetin cofactor assay, nowadays can be assessed with alternative methods. However, it's unclear whether there are differences in the evaluation of the inhibitory effect of caplacizumab using these assays.

*Aim:* To evaluate if the most common commercially available platelet-dependent VWF activity assays were equally capable to assess the *in vivo* inhibitory effect of caplacizumab on the VWF-GPIbα interaction.

*Methods:* We identified 14 patients with an acute episode of aTTP and a positive clinical response to caplacizumab. Patients were evaluated, along with 14 normal controls matched for sex/age, for the VWF antigen (VWF:Ag) and platelet-dependent VWF activity. We measured VWF:RCo (ristocetin and platelets), VWF:GPIbR (ristocetin and recombinant [r]GPIbα) using a turbidimetric and a chemiluminescent assays, VWF:GPIbM (gain-of-function rGPIbα without ristocetin) and VWF:Ab (monoclonal antibody directed against the GPIbα binding epitope of VWF).

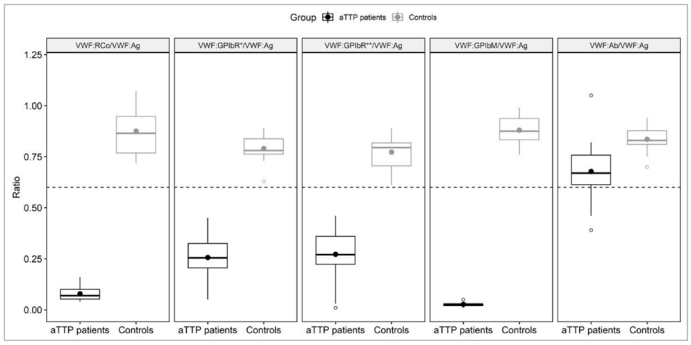
*Results:* The assays behaved differently in these patients, while consistent results were obtained in controls. Patients VWF activity was undetectable using VWF:RCo and VWF:GPIbM assays (the lowest activity/VWF:Ag ratios), whereas the VWF:Ab assay gave the highest activity/VWF:Ag ratios. Both VWF:GPIbR assays showed reduced activity/VWF:Ag ratios, although not to the extent of the VWF:RCo and VWF:GPIbM (Table 1 and Figure 1).

*Discussion:* The VWF:RCo and VWF:GPIbM appear to be the most suitable assays for the therapeutic drug monitoring of caplacizumab in aTTP patients. Both VWF:GPIbR assays could be useful, but the physicians should be aware of their limited capacity to detect the inhibitory effect of caplacizumab. The VWF:Ab assay should not be used for this purpose.

| | aTTP patients (N=14) | Controls (N=14) |
|---|---|---|
| **VWF:Ag** Median [Min, Max] | 87 [37, 137] | 132 [90, 210] |
| **VWF:RCo** Median [Min, Max] | 6+ [6, 6] | 115 [67, 197] |
| **VWF:GPIbR*** Median [Min, Max] | 26+ [3, 39] | 102 [68, 172] |
| **VWF:GPIbR**** Median [Min, Max] | 29+ [0.25, 47] | 99 [70, 152] |
| **VWF:GPIbM** Median [Min, Max] | 2+ [2, 2] | 121 [71, 179] |
| **VWF:Ab** Median [Min, Max] | 63 [17, 90] | 109 [75, 172] |

VWF:GPIbR* immunoturbidimetric assay; VWF:GPIbR** chemiluminescent assay; +For these analyses, test results below the lower limits of quantification were set at half that value (i.e. 6 IU/dL for VWF:RCo, 3 IU/dL for the VWF:GPIbR*, 0.25 IU/dL for VWF:GPIbR** and 2 IU/dL for VWF:GPIbM).

Table 1




VWF:GPIbR* immunoturbidimetric assay; VWF:GPIbR** chemiluminescent assay. Each box-plot represents the interquartile range with median value (horizontal line). Close circles indicate the mean value, the open circles indicate the outliers. The broken line indicates the cut-off value of 0.6.
Study groups were compared using the non-parametric Mann-Whitney test: the median comparison between aTTP patients vs controls for every ratio is statistically significant ($p < 0.05$).

Figure 1. Distribution of activity/VWF:Ag ratios in aTTP patients and controls using 5 different platelet-dependent VWF activity assays.

**PO 11**

### MEASUREMENT OF VON WILLEBRAND FACTOR LEVELS VIA FIBER OPTIC-SURFACE PLASMON RESONANCE

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*Background/Aims:* von Willebrand factor antigen (VWF:Ag) and functionality are key parameters in the diagnosis, subclassification and management of von Willebrand disease (VWD). Measurement of VWF:Ag and function (e.g. binding to collagen and platelet glycoprotein Ibα) requires different assays or platforms, which often take time and specific expertise to perform. Fiber optic – surface plasmon resonance (FO-SPR) may offer an easy, simple-to-use and fast technique to measure VWF parameters. Our aim was to develop an FO-SPR based measurement of VWF:Ag.

*Materials and Methods:* Gold-coated optical fibers containing a carboxyl self-assembling monolayer were functionalized through binding of our in-house monoclonal anti-VWF antibody 6D1. After blocking, a simple dip of the probe into the plasma sample allowed to capture VWF. Subsequent FO-SPR signal generation was performed using a biotinylated polyclonal anti-VWF antibody and signal amplification was done using goat anti-biotin coated gold nanoparticles. After full optimization of the assay, a calibration curve was constructed using normal human plasma. Next, using a VWD type 3 patient plasma sample, the lower limit of detection (LLOD) and quantification (LLOQ) were established. Finally, VWF:Ag levels of 11 VWD samples (all types included) were determined using the FO-SPR and compared to VWF:Ag levels measured in our in-house ELISA.

*Results:* All tested conditions revealed acceptable CV% values (<20%) and high signal to noise ratios (>10). LLOD and LLOQ were 0.68 and 1.24 µg/mL respectively. ELISA and FO-SPR-based measurements of the VWD samples (range: 1.38 – 7.46 µg/mL) showed a good correlation ($p = 0.5771$, $r = 0.9014$) with no systematic bias (mean bias: 2.417, 95%CI[-13.06, 17.89]).

*Conclusions:* A robust VWF:Ag FO-SPR-based assay was developed which showed a good correlation compared to our in-house ELISA. Currently, VWF:CB and VWF:RCo FO-SPR-based assays are being developed, which will ultimately allow a simultaneous fast (within 15-20 min) and easy determination of the most important VWF parameters.

## NON-REPLACEMENT THERAPIES

**PO 12**

### SAFETY OF FEIBA AND EMICIZUMAB (SAFE): DOSE ESCALATION STUDY EVALUATING THE SAFETY OF IN VIVO ADMINISTRATION OF ACTIVATED PROTHROMBIN COMPLEX CONCENTRATE IN HEMOPHILIA A PATIENTS ON EMICIZUMAB

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*Background:* Hemophilia A (HA) patients on emicizumab still experience breakthrough bleeding and may need treatment with activated prothrombin complex concentrate (aPCC). A concomitant drug reaction between emicizumab and aPCC resulting in thrombotic events was noted in the HAVEN 1 study which led to a reduction in the use of aPCC. Previous *in vitro* studies demonstrated excess thrombin generation (TG) when aPCC was spiked into simulated hemophilia inhibitor plasma with emicizumab.

*Aims:* To determine TG of *in vitro* spiking and *in vivo* administration of aPCC at escalating concentrations/doses in patients on emicizumab.

*Methods:* Patients with severe HA with inhibitors who are currently on emicizumab had TG performed at baseline and after spiking clinically relevant concentrations of aPCC *in vitro* into blood samples. Then, the same patients had TG performed at baseline and following an infusion of escalating doses of aPCC (5–75 U/kg) in the *in vivo* portion of the study. Once a patient's endogenous thrombin potential (ETP) was ≥90% of the pooled normal plasma's ETP, no further escalation was done.

*Results:* Nine patients with severe HA and inhibitors currently on emicizumab were enrolled in the study. The summary statistics are provided in Table 1.

*Conclusions:* This study demonstrates that spiking experiments of aPCC and emicizumab may be misleading. The *in vitro* portion of the study demonstrated that clinically relevant concentrations of aPCC resulted in excessive TG, however *in vivo* administration of aPCC to the same patients demonstrated significantly different results, with most of the patients (66%) having normal (not excessive) TG at the approved doses of aPCC (Figure 1). In conclusion, this data suggests that a single licensed dose of aPCC is safe for most patients on emicizumab and importantly calls into question the validity of *in vitro* spiking studies using TG in this setting.

| Patient number | Dose of aPCC at final visit | Lag time | ETP | Peak thrombin | Ratio of Patient's ETP to Pooled Normal Plasma's ETP | Adverse events |
|---|---|---|---|---|---|---|
| SAFE-01 | 50 U/kg | 4.17 | 724.62 | 47.7 | *71.85 % | No |
| SAFE-02 | 50 U/kg | 4.1 | 986.16 | 62.73 | *88.45 % | No |
| SAFE-03 | 75 U/kg | 4 | 1415.16 | 90.95 | 106.74 % | No |
| SAFE-04 | 50 U/kg | 4.94 | 1113.72 | 70.75 | 96.13 % | No |
| SAFE-05 | 25 U/kg | 3.64 | 1274.32 | 82.53 | 115.79 % | No |
| SAFE-06 | 10 U/kg | 5.00 | 1221.72 | 66.66 | 92.15 % | No |
| SAFE-07 | 75 U/kg | 4.1 | 897.43 | 60.28 | †80.49 % | No |
| SAFE-08 | 75 U/kg | 3.1 | 741.61 | 49.4 | †66.52 % | No |
| SAFE-09 | 75 U/kg | 3.1 | 623.95 | 47.89 | †55.96 % | No |

*Patient moved out of state, was eligible for the last visit but could not be enrolled.
†Patient reached maximum dose of 75 IU/kg of aPCC.

Table 1.

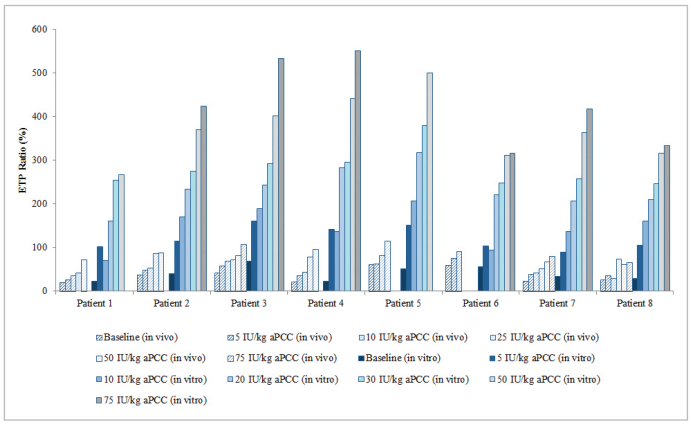


Figure 1.

## PO 13

### A PATIENT WITH FVIII INHIBITORS ON EMICIZUMAB PROPHYLAXIS SWITCHING TO IMMUNE TOLERANCE INDUCTION – LABORATORY ISSUES

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*Background/Aims:* Novel hemostatic agent emicizumab has a huge influence on standard laboratory assays and can lead in a misleading interpretation of coagulation results in emicizumab-treated patients. As quantification of inhibitors is the prerequisite for the successful immune tolerance induction (ITI), when switching from emicizumab prophylaxis to ITI, laboratory follow-up must be adequate due to long-term effect of emicizumab. The study aimed to present laboratory long-term follow-up results of a 9-year-old boy with severe hemophilia A and inhibitors (historical peak 21 BU/mL), treated with emicizumab and switched to ITI.

*Materials and Methods:* The boy received an emicizumab loading dose (3 mg/kg) once weekly for 4 weeks, followed by maintenance with lower doses (1.5 mg/kg) once weekly for 4 weeks. One month after the last dose, the boy was switched to ITI with daily administration of plasma-derived FVIII concentrate (Octanate 2500 IU/L). Laboratory follow-up included: aPTT (Actin FS), aPTT-based one-stage FVIII clotting assay with Actin FS (Siemens Healthcare Diagnostics, Marburg, Germany) and chromogenic FVIII activity assay with human reagents BIOPHEN FVIII:C (Hyphen BioMed, France). FVIII inhibitors were diagnosed using clot-based and chromogenic Bethesda assay with bovine reagents on AtellicaCOAG360 analyzer (Siemens) and chromogenic Bethesda assay with human reagents on SysmexCS-2000i (Sysmex, Kobe, Japan).

*Results:* Laboratory results obtained during a 4-month period (Table) showed remarkable shortening of aPTT results and high FVIII:C activities measured with clot-based assay up to 2 months after emicizumab discontinuation. Even low emicizumab activity (1.6 IU/dL) resulted in falsely low inhibitor titre (3.9 BU/mL) using clot-based assay, compared to chromogenic Bethesda assay with human and bovine reagents (57.6 and 58.9 BU/mL, respectively).

*Conclusions:* Residual emicizumab activity after discontinuation needs to be taken into consideration when performing clot-based coagulation assays in further follow-up of patients. Regarding inhibitor testing, unlike clotting assay, both chromogenic methods enabled reliable quantification.

| Date | Administered therapy/ dose | aPTT (sec) | aPTT-based one-stage clotting assay | | Chromogenic assay | | |
|---|---|---|---|---|---|---|---|
| | | | | | Human reagents (Biophen) | | Bovine reagents (Siemens) |
| | | | FVIII:C (IU/dL) | FVIII:C inhibitors (BU/mL) | FVIII:C (IU/dL) | FVIII:C inhibitors (BU/mL) | FVIII:C inhibitors (BU/mL) |
| January 7, 2019 | Emicizumab 4th dose/ 3 mg/kg | 19.5 | 616.0 | <0.6 | 29.0 | 2.2 | 2.6 |
| January 22, 2019 | Emicizumab 6th dose/ 1.5 mg/kg | 19.6 | 700.0 | <0.6 | 33.0 | 2.0 | 3.1 |
| February 5, 2019 | Emicizumab 8th dose/ 1.5 mg/kg | 19.9 | 439.0 | <0.6 | 28.0 | 2.3 | 2.6 |
| February 26, 2019 | No therapy | 20.0 | 263.0 | <0.6 | 19.0 | 2.6 | 2.5 |
| March 14, 2019 | Octanate/ 2500 IU daily | 23.6 | 130.0 | <0.6 | n.a. | 4.6 | 16.0 |
| March 22, 2019 | Octanate/ 2500 IU daily | 24.3 | 120.0 | 2.4 | 10.0 | 99.2 | 92.2 |
| March 28, 2019 | Octanate/ 2500 IU daily | 22.8 | 82.0 | 3.2 | 6.0 | 84.5 | 89.6 |
| April 5, 2019 | Octanate/ 2500 IU daily | 30.8 | 58.0 | 3.9 | 1.6 | 57.6 | 58.9 |
| April 29, 2019 | Octanate/ 2500 IU daily | 28.3 | 19.0 | 11.5 | 0.6 | 9.6 | 9.0 |
| May 15, 2019 | Octanate/ 2500 IU daily | 32.4 | 7.0 | 3.2 | <0.5 | 4.9 | 4.5 |
| Reference interval | Not applicable | 24.0-33.0 | 50.0-149.0 | <0.6 | 50.0-200.0 | <0.6 | <0.6 |

FVIII:C – FVIII activity; n.a. – not analyzed

Table. Laboratory results obtained during a 4-month period

## PO 14

### EMICIZUMAB FOR THE MANAGEMENT OF CHILDREN WITH SEVERE HEMOPHILIA A: EXPERIENCE FROM HEMOPHILIA CENTRES IN LITHUANIA

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*Background:* Recently non-factor therapy (NFT) has emerged for patients with severe haemophilia A (HA) with and without inhibitors. Real-world data on the safety and efficacy of emicizumab prophylaxis among the paediatric HA patients is scarce.

*Aim:* To review and report data available on the use of emicizumab prophylaxis in children in two Comprehensive/Treatment Hemophilia Centers.

*Methods:* A database of children with NFT was established for prospective registration. The information on haemophilia treatment was tracked in electronic health records. Data was collected from November 1, 2019 until March 31, 2021.

*Results:* Among 25 severe HA patients <18 years in Lithuania, 9 (36 %) were switched to NFT (Table). Median age at the commencement

of NFT was 5 years (range 16 months – 8 years). Two children started emicizumab prophylaxis after failure of immune tolerance induction (ITI), two have never attempted ITI due to poor family social circumstances, two after finishing ITI (one after partial success and one after successful ITI). Three patients without inhibitors started NFT with a median age of 17 months following 50 exposure days (ED) with FVIII therapy. COVID-19 triggered faster transition to NFT because of non-availability of home therapy. All patients received QW dosing regimen. None of them experienced bleeding episodes for a median follow-up of 8 months (range 3 – 17 months), except of one traumatic bleed in loading phase. One patient experienced recurrent FVIII inhibitors following partial success of ITI (half-life of FVIII 5h) being 2 months on NFT. One central venous line removal required post-interventional haemostatic coverage. Emicizumab prophylaxis has not been associated with significant adverse events.

*Conclusions:* Smooth and easy to implement switching to NFT processes. NFT was well tolerated in both inhibitors and non-inhibitor patients. Emicizumab was perfect salvage therapy for PTPs with persistent inhibitors. Patient and care-givers satisfaction was high.

**Inhibitor patients**

| Patient | Current age | Start of EMI | EMI dose | Previous treatment | ITI duration, last inhibitor titer | Reason for switching | Adjunctive therapy |
|---|---|---|---|---|---|---|---|
| 1. | 8 yr | Sep 2019 (3 years after ITI failure) | 1,5 mg/kg QW | aPCC prophylaxis | 19 months, 10.0 BU/ml | Hemophilic arthropathy, ITI falure | 0 (aPCC) |
| 2. | 6 yr | Jun 2020 (just after ITI failure) | 1,5 mg/kg QW | aPCC prophylaxis | >3 yr, 17. 6 BU/ml | Poor bleed control, ITI failure | 0 (aPCC) |
| 3. | 6 yr | Jun 2020 | 1,5 mg/kg QW | aPCC prophylaxis | - (73.6 BU/ml at diagnosis) 1.4 BU/ml | Hemophilic arthropathy, unable/unwilling to undergo ITI | 0 (aPCC) |
| 4. | 8 yr | Jun 2020 | 1,5 mg/kg QW | aPCC prophylaxis | - (92 BU/ml at diagnosis) 4.1 BU/ml | Hemophilic arthropathy, unable/unwilling to undergo ITI | 0 (aPCC) |

**Non-inhibitor patients**

| Patient | Current age | Start of EMI | EMI dose | Previous treatment | Reason for switching | Adjunctive therapy |
|---|---|---|---|---|---|---|
| 1. | 8 yr | Jul 2020 | 1,5 mg/kg QW | rFVIIa | POST-ITI, partial success; no venous access | 0 (rFVIIa) |
| 2. | 2 yr | Aug 2020 | 1,5 mg/kg QW | aPCC | POST-ITI, succesful; wish to reduce treatment burden | 0 (aPCC) |
| 3. | 2 yr | Jun 2020 | 1,5 mg/kg QW | rFVIII | PUP after 50 ED | 0 (rFVIII) |
| 4. | 1 yr | Dec 2020 | 1,5 mg/kg QW | rFVIII | PUP after 50 ED | 1 (rFVIII), traumatic bleed |
| 5. | 3 yr | Nov 2020 | 1,5 mg/kg QW | rFVIII | PUP after 50 ED, non compliant | 0 (rFVIII) |

Table 1. Patients' switch to emicizumab characteristics.

