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ORAL COMMUNICATIONS

POSTERS

ORAL COMMUNICATIONS

SESSION 1 - Hemophilia

OC-01

TARGETING OF HEPATOCYTE SUB POPULATION CONTRIBUTING TO LIVER POST-NATAL GROWTH IS CRUCIAL FOR MAINTENANCE OF TRANSGENE EXPRESSION IN LIVER-DIRECTED GENE THERAPY

Milani M.[1,2*], Canepari C.[1,2], Starinieri F.[1,2], Feo A.[1,2], Moalli F.[3], Ambrosi G.[3,2], Plati T.[1], Biffi M.[1], Covino C.[3], Iannacone M.[3], Cantore A.[1,2,4], Naldini L.[1,2,4]

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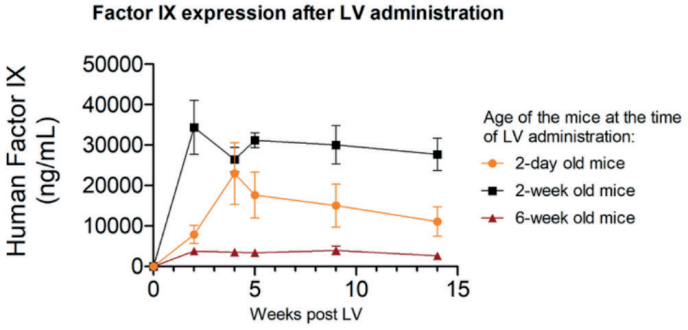
Introduction: Liver-directed gene therapy with adeno-associated viral (AAV) vectors delivering a clotting factor transgene into hepatocytes has shown successful results in adults with hemophilia. However, because AAV vectors do not actively integrate into the host cell genome, they are diluted upon cell division during liver growth, thus challenging their proficient use in pediatric patients. In contrast, lentiviral vectors (LV) integrate into the target cell chromatin and are maintained as cells divide.

Methods: We developed LV that achieve stable and therapeutic levels of coagulation factor IX (FIX) transgene expression in the liver of adult mice, dogs and non-human primates, after intravenous (i.v.) delivery. We then set out to evaluate the fate of LV-transduced liver cells during growth. We administered increasing doses of LV expressing marker genes (GFP or luciferase) under the control of hepatocyte-specific expression cassettes i.v. to newborn mice.

Results: Exploiting 3D imaging of cleared livers and bioluminescence, we show that transduced hepatocytes are maintained over time and proliferate locally. Unexpectedly, we observed an initial, promoter-independent, decrease in transgene expression due to dilution of transduced hepatocytes, followed by stable maintenance sustained by LV-targeted hepatocyte expansion, suggesting different growth phases supported by different cell populations within the mouse liver. We administered LV-FIX to 2-week old mice, right before the observed decrease in transgene expression, and showed 3-fold higher FIX output compared to newborn, suggesting targeting of the hepatocyte subpopulation contributing to liver growth at that time. Ongoing studies are underway to investigate the nature of transduced hepatocytes at the different time points.



Conclusion: Our work will inform about the extent and mechanism underlying long-term maintenance of LV-transduced hepatocytes, provide a rationale for application of LV-mediated liver gene therapy to pediatric patients and may shed light on the role of different cell populations involved in post-natal liver growth.

OC-02

TRANSPLANTATION OF FETAL LIVER CELLS INTO NEWBORN HEMOPHILIC MICE FOR THE TREATMENT OF HEMOPHILIA A WITHOUT INHIBITORS FORMATION

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Background: Hemophilia A cell therapy approaches in pediatric individuals require suitable FVIII-producing cell populations presenting stable engraftment potential. We previously showed that adult-derived FVIII-producing cells, i.e. liver sinusoidal endothelial cells (LSEC) and hematopoietic stem cells (HSC), can be used for the treatment of adult hemophilia A (HA) mice. However, after transplantation in busulfan-conditioned newborn mice, adult LSEC/HSC cannot efficiently engraft, while murine fetal liver (FL) hemato/vascular cells from day 11-13 of gestation (E11-E13) strongly reconstitute the hematopoietic compartment and showed multiorgan endothelial reconstitution potential while secreting FVIII.

Aims: To investigate the engraftment of FL cells in newborn HA mice as a new strategy to develop an experimental treatment for HA in neonates.

Materials and Methods: We transplanted E11-E13 GFP+ FL cells using intravenous cell transfer into myelo-ablated newborn syngeneic HA mice. The engraftment level as well as the FVIII production and activity was assessed in HA recipients starting from 4 weeks and followed-up to 1 year after transplantation. Moreover, we evaluated the presence of anti-FVIII antibodies/inhibitors in plasma of transplanted mice.

Results: We transplanted FL cells from E11-E13 GFP+ mice into newborn HA mice pre-treated with busulfan (FLE11-E13+BU). Control groups received FL E11-E13 GFP+ cells without busulfan pretreatment (FLE11-E13noBU) or PBS ± busulfan. In FLE11-E13+BU group we observed >60% chimerism in peripheral blood with a concomitant FVIII activity (up to 16%) which remained stable up to 1 year after transplantation. In FLE11-E13noBU we observed low level of engraftment (≤10%) and a consequent low FVIII activity (≤3.5%). Both groups of mice, FLE11-E13+BU and E13noBU, did not develop anti-FVIII antibodies, not even after FVIII immunization.

Conclusions: Transplantation of FL cells may provide a novel and highly promising neonatal preclinical model for HA treatment, paving the way for studies aiming at deriving long-term reconstituting "fetal-like" hemato/vascular progenitors from other sources (e.g. iPS).

OC-03

B-AMAZE, A PHASE 1/2 TRIAL OF A NOVEL INVESTIGATIONAL ADENO ASS OCIATED VIRUS (AAV) GENE THERAPY (FLT180A) IN SUBJECTS WITH SEVERE OR MODERATELY SEVERE HEMOPHILIA B (HB)

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Background: FLT180a is a novel recombinant AAV construct that is currently being evaluated in patients with severe hemophilia B. It includes a novel synthetic capsid, AAVS3, with a higher liver transduction efficiency than wild type AAV, and a codon optimised F9 gene with a gain of function mutation.

Methods: This is a first in human, phase 1/2, multi-centre, open-label study assessing the safety and clinical impact of systemic administration of ascending single doses of FLT180a with the aim of normalising levels of FIX (50-150%) ensuring patients are independent of FIX usage and can mount a normal haemostatic response following inflammation, trauma or surgery. Participants are adult males with severe (<1% FIX activity) or moderately severe (1-2%) HB with severe bleeding phenotype who are negative for neutralising antibodies to AAVS3. A prophylactic immunosuppression regimen including corticosteroids will be given to modulate AAV vector related transaminitis and maintain gene expression.

Results: Both patients treated in the lowest dose cohort achieved FIX levels >30%, within 4 weeks, peaking at 12 weeks with levels >40% without toxicity. Transaminitis was not observed in either patient.

Summary: FLT180a, a novel AAV gene therapy with higher potency shows promising results in the low dose cohort justifying further evaluation dose escalation with the aim of achieving a functional cure.

OC-04

DEVELOPING A NOVEL COAGULATION FACTOR VIII WITH REDUCED IMMUNOGENICITY BY A DIRECT DEIMMUNIZATION APPROACH

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Background: Up to 30 % hemophilia A patients develop inhibitory antibodies against therapeutic FVIII. Although several modified rFVIII products have been released, a deimmunized FVIII product, reducing the probability of inhibitor development, has not yet been reported.

Methods: We are developing a lower immunogenicity FVIII by reducing the number of FVIII-peptides presented on immune cells which leads to a reduction in naïve T cell maturation. Without activated FVIII-specific T helper cells, naïve FVIII-specific B cells cannot be activated to produce high-affinity inhibitory antibodies.

Using *in silico* tools we screened the FVIII sequence and identified FVIII-unique peptides predicted to bind with high affinity to the MHC class II. Amino acid exchanges were incorporated in order to reduce this binding. Only mutations leading to functional FVIII molecules were retained. Finally, 19 mutations were incorporated into the molecule, leading to a significant reduction in the predicted immunogenic potential. However, a total deimmunization was not possible, as regions important for activity, folding and binding could not be altered.

Results: Functional and structural analyses of this mutated FVIII variant revealed similarity to a control FVIII and other rFVIII products. An *in vitro* DC-T cell assay was applied to examine the reduced immunogenicity of the deimmunized FVIII variant. In this assay DCs are co-cultured with autologous, Treg-depleted, $CD4^+$ T cells. Regulatory T cells are depleted, in order to allow anti-FVIII immune response. The DCs are primed with the original and deimmunized FVIII products, and $CD4^+$ T cell proliferation is measured.

Conclusions: Using this assay, we demonstrated a significantly reduced immunogenicity of the mutated FVIII variant with cells derived from healthy donors.

Here we present the first deimmunized FVIII, which is functional and proven to be less immunogenic in an in vitro assay. This molecule could highly improve the hemophilia A therapy by reducing the risk of inhibitor development in patients.

OC-05

RECOMBINANT, PATIENT-DERIVED FVIII-NEUTRALISING ANTIBODIES: A PLATFORM FOR RESEARCH, PRODUCT TESTING, AND EX VIVO MODELLING OF HEMOPHILIA A

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Background: Congenital hemophilia A (HA) is a rare, sex-linked, hereditary condition that results in mild, moderate or severe bleeding due to a deficiency of endogenous factor VIII (FVIII). Exogenous FVIII can be administered to restore coagulation but 30% of severe HA patients develop alloantibodies that inhibit FVIII activity ('inhibitors') and render treatment ineffective. As part of our ongoing work developing a reference material to standardise detection and monitoring of inhibitors, we have developed a panel of recombinant antibodies with direct research applications.

Aims: To characterise recombinant anti-FVIII IgG4 antibodies and assess their applicability as research tools.

Methods: Recombinant IgG was expressed by and purified from ExpiCHO cells before being functionally assessed using microplate adaptations of the one-stage clotting assay (OSCA) or chromogenic assay. Binding was assessed by pulldown, ELISA, and surface plasmon resonance (SPR). *Ex vivo* activity was assessed using a microfluidic device (Figure 1).

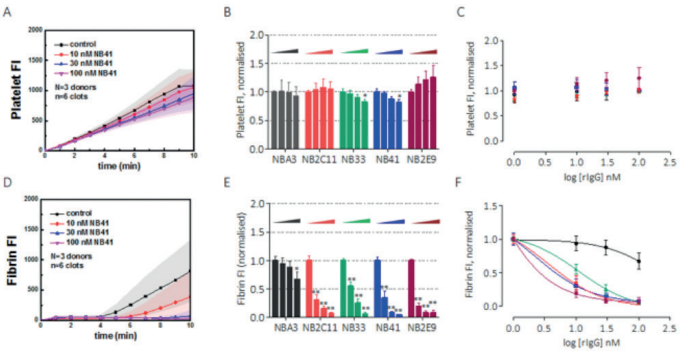

Figure 1. Ex vivo model for HA. Representative traces of platelet (A) and fibrin (D) deposition (quantification by fluorescence intensity, FI) on type I collagen/FXIa. Bar chart (B and E – triangles indicate increasing concentration, left to right 0, 10, 30 ad 100 nM) and dot plot (C and F) data summaries for all antibodies tested. Curves in F fitted using non-linear regression, three-way parameter. Data represent six intra-experimental replicates from three donors *p<0.05, **p<0.001 (Student's test). For C-F, NBA3 is black, NB2C11 is red, NB22 is green, NB41 is blue, NB2E9 is purple.

Results: Our panel of recombinant IgG4s contains 4 neutralising antibodies and two non-neutralising antibodies. These rIgGs bind to different parts of the FVIII molecule and have IC50s in the low nM range (Bethesda titres of between 1,000-10,000 BU/mg). They demonstrate different time- and concentration- dependent effects and we see evidence of type-1 and type-2 kinetics which, interestingly, can be assay-dependent. We observed differences in their neutralising activity against porcine FVIII both *in vitro* and *ex vivo*, highlighting their potential as a screening platform for new FVIII products.

Conclusion: This panel of recombinant anti-drug antibodies contains type-1 and type-2 inhibitors with time- and concentration-dependent neutralising activity with direct research applications. These regents can also be used to test the efficacy of FVIII products in the context of inhibitor patients both in vitro and *ex vivo*.

OC-06

TOWARDS THE TRANSPLACENTAL DELIVERY OF MATERNAL FVIII TO FVIII-DEFICIENT PROGENY FOR INDUCTION OF ACTIVE IMMUNE TOLERANCE TO THERAPEUTIC FVIII

Mimoun A.*, Daventure V., Delignat S., Bou Jaoudeh M., Christophe O.D., Lenting P., Denis C.V., Lacroix-Desmazes S.

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Background: The major complication of factor VIII (FVIII)-based therapy in hemophilia A patients is the development of inhibitory anti-FVIII antibodies. Using FVIII-deficient mice, we showed that the transplacental delivery of Fcγ1-fused A2 and C2 domains of FVIII during gestation induces immune tolerance to therapeutic FVIII in the progeny. Offspring from A2-Fc/C2-Fc-treated mothers developed regulatory T cells and were partially protected, albeit not completely, from FVIII inhibitors development. We hypothesize that transplacental delivery of whole FVIII should induce complete FVIII tolerance. To test this, we developed tools to capture and transplacentally deliver maternal FVIII to the fetus.

Methods: We designed Fc-fused molecules cross-linking FVIII and the neonatal Fc receptor (FcRn): monovalent FabFcs derived from the anti-C2 BO2C11 and anti-A2 BOIIB2 human IgG4k, and a D'D3-Fc derived from human von Willebrand factor (VWF). The molecules were produced in CHO cells and validated for FVIII binding and competition with VWF for FVIII binding by ELISA, and for transcytosis through mouse SC4235 syncytiotrophoblast cells.

Results: Protein sizes were confirmed by SDS-PAGE. BO2C11 FabFc, BOIIB2 FabFc and D'D3-Fc bound to FVIII with affinities of 0.4, 0.3 and 1-6 nM, respectively. Both BO2C11 FabFc and D'D3-Fc, but not the BOIIB2 FabFc, blocked the binding of FVIII to VWF. BO2C11 and

BOIIB2 FabFc were transported in a FcRn-dependent manner through the SC4235 cells.

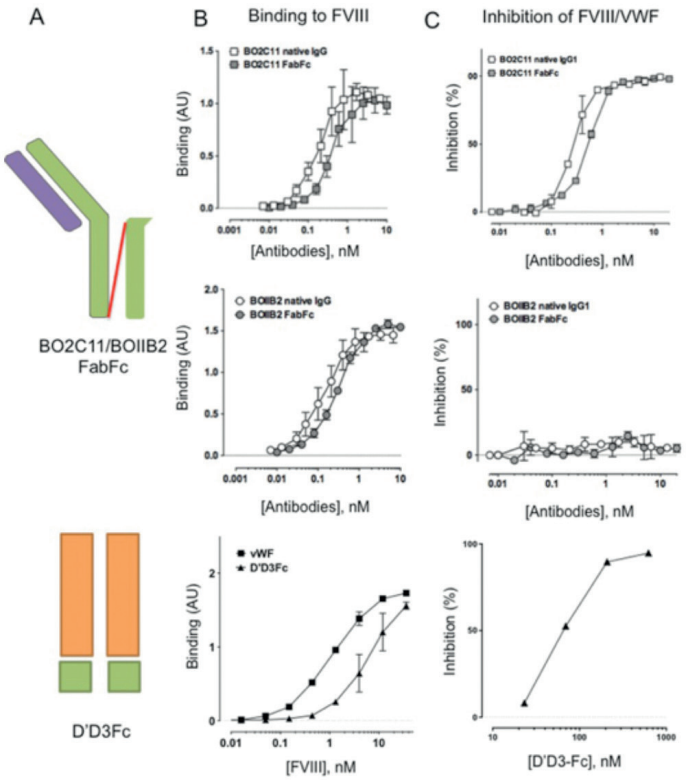

Figure. Validation of FVIII-specific FAbFC and D'D-3Fc. A. Structure of FAbFC and D'D-3Fc. B. Binding of native BO2C11 (white squares) or BOIIB2 (white circles) of BO2C11 FabFC (grey squares) or BOIIB2 FabFC (grey circles) and of D'D3-Fc (black triangles) to FVIII. C. Inhibition of VWF binding to FVII by native BO2C11(white squares) or BOIIB2 (white circles), of BO2C11 FAbFC (grey circles) and of D'D3-Fc (black triangles)

Conclusions: We have generated 3 recombinant proteins (exfiltrins) able to bind FVIII and demonstrating FcRn-dependent transcytosis. Our future work will validate the delivery of human FVIII through the SC4235 cell monolayer by the exfiltrins and will investigate whether VWF interferes with exfiltrin-mediated FVIII transcytosis. The latter will be confirmed in mice. Finally, the effect of transplacental transfer of mother FVIII during gestation on tolerance induction to therapeutic FVIII will be investigated in the offspring.

SESSION 2 - VON WILLEBRAND FACTOR & ADAMTS13

OC-07

VWF PRODUCTION AND SECRETION, AND ENDOTHELIAL CELL CHARACTERISTICS IN HEALTHY ECFCS; GENERATING A VALID EX VIVO MODEL FOR VWD

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Background: Endothelial colony forming cells (ECFCs) can be isolated from peripheral blood and represent a population of endothelial cells (ECs). These cells can be used to analyze the pathophysiology of von Willebrand disease (VWD). However, heterogeneity is observed between ECFC clones. This variability needs to be understood for ECFCs to be a robust cell model for application in vascular studies.

Aim: Determine normal reference ranges for ECFC characteristics to generate a VWD model.

Methods: Forty-seven ECFC clones, from 23 healthy donors, were isolated. ECFCs were analysed for von Willebrand factor (VWF) production and secretion, cell proliferation, and EC marker (CD31, CD34, CD51/61, CD144, CD146 and CD309) and hematopoietic cell marker (CD14, CD45 and CD133) expression by FACS analysis.

Results: Based on morphology, ECFCs were classified in three groups. Group 1 consisting of clones with classic cobblestone EC morphology and groups 2 and 3 containing less condensed ECFCs with either medium size or enlarged cells, respectively. VWF production and secretion, and cell proliferation were highest in group 1, and these characteristics decreased progressively within groups 2 and 3 (Figure 1). EC surface markers were present in all groups, with low expression levels of negative EC markers indicating that cells in all groups are ECs. However, a trend was observed in a group-related decrease in CD34 combined with a slight increase in CD14 and CD45 expression.

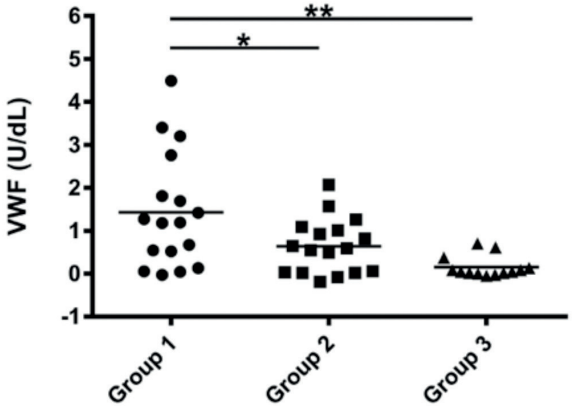

Figure 1. Absolute VWF release into media after histamine stimulation. ECFCs with classic EC morphology (group 1), showed the highest absolute VWF secretion (U/dL) in the medium when stibulated with 100 µM histamine. This stimulated VWF secretion decreased when EC morphology changes, with ECFCs in group 3 showing the lowest absolute secretion of VWF. Data shown as mean, Tukey's multiple comparisons test, *<p0.05, **p<0.001.

Conclusion: Even though all ECFCs show typical EC characteristics and cell surface markers, there is a decrease in VWF production and secretion when ECFCs lose the classic EC morphology. The changes of surface markers CD34, CD14 and CD45 in enlarged ECFCs could indicate a trend towards a mesenchymal phenotype. Therefore, for ECFCs to be used as a valid vascular disease model, patient ECFCs should be analysed with control ECFCs showing comparable morphology based on the groups discussed here.

OC-08

INHIBITION OF ADAMTS13 PREVENTS THE LOSS OF HIGH MOLECULAR WEIGHT VON WILLEBRAND FACTOR MULTIMERS IN AN IN VITRO LEFT VENTRICULAR ASSIST DEVICE

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Background: The bleeding diathesis observed in patients implanted with a left ventricular assist device (LVAD) is linked to the acquired von Willebrand syndrome as all patients have a loss of high molecular weight (HMW) VWF multimers. The loss of HMW VWF multimers might be explained by an increased shear-induced proteolysis of VWF by ADAMTS13. Hence, specifically blocking ADAMTS13 might be an efficient way to rescue the loss of HMW VWF multimers in LVAD patients.

Aim: To investigate if blocking ADAMTS13 using mAb 17C7 can prevent the loss of HMW VWF multimers in an in vitro LVAD circuit using human, ovine and bovine blood.

Methods: Human, ovine and bovine blood (750 mL) was circulated through an in vitro Impella CP® circuit with inhibiting anti-ADAMTS13 mAb 17C7 (n=4) or non-inhibiting mAb 5C11 (n=4) or PBS (n=4). Plasma samples were analysed for VWF multimers, VWF antigen (VWF:Ag) and VWF collagen binding activity (VWF:CB).

Results: Blocking ADAMTS13 using 17C7 prevented the loss of HMW VWF multimers and hence a decrease in VWF:CB/VWF:Ag in the in vitro Impella CP® system with human blood (Figure 1A and 1B) showing the therapeutic potential of 17C7 to rescue the loss of HMW VWF multimers in LVAD patients. However, blocking ovine ADAMTS13 with 17C7 did not prevent the loss of HMW VWF multimers in vitro (Figure 1C). In contrast, when using bovine blood in an in vitro Impella system, the decrease in HMW VWF multimers could be prevented by adding 17C7 (Figure 1D), similar to what was observed with human blood.

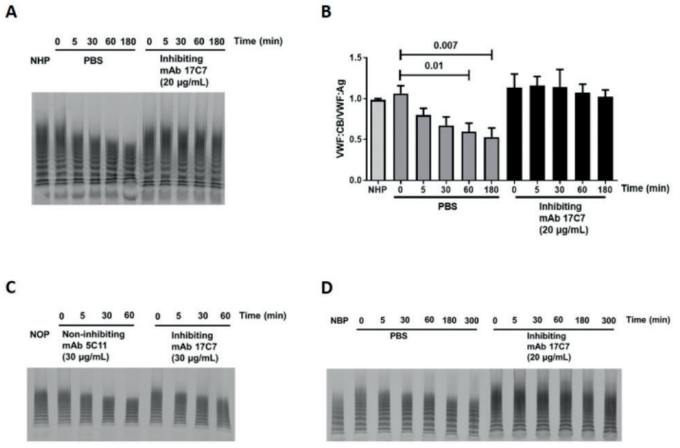

Figure 1. Inhibition of ADAMTS13 in an in vitro LVAD system using human, ovine and bovine blood.

Conclusion: Blocking ADAMTS13 is a promising therapeutic strategy to prevent the loss of HMW VWF multimers in LVAD patients. The loss of HMW VWF multimers is also ADAMTS13 dependent in calves, but not in sheep, making the calve the ideal preclinical animal model to study the in vivo effect of this novel therapy.

OC-09

CONFORMATION OF ADAMTS13 IN THE FRENCH COHORT OF CHILD-ONSET THROMBOTIC THROMB OCYTOPENIC PURPURA

Joly B.S.[1,2*], Roose E.[3], Boisseau P.[4], Benghezal S.[1,2], Schelpe A.S.[3], Vandeputte N.[3], Vandenbulcke A.[3], Stepanian A.[1,2], Vanhoorelbeke K.[3], Coppo P.[5], Veyradier A.[1,2], on behalf of the French Reference Center for Thrombotic Microangiopathies

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Background: Thrombotic thrombocytopenic purpura (TTP) is a rare and life-threatening thrombotic microangiopathy (TMA) related to a severe ADAMTS13 deficiency (activity <10 IU/dL). Child-onset TTP (~10% of all TTP cases) may be either congenital (cTTP, ~1/3) or acquired (aTTP, ~2/3). ADAMTS13 conformation is folded by an interaction between its spacer and CUB domains. When this interaction is abrogated by von Willebrand factor or activating anti-CUB antibody, ADAMTS13 conformation is changed and cryptic epitopes localized in its spacer domain are exposed. ADAMTS13 adopts specifically an open conformation in adult-onset idiopathic aTTP.

Objective: To determine if ADAMTS13 conformation is altered in child-onset TTP (both cTTP and aTTP).

