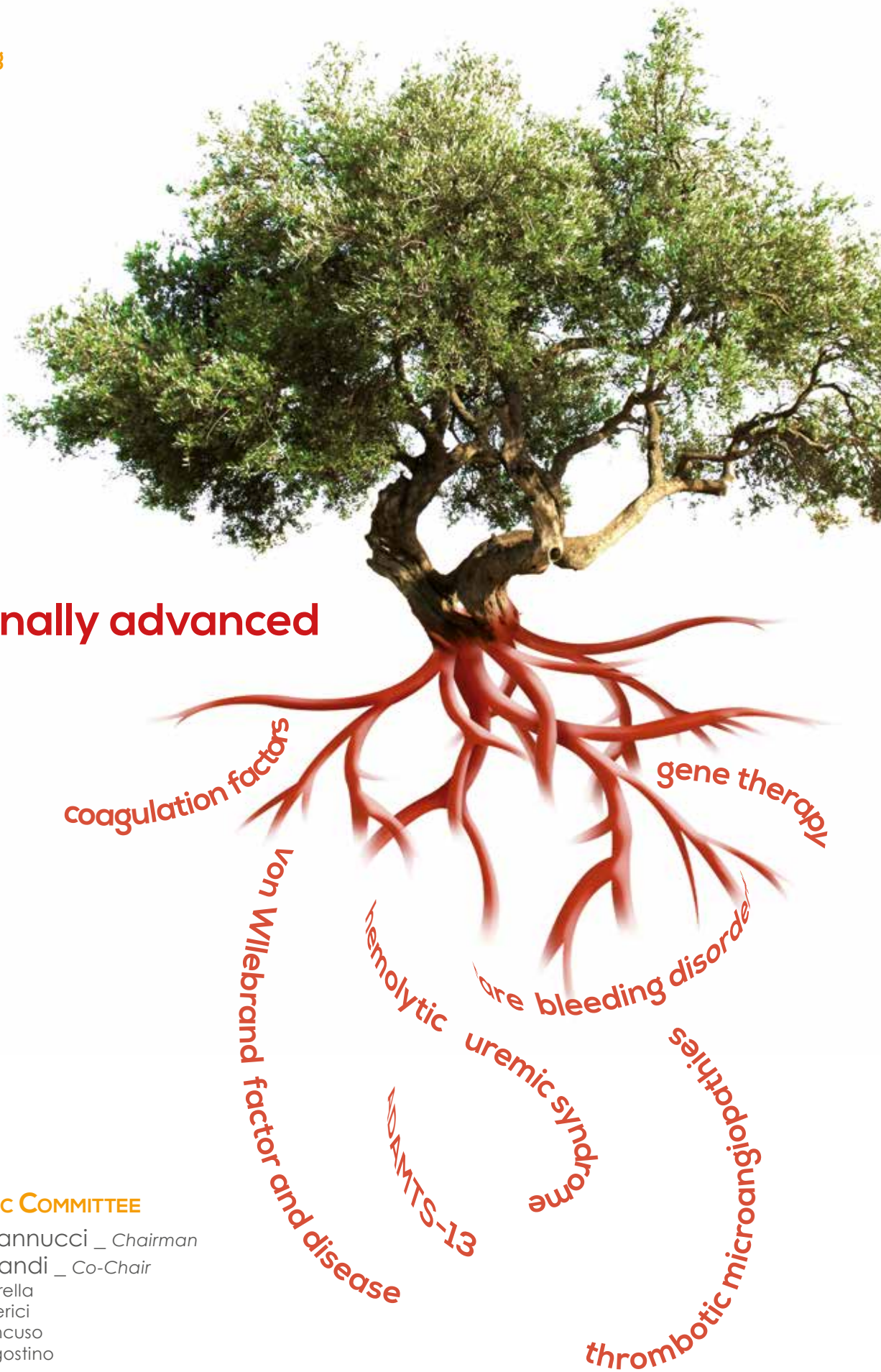
8th BIC

Bari International Conference

Bari • Italy • 3-5 October 2014

Villa Romanazzi Carducci Conference Center

www.bic2014.org



Abstract book

Scientific Committee

- P.M. Mannucci _ *Chairman*
- F. Peyvandi _ *Co-Chair*
- N. Ciavarella
- A.B. Federici
- M.E. Mancuso
- E. Santagostino

OC.01 - 96 COMPARISON OF BASELINE THROMBOELASTOGRAPHY AND THROMBIN GENERATION ASSAY IN FACTOR VIII DEFICIENT PATIENTS WITH AND WITHOUT INHIBITORS

V. Salinas[1], R. Carmona[1], B. M. Mohammed[2], E. J. Martin[2], D. F. Brophy[2], G. A. Young[1]

[1]Hemostasis and Thrombosis Center, Division of Hematology/Oncology, Children's Hospital Los Angeles, Los Angeles, CA, USA, University of Southern California Keck School of Medicine; [2]Coagulation Advancement Laboratory, Department of Pharmacotherapy and Outcomes Science, Virginia Commonwealth University, Richmond, VA, USA.

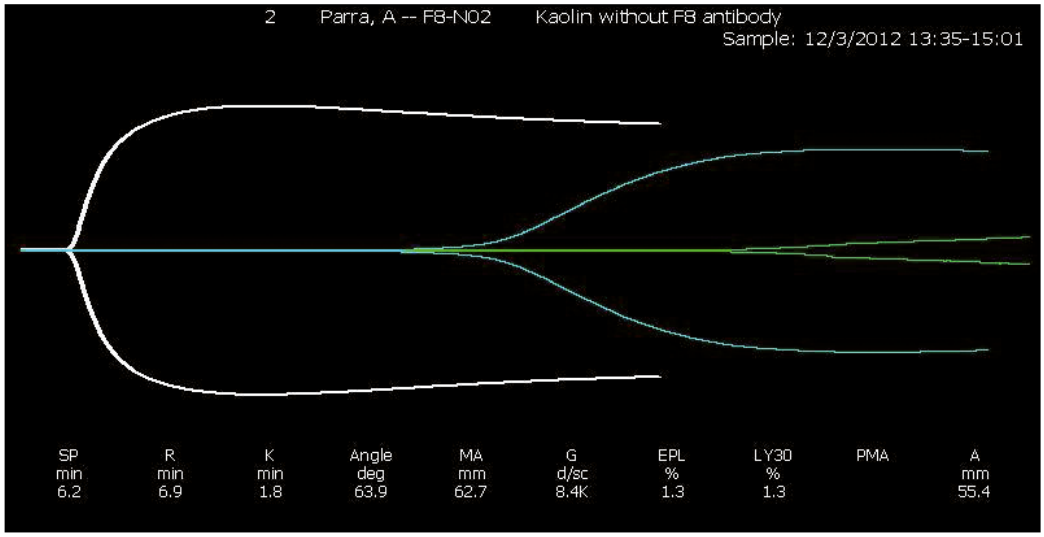
Background: Severe hemophilia A (SHA) patients with inhibitors have worse morbidity due to bleeding than patients without inhibitors. This study was designed to determine the coagulation kinetics in SHA with and without inhibitors using two global coagulation assays: thromboelastography (TEG) and thrombin generation assay (TGA), and to determine whether these differences can be correlated with clinical outcome.

Methods: Patients with SHA with and without inhibitors and controls (15 per group) provided a blood sample after a factor washout in sodium citrate tubes, some pre-loaded with corn trypsin inhibitor (CTI). Assays performed: FVIII activity, FVIII inhibitor, TEG on whole blood using kaolin or tissue factor, and TGA on platelet-poor plasma. TEG assays activated with TF and TGA used blood from the CTI-loaded tubes. Polyclonal anti-FVIII antibody was added to all above assays on subjects without inhibitors and controls. Clinical information was collected from medical records.

Results: Kaolin-activated TEG (but not TF-activated TEG) demonstrated significant differences between patients with and without inhibitors. These differences were statistically significant for R, K and angle ($p < 0.004$, < 0.01 and < 0.01 respectively). Addition of anti-factor VIII antibody to samples from control and non-inhibitors eliminated the difference, confirming that these differences were due to the presence of the inhibitor. Analysis of TGA parameters showed thrombin generation as markedly decreased in all patients with SHA, but no difference was demonstrated between patients with and without inhibitors. We could not demonstrate a correlation between any TEG parameter and the annualized bleeding rate (ABR).

Conclusions: Our study demonstrated that kaolin is a more sensitive predictor of coagulation kinetics than TF, and that kaolin-activated TEG can detect significant differences in clot formation kinetics between SHA patients with and without inhibitors. In addition, TGA could not demonstrate differences between inhibitor and non-inhibitor patients.

Corresponding Author
G. A. Young, *gyoung@chla.usc.edu*



OC.01 - 96
Figure 1

OC.02 - 109 HEMOPHILIC PATIENTS WITH INHIBITORS UNDERGOING ORTHOPAEDIC SURGERY: THE CONTRIBUTION OF THROMBIN GENERATION ASSAY (TGA) AS LABORATORY MONITORING TOOL

M. E. Mancuso, M. R. Fasulo, V. Chantarangkul, M. Clerici, E. Scalambrino, L. Padovan, F. Peyvandi, A. Tripodi, E. Santagostino

Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy.

Background and aims: No routine coagulation monitoring is available for hemophilic patients with inhibitors treated with by-passing agents. The aims of this study were to assess if TGA is able to predict the hemostatic and clinical response to by-passing agents.
Materials and Methods: TGA was assessed in platelet-rich (PRP) and platelet-poor (PPP) plasma with the addition of corn trypsin inhibitor (CTI) in 17 patients with severe hemophilia A (9 with high-responding inhibitors and 8 without inhibitors) aged 5-59 yrs (median: 39) undergoing orthopaedic surgery. TGA was assessed once daily prior and 30 minutes after either FVIII or by-passing agents injection starting from the pre-operative bolus and for 4 post-operative days. Haemostatic treatment to cover surgical procedures was established irrespective of TGA measurements.
Results: in the group of 8 non-inhibitor patients TGA values increased after the first FVIII infusion, however during the post-operative period (FVIII trough levels >50 IU/dL) TGA was scarcely sensitive to the significant variation observed in FVIII levels prior (median: 74%) and after daily infusions (median: 155%; $p<0.001$). In the group of 9 inhibitor patients TGA increased after by-passing agents injection however TGA did not reveal different responses related to the type of drug, the dose used and/or the occurrence of bleeding complications (n=5). Moreover, a lack of response of the TGA curve was observed during the post-operative period irrespective of treatment regimen modifications in all inhibitor patients.
Conclusions: our results indicate that TGA is not a suitable tool to monitor hemostatic response during surgery in haemophiliacs. In non-inhibitor patients TGA is moderately sensitive to FVIII levels variations and does not provide additional information on coagulation activation during replacement therapy and in inhibitor patients TGA is not able to predict either the hemostatic response to different by-passing agents used at different doses nor the risk of bleeding complications.

Corresponding Author
M. E. Mancuso, *elisamancuso@tiscali.it*

OC.03 - 129 INDUCED PLURIPOTENT STEM CELL (IPSC) - BASED STRATEGY TO CORRECT THE BLEEDING PHENOTYPE IN HEMOPHILIA A

M. Talmon[(1)], C. Olgasi[(1)], S. Merlin[(1)], A. Lombardo[(2)], L. Naldini[(2)], A. Raya[(3)], F. Valeri[(4)], M. Messina[(5)], P. Schinco[(4)], A. Follenzi[(1)]

[(1)]Department of Health Sciences, Università del Piemonte Orientale, Novara, Italy; [(2)]Tiget, San Raffaele Hospital, Milan, Italy; [(3)]Institute for Bioengineering of Catalonia (IBEC), Barcelona, Spain; [(4)]Haemostasis and Thrombosis Unit-Haemophilia Center, Department of Experimental Medicine and Oncology, Academic Hospital S. Giovanni Battista, Turin, Italy; [(5)]Haemostasis and Thrombosis Unit-Haemophilia Center for Pediatric Age, Regina Margherita Hospital, Torino, Italy.

Background/Aim: Hemophilia A (HA) is a bleeding disorder caused by factor VIII (FVIII) gene mutations. Somatic cells can be reprogrammed to generate autologous, disease-free iPSCs, then differentiated into cell targets relevant for gene and cell therapy. Our aim is to develop a novel HA treatment strategy generating FVIII-corrected patient-specific iPSCs from peripheral blood cells and differentiating them into functional endothelial cells (ECs), secreting FVIII after transplantation.
Methods: Mononuclear (MNC) and CD34+ cells were isolated from peripheral blood of healthy and hemophilic donors and reprogrammed with a Cre-excisable polycistronic LV carrying OCT4, SOX2 and KLF4. iPSCs were characterized by Alkaline Phosphatase (AP), immunofluorescence staining, telomeres length, RT-PCR for stem cell markers in iPSCs and three germ layers markers in embryoid bodies (EBs).The differentiation potential was assessed by adipogenic, osteogenic and chondrogenic differentiation. iPSCs were differentiated in ECs using hVEGF-supplemented EB medium. Endothelial markers expression was evaluated by FACS and RT-PCR. ECs, transduced with LV carrying GFP under the control of endothelial-specific promoters: Flk-1, Tie2 and VEC, were transplanted in NSG-HA mice. Cells engraftment was analyzed by GFP-staining of liver sections.
Results: Reprogrammed MNC and CD34+ cells gave rise to ESC-like-iPSCs colonies positives for AP staining and stem cell markers. EBs differentiated in osteogenic, chondrogenic and adipose tissues, and expressed three germ layers markers. Increased iPSCs telomeres length suggested telomerase reactivation. RT-PCR on FVIII-corrected HA-iPSCs showed hBDD-FVIII expression, confirming HA-MNC genetic correction by LV transduction. iPSCs differentiated into ECs, acquiring endothelial-like morphology, expressing ECs markers and formed tubules when cultured in matrigel. Flk-1/Tie2/VEC-GFP-LV transduced ECs were transplanted in NSG-HA mice and detected in liver sections up to 1 month after transplantation.
Conclusion: These data will be instrumental to assess engraftment, proliferation and FVIII expression levels from differentiated, gene corrected and reprogramming factor-free iPSCs to confirm the suitability of this approach for HA gene-cell-therapy.

Corresponding Author
A. Follenzi, *antonia.follenzi@med.unipmn.it*

OC.04 - 134 RESTORING HEMOSTATIC BALANCE IN HEMOPHILIA BY RNAi TARGETED SILENCING OF ANTITHROMBIN

B. Sørensen[1], A. Sehgal[1], J. Quin[1], T. Racie[1], S. Barros[1], L. Ivanciu[2], R. Camire[2], Y. Dargaud[3], C. Négrier[3], A. Akinc[1]
[1]Alnylam Pharmaceuticals, Cambridge, MA, USA; [2]Children's Hospital of Philadelphia, USA; [3]Hopital Edouard Herriot, Lyon, France.