## PREVIOUSLY UNTREATED PATIENTS (PUPS)

**PO 15**

### IGG2 AS HALLMARK OF INHIBITOR PERSISTENCE IN PUPS: A LONGITUDINAL ANALYSIS ALONG EXPOSURE TO FVIII

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*Background:* The immune response to FVIII is polyclonal and consists of the production of IgG antibodies (IgG1, IgG2, IgG3, IgG4). Previous findings suggest that a switch from IgG1 to other subclasses is detectable in patients who develop persistent inhibitors (*Reipert Blood Adv. 2020*)[1]. A longitudinal analysis of the IgG subclass profile over 6 months post inhibitor development in previously untreated patients (PUPs) reported IgG2 as the only subclass that could be considered a hallmark of inhibitor persistence (*Peyvandi Br J Haematol. 2021*)[2].

*Aims:* To investigate how the anti-FVIII IgG subclass profile evolves along FVIII exposure and whether there is any subclass that has a particular signature in persistent inhibitors.

*Methods:* From the SIPPET cohort (*Peyvandi N Engl J Med. 2016*)[3], 20 PUPs with severe haemophilia A who developed inhibitors and had available plasma samples at baseline, 5 exposure days (EDs), 10 EDs, 20 EDs, and 50 EDs were included in this analysis (Figure 1A). Inhibitors were characterised based on persistence (Figure 1B). Transient inhibitors were those tolerised endogenously within 6 months from development and persistent inhibitors were those remaining at 6 months. Anti-FVIII IgG subclasses were measured with an ELISA assay as reported previously[2].

*Results:* Nineteen patients developed inhibitors by 20 EDs (T3) and one at ED 38. IgG1, IgG3, and IgG4 antibody profiles did not allow to distinguish between transient and persistent inhibitors. IgG2 was the only subclass that distinguished persistent inhibitors from transient ones, in that IgG2 antibodies started to appear only in the persistent inhibitor group from inhibitor development onwards (Figure 2).

*Conclusions:* This is the first longitudinal analysis along FVIII exposure in the SIPPET cohort suggesting that the detection of the anti-FVIII IgG2 subclass development is a hallmark of inhibitor persistence not only during the 6 months post inhibitor development but also early after detection of the inhibitor.

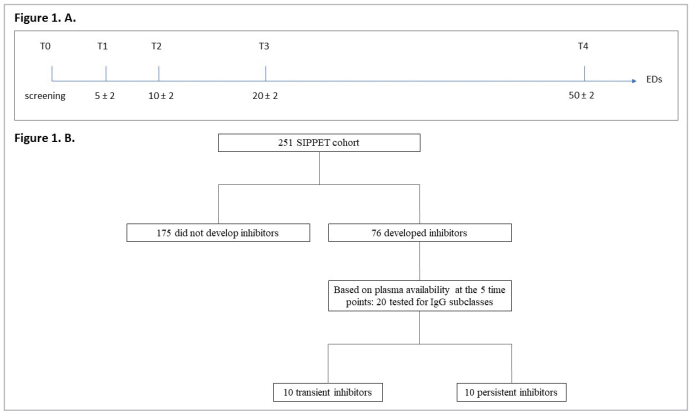


Figure 1.A: Study design. Figure 1.B: Flowchart.

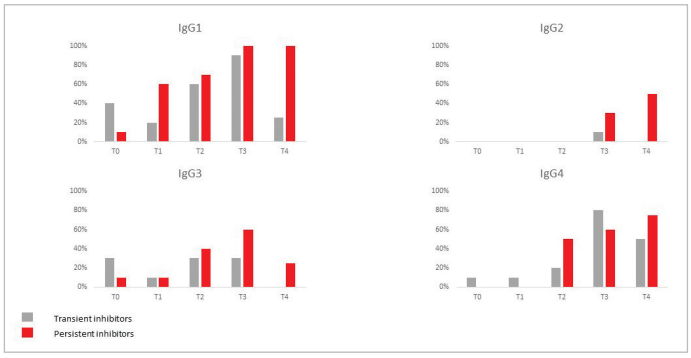


Figure 2: Anti-FVIII IgG subclass profile at the 5 study time points in transient and persistent inhibitors. Bars represent the proportion of patients positive for each subclass over the total number of patients, therefore the prevalence of that subclass in each of the 2 subgroups.

## PO 16

### A POST-HOC ANALYSIS OF TEMPORARILY DECREASED INCREMENTAL RECOVERY (IR) OBSERVED IN A SUBSET OF PREVIOUSLY UNTREATED PATIENTS (PUPS) WITH HAEMOPHILIA A TREATED WITH N8-GP

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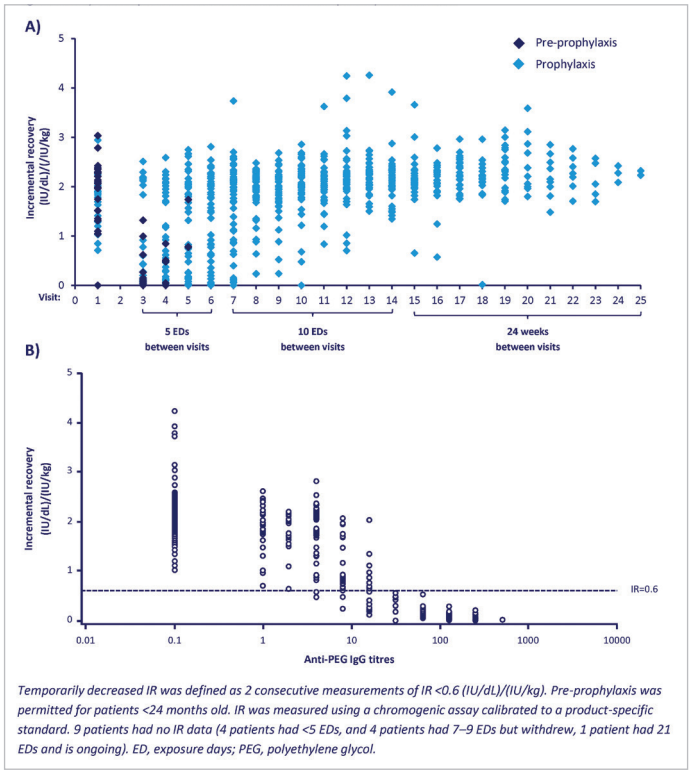
*Background/Aims:* N8-GP is a glycoPEGylated, extended half-life, recombinant factor VIII replacement product approved for the treatment of haemophilia A. During the pathfinder6 study, PUPs were treated with N8-GP. Temporarily decreased IR in the absence of factor VIII inhibitors was observed in a subset of patients. Here, we present an extensive post-hoc analysis of this observation.

*Methods and Materials:* Pathfinder6 (NCT02137850) is an ongoing, multinational, phase 3a trial investigating N8-GP treatment in male PUPs (<6 years old) with severe haemophilia A. We present a post-hoc analysis of the observed temporarily decreased IR – defined as 2 consecutive measurements of IR <0.6 (IU/dL)/ (IU/kg) without inhibitors. The main study results are presented separately.

*Results:* Overall, 80 PUPs were exposed to N8-GP. Temporarily decreased IR was observed in 17 patients (without inhibitors) within 5 exposure days (Figure 1A). IR returned to expected range in 13 patients with continued N8-GP dosing (9 patients within 30 exposure days), and 4 patients withdrew from study. Temporarily decreased IR had a strong temporal correlation with anti-PEG IgG (Figure 1B).

Although anti-PEG IgM and anti-N8-GP binding antibodies were observed, anti-PEG IgG accounted for 58.4% of the within-patient IR variance (the explained variation of titres was 62.3%). During the trial, patients with observed temporarily decreased IR had an estimated ABR (95% CI) of 2.41 (1.64; 3.52), compared with 2.75 (1.97; 3.84) in other non-inhibitor patients. During the temporarily decreased IR period, the success rate for treatment of bleeds (83.2%) was similar to the overall population (86.5%) and the ABR was only slightly higher (4.14 [2.45; 7.00]).

*Conclusions:* Temporarily decreased IR associated with transitory anti-PEG IgG antibodies was observed at treatment initiation in a subset of PUPs without inhibitors exposed to N8-GP and little to no apparent clinical impact or hypersensitivity was observed. IR returned to expected ranges with continued N8-GP dosing.




*Temporarily decreased IR was defined as 2 consecutive measurements of IR <0.6 (IU/dL)/(IU/kg). Pre-prophylaxis was permitted for patients <24 months old. IR was measured using a chromogenic assay calibrated to a product-specific standard. 9 patients had no IR data (4 patients had <5 EDs, and 4 patients had 7–9 EDs but withdrew, 1 patient had 21 EDs and is ongoing). ED, exposure days; PEG, polyethylene glycol.*

Figure. Incremental recovery (IR) in previously untreated patients A) measured at study visits, and B) IR vs anti-PEG IgG antibody titre in patients with an observation of temporarily decreased IR.

**PO 17**

## INHIBITOR DEVELOPMENT UPON SWITCH FROM PLASMA-DERIVED TO RECOMBINANT FVIII IN PUPS WITH SEVERE HAEMOPHILIA A: PRELIMINARY ANALYSIS OF THE PUP-SWITCH STUDY

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*Background:* In 2016, the SIPPET study reported that in previously untreated patients (PUPs) with severe haemophilia A (HA) the choice of plasma-derived FVIII (pdFVIII) products in the first 50 exposure days (EDs) is associated with a significantly lower incidence of inhibitors compared to recombinant FVIII (rFVIII) products[1]. However, the choice of FVIII product class after this high-risk period has been subject of debate. It has been established that switching products in multi-transfused patients (> 150 EDs) does not affect the risk of inhibitor development which becomes approximately 100 times lower in these previously treated patients (PTPs).[2-9]

*Aims:* To investigate whether a switch from pdFVIII to rFVIII in PUPs after the early high-risk period would follow the expected low-risk pattern of inhibitor incidence as previously reported in PTPs, or a novel peak of inhibitors would appear.

*Methods:* We designed a survey on the ISTH REDCap platform2 to investigate the rate of novel inhibitors after the switching of PUPs from pdFVIII to rFVIII between 50 and 150 EDs (Figure 1A). Centres who did the switch after the SIPPET publication were identified and invited to participate in this observational cohort study on PUPs under 6 years old with severe HA, who have been switched according to this strategy.

*Results:* To date, 11 Turkish centres, 1 Iranian centre, and 1 German centre have participated in this study. 47 surveys were analysed, among which 39 patients satisfied all eligibility criteria (Figure 1B). Patients were switched at a median of 60 (54 – 71) EDs and received a median of 111 (84 – 222) EDs with rFVIII after the switch. None of these 39 patients developed an inhibitor after the switch (Table 1).

*Conclusions:* Preliminary results from the first 39 patients enrolled in the PUP-SWITCH study did not show an additional risk of inhibitor incidence upon switch from pdFVIII to rFVIII.

**Figure 1. A.**

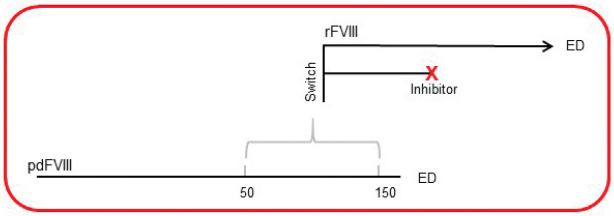


**Figure 1. B.**

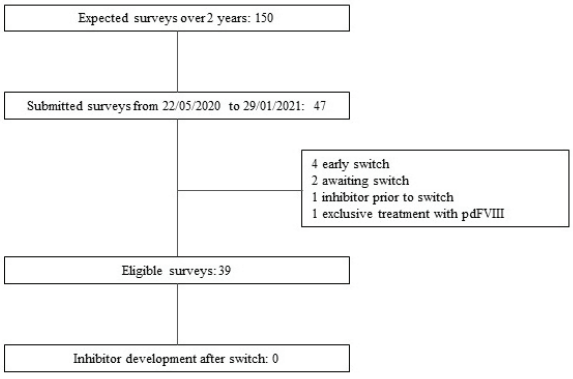


Figure 1.A: Study design. Figure 1.B: Flowchart

| Eligible surveys | ED of switch | | EDs with rFVIII after switch | | Total EDs at survey retrieval | | Inhibitor after switch |
|---|---|---|---|---|---|---|---|
| n | Median | IQR | Median | IQR | Median | IQR | n |
| 39 | 60 | 54 – 71 | 111 | 84 – 222 | 178 | 144 – 279 | 0 |

Table 1. Summarised data of the 39 switched patients

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## PO 18

### GEPHARD – THE PUP COHORT OF THE "STANDING COMMISSION PAEDIATRICS OF THE SOCIETY FOR THROMBOSIS AND HAEMOSTASIS RESEARCH" - A PROGRESS REPORT

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In Germany, approximately 40-60 newborns are newly diagnosed every year with haemophilia A or B. Haemophilia causes recurrent bleeding, leading to increased morbidity and mortality. Prophylaxis with clotting factor concentrates is the standard therapy for the prevention of bleeding and sequelae including haemophilic arthropathy. The optimal time or regime for starting prophylaxis to avoid the development of joint disease or neutralizing antibodies to clotting factors is still under debate. In Germany, only a limited set of data is documented by the German haemophilia database so far. Data on incidence, treatment, clinical course and outcome of newly diagnosed haemophilia patients, have not been documented yet.

The German Paediatric Haemophilia Research Database (GEPHARD) includes all children and adolescents (<18 years) that have been diagnosed with haemophilia A or B (FVIII or FIX levels <25%) after January 1st, 2017. This prospective registry is open to all centres and documents variables related to diagnosis and therapy. The registry concentrates on outcome in haemophilia, including inhibitor development, offers quality assurance and serves as a base for future studies.

The database has been established and will be used on the same platform as PedNet to allow international collaborations and data analyses. Since January 1st 2017 240 children and adolescents were enrolled from 37 participating centres. Two hundred and three children were diagnosed with haemophilia A including 120, 23 and 58 with a severe, moderate or mild phenotype, respectively. Thirty-seven children were diagnosed with haemophilia B including 24, 7 and 6 with a severe, moderate or mild phenotype, respectively. The median of the age of diagnosis for severe (n=112), moderate (n=20) and mild (n=55) haemophilia A is 0.38, 0.04 and 1.5 years. For haemophilia B the median of the age of diagnosis is 0.17, 2.75 and 3.21 for severe (n=19), moderate (n=7) and mild (n = 6). Since 2017, between 50 and 66 patients have been recruited every year. Longitudinal documentation is being initiated to document and analyze clinical parameters and outcome.

The GEPHARD community has already 240 children included. Due to the current pandemic, longitudinal documentation was initiated later than planned and has started now in a large number of newly diagnosed, previously untreated children in Germany.

## PO 19

### REAL-WORLD EXPERIENCE WITH EMICIZUMAB IN AN ITALIAN PAEDIATRIC COHORT: FOCUS ON PUPS AND TOLERIZED CHILDREN

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*Background/Aims:* Data on the use of emicizumab in children under 12 years without inhibitors are limited to case series and reports; in particular data are lacking for previously untreated patients (PUPs) and in infants. Emicizumab has been available in Italy for patients without inhibitors since February 2020. The aim is to share the experience of the pediatric center of Turin, Italy on emicizumab prophylaxis with a particular focus on PUPs and on tolerized children.

*Materials and Methods:* All children who switched to emicizumab underwent the following systematic evaluations: physical examination, physiatrist examination, inhibitor research, ABR, (HJHS, HEAD-US for children over 5 years of age).

*Results:* 14 children of which only three with inhibitors are being treated with emicizumab. From February 2020 11 children including 5 PUPs started emicizumab prophylaxis. 4 children tolerized for more than three years did not have recurrences of inhibitors with the discontinuation of factor VIII. Children's ABR improved or remained 0; no children showed worsening of ABR. With a total follow-up of 10,1 months/person, only one post-traumatic hemarthrosis and one bleeding from nasal varices were recorded. We did not observe any bleeding during 2 minor surgeries (catheter removal and cauterization of nasal varices). PUPs data are described in Table 1. 1 PUP developed high titer inhibitors after intensive treatment for iliopsoas hematoma before starting emicizumab.

*Conclusions:* The real-world experience of Turin confirms the efficacy and safety of emicizumab also in children under 12 years including 5 PUPs. In patients with a previous history of inhibitors eradicated for at least 3 years, although the follow-up is limited, we have not seen a recurrence of the inhibitor. No side effects nor anti-drug antibodies were observed.

| Age of diagnosis | Age of initiaion of emicizumab | Previous treatment with factor VIII (Exposure days EDs) | Inhibitor development | ABR pre-emicizumab | Follow-up | Bleeding episodes during emicizumab |
|---|---|---|---|---|---|---|
| At birth for cephalo-ematoma | 9 months | Yes (10 EDs) | No | 1 | 8 months | 0 |
| 4 months for iliopsoas hematoma | 6 months | Yes (11 EDs) | Yes High responder | 1 | 12 months | 0 |
| 8 months for bruising | 8 months | No | - | - | 2 months | 0 |
| 12 months for buising | 12 months | No | - | - | 1 month | 0 |
| At birth for bruising | 26 months | Yes (50 EDs) | No | 1 | 3 months | 0 |

Table 1.

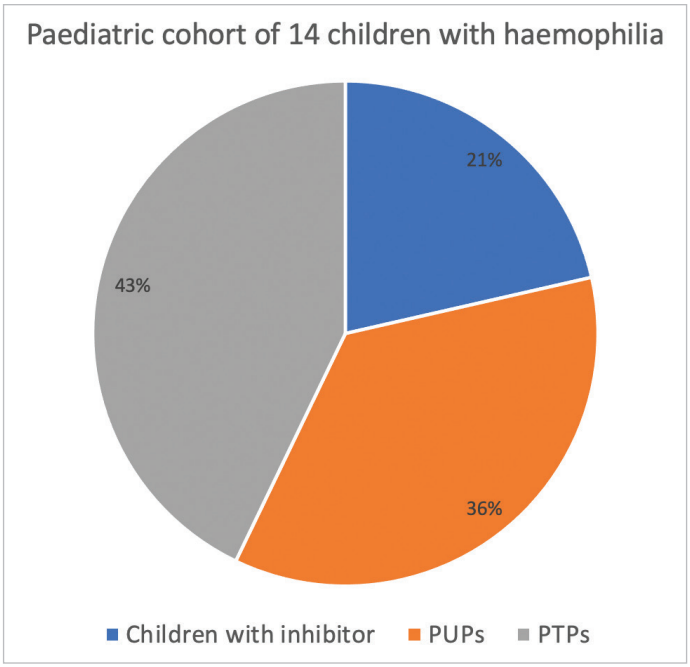


Figure 1.

## RARE COAGULATION DISORDERS

### PO 20

#### CATRIDECACOG IN THE TREATMENT OF AN ITALIAN POPULATION OF PATIENTS WITH FXIII DEFICIENCY: FROM PHARMACOKINETICS TO CLINICAL OUTCOMES (THE ITALIAN RFXIII STUDY)

Zanon E.[1], Pasca S.[2]*, Sottilotta G.[3], Molinari A.C.[4], Banov L.[4], Ferretti A.[5], Di Gregorio P.[6], Pollio B.[7], Siboni S.M.[8], Palla R.[8], Peyvandi F.[8], Pizzuti M.[9], Notarangelo L.D.[10], Cojutti P.[11], Biasioli C.[12], Simioni P.[1], Pea F.[13]

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*Background:* FXIII deficiency is a very rare coagulation disorder. Bleeds are usually mucocutaneous, but life-threatening intracranial hemorrhages (ICHs) can occur in cases of severe disease. Currently the best treatment consists in the use of FXIII replacement concentrates in prophylaxis since birth. Data concerning the use of catridecacog in the real-life is scarce.