Patients and Methods: All child-onset TTP patients included in the French registry for TMAs (from January 1st, 2000 to December 31st, 2017) were investigated for both ADAMTS13 antigen (3H9-ELISA) and conformation (1C4-ELISA). Child-onset aTTP were compared with adult-onset aTTP.

Results: Eighty-three TTP children (34 cTTP, 49 aTTP) were enrolled and 113 samples were tested for ADAMTS13 antigen (33 cTTP, 43 acute aTTP, 37 aTTP in remission). ADAMTS13 conformation was studied in 21 child-onset acute TTP (5 cTTP, 16 aTTP) and 36 aTTP in remission, whose ADAMTS13 antigen levels were detectable (>0.03 µg/mL). No significant difference was reported in ADAMTS13 antigen levels between idiopathic and non-idiopathic aTTP during both the acute phase (n=26 and n=17, respectively) and the remission (n=22 and n=15, respectively).

All 5 cTTP patients exhibited an altered ADAMTS13 conformation. ADAMTS13 sequence variations may interfere with the CUB-spacer domain interaction. In aTTP, ADAMTS13 conformation was spontaneously altered during the acute phase (11/16, 69%) and restored during remission (21/36, 58%). The idiopathic or non-idiopathic presentation of acute aTTP did not influence ADAMTS13 conformation.

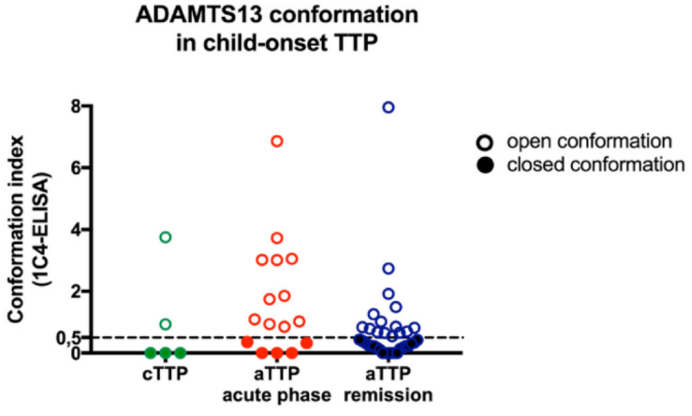

Figure. ADAMTS13 conformation in child-onset TTP

Conclusion: ADAMTS13 conformation is also altered in child-onset TTP, with a less pronounced "closed/open" dichotomy in aTTP when compared to adult TTP patients.

OC-10

PATIENT ANTI-ADAMTS13 AUTOANTIBODIES INDUCE AN OPEN ADAMTS13 CONFORMATION IN IMMUNE MEDIATED THROMBOTIC THROMB OCYTOPENIC PURPURA

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Background: Recently we showed that acute immune-mediated thrombotic thrombocytopenic purpura (iTTP) patients have an open ADAMTS13 conformation, while the majority of iTTP patients in remission with ADAMTS13 activity (TS13:act) >50% have a folded ADAMTS13. However, the ADAMTS13 conformation in iTTP patients in remission with a persistent (<10%) or moderately restored (10-50%) TS13:act as well as the cause of the open ADAMTS13 conformation is unknown.

Aim: Determine ADAMTS13 conformation in plasma of iTTP patients during acute iTTP and remission with TS13:act <10%, 10-50% and >50%. Investigate if anti-ADAMTS13 autoantibodies induce conformational changes in ADAMTS13.

Methods: ADAMTS13 conformation and presence of anti-ADAMTS13 autoantibodies was determined in ELISA in 118 iTTP plasmas from acute (n=44) or remission phase (n=74). Additionally, purified IgG's from 18 acute iTTP samples was added to folded ADAMTS13 from healthy donor (HD) plasma and tested for ADAMTS13 conformation.

Results: ADAMTS13 was open in 98% (43/44) of the acute samples and folded in 71% (29/41) of the remission samples with TS13:act >50%, confirming our previous results. Interestingly, ADAMTS13 was open in 93% and 89% of remission samples with TS13:act 10-50% and <10%, respectively. Next, we could demonstrate that open ADAMTS13 was linked with presence of anti-ADAMTS13 autoantibodies (chi square, P<0.0001). Finally, we identified that patient anti-ADAMTS13 autoantibodies can open the ADAMTS13 conformation. Indeed, folded ADAMTS13 in HD plasma could be opened after addition of purified IgGs from 14 of the 18 (78%) acute iTTP plasmas, demonstrating that patient anti-ADAMTS13 autoantibodies can indeed induce conformational changes in ADAMTS13.

Conclusion: We show that besides acute iTTP patients, also iTTP patients in remission have an open ADAMTS13 conformation when TS13:act is <10% or 10-50%. In addition, we showed that anti-ADAMTS13 autoantibodies cause conformational changes in ADAMTS13 in iTTP. Hence, an open ADAMTS13 conformation could be a novel biomarker for subclinical iTTP and the presence of undetectable anti-ADAMTS13 autoantibodies.

Conflict of interest: All authors have no conflict of interest.

OC-11

TILTING THE BALANCE: A TRADE-OFF BETWEEN TTP PATIENTS AUTOANTIBODY BINDING AND PROTEOLYTIC ACTIVITY OF ADAMTS13 VARIANTS

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Background/Aims: In immune thrombotic thrombocytopenic purpura (iTTP), patients' autoantibodies commonly target the spacer epitope R568/F592/R660/Y661/Y665 (RFRYY) of ADAMTS13 exosite-3. Each individual residue contribution for patients' autoantibodies binding was studied in full-length context.

Materials and Methods: A large panel of mutations was introduced into this epitope to create an extensive collection of full-length ADAMTS13 variants comprising conservative (Y←→F), semi-conservative (Y/F→L), non-conservative (Y/F→N) or alanine (Y/F/R→A) substitutions. Previously reported Gain-of-Function (GoF, KYKFF) and truncated MDTCS variants (wild-type and 5x alanine) were also included. All variants were expressed and quantitated in CHO cell line supernatants and used in an in-house sandwich ELISA to assess binding towards autoantibodies in the sera of 18 patients. The proteolytic activity of these variants was assessed through the FRETS-VWF73 assay.

Results: In 89% of patients screened, autoantibodies targeting the spacer RFRYY epitope have preponderance compared to other epitopes. In 67% of patients the autoantibody mixture was almost exclusively composed of autoantibodies targeting the RFRYY. Conservative mutations of the aromatic residues did not reduce the binding of autoantibodies. Moderate resistance to autoantibodies was achieved by replacing R568 and R660 by lysines in the GoF. Semi-conservative mutations of aromatic residues show a moderate effectiveness in autoantibody resistance. Non-conservative asparagine or alanine mutations of residues were the most effective. The proteolytic activity of ADAMTS13 was negatively affected in practically all full-length mutant variants, in varying degrees. The greatest activity reductions were observed in the most autoantibody-resistant mutant variants, appearing to be unavoidable. However, the most resistant variants maintained important levels of activity (15-35%).

Conclusions: Non-conservative modifications of the exosite-3 spacer RFRYY epitope in full-length ADAMTS13 allow it to escape the binding of autoantibodies of iTTP patients, simultaneously retaining important proteolytic activity. We provide a framework for the design of autoantibody-resistant ADAMTS13 variants for further clinical development.

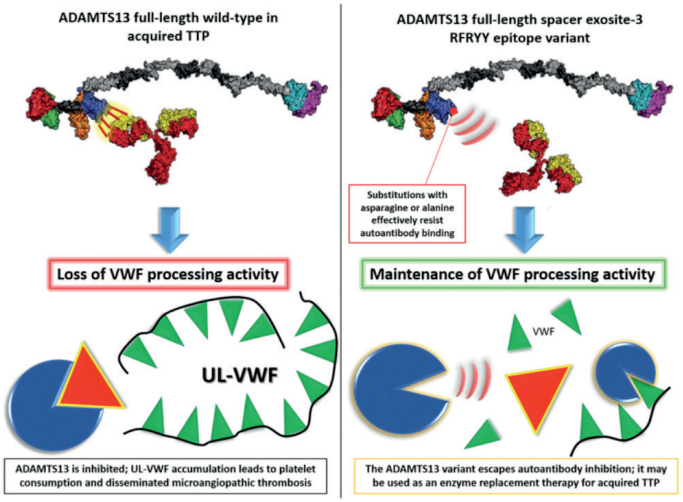

Figure 1. In acquired TTP, autoantibodies bind to ADAMTS13, preventing it from cleaving VWF multimers (left panel). By inserting mutations into the spacer domain exosite-3 of ADAMTS13, autoantibody-resistant variants were created, which maintain VWF proteolytic processing capability (right panel). These variants may potentially be used for treating patients with acquired TTP.

OC-12

IN-DEPTH EPITOPE MAPPING OF ANTI-ADAMTS13 AUTOANTIBODIES IN IMMUNE-MEDIATED THROMBOTIC THROMB OCYTOPENIC PURPURA PATIENTS USING A LARGE LIBRARY OF ADAMTS13 FRAGMENTS

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Background/Aims: In immune mediated thrombotic thrombocytopenic purpura (iTTP), patients develop an immune response against the multidomain enzyme ADAMTS13. ADAMTS13 consists of a metalloprotease (M), and disintegrin-like (D) domain, eight thrombospondin type 1 repeats (T1-T8), cysteine-rich (C), spacer (S), and 2 CUB domains (CUB12). Epitope mapping using relatively large fragments of ADAMTS13 revealed that almost all iTTP patients have anti-CS antibodies while up to 60% of the patients also have autoantibodies against other ADAMTS13 domains. In this project we aimed at developing a large library of ADAMTS13 fragments to profile the anti-ADAMTS13 autoantibodies in iTTP patients.

Materials/Methods: A library of 16 ADAMTS13 fragments, comprising domains of ADAMTS13: M, MDT, MDTC, DTC, DT, CS, C, S, T2T5, T6T8, T2T8, CUB1, CUB2, CUB12, MDTCS, T2C2, and ADAMTS13 was created. All ADAMTS13 fragments were expressed as a fusion protein with albumin domain 1 in Chinese hamster ovary cells and purified using affinity chromatography. Second, the correct folding of the fragments was tested using 18 anti-ADAMTS13 monoclonal antibodies (mAbs) with known epitopes using ELISA. Finally, the anti-ADAMTS13 autoantibody profile of 19 iTTP patients was fine mapped using this large library of ADAMTS13 fragments.

Results: Of the 16 ADAMTS13 fragments, only the C, S and CUB1 fragments were not correctly folded as identified in ELISA using the 18 mAbs. In-depth epitope mapping of iTTP patient samples using this collection of 14 ADAMTS13 fragments, showed that all patients have antibodies against MDTCS, and 88.2% against T2C2. Additionally, the fine mapping revealed that 11.7% of the patients had anti-M, 23.5% anti-DT, 64.7% anti-CS, 23.5% anti-T2T5, 29.4% anti-T6T8, and 76.5% anti-CUB12 antibodies.

Conclusion: We have developed a powerful tool to profile the iTTP patients according to their anti-ADAMTS13 antibodies. In-depth epitope mapping of the anti-ADAMTS13 autoantibodies allows to get a better insight in the immune response in iTTP patients.

POSTERS

SESSION 1

PO-01

NATURE OF FVIII-CONTAINING IMMUNE COMPLEXES AND INDUCTION OF IMMUNE TOLERANCE IN PATIENTS WITH HEMOPHILIA A

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Background: Immune tolerance induction (ITI) by repeated injections of high-dose factor VIII (FVIII) is the only strategy to eradicate inhibitory anti-FVIII IgG in hemophilia A patients. The epitope specificity and subclass of anti-FVIII IgG at initiation of ITI were proposed as predictors of ITI outcome. FVIII injection to patients with circulating anti-FVIII IgG leads to the formation of FVIII-containing immune complexes (FVIII-IC). We hypothesize that the nature of the FVIII-IC that form at the start of ITI dictates ITI outcome.

Methods: We produced the inhibitory human monoclonal anti-A2 (BOIIB2), anti-C1 (LE2E9) and anti-C2 (BO2C11) IgG in IgG1k and IgG4k formats. The capacity of the IgG to bind to FVIII, inhibit VWF-binding to FVIII and neutralize FVIII pro-coagulant activity was assessed by ELISA and Bethesda assay. The effect of the composition of FVIII-IC (domain specificity, mono/oligoclonality, subclass) on FVIII endocytosis by monocyte-derived dendritic cells (MODC) is being investigated.

Results: IgG size was confirmed by SDS-PAGE. Binding to FVIII and blockade of VWF-binding to FVIII (confirmed for BO2C11 and LE2E9) were not affected by IgG subclass change. The inhibitory activity of IgG1k was greater than that of IgG4k. BO2C11 IgG4k was confirmed to block FVIII endocytosis. We are now investigating the effect of single IgG/FVIII-IC and pooled IgG/FVIII-IC on FVIII uptake.

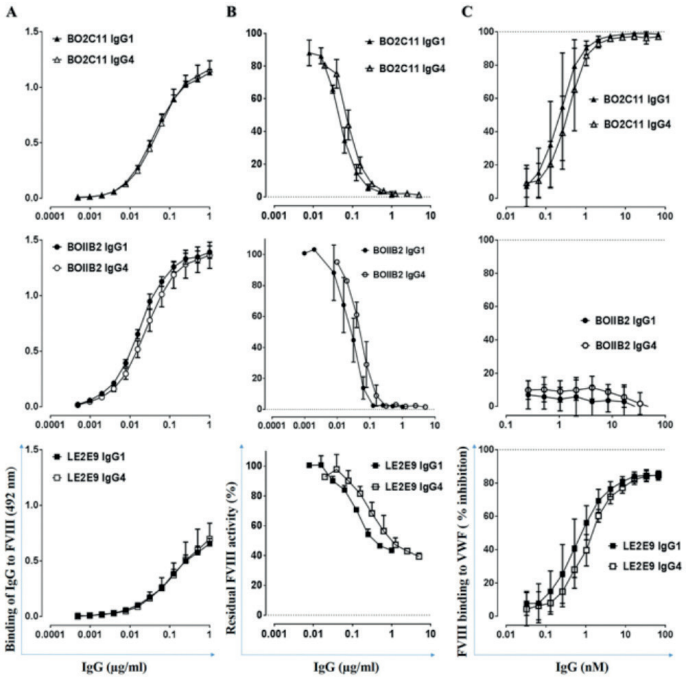

Figure. Validation of human monoclonal anti-FVIII IgG1k and IgG4k. A. Binding of BO2C11 (triangles), BOIIB" (circles) or LE2E9 (squares) to FVIII. B. Inhibition of FVIII functional activity in chromogenic assays. C. Inhibition of FVIII binding to VWF. IgG1k and IgG4k formats are represented in full and empty symbols respectively.

Conclusions: The present work validates the production and functions of human recombinant anti-FVIII antibodies in IgG1k and IgG4k formats. The change in IgG subclass was not associated with drastic alteration of IgG functions. Future work includes the analysis of the binding of IgG to Fc receptors, the validation of FVIII-IC formation and the repercussion of the nature of FVIII-IC on FVIII uptake by MODCs, MODC maturation and polarization of $CD4^+$ T-cell responses. At term, our findings will foster the rational design of FVIII-IC with tolerogenic properties for the treatment of inhibitor-positive patients.

PO-02

PERFORMANCE OF A CLINICAL RISK PREDICTION MODEL FOR INHIBITOR FORMATION IN SEVERE HEMOPHILIA A

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Background: There is a need to identify patients with hemophilia who have a high risk of developing inhibitors. High-risk patients could be candidates for preventive treatment strategies.

Aims: The aim of this study was to externally validate two previously published prediction models for inhibitor development and to develop a new prediction model that incorporates novel predictors.

Methods: The population consisted of 251 previously untreated or minimally treated patients with severe hemophilia A enrolled in the SIPPET study. The outcome was high-titer inhibitor formation. Discrimination was measured using the C-statistic and calibration was assessed visually with a calibration plot. The new models were internally validated using bootstrap resampling. Missing values in the dataset were imputed using multiple imputation.

Results: The 2008 prediction model consisted of three variables; family history of inhibitor development, *F8* gene mutation and intensity of first treatment with FVIII. The C-statistic was 0.53 (95%CI: 0.50-0.60) and calibration was moderate/poor. The 2015 prediction model contained the same predictors, slightly recoded. The C-statistic was 0.58 (95%CI: 0.50-0.67) and calibration was also moderate/poor.

Two new models were developed; a full model and a reduced model. The following predictors were included in the full model; F8 gene mutation, intensity of first treatment with FVIII, the presence of non-neutralizing antibodies before first treatment, FVIII product type (recombinant vs. plasma-derived), age at first treatment and the type of bleed at first treatment. The C-statistic was 0.65 (95CI: 0.55-0.75) and calibration was moderate. The reduced model only included F8 gene mutation and the presence of non-neutralizing antibodies before first treatment. The C-statistic was 0.61 (95CI: 0.52-0.70) and calibration was also moderate.

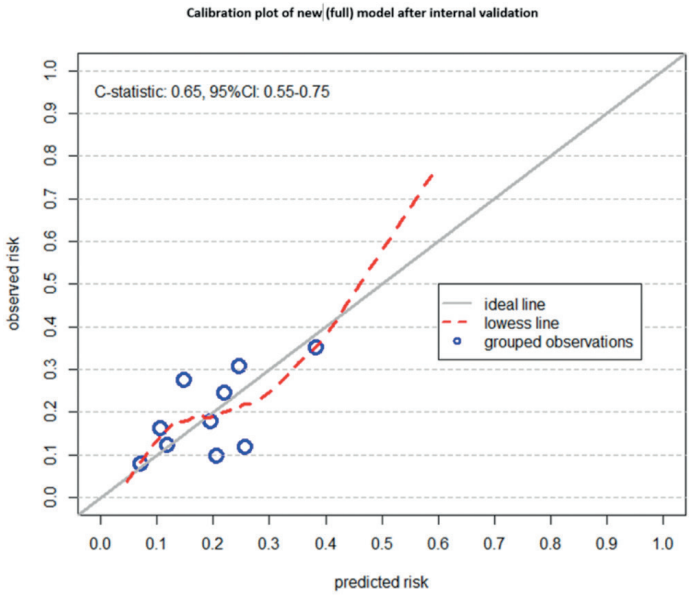

Figure. Calibration plot of new (full model after internal validation)

Conclusion: Performance of previously published prediction models after external validation in the SIPPET cohort was not informative. New risk prediction models using genetic and environmental variables seem to be useful for identifying patients at high risk for high titer inhibitor development.

PO-03

RFVIIIFC FOR FIRST-TIME IMMUNE TOLERANCE INDUCTION (ITI) THERAPY: INTERIM RESULTS FROM THE GLOBAL, PROSPECTIVE VERITI-8 STUDY

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Background: ITI is standard of care for inhibitor eradication and restoration of factor VIII (FVIII) responsiveness in subjects with severe hemophilia who develop high titer inhibitors (HTI). Retrospective data support use of recombinant FVIII Fc fusion protein (rFVIIIFc) in ITI1 but have yet to be confirmed in prospective studies. This study presents preplanned interim results of verITI-8 (NCT03093480).

Methods: Single-arm, nonrandomized, open-label, ethics–approved study of rFVIIIFc for first-time ITI. Eligible subjects had history of HTI (historical peak ≥5 BU/mL) and provided informed consent. Primary endpoint is time to tolerization, defined by negative inhibitor titer (<0.6 BU/mL) at 2 consecutive visits; incremental recovery (IR) ≥66% of expected at 2 consecutive visits; and rFVIIIFc half-life ≥7 hours. ITI failure is defined as not meeting above criteria by Week 48. This analysis was planned when ≥10 subjects had received ≥6 months of rFVIIIFc ITI.

Age at screening, years	Historical peak inhibitor titer, BU/mL	Inhibitor titer pre-rFVIIIFc ITI, BU/mL	Time from inhibitor diagnosis to start of ITI, wks	ITI outcome	Time to			Time on study, wks
					Negative inhibitor titer,[a] wks	Normal IR,[b] wks	Tolerization, [c] wks	
1	14.1	9	12.14	ITI success	2.29	4.29	8.14	10.86
3	8.11	0.64	18.86	ITI success	2.29	6.86	9.86	31.86
1.7	6.2	0.8	28.43	ITI success	1.71	5.43	11.43	32.14
16	40	2	747.43	ITI success	1.71	6	12	26.57
2.9	9	3.25	71.86	ITI success	2.29	6	12.43	36
1.3	7.1	20	3.86	ITI success	15.57	28.14	32	37.29
2	19.2	11	20.43	Ongoing	NA	NA	NA	0.14
10	150	4.2	>156	Ongoing	NA	NA	NA	2.14
0.8	11.3	5.1	23.14	Ongoing	NA	NA	NA	7.57
1.3	132.2	94	4.14	Ongoing	NA	NA	NA	16
5	48	40	71.14	Ongoing	NA	NA	NA	21
5	256	25	55.71	Ongoing	NA	NA	NA	21.43
2.2	105	12.2	64	Ongoing	NA	NA	NA	35.57
6	232	144	64.57	ITI failure	NA	NA	NA	49

BU, Bethesda unit; IR, incremental recovery; ITI, immune tolerance induction; rFVIIIFc, recombinant factor VIII Fc fusion protein.
[a]Time to negative inhibitor titer is defined as the time interval (weeks) from the start date of rFVIIIFc ITI treatment to the date of first negative inhibitor titer which is subsequently confirmed by a sample from a consecutive visit 2 weeks later.
[b]Time to normal IR is defined as the time interval (weeks) from the start date of rFVIIIFc ITI treatment to the date of the first normal IR (≥66%) which is subsequently confirmed by a sample from a consecutive visit 2 weeks later.
[c]Time to tolerization is defined as the time interval (weeks) from the start date of rFVIIIFc ITI treatment to the time meeting all 3 criteria of negative inhibitor titer (<0.06 BU/mL at 2 consecutive visits, normal IR at 2 consecutive visits, and half-life ≥7 hours).

Table. ITI outcomes as of December 5, 2018

Results: Results are as of December 5, 2018. 15 subjects were screened; 14 enrolled and received ≥1 dose of rFVIIIFc for ITI. Median (range) age at start of ITI was 2.6 (0.8–16.0) years and historical peak inhibitor titer was 29.6 (6.2–256.0) BU/mL. Six subjects have been successfully tolerized (Table), with median (range) time to first negative titer, normal IR, and tolerization of 2.3 (1.7–15.6), 6.0 (4.3–28.1), and 11.7 (8.1–32.0) weeks, respectively. Seven subjects continue to receive rFVIIIFc ITI (Table) (median time on ITI [range]: 16.0 [0.1–35.6] weeks) and one has failed. No adverse events related to rFVIIIFc have been reported.

Conclusions: Early results from this prospective/ongoing study of first-time ITI indicate that rFVIIIFc may offer rapid time to tolerization in some subjects with severe hemophilia A and HTI. Achieving tolerance faster can improve quality of life and reduce costs.

References:

1. Carcao, Haemophilia, 2018.

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PO-04

REAL-WORLD EFFICACY AND SAFETY DATA OF PATIENTS WITH HEMOPHILIA AND INHIBITORS TREATED WITH APCC: "FEIBA GLOBAL OUTCOME STUDY (FEIBA-GO)", RESULTS FROM >12 MONTHS FOLLOW-UP

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Background/Aims: The primary objective of the FEIBA Global Outcome study (FEIBA-GO) is to describe the long-term, real-world effectiveness of activated prothrombin complex concentrate (aPCC, Baxalta, a Takeda company, USA) for preventing and managing bleeding in patients with hemophilia A (HA) or B (HB) with inhibitors (PwHI) across different clinical settings. This interim >12 month analysis (May 2018) reports efficacy and safety data from patients participating in FEIBA-GO.

Materials and Methods: FEIBA-GO (EUPAS6691) is an ongoing post-authorization, prospective, observational, multicenter cohort study. Males with high-responding inhibitors of any titer diagnosed before study entry and prescribed treatment with aPCC will be followed over 4 years, treatment regimens (prophylaxis, on demand, or immune tolerance induction [ITI]) are prescribed at the physician's discretion.

Results: Enrollment was completed on Dec 31, 2017, with 53 PwHI from 27 sites in 11 countries (52 HA, 1 HB, median age at baseline of 18-years [range: 2–71]). For patients with >12 months follow-up data (n=28), mean annualized bleeding rate (ABR) and mean annualized joint bleeding rate (AJBR) for total bleeds (treated and nontreated) are shown in Table 1. Of 21 patients on aPCC prophylaxis, seven (33.3%) and 10 (47.6%) reported ≤3 ABR and ≤3 AJBR, respectively. Of the total 28 patients, 65 nonserious AEs (1 hemarthrosis, probably-related) were reported in 13 patients and 30 serious AEs were reported in 19 patients (1 possibly-related SAE of acute myocardial posterior wall infarction with coronary artery embolism was reported in an 18-year old male with a port catheter and 1 probably-related SAE of hemarthrosis). No thrombotic microangiopathies were reported.