Introduction: Hemophilia A or B are congenital bleeding disorders caused by dysfunctional propagation of thrombin generation due to deficiency in factors VIII or IX in the presence of normal levels of anticoagulants resulting in an imbalance of the hemostatic system toward a bleeding phenotype. We are currently investigating the use of RNA interference (RNAi) to target the natural anticoagulant antithrombin (AT) as a strategy to rebalance the hemostatic system and improve thrombin generation, and therefore hemostasis, in hemophilia. ALN-AT3, a subcutaneously administered RNAi therapeutic targeting AT, is currently being developed for the treatment of hemophilia.
Material and methods: Preclinical studies in hemophilia mouse models have investigated the ability of ALN-AT3 to silence AT and thereby correct thrombin generation as measured by Calibrated Automated Thrombin (CAT) generation assay, restore hemostatic plug formation in real-time laser injury clot formation visualization, and control traumatic bleeding in a saphenous vein bleeding model. Preclinical studies were also conducted in vehicle and ALN-AT3 treated non-human primates followed by infusion of high-dose anti-factor VIII antibody to induce inhibitor hemophilia A and measurement of thrombin generation. The association between AT reduction and factor VIII and IX equivalence was assessed by *in vitro* thrombin generation studies using human hemophilia A and B plasma samples. Finally, a phase 1 clinical study in healthy volunteers and severe/moderate hemophilia A or B has been initiated. Part A in healthy volunteers have been completed and Part B in hemophilia patients is ongoing.
Results: ALN-AT3 treatment targeting residual AT levels of 20-40% in hemophilia A and B mouse models increased thrombin generation, restored real-time localized hemostatic plug formation in the laser-injury model comparable to treatment with full-length recombinant factor VIII. ALN-AT3 controlled traumatic bleeding in the saphenous vein model with an increase in number of hemostatic events equivalent to that achieved with infusion of 25IU/kg full-length recombinant factor VIII. ALN-AT3 treatment targeting 20% residual AT levels normalized thrombin generation in non-human primates with induced high titer inhibitor hemophilia A. *In vitro* titration studies with factor VIII or IX in human hemophilia A or B plasma as well as decreasing AT showed that targeting residual AT levels of 40-60% is equivalent to factor VIII or IX trough levels ranging from 10-15%. In Part A of the Phase 1 study, human volunteer subjects received a single subcutaneous dose of ALN-AT3 and, per protocol, the maximum allowable level of AT knockdown was set at 40%. Initial results show that a single, low subcutaneous dose of ALN-AT3 at 0.03 mg/kg resulted in an up to 28-32% knockdown of AT at nadir that was statistically significant relative to placebo ($p < 0.01$ by ANOVA). This led to a statistically significant ($p < 0.01$) increase in peak thrombin generation, that was temporally associated and consistent with the degree of AT knockdown. ALN-AT3 was found to be well tolerated with no significant adverse events reported.
Conclusion: Collectively, these data suggest that the use of a novel RNAi therapeutic targeting AT is a promising approach for restoring hemostatic balance in hemophilia, and potentially, other bleeding disorders. Further, the subcutaneous route of administration, infrequent dosing, and applicability to persons with hemophilia who have inhibitors, make this a particularly encouraging potential therapy.

Corresponding Author
B. Sørensen, *bsorensen@alnylam.com*

OC.05 - 54 INDUCTION OF TOLERANCE TO FVIII USING NANOPARTICLES IN A MURINE MODEL OF HEMOPHILIA A

A. Zhang[1], R. Rossi[1], A. Griset[2], R. Maldonado[2], T. K. Kishimoto[2], D. W. Scott[1]
[1]Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD, USA; [2]Selecta Bioscience, Inc., Watertown, MA, USA.

Introduction/Background: Anti-FVIII inhibitor development is currently considered the most serious side effect in the treatment of hemophilia A patients. In this study, we sought to address this problem by determining the efficacy of a novel tolerogenic protocol utilizing nanoparticle formulations provided by Selecta Biosciences.
Methods: We treated hemophilia A mice (FVIII KO) weekly for five weeks with a highly immunogenic dose of 1 µg recombinant human FVIII (rFVIII), plus tolerogenic nanoparticles or control nanoparticles (coded F1 or F2 or F3), both given together intravenously.
Results: As expected, all the mice in the control group (later revealed to be F3) developed high levels anti-FVIII IgG and Bethesda titer after the five treatments. Both the F1 and F2 nanoparticle treatments effectively prevented the anti-FVIII inhibitory antibody development throughout the course of the 5-week treatment, a result that was stable for over 200 days even with repeated FVIII challenge. Moreover, mice treated with high dose IVIG were suppressed temporarily only when IVIG was given. The tolerogenic effect of the nanoparticles was specific as those mice responded normally to unrelated antigens, like bacteriophage viruses and foreign RBC. In a therapeutic protocol using mice pre-immunized with FVIII, nanoparticle treatment also led to substantial reductions in anti-FVIII titers but required additional treatment. The mechanism(s) of this tolerance process are under study.
Conclusion: In conclusion, the concomitant Selecta F2 nanoparticle treatments not only significantly prevented the anti-FVIII inhibitory antibody development during the treatments, but also resulted in long-term FVIII-tolerance, suggesting a potential clinical application.
(AZ is supported in part by a grant-in-aid from Selecta Bioscience and by HL061883)

Corresponding Author
T. K. Kishimoto, *KKishimoto@selectabio.com*

OC.06 - 78 DISTINCT CELLULAR VWF PHENOTYPES OBSERVED IN BOEC FROM TYPE 3 VWD PATIENTS WITH VWF PROPEPTIDE MUTATIONS COMPARED TO NON-PROPEPTIDE MUTATIONS

M. L. Bowman[1], L. Casey[1], A. Tuttle[1], P. James[2]
[1]*Department of Pathology & Molecular Medicine, Queen's University, Kingston, Canada;* [2]*Department of Medicine, Queen's University, Kingston, Canada.*

Background/Aim: We previously showed in the Canadian type 3 VWD population that 42% of mutations identified were located in the VWF propeptide, and index cases (IC) with mutations here had higher bleeding scores (BS) than IC with non-propeptide mutations (median BS=22 vs.13, p=0.012). The aim of this study was to use patient-derived blood outgrowth endothelial cells (BOEC) from type 3 VWD IC to make comparisons between the molecular pathogenesis of VWF propetide and non-propeptide mutations.
Methods: Peripheral blood samples were collected for BOEC isolation. Endothelial cell phenotype of isolated cells was confirmed. VWF secretion, multimerization, storage, and stimulated VWF release were examined.
Results: BOEC were isolated from eight type 3 VWD IC (seven different genotypes; Table 1) and nine family members (affected and unaffected). Despite a virtual lack of VWF in the plasma, confocal immunofluorescence microscopy showed two different patterns of intracellular VWF staining. IC who were homozygous or compound heterozygous for VWF propeptide mutations (n=5), showed a complete lack of VWF storage, with only diffuse VWF staining. The diffuse VWF co-localized with the ER marker, calnexin, indicating ER retention. In contrast, IC with mutations in the mature VWF molecule (n=3) showed both diffuse VWF staining and limited VWF stored in qualitatively abnormal Weibel-Palade bodies (WPB). The stored VWF co-localized with other proteins stored in WPB, such as P-selectin.
Conclusions: Two distinct cellular phenotypes are present in BOEC from type 3 VWD IC with VWF propeptide mutations and non-propeptide mutations. This may account for the differences in BS we previously noted. Patient-derived BOEC are a valuable cellular model for investigating the pathobiology of VWF mutations which is reflective of the native vascular environment. Additionally these cells may provide a tool to study the effect of novel treatments for VWD, leading the way for personalized treatment of VWD.

Corresponding Author
M. L. Bowman, *6mlb6@queensu.ca*

OC.07 - 94 ADAMTS-13 DESTABILIZES THROMBI IN A MOUSE MODEL OF THROMBOTIC FOCAL CEREBRAL ISCHEMIA

F. Denorme[1], F. Langhauser[2], C. Kleinschnitz[2], K. Vanhoorelbeke[1], S. De Meyer[1]
[1]*Laboratory for Thrombosis Research, IRF Life Sciences, KU Leuven Kulak, Kortrijk, Belgium;* [2]*Department of Neurology, University Clinic of Würzburg, Würzburg, Germany.*

Background/Aims: Ischemic stroke occurs when blood thrombi occlude major or multiple smaller intracerebral arteries. Currently, rapid thrombolysis using tissue-plasminogen activator (t-PA) is the only approved therapeutic treatment, but it has many serious limitations. ADAMTS-13 is a metalloprotease that cleaves von Willebrand factor (VWF), a crucial factor in thrombus formation. By cleaving ultra-large and hyper-reactive VWF multimers into smaller, less thrombogenic ones, ADAMTS-13 has been shown to have both antithrombotic and anti-inflammatory properties. In this study, we analyzed whether ADAMTS-13 can destabilize blood thrombi in the setting of ischemic stroke by cleaving VWF that is linking platelets together.
Materials and Methods: We used a mouse model of focal cerebral ischemia in which an occluding thrombus is generated in the right middle cerebral artery (MCA) by local application of FeCl3.
Results: A substantial difference between ADAMTS-13 knock-out (KO) and wild type mice was observed. In ADAMTS-13 KO mice an occlusive thrombus was formed faster than in wild type animals (4.2min ± 0.5min versus 6.4min ± 0.5min respectively, p= 0.0034). Moreover, ADAMTS-13 KO mice also developed significantly larger cerebral infarcts 24h after induction of focal cerebral ischemia (10.3mm2 ± 1.8mm2 versus 2.8mm2 ± 1.2mm2 respectively, p=0.0022). The origin of this discrepancy was found in the early stages after thrombus formation. In ADAMTS-13 KO mice, a stable thrombus was able to form which occluded blood flow in the MCA. Wild type mice on the other hand showed more spontaneous recanalization, rescuing the brain from cerebral ischemia.
Conclusions: These results point towards a destabilizing role for ADAMTS-13 in cerebral thrombotic occlusions. Hence, ADAMTS-13 may have prothrombolytic potential that could be exploited in the management of acute ischemic stroke. Possibly, ADAMTS-13 could also limit new thrombus growth onto thrombi that are being lyzed by t-PA, facilitating overall thrombus dissolution.

Corresponding Author
F. Denorme, *frederik.denorme@kuleuven-kulak.be*

Patient ID	Gender/age (M/F year)	Bleeding Score	VWF:Ag (IU/ml)	VWF:RCo (IU/ml)	FVIII:C (IU/ml)	Nucleotide Change, HGVS	Amino Acid Change, HGVS	Exon	Genotype
Propeptide Mutations									
T001	M/42	19	0.01	0.02	0.02	c.221-977_532+7059del	*p.Asp75_Gly178del*	4-5	Homozygous
T141	F/20	15	0.06	0.04	0.07	c.817C>T	*p.Arg273Trp*	7	Homozygous
T050	F/21	24	0.01	0.00	0.01	c.876delC, c.1255C>T	*p.Ser293fs, p.Gln419**	8/11	Compound heterozygous
T076	M/17	23	0.01	0.03	0.01	c.1897T>C	*p.Cys633Arg*	15	Homozygous
T076-S	F/11	9	0.01	0.03	0.01	c.1897T>C	*p.Cys633Arg*	15	Homozygous
Non-Propeptide Mutations									
T151	M/27	29	0.02	0.04	0.01	c.3939G>A, c.5842+1 G>C	*p.Trp1313**, _	28/34	Compound heterozygous
T105	F/62	18	0.02	0.00	0.03	c.8419_8422dupTCCC	*p.Pro2808Lysfs*24*	52	Homozygous
T154	M/16	24	0.01	0.03	0.01	c.1657dupT, c.8419_8422dupTCCC	*p.Trp553Lysfs*97, p.Pro2808Lysfs*24*	14/52	Compound heterozygous

OC.06 - 78
Table 1: VWF Phenotype and Genotype of the 8 Type 3 VWD Index Cases

OC.08 - 123 PREGNANCY COMPLICATIONS IN ACQUIRED THROMBOTIC THROMBOCYTOPENIC PURPURA

B. Ferrari[1], L. A. Lotta[1], A. Maino[1], A. Artoni[1], S. Pontiggia[1], S. M. Trisolini[2], A. Malato[3], F. R. Rosendaal[5], F. Peyvandi[1,5]

[1]Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico and Luigi Villa Foundation, Milan, Italy; [2]Cellular Biotechnologies and Hematology, Sapienza University, Rome, Italy; [3]UOC di Ematologia con UTMO, Ospedali Riuniti Villa Sofia-Cervello, Palermo, Italy; [4] Department of Clinical Epidemiology and Department of Thrombosis and Haemostasis, Leiden University Medical Center, Leiden, The Netherlands; [5]Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy.

Background/aims: Pregnancy is considered an important risk factor for relapse of acquired thrombotic thrombocytopenic purpura (TTP). The risk of miscarriage could also be increased in these women, similar to other autoimmune disorders. However, the exact entity and causes of these risks are unknown. The aim of this study was to evaluate risk factors associated with gravidic TTP relapse and miscarriage in women with a history of acquired TTP.

Materials and Methods: We conducted a nested case-control study in women with a history of acquired TTP enrolled in the Milan TTP registry from 1994 to October 2012. Sixteen out of 254 women had a pregnancy after diagnosis of acquired TTP. We contrasted women with a complicated pregnancy (i.e., cases of either gravidic TTP or miscarriage) with women with uncomplicated pregnancy (i.e., controls). Clinical variables (age at pregnancy, gravidity, time from the last TTP episode, TTP recurrence) and laboratory features (ADAMTS-13 activity, anti-ADAMTS13 antibody) were studied. We used odds ratios as an approximation of relative risks for these variables.

Results: According to pregnancy outcome, 4 cases with gravidic TTP, 5 with miscarriage and 7 controls with uncomplicated pregnancy were included. ADAMTS-13 activity levels in the first trimester were reduced in the cases, severely (median <3%) in gravidic TTP and moderately (20%, range 14-40%) in miscarriage; in the controls, median ADAMTS-13 activity level was 77% in the first trimester (range 40-129%) and remained above 39% until delivery, in the absence of detectable anti-ADAMTS13 antibodies. The presence of anti-ADAMTS13 antibodies during pregnancy was associated with an over 5-fold increase in the risk for both gravidic TTP and miscarriage (lower boundary of the confidence interval of the odds ratio).

Conclusions: ADAMTS-13 activity levels and evaluation of anti-ADAMTS13 antibody may help to predict the risk of complications in pregnant women with a history of acquired TTP.

Corresponding Author
B. Ferrari, *barbara.ferrari@policlinico.mi.it*

OC.09 - 125 CORRECTION OF MURINE ADAMTS-13 DEFICIENCY AND TTP-LIKE SYMPTOMS USING THE 'SLEEPING BEAUTY' TRANSPOSON SYSTEM

S. Verhenne[1], N. Vandeputte[1], I. Pareyn[1], Z. Izsvák[2], H. Rottensteiner[3], H. Deckmyn[1], S. De Meyer[1], K. Vanhoorelbeke[1]

[1]Laboratory for Thrombosis Research, IRF Life Sciences, KU Leuven Kulak, Kortrijk, Belgium; [2]Max Delbrück Center for Molecular Medicine, Berlin, Germany; [3]Baxter Innovations GmbH, Vienna, Austria.

Background/Aims: In humans, congenital deficiency of A Disintegrin And Metalloprotease with ThromboSpondin type 1 motif (ADAMTS-13) may cause the life-threatening disease thrombotic thrombocytopenic purpura (TTP). The current treatment of choice is plasma infusion. However, further research is required because plasma infusion exposes patients to the risk of allergies, infections, and fluid volume overload. We tested the use of the non-viral 'Sleeping Beauty' (SB) transposon system to integrate the murine ADAMTS-13 (muADAMTS13) gene and to correct the prothrombotic phenotype of Adamts13-/- mice.

Methods: The SB transposon system (muADAMTS13-transposon and transposase expressing plasmid) was delivered by hydrodynamic tail vein injection in Adamts13-/- mice. Transgene muADAMTS13 present in plasma was measured using a muADAMTS13:Ag ELISA, its proteolytic activity was assessed using a FRETS-VWF73 assay and VWF multimers were analyzed using SDS agarose gelelectrophoresis. Mice expressing transgene muADAMTS13 were challenged with recombinant human VWF (rhuVWF) at different time points to induce TTP. The TTP phenotype was evaluated by the occurrence of severe thrombocytopenia. Adamts13-/- mice hydrodynamically injected with 0.9% NaCl were used as controls (non-treated mice).

Results: The co-injection of the transposon plasmid with the transposase-expressing plasmid resulted in high levels of active transgene muADAMTS13 (350±38%, 192±55%, and 91±47% at day 7, 28 and 70 days after injection respectively). Moreover, transgene muADAMTS13 was active as it digested FRETS VWF73 *ex vivo* and ultra-large VWF multimers *in vivo*. Interestingly, the presence of transgene muADAMTS13 protected Adamts13-/- mice from TTP after challenge with rhuVWF at 7 or 28 days as no thrombocytopenia was observed in SB-treated mice compared with non-treated mice.