*Aim:* Aim of this study was to assess the different pharmacokinetics (PK) profile of rFXIII for each patient using a population statistics model, and to evaluate the clinical outcomes of prophylaxis

*Methods:* This study enrolled all patients presenting FXIII deficiency treated with catridecacog at ten Italian Hemophilia Centers. PK-profiles were evaluated at the Pharmacology Department of Bologna University Hospital. All clinical data and outcomes were collected and analyzed.

*Results:* Overall 20 patients with FXIII deficiency were collected, 75% presenting severe disorder. 11/20 were females. Mean age at diagnosis was 15 years (range birth-74 years). 60% had a known family disorder. Pharmacokinetics was assessed in 18/20 of cases before starting prophylaxis. Mean age at PK-evaluation was 36.4 years (6-74 years), mean dose of drug infused for PK was 33.9 IU/kg (25-50 IU/kg). All obtained PK parameters are showed in Figure 1. Prophylaxis was subsequently started on 65% of patients at a mean dosage of 33.8 IU/kg (range 25.0-80.0 IU/kg), on average every 4.0 weeks (range 3.0-8.0 weeks). During a mean follow-up of 43 months, one ileo-psoas hematoma which quickly resolved, one muscular hematoma, and two minor surgeries were reported. One severe patient who remained on demand treatment experienced a severe intracranial hemorrhage.

*Conclusion:* Efficacy and safety of prophylaxis with catridecacog was proven in all patients, also in preventing severe bleeding. The cumulative PK profile was similar to that reported in the MENTOR studies, but dosage and infusion timing for each patient were in some cases very different.

## VON WILLEBRAND DISEASE

### PO 21

#### LIVER GENE THERAPY WITH LENTIVIRAL VECTORS CORRECTS HEMOPHILIA A IN MICE AND ACHIEVES NORMAL-RANGE FACTOR VIII ACTIVITY IN NON-HUMAN PRIMATES

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Liver-directed gene therapy with adeno-associated viral (AAV) vectors delivering a clotting factor transgene into hepatocytes has shown successful results in hemophilia patients. However, AAV-vector non-integrating nature challenges their use in pediatric patients. We developed integrating lentiviral vectors (LV) for liver gene therapy and showed efficient gene transfer into hepatocytes in preclinical models (mice, dogs, non-human primates, NHP). To evaluate LV-mediated gene therapy for hemophilia A, we generated LV expressing engineered versions of human factor VIII (FVIII): codon optimized (coFVIII) and including a XTEN polypeptide (coFVIII.XTEN), known to increase half-life and secretion of the payload protein. We administered LV.FVIII, LV.coFVIII or LV.coFVIII.XTEN intravenously (i.v.) to newborn hemophilia A mice and observed long-term FVIII activity up to 2 U/mL and restoration of hemostasis in mice treated with LV encoding for engineered transgenes. We then administered 1e9 transducing units (TU)/Kg (n=2) or 3e9 TU/Kg (n=3) for LV.coFVIII.XTEN or 3e9 TU/Kg (n=2) or 6e9 TU/Kg (n=3) for LV.coFVIII to NHP. A corticosteroid immune-suppression regimen was applied from day -1/3 to day +7/9, since human FVIII is known to be immunogenic in NHP. I.v. administration of LV was well tolerated and we observed only a self-limiting leukopenia and limited serum aspartate aminotransferases (AST) elevation. Therapeutic FVIII amounts were observed in all treated animals, reaching 0.6-1 U/mL at 3e9 TU/Kg of LV.coFVIII.XTEN. We monitored anti-FVIII antibody (Abs) formation, acute cytokine response and T cell responses. Upon corticosteroids discontinuation, all NHP developed anti-FVIII Abs, but 4/5 LV.coFVIII.XTEN treated NHP, compared to 1/5 in LV.coFVIII treated NHP, maintained LV-positive hepatocytes in the liver and their splenocytes did not respond to *ex vivo* FVIII stimulation. Overall, our data show efficient and well-tolerated liver gene transfer in NHP by LV, with an improved therapeutic index for FVIII.XTEN transgene, supporting further development and potential clinical evaluation of this gene therapy strategy.

### PO 22

#### GENETIC DETERMINATION OF ENHANCED VON WILLEBRAND FACTOR CLEARANCE

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*Background:* Enhanced clearance of von Willebrand factor (VWF) is associated with the von Willebrand disease (VWD) however, the genetic background of this issue is not well defined.

*Aim*: To identify natural mutations in the VWF that are associated with reduced VWF survival.

*Methods*: We enrolled 230 (type 1=33, type 2=197) patients with VWD and 120 healthy controls. All patients were well-characterized according to the ISTH guidelines at the phenotypical level. The ratio of VWF propeptide/VWF:Ag (VWFpp ratio) was used for the evaluation of VWF clearance. Sanger sequencing was performed on exons encoding the defective domains (according to the laboratory results) in all patients.

*Results*: The baseline characteristics for the study population are summarized in Table 1. Eighty-six different mutations were found. The number of mutations with a VWFpp ratio between 1.7-2.2 was 22, for a ratio of 2.3-2.9 was 20 and it was 16 with a ratio ≥3 (Figure 1). The median (range) of VWFpp ratio for the healthy controls was 0.98 (0.55-1.6). Patients with type 1 (Vicenza not included) showed a significantly higher median of VWFpp ratio than controls (1.5 vs 0.98, p<0.001). Type 2 patients had a higher ratio (2.3) than both type 1 and healthy controls (p<0.001, p<0.001 respectively). A cut-off >1.6 for VWFpp ratio was defined as an indicator of accelerated clearance of VWF. Thus an enhanced clearance was observed in 44% of type 1, 100% of Vicenza, 80% of 2A, 73% of 2B, 88% of 2M, and 30% of 2N. Following the Vicenza variant, type IIA(IIE), and 2M showed the highest rate of clearance (Table 1).

*Conclusions:* Accelerated VWF clearance was found in the vast majority of type 2A, 2B, and 2M whereas it was found in a lower proportion of type 1(44%) and 2N(30%). 58 different mutations were associated with an increased VWFpp ratio. The mutations with the highest VWFpp ratio were mostly located at D3 and A1 domains.

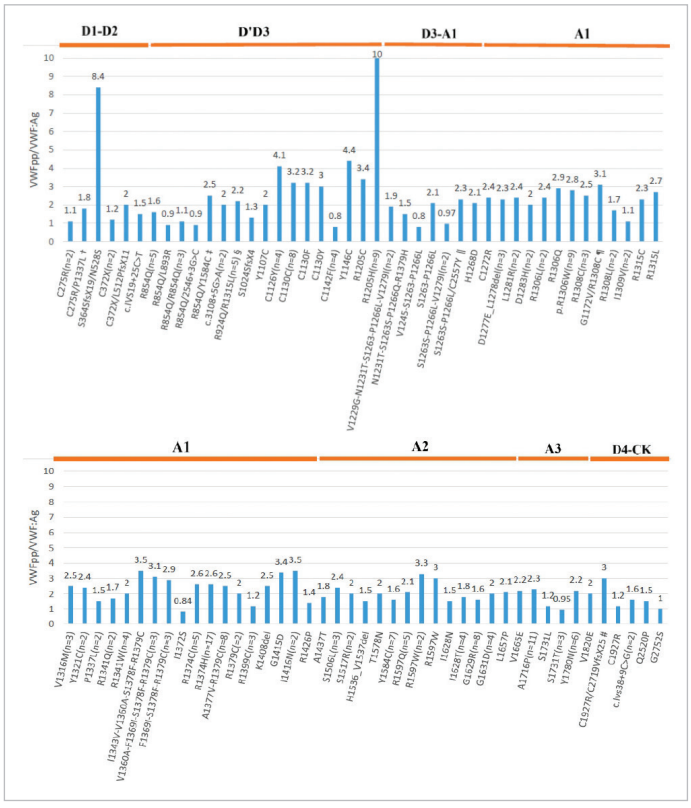


Figure 1. The ratio of VWFpp/VWF:Ag for all the individual identified mutations and the location of variants at the VWF domains. Mutations are ordered according to the protein position. If a patient carry two mutations, these were depicted based on the first variant. †(C275R at D1, P1337L at A1), ‡(R854Q at D', Y1584C at A2), §(R924Q at D3, R1315L at A1), ‖(S1263S-P1266L at D3, C2557Y at C4), ¶(G1172V at D3, R1308C at A1), #(C1927R at D4, C2719VfsX25 at C6).

| Type | N | FVIII:C (IU/dL) | VWF:Ag (IU/dL) | VWF:RCo (IU/dL) | VWFpp (IU/dL) | VWFpp/VWF:Ag ratio |
|---|---|---|---|---|---|---|
| Type 1 | 23 | 68 (20-110) | 37 (3-77) | 30 (3-57) | 57 (6-128) | 1.5 (0.68-3) |
| Vicenza | 10 | 17 (10-51) | 8 (3-31) | 8 (3-20) | 75 (40-128) | 8.7 (3.4-16) |
| 2A(IIA) | 36 | 50 (21-91) | 43 (10-109) | 9 (3-24) | 82 (18-174) | 2 (0.78-8) |
| 2A(IIE) | 24 | 41 (25-77) | 22 (13-115) | 11 (6-45) | 66 (33-88) | 2.8 (0.7-5.2) |
| 2B | 34 | 60 (6-132) | 47 (18-140) | 11 (4-90) | 102 (33-376) | 2.3 (0.85-3.8) |
| 2BNY | 7 | 62 (50-75) | 45 (21-80) | 26 (10-41) | 56 (35-126) | 1.8 (0.8-2.3) |
| 2M | 62 | 47 (23-124) | 28 (12-130) | 10 (3-44) | 67 (32-137) | 2.5 (0.8-4.1) |
| 2MCB | 23 | 51 (35-133) | 30 (18-147) | 25 (14-101) | 57 (43-302) | 2.1 (0.8-3.2) |
| 2N | 10 | 35 (15-50) | 52 (15-150) | 31 (15-112) | 76 (25-132) | 1.1 (0.8-2.4) |
| Healthy Controls | 120 | NA | 96 (45-169) | NA | 93 (47-184) | 0.98 (0.55-1.6) |

Table 1. Baseline characteristics of the study population. Data are reported by Median (range).

**PO 23**

## DIFFERENTIAL RELEASE OF VWF AND VWF-PROPEPTIDE FROM PLATELET ALPHA-GRANULES

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*Background/Aims:* Platelets bud off from megakaryocytes into the circulation and contain different types of granules. Alpha-granules contain many hemostatic proteins, including Von Willebrand Factor (VWF) and a processed part of the protein, VWF-propeptide (VWFpp). While multimerization, storage and release of VWF has been extensively studied in endothelial cells, regulation in megakaryocytes and platelets is unclear. Studying these processes in platelets will help us better understand how this key hemostatic protein contributes to adequate hemostasis from different compartments. Thus, we aimed to characterize the storage and release of VWF and VWFpp in platelet alpha-granules.

*Materials and Methods:* Healthy platelets were stimulated with PAR-1 activating peptide (PAR-1-ap). We employed super-resolution light microscopy and semi-automated image analysis to generate quantitative imaging data. Slides were stained for alpha-tubulin, VWF and VWFpp, SPARC or fibrinogen. Data are normalized to resting platelets as percentage of granule numbers ± SEM.

*Results:* We observed extensive, but not perfect (~85-95%) overlap in VWFpp+ and VWF+ granules in hundreds of resting platelets, implying that these proteins are stored in similar eccentric fashion in platelet alpha-granules (Figure 1A). In comparison to unstimulated platelets (100±1.7% granules over 3 donors), we observed less VWFpp+ (64.3±3.1%) and VWF+ granules (75.3±1.4%) at 0.6 µM of PAR-1-ap, signifying rapid release of a subset of granules (Figure 1A-B). Higher concentrations of PAR-1-ap triggered more pronounced differential release of VWFpp (leftover granules: 14.7±1.6% at 20 µM, p <0.0001) compared to VWF (62.4±1.4%, p=0.03). Release of other alpha-granule

proteins was intermediate at 20 µM PAR-1-ap (SPARC: 37.8±1.4%, fibrinogen 48.1±2.9%; p<0.001), providing further evidence for differential exocytosis of alpha-granule cargo.

*Conclusions:* Our findings show that VWF and VWFpp are stored in a similar compartment of alpha-granules but are differentially released from activated platelets. This may affect how platelet-derived VWF and VWFpp contribute to hemostatic clots.

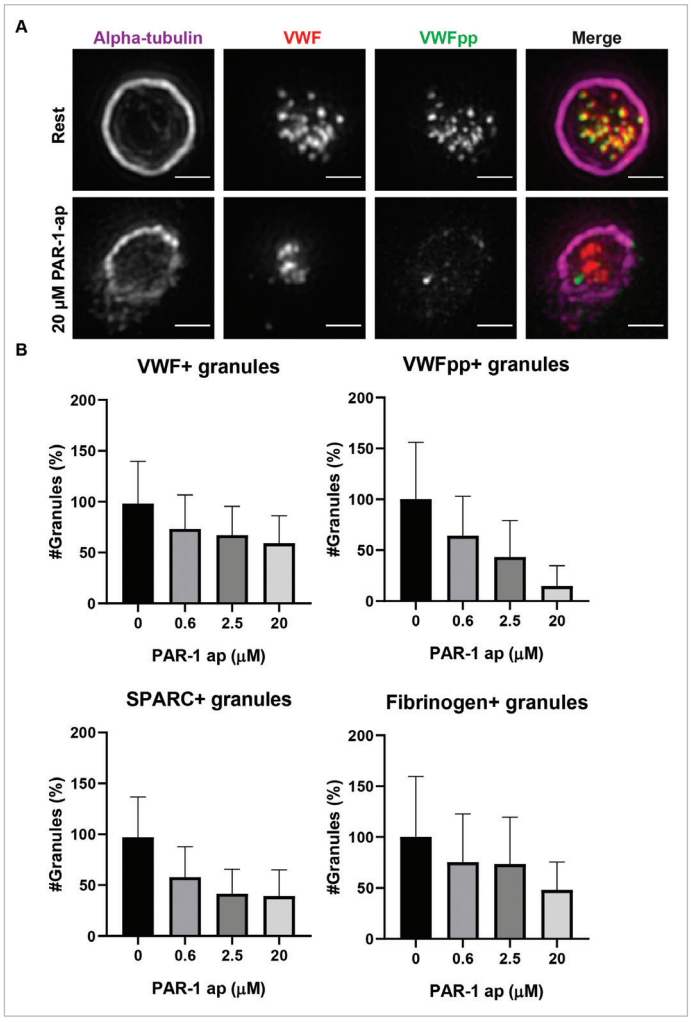


Figure 1. Storage and release of VWF and VWFpp in resting and stimulated platelets. Representative images are shown of resting and PAR-1 stimulated platelets (1A); stained for alpha-tubulin (magenta), VWF (red) and VWFpp (green). Differential release of VWF, VWFpp, SPARC and fibrinogen after PAR-1 stimulation was estimated through leftover granule numbers (1B). Mean±SD are shown

## PO 24

### GENETIC CHARACTERIZATION OF VON WILLEBRAND DISEASE TYPE 2 IN MILAN COHORT PATIENTS

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*Background:* Von Willebrand disease (VWD) type 2 is caused by qualitative defects of von Willebrand factor (VWF) for binding to Glycoprotein Ib, collagen, or factor VIII.

*Aim:* Genetic characterization of a large VWD type 2 cohort in Milan.

*Methods:* We enrolled 320 patients (female/male= 177/143) from 147 unrelated families with VWD type 2 diagnosis. Patients were characterized with full laboratory phenotype tests and their diagnosis was confirmed by target genetic analysis using Sanger sequencing following the ISTH guidelines.

*Results:* Patients were diagnosed with type 2A (n=95), 2B (n=85), 2M (n=114) and 2N (n=26) (Figure 1). Eighty-one different VWF variants including 10 novels (p.L893R, p.C1126Y, p.C1142F, p.L1281R, p.R1379H, p.R1426P, p.V1604V, p.L1657P, p.S1731L, p.C2557Y) were found. Most patients were heterozygous for a single variant (n=257), whereas 36 cases had 2 mutations: 4 were homozygous, 16 compounds heterozygous (in trans), and 16 in cis position. Twenty-seven patients had ≥3 variants, all due to gene conversion except one. Among the eighty-one distinct variants identified, five mutation types were observed: missense (n=61, 75.3%), gene conversion (n=13, 16%), synonymous (n=2, 2.5%), deletion (n=4, 5%) and splice (n=1, 1.2%). In type 2A, 59% of mutations were located in the A2 domain (IIA), 26% and 7.5% were respectively at the D3 and A1 domains (IIE). In type 2B, the variants were at the A1 domain (85%) and the D3-A1 junction (15%). In type 2M, 77% were located at the A1 domain, whereas 23% were at the A3 domain. In type 2N, all patients had p.R854Q (D' domain) in either homozygous, heterozygous (carrier), or compound heterozygous with VWF quantitative variants. The most common mutations for each VWD type 2 are shown in red in Figure 2.

*Conclusion:* Genetic analysis of a large cohort of VWD type 2 in Milan showed that the vast majority of patients (87.8%) had missense variants located in specific domains in each type.

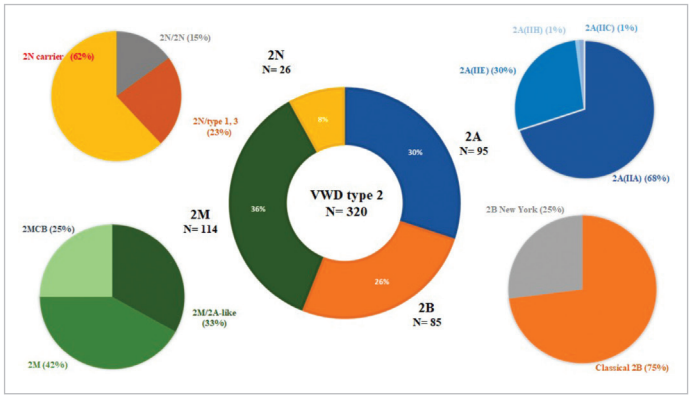


Figure 1. The epidemiologic picture and frequency of different VWD type 2.