Conclusions: These real-world data, >12 months follow-up, are consistent with previous experience on the effectiveness and safety of aPCC as monotherapy. Long-term real-world follow-up over 4 years from FEIBA-GO will provide further information on the real-world effectiveness and safety of aPCC.

	Treatment Regimen		
	Prophylaxis n=21	On-demand n=6	ITI n=1
ABR, Mean (SD)	7.1 (9.3)	11.4 (12.8)	41.3
ABR, Median (Range)	5.0 (0.0-42.1)	6.8 (0.0-28.9)	
ABR, Categorical, n (%)			
0	3 (14.3)	2 (33.3)	0
>0 - ≤3	4 (19.0)	0	0
>3 - ≤6	5 (23.8)	0	0
>6	9 (42.9)	4 (66.7)	1 (100.0)
AJBR, Mean (SD)	4.2 (5.1)	7.3 (7.9)	18.3
AJBR, Median (Range)	3.3 (0.0-18.4)	5.4 (0.0-19.5)	
AJBR, Categorical, n (%)			
0	5 (23.8)	2 (33.3)	0
>0 - ≤3	5 (23.8)	0	0
>3 - ≤6	6 (28.6)	1 (16.7)	0
>6	5 (23.8)	3 (50.0)	1 (100.0)

Table 1: Total Annualized Bleeding Rates (ABR) and Annualized Joint Bleeding rates (AJBR) in Patients followed for >12 months Total Annualized bleed rates (ABR) and annualized joint bleed rates (AJBR) are calculated as all bleeding standardized to 12 months, SD=standard deviation

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* a Takeda company

PO-05

THE NATURAL HISTORY OF NONSEVERE HEMOPHILIA A: WHAT IS THE EXPECTED TIMING OF THE INITIAL EX POSURES TO FACTOR VIII TREATMENT?

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Background and Aim: Currently, we lack detailed information on the natural history of nonsevere hemophilia A. This information is urgently required, as we are entering a new era in which novel treatment strategies, such as non-replacement therapies, are becoming available for severe hemophilia A. These novel therapies have the potential to convert a severe bleeding phenotype into a nonsevere bleeding phenotype, thereby postponing initial Factor VIII (FVIII) exposures. Therefore, the aim of this study is to assess the timing of the initial exposures to FVIII treatment in a large, consecutive and international nonsevere hemophilia A cohort.

Methods: We analyzed data from the INSIGHT study, including patients with nonsevere hemophilia A (baseline FVIII level [FVIII:C] 2-40 IU/dl) born after 1980. In the total cohort we assessed the median age at the first exposure day (ED) to FVIII treatment, according to 6 FVIII:C categories (2-5/5-10/10-15/15-20/20-25/25-40 IU/dL). For a sub-group of patients, we assessed the median age on each of the first 20 EDs, according to 3 FVIII:C categories (2-5/5-15/15-40 IU/dL).

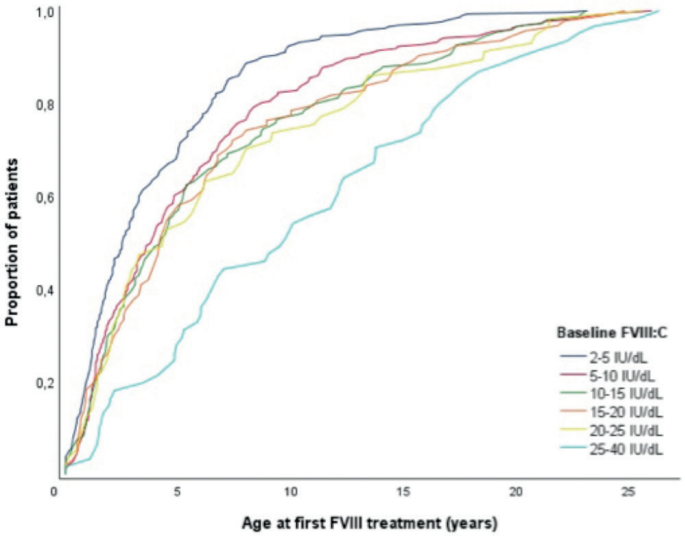

Figure 1. Age at first exposure to FVIII treatment in the total nonsevere hemophilia A cohort (n=1013) stratified for baseline FVIII categories.

Results: In the total cohort (n = 1013) the age at ED 1 was 2.5 years (IQR 1.2-5.7) for patients with a FVIII:C of 2-5 IU/dl, increasing to 9.7 years (IQR 4.8-16.0) for patients with a FVIII:C of 25-40 IU/dL (Figure 1). The sub-study (n = 104) demonstrated in patients with a FVIII:C of 5-15 IU/dl, that the age was 3.4 years (IQR 1.2-7.0), 5.6 years (IQR 2.6-8.8), 8.5 years (IQR 5.7-11.1) and 9.0 years (IQR 6.6-14.3) at respectively ED 1, ED 5, ED 10 and ED 20 (Table 1).

Baseline FVIII:C, IU/dL	Age at first exposures to FVIII treatment, years				
	ED 1	ED 5	ED 10	ED 15	ED 20
2 – 5	4.5 (1.4-7.5)	5.2 (3.2-9.4)	5.9 (3.8-10.7)	7.6 (3.8-11.2)	10.9 (4.9-14.2)
5 – 15	3.4 (1.2-7.0)	5.6 (2.6-8.8)	8.5 (5.7-11.1)	8.9 (5.8-11.9)	9.0 (6.6-14.3)
15 – 40	4.1 (2.5-8.9)	8.5 (3.0-11.6)	9.9 (4.2-12.6)	10.0 (5.3-15.3)	10.5 (7.9-15.5)
Patients under observation	*104*	*95*	*82*	*68*	*52*

Table 1. Age at first 20 exposure days to FVIII treatment in the sub-group (n=104) stratified for baseline FVIII level in categories. Values are given in medians and interquartile ranges. ED, exposure day.

Conclusions: The timing of the first exposure to FVIII treatment in nonsevere hemophilia A ranges from 2.5 to 9.7 years, depending on the baseline FVIII:C. In patients with a FVIII:C of 5-15 IU/dl, the 20th ED occurred at 9.0 years.

PO-06

FINAL RESULTS FROM THE NUPROTECT STUDY OF NUWIQ® (SIM OCT OCOG ALFA) TREATMENT IN PREVIOUSLY UNTREATED PATIENTS WITH SEVERE HEMOPHILIA A

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Background/Aims: Approximately 35% of previously untreated patients (PUPs) with severe hemophilia A develop inhibitors to recombinant FVIII replacement therapy. Nuwiq® (simoctocog alfa) is a 4th generation recombinant FVIII, produced in a human cell-line without chemical modification or protein fusion, which has the potential to reduce inhibitor development. An interim analysis of the NuProtect study in 66 PUPs treated with Nuwiq® for ≥20 exposure days showed cumulative incidences (95% confidence intervals) of 20.8% (10.7%, 31.0%) for all inhibitors and 12.8% (4.5%, 21.2%) for high-titre inhibitors, with effective prevention of bleeding (Leisner et al. Haemophilia 2018; 24: 211-20). No inhibitors developed in patients with non-null F8 gene mutations.

Patients and methods: The NuProtect study (NCT01712438) was a prospective, multicenter, multinational, open-label, non-controlled, phase III study. The study enrolled patients with severe hemophilia A (FVIII coagulant activity <1%) of any age and ethnicity with no previous exposure to FVIII concentrates or other blood products containing FVIII ('true' PUPs). Patients received Nuwiq® for standard prophylaxis or on-demand treatment, and for treatment of breakthrough bleeds or surgical prophylaxis, with the type of treatment and the dosage determined by the investigator based on the clinical situation of the patient. Treatment duration was 100 exposure days or a maximum of 5 years. The primary endpoint of the study was the incidence of FVIII inhibitors, as measured by the modified Bethesda assay (Nijmegen modification; cut-off 0.6 Bethesda units per mL).

Results and conclusions: The NuProtect study has enrolled a total of 110 true PUPs (of whom 108 received study drug) with severe hemophilia A treated with Nuwiq®. The final results of the study will be analysed and presented.

PO-07

THE COMBINATION OF PLASMA-DERIVED FVIII/VWF WITH EMICIZUMAB HAS NON-ADDITIVE EFFECTS ON THROMBIN GENERATION ASSAY INDEPENDENTLY OF THE PRESENCE OF INHIBITORS IN HEMOPHILIA A PLASMA

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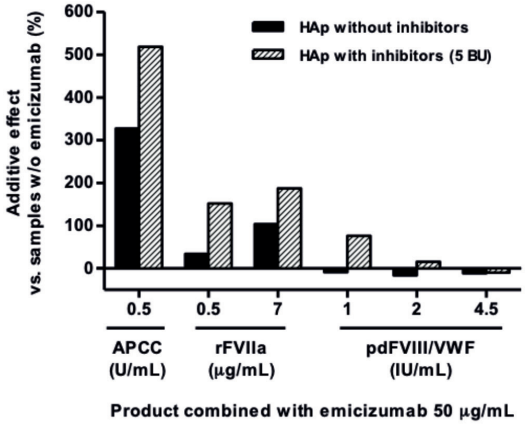
Background: Emicizumab (Hemlibra®, Chugai-Roche) is used to bridge aFIX and FX to function as missing aFVIII in hemophilia A (HA) patients. Although limited clinical results are available, fatal thrombotic complications have been reported when using emicizumab combined with aPCC but not with rFVIIa or FVIII. To provide a complementary insight to an abstract presented at ISTH, the percentage of additive effect on thrombin generation (TG) is proposed.

Methods: TG (tissue factor: PPP-Reagent Low, Stago; reaction parameter: TP [thrombin peak]) was assessed using CAT software on pooled HA plasma samples without inhibitors (HAp) or individual HA plasma samples with inhibitors (HAp-i) (5 BU), either alone or containing emicizumab (50 µg/mL) combined with: aPCC 0.5 U/ml (≈25 U/kg), rFVIIa 0.5 and 7 µg/ml (≈50 and 700 µg/kg), or plasma-derived (pd) pdFVIII/VWF (Alphanate®/Fanhdi®, Grifols) 1 to 4.5 IU/ml (≈50 to 200 IU/kg).

Results: TG for 13 individual healthy plasma samples was evaluated to establish TP normal ranges (47-104 nM). TP for both HAp and HAp-i in presence of emicizumab showed that addition of aPCC 0.5 U/ml induced a synergic response on TP even at low doses, while rFVIIa moderately increased TP in a dose-related manner but within normal ranges. In contrast, when pdFVIII/VWF was added to HAp or HAp-i, TP was similar to that observed without emicizumab once reached the normal levels (See Table). The absence of additive effects of the concomitant use of pdFVIII/VWF and emicizumab is shown as the percentage of TP increase versus samples without emicizumab (See Figure).

Products	Dose	Emicizumab (µg/mL)			
		HAp without inhibitors		HAp with inhibitors (5 BU)	
		TP (nM) Mean ± SD		TP (nM) Mean ± SD	
		0	50	0	50
aPCC (U/mL)	0.5 (~25U/Kg)	102 ± 6	436 ± 53	49 ± 5	303 ± 32
rFVIIa (µg/mL)	0.5 (~50µg/Kg)	59 ± 2	79 ± 1	25 ± 1	63 ± 0
	7 (~700µg/Kg)	83 ± 6	169 ± 12	38 ± 4	109 ± 10
pdFVIII/VWF (IU/mL)	1 (~50IU/Kg)	127 ± 20	116 ± 14	25 ± 4	44 ± 4
	2 (~100IU/Kg)	186 ± 27	156 ± 25	60 ± 6	69 ± 9
	4.5 (~200IU/Kg)	275 ± 34	244 ± 30	183 ± 16	165 ± 30



Conclusions: After reaching a normal TG range, emicizumab would have a limited ability to promote FX activation in the presence of pdFVIII/VWF, thus reducing the risk of overdosing. Our data support that pdFVIII/VWF has non-additive effects when combined with emicizumab.

PO-08

ISOTYPE PROFILING THE IMMUNOLOGICAL RES PONSE IN PREVIOUSLY UNTREATED HEMOPHILIA A PATIENTS TREATED WITH TUR OCT OCOG ALPHA

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Introduction: The aim of this exploratory study was to investigate the development of the immunological response during inhibitor development and immune tolerance induction (ITI) in previously untreated patients with severe hemophilia A, specifically describing isotype development over time.

Materials and Methods: In the clinical trial Guardian™ 4, 'Safety and Efficacy of turoctocog alfa in Prevention and Treatment of Bleeds in Paediatric Previously Untreated Patients with Haemophilia A', 46 previously untreated patients gave informed consent that residual samples could be used for exploratory analysis. More than 600 samples were collected from both positive and inhibitor negative patients throughout the trial. These were submitted to isotype analysis (IgM, IgG1 and IgG4).

Results: We confirm the correlation between inhibitor status and IgG4. However, the IgG4 response came later than the IgG1 response. Surprisingly, we detected IgM at several stages during both inhibitor development and clearance, and saw weak immune responses in patients that did not develop inhibitors.

Conclusions: Several studies have investigated the correlation of IgG4 with inhibitor status, but none have published a longitudinal study of this magnitude, both with regards to patient numbers and profile details. We observed a delay in the response from IgG1 to IgG4, indicating that IgG4 is a sign of maturation of the response, correlating with the presence of IgG4 in inhibitor patients. We believe that our data might support further elucidation of markers for inhibitor development or ITI success.

PO-09

USEFULNESS OF GLOBAL HEMOSTASIS ASSAYS IN HEMOPHILIA A PATIENTS WITH DISCREPANT BLEEDING PHENOTYPE

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Background: Traditionally used laboratory methods, i.e. one-stage or chromogenic assays for FVIII activity determination do not always and accurately reflect bleeding severity in hemophilia A (HA) patients. As global hemostasis assays provide overall hemostatic status estimation, we investigated the ability of three global assays for identifying bleeding phenotype both in severe and non-severe HA patients, as well as their usefulness in laboratory management of HA patients with discrepant bleeding phenotype.

Materials and Methods: Overall hemostasis potential (OHP), aPTT-clot waveform analysis (aPTT-CWA), endogenous thrombin potential (ETP) and FVIII activity were measured in 30 severe and 32 non-severe HA patients and 27 male controls. For classification of HA patients regarding bleeding phenotype, we used a scoring method that included three clinical parameters: age at first joint bleed, number of target joints and number of joint/muscle bleeds per year. Bleeding scores ≤4 and ≥5 suggested mild and severe bleeding phenotype, respectively.

Results: All global assays correlated significantly with FVIII activity ($P<0.001$), enabling clear discrimination between severe, non-severe

HA patients and controls. ROC analysis performed for distinguishing patients with severe and mild bleeding phenotype yielded the following AUC values: 0.891 for aPTT-CWA; 0.769 for OHP; 0.634 for ETP. Discrepant bleeding phenotype was identified in 11/62 HA patients: 3/30 severe patients presented with mild bleeding phenotype, whereas 8/32 non-severe patients demonstrated severe bleeding phenotype. Global assays allowed detection of all 11 patients with discrepant bleeding phenotype. The best discriminating ability was demonstrated by OHP and DELTA (10/11 and 9/11 discrepant results, respectively) followed by ETP (6/11 discrepant results).

Conclusions: Global assays are superior bleeding phenotype determinants in a majority of individual HA patients, compared to FVIII alone, and should be included in the diagnostic algorithm at least in patients with discrepant results between FVIII activity and calculated bleeding score.

PO-10

COMPARISON OF THE EFFECT OF CATIONIC AND ANIONIC POLYAMIDOAMINE DENDRIMERS ON COM PONENTS OF COAGULATION SYSTEM

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Background: Polyamidoamine (PAMAM) dendrimers, monodispersed polymers with highly branched three-dimensional globular structure and surface functional groups (-NH2, -OH, or -COOH), are of growing interest in nanomedicine, notably in systemic administrations as drug carriers. Charged surface groups of PAMAM dendrimers can to form non-covalent complexes with drugs, and are available for covalent attachment of biomacromolecules. PEGylation of FVIII doubles its lifetime in bloodstream while maintaining procoagulant activity. Modification of FVIII with PAMAM-dendrimers of various generation and surface charge can be used to obtain conjugates with desired properties.

Aims: To study the effect of PAMAM-NH2 dendrimers (G1-G3) and PAMAM-COOH (G1.5-G3.5) on blood coagulation components to select optimal polymer for modifying target factors.

Materials and Methods: Effect of the PAMAM-dendrimers concentration on RBC hemolysis, overall hemostatic potential, thrombin generation and activity, prothrombin time, zeta potential and CD spectrum of fibrinogen was studied.

Results: Cationic (G1-G3) and anionic (G1.5-G3.5) up to 200 μM had a little effect on thrombin activity and caused less than 8% RBC hemolysis. Cationic G3 and anionic G2.5, having 32 surface charged groups and diameter 3.6 nm, were used to compare the effect of PAMAM-dendrimers charge on clotting factors. Unlike G2.5, G3 significantly reduced the rate of formation and the amount of plasma clot induced by added thrombin. G2.5 had no effect, whereas even 7 μM G3 completely inhibited thrombin generation in plasma (Fig.1). G2.5 had no effect, while G3 (up to 200 μM) significantly prolonged the prothrombin time. Changes in the zeta potential and CD-spectrum of fibrinogen showed that G3, unlike G2.5, binding to negatively charged fibrinogen, changes the structure and impairs its polymerization.

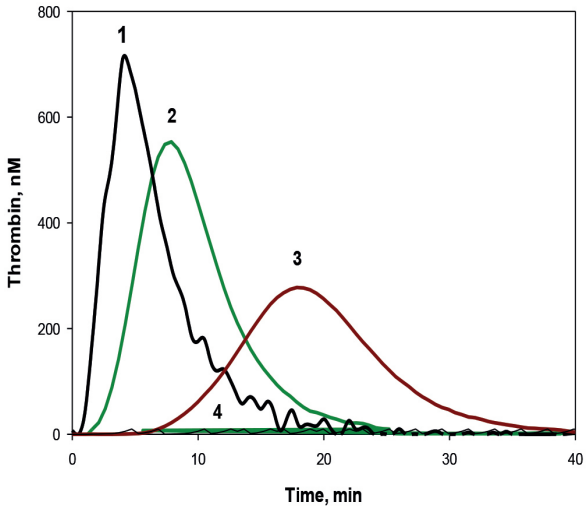

Figure 1. Influence of G3 PAMAM-NH2 dendrimers on the thrombin generation by rTF in the presence of phospholipid micelles (37oC). Concentration of cationic G3 dendrimer: 0 (1); 3 (2), 5 μM (3), and 7 μM. (4).

Conclusions: Anionic PAMAM dendrimers, not cationic dendrimers, are promising for modification of the factor FVIII amino groups, because unlike linear PEG, compact dendrimer molecules do not cover the protein, but can block its degradable bonds and antigenic epitopes.

PO-11

ACCURATE FACTOR VIII CONCENTRATE DOSING BY IDEAL BODY WEIGHT IN OVERWEIGHT AND OBESE HEMOPHILIA A PATIENTS

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Background/aims: Underdosing and especially overdosing of replacement therapy in hemophilia A patients may be prevented using other morphometric variables than actual body weight to dose factor VIII (FVIII) concentrates. Therefore, the extent to which morphometric variables describe inter-individual variability of FVIII concentrate PK parameters in normal weight, overweight and obese hemophilia A patients was explored.

Material and methods: PK profiling was performed by measuring three FVIII levels after a FVIII concentrate dose of 50 $IUkg^{-1}$. A population PK model was constructed, in which inter-individual variability (IIV) for clearance (CL) and central volume of distribution (V1) was quantified. Relationships between CL, V1 and five morphometric variables e.g. body weight, ideal body weight (IBW), lean body weight, adjusted body weight, and body mass index (BMI) were evaluated.

Results: Fifty-seven hemophilia A patients (FVIII ≤0.05 $IUmL^{-1}$) were included with a median (range) body weight of 83 kg (53–133) and age of 48 years (18–77). Patients were grouped into BMI categories:

26 patients with normal body weight (BMI <25kgm^{-2}), 21 overweight (BMI 25-30kgm^{-2}), and 10 obese patients (BMI >30kgm^{-2}). IBW best explained the observed variability between patients, as IIV for CL and V1 was reduced from 45.1% to 37.6% and 26.8% to 14.1%, respectively. Moreover, CL, V1 and half-life were similar in normal weight, overweight and obese patients. FVIII trough and peak levels in patients with obesity were similar to normal body weight patients when dosed based on IBW (Figure 1).

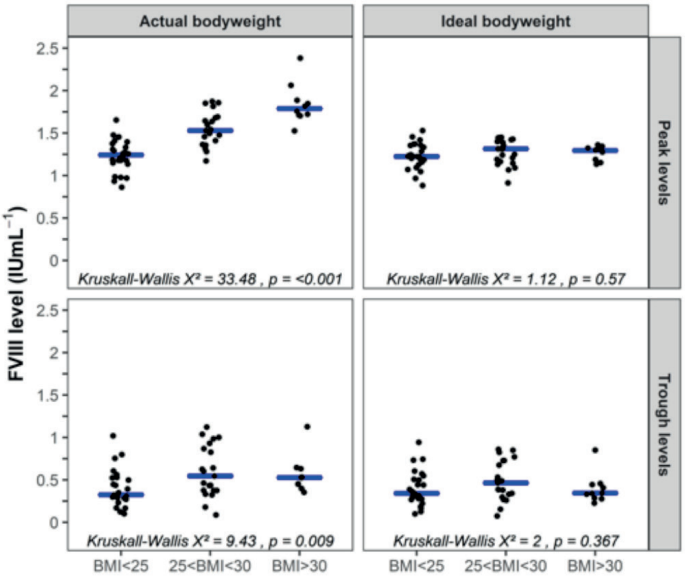

Figure 1. Boxplots of the peak and trough FVIII level in steady-state. The FVIII peak levels (upper panels) were obtained 5 minutes after the simulated loading-dose of 50 IUkg^{-1} that was followed by twice daily dosing of 25 IUkg^{-1} to treat a life-threatening bleed. The trough FVIII levels (lower panels) were obtained immediately before the 6th dose, which corresponded with 72 hours after administration of the loading-dose. The individual FVIII levels were simulated using the individual PK parameters from each patient of the studied population. The blue bar depicts the median FVIII level. To enhance the visibility for the number of FVIII levels in each category, the FVIII levels were scattered horizontally.

Conclusion: Ideal body weight most accurately calculates doses of FVIII concentrate in overweight and obese hemophilia A patients.

PO-12

CD152 IN HEMOPHILIA A PATIENTS: A USEFUL METHOD TO FOLLOW THE IMMUNOLOGICAL RES PONSE TO FVIII?

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Background: Indoleamine2,3-dioxygenase/IDO1 immune checkpoint pathway and T regulatory (Treg) lymphocytes are involved in immune response towards Factor VIII (FVIII) in hemophiliacs. CTLA-4/CD152 has an immunological role in downregulation of effector T and in enhancement of Treg activity via IDO1 pathway. By flow cytometry (FC) we analyzed in CD4$^+$T lymphocytes incubated with plasma-derived FVIII (pdFVIII) the CTLA-4/CD152 expression, the levels of IL-10 (anti-inflammatory/regulatory) and IFNϒ (pro-inflammatory) cytokines in a group of HA hemophilic patients with (INH+) and in a group without (INH-) inhibitors. All patients were inv22 positive.

Materials and methods: Peripheral blood of 3 HA patients INH+ and 3 HA INH- were collected to obtain peripheral blood mononuclear cells (PBMCs). These cells were incubated for 5 days (37° in a 5% CO2 humidified incubator) with IL2 and pdFVIII (5U/ml) and fixed-permealized cells were stained with antibodies to intra-cytoplasmatic CD152, IL10, IFNϒ and run in a BD FACSCantoII.