Conclusion: We successfully used the non-viral SB transposon system to realize long-term expression of transgene muADAMTS13 in Adamts13-/- mice. This transgene muADAMST13 was active, capable of digesting UL-VWF multimers *in vivo*, and able to prevent the onset of TTP-like symptoms in Adamts13-/- mice.

Corresponding Author
S. Verhenne, *sebastien.verhenne@kuleuven-kulak.be*

01. HEMOPHILIA B AND RARE COAGULATION DISORDERS

PO.01.01 - 149 CELL THERAPY MODEL IN HEMOPHILIA B BY GENETICALLY MODIFIED KERATINOCYTES BY LENTIVIRAL VECTORS TO PRODUCE HUMAN CLOTTING FACTOR IX

I. A. Gonzalez Ramos[1,2], I. J. Lara Navarro[2,3], L. F. Jave Suarez[4], J. A. Marchal Corrales[5,6], A. Bernad Miana[7], A. R. Jaloma-Cruz[4]

[1]Guadalajara University, University Center of Health Sciences, Guadalajara, Jalisco, Mexico; [2]West Biomedical Research Center of Social Security Mexican Institute, Department of Genetics, Tissue Culture, Guadalajara, Jalisco, Mexico; [3]Guadalajara University, University Center for agricultural biosciences, Guadalajara, Jalisco, Mexico; [4]West Biomedical Research Center of Social Security Mexican Institute, Department of Immunology Guadalajara, Jalisco, Mexico; [5]Department of Anatomy and Embryology Faculty of Medicine, Institute of Biopathology and Regenerative Medicine; [6]Biomedical Research Centre (CIBM), University of Granada, Granada, Spain.; [7] Head of Department of Regenerative Cardiology Foundation National Centre for Cardiovascular Research Carlos III, Madrid, Spain.

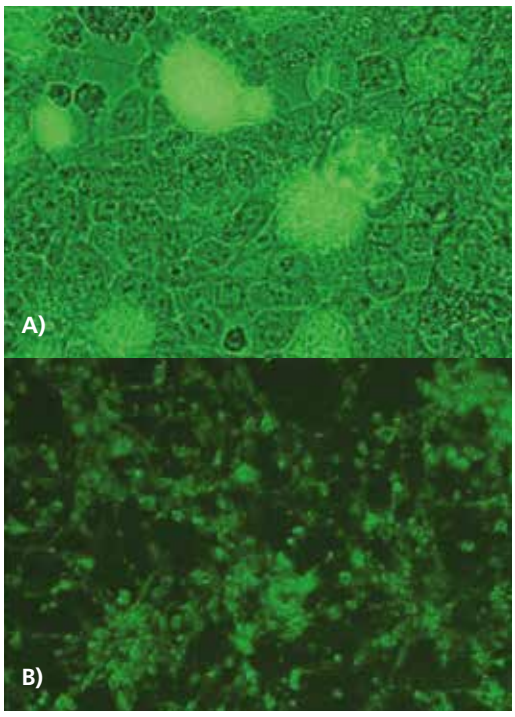
Background/Aims: Hemophilia B is a key model for gene therapy as replacement treatment. Keratinocytes are highly attractive as "target" of *ex vivo* gene therapy by their physiological properties and bio-safety traits as monitorable transplant. Self-inactivated (SIN), third-generation lentiviral vectors, show bio-security advantages and higher efficiency of gene expression. Dr. Ángeles Escartí (2006) proved the capacity of lentivirus-transduced keratinocyte graft to secrete biologically active human FIX (hFIX) into the bloodstream in a FIX-deficient transgenic mouse. Dr. Antonio Bernad transferred us the three lentiviral vectors of hFIX, tested by Escartí, to scale the approach to porcine model. We show preliminary results of the transduction of the vectors into a human cell line DOK (dysplastic oral keratinocytes). The aim of this study is optimizing the genetic modification of keratinocytes culture by SIN lentiviral vectors for hFIX production.

Material and Methods: Fibroblasts were obtained by enzymatic digestion with collagenase-dispase and trypsin-EDTA from porcine skin biopsies; the DOK cell line was kindly donated by Dr. Juan-Antonio Marchal. Plasmids of lentiviral constructs were transfected into 293-LentiX cells with the HT-Packaging-kit Clontech™ to produce lentiviral particles which in turn transduced keratinocytes to express hFIX as therapeutic gene and EGFP as marker. Transduction of DOK keratinocytes cell line was performed in 8-16 cell colonies and the efficiency was evaluated at 48 hours and after a week of culture, by EGFP incorporation and hFIX production assessed by flow cytometry. Transduced cells were harvested and sub-cultured in two conditions: with specific medium DMEM/Hydrocortisone and on a gel biomembrane composed of porcine plasma and fibroblasts.

Results: The transduction efficiency, formerly evaluated on human keratinocytes by EGFP production reached >80% with the three vectors (*figure 1*). The amount of hFIX protein in the supernatant of the DOK cells culture was similar among the three vectors at 48 hours (p=0.94) and after one week of transduction (p=0.7). A higher production of hFIX was demonstrated in cultured DOK on the biomembrane at 48 hours (p=0.38) and a significant increase was reached after one week of culture (p = 0.00).

Conclusions: Our findings did not show significant differences between the three vectors, because the *in vitro* model probably was not suitable to test the secretion of therapeutic protein under physiological conditions. A statistically significant difference in the increased production of supernatant hFIX in the gel-cultures, suggests that the bio-matrix provides a more similar physiological environment and promotes the hFIX secretion to the supernatant. Genetically modified keratinocytes for therapeutic gene production by lentiviral vectors is a promising model for several monogenic diseases, including hemophilia. According to our results *in vitro*, next step will include the graft of genetically modified skin into porcine model to test the efficiency of systemic production of hFIX transgene.

Corresponding Author
A. R. Jaloma-Cruz, *arjaloma@gmail.com*



PO.01 - 01 - 149
Figure 1. A) EGFP positive signal of transfection into 293-LentiX cells to produce lentiviral vector for hFIX, 40X. B) EGFP positive signal of transduction into keratinocytes (>80%) with lentiviral vector, 10X. Fluorescence-contrast by inverted microscopy.

PO.01.02 - 161 INFLUENCE OF APTT REAGENTS ON THE 1-STAGE CLOTTING ASSAY ACTIVITY IN HUMAN PLASMA DETERMINED FOR RECOMBINANT FIX PRODUCTS

H. Gritsch, S. Romeder-Finger, F. Scheiflinger, P. L. Turecek
Baxter Innovations GmbH, Vienna, Austria.

Background/Aims: Baxter recently introduced into the market in the US RIXUBIS, a new recombinant factor IX product produced from CHO cells by Baxter's protein-free manufacturing technology. Potency assignment for FIX products is performed with a 1-stage clotting assay based on the activated partial thromboplastin time assay (aPTT). Potency labeling should be traceable to the WHO standard for FIX concentrates. RIXUBIS and another rFIX product available on the market were analyzed for FIX potency using a panel of aPTT reagents from various manufacturers and the results were compared with the labeled potency. The impact from type and source of the aPTT reagent was investigated.
Materials and Methods: The 1-stage clotting assay was run on a coagulation analyzers (BCS/XP, Siemens Healthcare Diagnostics GmbH, Vienna, Austria), FIX activities were calculated relative to a secondary in-house standard, traceable to the WHO standard for FIX concentrates. In addition, the chromogenic activity was also analyzed using test kits from two manufacturers (Biophen Factor IX, Aniara/Hyphen Biomed, Coachrom, Vienna, Austria) and Rox Factor IX (Rossix, Haemachrom Diagnostica GmbH, Essen Germany).
Results: The FIX potency of both recombinant products was dependent on the type of aPTT reagent used, discrepancies up to 40% were found. The dependency was similar for both rFIX concentrates. For RIXUBIS, good agreement between the labeled potency and the FIX potency obtained with two different FIX chromogenic assays was found. The comparator product resulted in lower chromogenic activities as compared to the label. When RIXUBIS and the comparator rFIX product were spiked *in vitro* into plasma from hemophilia B patients the resulting FIX activity was also dependent on the type of aPTT reagent used for the 1-stage clotting assay.
Conclusion: Potency of rFIX products is dependent on the aPTT reagent used for the 1-stage clotting assay. Both RIXUBIS and the comparator rFIX product are affected in a similar way. For RIXUBIS the labeled 1-stage clotting potency is in good agreement with the chromogenic activity while for the other rFIX product lower chromogenic data were found.

Corresponding Author
P. L. Turecek, *peter_turecek@baxter.com*

PO.01.03 - 160 DOES THE FACTOR IXA CONTENT OF FACTOR IX PRODUCTS IMPACT FUNCTION, SAFETY AND EFFICACY?

G. Schrenk, W. Hoellriegl, A. Schiviz, H. Rottensteiner, F. Scheiflinger, H. Schwarz, P. L. Turecek, E. Muchitsch
Baxter Innovations GmbH, Vienna, Austria.

Background/Aims: Baxter has developed RIXUBIS, a new recombinant factor IX (rFIX) product for the treatment of patients with hemophilia B. The presented studies were designed to evaluate the function, safety and efficacy of RIXUBIS and other commercially available rFIX and pdFIX products with regard to differences in activated FIX (FIXa) content.
Materials and Methods: The FIXa concentration of RIXUBIS was 0.009 IU/mL and that for the commercially available rFIX was 0.106 IU/mL. As control, RIXUBIS samples with an increased FIXa content were prepared. Samples were analyzed *in vitro* by one-stage clotting assay, non-activated partial thromboplastin time assay and thrombin generation assay. The thrombogenic potential of RIXUBIS was assessed *in vivo* using a modified Wessler Test in rabbits at 750 IU/kg (10-fold human clinical dose). Efficacy of the rFIX products was studied in hemophilia B (FIX ko) mice that received prophylactic treatment with 75 IU/kg of RIXUBIS or commercially available FIX. Finally, FIX-deficient mice were treated with RIXUBIS or commercial FIX and analyzed in a carotid occlusion model and one using thrombelastography to study primary pharmacodynamics of the two products.
Results: In all three functional *in vitro* assays, spiking of FIX with FIXa caused an increase in the measured FIX activity, with thrombin generation assay being affected most. No thrombogenic potential was observed with RIXUBIS (individual scores of 0), whereas the mean score for the commercially available rFIX was 0.5. After increasing the FIXa concentration of RIXUBIS to equalize it with the commercially available rFIX, a mean score of 0.42 (individual scores 0 – 0.5) was determined. Efficacy was comparable for the two recombinant FIX products in both pharmacodynamic models.
Conclusion: The *in vitro* results demonstrated that FIXa interferes with potency assignment of FIX. The results obtained in the thrombogenicity model strongly suggested that the differences in preclinical thrombogenicity were caused by the higher FIXa content of the commercially available FIX, thereby confirming earlier findings. The efficacy studies demonstrated that despite its lower FIXa content, RIXUBIS was as efficacious as a commercially available rFIX at 75 IU/kg ($p<0.0076$), indicating that FIXa does not contribute to the efficacy of a FIX product. The gathered data thus indicate that FIX products should preferentially contain a low FIXa content.

Corresponding Author
P. L. Turecek, *peter_turecek@baxter.com*

PO.01.04 - 72 ASSOCIATION OF MIR-132 AND MIR-185 GENES METHYLATION AND THEIR EXPRESSION PROFILE WITH RISK CONGENITAL FACTOR XIII DEFICIENCY

M. Naderi[1], A. Dorgalaleh[2], D. Kordi-Tamandani[3], Z. Rezaei[3], S. Alizadeh[2], P. Eshghi[4], S. Tabibian[2]

[1]Genetic Researcher Center in Non-Communicable Disease, Zahedan University of Medical Sciences; [2]Department of Hematology, Allied Medical School, Tehran University of Medical Sciences, Tehran, Iran; [3]Department of Biology, University of Sistan and Baluchestan, Zahedan, Iran; [4]Pediatric Congenital Hematologic Disorders Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Abstract: Congenital factor XIII deficiency is a very rare bleeding disorder but because of high rate of consanguineous marriages, is common in Sistan and Baluchestan province of Iran. The discovery of promoter hypermethylation of numerous miRNAs in human diseases has demonstrated an epigenetic mechanism for aberrant miRNA expression. The present study was about analyzing methylation and expression status of miR-185 and miR-132 genes in patients with inherited FXIII deficiency in a sample of South-Eastern Iranian population.

Materials and Methods: Promoter methylation of miR-185 and miR-132 were investigated by Methylation Specific Polymerase Chain Reaction in the blood samples of 75 FXIII deficiency individuals and 74 healthy controls. Expression level of these genes were also assessed in 15 blood samples of patients and 15 healthy controls using real-time quantitative reverse transcriptase PCR.

Results: Analysis of miR-132 and miR-185 promoter hypermethylation does not show significant difference between cases and controls,relative gene expression analysis in cases (n=15) with congenital FXIII deficiency and healthy controls (n= 15) revealed no statistically significant relationship for miR-132 (p=0.126) and miR-185 (p=0.165) genes.

Conclusion: Our findings indicate that promoter methylation of miR-132 and miR-185 have no significant effect on etiology of this disease.

Corresponding Author
A. Dorgalaleh, *dorgalaleh.1390@yahoo.com*

PO.01.05 - 73 DISSEMINATED INTRAVASCULAR COAGULATION A CONTROVERSIAL CLINICAL FEATURE IN CONGENITAL FACTOR XIII DEFICIENCY IN SOUTHEAST OF IRAN

M. Naderi[1], A. Dorgalaleh[2], S. Alizadeh[2], S. Tabibian[2], M. Menegatti[3]

[1]Genetic Researcher Center in Non-Communicable Disease, Zahedan University of Medical sciences; [2]Department of Hematology, Allied Medical School, Tehran University of Medical Sciences, Tehran, Iran; [3]Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS, Ca' Granda Ospedale Maggiore Policlinico, Department of Pathophysiology Transplantation, University of Milan and Luigi Villa Foundation, Milan, Italy.

Background: Disseminated intravascular coagulation (DIC) is an extremely rare coagulopathy in rare factor XIII deficiency. Compensated DIC occurs due to injuries that lead to systemic coagulation activation and amplified by impaired fibrinolysis. This challenge translates into widespread deposition of fibrin degradation products in circulation. The aim of this study is to report 3 cases with FXIII deficiency who presented with DIC.

Methods: Data collection on patients with a diagnosis of FXIII deficiency, based on history of bleeding, positive urea clot solubility or 1% monochloroacetictest and molecular analysis for FXIII_A subunit Trp187Arg polymorphism, associated to DIC, diagnosed due to prolonged PT and PTT, low platelet count and fibrinogen level and high concentration of FDP and D-dimer.

Results: Three patients resulted to be affected by both FXIII deficiency and DIC. Two out of 3 (66.7%) were males, while 1 (33.3%) was female. The mean age was 3.6 years. Familial history of FXIII deficiency was positive in all patients. Umbilical cord bleeding was the first presentation of FXIII deficiency in all patients and they also were experienced ecchymosis (all patients), and delay wound bleeding (2 patients). DIC occurred in two patients simultaneously with intracranial haemorrhage (ICH) whereas another patient experiences DIC following extensive haematoma. D-dimer measured in all patients was comprised between 5 and 20 µg/ml, while FDP was between 4 to 8 µg/ml.

Conclusion: Consumptive coagulopathy can cause DIC even in patients with severe FXIII deficiency.

Corresponding Author
A. Dorgalaleh, *dorgalaleh.1390@yahoo.com*

PO.01.06 - 80 GLOBAL DEVELOPMENT PLAN FOR A DOUBLE VIRUS INACTIVATED FIBRINOGEN CONCENTRATE FOR THE TREATMENT OF CONGENITAL FIBRINOGEN DEFICIENCY

F. Peyvandi[(1)], B. Schwartz[(2)], S. Knaub[(3)], O. Hegener[(3)]
[(1)]University of Milan, Milan, Italy; [(2)]Octapharma USA, Inc., Hoboken, USA; [(3)]Octapharma AG, Lachen, Switzerland.