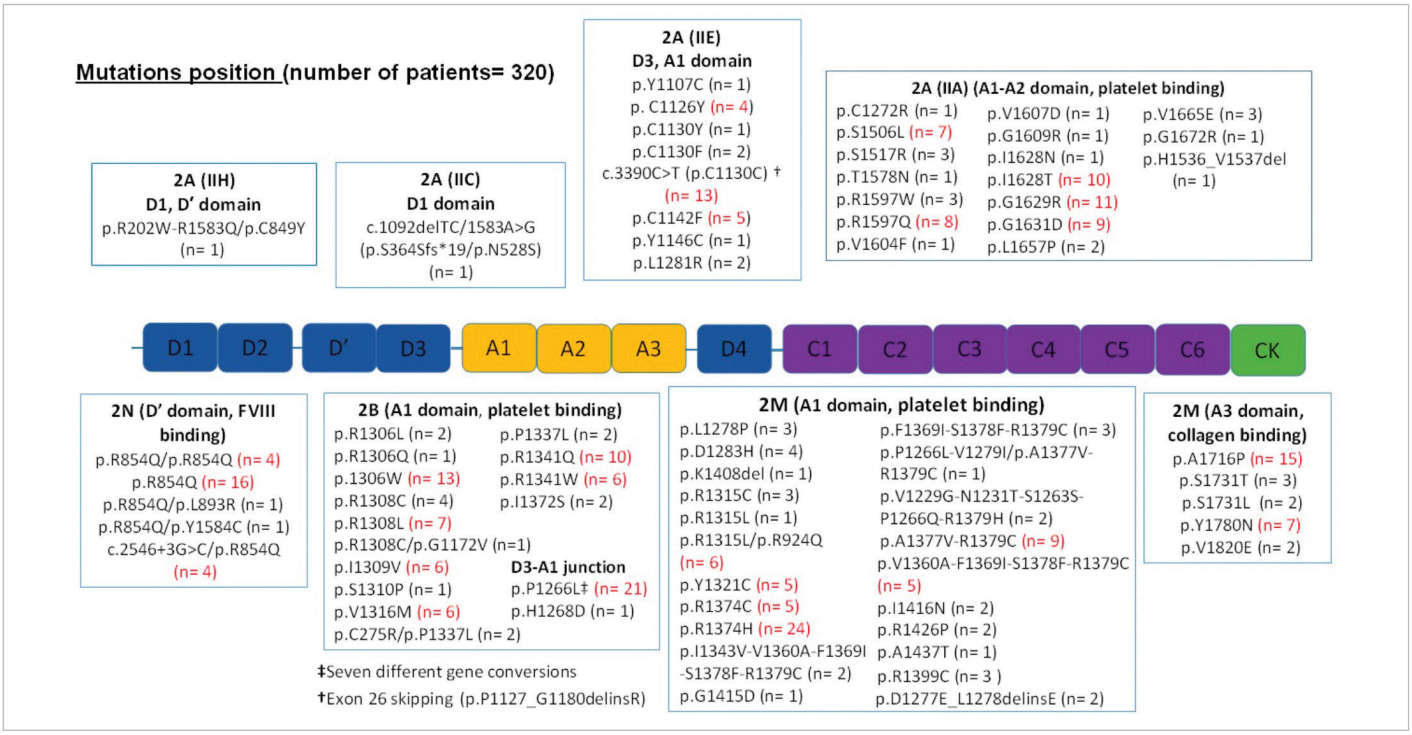


Figure 2. Location of type 2 VWD mutations on the pro-VWF coding region.

## PO 25

### IDENTIFICATION OF SMALL INTERFERING RNAS FOR ALLELE-SELECTIVE SILENCING OF MURINE VON WILLEBRAND FACTOR

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*Background/Aims:* High von Willebrand factor (VWF) plasma levels are associated with arterial thrombosis. Current antiplatelet therapy to prevent arterial thrombosis increases bleeding risk and often fails to prevent thrombosis. An alternative therapeutic approach would be to lower VWF by allele-selective silencing of VWF. This approach averts complete knockdown of VWF and thus minimizes bleeding risk. We aimed to identify small interfering RNAs (siRNAs) that can distinguish between strain-specific differences in murine *Vwf* to be used in allele-selective knockdown studies in heterozygous mouse models.

*Materials and Methods:* Two commonly used mouse inbred strains, C57BL/6J and 129S1/SvImJ, were selected based on genetic differences between their *Vwf* genes and comparable plasma VWF levels. siRNAs were designed to target one or two of 11 genetic differences between these mouse strains. *In silico* analysis predicted 14 siRNAs to be active, meaning that they effectively inhibit either of the strain-specific *Vwf* alleles. All selected siRNAs were identically chemically modified to increase stability, which included 2'O-Methyl and phosphorothioate backbone modifications. Activity and allele/strain-selectivity of these siRNAs were determined, dose-dependently, in HEK293 cells transiently expressing either C57BL/6J or 129S1/SvImJ *Vwf* or both.

*Result:* 7 out of 14 siRNAs effectively inhibited the targeted allele (≥80% at 1 nM siRNA), with minimal inhibition of the untargeted allele. The other siRNAs were either non-selective or showed limited inhibition. The 7 siRNAs were further tested at concentrations as low as 62.5 pM to find the most potent siRNAs. Two lead candidates were chosen based on potency (good inhibition at 62.5 pM), strain-selectivity and ability to target one nucleotide difference.

*Conclusions:* We have identified strain-selective siRNAs that can distinguish between C57BL/6J and 129S1/SvImJ *Vwf* based on one or two nucleotide(s) difference between their *Vwf* genes. The selected lead compounds will be tested in F1 hybrids of cross-bred C57BL/6J and 129S1/SvImJ mice.

## PO 26

### EVALUATION OF HEALTH-RELATED QUALITY OF LIFE IN PATIENTS WITH VON WILLEBRAND DISEASE ON LONG-TERM PROPHYLAXIS: WISH-QOL INTERMEDIARY RESULTS

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*Background/Aims:* WiSH-QoL is a French prospective non-interventional study conducted to assess health-related quality of life (HRQoL) over 24 months in patients with von Willebrand disease (VWD) regardless of VWD type, age and therapeutic strategy. We present here data of patient cohort treated with long-term prophylaxis (LTP) regimen.

*Materials and Methods:* HRQoL is assessed with SF-36 (8 items aggregated into 2 physical and mental scores) in adults, Disabkids in adolescents (12 items aggregated into 3 physical, mental and social scores) or children, and a VWD-specific-QoL tool in all patients.

*Results:* 355 patients were recruited in this study. Among them, 19 (5.4%) patients (median age of 32.3 years [4.7-70.4]) were treated with LTP: 14 adults, 3 adolescents and 2 children; 8/11 females were of childbearing age.

At inclusion, 15/19 (78.9%) had been on LTP for 3.7 years [0.35-15.6] with VWF median dose of 50 IU/kg 2.3 times/week. Four patients (21.1%) had been treated with continuous and/or intermittent prophylaxis for 9.8 years [0.5-25] at 54 IU/kg median dose 3 times/week.

LTP was mainly prescribed in type 3 patients (13/27, 48.1%) for

prevention of hemarthrosis and/or severe mucosal bleedings. It was rarely prescribed in type 2 (4/226, 1.8%) and type 1 (2/75, 2.7 %) patients, mainly for gastrointestinal haemorrhage, recurrent epistaxis or menorrhagia.

SF36 and Disabkids, completed at inclusion (Table 1), show lower scores in adult and adolescent LTP patients versus the overall cohort. Similar trends are found with VWD-specific-QoL tool. Despite the small number of children (2 type 3), the benefit of LTP seems to be better in this age group before the onset of joint damage.

*Conclusions:* These data suggest a negative impact of the disease severity on mental and physical health in adults and adolescents. The benefit of LTP on HRQoL must also consider the constraints of such a prophylactic treatment.

| | **Adults [≥18 years]** | | **Adolescents [8-17 years]** | | | **Children [4-7 years]** |
|---|---|---|---|---|---|---|
| **Long-term prophylaxis patients (M0)** | | | | | | |
| **GENERIC-QoL** Mean (SD) *[N patients]* | **SF36 V2** PCS* **38.9 (12.6)** *[11]* | MCS* **37.0 (12.6)** *[11]* | **Disabkids** PS** **66.7 (7.2)** *[3]* | MS** **60.4 (3.6)** *[3]* | SS** **64.6 (18.0)** *[3]* | **Disabkids** $N^{alized}$ score **93.8 (3.0)** *[2]* |
| **VWD-SPECIFIC***** Mean (SD) *[N patients]* | $N^{alized}$ score **37.0 (10.4)** *[12]* | | $N^{alized}$ score **40.3 (10.5)** *[3]* | | | $N^{alized}$ score **31.3 (8.8)** *[2]* |
| **Global cohort (LTP and on demand treatment) (M0)** | | | | | | |
| **GENERIC-QoL** Mean (SD) *[N patients]* | SF36 V2 PCS* **51.2 (9.3)** *[166]* | MCS* **44.0 (10.7)** *[168]* | **Disabkids** PS** **83.2 (15.8)** *[55]* | MS** **80.1 (19.3)** *[55]* | SS** **90.7 (13.3)** *[55]* | **Disabkids** $N^{alized}$ score **78.3 (15.0)** *[15]* |
| **VWD-SPECIFIC***** Mean (SD) *[N patients]* | $N^{alized}$ score **21.4 (12.8)** *[172]* | | $N^{alized}$ score **24.9 (10.0)** *[55]* | | | $N^{alized}$ score **24.9 (12.3)** *[15]* |

*SF36 V2: Higher score representing better health (PCS: Physical Component Score, MCS: Mental Component Score)
**Disabkids Adolescents: Higher score representing better health (PS: Physical Score, MS: Mental Score, SS: Social Score)
Disabkids Children: Higher score representing better health ($N^{alized}$:score: Normalized score i.e. re-calibrated from 0 to 100)
***VWD-specific: Lower score representing better health ($N^{alized}$:score: Normalized score i.e. re-calibrated from 0 to 100)

Table 1: Health-related quality of life scores at study inclusion (M0) according to therapeutic strategy and age group (all VWD types included)

## VARIOUS

### PO 27

#### PAD4-MEDIATED CITRULLINATION IN SEPSIS AND IMMUNE-MEDIATED TTP

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*Background/Aims:* Peptidyl Arginine Deiminase-4 (PAD4) mediated citrullination (irreversible conversion of arginine to citrulline) of proteins is a known driver of autoimmune disease, as exemplified by findings in rheumatoid arthritis research. Citrullinated proteins are capable of breaking tolerance-to-self. Recently, citrullinated ADAMTS13 has been found in sepsis patients, as well as in patients with cardiovascular comorbidities. Importantly, PAD4 is released in large quantities from neutrophil extracellular traps (NETs), which have been linked to autoimmune diseases as well as thrombo-inflammatory disorders[1]. In this project, we investigate the role of citrullination in immune-mediated Thrombotic Thrombocytopenic Purpura (iTTP) as a link between inflammation and iTTP.

*Methods:* We are developing a mass-spectrometry based assay, which uses biotin-PG as a covalent citrulline-specific linker. This modification allows for streptavidin-mediated enrichment of citrullinated peptides. After process optimization, we will use this method to determine the iTTP citrullinome. In parallel, we are developing an *in vitro* ADAMTS13 citrullination assay based on neutrophil NET formation.

*Results:* Covalent linking of citrullinated recombinant ADAMTS13 and subsequent streptavidin-mediated purification was successful. Current efforts are focused on developing the mass spectrometry assay to analyze citrullinated proteins from plasma. Additionally, using ionomycin we have successfully established a protocol for producing NETs *in vitro*. NET-formation coincided with citrullination of histone H3 and vimentin. We will employ this assay to monitor which neutrophil-derived proteins are modified by citrullination and also explore citrullination of ADAMTS3 under these experimental conditions. In parallel we are tailoring the biotin-PG approach to quantitatively assess whether citrullinated ADAMTS13 is present in plasma samples of patients with iTTP and sepsis.

*Conclusions:* We have successfully established novel assays to monitor PAD4 mediated citrullination of histone H3 and vimentin during in vitro formation of NETs. In parallel we are utilizing biotin-PG modified biological samples to quantitatively assess citrullination of ADAMTS13 in patients with sepsis and iTTP.

References:
1 Liu X, Arfman T, Wichapong K, Reutelingsperger CPM, Voorberg J, Nicolaes GAF. PAD4 takes charge during neutrophil activation: impact of PAD4 mediated NET formation on immune-mediated disease. Journal of Thrombosis and Haemostasis.

### PO 28

#### INTERACTION OF FACTOR XI WITH LIGANDS STUDIED WITH NOVEL ANTI-FACTOR XI NANOBODIES

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*Background/Aims:* Factor (F) XI is a pivotal player in the coagulation cascade whose deficiency causes a mild bleeding disorder, haemophilia C. The factor has a dual role in coagulation. Its activation by FXIIa is associated with prothrombotic characteristics, whereas thrombin-mediated activation of FXI is thought to mainly promote thrombin formation to stabilize the fibrin clot in haemostasis. FXI is a dimer consisting of two monomers composed of a catalytic domain supported by a base of four apple domains. These domains mediate

the interactions of FXI with its ligands. Various interactions have been mapped to a specific apple domain. Thrombin binds the apple 1 domain, HK binding is centred around the apple 2 domain, binding of FIX occurs on the apple 3 domain and the apple 4 domain mediates dimerization.

*Materials and Methods:* We tested the effect of 54 unique anti-FXI binding nanobodies on the activation of FXI or activity of FXIa in both purified and plasma-based assays. This resulted in the selection of 24 nanobodies with modulatory abilities. Epitope binning was performed with surface plasmon resonance to assess the correlation between binding site and their effect on FXI function.

*Results:* We have identified 24 nanobodies with varying binding epitopes that have an inhibiting effect on either FXIa activity, FXI activation by thrombin or FXIIa, or FXI-HK binding. These results could be related to the nanobody binding epitopes. Nanobodies binding to the FXI apple 2 domain mainly inhibited FXI-HK binding. Nanobodies with apple 3 as binding epitope influenced FIX activation by FXIa as well as FXIIa-mediated FXI activation. Apple 4-binding nanobodies served as general inhibitors of FXI activation by thrombin and FXIIa.

*Conclusions:* We have produced nanobodies with different binding epitopes on FXI as tool compounds to better understand the interactions of FXI with FIX, HK, FXIIa and thrombin.

## PO 29

### THE EXTRACELLULAR PROTEASE EPIP FROM *S. AUREUS* TRIGGERS BLOOD COAGULATION BY PROTEOLYTICALLY ACTIVATING PROTHROMBIN AND PLATELET PROTEASE-ACTIVATED RECEPTOR 1

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*Background: Staphylococcus aureus* is a Gram+ bacterium known for being responsible for both mild and systemic bactereamia and sepsis. During the last decades, clinical evidence correlates S. aureus infections to thrombotic complications, such as DIC. Among seeral virulence factors, extracellular proteases might play a role in triggering thrombotic events in infectious diseases, whereby bacterial proteases could activate the coagulation cascade by proteolytically converting ProT zymogen into thrombin species and stimulating platelets aggregation.

*Aims:* In this work, we investigated if the serine protease EpiP from *S. aureus* is involved in thrombus formation during staphylococcal infections, directly activating ProT and platelets.

*Methods:* Biochemical techniques: limited proteolysis, enzymatic chromogenic assay, mass spectrometry; coagulation assays: fibrin generation and platelet aggregation; fluorescence microscopy (FM).

*Results:* Staphylococcal EpiP converts ProT into an active species that can hydrolyze the thrombin-specific substrate S2238. The time-course analysis of ProT activation allowed to identify the EpiP cleavage sites, identical to those hydrolyzed by factor Xa under physiological conditions. The activation products of ProT by EpiP can induce fibrin clot formation and platelet aggregation. Surprisingly, EpiP can proteolyze PAR1(38-60) peptide at the same site of thrombin cleavage and electrostatically interact with GpIbα(268-282) peptide as demonstrated by SPR. Ultimately, we directly observed EpiP-mediated platelets agglutination by FM.

*Conclusions:* EpiP can proteolyze the inactive ProT into an active thrombin species which is able to trigger blood clotting, and directly induces platelet aggregation activating PAR1 receptor after binding to GpIbα on platelets. These results widen our understanding of the biochemical mechanisms whereby *S. aureus* proteases can initiate coagulation, establishing a direct link between infections and higher thrombotic risk.

## PO 30

### EXPLORING THE ROLE OF FACTOR VIII AS A POTENTIAL PLAYER IN CANCER CELLS

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*Background/Aims:* Presently, venous thromboembolism (VTE) is 5-6 times more likely to occur in cancer patients, while there is a greater risk of cancer diagnoses following thromboses. In considering novel players, factor VIII (FVIII), an essential coagulation co-factor with emerging extra-coagulative functions, has been identified as an independent VTE risk factor in cancer, however, the basis of this increase, is unknown. The aim of the study was to investigate the possible direct expression and secretion of FVIII by cancer cells.

*Materials/Methods:* Bladder cancer, which has a high-VTE risk, and normal bladder tissue epithelium, was used to investigate FVIII mRNA and protein expression (n=14). Factor FVIII protein and secretion was examined in bladder cancer cell lines (n=2). Expanding to other cancers, immunohistochemistry was performed of biopsies (n=90), and the Cancer Cell line Encyclopedia (CCLE) database was used to analyze FVIII, Tissue Factor (TF), FV, FVII, FIX, FX and von Willebrand factor (VWF) mRNA in 811 cell lines subdivided according to origin (n=16 groups). To reinforce mRNA findings, FVIII protein synthesis and secretion was investigated in a profile of cancer cell lines (n=42) representing the different origins.

*Results:* While expressed in the normal bladder epithelium, FVIII mRNA and protein was seen to be higher in matched bladder neoplasms, with synthesis and secretion of FVIII evident in bladder cancer cell lines (n=2) *in vitro*. Further, these observations could be extended to other cancers, with a pattern of FVIII expression reflecting the tumor origin, and one which was independent of VWF and other relevant players in the coagulation cascade. Finally, FVIII intracellular protein expression and secretion was detected, demonstrating a pattern related to tumor origin.

*Conclusion:* Herein, evidence is provided of a possible independent role for FVIII in cancer-related pathophysiology. It remains to be understood the functional significance of cancer derived FVIII expression.

## PO 31

### TRANSPLACENTAL DELIVERY OF RECOMBINANT FC-FUSED FACTOR VIII (RFVIIIFC) IN FVIII-DEFICIENT MICE.

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*Background:* The immune response against FVIII is a major complication of replacement therapy in hemophilia A. In FVIII-KO mice, the transplacental delivery of Fc-fused A2 (A2Fc) and C2 (C2Fc) domains of FVIII induces partial tolerance to therapeutic FVIII in the offspring. Placental transfer of the entire FVIII should induce complete tolerance to FVIII. rFVIIIFc however crosses the placenta to much lesser extents than IgG1 and C2Fc, in amounts insufficient to foster FVIII-specific immune tolerance. The inefficient placental transfer of rFVIIIFc is not due to its binding to VWF. Understanding the properties of rFVIIIFc associated with its reduced transplacental delivery should allow optimization of placental transfer to levels compatible with tolerance induction.

*Aims:* To compare FcRn binding and endocytosis by human trophoblast BeWo cells of C2Fc, IgG1 and rFVIIIFc.

*Methods:* rFVIIIFc, human (hu) IgG1 and C2Fc (114 nM) were incubated with BeWo cells for 4 hours at 4°C or 37°C to study binding and endocytosis, revealed using an anti-human IgG AF 647 and analyzed by fluorescence microscopy. Binding and dissociation kinetics of C2Fc, rFVIIIFc and huIgG1 were analyzed at pH6 towards biotinylated human and murine (mu) FcRn (Immunitrack®) by Biacore.

*Results:* BeWo cell-bound (4°C) and endocytosed (37°C) rFVIIIFc and C2Fc presented a puncta pattern, with C2Fc showing a more diffused pattern than rFVIIIFc. rFVIIIFc, huIgG1 and C2Fc bound with higher affinities to muFcRn than to huFcRn. At pH6, binding affinities of the three molecules were similar for muFcRn, but greater for C2Fc towards huFcRn.

*Conclusions:* Both C2Fc and FVIIIFc bind to and are captured by BeWo cells. As compared to FVIIIFc, C2Fc binds with higher affinity to huFcRn, and with identical affinity to muFcRn. Future work will compare the intracellular routing of rFVIIIFc and C2Fc and identify structural modifications to be introduced in rFVIIIFc to foster its transplacental delivery.