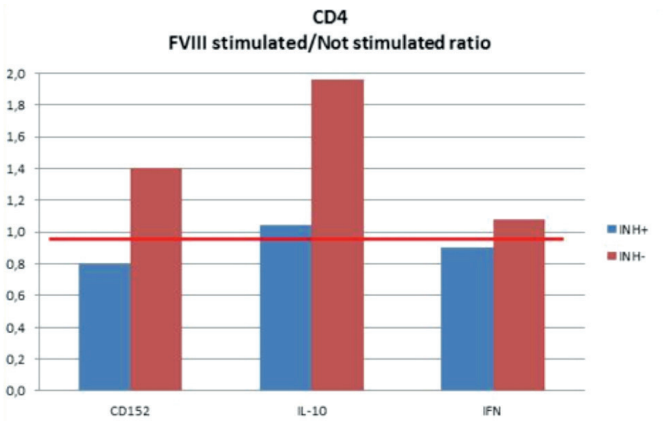

Figure. Peripheral blood samples of 2 HA patients (blue) with a positive story of inhibitor (INH+) and of 3 HA patients (red) without inhibitor (INH-) were collected to obtain PBMNC. The PBMNC were incubated for 5 days (37°C in a 5% CO2 humidified incubator) in presence of IL-2 and pdFVIII (5U/ml) and then fixed-permeabilized cells were stained with antibodies against intracitoplasmic CD152, IFN-γ and IL-10 and run in a BD FACSCanto II. Normalized values of median % of CD4+CD152+, CD4+IL-10+ abd CD4+IFN-γ+c cells were obtained as rations between the values obtaioned in cells stimulated with pdFVIII and the values obtained in not stimulated cells. Ratio 1 line is outlined in red.

Results and conclusions: The results were expressed as normalized values of median % of CD4+CD152+, CD4+IL10+ and CD4+IFNϒ+ cells calculated as ratios between the values obtained in cultures with pdFVIII and those in not stimulated ones (figure). Comparing hemophiliacs with and without inhibitors, we observed a greater different expression in CD152 and IL-10 level. Then our data would demonstrate in HA/INH- patients an anti-inflammatory/regulatory immune response characterized by a higher IL-10 level in CD4+T. Moreover, the increased expression of CD152 in HA/INH- would suggest a possible IDO1 pathway and therefore a greater tolerance towards FVIII. Our experience could suggest that CD152 evaluation by FC in hemophiliacs could provide a simple method to evaluate the immunological response to FVIII determined on the IDO1-dependent induction of Treg.

PO-13

OBSERVATIONAL IMMUNE TOLERANCE INDUCTION STUDY (OBSITI): IMMUNE TOLERANCE INDUCTION WITH A SINGLE FACTOR VIII/VON WILLEBRAND FACTOR CONCENTRATE FOR TREATMENT OF HEMOPHILIA A PATIENTS WITH INHIBITORS

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Background/Aims: FVIII inhibitors increase morbidity, mortality and socioeconomic burden in patients with hemophilia A. Immune tolerance induction (ITI) with replacement FVIII is the only proven strategy for eradication of inhibitors and is recommended as the primary treatment option in European and US guidelines. The ongoing, prospective and retrospective, international, multicenter, open-label, uncontrolled, observational ITI (ObsITI) study (NCT02207894) is evaluating ITI with a range of FVIII products and the high-dose Bonn protocol as the recommended ITI regimen.

Patients and methods: As of 10 January 2019, 205 patients from 20 countries undergoing ITI have been recruited in ObsITI. This analysis reports interim data for 100 prospective patients (the majority of whom had poor prognosis based on known risk factors for poor ITI outcome) treated exclusively with a single plasma-derived, von Willebrand factor-stabilized, FVIII concentrate (pdFVIII/VWF, octanate®) between December 2005 (study initiation) and January 2019. Success of ITI was defined as achievement of 2 of the following 3 criteria: inhibitor tire <0.6 Bethesda units [BU]/mL; FVIII recovery ≥66%; FVIII half-life ≥6 h.

Results: Of the 100 patients, 14 were low responders and 86 were high responders. Success was achieved by 70/100 (70%) patients in a

median time of 12.5 months [interquartile (IQR) range 7.0-25.9]. Negative inhibitor titres (<0.6 BU/mL) were achieved in 71 (71%) patients in a median time of 4.0 months (IQR 1.6-9.0). None of the patients reported a relapse within 12 months of achieving complete success. ITI outcome correlated with ITI group (primary or rescue ITI), age at ITI start, inhibitor titre at ITI start, responder type (low or high responder) and peak inhibitor titre during ITI. There were no treatment-related safety concerns.

Conclusions: ITI with octanate® led to rapid, sustained eradication of FVIII inhibitors and normalisation of FVIII pharmacokinetics and has the potential to reduce the length of ITI treatment.

PO-14

IMMUNE TOLERANCE INDUCTION WITH NUWIQ® (SIM OCT OCOG ALFA) IN NINE PATIENTS WITH SEVERE HEMOPHILIA A AND INHIBITORS TO FVIII

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Background/Aims: The development of inhibitors to coagulation factor VIII (FVIII) represents a serious concern in the treatment of children with hemophilia A. The only clinically proven strategy to eradicate such inhibitors is immune tolerance induction (ITI) using FVIII. Our aim was to assess ITI outcomes with Nuwiq®, a human cell line-derived recombinant FVIII, in patients who developed inhibitors during Nuwiq® treatment.

Patients and methods: Nine previously untreated patients with severe hemophilia A who developed inhibitors on Nuwiq® treatment received ITI with Nuwiq®. ITI success was determined based on achievement of an undetectable inhibitor titre (<0.6 BU/mL), FVIII recovery ≥66% and half-life ≥6 hours. Further assessments included bleeding rates, tolerability and safety.

Results: Patients were Caucasian (n=5), African (n=2), Arabic and Pakistani (n=1 each), aged 9–193 months at ITI start, and had had 9–33 exposure days prior to inhibitor detection. All patients had *F8* null mutations and peak inhibitor titres were 0.9–114 BU/mL. Six patients started ITI with 100 IU/kg Nuwiq} daily and one patient each received 100, 90 and 50 IU/kg every other day. Two patients achieved all three success criteria after 9 and 27 months of ITI. Four other patients achieved inhibitor titres <0.6 BU/mL after 1, 1, 3.5 and 6 months, and normalised FVIII recovery. One patient discontinued Nuwiq® ITI after 15 months due to an increasing inhibitor titre. Titres are declining in the remaining two patients. Seven patients experienced no bleeds during ITI, one patient had one bleed and one had two bleeds. Nuwiq® was well-tolerated and no adverse drug reactions were documented.

Conclusions: Six of nine patients receiving Nuwiq® ITI have achieved an undetectable inhibitor titre to date. These data suggest that Nuwiq® may be effective as ITI treatment in patients with hemophilia A and inhibitors.

Conflict of interests: RL is a clinical study investigator for the NuProtect Study (Octapharma-sponsored). She has received grants/research support from Octapharma, Bayer, Baxalta, Novo Nordisk and Roche and has acted as a consultant for Octapharma, Bayer and Baxalta. She has participated in speaker bureaus for Octapharma, SOBI and Novo Nordisk. MM has received grants/research support from CSL and Novo Nordisk and has acted as a consultant for Baxalta. She has participated in speaker bureaus for SOBI, Novo Nordisk and Baxalta. GWH has been a symposium speaker for Octapharma. AW and NB have no conflicts of interest to declare.

PO-15

POPULATION PHARMACOKINETIC MODEL FOR RECOMBINANT FACTOR VIII FC FUSION PROTEIN (RFVIIIFC) VALIDATED AND OPTIMIZED FOR USE IN CHILDREN

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Background: Pharmacokinetic (PK)-guided dosing can aid in individualizing replacement therapy for hemophilia patients. The current population PK models used for PK guided treatment are based on clinical trial data, whereas real clinical patients may differ significantly from carefully selected clinical trial populations. The aim of this study was to validate and potentially optimize a previously published rFVIIIFc population PK model with independent real-world clinical data.

Methods: Clinical data of hemophilia A patients treated with rFVIIIFc concentrate participating in the United Kingdom Extended Half-Life Outcomes Registry were collected from ten different UK treatment centers. FVIII levels were determined by one-stage or chromogenic assay. The predictive performance of the published model was assessed using mean percentage error (MPE, bias) and mean absolute percentage error (MAPE, inaccuracy). An alternative extended population PK model was developed using nonlinear mixed-effects modelling (NONMEM).

Results: Data of 43 hemophilia A patients (FVIII ≤2%), aged 5 to 70 years, were collected. The model could predict the 244 rFVIII-FC concentrations without significant bias (-4.3 ± 8.2%) and with sufficient accuracy (12.3 ± 2.2 %). However, when analyzing data in more detail, clearance and central distribution volume were under predicted in patients <12 years. An alternative model was created by modifying the relationship between bodyweight and both clearance and central volume and by re-estimating the PK parameters. The importance of this newly developed model was underlined by a clinical patient of 2 years, as only the new model showed reliable results.

Conclusions: The published rFVIIIFc model was developed for patients ≥12 years. Our study demonstrated that, this model will give erroneous results when applied in patients younger than 12 years. The developed alternative model allowed a better description for younger patients as well. This study demonstrates the importance of validating population PK models used in clinical routine.

PO-16

BRAZILIAN REGISTRY OF PERSONS WITH HEMOPHILIA A RECEIVING EMICIZUMAB (EMICIZUMAB CASES, EMCASE PROJECT)

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Emicizumab (MC-Ab) is a humanized bispecific antibody which binds to activated factors IX and X, speeding up the activation of factor X. It has bypassed some unmet needs in hemophilia A (HA) treatment, such as regimen (once weekly up to once monthly infusion) and route of administration (subcutaneous). Although it is an effective non-replacement alternative in the prophylaxis of people with HA (PwHA) with (PwHAi) or without inhibitor, its safety has not been clarified yet, and a few cases of thrombosis and development of anti-MC-Ab antibody have been described.

The aim of this project is to create a national registry to follow up PwHA receiving MC-Ab.

EMCase is an observational study and any PwHA receiving MC-Ab can be included. The treatment will be decided among the patient, the physician and the interdisciplinary team of the hemophilia treatment center (HTC). The research group will develop a brochure with suggestions on classical outcome assessment tools (e.g., bleeding rate, joint health, absenteeism, adherence, quality of life and mortality) which can be evaluated as the judgement of the HTC team. Outcome data, laboratory results and therapeutic progression will be compiled yearly over 10 years. Pharmacovigilance and economic analyses will also be included. Finally, a national guidance will be developed.

In Brazil, MC-Ab was approved in 2018 only for treatment of PwHAi. In 2015, there were 250 patients on immune tolerance and the failure rate of this treatment has been described about 20%. Consequently, we expect to register at least 50 PwHAi. The registry of non-inhibitor PwHA will depend on the approval of MC-Ab for the treatment such patients.

We expect to establish some outcome assessment tools and laboratory tests to aid the interdisciplinary team to manage hemophilia treatment with MC-Ab as well as to help to clarify the safety of this bispecific antibody.

PO-17

BASELINE PATIENT CHARACTERISTICS IN REITIRATE - A PROSPECTIVE STUDY OF RESCUE ITI WITH RECOMBINANT FACTOR VIIIFC (RFVIIIFC) IN PATIENTS WHO HAVE FAILED PREVIOUS ITI ATTEMPTS

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Background/Aims: Inhibitor development is the most serious complication of hemophilia A (HA) therapy. Immune Tolerance Induction (ITI) is the gold standard for inhibitor eradication, restoring factor VIII (FVIII) responsiveness. Retrospective data on ITI therapy using recombinant FVIIIFc fusion protein (rFVIIIFc) have been reported (Carcao, et al. Haemophilia. 2018). The ReITIrate study (NCT03103542) was designed to prospectively evaluate success of rescue ITI with rFVIIIFc.

Materials and Methods: ReITIrate, a prospective, interventional, multicentre, open-label study, enrolled severe HA inhibitor patients, who failed previous ITI attempts. The primary objective is to describe the outcome of ITI performed with rFVIIIFc (200 IU/kg/day) within a maximum of 60 weeks. Here, patient baseline characteristics are reported using descriptive statistics and listing.

Results: Sixteen subjects were included in the study between Nov 2017 and Dec 2018 (Table 1). The median (range) age at study enrolment was 7.5 (2–46) years. The median (range) number of prior ITI attempts was 1 (1–3) and the total median (range) ITI duration 51.5 (12-155) months. The median (range) inhibitor titre at screening and historical peak were 11 (0.9–635) BU/dl and 127 (8-3000) BU/dl, respectively. During the 12 months prior to enrolment the median (range) number of bleeds was 5 (0-24), 11 subjects used aPCC for treatment of bleeds, 5 received rFVIIa and 1 each FVIII/VWF, rFVIII, and tranexamic acid. Twelve subjects received prophylaxis with bypassing agents during this period (10 aPPC, 1 rFVIIa and 1 both products).

Patient No.	FVIII genotype	Family history for inhibitors	Age at enrolment (years)	Historic peak inhibitor titre (BU/ml)[a]	Products used for previous ITI (recombinant, plasma, or both)	Number of previous ITI attempts	Total duration of previous ITI (months)	Previous ITI with high dose	Previous immuno-modulation used	Inhibitor titre screening (BU/ml)[b]
1	Not available	Unknown	14	90	Recombinant	1	155	Yes	No	12
2	Not available	No	8	760	Both	1	74	Yes	No	21
3	Intron 22 inversion	Unknown	13	500	Both	3	26	Yes	Yes	25
4	Not available	Yes	7	3000	Recombinant	1	20	Yes	No	635
5	Big deletion	Unknown	11	1100	Plasma	2	65	Yes	No	126
6	Intron 22 inversion	No	7	1000	Both	2	62	Yes	Yes	4
7	Not available	Yes	4	20	Recombinant	1	57	Yes	No	1
8	Intron 22 inversion	Unknown	7	870	Both	1	67	Yes	No	85
9	Not available	Yes	4	20	Recombinant	1	44	Yes	No	17
10	Intron 22 inversion	Yes	46	100	Plasma	1	63	Yes	No	10
11	Not available	Yes	4	10	Both	2	44	Yes	No	1
12	Intron 22 inversion	No	16	110	Recombinant	1	12	Yes	No	46
13	Intron 22 inversion	No	2	140	Both	1	15	Yes	No	10
14	Frameshift	No	5	30	Recombinant	2	29	Yes	Yes	5
15	Frameshift	Yes	8	70	Plasma	1	46	Yes	Yes	1
16	Nonsense	Yes	12	210	Both	1	73	Yes	No	9

BU, Bethesda unit; ITI, immune tolerance induction.
[a]Rounded to the nearest 10; [b]Rounded to the nearest integer

Table 1. Baseline patient characteristics in ReITIrate

Conclusions: This is the first prospective study describing rescue ITI with an extended half-life rFVIII product. Enrolled subjects have multiple risk factors for poor ITI outcome and long duration of previous ITI. There is an unmet need for successful tolerisation in such patients, allowing regular FVIII prophylaxis and potentially leading to improved clinical outcomes and quality of life.

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PO-18

VISUAL INSPECTION OF THE APTT REACTION CURVE AS A RELIABLE SCREENING TOOL FOR DETECTING THE PRESENCE OF FVIII AND FIX INHIBITORS

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Background: Specific shapes and slopes of the aPTT reaction curve are characteristic for patients with congenital or acquired FVIII/FIX deficiency. Questionable aPTT results are flagged by the analyzer and should be visually inspected.

Aims: To investigate the possibility to detect the presence of FVIII/FIX inhibitors by analyzing flagged aPTT results after visual inspection of reaction curves and manual measurement of the reaction curve angle (RCA).

Materials and Methods: The study included 131 patient plasma samples with request for the determination of FVIII/FIX inhibitors. aPTT was performed using Actin FS on Atellica COAG 360 (Siemens, Germany) at 405 nm with manufacturer's proposed evaluation method DRIFTING BASELINE (DB) and checking method ANGLE DB that flagged aPTT results with RCA<30 degrees. All aPTT reaction curves were visually inspected and RCA were manually measured. FVIII and FIX activities were determined by one-stage clotting assay. Inhibitor titers were determined using Nijmegen modified Bethesda assay. Patients were divided according to the absence (group I, n=103) or presence (group II, n=28) of inhibitors.

Results: Excellent correlation between FVIII activities (median:11.0 U/dL, range:0.9–139 U/dL) and RCA (median:53 degrees; range:13.0–78.0 degrees) was obtained for all tested samples (r=0.932; P<0.001). Statistically significant difference of RCA (P<0.001) was identified between group I (median:58 degrees; range:13.0–78.0 degrees) and group II (mean:31.6 degrees; range:19.0–56.0 degrees). ROC analysis revealed that RCA of 51 degrees allowed distinguishing between two groups of patients with sensitivity of 96.4% and specificity of 67.0%, while predefined RCA of 30 degrees showed the sensitivity of 57.1% and specificity of 81.6%.

Conclusions: Based on obtained results, it may be concluded that ANGLE DB checking method, together with visual inspection of aPTT

reaction curve and angle measurement, provides a simple and reliable tool to screen for the presence of FVIII/FIX inhibitors.

PO-19

PROSPECTIVE STUDY OF THE IMMUNOLOGICAL RES PONSE TO FACTOR VIII IN SEVERE HEMOPHILIA A PATIENTS DURING IMMUNE TOLERANCE INDUCTION TREATMENT

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Background/Aims: The development of an immunogenic response to therapeutic FVIII, with the appearance of neutralizing antibodies ("inhibitors"), is the main adverse event in hemophilia A treatment. The only proven and currently available strategy to eliminate inhibitors is ITI, based on the long-term, intravenous administration of high-dose FVIII. The success rate of ITI is about 60-70 % and not much information is available about the potential factors of success and failure. The aim of our study is to investigate the immunological events that develop in these patients undergoing ITI and to understand which parameters influence the achievement of FVIII tolerance.

Materials and Methods: This is a multicenter, prospective cohort study that enrolled 20 severe hemophilia A patients with high-titer inhibitors, candidate to ITI. Blood samples were collected at different time points and total PBMCs were cultured with the FVIII product administered during ITI. Studies of gene expression profiling and phenotypic characterization were performed using immune cells. Multiplex cytokines arrays were also conducted to measure the cytokine levels in plasma samples and in cell culture supernatants. Plasma samples were also tested for anti-FVIII antibodies determination.

Results: We report the results of 7 patients that completed the ITI treatment, 3 of them successfully achieved tolerance while 4 failed to eradicate inhibitors. We found a sustained IgG4 anti-FVIII response, a production of pro-inflammatory cytokines and a consistent immune gene expression in patients who failed to induce FVIII-tolerance during the ITI. Notably, successfully tolerized patients experienced a decreasing anti-FVIII IgG4 response and an increased production of regulatory cytokines, such as TGF-β.

Conclusions: This ongoing study will provide us with more knowledge about potential biomarkers of success or failure and about which immune-regulatory mechanisms are missing in failure's cases.

PO-20

LIFE-ACTIVE: OBSERVATIONAL STUDY EVALUATING THE PHYSICAL ACTIVITY IN A SUBSET OF DAM OCT OCOG ALFA PEGOL TREATED HEMOPHILIA A PATIENTS WHO ARE ENROLLED IN THE HEM- POWR STUDY

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Background: BAY 94-9027, a site-specifically PEGylated recombinant factor VIII with an extended half-life, is approved for use in previously-treated patients aged ≥12 years with hemophilia A in Canada, Japan, the EU and the USA. The HEM POWR study will assess real-world clinical use, and the LIFE-ACTIVE sub-study will utilise an innovative new method to measure and precisely record patient activity. Here we describe the LIFE-ACTIVE rationale and design.

Materials & Methods: Patients within the HEM POWR multinational, non-interventional, open label study (N ≥200 planned) will be given the option of participating in LIFE-ACTIVE. Patients will wear an ActiGraph CP Insight activity-tracking smart watch continually for 30 day periods, from their initial visit, and then at months 12, 24 and 36. Measurements will include sleep quality and duration, physical activity intensity and duration and general mobility measurements. All data will be transferred to the secure, cloud-based CentrePoint system and patients will not be aware of the values measured by the device.

Results: It is expected that this innovative data collection approach will provide robust data in near real time. Proposed endpoints include physical activity, total steps (per minute/per day) and energy expenditure. This sub-study will investigate the relationship between daily regular activity, as measured by ActiGraph, and efficacy parameters collected from the HEM-POWR study (including bleeding rates). It is expected that positive changes, such as an increase in sleep length and increases in physical activity would correlate with an increased quality of life and improvement in clinical parameters.

Conclusions: ActiGraph will provide thorough, robust activity measurements, helping to accurately assess quality of life. LIFE-ACTIVE will provide insights into patterns of physical activity and sleep, and their relationship with clinical outcomes. This sub-study will also demonstrate how technology such as smart watches can improve data measurements and may positively influence patient compliance.

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PO-21

COMBINATION OF EMICIZUMAB WITH SIM OCT OCOG ALFA FOR PROPHYLAXIS IN PREVIOUSLY UNTREATED/ MINIMALLY TREATED HEMOPHILIA A PATIENTS, AND FOR MANAGING INHIBITOR PATIENTS

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Background/Aims: Development of neutralising antibodies to FVIII (inhibitors) is the major complication of hemophilia A (HA) treatment with FVIII concentrates. Simoctocog alfa (Nuwiq®) is a 4th generation recombinant FVIII manufactured in a human cell-line, with a low reported rate of inhibitors in previously untreated patients. Emicizumab is a non-factor bispecific monoclonal antibody for prophylactic treatment of HA patients with/without FVIII inhibitors. This study will investigate a) the combination of emicizumab and simoctocog alfa to determine the rate of inhibitor development while providing adequate bleeding protection in children with HA who have had little to no previous exposure to FVIII; and b) the use/outcome of immune tolerance induction (ITI) with simoctocog alfa in combination with emicizumab prophylaxis in patients with HA and inhibitors.

Materials and methods: This is a multicentre, US-based study. Part A will enrol 40 previously untreated/minimally treated patients <3 years of age with severe HA without an inhibitor. Participants will receive monthly emicizumab followed 3-6 months later by the addition of simoctocog alfa at low dose (25 IU/kg) every 2 weeks for up to 50

exposure days or 3 years. Part B will enrol 20 participants <21 years old with HA of any severity, with an existing inhibitor. Participants will receive weekly/biweekly emicizumab injections followed by ITI with simoctocog alfa at 100 IU/kg 3x/week for up to 12 months. If a participant in Part A develops an inhibitor, they may enter Part B.

Results: The primary endpoint in Parts A and B is the hemostatic efficacy of emicizumab based on the number of bleeding events requiring alternative haemostatic therapy. Secondary endpoints for Part A include immunogenicity of combination treatment and for Part B ITI success rates, safety, efficacy, and protocol feasibility.

Conclusions: This study will provide insight into novel combination treatment for prevention and eradication of FVIII inhibitors.

PO-22

TESTING CLOT GROWTH AND THROMBIN WAVE PATTERNS IN HEMOPHILIA PATIENTS

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Background and Methods: A new pharmacodynamic test system for coagulation has been developed in which simultaneously clot growth rate and thrombin wave patterns in space can be documented with a video-microscopic system/fluorogenic substrate: Thrombodynamics. Coagulation is activated from the surface by immobilised tissue factor with a density resembling cell-bound tissue factor.

Two systems regulate the thrombin waves and clot growth. One concerns the TF-VII activation of factor X which results in a thrombin wave pattern and clot growth that gradually dampens. The other concerns the cooperative action of factor VIII, IX and XI and clot growth is stationary for 1 ½ hr and clot waves are stable. The continued stable triggering for clotting in normal plasma is derived from the thrombin-factor XI.

Results: Suppletion of deficient factors in hemophilia plasma results in congruent curves for plasma derived, recombinant and modified products (extended half-life molecules and Hemlibra). An advantage of the thrombodynamics is that factor XII/prekallikrein are not involved. It avoids artefacts of calcium-independent and dependent effects of kallikrein in the two step OCT test for some of the extended half-life products.

Inhibitors of factors VIII, IX and XI can be quantified with the Bethesda principle. Modified preparations of factor VIII or IX show reduced inhibitory effect by acquired inhibitors.

Unlike chromogenic and OCT, designed for evaluating a single factor, the clot growth results are a cooperative effect of all three factors VIII, IX and XI as shown in simulation experiments.

Conclusions: Clot growth and thrombin wave analysis provide a new promising pharmacodynamic test for assessing several aspects of the clotting and replacement treatment in hemophilia patients. The determination of the combined effect of factor VIII, IX and XI may provide a measure that correlates better with bleeding and would be of high interest to test in practice.

PO-23

USE OF CHROMOGENIC FACTOR VIII ACTIVITY DETERMINATION IN HEMOPHILIA A PLASMA OF PATIENTS UNDER EMICIZUMAB TREATMENT

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Background: Factor VIII activity (FVIII:C) assays of samples containing recombinant FVIII or the new bispecific antibody emicizumab (Hemlibra) can be associated with differences in FVIII recovery in vitro between one-stage-clotting-assays and chromogenic assays. Chromogenic assays designed with human proteins, offer the possibility to measure the activity and recovery of this new drug in patient samples.