Background: Patients with congenital afibrinogenaemia and hypofibrinogenaemia, present with frequent severe bleeding episodes starting at birth or early childhood. Bleeding may occur after a minor trauma or a small surgical intervention, into the skin, mucosa, muscles, gastrointestinal tract, or the brain. Therapeutic substitution with human fibrinogen concentrate can correct the haemostatic defect and arrest the bleeding in patients with these fibrinogen deficiencies. Octafibrin is a highly purified, plasma derived lyophilized, fibrinogen concentrate, double virus inactivated using 2 dedicated virus inactivation / removal steps.
Materials and Methods: The development plan calls for a prospective, randomized, open label, multinational, pivotal PK comparison of Octafibrin to an existing marketed product in 18 adult and adolescent patients, including comparison of a surrogate efficacy endpoint measured by TEG. In a second study the efficacy and safety of the product in bleeding and invasive procedures will be assessed in 24 adult and adolescent patients. Finally a pediatric PK, efficacy and safety study in patients below 6 years will be performed but because of the rarity of these patients this study will not need to be completed before review and approval by the regulatory agencies.
Results: Nineteen patients have been enrolled in the FORMA 01 PK study as of May 2014. There have been no reports of adverse events related to the infusion of this new concentrate. Data of a planned interim analysis of 9 patients will be presented. The efficacy study is about to start. Pivotal comparative PK data and interim efficacy and safety data will be available at time of regulatory submissions while the finalization of the pediatric study is deferred.
Conclusion: The aim of this clinical program is to show that Octafibrin is safe and effective in patients with congenital fibrinogen deficiency. So far, no related serious adverse events have been reported.

Corresponding Author
O. Hegener, *oliver.hegener@octapharma.ch*

PO.01.07 - 88 CORRELATION OF GENOTYPE AND PHENOTYPE IN PATIENTS WITH RARE BLEEDING DISORDERS IN PAKISTAN

M. Borhany[(1)], H. Handrkova[(2)], A. Cairo[(3)], N. Fatima[(4)], H. Fatima[(4)], A. Naz[(1)], T. Shamsi[(1)], F. Peyvandi[(3)], H. Kohler[(2)]
[(1)]Department of Haemostasis & Thrombosis, National Institute of Blood Disease & Bone Marrow Transplantation (NIBD), Karachi Pakistan; [(2)] University Clinic of Haematology, Haemostasis Research Laboratory, University Hospital & University of Bern, Bern, Switzerland; [(3)]U.O.S. Dipartimentale per la Diagnosi e la Terapia delle Coagulopatie, A. Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione I.R.C.C.S. Cà Granda Ospedale Maggiore Policlinico, Università degli Studi Milano, Italy; [(4)]Department of Research and Development, National Institute of Blood Disease & Bone Marrow Transplantation (NIBD), Karachi Pakistan.

Background: Consanguinity remains common in several populations around the world, and varies from country to country. In Pakistan, close consanguineous unions continue to be extremely common as in South West Asia. The aim of the study was to describe the frequency of rare bleeding disorders (RBDs) with phenotypic features and their genotype.
Materials & Methods: Pre-designed data sheets were filled by incorporating patients' demographics, family history, present and past history of bleeding episodes with the associated signs and symptoms. In female cases, maternal and obstetrical history was taken. Blood samples were collected for complete blood count (CBC), coagulation assays and genetic characterization.
Results: Out of 600 patients diagnosed with inherited coagulation bleeding disorders, 64 subjects had RBDs (11%). Among them, 35(55%) were male and 29(45.31%) were female. Median age of patients was 9.8 years, (range, 12 days to 37 years). History of consanguinity was present in 85% of cases and significant family history of bleeding in 54% of patients. The most common deficiency was FXIII (n=18, 28%) and FVII deficiency (n=18, 28%) followed by fibrinogen deficiency (n=15, 23%), FV deficiency (n=5, 8%), FX deficiency (n=4, 6%), FXI deficiency (n=2, 3%) respectively. There was one case of each combined FV and VIII deficiency and vitamin K dependent factor deficiency (2%).In FI deficiency all patients were afibrogenemia. Clinical phenotype ranged from grade II to grade III bleeding symptoms. Some patients were found to have both grades (II & III).
Genetic characterization was identified so far in 31/64 (48%) patients. Genetic mutations were lined out in which 22/31 (71%) were novel in our RBD patients, only 09/31 (29%) were previously reported in the literature.
Conclusion: The study shows that autosomal recessive disorders are common in the setting of consanguineous marriages. Further studies of the association between phenotype and genotype in this subset of patients are needed.

Corresponding Author
N. Fatima, *dr.naveena@hotmail.com*

PO.01.08 - 144 GENOTYPE AND PHENOTYPE RELATIONSHIPS IN 10 PAKISTANI UNRELATED PATIENTS WITH INHERITED FACTOR VII DEFICIENCY

M. Borhany[1], H. Boijout[2], J. Pellequer[2], N. Fatima[3], T. Shamsi[1], G. Moulis[2], P. A. Martinez[2], J. Schved[2], M. G. Blaizot[2]

[1]Department of Haemostasis & Thrombosis, National Institute of Blood Disease & Bone Marrow Transplantation, Karachi, Pakistan; [2] CHU Montpellier, Département d'Hématologie Biologique, Hopital Saint-Eloi, Montpellier, France; and Cea, Ibeb, Service de Biochimie et Toxicologie Nucléaire, Bagnols sur Cèze, France; [3]Department of Research and Development, National Institute of Blood Disease & Bone Marrow Transplantation, Karachi, Pakistan.

Backgroud/Aim: Inherited factor VII (FVII) deficiency is one of the commonest rare bleeding disorders. It is characterized by a wide molecular and clinical heterogeneity and an autosomal recessive pattern of inheritance. Factor VII-deficient patients are still scarcely explored in Pakistan although rare bleeding disorders became quite common as a result of traditional consanguineous marriages. The aim of the study was to give a first insight of F7 gene mutations in Pakistani population.

Material and Methods: Ten unrelated FVII-deficient patients living in Pakistan were investigated (median FVII:C= 2%; range = 2–37%). A clinical questionnaire was filled out for each patient and direct sequencing was performed on the coding regions, intron/exon boundaries and 5′ and 3′ untranslated regions of the F7 gene.

Results: Nine different mutations (eight missense mutations and one located within the F7 promoter) were identified on the F7 gene. Five of them were novel (p.Cys82Tyr,p.Cys322Ser, p.Leu357Phe, p.Thr410Ala, c-57C>T, the last being predicted to alter the binding site of transcription factor HNF-4). Half of the patients had single mutations in Cys residues involved in disulfide bridges. The p.Cys82Arg mutation was the most frequent in our series. Six of seven patients with FVII:C levels below 10% were homozygous in connection with the high percentage of consanguinity in our series. In addition, we graded the 10 patients according to three previously published classifications for rare bleeding disorders. The use of the bleeding score proposed by Tosetto and co-workers in 2006 appears to well qualify the bleeding tendency in our series.

Conclusion: Molecular analysis of an even small series of 10 FVII-deficient patients allowed us to report five novel mutations. The p.Cys82Arg mutation appears to be very common in Pakistan. Moreover, using the Tosetto's bleeding score should be a promising tool to classify patients with rare bleeding disorders.

Corresponding Author
N. Fatima, *dr.naveena@hotmail.com*

02. VON WILLEBRAND FACTOR

PO.02.01 - 95 ELUCIDATION OF THE MECHANISM OF VON WILLEBRAND FACTOR INCORPORATION INTO A FIBRIN NETWORK

L. Pelkmans[1,2], A. Miszta[1,2], T. Lindhout[1,2], G. Krishnamoorthy[1,2], P. G. de Groot[3], C. Hemker[1,2], J. Heemskerk[1,2], H. Kelchtermans[1,2], B. de Laat[1,2]

[1]Department of Biochemistry, Cardiovascular Research Institute Maastricht, Maastricht University, the Netherlands; [2]Synapse BV, Maastricht, the Netherlands; [3]Clinical Chemistry and Hematology, University Medical Center Utrecht, Utrecht, the Netherlands.

Background/Aims: Attachment of platelets from the circulation onto a growing thrombus is a process involving multiple platelet receptors, endothelial matrix components and coagulation factors. It has been shown previously that during a transglutaminase reaction activated factor XIII (FXIIIa) covalently crosslinks von Willebrand factor (VWF) to polymerizing fibrin. Bound VWF further recruits platelets via interactions with the platelet receptor complex glycoprotein Ib. The aim of this study was to investigate the mechanism of incorporation of VWF into a growing fibrin network and the incorporation of platelets into a growing thrombus under high shear.

Materials and Methods: The interactions of VWF with fibrinogen, fibrin monomers and polymerized fibrin were investigated using different surface techniques including ellipsometry and Surface Plasmon Resonance. Furthermore, the influence of FXIIIa on VWF binding to fibrin monomers was determined using a domain deletion mutant FXIIIa inhibitor. Finally, we used a perfusion system to study the effect on platelet adhesion of VWF incorporation into a fibrin network.

Results: We established binding of VWF to a fibrin monomer layer during the process of fibrinogen-to-fibrin conversion in the presence of thrombin, arvin or a snake venom from Contralux atrox. Using a domain deletion mutant we demonstrated that VWF binds fibrin monomers via its C1C2 domain. In the presence of the FXIIIa inhibitor, K9-DON, the binding of VWF to fibrin monomers was decreased, but not completely abolished. Under high flow conditions, platelet adhesion to fibrin was promoted in the presence of VWF and thrombin.

Conclusions: We have provided evidence that the C1C2 domain of VWF and the E domain of fibrin monomers are involved in the incorporation of VWF multimers and subsequent platelet incorporation into a growing thrombus. Our findings help to elucidate the mechanism of thrombus growth and platelet adhesion under conditions of arterial shear rate.

Corresponding Author
L. Pelkmans, *l.pelkmans@thrombin.com*

PO.02.02 - 99 DEFINING THE MOLECULAR BASIS UNDERLYING THE PHYSIOLOGICAL INTERACTION BETWEEN VON WILLEBRAND FACTOR AND GALECTINS IN NORMAL PLASMA

O. Rawley[1], J. M. O'Sullivan[1], A. Chion[1], N. O'Regan[1], P. V. Jenkins[1], T. M. Brophy[1], J. S. O'Donnell[2]

[1]Haemostasis Research Group, Institute of Molecular Medicine, St. James's Hospital Campus, Trinity College Dublin, Ireland; [2] Haemostasis Research Group, Institute of Molecular Medicine, St. James's Hospital, Trinity College Dublin, Ireland.

Background: Although the molecular mechanisms through which glycans modulate VWF biology remain poorly understood, recent studies have demonstrated that plasma VWF circulates in complex with specific members of the galectin family. Moreover, these galectin interactions modulate VWF-mediated thrombus formation *in vivo*.

Aims: In this study, we sought to define the molecular basis underlying the interactions between VWF and galectins -1 and -3.

Methods: VWF was purified from human plasma (pdVWF) by cryoprecipitation and gel filtration. VWF glycosylation was then modified using specific exoglycosidases. Recombinant wild-type and mutant VWF were transiently expressed in HEK293T cells. Recombinant galectin-1 and -3 were expressed in E. coli and purified via nickel affinity chromatography. Binding interactions were subsequently characterized using modified immunosorbent assays.

Results: Galectin-1 and galectin-3 bound to pdVWF in a dose-dependent manner. Pre-incubation with PNGase F markedly decreased binding to both galectin-1 and galectin-3 (13±1% and 57±2%, $p<0.001$). Moreover, removal of both N- and O-linked glycans (PNGase F and O-glycosidase treatment) further attenuated galectin-3 binding (21±1%, $p<0.0001$). Terminal sialic acid and sub-terminal galactose expression on VWF also modulated galectin interaction. For example, enzymatic desialylation of pdVWF with α2-3,6,8,9 neuraminidase markedly enhanced binding to galectin-1 and galectin-3 (231±6% and 136±6%, $p<0.05$). Furthermore, ABO blood group antigen expression significantly influenced interaction with both galectins. In particular, group AB VWF bound to both galectin-1 and galectin-3 significantly better compared to group O VWF. Interestingly, targeted removal of the individual N-linked glycans located at N1515 and N1574 in the VWF A2 domain via site directed mutagenesis led to dramatically decreased binding to galectin-1 and -3. Conversely, modification of pdVWF via ristocetin treatment, or introduction of the type 2B mutant R1450E, markedly enhanced binding to both galectins.

Conclusions: These novel data define the molecular basis underlying the physiological VWF-galectin interaction. In particular, we have demonstrated that both N- and O-linked glycan determinants modulate VWF-galectin binding through terminal sialic acid and ABO blood group expression, with an additional role for specific N-linked glycans. Furthermore, we have identified a critical role for the VWF A domains in modulating these interactions.

Corresponding Author
O. Rawley, *rawleyo@tcd.ie*

PO.02.03 - 111 THE INFLUENCE OF RECOMBINANT VON WILLEBRAND FACTOR OF DIFFERENT MULTIMER SIZES ON THE ACTIVITY OF FACTOR VIII IN THROMBIN GENERATION AND CHROMOGENIC ASSAY

S. Knappe, S. Till, F. Scheiflinger, M. Dockal
Baxter Innovations GmbH, Vienna, Austria.

Background/Aims: Von Willebrand factor (VWF) is a multimeric glycoprotein found in plasma as a single dimer or as multimers consisting of as many as 50-100 dimers. One important function of VWF is the binding and stabilization of factor VIII (FVIII) in plasma. FVIII-VWF complex formation prevents FVIII from interaction with lower affinity binding partners such as FIXa, phospholipids or clearance receptors and enzymatic (in)activation. Thus, FVIII survival in the circulation depends on its chaperone VWF. In this study we investigated the effect of a recombinant VWF (rVWF; BAX 111) and fractions thereof on FVIII in the absence of platelet binding and activation.
Material/Methods: The activity of recombinant FVIII (ADVATE) was determined by calibrated automated thrombography (CAT) with different coagulation triggers and by chromogenic assay (TECHNOCHROM® FVIII:C kit) in FVIII/VWF-double deficient platelet-poor human plasma. The experiments were performed in the absence and presence of rVWF of ultrahigh, high, medium and low multimer size.
Results: Thrombin generation in FVIII/VWF-deficient plasma was only partially restored by supplementation with 1 IU/mL FVIII. Addition of rVWF further increased thrombin formation reaching a normal plasma control. The activity increase was dependent on rVWF concentration, reaching saturation at about 0.4 IU/mL rVWF, which corresponds to a 20-fold molar excess of rVWF monomers. rVWF fractions with reduced molecular weight showed a lower effect on FVIII procoagulant activity. Thus, rVWF size-1dependently protects or stabilizes FVIII in plasma in the absence of VWF's platelet-mediated effects. Similar results were obtained by chromogenic assay.
Conclusions: In conclusion, we established thrombin generation as a new tool to characterize FVIII/VWF complexes. We showed that FVIII activity is influenced by rVWF depending on its size and concentration, and that standard clinical assays may be affected by VWF plasma levels.