# PUBLICATION ONLY

## PUBLICATION ONLY

### ABS 01

#### FUNCTIONAL TRANSITORY ANTI EMICIZUMAB ANTIBODY CREATING BLEEDING EPISODES

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*Background:* The development of anti-drug antibody in patients with hemophilia A treated with emicizumab is a rare event. Nevertheless, as already described, patients may develop neutralizing anti-emicizumab antibody which could be associated with unexpected bleeding

*Aim:* To describe a new case of partially neutralizing anti-emicizumab antibody, developed in a hemophilia A patient with no FVIII inhibitor treated with emicizumab which disappeared with continuous use of drug.

*Case:* The patient is a 33-years-old male with severe HA without FVIII inhibitor, previously in prophylaxis with 2000U of recombinant FVIII every other day. His replacement therapy was switched to emicizumab receiving the initial four standard loading doses with 3 mg/kg/week followed by the 1.5 mg/kg/week regimen. After receiving the 5th dose, the patient developed an acute bleeding episode at his right elbow confirmed with ultrasound. This acute bleeding required additional treatment of FVIII. Emicizumab plasma level and development of anti-drug antibody has been controlled.

*Results:* The bleeding episode in our patient at the 5th dose of emicizumab was associated with a modest reduction of emicizumab concentration from 49 to 31.7 ug/mL and presence of anti-emicizumab antibody tested with western blot analysis on total IgG fraction. Since the neutralization was not complete the patient has continued his regular administration using emicizumab and a reduced dose of FVIII (4 total infusions) with a close clinical (ultrasound) and laboratory (emicizumab level and anti-emicizumab antibody) monitoring. The intensity of anti-drug antibody was reduced at 7th ED and completely disappeared at 8th ED with a gradual recovery of emicizumab concentration up to 41 ug/mL in patient's plasma.

*Conclusion:* During the initial phase of emicizumab treatment (<20 exposure days) patients need particular attention with an accurate clinical and laboratory monitoring. Some transitory anti-drug antibody could have a functional effect creating bleeding episodes, and this need to be properly treated.

### ABS 02

#### D-DIMER AS A PREDICTIVE MARKER FOR MORTALITY IN HOSPITALIZED COVID-19 PATIENTS

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*Background:* The novel coronavirus disease 2019 (COVID-19) presents an important and urgent threat to global health. Identifying strong predictors of mortality could assist medical staff in treating patients and allocating limited healthcare resources.

*Aims:* The primary aim of this paper was to study the effect of d-dimer levels at admission as a predictive marker for in-hospital mortality.

*Methods:* This was a retrospective cohort study evaluating hospitalized patients (age> 18 years), who were positive for COVID-19 based on real-time PCR at one of nine COVID-19 units during the period of the first COVID-19 wave in Lombardy, Italy. The primary endpoint was in-hospital mortality. Information was obtained from patient records. Statistical analyses were performed using a Fine-Gray competing risk survival model. Predictive power was assessed using Harrell's C-index.

*Results:* Out of 1049 patients that were admitted to the emergency department and subsequently hospitalized, 501 patients had evaluable data for d-dimer. Of these 501 patients, 96 did not survive. Cumulative incidence of in-hospital mortality within 30 days was 20%, and the majority of deaths occurred within the first 10 days. (Figure 1) When compared to patients in the lowest quartile of d-dimer blood concentration, the hazard ratio of in-hospital mortality for patients in the 2nd, 3rd and 4th quartile was 3.9 (95CI: 1.5-10.0), 5.8 (95CI: 2.3-14.7), and 4.6 (95CI: 1.8-11.5) respectively, after multivariable adjustment for age, sex and number of comorbidities. The C-statistic of d-dimer for in-hospital mortality was 0.67 (95CI: 0.62-0.71). (Table 1)

*Conclusion:* Higher d-dimer levels were strongly associated with in-hospital mortality. However, the predictive power of d-dimer alone was not high enough to be useful as a risk prediction score. Future research should focus on the added value of d-dimer as part of a larger risk prediction score.

| | N | n | Observed incidence after 30 days | Univariate model | Multivariable model[1] |
|---|---|---|---|---|---|
| D-dimer | | | | | |
| < 538 ng/mL | 126 | 5 | 0.04 | ref | ref |
| 538-957 ng/mL | 124 | 20 | 0.16 | 4.2 (1.6-11.1) | 3.9 (1.5-10.0) |
| 957-1764 ng/mL | 124 | 36 | 0.30 | 8.6 (3.4-21.8) | 5.8 (2.3-14.7) |
| > 1764 ng/mL | 127 | 35 | 0.28 | 7.6 (3.0-19.3) | 4.6 (1.8-11.5) |
| C-statistic (95%CI) | | | | 0.67 (0.62-0.71) | - |

1: Model, adjusted for age and sex. 2: Model, corrected for age, sex and Charlson comorbidity index score.

Table 1: Association between d-dimer values and in-hospital mortality.

## ABS 03

### A COMBINATION OF COMPLEMENT-RELATED VARIANTS PREDICTS SEVERE COVID-19 WITH HIGH SENSITIVITY AND SPECIFICITY

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*Background:* Complement activation as part of the innate immunity is considered a key driver of hyper-inflammation in severe coronavirus disease-19 (COVID-19). We have previously shown genetic susceptibility in complement-related variants that was independently associated with severe COVID-19. In this study we investigated which combination of complement-related variants predicts severe COVID-19 with high sensitivity and specificity.

*Materials and Methods:* We studied consecutive patients hospitalized for severe COVID-19 (March 2020-October 2020). Genomic DNA was isolated from peripheral blood samples and analyzed using next generation sequencing (NGS) with a customized complement-related gene panel (*complement factor H/CFH, CFH-related, CFI, CFB, CFD, C3, CD55, C5, CD46, thrombomodulin/THBD*), including TMA-associated ADAMTS13 (*A Disintegrin and Metalloproteinase with Thrombospondin motifs*). To find the variants that predict severe disease, a rigorous algorithm, based on laws and rules of logic, has been developed and trained for disease risk prediction.

*Results:* We analyzed genetic and clinical data from 97 patients hospitalized for COVID-19: 63 with moderate disease hospitalized in COVID-19 general ward, and 34 with severe disease hospitalized in ICU. Analysis of almost infinite variant combinations (more than yotta=$10^{24}$) showed that patients with rs1042580 in *thrombomodulin* and without rs800292 in *complement factor H* did not require ICU hospitalization (favorable combination). The opposite was true for ICU patients, the majority of whom did not have rs1042580 in *thrombomodulin* and had rs800292 in *complement factor H* (unfavorable combination). Figure shows the percentage of patients with the favorable combination among those that did not require ICU, and percentage of patients with the unfavorable combination in ICU.

*Conclusions:* We highlight a combination of complement-related variants that predicts severe COVID-19 with high sensitivity and specificity. In line with our approach, results from ongoing trials of complement inhibitors in COVID-19 suggest that patient stratification with complement-specific markers might be necessary to achieve optimal results.

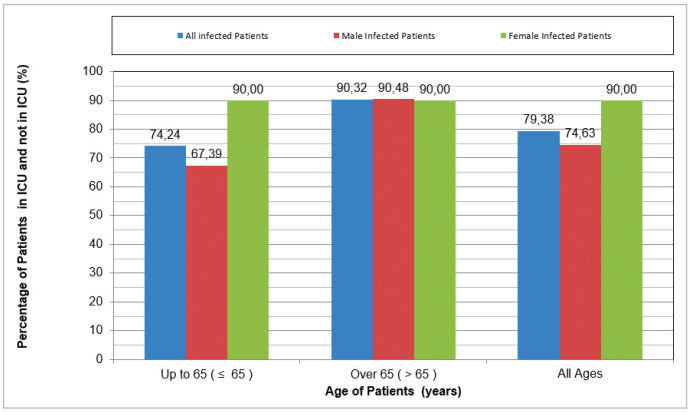


Figure 1.

## ABS 04

### INVESTIGATION OF ADAMTS13-INDEPENDENT CLEAVAGE AND REGULATION OF VON WILLEBRAND FACTOR

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*Background:* Interactions of von Willebrand factor (VWF) and its cleaving protease, ADAMTS13 play an important role in the maintenance of capillary flow. ADAMTS13 regulates the biological activity of VWF by cleaving prothrombotic ultra-large multimeric forms into hemostatically active high molecular weight forms. ADAMTS13-independent cleavage of VWF under in-vitro conditions by multiple leukocyte proteases including matrix metalloprotease (MMP-9), polymorphonuclear (PMN) elastase and proteinase 3 (PR3) was reported. However, the biological relevance of these interactions was not investigated. In this work, we aimed to evaluate the ADAMTS13-independent regulation of VWF in biological context by using sickle cell disease (SCD) patient plasma.

*Materials and Methods:* Plasma was obtained from steady state adult SCD patients (n=20) and healthy control patients (n=14). ADAMTS13 activity was assessed by using both full length VWF cleavage under shear stress or static conditions and peptidyl-based assays. Plasma concentrations of proteases capable of cleaving VWF were measured using ELISAs. The extent of ADAMTS13-independent VWF cleavage was assessed using an inhibitory anti-ADAMTS13 antibody (3H9). Recombinant VWF (Baxalta), ADAMTS13 (R&D Systems) and MMP-9 (R&D Systems) were employed.

*Results:* In the peptidyl-based assays, significantly lower ADAMTS13 activity was observed in SCD plasma. By contrast, the cleavage of the full length VWF molecule was normal. Among leukocyte proteases, MMP-9 levels were found to be significantly higher in SCD samples. Recombinant MMP-9 showed similar VWF cleavage to recombinant ADAMTS13 in the FRETS-VWF73 assay but enhanced cleavage of full length VWF. Finally, SCD plasma showed VWF degradation with inhibition of ADAMTS13.

*Conclusions:* ADAMTS13-independent cleavage of VWF occurs in SCD and possibly helps limit the pro-thrombotic capacity of VWF. Further research will identify the proteases with the greatest capacity for VWF cleavage. Recombinant proteases (e.g. MMP-9) may be evaluated for their potential to reverse the course of thrombotic disorders caused by VWF-ADAMTS13 imbalance.

## ABS 05

### DEVELOPMENT OF A NEW ELISA METHOD FOR ANTI-FVIIA ANTIBODY MEASUREMENT

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*Background:* Recombinant FVIIa has been shown to be efficacious for treatment of bleeding in congenital and acquired hemophilia A with inhibitor and in congenital FVII deficiency as well as in Glanzmann's thrombasthenia. As for other recombinant therapeutic proteins, administration of rFVIIa may induce the development of anti-FVIIa antibody that could be associated with a decrease of the treatment efficacy. Very limited data are available on development of anti-FVIIa antibody. Surveillance of rFVIIa immunogenicity is then required.

*Aim:* To develop a method for measuring the anti-FVIIa antibody in patients treated with rFVIIa.

*Method:* An ELISA bridging method has been developed by using rFVIIa (NovoSeven®) for both coating and detection. The best experimental conditions were a high-density of immobilized antigen, acid dissociation and long incubation time with plasma samples.

Commercial (Affinity Biological) FVII deficient plasma and FVII Inhibitor plasma (4.5 and 67.1 BU) have been used as negative and positive controls with sheep anti-human FVII IgG used as a reference standard.

*Results:* Several experiments have been performed and a significant improvement in IgG recovery has been achieved with FVII depleted plasma containing 1 ug/mL rFVIIa and 1.25 or 0.6 ug/mL of anti-human FVII IgG, by the acid dissociation and the long incubation time (Figure 1).

By increasing the concentration of immobilized antigen from 1 to to 5ug/mL further improvement has been achieved in the IgG recovery.

*Conclusion:* Monitoring the patient immune-response to a therapeutic protein is important to assess the drug efficacy and its immunogenicity. However, the antibody detection could be complicated by the presence of the antigen in the plasma sample. Here we describe a novel method unaffected by the presence of rFVIIa that could detect the incidence of the anti-FVIIa antibodies during the treatment with recombinant FVIIa.

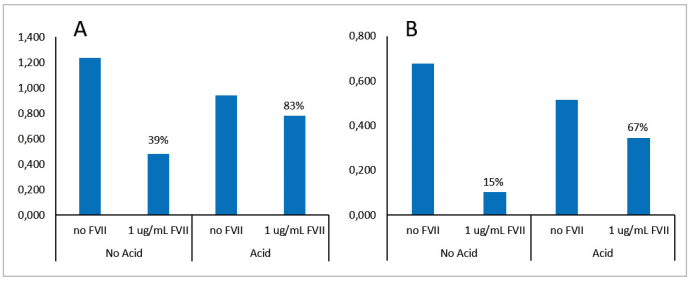


Figure 1. Improvement of IgG recovery obtained by acid dissociation with FVII depleted plasma containing 1 ug/mL of rFVIIa and 1.25 (A) or 0.6 ug/mL (B) of anti-human FVII IgG.

## ABS 06

### DEEP MOLECULAR MECHANISMS OF *F8* EXON 19 VARIANTS AND TRANSLATIONAL APPROACHES IN HEMOPHILIA A

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*Background:* A major aim of Hemophilia A (HA) studies is the definition of *F8* variants' causative role and the association with bleeding phenotypes. The pathogenic significance of missense variants is generally attributed to quantitative or qualitative changes in protein expression. Nevertheless, these changes may exert pleiotropic effects and also impair mRNA splicing due to the overlapping of the amino acid and splicing codes.

*Aims:* To combine and compare *in silico* and *in vitro* analyses to systematically characterize the pleiotropic effects of *F8* exon 19 variants on both protein biology and mRNA splicing.

*Methods:* Analysis of nucleotide variants through bioinformatic tools, combining results from multiple algorithms, and transient expression of minigenes and recombinant FVIII variants in cellular models, to evaluate their impact on splicing and protein features.

*Results:* Data on thirty variants provided evidence for higher prediction ability of *in vitro* assays. Whereas bioinformatics provided qualitative indications, recombinant expression provided better quantitative prediction (60% vs 90%), essential for relationships with the degree of bleeding severity. Importantly, the knowledge of the specific pathogenic molecular mechanisms led to the development of tailored correction approaches. In particular, a single engineered U1snRNA rescued mRNA splicing of nine different variants and the use of a chaperone-like drug resulted in improved factor VIII protein secretion for four missense variants.

*Conclusions:* We extensively characterized and provided molecular insights for a large panel of HA-causing variants by combining *in silico* and *in vitro* analysis, demonstrating the pleiotropic effects of several exonic changes. Our data suggest caution during variants classification based on nucleotide location or bioinformatic prediction and highlight the importance of experimental characterization to dissect the molecular mechanisms underlying HA, which might pave the way for the development of new individualized therapeutic strategies, also translatable to other genetic diseases.

## ABS 07

### TIME FROM THE INHIBITOR DETECTION TO THE START OF IMMUNE TOLERANCE INDUCTION AND THE OUTCOME OF IMMUNE TOLERANCE INDUCTION: RESULTS FROM THE BRAZILIAN IMMUNE TOLERANCE (BRAZIT) STUDY

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Immune tolerance induction (ITI) is the treatment of choice for eradication of anti-factor VIII (FVIII) antibodies (inhibitors) in people with inherited haemophilia A and inhibitors (PwHAi). Previous studies (most deriving from registries) have shown that a longer time elapsed between inhibitor development and the start of ITI ($\Delta t_{inhi\text{-}ITI}$) associates with a lower ITI success rate among PwHAi. We aimed to evaluate this association among PwHAi included in the BrazIT Study. PwHAi (n = 133) were enrolled from 15 haemophilia treatment centres in Brazil. PwHAi were treated according to the Brazilian ITI Protocol, using a low-dose regimen (50 IU/kg three times weekly). Upon absence of a decline in inhibitor titre, a high-dose regimen (100 IU/kg daily) was indicated. Total success was defined as a negative inhibitor titre (< 0.6 BU/mL) and a normal FVIII pharmacokinetics (recovery 66% or higher of expected values, and half-life of 6 h or higher). Partial success was defined as inhibitor titre between 0.6 and 2 BU/mL; and/or abnormal FVIII pharmacokinetics (recovery below 66% of expected values; and/or half-life less than 6 h). Patients who did not achieve success parameters after 33 months were considered as failure. The median ages at inhibitor diagnosis and at ITI start were 3.6 years [IQR,1.6-7.2] and 7.0 years [IQR,2.6-19.2], respectively. PwHAi were stratified among four $\Delta t_{inhi\text{-}ITI}$ quartiles: first (0.00 to 0.63 year), second (0.64 to 1.75 years), third (1.76 to 9.48 years), and fourth quartile (9.49 to 24.52 years). The overall success rate was 67.7% (90/133) of which first, second, third and fourth were 69.7% (23/33 patients), 69.7% (23/33 patients), 64.7% (22/34 patients), and fourth 66.7% (22/33 patients), respectively (p = 0.965). In this study, about 70% of PwHAi responded to the Brazilian ITI Protocol. A longer $\Delta t_{inhi\text{-}ITI}$ was not associated with a lower success rate of ITI in PwHAi.

## ABS 08

### ELEVATED SOLUBLE C5B-9 AS A ROUGH MARKER OF COMPLEMENT ACTIVATION IN COMPLICATIONS OF SICKLE CELL DISEASE

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*Background:* Sickle cell disease (SCD) leads to complications with increased rate of morbidity and mortality, even in the era of hydroxyurea. Although our group and others have suggested increased complement activation in SCD, the role of complement in common complications such as vaso-occlusive crises (VOC) remains unknown.

*Aims:* We aimed to prospectively investigate complement activation in patient sera during steady state and VOC, along with potential associations of complement levels with clinical characteristics.

*Materials and Methods:* We studied SCD patients regularly monitored at our Thalassemia Units by measuring soluble C5b-9 (Quidel ELISA) as a rough marker of complement activation.

*Results:* We studied 56 SCD patients, aged 43 years (range 19 – 69), 36 female:20 male, 47 with S/β and 9 with SS genotype. Cholecystectomy had been performed in 39, splenectomy in 16; while 43 were on hydroxyurea (10-15mg/kg). Among them, 21 had increased C5b-9 during steady state compared to upper normal limits established in our laboratory (245 ng/ml). At steady state, median C5b-9 values were 218 ng/ml (range 91-889), showing a significant association with HbS levels (r=0.292, p=0.048). During VOC, C5b-9 levels were significantly increased (median 404, range 213-1218, p=0.037, Figure), in line with the increase of HbS (from 70% to 83%). In this rather limited population, no further associations with clinical characteristics were found.

*Conclusions:* We have shown for the first time increased C5b-9 levels during VOC. There have only been a limited number of studies in the field. The high number of S/β patients and previous or current treatments (i.e. splenectomy, hydroxyurea) should be taken into consideration. Future prospective studies are needed to better understand the role of complement in the pathophysiology of SCD and possible new therapeutic approaches through complement inhibition.

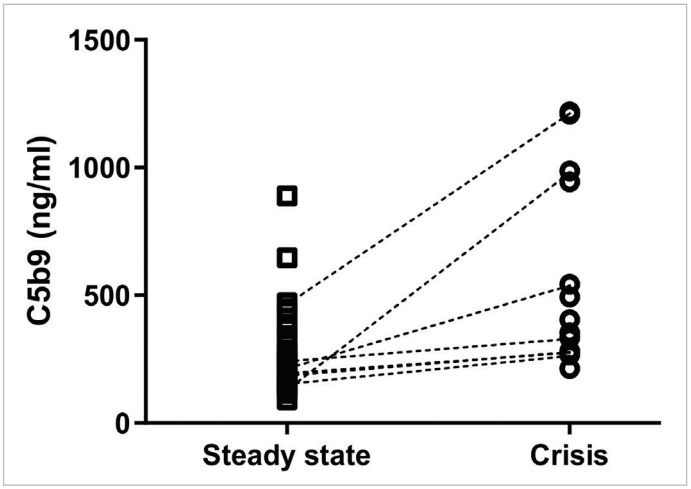


Figure 1.