To monitor FVIII-INH titers with Nijmegen-Bethesda-Assays in hemophilic patients treated with emicuzumab, it was shown that only chromogenic assays with bovine proteins can be used.

Aim: Aim of this study was to investigate if chromogenic assay containing both, human and bovine proteins, can be used to measure the recovery of emicizumab in patient samples or the assay can be used in FVIII-INH determination by Nijmegen-Bethesda-Assay.

Methods: Plasma from Hemophilia-A patients was used for spiking with Refacto, Fanhdi and emicizumab. The FVIII activity was determined with one-stage-clotting-assay and a chromogenic assay with human FIXa and bovine FX protein. Both assays were calibrated with reference plasma prepared from normal pooled plasma. FVIII-INH was determined by modified Nijmegen-Bethesda-Assay.

Results: The regression parameters of the chromogenic FVIII activity determination of Hemophilia-A sample spiked with increasing concentrations of Fanhdi and Refacto showed a good correlation with a slope of 0.91 and r2= 0.9951 for Fandhi and slope 0.71 and r2 = 0.995 for Refacto. As expected the one-stage-clotting-assay underestimates Refacto in patient plasma, as shown by a slope of 0.4.

Chromogenic FVIII assay showed no significant sensitivity to emicizumab concentrations within the expected therapeutic range and recovery of FVIII-INH titer was 100±5%.

Conclusion: Chromogenic assay with both human FIXa and bovine FX protein can be used for therapy monitoring of full length and truncated FVIII preparation but is insensitive to emicizumab concentrations in therapeutic range. FVIII-INH titer in chromogenic Nijmegen-Bethesda-Assay is insensitive to emicizumab.

PO-24

VERY LOW RATE OF INHIBITOR IN IVOIRIAN PUPS AND MTPS AFTER INITIATION OF PROPHYLAXIS WITH EXTENDED HALF-LIFE FACTOR VIII AND IX IN THE SETTING OF THE WFH DONATION PROGRAM

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In 2019, 43 boys with severe hemophilia under 12 years of age are regularly followed at the Hemophilia Treatment Centre of Yopougon, Abidjan. In Côte d'Ivoire there is limited access to clotting factor concentrates (CFC).

World Federation of Hemophilia (WFH) humanitarian aid program allowed to initiate prophylaxis in PUPs and MTPs in Côte d'Ivoire since January 2018. Candidates were selected according to the following criteria: young age, absence of advanced arthropathy, severe bleeding phenotype (annual joint bleeding rate (AJBR) > 10), absence of inhibitor, parents' motivation, access for regular inhibitor screening, and peripheral venous access. The regimen was once a week 15-30 U/kg Fc-FVIII for HA and once every 10 days 20-30 U/Kg for Fc-FIX. Inhibitor screening by mixing studies was repeated every 5 exposure days (ED).

Between January 2018 and January 2019, 22 boys (19 HA - 3 HB), median age 52 months, started primary prophylaxis. One boy aged 10 years old started secondary prophylaxis because of high bleeding rate and had approximately 50 ED before initiation of prophylaxis. Thirteen have already been on prophylaxis for 15 months. More than 50 % achieved an AJBR of 0 during the follow-up period.

Among primary prophylaxis group, none developed an inhibitor after 15 months. A low titer (1 Bethesda Unit) inhibitor was detected after 4 months in the patient on secondary prophylaxis, despite a significant clinical improvement (no spontaneous bleeds). In this subject, the presence of an inhibitor could not be detected at inclusion. However, detection of low titer inhibitors can be challenging when using manual technique.

This study demonstrates the very low rate of inhibitor development in African PUPs and MTPs treated with a low dose primary prophylaxis with extended half-life FVIII and IX. Although preliminary and limited, these results are encouraging in promoting prophylaxis using donations of the WFH.

PO-25

GLOBAL HEMOSTATIC METHODS IN PATIENTS WITH INHERITED HEMOPHILIA WITH INHIBITORS AND WITH ACQUIRED HEMOPHILIA; A TWO-CENTRE STUDY

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Background/Aims: Since there are no reliable methods that assess the effects of bypassing agents in patients with hemophilia with inhibitors, the present study aims to investigate the utility of global hemostatic methods in this context.

Materials and methods: This is a non-interventional study, NCT02453542. All patients aged 6 years and above with hemophilia with inhibitors and acquired hemophilia that are followed up at the Coagulation Department or the Pediatric Coagulation Unit of the Karolinska University Hospital and the Clinical Centre Hospital, Zagreb, Croatia respectively, were eligible for inclusion. Blood samples were drawn prior (baseline) and following administration of bypassing agents and factor concentrates. Baseline samples were spiked with bypassing agents in increasing concentrations (aPCC, FEIBA® 50 U/kg, 100 U/kg, 150 U/kg and rFVIIa, NovoSeven® 90 µg/kg and 270 µg/kg). All samples were subsequently analysed by global hemostatic methods (Overall hemostatic potential, OHP, Endogenous thrombin potential, ETP and Calibrated Automated Thrombogram, CAT).

Results: Overall, ten patients with inherited (median age 10.5 years (6-79 years)) and sixteen patients with acquired (median age 66 years (51-81 years) hemophilia were included. The results are presented as difference in the coagulation potential before and after spiking due to the different wash-out periods and treatments. The results for OHP are presented in Table 1. None of the differences were statistically significant. CAT was more reliable in exhibiting the coagulation potential at baseline and following spiking, whereas ETP showed higher baseline hemostatic potential and displayed the increase following administration of aPCC but not rFVIIa.

Inherited	BL-F_50	BL-F_100	BL-F_150	BL-N7_90	BL-N7_270
OCP	19.87	18.43	17.95	17.34	17.95
OHP	12.22	13.67	14.04	9.09	13.32
OFP%	-26.03	-32.35	-33.45	-34.80	-32.70
Acquired	**BL-F_50**	**BL-N7_90**			
OCP	165.6	132.88			
OHP	43.65	18.11			
OFP%	-53.5	-43.4			

Table. Difference in Overall Coauglation Potential (OCP), Overall Hemostatic Potential (OHP) and Overall Fibrinolysis Potential (OFP) before (BL) and after spiking with bypassing agents in patients with inherited haemophilia with inhibitors and acquired haemophilia. BL: baseline, F: FEIBA® , N7; NovoSeven® . Final concentrations for FEIBA® : 50 U/kg, 100 U/kg, and 150 U/kg. Final concentrations for NovoSeven® : 90 µg/kg, 270 µg/kg.

Conclusions: Both rFVIIa and aPCC normalized the coagulation potential in a similar way and already at the lowest concentrations. CAT, but not ETP was indicative of the baseline coagulation and its increase following spiking, which could reflect differences in preanalytical variables and the concentrations of tissue factor used in the assays. The more profound increase observed in OCP in patients with acquired hemophilia is probably secondary to higher baseline factor levels. OHP and CAT have the potential to be used as complement tools when making decisions on treatment changes.

PO-26

CREATION OF TECHNOLOGY PRODUCTION OF COAGULATION FACTOR VIII: THE CHOICE OF METHODS

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Background: Nowadays, protein concentrates prepared from human plasma play a vital role in the treatment of patients with different diseases. The importance of factor VIII (FVIII) is obvious through a series of clinical problems in the FVIII deficiency diseases, in particular classic hemophilia A and von Willebrand's disease.

Aims: to investigate a comparative study activity of FVIII in combination of different stages of purification.

Methods: pre-fractionation of plasma; ion-exchange chromatography; dye-ligand affinity chromatography.

Results: Research demonstrated that the process of purification of FVIII by the use dye-ligands is due to the phenomenon of the negative affinity adsorption. In particular, the application of the affinity chromatography directly (Experiment I, Table 1) allowed to achieve a maximum purification approximately 8.33 fold.

We reached hundredfold clearing of the FVIII by the use of the pre-fractionation with the method of negative affinity sorption (Experiment II, Table 1).

When combined with ion-exchange chromatography and affinity chromatography, FVIII has been purified by 129 to 242-fold (Experiment III, Table 1).

It has been found that the combination of pre-fractionation, ion-exchange and affinity chromatography stages provided a purification rate approximately 239–700-fold (Experiment IV, Table 1).

Sorbents	Experiment I	Experiment II	Experiment III	Experiment IV
	Degree of purification, fold			
Diasorb-Active purple 4GT	5.78	110.59	242.10	702.05
Diasorb-Procion Blue HB	3.92	100.59	129.44	343.06
Diasorb-Procion Gelb M4R	6.28	93.35	166.52	499.44
Diasorb-Procion Blue MXR	8.33	94.67	154.60	612.92
Diasorb-Active bright K	5.92	97.05	179.54	609.02

Table 1. Comparison of FVIII purification depending on receiving methods

Conclusion: A new procedure that includes stages of preliminary fractionation, ion exchange and affinity chromatography has been developed to produce a highly purified FVIII concentrate from plasma of blood. FVIII has been purified from 343.06 to 702.05-fold depending on the type of selected sorbents.

PO-27

PREVALENCE OF FVIII AND FIX INHIBITORS IN ONE POPULATION OF THE WEST ALGERIA BETWEEN 2011 AND 2017

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Background: The development of inhibitors against factor VIII (FVIII) replacement therapy remains the most important challenge for clinicians in the treatment of hemophilia patients. This study aimed to determine in a population of West Algeria the prevalence of FVIII and IX inhibitors. This cross-sectional study evaluated the prevalence of hemophilia A and B in the population of West Algeria.

Methods: We included 80 patients with hemophilia (2 to 55 years),

62 patients with hemophilia A (77.5%), and 18 patients with hemophilia B (22.5%). The patients exhibited severe factor VIII or IX activity (<1%; 53 patients 66.5%), moderate activity (1–5%; 23 patients; 28.75%), and mild activity (4 patients; 5%). Among the patients with care-related data, most patients were treated for episodic bleeding or received prophylaxis (16.5%). Among the patients with source-related data, the factor replacements were derived, recombinant concentrates VIII or IX (22.9%), Novoseven (2.5%).

Results: FVIII inhibitors were observed in 12 (19.3%) of the 62 patients with hemophilia A, and none of the 18 patients developed FIX inhibitors. Most patients who developed inhibitors had severe hemophilia, and inhibitors were also common among patients who received recombinant products. DNA was isolated from peripheral blood in severe hemophilia A with inhibitors and the target gene fragment was amplified by PCR, in combination with the second-generation sequencing, 22 and 1 introns were detected in 50%.

Conclusion: The prevalence of factor inhibitors in West Algeria was similar to those among other ethnic populations.

PO-28

FREQUENCY OF FVIII INHIBITOR AMONG JORDANIAN CHILDREN WITH HEMOPHILIA A

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Aims: The aim of the study is to determine the frequency of factor VIII (FVIII) inhibitor, among children with hemophilia A (HA), at the Jordanian royal medical services.

Materials and methods: A total of 165 (age range: 1 month-16 years) previously treated HA patients were tested for FVIII inhibitor, between 2003 -2018. Venous blood samples were collected in 0.109M (3.2%) tri -sodium citrate tubes. FVIII inhibitor screening assay, using APTT based mixing study, followed by Bethesda assay in inhibitor positive samples, to quantify FVIII inhibitor titre in Bethesda units (BU) were performed. Samples were labeled as low-titre and high-titre when BU less than or equal 5 BU/ml and more than 5 BU/ml, respectively.

Results: Of the total 165 HA patients, 111(68.3%), 27(15.7%), and 28(16%) had severe, moderate and mild type of the disease, respectively. Twenty (12.1%) out of the total 165 patient were tested positive for FVIII inhibitor. The mean age was (5.1± 4.89). Of the 20 patients 18 (90%) had severe disease and 2 (10%) had moderate disease. None of the mild type patients were tested positive. FVIII inhibitor levels ranged between 1.8 to 563 BU/ml, 90% of which were high-titre and 10% were low-titre. Three families in the study were having two siblings with severe disease who were tested positive for FVIII inhibitor.

Conclusions: The frequency of FVIII inhibitor in our study is lower than in other children. Severity of disease was found to be a main contributing factor for FVIII inhibitor development.

PO-29

IMMUNE TOLERANCE INDUCTION RESCUE (ITI-R) WITH HUMAN-CL RHFVIII IN HEMOPHILIA A PATIENTS AND HIGH-TITRE INHIBITORS

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Background: The appearance of inhibitors is the most serious complication in hemophilia A. The primary objective is their eradication. When primary ITI fails, another ITI-R is often started. We report three cases of ITI-R with the human cell line-derived recombinant FVIII (human-cl rhFVIII), simoctocog-alfa.

Methods: Three patients with severe hemophilia A who developed high-titre inhibitors to different FVIII concentrates were treated with simoctocog-alfa for ITI-R. Complete success of ITI-R was assessed based on achievement of an undetectable inhibitor titre (<0.6 BU/mL), FVIII in vivo recovery (IVR) ≥66% and half-life ≥6 hours.

Results: At the onset of ITI-R with simoctocog-alfa two patients were children and one was a 48-year-old adult. The children had failed a previous ITI with the same FVIII concentrate that caused the inhibitor development, while the adult patient had failed two consecutive ITI performed with plasmaderived FVIII enriched by von Willebrand factor (pdFVIII/vWF) and with moroctocog-alfa, respectively. All patients presented at least three risk factors for a poor-prognosis ITI-R: previous ITI failed, age >7 years, more than 2 years since inhibitor diagnosis and ITI start. In all cases the historical peak was ≥10 BU/mL, ITI-R treatment consisted of 200IU/kg simoctocog-alfa daily for all three patients. One child has achieved complete tolerisation, with an undetectable inhibitor titre, an IVR≥66% and a half-life ≥6 hours after 6 months. The remaining two patients presented an initial disappearance of inhibitors after 7 and 22 months, respectively, without IVR and half-life normalisation. The inhibitor titre subsequently increased, reaching 1.9 BU/ml and 1.0 BU/ml, respectively. In these two cases the ITI-R were then considered as a partial response to treatment.

Conclusions: A treatment with human-cl rhFVIII could be an important option in achieving a complete or a partial immune tolerance also in a population of patients with high-titre inhibitors, and presenting some risk factors for poor ITI prognosis.

PO-30

MANAGEMENT OF ACQUIRED HEMOPHILIA A: A CASE SERIES

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Introduction: Acquired hemophilia A (AHA) is a rare hematologic disorder potentially leading to life-threatening bleeding. AHA is caused by inhibitors against factor VIII (FVIII) and it typically manifests itself through spontaneous or post-surgery bleeding in patients with no personal history of haemorrhages. AHA is notoriously associated to specific risk factors, such as pregnancy and malignancies, but its aetiology remains unclear in half of the cases. The therapy is based on management of underlying conditions, immunosuppressive drugs and, in acute haemorrhagic episodes, on haemostatic treatments.

Material and methods: Data about patients with newly diagnosed AHA visiting our Unit in a period ranging from January 2009 and December 2018 were retrospectively collected.

Results: Twelve patients (M:6, F:6) were included in the research. Baseline characteristics and treatments are described in Table 1. The median age at diagnosis was 75 years old (range 38-92). Eleven patients were older than 70 years. The only young patient in the cohort was a woman presenting post-partum FVIII inhibitors. Eleven patients showed symptoms of haemorrhages at diagnosis and major bleedings were observed in 83% of cases. Associated medical conditions were identified in four cases, whereas eight cases were idiopathic. All patients were treated with immunosuppressive treatment (steroids alone or in association with other immunosuppressant). Haemostatic therapy with bypassing agents (aPCC or rFVIIa) was administered to seven patients. No patient was treated with rpFVIII. Symptomatic relapse during or after treatment occurred in three patients. One suspected thrombotic event was reported during therapy with rFVIIa. Treatment failure and death for bleeding causes were described in two cases.

Conclusions: AHA still represents a therapeutic challenge. Treatment failure, relapses and thrombotic side effects of bypassing agents are common issues. The development of new therapeutic strategies, such as rpFVIII, represent a great progress in treating AHA, especially in patients experiencing these complications.

PO-31

MOTIVATE: MODERN TREATMENT OF INHIBITOR- POSITIVE PATIENTS WITH HEMOPHILIA A – AN INTERNATIONAL OBSERVATIONAL STUDY

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Background/Aims: Development of neutralising inhibitors against factor VIII (FVIII) is the most serious complication of hemophilia A (HA) treatment. Immune tolerance induction (ITI) with FVIII remains the only clinically proven strategy for eradicating FVIII inhibitors. Emicizumab, a bispecific monoclonal antibody to factors IXa and X, was recently approved for bleeding prevention in people with HA with and without inhibitors. This study aims to capture different approaches to the management of patients with HA and inhibitors, document current ITI approaches, and evaluate efficacy and safety of ITI and/or emicizumab.

Patients and Methods: MOTIVATE is an international observational study in patients with HA and FVIII inhibitors. Target enrolment is ≥120 patients. Patients will be allocated to three groups, according to treatment, and followed for 5 years. All treatment is at the investigator's discretion. Group 1 will be treated with standard ITI protocols using the recombinant FVIII simoctocog alfa (Nuwiq®) or plasma-derived FVIII containing von Willebrand factor (octanate® or wilate®). Group 2 will receive ITI with one of these products alongside emicizumab prophylaxis. Group 3 will receive prophylaxis with emicizumab or bypassing agents (BPAs), without ITI.

Results: The primary endpoints are ITI outcomes with and without emicizumab, and annualised bleeding rates across all treatment groups. Secondary endpoints include time to ITI outcome, ITI relapse rate, frequency and severity of bleeding, use of BPAs and Hemophilia Joint Health Score. Safety will be assessed by monitoring adverse drug reactions, particularly thrombotic events. Optional sub-studies include activated protein C plasma levels, VWF antigen/activity, D-dimer plasma levels, joint health biomarkers, *F8* gene mutations, FVIII inhibitor epitope mapping, thrombin generation and batch selection.

Conclusions: MOTIVATE will collect real-world clinical experience on approaches for the management of patients with HA and inhibitors. The results will facilitate discussions on future standard of care in this population.

PO-32

STRUCTURAL EVIDENCE OF AN ANTI-FACTOR VIII C1 DOMAIN ANTIBODY BOUND TO B DOMAIN-DELETED FVIII

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The most common epitopes for neutralizing anti-factor VIII (FVIII) antibodies (inhibitors) are localized to the A2 and C2 domains, which are shared amongst both acquired hemophilia A and congenital hemophilia A patients. These inhibitors commonly interfere with binding of FVIII to activated factor IXa, platelet membrane surfaces (PS) and von Willebrand factor (VWF). By contrast, recent evidence indicates that the C1 domain contributes to the inhibitor response, potentially disrupting the ability of FVIII to bind PS and/or VWF, blocking the endocytosis of FVIII by antigen presenting cells, or serving as a weak inhibitor that rapidly accelerates FVIII clearance. Epitope localization for anti-FVIII inhibitors has largely been characterized through ELISA-based detection with human/porcine chimeric FfVIII hybrids or H/D exchange protection patterns with LC-MS/MS detection. Previous structural findings of antibody epitopes have been limited to complexes between anti-C2 domain inhibitors and the isolated FVIII C2 domain. In this study, we have determined the structure of an anti-C1 domain pathogenic inhibitor (2A9) in complex with a bioengineered construct of FVIII (ET3i). The 2A9 inhibitor shows weak inhibition of FfVIII binding to both PS and VWF, and mutational analysis localizes the epitope to F2068, which overlaps with anti-C1 antibodies present in hemophilia A patients. This structure shows that the epitope directly interacts with F2068 in the C1 domain, but also makes specific contacts with the adjacent A3 domain. Overall, this study represents the first antibody complex with an intact construct of B domain-deleted FVIII and lends structural evidence to the anti-C1 domain pathogenic immune response.

SESSION 2

PO-33

APAC, A DUAL ANTIPLATELET AND ANTICOAGULANT - TOWARDS A L OCAL, VASCULAR TARGETING ANTITHROMBOTIC AGENT?

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Background: APAC, developed as a mimic of heparin proteoglycans, has antiplatelet and anticoagulant action (collagen and thrombin) in several *in vitro* and *vivo* models(1). APAC alone inhibits both platelet and fibrin accumulation on collagen and tissue factor under a high shear rate (1000 1/s), and retained primary adhesion occurs. Moreover, APAC protects kidneys from ischemic reperfusion injury.

Aims: We present APAC and its antithrombotic data on platelet aggregation and global coagulation in vitro, vascular targeting and antithrombotic role in vivo, including toxicology margins.

Methods: APAC was studied in platelet aggregation in response to collagen and ristocetin in whole blood (Multiplate). platelet-rich plasma (PRP) and in rotational thromboelastometry (ROTEM) compared with citrated blood and spiking. Porcine balloon and arterio-venous fistula sites were exposed to labelled APAC. Single and repeated dose toxicology of rodents and monkeys was assessed.

Results: APAC dose-dependently attenuates aggregation to collagen and ristocetin, unlike heparin, in citrated blood at 150 µg/ml by 58 ±15% (n=6) (mean ± SD) and by 25 ±2%, respectively. In PRP inhibition of collagen-induced aggregation was reached at 1 µg/mL by 55±31% to 30 µg/mL by 85±11% (n=9). APAC alone, also dose-dependently attenuated aggregation and ROTEM variables in whole blood. In ROTEM APAC alone (3-16 µg/mL) reduces INTEM, and acts as a broad platelet-dependent procoagulant. APAC co-localized with Von Willebrand Factor and laminin in porcine arterial injuries, but not with intact endothelium. Upon targeting vessels, APAC locally decreased platelet accumulation and overt thrombosis in a rat model of anastomosis but retained at anastomosis sites despite high shear blood flow. Single and multiple (7-14 d) doses (3-20 mg/kg in monkeys and rodents) indicate wide safety without accumulation.

Conclusions: With APAC's unique profile, local integration and inhibition of platelet-mediated thrombosis may benefit vascular interventions.

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PO-34

SINGLE-SAMPLE DETECTION OF ANTI-ADAMTS13 AUTOANTIBODIES USING FIBER-OPTIC SURFACE PLASMON RESONANCE TECHNOLOGY

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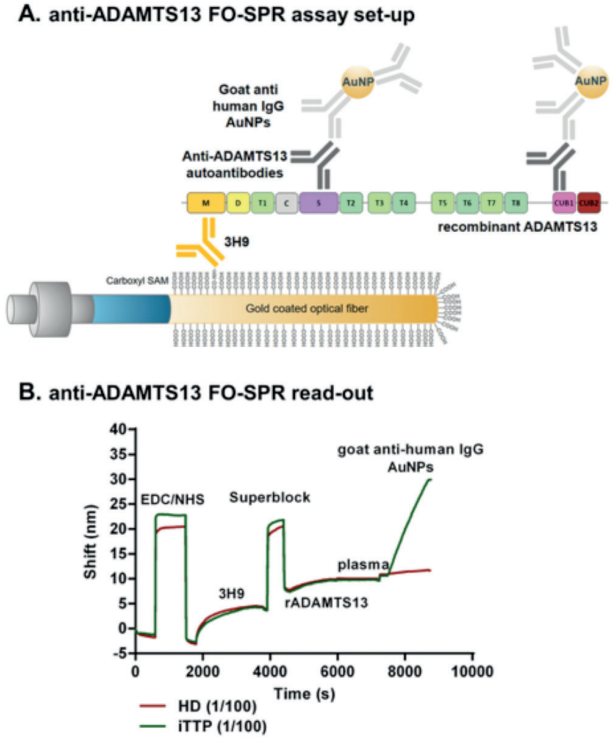
Background/Aim: Determination of ADAMTS13 activity, antigen and anti-ADAMTS13 autoantibodies is needed for the diagnosis, prognosis and treatment of the rare disease thrombotic thrombocytopenic purpura (TTP). ADAMTS13 parameters are, however, not always available on demand as current assays are complex and therefore only performed in expert centers and when multiple samples are available. Our goal is to develop fast and easy-to-use ADAMTS13 assays that allow single sample measurements using the fiber-optic surface plasmon resonance (FO-SPR) technology (White Fox 1.0 device). In this study we aimed at developing the anti-ADAMTS13 autoantibody assay.

Methods: Using the White Fox 1.0 device, anti-ADAMTS13 antibody 3H9 was covalently coupled to carboxyl self-assembling monolayer gold-coated optical fibers using EDC/NHS chemistry. After blocking, recombinant (r)ADAMTS13 was captured. Next, iTTP plasma was added and signal amplification of the bound autoantibodies was generated using anti-human IgG gold nanoparticles. Anti-ADAMTS13 autoantibody titers were determined in six iTTP plasma samples using both our FO-SPR assay and our in-house ELISA. The calibration curve in both assays was established using different dilutions of a high titer iTTP plasma sample (set at 100%). Healthy control plasma was used to determine the detection limit of the FO-SPR assay. The inter-assay %CV was assessed by performing four repeats of one iTTP sample.