Corresponding Author
S. Knappe, *sabine_knappe@baxter.com*

PO.02.04 - 158 COMPARISON OF AN RVWF DRUG CANDIDATE HAVING AN INTACT MULTIMER SPECTRUM WITH PDVWF TO PROMOTE PLATELET ADHESION UNDER SHEAR STRESS

G. Schrenk, J. Schreiner, H. Gritsch, F. Scheiflinger, P. L. Turecek
Baxter Innovations GmbH, Vienna, Austria.

Background/Aims: Baxter has developed a recombinant von Willebrand factor (rVWF) for treatment von Willebrand disease which completed phase 3 clinical development. rVWF is characterized by containing the hemostatic most active ultra-large and high molecular weight multimers, thus showing higher specific activity than commercially available plasma-derived VWF (pdVWF) products, in terms of VWF:RCo and VWF:CB activity. To investigate the contribution of VWF multimer size on rVWF´s hemostatic activity, fractions containing distinct portions of VWF multimers were generated and analyzed regarding their ability to mediate platelet adhesion under shear stress *in vitro*. The rVWF drug candidate was compared with pdVWF.
Materials and Methods: rVWF fractions of different VWF multimer size were generated by size-exclusion chromatography, purified and size distribution was determined using low and high resolution agarose gel electrophoresis. A parallel-plate perfusion chamber system was used to investigate adhesion of platelets from human blood to fibrillar collagen type I under shear mediated by rVWF, rVWF fractions and pdVWF. For this, 1.0 IU/mL VWF:Ag of VWF containing sample was added to blood and time course of surface coverage was evaluated by microscopy using fluorescent labeled platelets. Multiple pictures were taken every 5 seconds. The perfusion was performed for 3 minutes at +37°C and at a defined wall shear rate of 1500 s-1.
Results: Endogenous VWF present in human whole blood already mediated initial platelet adhesion, which was approximately 5% surface coverage after 120 seconds perfusion. Spiking human blood with 1.0 IU/mL of VWF and its fractions of different multimeric size resulted in increased, time-dependent platelet binding. The amount of platelets attached to collagen clearly dependent on the size of rVWF. While highly multimerized rVWF very effectively promoted platelet binding, lower-sized rVWF fractions containing VWF multimers of lower molecular weight showed decreased platelet adhesion properties. As such rVWF had a more pronounced effect than pdVWF.
Conclusion: The results showed that the improved hemostatic activity of rVWF over pdVWF is a result of the presence of high molecular weight multimers and confirmed the importance of these to mediate intact platelet binding.

Corresponding Author
P. L. Turecek, *peter_turecek@baxter.com*

PO.02.05 - 112 FACTOR VIII BINDING CAPACITY AND AFFINITY OF RECOMBINANT VON WILLEBRAND FACTOR OF DIFFERENT MOLECULAR WEIGHT RANGES

S. Knappe, G. Schrenk, M. Palige, R. Hartmann, E. Panholzer, M. Billwein, G. Gerstenbauer, F. Scheiflinger, P. L. Turecek, M. Dockal

Baxter Innovations GmbH, Vienna, Austria.

Background/Aims: Von Willebrand factor (VWF) is a plasma glycoprotein assembled from up to 100 dimers. VWF forms a non-covalent complex with factor VIII (FVIII) and thereby stabilizes FVIII. Each VWF monomer contains one potential FVIII-binding site. However, *in vivo* the molar ratio of FVIII:VWF monomer is 1:50 and consequently, VWF is not saturated with FVIII. BAX111, Baxter's recombinant VWF (rVWF), contains ultra-high molecular weight (Mw) multimers. Here, we compared the rFVIII binding capacity and affinity of BAX111 with VWF preparations of lower Mw ranges.
Material/Methods: The interaction of full-length rFVIII (ADVATE) with BAX111 (11000 kD) or rVWF with an average Mw of 6000, 2500, and 1300 kD was studied. The rFVIII binding capacity of rVWF in solution was investigated by separating preformed FVIII-VWF complexes from non-bound rFVIII by size exclusion chromatography (SEC). In an ELISA based assay, preformed FVIII-VWF complexes were captured by a polyclonal anti-human VWF antibody, followed by activation, release, and detection of rFVIII by its FIXa cofactor activity. Binding studies were carried out on a Biacore 3000 instrument with VWF immobilized on a C1 sensor chip and rFVIII as analyte.
Results: Calculation of the FVIII:VWF binding stoichiometry by SEC revealed that BAX111 had the highest FVIII capacity and was near the theoretical maximum: 0.84 FVIII molecules per VWF monomer. This capacity declined with decreasing Mw of rVWF. Similar observations were made using ELISA. FVIII binding capacity of BAX111 was 112% and 76-21% for VWF with lower Mw than a normal plasma reference. Biacore binding studies confirmed the differences in FVIII binding capacity, while the FVIII binding affinities of the different VWF fractions were similar (KD= 2*10-10 – 7*10-11M).
Conclusions: Together, these data demonstrate that BAX111 has optimal FVIII binding properties and that a reduction in VWF multimer size lowers the FVIII binding capacity.

Corresponding Author
S. Knappe, *sabine_knappe@baxter.com*

PO.02.06 - 133 SINGLE NUCLEOTIDE VARIANTS RS1063857 AND RS1063856 ARE ASSOCIATED WITH INCREASED VWF PLASMA LEVELS

A. H. Mufti, A. C. Goodeve, D. J. Hampshire

Haemostasis Research Group, Department of Cardiovascular Science, University of Sheffield, Sheffield, UK.

Introduction: von Willebrand factor (VWF) plasma levels vary considerably; between 50-200IU/dL in 95% of the general population. Several factors have been associated with this variation including ABO blood group and VWF single nucleotide variants (SNV). Several studies highlighted significant association between SNV rs1063857 (c.2385T>C; p.Tyr795=) and rs1063856 (c.2365A>G; p.Thr789Ala) with VWF level.
Aims: To further investigate this association between SNV rs1063857 and rs1063856 with VWF level and elucidate the mechanism(s) involved.
Methods: In silico analysis was used to determine linkage disequilibrium (LD) between SNV and to predict SNV effect at the RNA and protein level. SNV were genotyped in healthy controls (HC) recruited by the MCMDM-1VWD study. Association of SNV with VWF level was determined using Mann-Whitney and Kruskal-Wallis tests. *In vitro* expression of VWF containing SNV was performed in HEK293T cells, followed by measurement of VWF:Ag via ELISA and mRNA expression using TaqMan quantitation.
Results: In silico analysis of both SNV showed no major effect on VWF protein structure or mRNA splicing. However, genotype analysis of HC showed that both were significantly associated with increased VWF level (c.2385T>C TT: 96.4IU/dL; TC: 99.5IU/dL; CC: 113.9IU/dL; p<0.0001; n=1109; c.2365A>G AA: 96.37IU/dL; AG: 99.65IU/dL; GG: 114.33IU/dL; p<0.0001; n=1095). Both SNV are in strong LD (r=99.01%). *In vitro* expression of SNV (n=3) showed similar results (c.2385T>C TT: 100%; TC: 104.4%; CC: 128%; p=0.006; c.2365A>G AA: 100%; AG: 115%; GG: 131.2%; p=0.0009; both SNV in trans WT: 100%; heterozygous: 113.1%; homozygous: 125.1%; p=0.03). mRNA expression (n=3) was also increased (c.2385T>C TT: 100%; TC: 188%; CC: 282%; p=0.002; c.2365A>G AA: 100%; AG:135%; GG: 184%; p>0.05; both SNV WT: 100%; heterozygous: 176%; homozygous: 192%; p>0.05).
Conclusions: SNV rs1063857 and rs1063856 were associated with similarly increased VWF mRNA and protein expression levels. Increased mRNA expression from the variant allele may be responsible for increased protein secretion.

Corresponding Author
A. H. Mufti, *ahmufti1@sheffield.ac.uk*

PO.02.07 - 127 VON WILLEBRAND FACTOR IN RELATION TO CORONARY PLAQUE CHARACTERISTICS AND CARDIOVASCULAR OUTCOME

M. A. H. Sonneveld[1], J. M. Cheng[2], R. M. Oemrawsingh[2], M. P. M. de Maat[1], I. Kardys[2], H. M. Garcia-Garcia[2], R. van Geuns[2], P. W. Serruys[2], E. Boersma[2], M. Akkerhuis[2], F. W. G. Leebeek[1]

[1]Department of Hematology, Erasmus University Medical Center, Rotterdam, the Netherlands; [2]Department of Cardiology, Erasmus University Medical Center, Rotterdam, the Netherlands.

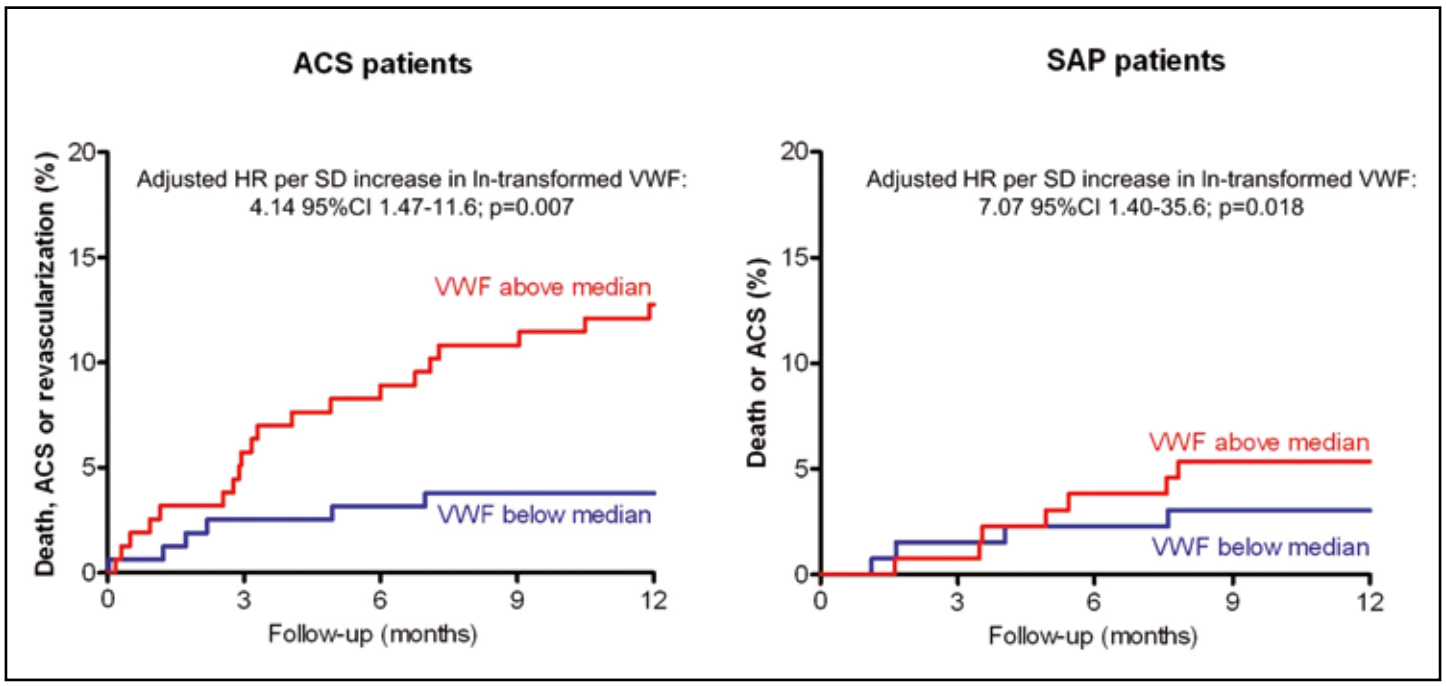
Background / Aims: It is well established that high VWF plasma levels are associated with an increased risk of coronary artery disease. However, it is still unknown whether VWF levels are related to coronary plaque characteristics, such as high risk lesions. Our aim was to investigate the relationship between VWF levels and coronary plaque burden, the presence of high-risk coronary lesions as measured by intravascular ultrasound (IVUS), and cardiovascular outcome.

Materials and Methods: Between 2008 and 2011 IVUS virtual histology imaging of a non-culprit coronary artery was performed in 577 patients undergoing coronary angiography for acute coronary syndrome (ACS) (n= 315) or stable angina pectoris (SAP) (n= 262). Arterial blood was sampled prior to the coronary angiography. VWF antigen (VWF:Ag) levels were measured using ELISA.

Results: Patients with acute coronary syndrome (ACS) had significantly higher VWF:Ag levels than stable angina pectoris (SAP) patients (p<0.001). High VWF:Ag levels were associated with a higher coronary plaque burden (p=0.027) in SAP patients, but not in ACS. VWF:Ag levels were not associated with characteristics of plaques. The cumulative incidence of all-cause death, hospitalisation for ACS or unplanned coronary revascularisation (MACE) at 1-year follow-up was 9.7%. In ACS patients, the VWF:Ag levels predicted incidence of 1-year MACE (HR 4.14 per SD increase in lnVWF:Ag, 95% CI 1.47-11.6), whereas in SAP patients this was only seen for 1-year all-cause death and hospitalisation for ACS (HR 7.07 95% CI 1.40-35.6).

Conclusions: VWF:Ag levels are associated with coronary atherosclerosis in SAP patients undergoing coronary angiography. In ACS and SAP patients, high VWF levels are predictive of adverse cardiovascular outcomes and death during 1-year follow-up.

Corresponding Author
M. A. H. Sonneveld, *m.a.h.sonneveld@erasmusmc.nl*



PO.02.07 - 127
Figure 1

PO.02.08 - 52 PLASMA LEVELS OF ACTIVE VWF ARE INCREASED IN PATIENTS WITH FIRST ST-SEGMENT ELEVATION MYOCARDIAL INFARCTION: A MULTICENTER AND MULTIETHNIC STUDY

B. Rutten[1], A. Maseri[2], D. Cianflone[3], A. Laricchia[3], N. A. Cristell[3], A. Durante[3], M. Spartera[3], F. Ancona[3], L. Limite[3], D. Hu[4], H. Li[4], N. G. Uren[5], P. G. de Groot[1], P. M. Mannucci[6], M. Roest[1]

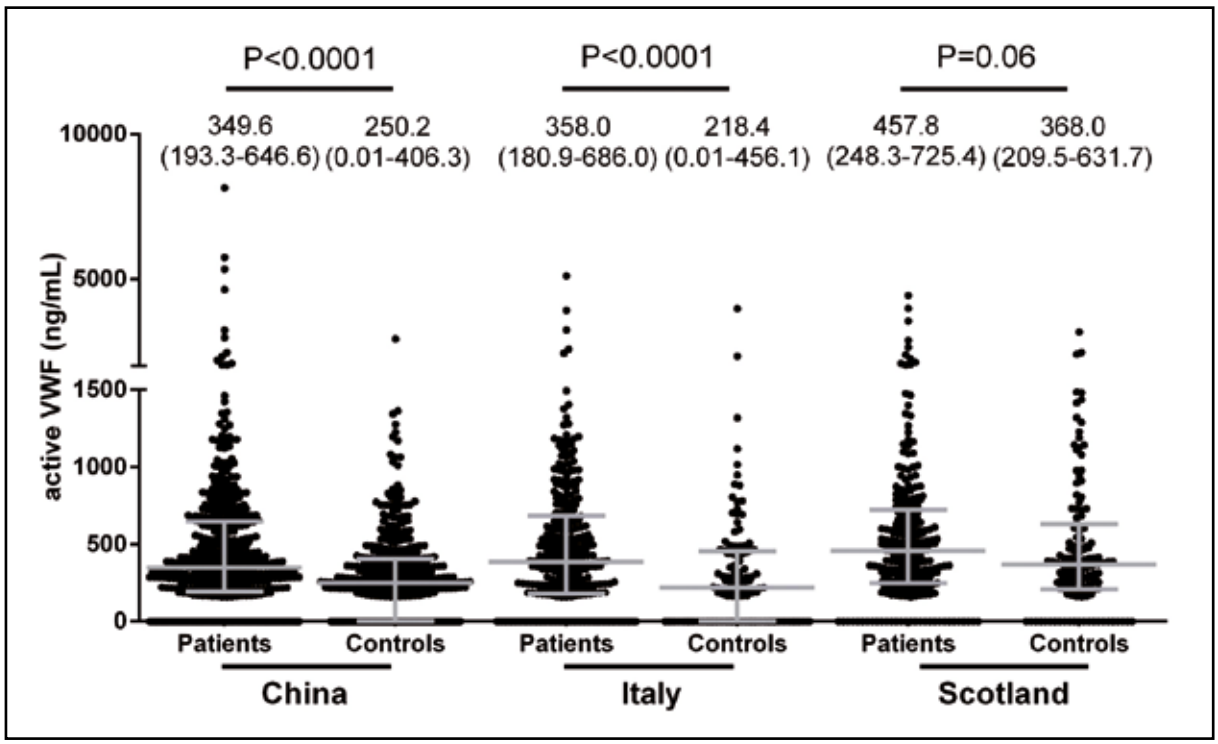
[1]Laboratory of Clinical Chemistry and Haematology, University Medical Center Utrecht, The Netherlands; [2]Heart Care Foundation, Florence, Italy; [3]Clinical Cardiovascular Biology Centre, Universita Vita-Salute San Raffaele, Istituto Scientifico San Raffaele, Milan, Italy; [4]The Heart Center, People's Hospital of Peking University, Beijing, China; [5]Department of Cardiology, Edinburgh Heart Center, Royal Infirmary of Edinburgh, United Kingdom; [6]Scientific Direction Fondazione IRCCS Ca'Granda, Ospedale Maggiore Policlinico and A. Bianchi Bonomi Hemophilia and Thrombosis Center, Milan, Italy.