## ABS 09

### AUTOMATIC SEGMENTATION OF ARTERIES, ARTERIOLES AND GLOMERULI ON KIDNEY BIOPSIES WITH THROMBOTIC MICROANGIOPATHIES

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*Background and Aims:* Thrombotic microangiopathies (TMAs) manifest themselves in arteries, arterioles and glomeruli. Nephropathologists need to differentiate TMAs from mimickers like hypertensive nephropathy and vasculitis which can be problematic due to interobserver disagreement and poorly defined diagnostic criteria over a wide spectrum of morphological changes with partial overlap.

As a first step towards a machine learning analysis of TMAs, we developed a model for arteries, arterioles and glomeruli in TMA and mimickers.

*Method:* We manually segmented n=1,394 arteries, n=9,673 arterioles, n=7,116 glomeruli on whole slide images (WSIs) of 42 renal biopsies and their HE, PAS, trichrome and Jones sections (21 TMA, and 21 mimickers of TMA including hypertensive nephropathy and vasculitis with preglomerular involvement). As a segmentation model we used DeepLab V3, pretrained on 61,734 segmented glomeruli from 768 WSIs. 58 randomly chosen WSIs served as the intra-institutional holdout testing set after training of the model on the remaining slides. Automatic segmentation accuracies were reported as Cohen's kappa, intersection over union (IoU) and Matthews correlation coefficient (MCC) against the nephropathologist's segmentation as ground truth.

*Results:* Over all classes (arteries, arterioles, glomeruli) Cohen's kappa was 0.874.

IoU was 0.751 for artery, 0.03 for arteriole and 0.847for glomerulus.

MCC was 0.858 for artery, 0.669 for arteriole and 0.917 for glomerulus.

*Conclusion:* We achieved satisfactory automatic segmentation of arteries, arterioles and glomeruli, even with severe histopathological distortion on routine histopathological slides. We will further improve this segmentation technology in order to enable the bulk analysis of these decisive tissue compartments in large clinicopathological repositories of native kidney biopsies with TMA using supervised and unsupervised machine learning algorithms.

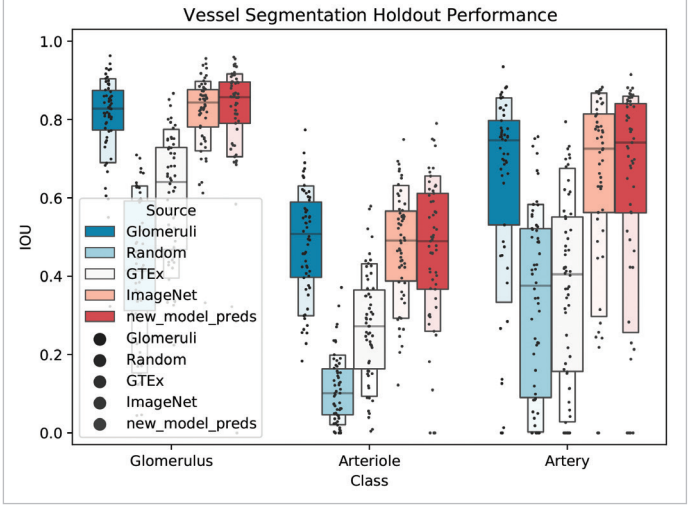


Figure 1

## ABS 10

### COST-EFFECTIVENESS ANALYSIS OF THE BRAZILIAN IMMUNE TOLERANCE INDUCTION PROTOCOL WITH PROPHYLAXIS WITH BYPASSING AGENTS OR EMICIZUMAB

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Treatment of people with haemophilia A and inhibitors (PwHAi) has improved considerably in Brazil since the introduction of immune tolerance induction (ITI) in 2012. Recently, emicizumab (EMI) was incorporated by the Brazilian Public Healthcare System (SUS) for prophylaxis of PwHAi who failed ITI. We aimed to compare the costs and the outcomes of the Brazilian ITI Protocol (BIP) with recombinant factor VIII concentrate (FVIII; α-octocog) and prophylaxis with bypassing agents (BPA; ITI+BPA) or EMI (ITI+EMI), using a decision-three model, from the SUS (payer) perspective. The BIP recommends starting ITI at low-dose rFVIII regimen (50IU/kg 3x/week) for all PwHAi and, upon poor response, increasing rFVIII dose to 100IU/kg/day. BPA can be prescribed to treat or prevent bleeding during ITI. Outcomes were success (inhibitor titre <2 BU/mL and FVIII responsiveness) and failure. The success rate of the BIP was 71%. In this analysis, the price of EMI was considered as the amount paid by the Ministry of Health in the last purchase, which is almost twice as high as the price proposed by the industry for incorporation. In the cost-effectiveness analysis, the costs were US$414,773.84 (ITI+BPA) and US$243,244.25 (ITI+EMI), generating an additional cost of US$171,529.59 for PwHAi receiving ITI. ITI+BPA resulted in 8.25 bleeds over ITI+EMI, and each additional bleed cost, US$20,799.28. By deterministic sensitivity analysis, the most impacting variable to the incremental cost was the EMI price: if the incorporation proposed price was used, the savings generated using EMI could reach US$254,319.20 for each PwHAi on ITI. In conclusion, our model showed ITI+BPA is more expensive and people in this arm are more likely to experience bleeding than ITI+EMI. The inclusion of EMI in the BIP can reduce costs for the SUS and bleedings among PwHAi.

| Treatment | Cost (US$) | Incremental cost (US$) | Treated bleedings | Incremental treated bleedings | Incremental cost per treated bleeding (US$) |
|---|---|---|---|---|---|
| **Current EMI price (US$ 64.84/mg)** | | | | | |
| ITI+EMI | 243,244.25 | | 0.65 | | |
| ITI+BPA | 414,773.84 | 171,529.59 | 8.9 | 8.25 | 20,799.28 |
| **Previously proposed EMI price (US$ 30.99/mg)** | | | | | |
| ITI+EMI | 160,454.64 | | 0.65 | | |
| ITI+BPA | 414,773.84 | 254,319.20 | 8.9 | 8.25 | 30,838.16 |

BPA, bypassing agent; EMI, emicizumab; ITI, immune tolerance induction

Table 1. Cost-effectiveness analysis of the Brazilian Immune Tolerance Induction Protocol using α-octocog and prophylaxis with bypassing agents or emicizumab.

## ABS 11

### FACTOR V DEFICIENCY AND DEEP VENOUS THROMBOSIS: A CASE REPORT

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Background/Aims: Factor V (FV) is a coagulaion factor produced by liver and stored within platelets. FV deficiency is a rare autosomal recessive disorder with the incidence of one per 1,000,000 people. Active FV leads to active Factor X which is an extremely important coagulation factor to convert prothrombin into thrombin. Hemorrhagic episodes are commonly expected for FV deficiency however rarely it could cause thrombotic events.

*Materials and Methods:* We present the management of a pregnant

patient with FV deficiency who developed deep venous thrombosis when her international normalized ratio (INR) level was 3.

*Results:* A 30 year-old lady evaluated for increased INR level when she was 9 weeks pregnant. On her history, this is her first pregnancy and she had no bleeding history but she had two sisters with FV deficiency. Her coagulation screen was performed and the prothrombin time and activated partial thromboplastin time were 34,4 seconds (10.9-14.7) and 38 seconds (22.5-31.3) respectively. INR level was 3,3 and factor levels were examined; FV: < %5 (70-120); FVIII: >144,6 (70-150); FX : 113,3(70-120). She was diagnosed with FV deficiency and followed every other week at clinic. When she was 16 weeks pregnant, she was admitted sudden left leg swelling. Work-up revealed deep vein thrombosis of the distal external iliac, common femoral, deep femoral and popliteal veins. Treatment with low molecular weight heparin (LMWH) was done and INR level was checked regularly. Simultaneously, plasma transfusions were done when INR level was greater than 2.5. Thrombophilia testing demonstrated normal activities of antithrombin and protein C, S and she did not have FV leiden mutation.

*Conclusions:* FV defects can cause bleeding and thrombosis at the same time and FV deficiency presents with variable clinical symptoms. Although prolonged coagulations tests were detected, thrombosis is a challenge in patients with FV deficiency.

## ABS 12

### ACQUIRED HAEMOPHILIA: CLINICAL MANIFESTATIONS AND MANAGEMENT EXPERIENCE FROM A SINGLE CENTRE

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*Introduction:* Acquired haemophilia (AH) is a rare, often life-threatening haemorrhagic disorder, in individuals with no prior history of clinical bleeding. AH is caused by autoantibodies commonly against coagulation factor VIII, rarely to other coagulation factors. Management of this disorder consists in rapid accurate diagnosis, control of bleeding and eradication of the inhibitor by immunosuppression.

*Aim:* We report a retrospective cohort of 44 patients with AH A and 1 patient with acquired FXIII deficiency.

*Methods and Materials:* Inhibitor presence was suspected by the prolongation of aPTT, in patients with acquired bleeding tendency and confirmed by detection of FVIII inhibitor measured by NIijmegen modification of the Bethesda assay (cut off <0.5 BU). Complete response (CR) to therapy was defined as negative inhibitor title at the end of immunosuppressive regimen.

*Results:* Between 1997 and 2020, 45 patients with acquired inhibitors, were diagnosed or referred to our Centre. The most common symptoms in admission were muscle or soft tissue haematomas (85%). Autoimmune disorders were found most frequently as the underlying cause of inhibitor appearance. In 20/45 patients (44.5%) no underlying disease could be identified. By passing agents for bleeding control were used in 31/45 pts. 41/45 patients received immunosupressive therapy : Prednisolone (12 pts), prendisolone + cyclophosphamide (29 pts). Rituximab was added to treat 3 patients with very high inhibitor titles (2 pts with post-partum and 1 pt with MGUS).

Median time to a negative inhibitor detection was 4 months (range 0.5-10).

32/45 (71%) had a CR, while 13 patients did not achieve a negative inhibitor title. 12 pts (26.6%) relapsed 1-4 months after interruption of immunosupression.

*Conclusion:* Prompt recognition and early initiation of immunosuppressive treatment together with control of severe bleeding using by passing agents, are essential for the outcome of AH. Inhibitor eradication strategy can be adapted to clinical severity and patient's comorbidities.

## ABS 13

### ALTERATION OF THE INTRINSIC PROLIFERATION CAPABILITY OF HAEMOPHILIC CD8 T CELLS

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Background/Aims: Haemophilia A (HA) is a recessive X-linked bleeding disorder caused by the lack of coagulation factor VIII (FVIII). One of the main complications of the current treatment is the appearance of specific anti-FVIII antibodies (inhibitors) due to the B and T cells activation upon FVIII administration in haemophilic patients. Instead, little is known about any intrinsic T cell alteration especially prior to any FVIII exposure. Moreover, the spontaneous bleedings occurring in haemophilic patients could favor the establishment of an inflammatory microenvironment which could influence the immune cell activation threshold. Here we sought to investigate in a preclinical murine model of HA the CD8 T cell proliferation capability.

*Materials and Methods:* Splenic CD8 T cells from HA and wild-type (wt) Balb/c mice were isolated and labelled with a fluorescent proliferation dye. An *in vitro* assay was performed upon anti-CD3/CD28 stimulation while the *in vivo* test was run by adoptively transferring the cells into immunodeficient recipient NSG HA and NSG mice.

*Results:* The haemophilic CD8 T cells showed an increased proliferation rate both *in vitro* and *in vivo*. After 72 hours in culture the total number of HA CD8 T cells in the wells was greater (18410±3920) than the wt ones (10298±1968). Similarly, 72 hours after the adoptive transfer, the splenic CD8 T cells recovery from the mice was 1,75 higher for the HA compare to the wt.

*Conclusions:* Our preliminary results showed a higher proliferation capability of HA than wt CD8 T cells, supporting the hypothesis of a possible intrinsic T cell alteration and/or a tissue microenvironment involvement in haemophilic patients that would require further studies. The definition of a possible alteration in the HA immune system prior to FVIII treatment could be helpful in the development of new strategies for preventing inhibitor formation or for tolerance restoration.

## ABS 14

### OFF LABEL USE OF MUTLIPLE AGENTS IN ORDER TO CONTROL BLEEDING EPISODES IN A BOY WITH WISKOTT-ALDRICH SYNDROME

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*Introduction:* Wiskott-Aldrich syndrome (WAS) is an X-linked hereditary disease characterized by small platelet thrombocytopenia, eczema and increased susceptibility to infections. Platelet defect occurs as a result of cytoskeletal changes. Patients are in great risk of serious bleeding, including intracranial hemorrhage, due to dysfunctional platelets. Besides the traditional approach of platelet transfusion in order to control bleeds, alternative therapeutic options are now aiming either at treating or at preventing bleeds in WAS patients.

*Aim:* To report on off label use of activated recombinant factor VII (rFVIIa) and eltrombopag in a patient with WAS and severe bleeding phenotype.

*Case presentation:* A four-year old boy with a known history of WAS, diagnosed during infancy, was referred for follow up. Reported platelet count varied between 4,000-20,000/μl. Repeated mucosal bleeding and frequent presentation of subcutaneous hematomas, leading to severe posthemorrhagic iron deficiency, were reported. Before referral, the patient had received multiple platelet transfusions in order to control bleeding episodes.

Permission from regulatory authorities to administer rFVIIa was granted and following mucosal bleeds were effectively treated with one infusion (90IU/kg). Meanwhile, permission was granted for initiating eltrombopag treatment. Administered dose was gradually titrated to the maximum of 75mg daily, leading to a stable platelet count of over 20,000/μl and to a dramatic reduction of bleeding episodes. The patient has been on eltrombopag for 7 months, well tolerating the drug and not presenting any adverse effects.

*Conclusion:* Off label use of rFVIIa in order to treat and eltrombopag in order to prevent bleeds in a child with WAS demonstrated a safe and effective profile. The two agents can be used as a bridge until indicated cure with hematopoietic stem cell transplantation is achieved, minimizing need for hospitalization and limiting risk of alloimmunization due to platelet transfusions.

## ABS 15

### COAGULATION DISORDERS IN PAEDIATRIC PATIENTS HOSPITALIZED DUE TO SARS-COV-2 INFECTION

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*Background:* Haemostatic impact of SARS-CoV-2 infection and multisystem inflammation syndrome (MIS-C) in children is still under consideration.

*Aim:* To investigate COVID-19 coagulopathy in hospitalized children due to SARS-CoV-2 infection.

*Materials and Methods:* A retrospective review on coagulation parameters of children with SARS-CoV-2 infection and MIS-C hospitalized from August 2020 until April 2021 was conducted in our Haemostasis and Thrombosis Centre. Values of D-dimers, fibrinogen, antithrombin, protein C, PT, APTT and the respective coagulation factors were recorded at hospital admission, in multiple time points during hospitalization and upon hospital discharge. Statistical analysis was performed with Mann-Whitney test and t-test.

*Results:* In total, 226 children (boys: 58.8%) of mean age 3.7±3.0 years (0-18) were investigated. Patients were categorized as follows: patients with mild/moderate (92.9%) or severe COVID-19 (2.2%) and patients with MIS-C (4.9%). Severe, in comparison with mild/moderate patients, had statistically increased admission D-dimer values (15.3 vs 4.3, μg/ml), PT (14.9 vs 13.5, sec), APPT (39.5 vs 32.9, sec) and decreased antithrombin values (63.7 vs 100, %). MIS-C children had significantly increased admission values of PT (15.6 sec), fibrinogen (529 vs 265, mg %), factor VIII (204 vs 128, %) and decreased factor VII values (38.8 vs 57, %) in comparison to mild/ moderate patients. Interestingly, MIS-C children had markedly increased fibrinogen values even in comparison with severe patients (529 vs 286, mg %). Additionally, at least 12 patients had D-Dimmer >36 μg/ml. In most of them, D-dimer values almost normalized in 1-3 days. During hospitalization, PT, aPTT, fibrinogen, D- dimer and factor VIII values were markedly decreased but without statistical significance, due to small number of samples of mild/moderate patients in multiple time points.

*Conclusions:* Our COVID-19 hospitalized children showed a mild deterioration of coagulation parameters. However, in children with MIS-C and severe COVID-19, these coagulation parameters were found mainly affected.

## ABS 16

### HEAVY MENSTRUAL BLEEDING IN VON WILLEBRAND PATIENTS – THE REALITY OF A TERTIARY CARE HOSPITAL.

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*Background/Aims:* Von Willebrand disease (VWD), the most common inherited bleeding disorder, is a common cause of heavy menstrual bleeding (HMB). Women with VWD are more prone to develop HMB, to be symptomatic and present with bleeding, in association with gynecological problems. We aim to analyze characteristics and treatment of our patients who presented with HMB.

*Materials and Methods:* We reviewed medical files of female von Willebrand patients followed in our institution and collected data of their diagnosis, occurrence of heavy menstrual bleeding and treatment established.

*Results:* From 62 female patients with VWD, 24 (median age of 40,3 ±20,9 years) experienced HMB. There were 15 patients with VWD type 1, 3 with VWD type 2B, 2 with 2A, 2 without a clear subtype defined,1 with type 2M and 1 with VWD type 3. Treatment with oral iron supplementation was necessary in 6 patients and intravenous iron supplementation in 3 patients. Only 1 of these patients needed blood transfusion. Of note, from those 24 patients with these bleeding symptoms, only one was referred to our Congenital Coagulopathies Reference Centre due to HMB.

*Conclusions:* HMB can be a debilitating symptom, leading to absenteeism at work and school and in some cases, even needing hospital care and admission. A multidisciplinary approach should manage these patients in order to provide the best care in order to prevent bleeding, improving their quality of life and reduce the need for more invasive and risk associated treatments, such as intravenous iron supplementation and transfusion.

## ABS 17

### COAGULATION PROFILE AND LABORATORY DATA IN COVID-19 PATIENTS ON CHRONIC ANTICOAGULATION OR ANTIPLATELET THERAPY.

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*Background:* Coronavirus 2019 (COVID-19) has resulted in a global pandemic. Elevated D-dimer levels and other laboratory markers are prognostic indicators in Covid-19 patients.

*Aims:* To compare coagulation profile and laboratory data in Covid 19 patients on chronic anticoagulation versus no chronic anticoagulation or antiplatelets. To find an association between laboratory data and composite variables such as length of stay, ICU admission, and 3-months mortality.

*Methods:* This is a retrospective cohort study of COVDI-19 hospitalized patients over 18 years who presented to the Trinity Health hospitals from March 8, 2020, to May 15, 2020. The exposed group was defined as patients who received chronic anticoagulation (warfarin, direct oral anticoagulant) therapy (for more than one-month duration) for reasons other than COVID-19 disease. In contrast, control group patients were defined as those who did not receive these therapies before admission. Laboratory data include D-dimer levels, LDH, ferritin, procalcitonin, platelets. The chi-square test and the student's t-test were used to compare the outcome in both groups. Statistical analysis was performed using SPSS version 25.

*Results:* 3180 patients were COVID-19 positive during the study period; 452 patients met the inclusion criteria. There were 62 patients in the exposed group and 383 patients in the control group. Those on home anticoagulation had a lower D-dimer peak (633.2 ± 540.8) than did those not on home anticoagulation (1303.2 ± 1446.5), $p<0.0005$. Patients on home anticoagulation with 90 days mortality had a higher D-dimer peak (1740.9 ±1409.1), LDH peak (407.2 ±307.0). There was a positive correlation between hospital length of stay and D-dimer peak for those on home anticoagulation, $r=0.292$, $p=0.002$.