Results: Effective immobilization of 3H9 and subsequently rADAMTS13 on the optical fiber was obtained, followed by specific signal amplification after addition of iTTP plasma. The detection limit of the assay was 1.14% and inter-assay %CV was 12.49%. Anti-ADAMTS13 autoantibody titers (ranging from 3.16 to 95.40 %) obtained via FO-SPR showed significant correlation with titers obtained in ELISA ($p < 0.05$, $r = 0.94$).

Conclusions: A new FO-SPR anti-ADAMTS13 autoantibody assay has been established, which allows fast and easy single sample measurements. We are currently developing the ADAMTS13 activity and antigen assays on this device.

PO-35

LONG-TERM NEUROPSYCHOLOGICAL SEQUELAE, EMOTIONAL WELLBEING AND QUALITY OF LIFE IN PATIENTS WITH ACQUIRED THROMBOTIC THROMB OCYTOPENIC PURPURA

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Background: Neurological symptoms related to microthrombosis are the hallmark of acute manifestations of acquired thrombotic thrombocytopenic purpura (aTTP). Despite the achievement of hematological remission, patients may report persisting neurological impairment that affects their quality of life.

Aims: To assess the long-term neuropsychological consequences, emotional wellbeing and Health-related Quality of Life (HrQoL) in survivors of acute aTTP.

Methods: Thirty-five patients who experienced an acute aTTP episode between 2004 and 2016 (29 first and 6 relapsing episodes) underwent a comprehensive psychological evaluation of memory and attentional functions, emotional wellbeing and HrQoL at least 3 months after the event (median 36 months, interquartile range 17-54). Memory functions were evaluated with the digit span and the word Rey list tests, attentional functions with the Trail Making Test A and B. Emotional wellbeing was assessed with the Hamilton anxiety and depression scales, HrQoL with the Short-Form 36.

Results: During the psychological consultation, 17 (49%) patients referred persisting subjective neurological impairment in remission phase, with at least one symptom as confusion, loss of concentration, dizziness, lack of balance, headache, and diplopia. Neuropsychological assessment revealed lower scores than the Italian general population pertaining to direct, indirect and deferred memory (Table 1). A higher degree of impairment of memory domains was found in the 22 patients with neurological involvement at the time of presentation of the first acute TTP episode. Anxiety and depression symptoms were detected in 7 (20%) and 15 (43%) patients, respectively. HrQoL was lower than the Italian general population, with mental domains more impacted than physical domains (mean difference 58.43, 95% confidence interval 71.49 to -45.37).

Test	TTP patients Mean (SD)	General population Mean	Mean difference (95% CI)
Memory			
Digit span (direct)	5.7 (1.1)	7.0	-1.3 (-1.6 to -0.9)
Digit span (backward)	4.5 (1.5)	6.0	-1.5 (-2.0 to -1.0)
Rey word list (direct)*	26.4 (7.7)	32.2	-5.9 (-8.6 to -3.2)
Rey word list (deferred)*	4.0 (1.9)	5.7	-1. 7 (-2.3 to -1.0)
Attention			
Trail making A, seconds	34.4 (15.1)	45.0	-10.6 (-15.8 to -5.4)
Trail making B, seconds	214.1 (52.0)	149.0	65.1 (47.2 to 82.9)

In memory tests a lower score indicates a worse performance, in attention tests a higher score indicates a better performance.

*Available in 34 TTP patients.

Abbreviations: CI, confidence interval; SD, standard deviation; TTP, thrombotic thrombocytopenic purpura.

Conclusions: Survivors of acute aTTP showed compromised memory and attention functions, anxiety/depression symptoms and a generally reduced quality of life. New clinical strategies should be considered to improve these symptoms.

PO-36

TYPE 1 VWD CAUSED BY A NOVEL DOMINANT P.THR274PRO MUTATION L OCALIZED IN *VWF* PROPEPTIDE

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Background: VWF propeptide (VWFpp) has an important role in the intracellular processing of VWF. After secretion into plasma it circulates independently from VWF. VWFpp mutation have been previously associated with types 1, 3 and 2A(IIC) VWD.

We herein describe the characterization of two unrelated Italian patients affected with type 1 VWD (VWD1), carrying the same novel variant localized in the VWFpp.

Aim: Molecular and functional characterization of two patients carrying the p.Thr274Pro variant in the VWFpp.

Materials and methods: Conventional phenotype tests were carried out to evaluate patients' plasma and platelets as recommended by ISTH-SSC guidelines. Molecular analysis was performed using next-generation sequencing. *In silico* tools (SIFT, polyphen2.0, SNP&GO, MutationTaster and Pmut) were used to predict the likely damaging effects of this substitution. For the *in vitro* expression studies the pcDNA3.1-VWF-WT and mutant pcDNA3.1-VWF-Thr274Pro expression vectors were transiently transfected into HEK293 cells. The amount of mutant Thr274Pro recombinant (r)VWF and hybrid Thr274Pro/WT-rVWF, which mimics patients' heterozygous state, were reported as a percentage of the WT-rVWF set as 100% ±SD.

Results: Biochemical analysis of plasma VWF indicated a VWD1 diagnosis (Table 1). A novel VWF variant in heterozygous state was identified in patients' VWFpp (c.820A>C; p.Thr274Pro). The potential damaging effect of p.Thr274Pro was predicted by in silico tools and then confirmed with in vitro expression studies. The amount of secreted mutant Thr274Pro-rVWF was reduced to 21±2%, whereas the hybrid Thr274Pro/WT-rVWF showed a secretion reduction of 36±4% in accordance to patients' plasma VWF:Ag levels. The amount of rVWF in cell lysates was nearly normal for both Thr274Pro-rVWF and Thr274Pro/WT-rVWF (62±17% and 72±23% respectively).

Pt	ABO	FVIII:C (IU dL^{-1})	VWF:Ag (IU dL^{-1})	VWF:RCo (IU dL^{-1})	VWF:CB (IU dL^{-1})	VWFpp/VWF:Ag	Multimer analysis	Platelet VWF:Ag (IU/10^8 platelets)	Platelet VWF:RCo (IU/10^8 platelets)
I	Non-O	69	32	27	26	1.2	Normal	0.20	0.10
II	O	109	35	34	29	0.9	Normal	0.10	0.10
N.R.	-	50-150	40-169 * 55-165 †	41-160 * 53-168 †	45-170* 56-174†	0.6-1.6	-	0.20-0.54	0.16-0.57

Pt, patient; **ABO**, blood group system; **FVIII:C**, factor VIII coagulant activity; **VWF:Ag**, von Willebrand factor antigen; **VWF:RCo**, von Willebrand factor ristocetin cofactor activity; **VWF:CB**, von Willebrand factor collagen-binding activity; **VWFpp**, von Willebrand factor propeptide; **N.R.**, normal range.
Plasma multimer analysis was performed both at low (HGT 1.2%) and intermediate (LGT 1.6%) resolution.
* range values of normal individuals with blood group O; † range values of normal individuals with blood group non-O;
Values are shown as a mean of three measurements in three different samples. Patients' platelets were isolated once.

Table 1. Biochemical data of the two patients.

Conclusions: We identified a novel VWF mutation localized in the VWFpp region in two unrelated Italian patients. In vitro expression studies showed the dominant effect of p.Thr274Pro and supported the VWD1 diagnosis of the patients.

PO-37

EXPLORATION AND TREATMENT STRATEGIES FOR GASTROINTESTINAL BLEEDING IN VON WILLEBRAND DISEASE OVER A 3-YEAR PERIOD: A RETROSPECTIVE NATIONAL SURVEY FROM THE FRENCH REFERENCE CENTER FOR VWD

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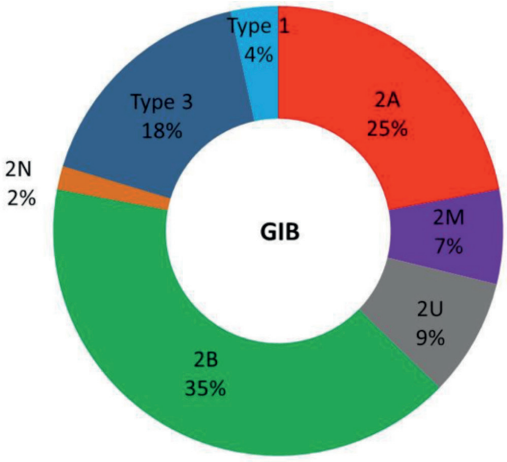
Background: Gastrointestinal bleeding (GIB) has been reported as the 1st cause of hospitalization in VWD patients with recurrences even under VWF prophylaxis. However, guidelines for exploration and treatment of GIB are lacking in VWD.

Aim: To investigate over a 3 year-period (2015-2018) the current clinical practice regarding GIB exploration and treatment among the network of French comprehensive care centers for VWD.

Methods: A 15-item mail-based survey was sent to 30 centers between December 2017 and February 2018.

Results: A total of 21 centers (70%) completed the survey. All centers had access to a digestive intensive care unit and to the full set of endoscopic exploration including video capsule endoscopy (VCE). During the last 3 years, 137 GIB episodes needing exploration were reported in 54 patients (mean = 0.85/patient-year). VWD2A, 2B and 3 were more frequently associated with both GIB (78% of episodes; Figure) and a younger age when compared to other subtypes, even after exclusion of VWD type 3 patients (p< 0.01). Pediatric cases were only observed in VWD2A, 2B and 3 (n=6). 32 of 54 patients were receiving VWF concentrates prophylaxis, including 11 for whom GIB was the reason to initiate prophylaxis. In patients without any gastrointestinal bleeding identified after conventional endoscopy, a VCE was performed either at 1st episode or after 1st recurrence of GIB in 40% and 70% of cases, respectively. The presence of angiodysplasia was confirmed after VCE in 19 of 54 patients of whom 15 had VWD2A, 2B or 3. 7 VWD-patients with angiodysplasia were receiving prophylaxis alone or with antiangiogenic drugs (n=4). Of the 16 VWD-patients remaining without any identified cause of GIB after conventional +/- video capsule endoscopy, 15 had VWD2A, 2B or 3.



Conclusion: This French national survey addressing how VWD-patients with GIB are currently managed indicates a high inter-centers heterogeneity regarding both the use of VCE and the therapeutic management.

PO-38

QUANTIFICATION OF VWF PROPEPTIDE RELEASE BEFORE AND AFTER DESMOPRESSIN IN VON WILLEBRAND DISEASE AND HEMOPHILIA A

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Background: Von Willebrand factor (VWF) plasma levels represent a balance between synthesis, secretion and clearance of VWF. VWF propeptide (VWFpp) is a marker of VWF synthesis. Increased VWFpp/VWF antigen (VWF:Ag) ratio reflects increased VWF clearance. Adding VWFpp to population pharmacokinetic models for therapy in VWD may allow for more precise estimation of the endogenous VWF fraction released after DDAVP or trauma or during surgery.

Aims: To measure VWFpp concentrations in von Willebrand disease (VWD) and "low VWF" (VWF 0.30-0.50 IU/ml) patients, with hemophilia A patients as controls, before and after desmopressin.

Methods: VWFpp concentrations were measured with an enzyme-linked immunoassay using antibodies from Sanquin (Amsterdam, the Netherlands) in plasma samples from patients with bleeding disorders undergoing a desmopressin test with an intravenous dose of 0.3 µg/kg. Pearson's correlation coefficient was calculated for VWFpp/VWF:Ag ratio and fold VWF:Ag increase.

Results: Plasma samples of 77 subjects were analyzed (for diagnosis and baseline values, see table 1). One hour (t=1) after desmopressin administration, median VWFpp levels had increased 6.8 [IQR 3.2]-, 5.7 [2.9]- and 6.4 [3.4]-fold in VWD type 1 and 2 and low VWF patients respectively and 7.9 (IQR 4.89] fold in hemophilia A patients. VWFpp half-life was shorter in low VWF and VWD than in hemophilia A (median 3.1 vs. 5.6 hours). Baseline VWFpp/VWF:Ag ratio inversely correlates with VWF:Ag half-life (r = -0.657, p<0.05).Baseline VWFpp/VWF:Ag ratio correlates moderately with fold VWF:Ag increase at t=1 (r = 0.532, p = 0.000), but only weakly with VWFpp/VWF:Ag ratio at t=1 (r = 0.322, p = 0.007). No correlation was found between VWFpp/VWF:Ag ratio at t=1 and fold increase in VWF:Ag.

Conclusions: Baseline VWFpp/VWF:Ag ratios indicate higher VWF clearance in VWD and low VWF, but also correlate with VWF:Ag increase 1 hour after desmopressin. Incorporation of VWFpp levels into population pharmacokinetic models will potentially improve model prediction.

	N(%)	Median FVIII [IQR]	Median VWF:Ag [IQR]	Median VWF:RCo [IQR]	Median VWFpp [IQR]	Median VWFpp/VWF:Ag ratio [IQR]
Hemophilia A	17 (22)	0.24 [0.08-0.40]	1.24 [0.96-1.53]	1.25 [1.02-1.48]	0.88 [0.74-1.02]	0.71 [0.54-0.89]
Low VWF	38 (50)	0.71 [0.59-0.84]	0.52 [0.42-0.63]	0.56 [0.46-0.67]	0.64 [0.54-0.74]	1.20 [0.96-1.45]
VWD type 1	15 (20)	0.66 [0.45-0.87]	0.35 [0.22-0.49]	0.34 [0.20-0.48]	0.66 [0.54-0.78]	1.78 [1.29-2.27]
VWD type 2	6 (8)	0.44 [0.29-0.60]	0.65 [0.49-0.82]	0.45 [0.14-0.56]	0.78 [0.68-0.88]	1.22 [0.90-1.54]

FVIII = Factor VIII; VWF = von Willebrand factor; Ag = antigen; RCo = Ristocetin Cofactor activity; pp = propeptide; VWD = von Willebrand disease

Table 1. Baseline values (before desmopressin)

PO-39

ADAMTS13 INHIBITOR ASSESSMENT WITH THE HEMOSIL ACUSTAR ADAMTS13 ACTIVITY ASSAY

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Background: Thrombotic thrombocytopenic purpura (TTP) is a rare hematological disease characterized by the severe deficiency of ADAMTS13 activity. In acquired TTP, reduced ADAMTS13 activity is caused by inhibitory autoantibodies blocking ADAMTS13 functional activity. ADAMTS13 activity assays can be used to detect and quantify ADAMTS13 inhibitors. The aim of this study was to assess the performance of the HemosIL AcuStar ADAMTS13 Activity assay for ADAMTS13 inhibitor assessment.

Materials and Methods: Sixty-five samples, including 15 from normal controls and 50 from patients with acquired TTP, in acute and remission phases, were tested for the presence of anti-ADAMTS13 inhibitors using a mixing procedure. Heat-inactivated samples were mixed 1:1 with HemosIL Normal Control (pooled normal plasma) and incubated for 30 min at 37°C. Residual ADAMTS13 activity was measured with the HemosIL AcuStar ADAMTS13 Activity assay. Results were compared with historical data obtained using a similar mixing procedure (heat-inactivated patient plasma mixed 1:1 with pooled normal plasma) but different incubation conditions, 2 hours at 25°C, followed by the measurement of the residual ADAMTS13 activity with an in-house FRET assay. For both procedures, residual activity values between 25% and 75% were used for inhibitor titer calculation, after sample dilution if required, following the Bethesda method.

Results: Full agreement between AcuStar and FRET assays was obtained regarding the detection of ADAMTS13 inhibitors (Table1). There was also a good correlation (r = 0.93), although inhibitor titers measured by the AcuStar assay were, on average, 2-fold higher than those of the FRET assay (Weighted Deming regression slope 2.0, intercept -0.44).

	FRET				
AcuStar	Normal	Acute TTP	Remission Negative	Remission Positive	Total
Normal	15	0	0	0	15
Acute TTP	0	22	0	0	22
Remission Negative	0	0	10	0	10
Remission Positive	0	0	0	18	18
Total	15	22	10	18	65

Conclusions: The HemosIL AcuStar ADAMTS13 Activity assay showed a good performance in identifying the presence of anti-ADAMTS13 inhibitors in TTP patients, compared with the in-house FRET assay. The short time of incubation of the mixture (30 min vs 2 hours) allows quick inhibitor results without affecting assay sensitivity and specificity.

PO-40

EVALUATION OF THE VON WILLEBRAND FACTOR (VWF) INHIBITOR IN A LARGE COHORT OF EUROPEAN AND IRANIAN PATIENTS PREVIOUSLY DIAGNOSED WITH TYPE 3 VON WILLEBRAND DISEASE (VWD3) ENROLLED INTO THE 3WINTER-IPS PROJECT

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Background/Aims: Patients with VWD3 have virtually complete deficiency of VWF and a very severe bleeding phenotype. A severe complication in VWD3 patients is due to the development of an inhibitor against VWF. This event not only complicates patients' therapy, but expose patients to the risk of a life-threatening anaphylactic shock. Our aim was to evaluate the presence of inhibitors against VWF in a cohort of European and Iranian patients with previously diagnosed VWD3 enrolled in the 3WINTERS-IPS project.

Materials and Methods: Among the 260 VWD3 patients enrolled in the project 205 had plasma VWF antigen (VWF:Ag) <5% and in 151 of these the VWF:Ag was undetectable (<1%). The Bethesda method was used to test all patients for the presence of inhibitors using the VWF collagen binding (VWF:CB) assay. Patients with VWF:CB inhibitors were also evaluated by Bethesda method using the VWF:Ag and an automated gain-of-function mutant glycoprotein Ib binding (VWF:GPIbM) assay.

Results: Neutralizing antibodies for VWF:CB were identified in 13 out of 205 (6%) patients and most of them have the VWF:Ag <1% (Table). Among them, 10 out 13 (77%) were positive to the Bethesda VWF:GPIbM and 6 out 13 (46%) for Bethesda VWF:Ag assays. All patients with inhibitors present mutations that results in null alleles with one exception, a case who appears to have only a homozygous missense mutation.

Table

Patient ID	VWF:Ag	Mutation	VWF:CB Inhibitors	VWF:Ag Inhibitors	VWF:GPIbM Inhibitors
IR05-01-I-04	< 1%	c.311_312delAG/c.311_312delAG	56 BU	7.6 BU	70 BU
IR03-04-II-15	< 1%	p.Gln1346Ter/unknown	23 BU	1.5 BU	52 BU
IT01-F19-I-P01	< 1%	del ex 1-52/del ex 1-52	15 BU	0.7 BU	5.9 BU
IR06-F11-I-P13	< 1%	c.4309delG/c.4309delG	13 BU	1.7 BU	28 BU
IT04-F04-II-P05	< 1%	del ex 1-52/del ex 1-52	10 BU	2 BU	3.8 BU
FI01-6-I-6	< 1%	p.Arg1659Ter/p.Arg1659Ter	5 BU	0.3 BU	2.8 BU
IT05-F01-I-P04	< 1%	c.6182delT/c.6182delT	3.8 BU	< 0.3 BU	0.4 BU
NL02-F03-I-P01	< 1%	p.Asn2546Tyr/p.Asn2546Tyr	1.8 BU	< 0.3 BU	< 0.3 BU
FR03-F02-II-P01	< 1%	del ex 1-52/del ex 1-52	1.8 BU	0.1 BU (< 0.3)	0.17 BU (< 0.3)
IT04-F04-II-P08	< 1%	del ex 1-52/del ex 1-52	1.3 BU	< 0.3 BU	0.7 BU
IR01-F07-I-P07	1.4%	del ex 35-52/del ex 35-52	1.2 BU	< 0.3 BU	0.5 BU
IT04-F01-II-P02	1.5%	c.8155+1G>T/c.8155+1G>T	0.4 BU	< 0.3 BU	< 0.3 BU
IT04-F04-II-P07	< 1%	del ex 1-52/del ex 1-52	0.3 BU	< 0.3 BU	0.3 BU

The inhibitors were considered to be present for values ≥ 0.3 Bethesda Units (BU)

Conclusions: The development of inhibitors in VWD3 patients is confirmed to be a rare event, only 6% of our patients were found to be positive. High-titer antibodies anti-VWF have been reported to be able to precipitate VWF in normal plasma, this would explain the positive results obtained in six of our patients using the VWF:Ag Bethesda assay. Development of inhibitors is mainly restricted to patients with null alleles and nearly half of them carry homozygous large VWF gene deletions.

PO-41

OVERVIEW OF EFFICACY AND SAFETY OF A PLASMA-DERIVED HUMAN VON WILLEBRAND FACTOR CONCENTRATE FOR PERIOPERATIVE MANAGEMENT AND DELIVERY IN PATIENTS WITH HEREDITARY VWF DEFICIENCY, UNRES PONSIVE TO DDAVP

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Background: Single-factor replacement therapy specifically for VWF-deficient patients was developed in France over 20 years ago with the aim of avoiding unnecessary infusions of FVIII. We present here clinical efficacy and safety data including those that led to regulatory approval of WILFACTIN/WILLFACT in surgery.

Methods: Data from five phase 2-3 clinical trials and one post-marketing study, carried out between 1999 and 2014, were pooled. Overall, 153 unique patients with VWD (31 were type 1, 83 type 2, 35 type 3 and 4 unspecified). Basal VWF:RCo ≤15 IU/dL and FVIII:C <40 IU/dL were respectively reported in 75.1% and 71.2%. Patients received VWF to prevent bleeding and achieve haemostasis in 240 surgical procedures. Investigator's assessment was requested for a total of 147 surgeries in 99 patients.

Results: Studies reported a broad range of high-risk procedures including 41 orthopaedic procedures and 24 childbirths. There were 17 interventions in obese and 32 in elderly patients. Haemostatic efficacy was rated for 146 procedures as excellent (119) or good (26) for 99.3% of surgeries, and moderate for one. The median daily dose was 55 IU/kg with half of procedures managed between 43 and 70 IU/kg (interquartile range, IQR). The median number of exposure days per surgery including the post-surgical prophylaxis at home was 7 (IQR 3-12). Correction of FVIII:C levels was obtained by FVIII in combination with the VWF preoperative dose in 31/147 (21.1%) surgeries. In 112 remaining procedures with detailed information, a dose was administered 12/24h before allowing endogenous FVIII:C correction (68), or FVIII:C levels were considered high enough to ensure haemostasis (44). There were no AEs pointing to thrombosis, and no inhibitors to VWF occurred during studies.

Conclusion: These results provide clinical evidence for the excellent efficacy and safety of WILFACTIN/WILLFACT when used in surgery or childbirth, including in patients with high risk factors.

PO-42

THE EFFECT OF A TRIPEPTIDE INHIBITOR ON THE ACTIVITY OF SERINE PROTEASES OF THE HEMOSTATIC SYSTEM

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Background: Enzymes that regulate blood coagulation and the dissolution of fibrin clots are serine proteases. Normally, their activity is regulated by protein inhibitors present in the blood. In pathology, imbalance of the protease-inhibitor can lead to an increase in protease activity. An example of such disorders is enhanced thrombin activity, leading to blood vessel thrombosis. Inhibitors of different selectivity are designed to regulate the activity of therapeutically significant proteases. However, such inhibitors in high doses can be toxic.

Aims: To evaluate the inhibitory effect of synthesized Ac-Ala-Phe-Lys-Pip AcOH (AFK) on the activity of enzymes of coagulation system and fibrinolysis system and its toxicity.

Materials and Methods: Inhibition of thrombin, FXa, APC, plasmin, urokinase (uPA) and tissue plasminogen activator (tPA) activities by AFK was studied using their specific substrates. We studied the effect of AFK on thrombin generation in plasma by rTF and on fibrin generation by thrombin spectrophotometrically. Toxicity of AFK was studied in a model of liver parenchymal bleeding of anesthetized mice and rats.