Aims: Von Willebrand factor (VWF), a key player in hemostasis and thrombosis, is released from endothelial cells during inflammation. Upon release, VWF is processed by ADAMTS-13 into an inactive conformation. The aim of our study was to investigate whether plasma levels of active VWF, total VWF, ADAMTS-13 and osteoprotegerin (OPG) are risk factors for first ST-segment elevation myocardial infarction (STEMI).

Methods and results: We assessed 1026 patients with confirmed first STEMI and 652 control subjects from China, Italy and Scotland, within 6 hours after cardiovascular event. Median plasma levels of total VWF, active VWF and OPG were increased, while plasma levels of ADAMTS-13 were decreased in patients compared to controls. The odds ratio (OR) of STEMI in patients with high plasma levels of active VWF was 2.3 (interquartile range (IQR): 1.8-2.9), total VWF was 1.8 (1.4-2.3), ADAMTS-13 was 0.6 (05-0.8) and OPG was 1.6 (1.2-2.0). The OR for total VWF and active VWF remained significant after adjustment for established risk factors, medical treatment, C-reactive protein, total VWF, ADAMTS-13 and OPG. When we adjusted for levels of active VWF, the significance of the OR for VWF disappeared while the OR for active VWF remained significant

Conclusions: We found evidence that plasma levels of active VWF are an independent risk factor for first STEMI in patients from 3 different ethnic groups. Our findings confirm the presence of VWF abnormalities in patients with STEMI and may be used to develop new therapeutic approaches.

Corresponding Author
B. Rutten, *bertrutten@gmail.com*



PO.02.08 - 52
Figure 1

PO.02.09 - 159 VISUALIZATION OF VWF-FVIII-COMPLEX FORMATION BY SINGLE MOLECULE IMAGING

H. Rottensteiner[1], B. Klaus[2], B. Seyfried[1], G. Schrenk[1], F. Scheiflinger[1], G. Allmaier[2], G. Friedbacher[2], P. L. Turecek[1]
[1]Baxter Innovations GmbH, Vienna, Austria; [2]Vienna University of Technology, Vienna, Austria.

Background/Aims: Complex formation between VWF and FVIII has been investigated for over three decades, and is known to be important in stabilizing FVIII in the circulation. We developed a new method to study interactions between coagulation factors using atomic force microscopy. The most challenging factors in studying this interaction were the high mobility of coagulation factors on a surface and the fact that the globular FVIII molecules did not differ significantly in size from the globular units of VWF.
Materials and Methods: A single molecule approach was used that allowed monitoring of identical VWF molecules before and after reaction with FVIII. The resulting morphological changes were assessed by visual inspection of the micrographs; a binding event was determined to have occurred when the height and size of a VWF globular domain had measurably increased. This increase was also quantified by recording height changes in cross sectional profiles.
Results: Complex formation between FVIII and VWF was discernible as globular structures appended to the N-terminal large globular domain of the VWF monomer, which contains the major FVIII binding site. The specificity of this approach was demonstrated in various control experiments. VWF was incubated either with or without FVIII, but in the presence of a high ionic strength buffer; in both cases, structures indicative for complex formation were virtually absent. The FVIII:VWF monomer ratio was found to be 1:4, confirming that, at least *in vitro*, VWF has a higher FVIII binding capacity than estimated from the measured ratio of normal human plasma. Finally, our method revealed that the morphology of VWF changed in a calcium-dependent manner.
Conclusion: We provide the first images which directly show the FVIII-VWF complex formation on a single molecule level.

Corresponding Author
P. L. Turecek, *peter_turecek@baxter.com*

PO.02.10 - 153 MAPPING VWF BINDING REGIONS IN FVIII BY HYDROGEN/DEUTERIUM EXCHANGE (HDX) MASS SPECTROMETRY: LIGHT CHAIN OF FVIIIFC SHOWS DIFFERENTIAL DEUTERIUM UPTAKE ON BINDING TO VWF-D'D3

E. Chhabra, G. Bou-Assaf, J. Kulman, J. Salas, R. Peters
Biogen Idec.

Introduction: More than 95% of circulating FVIII exists in a non-covalent complex with von Willebrand Factor (VWF). VWF prolongs FVIII half-life by protecting FVIII from degradation/clearance. The D'D3 domain of VWF has been reported to interact with the C1 and C2 domains of FVIII. However, detailed structural information about the binding region and conformational rearrangements associated with the interaction between these two proteins has been lacking. Here, we have used hydrogen/deuterium exchange (HDX)- mass spectrometry to define this binding interface.
Methods: HDX was initiated by dilution of rFVIIIFc (220kDa), VWF D'D3 (53kDa) or the rFVIIIFc-D'D3 complex in deuterated buffer. After deuterium labelling, the reaction was quenched (pH 2.3, 0ºC). The quenched protein samples were denatured, reduced and directly injected into Waters HDX Manager for online pepsin digestion. The eluting peptides were separated by HPLC, followed by introduction into the Synapt G2S mass spectrometer by electrospray ionization. Peptides were identified by ProteinLynx Global Server (PLGS) and deuterium uptake calculation was performed with DynamX (Waters Corp.).
Results/Conclusions: The peptic digestion of the rFVIIIFc-D'D3 complex generated 529 unique peptides that covered 93% of the rFVIIIFc primary sequence and 49 peptides that covered 41% of the D'D3 primary sequence. Preliminary data indicate that upon D'D3 binding, several regions in FVIII light chain (LC), but none in the heavy chain (HC), display significant differences in deuterium uptake. There were 7 peptides which showed decreased deuterium uptake. Some of this reduction is consistent with solvent exclusion from the binding interface. These peptides corresponded to residues 1670-1678 (a3), 1856-1875(A3), 2035-2051(C1), 2076-2096(C1), 2124-2164(C1), aa 2238-2247(C2) and aa 2267-2275(C2). These 7 peptides were dispersed throughout the light chain, but the most significant changes were observed in the C1 domain. These results indicate that several distinct regions in the FVIII light chain are directly or indirectly involved in VWF binding.

Corresponding Author
E. Chhabra, *ekta.sethchhabra@biogenidec.com*

PO.02.11 - 148 VON WILLEBRAND FACTOR DIRECTLY INTERACTS WITH PROTEIN DISULFIDE ISOMERASE PDI

M. A. Brehm[1], S. Lippok[2], T. Obser[1], R. Schneppenheim[1], J. O. Rädler[2]

[1]Department of Pediatric Hematology and Oncology, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany; [2]Faculty of Physics and Center for NanoScience, Ludwig Maximilian University, Munich, Germany.

Von Willebrand factor (VWF) is a multimeric glycoprotein essential for platelet-dependent primary hemostasis. The biosynthesis of VWF high molecular weight multimers is a highly sophisticated process requiring spatial separation of each step due to varying pH value requirements. Multimerization is facilitated in the acidic environment of the trans-Golgi apparatus by formation of inter-dimer disulfide bonds mediated by the VWF pro-peptide. Dimerization occurs at neutral pH in the endoplasmic reticulum (ER) by formation of disulfide bonds between the CK domains of two VWF monomers. Which protein catalyzes this C-terminal disulfide bond formation has not been elucidated yet. The protein disulfide isomerase PDI has previously been used to visualize colocalization of von Willebrand factor with the ER. However, if these two proteins are binding partners and the reason for this putative direct interaction have never been investigated in detail.

Using Microscale Thermophoresis (MST) and Fluorescence Correlation Spectroscopy (FCS), we were able to clearly show binding of PDI to recombinant wildtype VWF. The dissociation constants were determined to be KD = 236.0 ± 66 nM and KD = 282.4 ± 123 nM by MST and FCS, respectively.

Since the interaction of PDI and the isolated CK domain exhibits a similar KD value (258 ± 104 nM) our data indicate that a single PDI binding domain is located within the CK domain of VWF where dimerization occurs. Furthermore, we found that VWF mutations associated with von Willebrand disease type 2A phenotype IID, that lead to a disturbed VWF dimerization, show altered PDI interaction. Exemplary, reduced ER localization as well as decreased colocalization with PDI for mutant p.Ser2775Cys is shown in *Figure 1*. We therefore hypothesize that PDI is the protein that dimerizes VWF.

Corresponding Author
M. A. Brehm, *m.brehm@uke.de*

PO.02.12 - 93 REGULATION OF STIMULATED AND BASAL RELEASE OF WEIBEL-PALADE BODIES BY STXBP1 AND SYNTAXIN-3

D. van Breevoort[1], N. Hellen[2], A. Snijders[3], S. Weckhuysen[4], J. Voorberg[1], T. Carter[2], R. Bierings[1,2]

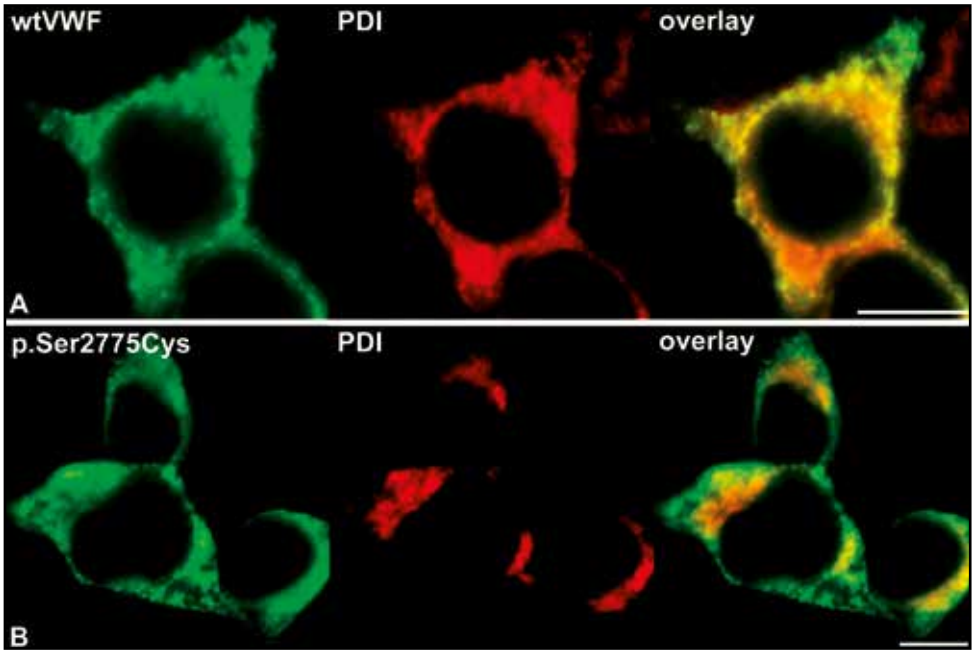
[1]Department of Plasma Proteins, Sanquin Blood Supply Foundation, Amsterdam, The Netherlands; [2]Division of Physical Biochemistry, MRC National Institute for Medical Research, London, United Kingdom; [3]Biomolecular Mass Spectrometry and Proteomics, MRC Clinical Sciences Centre, London, United Kingdom; [4]Molecular Genetics, University of Antwerp, Antwerp, Belgium.

Background/Aims: Vascular endothelial cells contain unique rod-shaped secretory granules, called Weibel-Palade bodies (WPBs), which contain a number of haemostatic, angiogenic and inflammatory mediators. Several components that are critical for regulated WPB exocytosis have been identified, including the small GTPase Rab27A and its effector synaptotagmin-like protein 4-a (Slp4-a), but the mechanism remains unclear. In this study we investigate the possible role of syntaxin binding protein 1 (STXBP1) and the SNARE-proteins syntaxin-2 and -3 in WPB exocytosis.

Results: Using a non-biased proteomic screen for targets for Slp4-a we have identified syntaxin binding protein 1 (STXBP1) and syntaxin-2 and -3 as endogenous Slp4-a binding partners in endothelial cells. Co-immunoprecipitation experiments showed that STXBP1 associates with synaxin-2 and syntaxin-3 in a mutually exclusive manner. The functional involvement of STXBP1 in WPB release was tested by siRNA mediated knockdown of gene expression: histamine and forskolin-induced VWF secretion were impaired in STXBP1 depleted cells, indicating that STXBP1 is involved in Ca2+- as well as cAMP-mediated release of WPBs. Blood outgrowth endothelial cells (BOECs) from an early infantile epileptic encephalopathy type 4 (EIEE4) patient carrying a de novo mutation in STXBP1 displayed significantly impaired histamine- and forskolin-stimulated VWF secretion. Interestingly, we found that the t-SNARE syntaxin-3 can be found on WPBs and our preliminary data suggest that syntaxin-3 regulates WPB pool size by controlling basal secretion of VWF.

Conclusions: Based on these findings, we propose that the Rab27A-Slp4-a complex on WPB promotes exocytosis through an interaction with STXBP1, thereby controlling the release of vaso-active substances in the vasculature. Our results position syntaxin-3 as a WPB-linked SNARE-protein that regulates basal secretion of VWF.

Corresponding Author
R. Bierings, *r.bierings@sanquin.nl*



PO.02.11 - 148
Figure 1

03. VON WILLEBRAND DISEASE

PO.03.01 - 75 AN ALTERNATIVE ASSAY (ELISA USING GPIB GAIN-OF-FUNCTION) TO RISTOCETIN INDUCED PLATELET AGGLUTINATION (RIPA) IN THE DIAGNOSIS OF TYPE 2B VON WILLEBRAND

F. Stufano[(1)], L. Baronciani[(1)], M. T. Pagliari[(1)], F. Franchi[(2)], G. Cozzi[(1)], I. Garcia-Oya[(1)], F. Peyvandi[(1,2)]

[(1)]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico Milano, A. Bianchi Bonomi Hemophilia and Thrombosis Centre, Milan, Italy; [(2)]Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy.

Background: diagnosis of VWD2 relies on the discrepancy between the ristocetin cofactor activity assay (VWF:RCo) and von Willebrand factor antigen (VWF:Ag). VWD2B patients could be discriminated from other qualitative VWD variants by RIPA. The drawback of RIPA is that is platelets number dependent, it cannot be performed in some VWD2B patients with low platelet count and must be performed shortly after blood sampling. We developed an ELISA using a recombinant gain-of-function (p.D235Y and p.M239V) platelet glycoprotein Ib (VWF:GpIbB), similar to Flood et al (Blood, 2011), able to discriminate type 2B from 2A and 2M.