*Conclusion:* Laboratory data in COVID-19 patients on antecedent anticoagulation might have some protection against the hypercoagulable state. These patients have lower D-dimer levels compared to patients not on antecedent anticoagulation.

## ABS 18

### RECURRENT INTRAVITREAL ANTI-VEGF TREATMENT IN HEMOPHILIA B PATIENT WITH OCULAR DEGENERATION

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*Background/Aims:* Haemophilia B (Christmas Disease) is a coagulation disorder due to factor IX deficiency. Hemophilia B is a rare and an X-chromosome-linked inherited bleeding disorder. Spontaneous joint or other bleedings may not be observed in moderate hemophilia B. Treatment may be administered in an episodic/on-demand to control bleedings, pre-operation whereby FIX is administered to prevent or reduce bleeding episodes.

*Materials and Methods:* This case report summarizes the successful

treatment of a 70-year-old man with moderate hemophilia B and recurrent intravitreal anti-VEGF, with the use of repeateddoses of factor IX and patient's adherence to therapy.

*Results:* He was treated on demand therapy for management of invasive procedures or bleedings. The patient presented in our Eye Disease department because of bleeding into his right eye in 2018. Eye Disease specialist planned an invasive treatment such as intravitreal anti-VEGF therapies for five times in 10 months. Patient with hemophilia B was referred to us for preparation of elective surgery. Laboratory studies revealed the following results: factor IX level was 2,5 %, factor IX inhibitor was <0,6 BU. Routine biochemical studies and blood counts were normal. The patient was hospitalized before surgery. Our target factor IX level was to maintain his FIX activity at 80% -100% for post-op days 0-3. He was discharged the day after surgery. All of his surgery was managed successfully with no complication by in consultation with a hematologist at hemophilia center.

*Conclusions:* Surgery procedures in hemophilia have been categorized as major or minor. Adjusted for the estimated risk of bleeding associated with the surgery, this classification is often used for the calculated factor replacement therapy doses. Major surgeries define the operations with significant risk of large volume blood loss. Planning should also involve assessment for presence of inhibitors, dose calculations which may include pre-operative pharmacokinetic studies and comprehensive clinical approach with hematologist and surgeon.

## ABS 19

### MANAGEMENT OF MAJOR SURGERY IN A PATIENT WITH TYPE 3 VON WILLEBRAND DISEASE WHO HAS LOW TITER INHIBITOR

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*Background/Aims:* Von Willebrand disease (vWD) is an autosomal inherited coagulation disorder arised from a deficiency in the quality or quantity of von Willebrand factor (VWF), a multimeric protein that is required for platelet adhesion. Plasma FVIII levels are also low because this protection is lacking. Antibodies to VWF in patients with vWD are rare; the prevalence in type III vWD patients is 6–10%.

*Materials and Methods:* In this article, we aimed to present our major surgical experience in a rare case with low titer inhibitor type 3 vWD.

*Results:* A 29-year-old female who has had vWD for years was consulted for pre-surgery evaluation of synovectomy. She had a life-long severe bleeding phenotype with extensive ecchymosis, epistaxis, oral cavity bleeding and joint bleedings. Arthroscopic bilateral synovectomy operation was planned because of the patient who had limited walking and climbing stairs and crouching movement to 90 ‘.

On Laboratory examinations: vW Ag: % 0.6, ristocetin cofactor activity: % 0.7 Factor VIII level: 1 %, Factor VIII inhibit r: 1,76 BU. Recovery test with Factor VIII/VWF complex in perioperative management of surgery was performed.

16 hours later after the operation, massive bleeding that was life-threatening and disrupted hemodynamics was observed in the bilateral knee joints of the patient. Recombinant factor VIIa (RF7a) was administered every 2 hours at a dose of 90 mcg / kg. Re-debridement was planned on the 4th day for the patient, whose treatment was administered by reducing the dose. Joint bleeding was controlled with rFVIIa at the 9th day of administration.

*Conclusions:* The prognosis of patients diagnosed with type 3 may be similar to patients with severe hemophilia A. In particular, patients who undergo prophylaxis should be followed closely in terms of inhibitor development, and major surgical operations should be performed in a multidisciplinary center with hematologists and surgeons experienced in hemophilia.

## ABS 20

### WORDS IN HEMOPHILIA: A PROJECT FOR A BETTER PATIENT ENGAGEMENT

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*Background and aims:* Greater patient engagement in therapeutic communication is crucial in prophylactic therapies in hemophilia. An improved patient-hematologist relationship leverages this objective. The aim of this project is to investigate differences between the patient's and hematologist's experience of care and therapeutic communication.

*Materials and methods:* The research adopts a mixed method design. A narrative based cross-sectional survey was conducted in parallel on a sample of patients and hematologist. 50 patients and 27 hematologists were involved.

Data collection was based on a self-report online questionnaire. Data were analysed both qualitatively and quantitively.

*Results:* Concerning the patient-hematologist relationship, trust and perceived acceptance are reported as positive elements but the shared decision making about therapies is more critical. Some misalignments have been highlighted: patients considered hemophilia partially limiting their daily lives while hematologists underestimate the patients' perceived impact of it. Drugs are considered as life-saving from patients, but this perspective is underestimated from hematologists. Patients barely think to abandon their therapies, while hematologists overestimate this intention.

*Conclusions:* These findings support the importance of improving medical communication to increase engagement for an effective management of prophylactic therapy in hemophilia.

## ABS 21

### HOW CAN WE BETTER UNDERSTAND COMPLICATIONS ARISING FROM FVIII SUBSTITUTE THERAPIES?

Aledort L.*

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FVIII replacement therapy for persons living with haemophilia A has long been the mainstay of management.

Personalizing FVIII replacement reduces and eliminates the morbidity of the disease and tailoring therapy to meet patient goals. Its safety record has been excellent with notable absence of thrombotic events in pivotal clinical trials. Persons with haemophilia (PwH) are now able to reach normal US lifespans.

Novel agents using different mechanisms of action have emerged as potential substitute therapies for Factor VIII deficient patients. In 2019, emicizumab was the first such molecule to be approved for those PwH without inhibitors. Within 6 months 13 deaths and multiple thrombotic events were reported. The lack of transparency has resulted in persistent uncertainty about causality and potential of reporting bias. However, several other substitute therapies in late-stage clinical trials have been associated with both arterial and venous thrombotic events, notably antithrombin knockdown fitusiran and Tissue Factor Pathway Inhibitor (TFPI) inhibition requiring study pause or abandonment. However, they have important global relevance given their ability to reduce spontaneous bleeding in these patients.

Increased thrombin generation from baseline with the substitutes has clearly been responsible for their good clinical effect. The mechanism(s) responsible for thrombosis are not fully understood. The coagulation system is a balanced system of pro and anti- coagulation processes which lead to activation/expression in the LOCAL environment in which vascular damage has occurred. “Bypassing” agents tip this balance to produce or allow a systemic active process intended to overcome a procoagulant deficiency. For example, proteases inhibitors

in the serpin family may play a role in this process. Thrombosis, a major issue for substitute therapies demand investigation to better understand the trigger for these clinical events. Technology needs to differentiate risk/benefit in the hemophilia individual.

## ABS 22

### VON WILLEBRAND DISEASE TYPE 2B AND DDAVP. STILL A TREATMENT DRUG TO AVOID?

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*Background/Aim:* Von Willebrand disease (VWD) type 2B is an inherited bleeding disorder caused by vWF-GPIb platelet binding pathologically enhanced, resulting in abnormal complexes between platelets and large adhesive forms of VWF.

The treatment is similar to other types of VWD and the mainstay remains VWF replacement and VWD adjunctive therapies. The potential to exacerbate bleeding or thrombocytopenia (in type 2B VWD), resulted in the longstanding practice of avoiding the use of 1-Desamino-8-d-arginine vasopressin (DDAVP) in these patients. The aim of this work was to look to the use of DDAVP in patients with type 2B VWD in our centre.

*Material and Methods:* A retrospective search of our registered patients with type 2B VWD submitted to a trial test of DDAVP. Our population has a median age of 38,0 [19,5;57,3] years. This study includes our experience with 10 patients (8 females) with confirmed diagnosis of type 2B VWD out of 121 VWD registered patients. An analysis was performed regarding platelet count (before/after DDAVP), hemorrhagic episodes and/or therapeutic effectiveness. Special attention was given to situations that can worsen thrombocytopenia and patients with thrombocytopenia at initial evaluation were excluded to trial test.

*Results:* Only 4 patients were submitted to a trial test of DDAVP. The initial platelet count in these patients submitted to the trial test was on average > 246,000/uL and none of them developed thrombocytopenia after DDAVP. Only 2 patients used DDAVP afterwards, for minor hemostatic challenges and had a competent response.

*Conclusion:* Type 2B is a subtype of VWD being difficult to identify and manage due to its clinical heterogeneity. The potential of DDAVP treatment could be an important tool in minor hemostatic challenges, with the advantage of being easier to manage and less expensive than factor concentrates.

## ABS 23

### THROMBOSIS IN INHERITED HYPOFIBRINOGENEMIA: A CASE REPORT

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*Background and Aims:* Inherited fibrinogen disorders (IFD) are primarily considered to be bleeding disorders. However, they can be associated with thrombotic events more often than other clotting factors deficiencies. When a thrombotic event occurs, the management of anticoagulant therapy is challenging, as it can increase the underlying bleeding risk.

*Materials and Methods:* We report a case of a 20 years-old woman with an unprovoked DVT, treated with rivaroxaban (15 mg bid for 3 weeks, followed by 20 mg od for 6 months). At the time of the event, she had been under oral contraceptives for more than 2 years and this medication was immediately interrupted. Her past medical history was uneventful, without abnormal bleeding events, even under rivaroxaban. After cessation of anticoagulation, thrombophilia screening was performed: routine clotting times, fibrinogen, antithrombin, protein C and S, FV Leiden and prothrombin gene mutations, antiphospholipid antibodies. A ROTEM analysis was also performed.

*Results:* All the results were normal, but fibrinogen: a hypofibrogenemia was diagnosed (30 mg/dL and 49 mg/dL, in 2 different samples collected 2 weeks apart). The ROTEM analysis results demonstrated an overall Clot Time and Clot Formation Time prolongation, combined with a decreased Amplitude at 10 minutes and Maximum Clot Firmness in the EXTEM, INTEM and FIBTEM tests. Samples of the patient sent to the genetic department are not yet available.

*Conclusions:* The balance of hemostasis can be difficult as bleeding and thrombosis risks are both present. Thrombotic and bleeding acquired risks must be avoided. If anticoagulation is warranted, fibrinogen replacement therapy can be necessary.

## ABS 24

### A CASE OF VITT (VACCINE-INDUCED IMMUNE THROMBOTIC THROMBOCYTOPENIA) WITHOUT CEREBRAL VEIN THROMBOSIS POST VAXZEVRIA VACCINE EXPOSURE: CLINICAL AND LABORATORY DIAGNOSIS AND FAVOURABLE OUTCOME.

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*Background:* Viral vectors SARS-CoV-2 vaccines may trigger an autoimmune thrombosis mimicking heparin-induced thrombocytopenia (HIT).

*Materials and Methods:* A healthy smoker 57 years-old female was referred to the Emergency Unit 11 days after her first Vaxzevria SARS-CoV-2 vaccine dose because of headache and onset of petechiae, Platelets were significantly decreased (10x109/L) and D-dimer increased (5.09 mg/L). A CT scan showed a presumably longstanding thrombotic apposition in the abdominal aorta. Brain MRI resulted negative. During the hospitalization the patient was treated with methylprednisolone 0.8 mg/Kg/die and immunoglobulins 1 g/kg on the first day. At discharging platelets were 70x109/L and d-dimer furtherly rose (37.8 mg/L). The patient continued steroid therapy at home (prednisone 1 mg/kg/die). Five days later she was readmitted because of abdominal pain; CT scans showed pulmonary embolism, portal vein thrombosis, increased thrombotic apposition in aorta, splenic artery thrombosis with infarcts of the spleen. Platelets were 55x109/L. Therapy with immunoglobulins 1 g/kg/die for two days and dexamethasone 40 mg/die for 4 days was promptly established. After replacement therapy for hypofibrinogenemia (86 mg/dL), we started therapy with fondaparinux 7.5 mg/die (later replaced with dabigatran 150 mg twice a day, once liver function tests improved) in association with acetylsalicylic acid 100 mg/die. Blood tests sent to Careggi Hospital (Florence) showed negative immunoassay for anti-PF4-heparin, but positive functional HIPA test (heparin-induced platelet activation), even in absence of heparin, thus confirming a diagnosis of VITT. We also performed thrombophilia screening, PNH (paroxysmal nocturnal hemoglobinuria) phenotype, ADAMTS-13 and Jak2V617F, CALR, MPL mutations, all resulting normal or negative.

*Results:* Platelets are firmly within the normal range. CT scan showed the resolution of pulmonary embolism and portal vein thrombosis. No indication was given for splenectomy at the moment.

*Conclusions:* Early diagnosis of VITT allowed us to provide promptly an adequate treatment. Undoubtedly the favourable outcome was determined by the absence of cerebral thrombosis.

## ABS 25

### EMICIZUMAB PROPHYLAXIS IN ADULT PATIENTS WITH SEVERE HAEMOPHLIA A INHIBITORS: EXPERIENCE FROM A GREEK HAEMOPHILIA CENTRE

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The development of inhibitors remains a challenging complication of haemophilia A treatment. By passing agents are used for bleeding management and immune tolerance induction (ITI) for inhibitor eradication. However, these approaches don't offer consistent protection, require intensive treatment regimens and have high-cost impact.
Emicuzimab, a bispecific monoclonal antibody restores haemostasis, mimicking FVIIIa action. Weekly subcutaneous (sc) administration, has shown good clinical response through clinical trials.

*Aim:* Aim of this study was to assess the effectiveness and safety of emicizumab administration in severe haemophilia A patients with inhibitors.
*Materials and methods:* 4 severe haemophilia A patients with high titre inhibitors, median age 44 (IQR 30-58), progressive haemophilic arthropathy and annual bleeding rate (ABR) 2-10, were given sc emicuzimab prophylaxis, 3mg/Kg/w for 4 weeks, following by 1.5mg/Kg/w. No patient had received previous ITI. Joint's function and QoL were evaluated by Haemophilia Joint Health Score (HJHS) and SF36 questionnaire respectively, before and 12 months
*Results:* Median follow up was 17.5 (11-24) months. During follow up period only one patient developed a knee hemarthrosis in previous target joint, which was treated efficiently with one dose of 90g/Kgr of rFVIIa iv bolus (ABR: 0-1)
One patient underwent minor surgery (dental cleaning) without receiving additional therapy with by passing factor. The reduction of swelling and pain leaded to significant improvement of joint mobility and HJHS (median score 33 (range 41-25) before vs 26 (7-40) after). Improvement of joint function and weekly sc administration had a positive impact of SF36. No side effects were observed during follow up.
*Conclusion:* Prophylactic therapy with emicuzimab in our haemophilia A patients with inhibitors, resulted in significant reduction of bleedings by increasing compliance, leading to better long term clinical outcome and improved quality of life. So far, no side effects are observed. Longer follow up will confirm the safety and efficacy of this novel therapy.

## ABS 26

### CLINICAL EFFICACY AND SAFETY OF PLASMA-DERIVED VON WILLEBRAND FACTOR- FVIII COMPLEX CONCENTRATES, IN PATIENTS WITH VON WILLEBRAND DISEASE IN ITALY: STUDY DESIGN.

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*Background/Aims:* The efficacy and safety of plasma-derived von Willebrand factor-FVIII complex concentrates (pdFVIII/VWF), Fanhdi® and Alphanate® (Grifols) in von Willebrand disease (VWD) have been reported in prospective clinical trials. To date, studies on use of pdFVIII/VWF concentrates in standard clinical practice are limited. An observational retrospective study has been designed to evaluate the efficacy and safety of Fanhdi® and Alphanate® in VWD patients treated in Italy: the RECLASFAWILL study.
*Materials and Methods:* This is a multicentric study of patients diagnosed with congenital VWD and treated with Fanhdi® or Alphanate® for the management of bleeding and surgery and for secondary long-term prophylaxis, when desmopressin is ineffective or contraindicated. Medical records of patients followed up from January 2007 to December 2019 will be retrospectively collected. Demographic data include VWD type, phenotypic tests, dosage, and bleeding score. The primary efficacy endpoint achieving hemostasis will be described in three clinical situations: bleeding complications, prevention of surgical bleeding, and secondary long-term prophylaxis, based on the following criteria: Excellent, Good, Poor, and No Response. Secondary endpoints will include the assessment of regimen, duration of treatment, and quantification of pharmacokinetic parameters. For safety analysis, adverse events, immunogenicity and thrombogenicity events related to the study drugs will be assessed.
*Results:* A total of 14 sites confirmed available data about patients treated with Fanhdi® or Alphanate® and approximately 100 patients will participate in the study. The protocol was notified to the competent authorities in February 2021. The patient recruitment period will be finalized in October 2021. Clinical study report is expected by March 2022.
*Conclusion:* In Italy, the efficacy and safety of the study concentrates has been already proven in patients with VWD. However, this retrospective study will provide further evidence supporting the use of highly purified pdFVIII/VWF concentrates Fanhdi® and Alphanate®, for treating VWD in routine clinical practice.

## ABS 27

### THE STUDY OF THE EFFECT OF SUBSTANCES ARE USED IN THE PROCESS OF PURIFICATION ON THE DETERMINATION OF FACTOR VIII ACTIVITY

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*Background:* The manufacturing process of Factor VIII (FVIII) concentrate requires a combination of different purification methods with factor level control at each stage. The one-stage clotting method can be adapted to most indication activity of FVIII for laboratory work.
*Aim:* To investigate the effect of substances used in the process of purification on the determination of FVIII activity.
*Methods of research:* The IMMUNATE (Baxter AG) was used initial material. The study was used the following chemicals in different concentrations: NaCl, Epsilon-aminocaproic acid (EACA) and iso-Propanol (iso-Pro). The activity of factors VIII was determined using a coagulation test.
*Results:* All these chemicals were used in the process of chromatographic purification of the FVIII. The control of the activity of FVIII must be carried out at each stage of purification, so is important to study their impact on the process determination of FVIII activity. In the study were used: 0.1 M NaCl, 0.5 M NaCl, 1.0 M NaCl; 5.0 % iso-Pro, 10.0 % iso-Pro, 25.0 % iso-Pro; 0.1 M EACA and 0.25 M EACA. The presence of these substances in the reaction mixture interferes determination of FVIII activity. In particular, in the presence of 1.0 M NaCl or 25.0 % iso-Pro activity was not determined (FVIII<1.0 %).
It has been found that the least influence on the definition of activity had: 0.1 M NaCl (FVIII about 59.0 %); 5.0 % iso-Pro (52.0 %) and 0.1 M EACA (33.0 %).
*Conclusion:* All these substances have a negative effect on the one-stage clotting method of determination the activity of the FVIII. This should be taken into account in technology of obtaining of FVIII. These substances it necessary to remove from the reaction mixture (dialysis) or to take into accounts the error of rate for each individual determination.