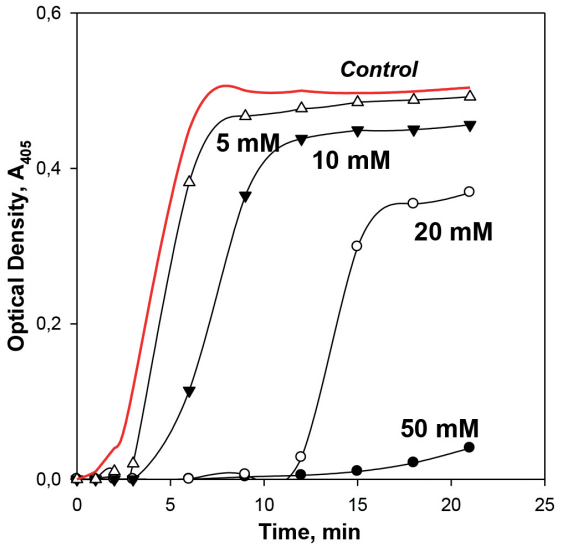

Figure 1. Influence of AFK on coagulation of fibrinogen (4 μM) by thrombin (0.1 IU/ml) at 25oC

Results: AFK inhibited the activity of coagulation enzymes more strongly than the activity of fibrinolysis enzymes: the IC_{50} values were 12, 50, 35, 100, 120 and 180 mM for thrombin, FXa, APC, tPA, plasmin and uPA, respectively. It inhibited competitively all enzymes except tPA, and thrombin most strongly (Ki 3.0 mM). AFK at concentrations > 50 mM almost completely suppressed thrombin generation in plasma. The increase in AFK concentration caused an increase in lag phase and a decrease in the fibrinogen clotting rate by thrombin, which was completely suppressed at concentration of 50 mM. AFK had low toxicity: LD_{50} in mice and rats was < 6.5 g/kg.

Sample	Lag phase	Peak height		Endogenous thrombin potential (ETP)
	Time, min	Thrombin, nM	Time, min	
Control	8.1 ±0.5	414±10	12.0±0.1	6579±70
AFK 50 mM	16.0±0.5	230±5	18.0±0.1	4203±85
AFK 100 mM	45.5±0.5	30.5±0.5	75.0±0.2	1570±95

Table. Influence of AFK inhibitor on thrombin generation test parameters in plasma.

Conclusions: AFK, which strongly inhibits thrombin activity, suppresses plasma thrombin generation and fibrinogen coagulation and has low toxicity, is promising for the treatment of thrombotic disorders.

PO-43

THROMBIN AND PLASMIN GENERATION IN PATIENTS WITH PLASMINOGEN OR PLASMINOGEN ACTIVATOR INHIBITOR TYPE 1 DEFICIENCY

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Introduction: Deficiencies of plasminogen and plasminogen activator inhibitor type 1 (PAI-1) are rare disorders of fibrinolysis. Conventional laboratory assays of fibrinolysis lack specificity and validation. Moreover, there is a weak correlation of the assays with clinical symptoms.

Methods: The Nijmegen Hemostasis Assay (NHA) was used to simultaneously measure thrombin and plasmin generation in five patients with plasminogen deficiency and ten patients with complete PAI-1 deficiency. Analysed parameters were: lag-time ratio, thrombin peak time ratio, thrombin peak height, thrombin potential (AUC), fibrin lysis time, plasmin peak height and plasmin potential. All parameters were expressed as a percentage compared to a reference value of fifty-three healthy individuals.

Results: Thrombin and plasmin generation curves are shown in Figure 1. Patients with plasminogen deficiency demonstrated a short lag

time and thrombin peak time, and normal thrombin peak height but high AUC. Plasmin generation was not detectable in all but one patient with a plasminogen activity of 23%. Patients with complete PAI-1 deficiency demonstrated a short lag time and thrombin peak time, low thrombin peak height and normal AUC. Plasmin generation showed an increased plasmin peak and plasmin potential, although with a large variation between individual patients.

Conclusion: Patients with plasminogen or PAI-1 deficiency show distinct abnormalities in plasmin generation as well as thrombin generation in the NHA. Plasmin generation may explain the differences between the two groups in propagation of coagulation. These results suggest that these disorders of fibrinolysis also affect coagulation differently and a global assay measuring both activities may better correlate with clinical outcome.

PO-44

IN VITRO AND IN VIVO MODULATION OF VON WILLEBRAND FACTOR GENE MUTATIONS WITH DOMINANT-NEGATIVE EFFECT

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Background: von Willebrand factor (VWF) is a multimeric protein that undergoes dimerization and multimerization processes during its biosynthesis. Dominant-negative mutations are associated with severe von Willebrand disease phenotypes but remain difficult to identify.

Aim: To study and modulate the expression of VWF carrying dominant-negative mutations.

Methods: We created in vitro and in vivo transient models to express VWF carrying the p.P1127_C1948delinsR (del) and p.C2773R dominant-negative mutations affecting multimerization and dimerization processes, respectively. COS-1 cells were transiently co-transfected with plasmids for wild-type (wt) and mutant VWF expression. Similarly, Vwf-deficient mice were subjected to hydrodynamic gene transfer of both plasmids. VWF multimers were evaluated in conditioned media and mouse plasma.

Results: As expected, co-expression of pC2773R- with wt-VWF was associated with absence of high molecular weight multimers but normal VWF antigen levels in conditioned media.

Co-expression of del- & wt-VWF also resulted in severe reduction of VWF multimers and high-resolution gel enabled the separation of heteropolymers formed by wt- and del-VWF subunits. We next created plasmids for the expression of VWF carrying both gene defects (p.del/C2773R) in cells and mice. Noticeably the double-mutant VWF was unable to interact with wt monomers leading to abolition of the dominant-negative effect of the single defects and rescue of the multimer profile.

The detrimental effect of the large deletion was also challenged in vivo by the administration of siRNA selectively directed against del-VWF. By interfering with the dominant-negative mechanism, the silencing treatment improved VWF antigen levels and multimer profile.

Conclusions: We established the first in vivo heterozygous mouse model of VWD associated with dominant-negative mutations. The association of other VWF mutations with del-VWF can be employed to investigate/antagonize their dominant-negative effect. An in vivo silencing approach was successfully applied to our von Willebrand disease mouse models.

PO-45

EVALUATION OF THE CHROMOGENIC ASSAY "BIOPHEN FACTOR IX" ON SYSMEX CS-2400 ANALYZER

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Background and aim: Measurement of factor IX is required for quantification of residual plasma fix levels with a diagnostic purpose (hemophilia B) or for performing recovery studies during treatment by replacement therapies. The one-stage clotting assay is the most used in routine laboratories; there are only two chromogenic assays commercially available at present. We aim to evaluate the analytical performance of the biophen FIX chromogenic assay on the Sysmex cs-2400 analyzer.

Materials and methods: Forty patients with hemophilia B (severe, moderate and mild), were enrolled together with 120 healthy Italian subjects (aged 18-69 years). Biophen FIX:C was compared with the one-sage assay using Actin FS and factor IX deficient plasma (Siemens healthcare diagnostics, Marburg, Germany) on Sysmex CS-2400. All the tests were performed on citrated platelet poor plasmas stored at -80°C.

Analytical performance and method comparison analysis were performed according to the Clinical & Laboratory Standards Institute (CLSI) Guidelines.

Results: Intra- and inter-assay coefficient of variation were < 3% and < 8%, respectively. Linearity was good up to 1/128 dilution (r=0.99); mean recovery was 97.3% and limit of detection was 0.2%.

The reference range calculated on the 120 healthy subjects was 60 to 138% (95% reference interval, double-sided). Biophen FIX:C assay showed a good correlation and diagnostic agreement with the one-stage assay: the spearmen's rank correlation coefficient was 0.94 and the inter-rate agreement k cohen coefficient was 0.83. The k coefficient was 0.89 when biophen FIX:C was compared with the historical classification of the patients, demonstrating an optimal diagnostic accuracy in hb.

Conclusions: Our results showed a good analytical performance of biophen FIX:C as a suitable chromogenic FIX functional assay for monitoring haemophilic patients treated with extended half-life products.

PO-46

EVALUATION OF PLATELET-DEPENDENT VWF ACTIVITY ON AN HETEROGENEOUS GROUP OF VON WILLEBRAND DISEASE PATIENTS USING FOUR DIFFERENT METHODS: TWO VWF:GPIBM AND TWO VWF:RCO ASSAYS

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Background: In the last decade alternative assays to evaluate platelet-dependent VWF activity have emerged as an alternative to the VWF:RCo assays that are known to have some limitations. We have previously found a good agreement of results between our VWF:GPIbM ELISA and VWF:RCo assays, with the exception of VWD type 2B patients who present loss of high molecular weight multimers (HMWM). In these patients the ELISA values were higher than those obtained using the aggregometric/automated VWF:RCo assays. This discrepancy has not been reported using the automated VWF:GPIbM assay. Additional investigations are required to clarify the automated VWF:GPIbM and VWF:RCo assays correlation using a larger VWD type 2B population. Aim: To evaluate platelet-dependent VWF activity with four different assays in an heterogeneous group of VWD patients.

Methods: 76 VWD patients classified as type 2A (26), type 2B (29), type 2M (9) and type 2A/2M (12) were tested using two VWF:GPIbM assays (an automated and an ELISA one) and two VWF:RCo assays (an automated and an aggregometric one).

Results: Correlation values between the assays are shown in the Table. The different assays exhibited a good agreement, as assessed by the method of Bland-Altman plots (Figure, A-B-C-D), although with some exceptions.

Conclusion: Concordant results were obtained with the two VWF:RCo assays in the evaluation of VWD variant groups. Consistent values were obtained between automated and ELISA VWF:GPIbM, automated VWF:RCo and automated VWF:GPIbM and automated VWF:RCo and ELISA VWF:GPIbM assays. The exceptions regard most of VWD type 2B patients, those who lack HMWM. In these subjects the ELISA VWF:GPIbM gave higher results than the automated method. In three of these patients, a discrepancy was observed also between the auto-

mated VWF:RCo and VWF:GPIbM assays, where the latter gave lower values. The eight VWD type 2B patients with HMWM did not present this discrepancy.

Methods comparison	r value
VWF:GPIbM ELISA *vs* VWF:GPIbM automated	0.83
VWF:RCo automated *vs* VWF:GPIbM ELISA	0.90
VWF:RCo automated *vs* VWF:RCo aggregometric	0.91
VWF:RCo automated *vs* VWF:GPIbM automated	0.95

Table. Comparision between alternative assays to evaluate platelet-dependent VWF activity.

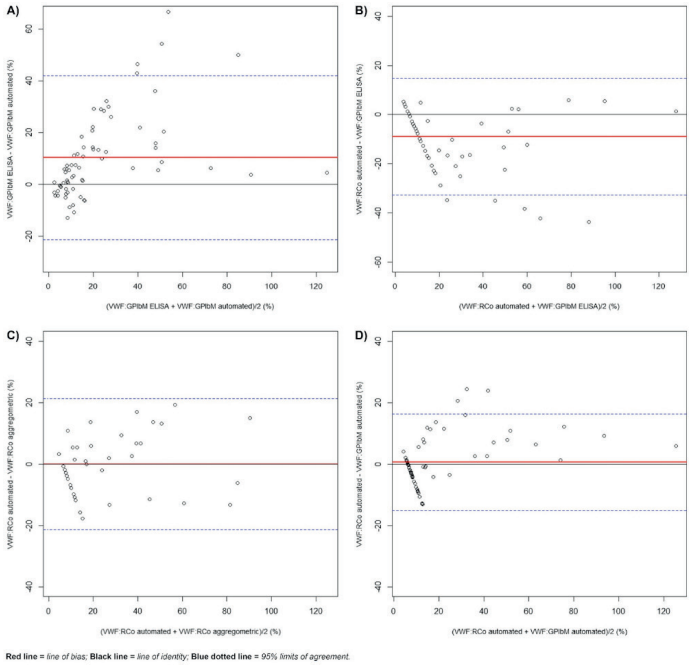

Figure. Bland-Altman plots

PO-47

FROM AUTOANTIBODIES TO THERAPEUTICS: EN ROUTE TO NOVEL TREATMENT FOR IMMUNE TTP

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Background/Aim: A cryptic epitope in the ADAMTS13 spacer domain is targeted by autoantibodies in most of the immune TTP (iTTP) patients. Our aim is to employ in silico approaches, cryo-EM and functional characterizations to map the interactions between the spacer domain and autoantibodies, such as to provide structure-function data that can be translated into therapeutic use for this rare but life-threatening disease.

Materials and Methods: A structural bioinformatics investigation pipeline was developed to model a 3D complex formation between the human ADAMTS13 spacer domain and autoantibodies II-1 and I-9. Binding free energies between the ADAMTS13 spacer domain and I-9 or II-1 were calculated from the final 3D-models, were found to be in accordance with experimentally determined binding affinities. Finally, 3D-models were subsequently exploited for epitope mapping to investigate the essential residues of autoantibody binding.

Results: Our final 3D-models were on full agreement with any mutagenesis study on the known epitope residues. Also, several other residues within the spacer domain were found to be contributing to autoantibody binding. These additional spacer domain residues were previously described in an autoantibody binding study where hydrogen/deuterium exchange mass spectrometry was utilized. Paratope residues from the final 3D-models were utilized to design peptides that bind to the epitope of the spacer domain to prevent autoantibody binding. These peptides were produced and functionally tested for their ability to compete with autoantibody binding. Such peptides may prevent the binding of autoantibodies to ADAMTS13 and become beneficial for iTTP patients.

Conclusions: In summary, we have used a unique structural bioinformatics pipeline to predict the nature of autoimmune antibody binding to ADAMTS13. The accuracy of the final 3D-models will be further verified by the use of cryo-EM. The derived knowledge will be employed to facilitate the design of novel therapeutic approaches for treatment of immune TTP.

PO-48

INVESTIGATION OF THE ADAMTS13 C.3178C>T MUTATION IN HEREDITARY AND ACQUIRED THROMBOTIC THROMB OCYTOPENIC PURPURA

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Introduction: Thrombotic thrombocytopenic purpura (TTP) is a thrombotic microangiopathy, characterized by the severe deficiency of ADAMTS13, which is responsible for cleaving ultra-large von Willebrand factor multimers. ADAMTS13 deficiency is caused by anti-ADAMTS13 autoantibodies in the acquired form of the disease, or by bi-allelic ADAMTS13 mutations in its hereditary form. The c.3178C>T (p.R1060W) mutation was described predominantly in adult-onset hereditary TTP cases, often associated with first disease manifestation in pregnancy. Interestingly, this pathogenic variation was reported to cause ADAMTS13 deficiency in heterozygous form, in the absence of other mutations; in some of these cases, anti-ADAMTS13 antibodies were detectable.

Aims: In order to better understand the mechanism how c.3178C>T (p.R1060W) causes ADAMTS13 deficiency in heterozygous form, and to investigate its association with anti-ADAMTS13 antibodies, the presence of the c.3178C>T mutation in a cohort of hereditary and acquired TTP patients, and its association with clinical parameters, other mutations, and antibody levels, was investigated.

Methods: The full *ADAMTS13* gene (29 exons) was sequenced in 8 hereditary TTP patients, whereas only exon 24 (containing c.3178) was analysed in 146 acquired TTP patients and 2 healthy relatives of a hereditary patient carrying the c.3178C>T mutation by Sanger sequencing.

Results: One (HUN994) out of the two adult-onset hereditary TTP patients in our cohort was found to carry the c.3178C>T mutation in compound heterozygous form together with the common ADAMTS13 c.4143-4144insA mutation. No other hereditary or acquired TTP patient nor one of the relatives of HUN994 carried the c.3178C>T mutation.

Conclusion: The c.3178C>T mutation was present in one adult-onset hereditary TTP patient in our cohort. In contrast with previous studies, the c.3178C>T mutation was neither found in the absence of another mutation nor in patients with detectable anti-ADAMTS13 antibodies in our TTP cohort.

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P0-49

THROMBOELASTOGRAPHY: A PROMISING ALTERNATIVE FOR MONITORING FRESH FROZEN PLASMA TRANSFUSION IN INTENSIVE CARE UNIT PATIENTS

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Background: Many of the FFP transfusions given are inappropriate and are based on abnormal coagulation tests. The objectives of our study were to determine whether TEG can be used as an alternate to guide and monitor FFP transfusions.

Methods: A prospective observational study was conducted and 28 patients were observed during their ICU stay. Blood samples were collected before and 18-24 hours post FFP transfusion. Platelet counts and conventional coagulation tests were manually measured and TEG were performed simultaneously.

Objectives:

- To compare results of conventional coagulation tests with TEG.
- To compare changes in conventional coagulation tests and TEG before and after FFP transfusion.
- To assess whether TEG can be used to monitor FFP transfusion.

Results: 20/28 patients showed abnormal conventional coagulation tests results. In contrast, only 13/28 showed abnormal TEG before FFP transfusion. 3/28 patients experienced bleeding and significant association of TEG parameters - R time, *α* angle and Maximum Amplitude, was observed ($p<0.05$). Post FFP transfusion, 5 patients were lost to follow-up. 11/23 patients had deranged coagulogram, while 13 patients had deranged TEG results. 1 patient experienced bleeding symptoms and showed abnormal TEG as well as conventional coagulation test results. Significant change in K time were found in all patients following FFP transfusion ($p<0.05$). MA showed weak correlation with platelet counts. With respect to bleeding, sensitivity of TEG was similar to conventional coagulation test results. However, the specificity of TEG was found to be higher (60%).

Conclusions: Using TEG can be considered as an effective tool to assess coagulation status of ICU-patients. Moreover, TEG is more specific and can therefore possibly decrease the use of inappropriate FFP transfusions.

Parameter (abnormal value)	Bleeding patient(s)		Patients without bleed		P value
	Coagulogram				
	Mean±SD	Median (IQR)	Mean±SD	Median (IQR)	
PT (>16s)	18.00±4.36	20 (13,21)	22.4±9.38	21 (18.5, 23)	0.23
PTI (<70%)	63.00±1.73	62 (62,65)	65.36±13.06	64 (61,74)	0.85
APTT (>40s)	39.00±2.65	38 (37,42)	39.92±5.45	40(35.5, 45)	0.68
INR (>1.5)	1.61±0.06	1.6 (1.5,1.6)	1.72±0.72	1.6 (1.4, 1.7)	0.85
PC (<1.5 lacs/mm^3)	140±36.05	150 (125, 160)	113.72±58.49	120 (70, 150)	0.09
	TEG				
R (>14m)	5.87±3.88	5.3 (2.3,10)	1.61±2.65	0.7 (0.7, 0.7)	0.003*
K (0, >6.5m)	6.0±3.4	5.5 (2.8,9.7)	3.33±4.22	0.5 (0.3, 6.2)	0.183
α (<23^0)	34.41±6.49	34.9(27.6,40.6)	71.39±17.39	81.1(63.4,82.8)	0.016*
MA (<48mm)	29.63±9.93	32.8(18.5,37.6)	59.12±21.06	56.6(46.4, 78.3)	0.013*

Table 1: Comparison of results of conventional coagulation tests and TEG in bleeding and non bleeding patients before FFP transfusion

Parameter	Pre FFP transfusion		Post FFP transfusion		p value
	Mean±SD	Median (IQR)	Mean±SD	Median (IQR)	
PT	20.95±2.94	21 (19,23)	20.4±4.64	20 (17,23)	0.373
PTI	65.87±8.13	64 (61, 70)	68.22±11.99	70 (61, 81)	0.291
APTT	39.87±4.95	40 (37,45)	38.39±5.94	37 (34, 42)	0.216
INR	1.61±0.20	1.6 (1.4, 1.7)	1.57±0.36	1.47 (1.2, 1.7)	0.354
R	1.46±1.76	0.7 (0.7,1)	1.1±1.46	0.7 (0.7, 0.7)	0.475
K	2.67±3.14	0.5 (0.3, 5.5)	5.63±6.03	1.7 (0.3, 11.2)	0.030*
α	70.14±18.63	81.16 (60.7,82.7)	73.05±16.99	79.77(68.2, 83.2)	0.414
MA	58.47±23.46	57.6 (43.3, 78.5)	59.24±21.34	59.5 (50, 70)	0.733

Table 2: Comparing results of conventional coagulation tests and TEG before and after FFP transfusion

P0-50

THE PRESENCE OF ANTI-ADAMTS13 AUTOANTIBODIES IN IMMUNE-MEDIATED THROMBOTIC THROMB OCYTOPENIC PURPURA PATIENTS DOES NOT HAMPER CORRECT DETERMINATION OF ADAMTS13 ANTIGEN LEVELS

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Background/Aim: The diagnosis of immune-mediated thrombotic thrombocytopenic purpura (iTTP) is made upon ADAMTS13 activity (ADAMTS13 activity < 10%) and anti-ADAMTS13 autoantibody determination, as the presence of thrombocytopenia, hemolytic anemia and organ failure is not specific for iTTP. Although ADAMTS13 antigen (Ag) levels are not routinely measured, studies have shown that ADAMTS13 Ag levels are associated with disease prognosis. However, whether the anti-ADAMTS13 antibodies present in plasma of iTTP patients hamper a correct determination of ADAMTS13 Ag levels using an immune assay has not been investigated in detail yet. The aim is to measure ADAMTS13 Ag levels in a large cohort of healthy controls and iTTP patients using our in-house ADAMTS13 Ag ELISA, and investigate whether these measurements are influenced by the presence of anti-ADAMTS13 autoantibodies.

Methods: ADAMTS13 antigen levels were determined in 424 healthy control samples and 112 acute iTTP samples using our in-house monoclonal antibody (mAb)-based ELISA. Whether the presence of anti-ADAMTS13 autoantibodies influenced ADAMTS13 Ag determination in our ELISA was tested by determining ADAMTS13 Ag levels in normal human plasma (NHP) with or without pre-incubation of purified IgGs from iTTP patients (19 samples).

Results: ADAMTS13 Ag levels were significantly reduced ($P < 0.0001$) in acute iTTP patients (14.88 ± 18.27 %) compared to healthy controls (100 ± 18.22 %). To prove that anti-ADAMTS13 autoantibodies do not interfere with our assay mAbs, we showed that ADAMTS13 Ag levels in NHP were not significantly reduced after pre-incubation with purified IgGs from iTTP patients.

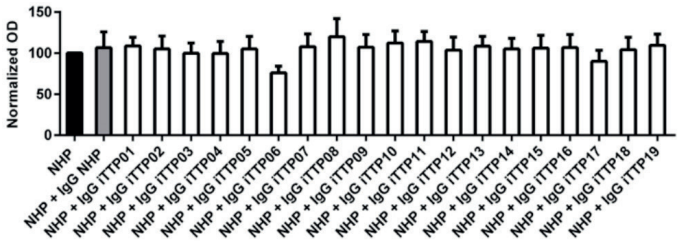

Figure. ADAMTS13 Ag levels in NHP after pre-incubation with IgGs from iTTP patients.

Conclusion: The presence of anti-ADAMTS13 autoantibodies in plasma of iTTP patients does not influence ADAMTS13 antigen determinations using our in-house ELISA. The latter is thus a powerful tool to correctly determine ADAMTS13 Ag levels in iTTP patients. These data support routine measurements of ADAMTS13 antigen levels in these patients to have a better insight in disease prognosis.

PO-51

SUCCESSFUL TREATMENT AND DURABLE REMISSION OF REFRACTORY THROMBOTIC THROMB OCYTOPENIC PURPURA (TTP) WITH BELIMUMAB

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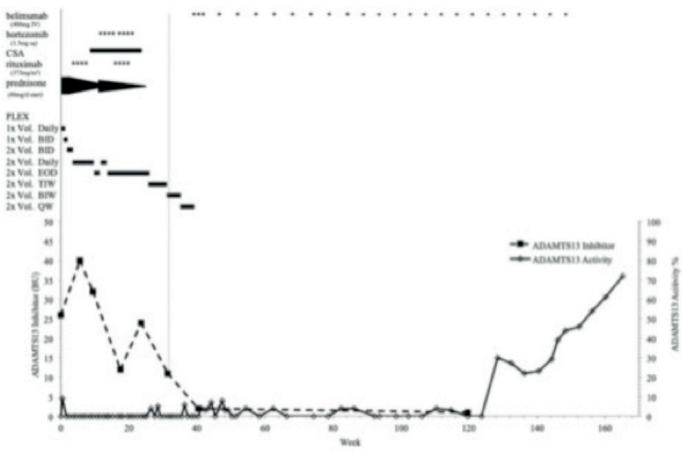
Introduction: We present a case of refractory TTP that ultimately recovered ADAMTS13 activity following treatment with belimumab. To our knowledge, this is the first report of this agent being used in TTP.

Case Description: A 14 year old male presented to a local ER with left facial and bilateral arm paresthesia and weakness. On initial laboratory examination, he was found to have thrombocytopenia (platelet count $13x10^3$/uL) and evidence of microangiopathic hemolytic anemia (hemoglobin 10.5g/dL, undetectable haptoglobin, elevated lactate dehydrogenase, and over 20 schistocytes/HPF). TTP was suspected, and the patient was started on plasma exchange (PLEX), steroids, antiplatelets, and folic acid pending confirmatory ADAMTS13 activity. ADAMTS13 was undetectable with a 26 BU inhibitor, ultimately climbing to 40 BU. The patient required escalation of PLEX for hematologic response prior to a durable improvement in hematologic parameters. Per our institutional preference, he was continued on PLEX taper with adjustments based upon weekly monitoring of pre- and post-pheresis ADAMTS13 activity trends. Despite multiple sequential immunosuppressive/immunomodulatory agents (including high-dose steroids, rituximab times two courses of four weekly doses, cyclosporine for 140 days, bortezomib times two monthly cycles) a 10 BU inhibitor remained at 36 weeks after presentation, with continued low ADAMTS13 activity level. At 40 weeks, the patient was started on belimumab 400 mg IV every two weeks times three doses followed by 400 mg monthly. A clinical remission was observed with ADAMTS13 activity between 3-5% for the next 80 weeks. Disappearance of the inhibitor and continued improvement of ADAMTS13 activity level was noted at 124 weeks. The patient remains well on maintenance belimumab without relapse with ADAMTS13 over 70% of normal.