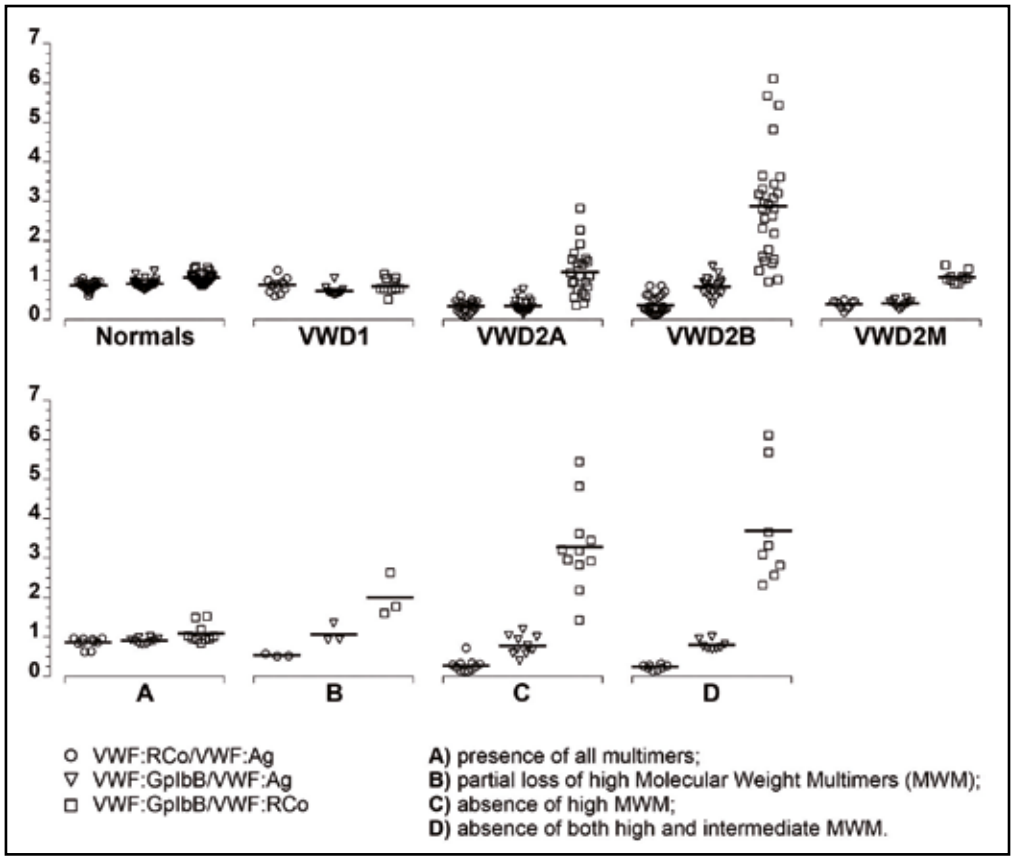
Aim: to confirm the use of VWF:GpIbB (VWF:GpIbB/VWF:RCo ratio) as an alternative assay to RIPA.

Patients and methods: 67 VWD patients (types: 1=9, 2A=22, 2B=26 and 2M=9) and 31 healthy subjects were evaluated for VWF:Ag, VWF:RCo and VWF:GpIbB.

Results: VWF:GpIbB/VWF:RCo ratio values obtained in different VWD variants showed that VWD2B patients had a significantly higher mean ratio compared with healthy controls, type 1, 2A and 2M (P<0.0001) (*Figure, upper panel*). VWD2B patients were divided into four groups on the basis of their different multimeric pattern: from A (presence of all multimers) to D (absence of both high and intermediate molecular weight multimers [MWM]) as previously reported by Federici et al (Blood, 2009). The mean value of the four groups shows a clear increasing trend (from 1.08 to 3.69) proportionally to the loss of HMWM (*Figure, lower panel*).

Conclusions: VWF:GpIbB/VWF:RCo ratio is able to discriminate VWD2B patients from other types 2, contrary to VWF:RCo/VWF:Ag and better than VWF:GpIbB/VWF:Ag ratios. However, in few VWD2B patients, with a full set of multimers, VWF:GpIbB/VWF:RCo ratio was similar to those of normal subjects and type 1 patients. Nevertheless, this new assay overcomes RIPA drawbacks being able to diagnose patients with a low platelet count, using a small amount of frozen plasma.

Corresponding Author
F. Stufano, *stufano.f@gmail.com*



PO.03.01 - 75
Figure 1

PO.03.02 - 77 TOWARDS PATIENT TAILORED THERAPY IN VON WILLEBRAND DISEASE PATIENTS IN THE PERIOPERATIVE SETTING

H. C. A. M. Hazendonk[(1)], H. C. Veerman[(1)], Y. V. Sanders[(2)], F. W. G. Leebeek[(2)], M. H. Cnossen[(1)]

[(1)]Department of Pediatric Hematology, Erasmus University Medical Center-Sophia Children's Hospital Rotterdam, Rotterdam, the Netherlands; [(2)]Department of Hematology, Erasmus University Medical Center Rotterdam, Rotterdam, the Netherlands.

Background: Von Willebrand Disease (VWD), the most common inherited bleeding disorder, is caused by a quantitative or qualitative defect of von Willebrand factor (VWF). Moderate/severe VWD patients are treated with VWF/FVIII clotting factor concentrate in case of acute bleeding or to prevent bleeding in the perioperative setting. Dosing is based primarily on bodyweight. Treatment with expensive clotting factor concentrates has a major impact on the National Health Care budget, therefore cost effectiveness is of importance. Data on perioperative VWF/FVIII concentrate consumption and clearance in VWD patients is limited.

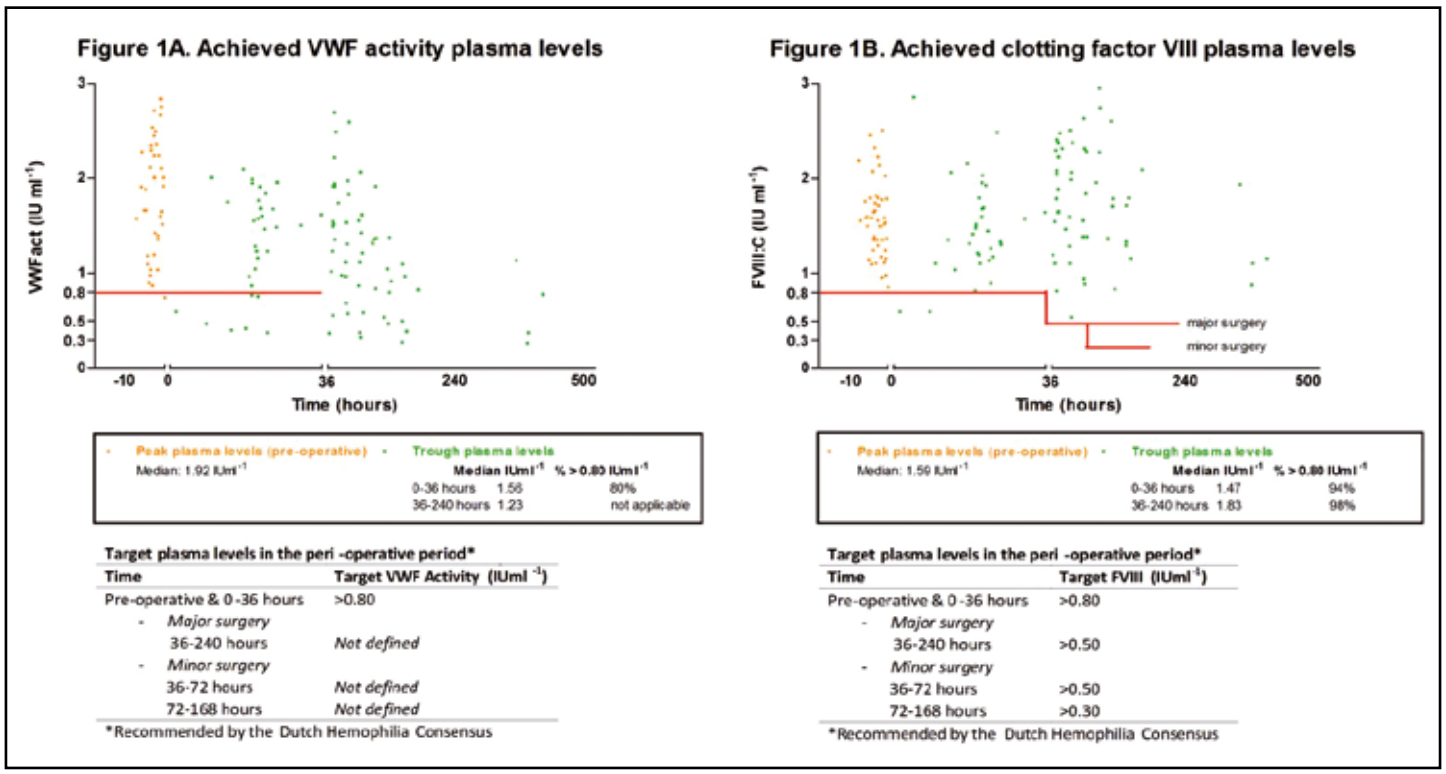
Aim: To evaluate current perioperative management of adults and children with VWD in relationship to VWF and FVIII target levels.

Patients and methods: In this retrospective observational study, we included VWD patients with severe or moderate VWD (historical VWF levels≤0.30IUml-1) undergoing surgery from 2000 until 2014 in the Erasmus MC, Rotterdam. VWF/FVIII concentrate HaemateP© was infused targeting VWF and FVIII clotting factor levels, defined by the Dutch Hemophilia Consensus. We collected patient and surgical characteristics, and achieved VWF activity (VWF:Act) and FVIII levels from medical files.

Results: 50 surgical procedures were performed in 32 patients: 28 adults (46 operations; median age 52 years; median weight 78kg) and 4 children (4 operations; median age 10.5 years; median weight 46kg). Mainly orthopedic (N=12;24%) and obstetric (N=12;24%) procedures were performed. During the first 36 post-surgical hours median VWF:Act was 1.56IUml-1 and median FVIII 1.47IUml-1, with 80% of achieved VWF:Act and 94% of achieved FVIII levels above 0.80IUml-1 (*figure1*).

Conclusion: Most perioperative VWF:Act and FVIII levels were above predefined target levels. Replacement therapy is often dosed according to FVIII levels and products such as HaemateP© contain more VWF than FVIII (2.5:1). These data support critical appraisal of peri-operative dosing strategies in VWD. Moreover, pharmacokinetic-guided dosing with iterative pharmacokinetic modeling may be a promising perspective, facilitating VWF/FVIII targeting.

Corresponding Author
H. C. A. M. Hazendonk, *h.hazendonk@erasmusmc.nl*



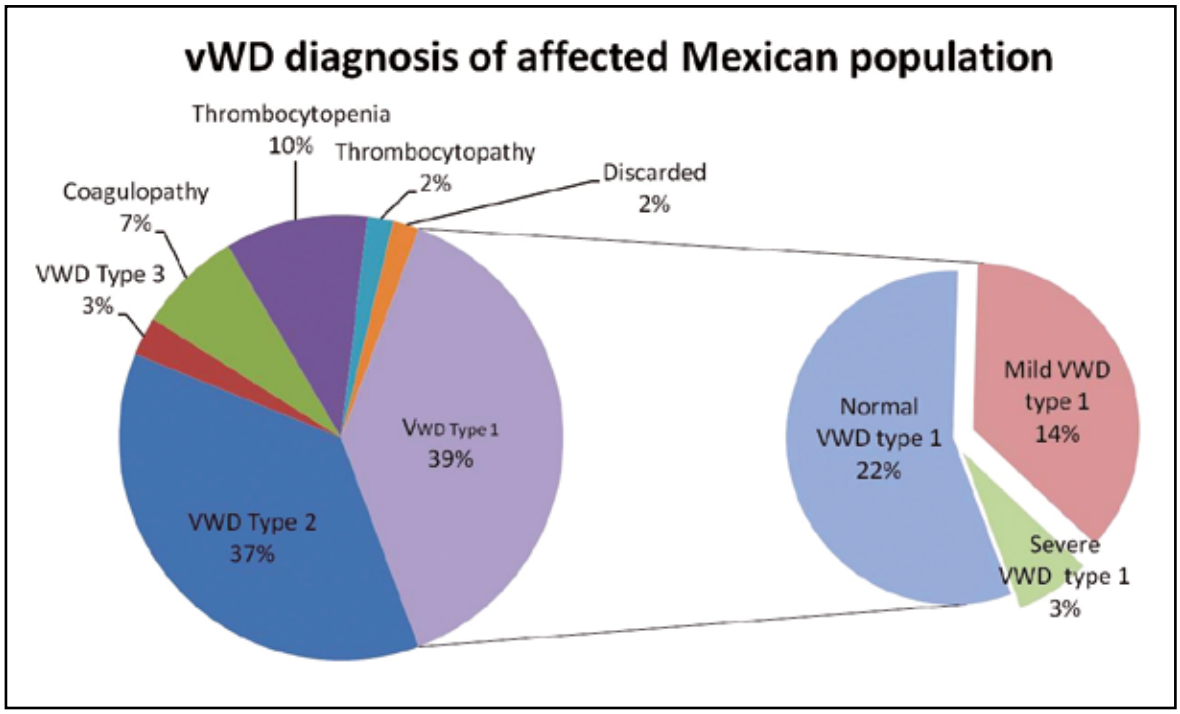
PO.03.02 - 77
Figure 1

PO.03.03 - 97 VON WILLEBRAND DISEASE IN POPULATION OF WESTERN MEXICO: BLOOD GROUP IMPACT ON DIAGNOSIS

A. R. Jaloma-Cruz[1], M. Z. Padilla-Romo[2], M. P. López-Montejo[3], A. Ibarra-Hernández[4], J. M. Soto-Padilla[3], L. B. Aguilar-López[4]
[1]Genetics Department, Western Biomedical Research Center, IMSS, Guadalajara, Jalisco, Mexico; [2]Doctorate in Human Genetics, Health Sciences Universitary Center, University of Guadalajara, Guadalajara, Jalisco, Mexico; [3]Hematology Department, Pediatrics Hospital, Western Medical Center, IMSS, Guadalajara, Jalisco, Mexico; [4] Hematology Department, General Hospital, Western Medical Center, IMSS, Guadalajara, Jalisco, Mexico.

Background / Aims: von Willebrand disease (vWD) is highly sub-diagnosed in Mexico because of the complexity of the tests and their unavailability across the country. The vWF is highly influenced by the blood group. The "O" hemotype confers susceptibility to bleeding. Since 63% of Mexicans are "O" group, this factor must be considered in vWD with predominant quantitative deficiencies.
The aims of this study are to diagnose vWD patients from Western Mexico by screening and confirmatory tests and to assess the phenotype analysis by weighing the ABO blood group.
Material and Methods: After obtaining consent letter, 129 patients from 106 independent families were recruited. They were referred because of mucocutaneous bleeding or asymptomatic with abnormal clotting times. Hematologists made a general physical examination and applied a standardized clinical questionnaire to assess the bleeding tendency (Federici, 2004). Plasma samples were obtained for screening (HC, blood group, PT, aPTT, fibrinogen, BT Ivy), initial confirmation tests (vWF:Ag, vWF:RCo, FVIII:C) and vWF multimers analysis.
Results: 129 patients were recruited (76 women, 53 men), aged from 1 to 77 years old, from 106 independent families (62% familial cases; 38% sporadic). 106 index cases were studied (82 pediatric, 24 adults). Screening and confirmatory tests were performed to obtain the diagnosis of VWD types. 83 patients (78%) had O blood group, in contrast to general Mexican population (63%), (p=0.024). Affected population was diagnosed by the screening tests and the ratio vWF:RCo/vWF:Ag and other clotting and platelets disorders were discarded (*figure 1*). vWF multimers analysis was done in 60 cases and an 80% of concordance rate was obtained respect to vWD diagnosis.
Conclusions: A considerable number of vWD patients showed O blood group, confirming higher bleeding risk related to this hemotype. Patients with normal vWF values and bleeding symptoms (moderate severity score), may still have vWD1. They had predominance of non O blood group (39%) in contrast to the vWD1 mild and severe (28%), (p=N.S.). Our findings highlight the importance of the hemotype in vWD diagnosis of Mexican patients.