## ABS 28

### THE CHALLENGING MANAGEMENT OF PREGNANCY IN TYPE 3 VON WILLEBRAND DISEASE: LABOR, DELIVERY AND POSTPARTUM. A CASE REPORT

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*Background/Aims:* Von Willebrand disease (VWD) is the most common inherited bleeding disorder, although VWD type 3 is the rarest, most severe form of VWD, with undetectable von Willebrand Factor (VWF). Women with VWD remain at risk of early pregnancy bleeding, and primary (PPPH) or secondary postpartum haemorrhage (SPPH).

*Materials and Methods:* A 25-year-old woman, followed in our Centre diagnosed VWD type 3 in infancy, history of heavy menstrual bleeding with need of oral iron supplementation, was admitted for delivery with 37-weeks gestation pregnancy. Her initial evaluation showed a normal platelet count, VWF activity and VWF Ag <0.1 IU/dL, FVIII: 0.03 IU/dL.

*Results:* The patient was proposed for elective caesarean section and a plasma-derived concentrate with a low VWF/FVIII ratio (60UI/Kg) and tranexamic acid was prophylactically administered, rising VWF:act (0.89), VWF Ag: 1.26, FVIII: 1.28 (IU/dL). The newborn weighted 3,140 kg, Apgar score of 5, 8, 9 at 1, 5, 10 minutes and FVW levels: VWF Ag 1.27, VWF RCo 1.55, FVIII 1.50 (IU/dL). Factor replacement of patient was adjusted according to factor levels and maintained tranexamic acid with progressive reduction. No major bleeding occurred after delivery until discharge on day 7 (Hb 9,4g/dL) and maintained FVW id for 3 more days. On evaluation 4 weeks after delivery: Hb 9,1g/dL, but after 3 more weeks, she returned to Centre due SPPH and presented anaemia (Hb 5.6 g/dL). She was transfused with 2 packed red blood cell concentrates, FVW/FVIII concentrate and began oral tranexamic acid for (1 more week). On next week, she had no more blood loss was recovering only needing oral iron supplementation and contraceptive pill.

*Conclusions:* Managing pregnant women with VWD type 3 requires a multidisciplinary approach, with close follow-up, as they are at increased risk of delayed postpartum haemorrhage, requiring factor replacement for prophylaxis or treatment.

## ABS 29

### MASSIVE HAEMOTHORAX IN HAEMOPHILIA A PATIENT WITH HIGH FACTOR VIII INHIBITORS TREATED SUCCESSFULLY WITH HIGH DOSE OF FACTOR VIII.

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*Introduction:* Haemophilia A (HA) is an X-linked disorder caused by a deficiency of factor VIII (FVIII) due to *F8* gene defect. Acquired coagulation inhibitors in congenital haemophilia results from immune-mediated depletion or inhibition of a coagulation factor VIII function (1). Development of FVIII inhibitors remains a major source of morbidity and mortality in the treatment of patients with haemophilia A. Spontaneous haemothorax is extremely rare and life-threatening has been reported in hemophilia A with inhibitors (HAWI) (2). Bypassing agents such as recombinant factor VIIa (rFVIIa)and activated prothrombin complex concentrates (aPCC) are the mainstays in treatment of patients with inhibitors (3). Here we report the case of a HA with inhibitor presenting with massive spontaneous haemothorax treated with factor VIII due to failure of bypass agents in controlling his bleeding event.

*Case report:* A 17-years-old patient, known to have HA since birth with family history of HA. He developed high titer FVIII inhibitors in 2014. On 24 June 2020 he presented to the emergency department with right sided pleuritic chest pain, started hours after he was having vigorous cough with no hemoptysis. He was conscious and vitally stable. He had decreased breath sound over the right infrascapular area.

Chest x-ray (CXR) showed obliterated right costal angle. His haemoglobin (Hb) dropped from a baseline of 150 gm/l to 130 gm/l. FVIII activity was suppressed >0.01 and FVIII inhibitors was 14 Bethesda unit (BU/ml), APTT 107 second, PT 14 second, fibrinogen 5.0. Given CXR finding and decreased Hb in patient with HAWI, a diagnosis of right haemothorax was made and patient started on factor VIII Inhibitor bypassing agent FEIBA 4000 unit (66.5 unit/kg every 8 hours and cefepime). He was not responding on FEIBA. Over 2 days CXR showed worsening opacification in right chest he had further significant Hb drop from 120 to 105. FEIBA was overlapped and the patient was started on rFVIIa 90 mcg every 2 hours. On day 5 Hb dropped from 108 to 77 gm/l CXR continued to show worsening progressive right lung opacification. Active bleeding had been ruled out by CT chest angiogram.

On day 6 Hb decreased from 9.6 to 5.8 gm/l. Patient started to be distressed and was moved to intensive care unit (ICU). He was able to maintain O2 saturation with high flow nasal cannula, so nor intubation neither inotropic support was needed. Pigtail was inserted about 1.5 litre of blood had been drained over 2 days without improvement. FVIII inhibitors level increased to 68 BU/ml. On day 8, the patient was started on high dose FVIII 180 unit/kg/dose every 8hrs along with rFVIIa every 2 hours with PRBCs transfusion support we able to insert chest tube. 4 liters of blood had been drained from chest tube in 24 hours. Bleeding was decreasing day by day, but the CXR was not improving.

Thoracoscopy with evacuation was done when FVIII level was 33%.

Serial CXR had been showing no recurrence of bleeding or re-accumulations of blood. Patient was discharged from ICU to the general ward, we started gradual decrease of aFVII then stopped while FVIII continued. Patient became stable, chest tube was removed seven days after ICU discharge and FVIII decreased to (90unit /kg) every 12 hours with no signs of bleeding Hb 12gm/L. Patient was discharged home on FVIII Q every 12 hours, with outpatient weekly rituximab.

*Discussion:* Haemothorax is a rare complication occurring in <1% of patients with HA. Haemothorax occurring in patients with HAWI is extremely rare and life-threatening. There is limited experience clinical in management. There are no guidelines in the literature regarding the optimal management of this rare presentation. rFVIIa and aPCC are used in treatment of patients with HAWI. Both rFVIIa and aPCC have shown ~80% haemostatic efficacy in patients with haemophilia with inhibitors in a variety of clinical settings with rare incidents of thromboembolism. Traditionally, the use of bypassing agents (BPAs) during ITI has been reserved for patients with inhibitor titre higher than 10 BU/mL and persistent bleeding symptoms despite high doses of rFVIIa replacement (3).

In the literature, 7 male patients with HAWI developed haemothorax have been reported since 1998. The median age was 20.8 years (4-56) median inhibitor titre level 288.8 BU (6-1690). 1 patient required pleurocentesis, 2 patients required chest tube insertion to control bleeding and endotracheal intubation with ventilator was done in 2 patients. 5 patients received monotherapy of rFVIIa and 2 patients received both rFVIIa and aPCC (2,4-7).

Our patient had high-titre inhibitors (>5 BU) before diagnosis of haemothorax. Initially he did not respond to rFVIIa and aPCC, subsequently his Hb level decreased from baseline of 150 to 58 and developed massive haemothorax that required high dose of FVIII (180 unit/kg/dose) to control bleeding along with chest tube insertion when FVIII level was 33%. 4 litres of blood had been drained from chest. nether endotracheal intubation nor mechanical ventilation required. Patient was discharged from ICU on day 11, 7 days later the chest tube was removed and the dose of FVIII decreased to 90 uni/kg BID without any recurrence of bleeding and patient was discharged. Follow-up chest CT scan and CXR revealed complete recovery. No recurrence or sequelae occurred during the 18 months follow-up period.

*Conclusion:* In conclusion, inhibitors remain a challenging complication of treatment in patients with haemophilia. Our patient achieved successful bleeding control by a combined therapy with high dose FVIII replacement and chest tube insertion. Because of the rarity of such difficult cases, we believe that our experience can help in clinical management of massive haemothorax in HAWI.

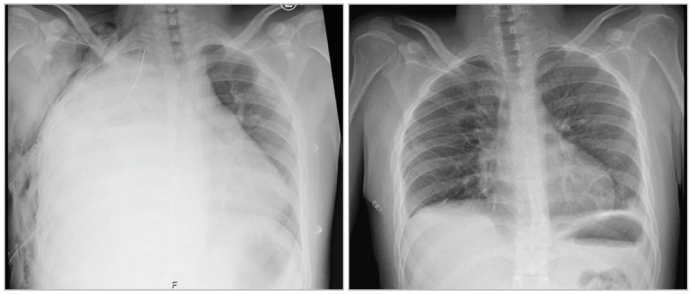

Figure. Chest X ray showing (right) opacification in right side of the chest (bleeding) and complete resolution of bleeding after high dose FVIII (left)

| Date | Clinical situation | Intervention/treatment |
|---|---|---|
| Day 1 | Hb decreased from 150 gm/l to 130 gm/l FVIII inhibitors 14 BU/ml | FEIBA, cefepime |
| Day 2 | Active bleeding (Hb dropped from 120 to 105) | FEIBA |
| Day 3,4 | Worsening of opacification in right side of chest | rFVIIa |
| Day 5 | Hb dropped from 108 to 77, CRX showed worsening opacity, right lung collapsed | rFVIIa |
| Day 6 | Respiratory distress, ICU admission, PRBCs transfusion, HGB from 96 to 58 | Pigtail inserted |
| Day 7 | PRBCs transfusion, FVIII inhibitors 68 BU/ml | CT chest angiogram, no active bleeding |
| day 8 | PRBCs transfusion, chest tube insertion | High dose FVIII |
| Day 10 | CXR still showing right side opacity, HGB stable | Thoracoscopy for evacuation of blood |
| Day 11,12 | Hb stable 102-104 gm/l; CXR: no new bleeding, discharged from ICU | rFVIIa every 4 hour, high dose FVIII |
| Day13-19 | Patient clinically stable, no bleeding | rFVIIa decreased to every 8 hours, chest tube removed on day 17, rFVIIa every 8 to 12 hours, high dose FVIII |
| Day18 | Patient clinically stable | FVIII decreased to (90unit /kg) rFVIIa stopped, high dose FVIII |
| Day20 | Patient was discharged | |

Table 1. Clinical situations and interventions

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## ABS 30

### APPLICATION OF THE ISTH-BAT IN CONGENITAL FIBRINOGEN DISORDERS

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*Background:* The ISTH bleeding assessment tool (ISTH-BAT) was developed to aid the evaluation of suspected patients for bleeding disorders. We aimed to assess the utility of ISTH-BAT in diagnosis, determining the bleeding severity in congenital fibrinogen disorders (CFDs) and diagnosing them according to the new ISTH classification.

*Methods:* A total of 70 (40 female, 30 male) Iranian patients with CFDs and 31 adult healthy controls (15 female, 16 male) were selected. Routine coagulation laboratory tests such as prothrombin time (PT), activated partial thromboplastin time (APTT), and thrombin time (TT) were performed. The fibrinogen antigen level (Fg:Ag) and its functional activity were measured using an immunoturbidimetric assay and the Clauss method (Fg:C), respectively. The ratio of Fg:C/Fg:Ag was calculated and ISTH-BAT was used to assess the bleeding score (BS) of patients and healthy controls.

*Results:* Laboratory and clinical parameters are summarized in table 1. Patients were identified according to the new classification: 1A (afibrinogenemia, n=37), 2B (moderate hypofibrinogenemia, n=6), 2C (mild hypofibrinogenemia, n=2), 3A (dysfibrinogenemia, n=19), 4B (moderate hypodysfibrinogenemia, n=2), 4C (mild hypodysfibrinogenemia, n=4). Sixty-seven (95.7%) patients experienced bleeding events and only 3 (4.3%) patients with dysfibrinogenemia were asymptomatic. Each group of patients showed a statistically higher median BS than healthy controls (Fig. 1). Overall median BS of all patients was 4 (range 0-9) and was statistically significantly higher than healthy controls (median 1, range 0-6, p <0.002).

The most common clinical symptoms in patients with CFDs were cutaneous bleeding (56%), menorrhagia (39%), and umbilical cord bleeding (29%). Umbilical cord bleeding was observed only in afibrinogenemia and one afibrinogenemia patient experienced eye bleeding. Miscarriage was reported in three patients (two dysfibrinogenemia, one hypodysfibrinogenemia), and two women (afibrinogenemia) had an ovarian cyst rupture.

*Conclusions:* This study showed that ISTH-BAT is a useful tool for the diagnosis of CFDs and identifies those patients who need further investigations.

| classification of disorders | N (%) | Age | Sex (M/F) | Fg:C (mg/dl) | Fg:Ag (mg/dl) | PT(s) | APTT (s) | TT(s) | ISTH-BAT |
|---|---|---|---|---|---|---|---|---|---|
| Afibrinogenemia | 37 (53%) | 13 (1-39) | 20/17 | Undetectable | Undetectable | >60 | >120 | >60 | 5 (2-9) |
| Hypofibrinogenemia | 8 (11%) | 23 (2-34) | 3/5 | 89 (75-117) | 110 (73-134) | 17 (11-19) | 38 (31-47) | 23 (23-29) | 4.5 (1-6) |
| Dysfibrinogenemia | 19 (27%) | 34 (4-63) | 5/14 | 50 (25-78) | 312 (228-370) | 15 (12-20) | 36 (32-47) | 35 (19-46) | 3 (0-5) |
| Hypodysfibrinogenemia | 6 (9%) | 27 (8-61) | 2/4 | 43 (40-90) | 125 (76-156) | 14 (11-20) | 36 (30-42) | 25 (17-39) | 4 (2-7) |
| Normal | - | - | - | 154-475 | 184-334 | 10-13 | 18-28 | 14-19 | male ≤3 female ≤5 children ≤ 2 |

-Data were reported as median (range)

Table 1: Laboratory and clinical characteristics of patients

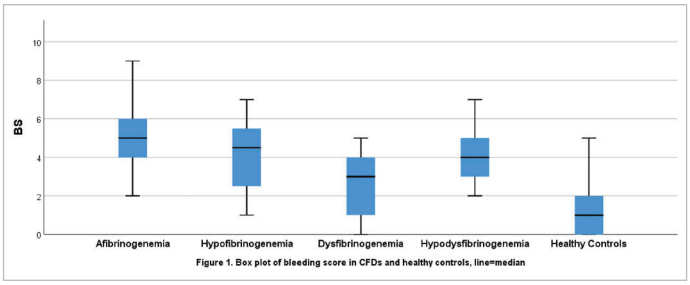


Figure 1

## ABS 31

### PRIOR PLASMA FRACTIONATION: CHOICE OF THE METHOD PRECIPITATION AND SEARCH FOR RAW MATERIALS

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*Background:* Plasma of blood as a source of various drugs contains a number of proteins in various concentrations. The factor VIII (FVIII) belongs to a class of macromolecule protein's that are in very low concentrations and unstable due to activation/inactivation processes.

*Aims:* To investigate the concentration of FVIII in different subfractions of plasma of blood and at different stages of precipitation.

*Methods:* The initial material was: fresh frozen plasma (FFP), cryoprecipitate, Cohn fraction I and II+III. As precipitants were used: barium chloride (0.125 M), aluminum hydroxide (III) (3.0%) and PEG-4000 (3.2%)

The activity of factors VIII and IX was determined using a coagulation test that measures the partial thromboplastin time; VWF – the cofactor activity of rystomycin; fibrinogen concentration – by the method of Klaus; the total concentration of proteins – the Bradford method.

*Results:* In Cohn fraction I the specific activity of FVIII is approximately 5 times higher than in Cohn fraction II+III (19.27±2.32) x10-4 and (4.40±0.76)x10-4 IU/mg of protein respectively).

It was found that the specific activity of FVIII in the cryoprecipitate is approximately 4.2 times higher than in FFP, 31.25 times higher than in Cohn fraction I and 156.25 times higher than in Cohn fraction II+III. All these fractions are a potential source of FVIII, but the main in the technology of FVIII are Cryoprecipitate and plasma.

A research of the effect of various precipitants on the content of coagulation factors (FVIII, VWF, FIX, thrombin and fibrinogen) and protein concentration was conducted. The obtained data are presented in Table 1.

*Conclusion:* The preliminary stages of precipitation of non-target proteins not only facilitate the process of isolation of the desired protein (FVIII), but also improve analytical characteristics of the final product.

| Indexes | Control | Indicators after precipitation | | |
|---|---|---|---|---|
| | FFP | $Al(OH)_3$ | PEG-4000 | $BaCl_2$ |
| Activity FVIII, IU/ml | 1.12±0.03 | 1.08±0.02 | 1.06 ±0.02 | 1.05±0.13 |
| Specific activity of FVIII, IU/mg protein, x $10^{-1}$ | 0.17±0.01 | 0.21±0.01 | 0.24±0.01 | 0.23±0.03 |
| Activity vWF, % | 104.71±2.31 | 108.57±3.40 | 99.71±1.57 | 98.57±1.38 |
| Activity FIX, IU/ml | 1.13±0.04 | 0.09±0.01 | 1.13±0.02 | 0.13±0.01 |
| Specific activity of FIX, IU/mg protein, x $10^{-2}$ | 1.70±0.10 | 0.17±0.01 | 2.60±0.30 | 2.00±0.02 |
| Thrombin, NIH/ml | 1.05±0.03 | not determinate | 1.04±0.03 | mot determinate |
| Fibrinogen, mg/ml | 4.87±0.21 | 3.23±0.19 | 2.05±0.03 | 2.05±0.04 |
| Protein concentration, mg/ml | 64.14±1.06 | 50.71±1.21 | 43.71±1.04 | 45.77±0.65 |

Table 1. Study of the effect of precipitants on the content of coagulation factors and protein concentration (M±m; n=7)

## ABS 32

### IMMUNE THROMBOCYTOPENIAPURPURA (ITP) RELAPSE OR VACCINE-INDUCED IMMUNE THROMBOTIC THROMBOCYTPOZOPENIA (VITT). WHAT CAN WE CONCLUDE FROM A CASE REPORT?

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*Background/Aims:* ITP is caused by a peripheral destruction of platelets caused by antibodies and associated to a variable bleeding risk. VITT shares features with heparin-induced thrombocytopenia; few cases have been reported after ChAdOx1 nCov-19 [Vaxzevria] vaccine administration.

*Materials and Methods:* A 67-years-old woman entered the emergency department due to petechiae, multiple ecchymosis, epistaxis, bleeding gums and frontal headache with one day of evolution. Four days before admission, she was vaccinated with the first dose of ChAdOx1 nCov-19, having mild asthenia and fever on day after. She has a past medical history of stable ITP after splenectomy in 2013, acute myocardial infarction in October 2020 with stent implantation and was treated with aspirin and apixaban.

*Results:* On physical examination there were no other findings than petechiae and multiple ecchymosis. The blood tests showed a platelet count of 13x109/L, normal hemoglobin, fibrinogen and D-dimers. A head computed tomography was normal. The test for antibodies against platelet factor 4 (PF4)/heparin complex (anti-PF4) was not performed at admission. Treatment with intravenous immune globulin (IVIG) and glucocorticoids was started. At day 2, anti-PF4 assay was performed and was negative; platelet count was79x109/L. At day 4, platelet count was 119x109/L, anticoagulation and aspirin were restarted.

*Conclusions:* VITT cannot be assumed in spite of timing after vaccination. The anti-PF4 assay was performed after treatment with IVIG, which can lead to a negative test; the patient was on anticoagulation (apixaban), probably protecting her from thrombosis. For either VITT or ITP in a patient with a clear indication for antithrombotic treatment, IVIG and glucocorticoids were indicated and necessary for treatment. This case report shows the importance to keep in mind that nowadays VITT should be considered when evaluating patients vaccinated with ChAdOx1 nCov-19 with either thrombocytopenia and/or thrombosis.

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