Discussion: The anti B-cell activating factor agent belimumab has proven efficacy in autoimmune syndromes, and is FDA approved for systemic lupus erythematosus. In TTP, Belimumab represents a novel, but logical immunomodulatory/immunosuppressive agent, and proved effective in this case. Additional clinical investigation is warranted.

PO-52

BMI IS AN IM PORTANT DETERMINANT OF VWF AND FVIII LEVELS AND BLEEDING PHENOTYPE IN PATIENTS WITH VON WILLEBRAND DISEASE

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Background: In the general population, BMI is associated with increased von Willebrand factor (VWF) levels. Higher VWF levels may have a protective effect on bleeding. The association between BMI, VWF levels and bleeding is unknown in patients with von Willebrand disease (VWD).

Aim: To investigate the association between BMI, VWF and FVIII levels, and bleeding phenotype in VWD patients.

Methods: We included patients from the WiN study, a nationwide cross-sectional study in moderate and severe VWD patients in the Netherlands. All patients completed a questionnaire (Tosetto bleeding score) on bleeding episodes during their life time, and in the year prior to study inclusion. VWF and FVIII levels were measured centrally. Informed consent was obtained and the study was approved by the medical ethics committee.

Results: We included 545 adult VWD patients. Table 1 shows the baseline characteristics. In type 1 VWD, higher BMI was associated with higher VWF antigen; 0.07 IU/mL (95% confidence interval 0.02-0.13) per 10 kg/m^2, adjusted for age, sex, blood group and comorbidities. Similar results were found for VWF collagen binding, VWF activity and FVIII activity (FVIII:C) (Figure 1a). In type 2 VWD, FVIII:C

was higher among patients with higher BMI; 0.09 IU/mL (0.00-0.17) per 10 kg/m^2 (Figure 1b). In the total VWD population, patients with normal weight had fewer bleeding episodes in the year prior to study inclusion than patients with overweight or obesity, respectively 25% (69/276) vs 30% (53/175) and 38% (31/81) (p=0.018). Adjusted for age, sex, blood group and presence of relevant comorbidities, this association was clear in type 1 VWD (OR=1.42, 1.00-2.03), but less evident in type 2 VWD (OR=1.18 (0.76-1.82)).

Conclusion: BMI is associated with higher VWF and FVIII levels in type 1 and type 2 VWD patients. Despite higher levels, patients with increased BMI did not have less bleeding episodes.

PO-53

THE PRESENCE OF INHIBITORS AGAINST VON WILLEBRAND FACTOR AND FACTOR VIII IN THE SAME PATIENT: A CASE RE PORT

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Background/Aims: The development of an inhibitor against von Willebrand factor (VWF) is a rare but serious treatment complication of von Willebrand disease (VWD). It has been reported that some VWF antibodies additionally inhibit factor VIII coagulation activity (FVIII:C). Hereby, we report the case of the presence of inhibitors against VWF and FVIII identified during the preoperative laboratory workup in a 50-year-old man with previously diagnosed VWD.

Materials and Methods: aPTT, one-stage FVIII:C, VWF activity (VWF:Ac) and VWF antigen (VWF:Ag) were measured using reagents from Siemens Healthcare Diagnostics (Marburg, Germany). Technozym vWF:CBA ELISA (Technoclone, Austria) was used for the measurement of VWF collagen-binding activity (VWF:CB). FVIII and VWF inhibitors were diagnosed using Bethesda-based assays (Nijmegen modification).

Results: Preoperative workup results were: aPTT 56.1s, FVIII:C 2%, VWF:Ac <4.0%, VWF:CB <0.01 IU/mL, VWF:Ag <2.0%, FVIII inhibitor 1.0 Bethesda Units (BU/mL) and neutralizing antibodies to VWF:Ac 22.1 BU/mL. No recovery of VWF:Ac, VWF:CB nor VWF:Ag was noted at 1, 3 and 5 hours after administration of 50 IU/kg Wilate (Octapharma, Switzerland), with a rise of FVIII:C (31%) observed 1 hour post Wilate administration, declining to 11% and 6% after 3 and 5 hours, respectively. Exams performed three weeks later revealed FVIII inhibitor 1.6 BU/mL, an increasing anti-VWF:Ac titer of 88.3 BU/mL, anti-VWF:CB titer 117.8 BU/mL, with persistently low FVIII:C (1%), VWF:Ac (<4.0%), VWF:CB (<0.01 IU/mL) and VWF:Ag (<2.0%). Same FVIII:C, VWF:Ac, VWF:CB and VWF:Ag levels were obtained on two consecutive control examinations during the next three-month period, with measured FVIII inhibitor of 1.3 and 0.9 BU/mL.

Conclusions: The presented rare case of inhibitor against VWF and FVIII:C, detected after extensive laboratory examination during the preoperative workup, prevented serious perioperative and postoperative bleeding and enabled the opportunity to find out the most appropriate treatment option for this patient.

PO-54

ANALYTICAL PERFORMANCE VALIDATION OF A NEW SCREENING ASSAY FOR THE DEFICIENCY OF ADAMTS-13 ACTIVITY

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Introduction: Severe deficiency in ADAMTS-13 activity levels (< 0.1 IU/ml) is a specific marker for thrombotic thrombocytopenic purpura (TTP). TTP is an acute life-threatening disorder with the highest mortality rate within the first 24 hours of admission. It would be beneficial to have a quick turnaround of ADAMTS-13 activity reporting. We have developed and validated a simple screening assay which allows the reporting within 1 hour of blood draw.

Method: The screening assay is based on flow through technology using a synthetic vWF substrate, tagged with GST. ADAMTS-13 in the patient sample cleaves the substrate, which binds to the specific antibody coated in the test well of the test device. In the control well the cleaved and non-cleaved substrate will bind via the GST tag. A secondary biotinylated anti-GST antibody is used to detect the membrane-bound complex and is revealed by reactivity with a streptavidin-gold conjugate, producing a red color. The color intensity of the test well is proportional to the level of ADAMTS-13 activity in the plasma sample and is compared with a color card, allowing semi quantitative analysis.

Results: The screening assay was validated using samples with differing activity levels and 3 kit lots. At least 3 different readers were used to interpret the results. There was excellent agreement between the different readers. The assay showed good repeatability and reproducibility. Lot-to-lot consistency was determined by running 40 samples including TTP patients in the different screen assay kit lots and the ELISA. Using a cut off of < 0.1 IU/ml sensitivity, specificity and predictive values were calculated for each lot, showing excellent correlation between the lots and the ELISA results.

Conclusion: We have demonstrated that this screening test is an excellent tool for determining deficient levels of ADAMTS-13 activity with a sensitivity >96%.

PO-55

PROGNOSTIC VALUE OF POLYMORPHISMS OF THE CYP2C19 GENE IN THE DEVELOPMENT OF STENT THROMBOSIS

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Background: The consequence of insufficient suppression of increased activity of platelets can be a repeated cardiovascular event. At the heart of the variability of the pharmacological response of clopidogrel are many factors, among which the most important is the carrier of the CYP2C19*2 and -*3 polymorphisms associated with resistance to antiplatelet agents.

Aim: To study the prognostic value of CYP2C19 gene polymorphisms in the development of stent thrombosis in patients after myocardial revascularization.

Material and methods: The study included 47 patients with acute coronary syndrome (ACS) after myocardial revascularization (percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG)), who took double anti-platelet therapy (DAPT): aspirin and clopidogrel. Pharmacogenetic testing to determine the allelic variants of CYP2C19*2, -*3 was carried out by the method of polymerase chain reaction (PCR). Statistical processing was performed using the SPSS program.

Results: A total of 47 patients were examined, with an average age of 67.1 (+/-8.8) years. Male 34 (72.3%) at the age of 55.7 (+/-9.3) years, women 13(27.7%) at the age of 61(+/-6.4) years (p=0.037). In the history: 44 (93.6%) patients had PCI, 3 (6.4%) had CABG. Stents with a drug coating 38 (80.9%), without drug coverage 6 (12.8%). For recurrent coronary events: no event 20 (42.6%), stent thrombosis 12 (25.5%), stent restenosis 3 (6.4%), incomplete revascularization 2 (4.3), "no reflow" 2 (4.3%), rhythm disturbance 1 (2.1%), heart failure 2 (4.3%), "clean vessels" 2 (4.3%), failure of shunts 3 (6.4%). Genetic testing was performed: normal genotype 29 (61.7%), the CYP2C19*1/*2 heterozygote 13 (27.7%), the CYP2C19*2/*2 homozygote 3 (6.4%), the heterozygote CYP2C19*1/*3 2 (4.3%). The odds ratio (OR) for carriers of CYP2C19*2 and -*3 for the development of repeated coronary events after myocardial revascularization is [OR 4.308; 95% CI; 1.139-16.296; p=0.031], stent thrombosis [OR 5.0; 95% CI, 1.225-20.409; p=0.025], stent thrombosis and shunt failure [OR 6.0; 95% CI; 1.573-22.889; p=0.009].

Conclusion: Carrying polymorphisms of the CYP2C19 *2, -*3 gene increases the risk of ischemic complications by 4-6 times, including stent thrombosis after myocardial revascularization.

PO-56

RARE BLEEDING DISORDERS IN THE PEDIATRIC POPULATION OF NORTHERN GREECE: A 10-YEAR SINGLE CENTER EXPERIENCE

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Background: Rare bleeding disorders (RBDs) represent approximately 3–5% of all inherited coagulation deficiencies and their clinical phenotype is characterized by considerable heterogeneity.

Aim: To report on the experience of a referral center following pediatric patients with RBDs.

Methods: Records of 18 children diagnosed with RBDs were retrospectively reviewed, and both clinical and laboratory data were reported, covering a 10-year period.

Results: 11 out of 18 patients (61.1%) were males. Diagnosis was made at a mean age of 7.4 years (2-16 years). In 4 patients (22.2%) comorbidities were reported (deafness, histocytosis, cystic nephropathy or leukodystrophy).

FVII deficiency was the most common RBD reported, observed in 7/18 patients (38.9%) presenting with a mean factor level of 15.6U/dL (3-36.4U/dL). FX and FXII deficiency were each reported in 3 patients (16.7%), who presented with a mean factor level of 36U/dL and 27U/dL, respectively. FV and FXI deficiency were each reported in 2 patients, with a mean factor level of 40U/dL and 35U/dL, respectively. Hypofibrinogenemia was diagnosed in 1 patient (fibrinogen levels 75mg/dl).

Diagnosis was made following bleeding manifestations in 4 patients (22.2%), due to abnormal pre-operative or routine laboratory screening in 7 (38.7%) and following investigation due to a positive family history in the rest (38.7%).

Bleeding phenotype was variable, while correlation between missing factor levels and bleeding tendency was not always clear.

Conclusion: This long-term single center study confirms clinical variability of RBDs. Multi-center registries may help towards better understanding and management of such rare diseases.

Disclosure of Interest: None Declared

PO-57

FEASIBILITY OF A NEW FULLY AUTOMATED ADAMTS-13 ACTIVITY ASSAY

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Background: ADAMTS-13 activity is a unique marker for thrombotic thrombocytopenic purpura (TTP) which helps differentiate TTP patients from those suffering other more common thrombotic microangiopathies (TMA) and also less frequent conditions such as hemolytic uremic syndrome. Treatment for TTP patients includes plasma exchange. However, for most TMA patients, PEX is an unnecessary and expensive treatment. In routine, it may take an external laboratory between days and weeks to return the results of ADAMTS-13 activity. The preventive treatment initiated for a high risk TTP patient amounts to thousands of dollars. Moreover, routinely available ADAMTS-13 diagnostic is essential for the monitoring of TTP treatment and control of relapse rates. Thus, an in-house laboratory delivering results on the same day, spares the cost of unnecessary treatments and improves the overall outcome of the patient care.

Aim: The aim of this study was to develop a fully automated ADAMTS-13 activity assay.

Methods: The assay principle is based on a modified FRETS-VWF73 Substrate. ADAMTS-13 from the diluted plasma sample cleaves the substrate in a dose dependent manner. The generated fluorescent signal is proportional to the activity of ADAMTS-13 signal.

Results: The newly developed assay covered an assay range 0.0-1.0 IU/ml with calibrators directly traceable to the WHO standard. In a method comparison with the chromogenic ELISA plasma samples covering the whole assay range were analyzed. The regression between the two assays exhibited a good correlation (r>0.90) with an intercept <0.01 IU/mL. In addition, the possibility of testing inhibitors for ADAMTS-13 was evaluated using an automated mixing test.

Conclusions: This feasibility study demonstrates that this developed assay enables the accurate assessment of ADAMTS-13 activity and also the presence of antigenic inhibitors. The assay is designed to be automatable on a new fully automated coagulation analyzer.

PO-58

CURRENT VIEW ON THE SAFETY OF PEGYLATED BIOLOGICS IN HEMOPHILIA TREATMENT

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Background/Aims: The addition of polyethylene glycol (PEG) to molecules (PEGylation) is a valuable tool for half-life extension. BAY 94-9027 is an extended-half-life, B-domain-deleted recombinant factor VIII (FVIII) that is site-specifically PEGylated with a 60-kDa (2×30-kDa) PEG. Here, we discuss the safety of PEG and address potential concerns surrounding its possible accumulation in the body.

Materials and Methods: Four main factors determine the safety profile of PEG in hemophilia treatment: the PEG pharmacokinetic profile, conjugated with protein and alone; the nonclinical safety profile demonstrated in studies of the distribution and excretion pathways of PEG; long-term clinical findings; regulatory indications and findings.

Results: PEG has been used in several clinical indications, as well as in biopharmaceuticals, with at least 15 PEGylated products currently approved in Europe and the United States. Cellular uptake and excretion are determined by PEG size, characteristics of the PEGylated protein, existing nonspecific or receptor-mediated uptake, PEG dose and dosing frequency, and turnover kinetics of the cells involved. Excretion processes for large PEG molecules >60 kDa have been demonstrated such that there is no further increase in blood and/or tissue concentrations. Nonclinical studies have demonstrated that PEG excretion is dose dependent, indicating that a steady state will be reached for a given dosing schedule. An important consideration is whether the level achieved at steady state is associated with adverse effects. The only effect indicated in a nonclinical toxicology study using high-dose PEG in rats was cellular vacuolation. However, two further toxicological studies in rats chronically exposed to PEGylated FVIII products have shown no evidence of vacuolation. Finally, clinical data on >5 years' use of BAY 94-9027 have demonstrated an acceptable safety profile.

Conclusions: Concurrent with our understanding of PEG PK, distribution and bioavailability, the safety profile of PEGylated BAY 94-9027 has been demonstrated to be acceptable in clinical trials.

Conflict of Interest: The author is an employee of Bayer.

PO-59

PSYCHOS OCIAL ISSUES CHALLENGING THE MANAGEMENT OF HEMOPHILIA IN MAHARASHTRA INDIA

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The issues of access and affordability of optimal treatment in hemophilia in India has been known and written about many times. However, little has been documented regarding psychosocial issues further demanding the life of PWH (persons with hemophilia). Poor early recognition of the disease is coupled with undefined laboratory diagnostics. In addition, routine inhibitor testing is not always done. In our center at Sion Hospital covering almost three hundred patients we have experienced many issues worth describing and illuminating what needs focusing.

Long distances need to be traveled not only for acute care but few patients capable of administering or having factor. Essentially most of these patients need one factor treatment at home with the ability to self-infuse. Few non obligate carriers are tested, nor counseled and their roles of delivering a child with not understood hemophilia.

The ability to finish education, maintain active employment is markedly affected by recurrent absences.

The culturally determined family unit is comforting but with issues facing the hemophilia child. Restrictions make these young men frequently isolated.

Hostility toward the daughter-in-law carrier has faced some couples to leave, make marriages for young men difficult and at times impossible.

The recent recognition of the government that support for the hemophilia treatment is needed and changes in delivery systems in some parts of India already offer hope that treatment options will be improved. Without reconciling the psychosocial issues PWH may have less morbidity but will remain happy.

PO-60

SIX YEARS OF EXPERIENCE WITH PLASMA FRACTIONATION INDUSTRIES AT A PRIVATE SUPER SPECIALITY HOSPITAL IN SOUTH INDIA: RENAISSANCE IN USAGE OF EXCESS PLASMA IN BLOOD BANKS

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Background: According to World Health Organization (WHO) each country must plan for its safe and consistent supply of blood and plasma products for regular clinical needs as well as in case of any disaster. Self-sufficiency ratio is the key parameter to assess the ability of a country to meet its need for plasma derived medicines, through utilization of locally collected plasma. It also helps in proper usage of surplus plasma in blood banks.

Aim: This study aims at evaluating the impact of plasma fractionation industries as an add on in proper utilization of plasma components in the current Indian scenario.

Materials and Methods: A retrospective observational study was conducted to analyze the data of 6 years in providing excess plasma for plasma fractionation (PF) industries at Blood Bank, Yashoda Hospitals, Hyderabad, India.

Results: A total of 25,361 whole blood donations were collected from qualified blood donors between January 2013 and December 2018. 23,448 plasma units were prepared from the donations which were negative for Transfusion Transmittable Infections (TTIs). Only 54.7% (12832) of the plasma units were transfused to various patients and 0.79% (187 units) was discarded. The remaining 44.3% (10,301) of the plasma units were sent to PF companies at regular intervals where they are used for preparing varied plasma derived medical products.

Conclusion: Six years of shipping plasma to PF interdicted wastage of 44.3% (10,301) TTI negative plasma units. Implementation of usage of plasma components for transfusion to patients along with shipping excess plasma for PF periodically improves the effective utilization of blood components as well as preparation of medical products in large numbers by PF industries. However, the blood donation process and plasma supplied should meet the specifications as per Drugs and Cosmetics Act, 1940 and Rules made thereunder.

Year	Plasma transfused to patients	Plasma sent for fractionation	Discarded plasma
2013	2,774	1,296	39
2014	2,506	952	25
2015	1,876	1,900	33
2016	1,669	1,900	19
2017	1,633	2,647	32
2018	2,374	1,706	39

PO-61

IFN-G, IL-10, CD152 EVALUATION BY FLOW CYTOMETRY IN CD4+ T LYMPH OCYTES OF AN HEMOPHILIA A (HA) PATIENT WITH INHIBITORS DURING TREATMENT WITH PDFVIII

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Background: The development of anti-FVIII inhibitors is dependent on activation of TCD4 and on the secretion of specific cytokines. On the other hand, kynurenine pathway of tryptophan catabolism performed by IDO1 is an important component of a regulatory system favoring a tolerance to the FVIII. In a HA patient with inhibitors treated with plasma derived (pdFVIII) we evaluated by flow cytometry (FC) in TCD4 the level of pro-inflammatory IFNγ and anti-inflammatory/regulatory IL-10 cytokines. Moreover, we analyzed by FC the expression of immune checkpoint CTLA-4/CD152 given its connected role to the IDO1-pathway.

Materials and Methods: C.G. is HA patient with inhibitors, high responder. He underwent a radiosynoviorthesis on left shoulder because of chronic synovitis. As the last inhibitor titer was < 5 BU/ml, he was treated with pdFVIII from the day of procedure until day +4. Before and during pdFVIII treatment, until day +20, his peripheral blood were collected for the short-term in vitro cultures with and without pdFVIII. After 5 days the cells were analyzed by FC.

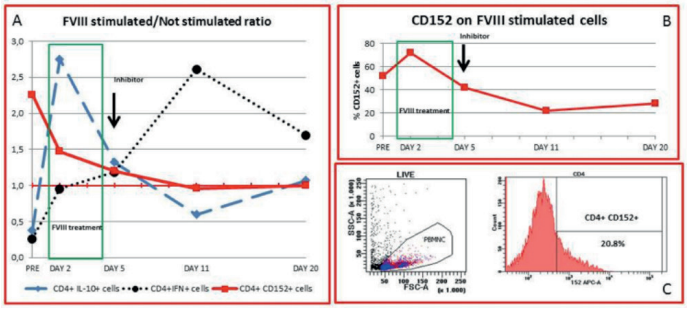

Figure. Before and during pdFVIII treatment and until the day 20, peripheral blood samples were collecte to perform anti-FVIII measurement and to obtain PBMCs. The PBMCs were incubated for 5 days (37°C in a 5% CO2 humidified incubator) in presence of IL-2 and pdFVIII and the fixed-permeabilized cells were stained with antibodies against intracitoplasmatic CD152, IFN-γ and IL-10 and run in a BD FACSCanto II. A. Normalized values of % of CD4+IL-10+, CD4+IFN-γ+ and CD4+CD152+ cells were obtained as ratios between the values in cells stimulated with pdFVIII and the values obtained in not stimulated cells. Ratio 1 line is outlined in red. FVIII treatment was performed in day PRE, 1, 2, 3, and 4; inhibitor appeared at day 5 and a significant increase was registered from day 11 (19,8 BU/ml) until day +20(24,5 BU/ml). B. Data were expressed as the percentage of CD152-positive cells among gated CD4T cells in 4 days culture with pfFVIII (50/ml). Inhibitor level remains at pre-treatment value until the increase at day 5 and increases in the following days. C. The CD152 analysis is performed in CD4T subsets after removing the dead cells.

Results and conclusions: The results were expressed as normalized values of median % of CD4+CD152+, CD4+IL10+ and CD4+IFNγ+ cells calculated as ratios between the values obtained in cultures with pdFVIII and those in not stimulated ones (figure). Inhibitor value remains as pretreatment value until day +5 and a significant increase was registered in the following days. Pretreatment ratio IL-10 and CD152 expression in TCD4+ was < 1; this ratio changes during the days coming to around 1 to the fifth day, and it maintain then ratio < 1. The synthesis of IFN-γ double up after day +5. According to our data (Figure 1) we had the opportunity to follow-up "in vitro" the response right after the stimulation with pdFVIII using FC in cells culture. The "in vivo" increase in inhibitor corresponds "in vitro" to a higher level of IFN-γ and a reduction in IL-10. We emphasize the reduction of CD152 expression during inhibitor production suggesting lower IDO1-dependent regulation to prevent anti-FVIII generation.

PO-62

NON-SPECIFIC NON-ANTIPHOSPHOLIPID INHIBITOR IN A 26-YEARS-OLD WOMAN: A CASE RE PORT WITH FEW ANSWERS AND MANY QUESTIONS

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A 26-years-old woman with slight asthenia and euthyroid autoimmune thyroiditis was admitted to the ambulatory of the "Miulli" Hospital of Acquaviva delle Fonti (Bari) for hypochromic microcytic anemia (Hb 7 g/L). She had neither personal nor familiar history of bleeding, but she referred heavy menstrual bleeding. The patient's anemia was promptly solved after intravenous iron infusions. However, routine coagulation tests showed prolonged PT ratio (1.29) and aPTT ratio (2.0). The patient was sent to a laboratory specialized in coagulation disorders which, following the laboratory tests results (aPTT ratio 1.92 with uncorrected mixing, SCT screening 3.74, SCT mixing 2.48, dRVVT screening 1.85, dRVVT mixing 1.53), made diagnosis of lupus anticoagulant. A second laboratory confirmed the previous tests, but in addition coagulation factors activity was measured: FVIII 7.6%, FXI 8.0%, FIX 4.0%, FX 64%, FII 48%, FVII 53%, FV 52%. Furthermore, TT was 1.34, fibrinogen (Clauss method) 198 mg/dL, von Willebrand antigen 92% and RCoF 72%, and anti-cardiolipin and anti-beta 2 glycoprotein 1 antibodies were negative. Unlike the first one, the second laboratory come to the diagnosis of a non-specific non-phospholipid type inhibitor. After 18 months, the patient was hospitalized for hemoperitoneum due to corpus luteal cyst rupture and she underwent an ovarian surgical excision without bleeding complications.

This case report highlights the difficulty of reaching a clear diagnosis in patients with acquired inhibitors. The case is still open because we have not isolated yet the inhibitor responsible for the patient's impaired laboratory tests results. Moreover, the real patient's bleeding risk remains unknown.

CSL Behring

Driven by Our Patients

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