Corresponding Author
A. R. Jaloma-Cruz, *arjaloma@gmail.com*



PO.03.03 - 97
Figure 1. Distribution of vWD diagnosis of Mexican patients according vWF:Rco/vWF:Ag ratio and screening testing. vWD type 2 was considered with a ratio <0.7, and there were identified by vWF multimer analysis subtypes 2A, 2B and normal patterns (probable 2M or type 1). Type 3 cases were also confirmed with the complete vWF absence.

PO.03.04 - 102 *IN VITRO* CHARACTERISATION OF MISSENSE MUTATIONS LOCATED IN THE VON WILLEBRAND FACTOR (VWF) D1 DOMAIN ASSOCIATED WITH QUANTITATIVE VWF DEFICIENCY

M. M. Dsouza[(1)], S. J. Webster[(1)], A. Cartwright[(1)], U. Budde[(2)], A. C. Goodeve[(1)], D. J. Hampshire[(1)]

[(1)]Haemostasis Research Group, Department of Cardiovascular Science, University of Sheffield, UK; [(2)]Hemostaseology Department, Medilys Hamburg, Germany.

Background/Aims: Quantitative deficiency of von Willebrand factor (VWF) manifests as either type 1 (VWD1; mild-moderate reduction) or type 3 (VWD3; severe reduction) von Willebrand disease (VWD). VWD1 is associated primarily with missense mutations altering VWF protein sequence, while VWD3 is primarily associated with mutations resulting in a null allele. Missense mutations located in the D1 domain of VWF have however been shown to cause both VWD1 and VWD3. This study aimed to investigate the cellular expression of D1 domain VWD1 and VWD3 missense mutations to elucidate the disease mechanism(s) involved and determine whether these differ dependent on VWD type.

Materials and Methods: Bacterial plasmid pcDNA3.1 expressing either wild-type VWF cDNA (WT) or a D1 missense mutation (p.R34G, p.D47H, p.S49R, p.L60P, p.R81G, p.S85P, p.V86E, p.Y87S, p.L129M, p.D141G, p.L150E, p.R273W) were transfected into HEK293T cells to mimic homozygous (Hom) inheritance, with co-transfections performed to mimic heterozygous (Het) inheritance. The quantity of mutant VWF expressed relative to WT only was assessed using ELISA. Multimer analysis of secreted VWF was performed via electrophoresis on 1.6% (w/v) SDS-agarose gels.

Results: Expressed mutants displayed significant reduction in VWF secretion compared to WT. VWD1 mutant p.S49R showed ~50% reduction in Hom and Het secretion, whereas VWD3 mutants p.R34G, p.D47H and p.R81G showed >90% reduction (Hom) and >75% reduction (Het) in secretion. Expression of varying WT:mutant ratios (25:75, 75:25) confirmed that p.R34G and p.R81G had a dominant-negative effect. p.L129M (reported in VWD1 and VWD3) and p.L60P when expressed alone (Hom) showed >90% reduction in secretion, but a milder ~50% reduction when co-expressed with WT (Het). Notably, none of these mutants showed increased intracellular retention.

Conclusions: Both VWD1 and VWD3 D1 missense mutations have been shown to cause reduced VWF secretion with varying severity. Lack of intracellular retention suggests abnormal cellular processing of VWF as a possible disease mechanism.

Corresponding Author
D. J. Hampshire, *d.hampshire@sheffield.ac.uk*

PO.03.05 - 103 RELATIONSHIP BETWEEN AGE AT FIRST BLEEDING AND BASELINE FACTOR VIII PLASMA LEVELS IN PATIENTS WITH SEVERE FORMS OF VON WILLEBRAND DISEASE (VWD)

S. M. Siboni[(1)], E. Biguzzi[(1)], C. Mistretta[(1)], V. Caiani[(1)], P. M. Mannucci[(2)], P. Bucciarelli[(1)], F. Peyvandi[(1)]

[(1)]Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico and University of Milan, Milan, Italy; [(2)]Scientific Direction, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan, Milan, Italy.

Background: VWD is characterized by muco-cutaneous bleeding, mostly of mild or moderate severity. Life-threatening bleedings can occur in patients with severe forms of VWD. Age at first bleeding is highly variable.

Aims: to evaluate the type of and the age at first bleeding in different types of severe VWD, and to assess the relationship between baseline FVIII:C plasma levels and age at first bleeding.

Material and methods: 103 patients with severe forms of VWD (i.e., VWF:RCo <6 IU/dL) undergoing a medical examination between September 2010 and September 2013 were analyzed (45 males, 58 females; 73 type 2, 24 type 3, 6 severe type 1).

The first bleeding event was defined as any bleeding occurring within 6 months before VWD diagnosis or necessitating a medical attention.

Results: A positive family history of bleeding was reported in 68 (66%) patients. Muco-cutaneous was the most common first bleeding symptom (n=66; 64%) followed by post-surgical (n=20; 19%) and gastrointestinal bleeding (n=11; 11%). The median age (IQR) at the first bleeding was 6 years (3-13). It was 7 years (5-15) for severe type 1, 8 years (5-18) for type 2 and 1 year (1-3) for type 3 VWD. A linear relationship between FVIII:C levels and age at first bleeding was found, the latter increasing 5 years for every 10 IU/dL increase of FVIII:C (β=4.95 [95%CI:2.02-7.87]), until levels of 30 IU/dl, after which the age at first bleeding did not raise further.

Conclusions: among patients with severe forms of VWD, the age at first bleeding was different in different types of VWD, being symptoms present at a very young age in type 3 VWD. The age at first bleeding is positively correlated with baseline FVIII:C plasma levels, which play a key role in the bleeding tendency at a very young age until levels of 30 IU/dL.

Corresponding Author
S. M. Siboni, *simona.siboni@guest.unimi.it*

PO.03.06 - 106 DEVELOPMENT AND VALIDATION OF ARRAY COMPARATIVE GENOMIC HYBRIDISATION (ACGH) IN VWD GENETIC ANALYSIS

S. J. Webster[1], D. J. Hampshire[1], B. Theophilus[2], R. Schneppenheim[3], D. Bellissimo[4], A. C. Goodeve[1]

[1]Haemostasis Research Group, Department of Cardiovascular Science, University of Sheffield, UK; [2]Dept. Haematology, Birmingham Children's Hospital, UK; [3]Department of Paediatric Haematology and Oncology, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany; [4]Blood Research Institute, Blood Center of Wisconsin, Milwaukee, WI, USA.

Background: von Willebrand disease (VWD) is caused by mutations in von Willebrand factor (VWF), a large multimeric glycoprotein, essential for platelet dependent primary haemostasis and the binding and transportation of factor VIII (FVIII). Mutations in the VWF gene (VWF) result either in defective (type 2) or deficient (types 1 and 3) VWF. In type 1 VWD, ~35% of patients have no causative VWF mutation identified following exon and intron-exon boundary sequencing. Copy number variation (CNV) consisting of large deletions/duplications within VWF exists, but little is understood about potential pathogenicity of these variants in VWD. In this study we hypothesise that CNV occurring in deep intronic and flanking untranslated regions (UTRs) contribute to type 1 VWD.
Aims: To use array comparative genomic hybridisation (aCGH) to identify potential pathogenic CNV across the entire VWF locus, including introns and 5' and 3' UTRs that may contribute to VWD pathogenesis.
Methods: A custom 8x15K microarray was designed (Agilent Technologies UK Ltd) containing ~1800 probes spanning the VWF locus. 18 patients with known CNV and 2 with no identified CNV were selected. Data analysis was performed using Agilent CytoGenomics software.
Results: Known CNV were detected in 13/18 patients, where array breakpoints mapped to within a minimum of ~100bp and a maximum of ~4.5kbp of known breakpoints. In two of these, a novel ~4kb 5'UTR deletion was also detected. The remaining 5 patients had CNV within the pseudogene region that were not reliably detected. In 2 patients, no clear CNV was found.
Conclusions: aCGH is a useful method for screening VWF for CNV. The analysis detected 13 of 18 known genetic imbalances and also a novel 5'UTR deletion approximately 11 kb 5' to the initiator ATG. Further refinement of pseudogene region probes is required to improve array performance and utility in VWD genetic analysis.

Corresponding Author
S. J. Webster, *sjwebster@sheffield.ac.uk*

PO.03.07 - 116 VON WILLEBRAND DISEASE (VWD) TYPE 1 MUTATION P.R1379C AND P.A1377V SYNERGISTICALLY DETERMINATE A 2M PHENOTYPE IN FOUR UNRELATED ITALIAN PATIENTS

M. T. Pagliari[1], L. Baronciani[1], F. Stufano[1], I. Garcia-Oya[1], F. Franchi[2], G. Cozzi[1], F. Peyvandi[1,2]

[1]Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center Milan, Italy; [2]Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy.

Background/Aim: We identified and fully characterized a small group of VWD patients carrying the VWD type 1 mutation p.R1379C (Goodeve et al, Blood 2007). One patient was classified as type1 and four as type 2M, on the base of their biochemical characterization. The four 2M patients also share the reported polymorphism p.A1377V. The aim of this study was to evaluate the effect of p.A1377V and p.R1379C, both alone and together, in order to explain patients' phenotype.
Materials and Methods: Conventional phenotype tests were used to evaluate patients' plasma and platelets as recommended by ISTH-SSC guidelines. Direct sequence analysis of exon 28 was carried out using genomic DNA of patients and 100 healthy subjects. WT and mutant (p.A1377V, p.R1379C and p.A1377V-R1379C) pcDNA3.1 VWF expression vectors were transiently transfected in HEK293 cells. Binding of recombinant (r)VWF to recombinant platelet glycoprotein Ibα (rGpIbα) was evaluated by an ELISA using increasing ristocetin concentrations (from 0 to 1.50 mg/ml).
Results: Biochemical and molecular results are reported in the Table. Platelets VWF levels were normal in patient 1 and strongly reduced in the others. Substitution p.A1377V was not found in 100 healthy subjects. Patient 5 had an additional mutation (p.S1378F) on the same allele with p.A1377V and p.R1379C.
The binding of p.A1377V-rVWF to rGpIbα was similar to that of the WT, whereas the p.R1379C-rVWF showed a slightly increased affinity to rGpIbα. The affinity of p.A1377V-R1379C-rVWF to rGpIbα was markedly reduced also at the highest ristocetin concentrations.
Conclusions: Only the presence of both p.A1377V and p.R1379C impairs the ability of rVWF to bind rGpIbα, which may explain the 2M phenotype of these patients.

Corresponding Author
M. T. Pagliari, *mtpagliari@gmail.com*

Pt	ABO	FVIII:C (IU/dL)	VWF:Ag (IU/dL)	VWF:RCo (IU/dL)	VWF:CB (IU/dL)	Multimeric Pattern[a] (HMWM)	Triplet structure[b]	RIPA	Nucleotide Change	Amino Acid Change
1	no-O	68	46	43	50	present	present	0.7	c.[4135C>T];[=]	p.[R1379C];[=]
2	O	53	38	< 6	18	present	present	1.4	c.[4130C>T;4135C>T]/ c.[3797C>T ;3835G>A]	p.[A1377V;R1379C]/ p.[P1266L;V1279I]
3	O	33	17	< 6	12	UL-VWF with smear	decreased	> 2	c.[4130C>T;4135C>T];[=]	p.[A1377V;R1379C];[=]
4*	no-O	37	28	8	17	UL-VWF with smear	decreased	> 2	c.[4130C>T;4135C>T];[=]	p.[A1377V;R1379C];[=]
5	no-O	36	24	7	16	UL-VWF with smear	decreased	> 2	c.[4130C>T;4133C>T;4135C>T];[=]	p.[A1377V;S1378F;R1379C];[=]
NR	n.a.	50-150	40-169[c] 55-165[d]	41-160[c] 53-168[d]	45-170[c] 56-174[d]	present	present	0.7-1.2 mg/ml	n.a.	n.a.

Values are shown as a mean of 3 independent measurements; *Measurements were peformed only once; n.a. not applicable;
N.R. normal range; Pt, Patient; HMWM, High Molecular Weight Multimers; UL-VWF, Ultra Large VWF multimers;
[a] Low resolution gel; [b] Intermediate resolution gel; [c] range values of normal individuals with blood group O;
[d] range values of normal individuals with blood group no-O;

PO.03.07 - 116
Table 1

PO.03.08 - 164 TYPE 2B/2M VON WILLEBRAND DISEASE MUTATIONS MISFOLD THE VWF A1 DOMAIN

M. Auton, A. Tischer, P. Madde, L. Moon-Tasson
Mayo Clinic Division of Hematology, Rochester MN, USA.

Background: We have surveyed the effect of Type 2B and Type 2M von Willebrand Disease (VWD) mutations the structure and rheological function of the Von Willebrand factor (VWF) A1 domain. These mutations have a dynamic range of clinical manifestations from a paucity of VWF-platelet interactions to severe thrombocytopenia.
Methods: To assess function, we have developed a real-time high-speed video microscopy analysis of platelet translocation dynamics under shear flow in a parallel plate microfluidic flow chamber chelated with recombinant A1 domains harboring these mutations. To assess structure, we have developed a number of solution biophysical and thermodynamic metrics that classify these mutational variants of the A1 domain as Native (with varying thermodynamic stability), Native-Like (having reduced secondary structure but retaining some thermodynamic stability), and Molten Globule (a complete lack of tertiary structure with residual secondary structure characterized by the absence of a urea and thermal unfolding transition).
Results: Our analysis of translocation dynamics results in statistical distributions of pause (residence) times that are proportional to the strength of the A1-GPIb interaction and quantitatively correlate to reported VWD patient platelet counts and the severity of thrombocytopenia. Our biophysical analysis demonstrates that the majority of these mutations cause the A1 domain to misfold to the Molten Globule conformation (12 of the 18 variants studied are not natively structured). The A1 domain remained misfolded and retained the correct functional phenotype when 2 extreme molten globule causing mutations from each phenotypic subtype were engineered into the VWF A1A2A3 tridomain.
Conclusions: Taken together, these mutations reveal a significant misfolding propensity of the A1 domain, A1 retains the functional and phenotypic properties of plasma VWF and the fact that misfolding can cause both phenotypes indicates specific secondary structural elements are required for the A1-GPIbα interaction while others regulate platelet adhesive strength.

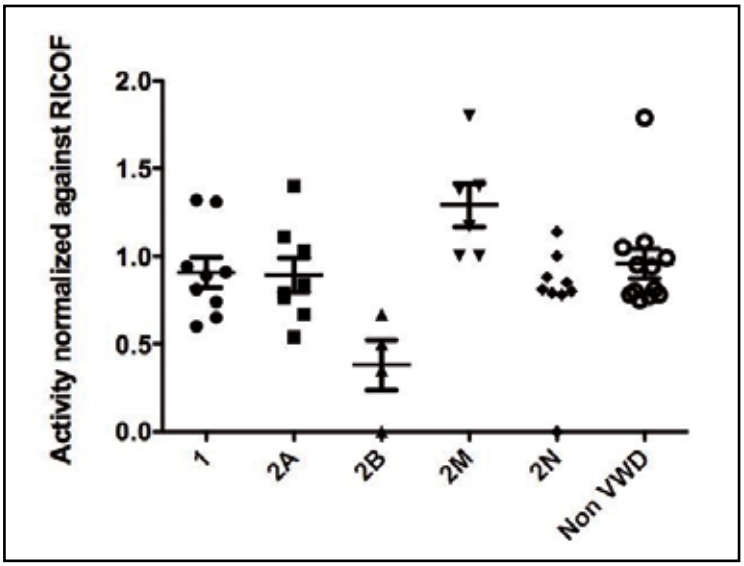
Corresponding Author
M. Auton, *Auton.Matthew@mayo.edu*

PO.03.09 - 132 DETERMINATION OF THE VWF SUBTYPES WITH THE RISTOCETIN INDEPENDENT GAIN OF FUNCTION GLYCOPROTEIN 1B INNOVANCE VON WILLEBRAND ACTIVITY ASSAY.

C. van Duren[(1)], S. Schoormans[(1)], B. A. P. Laros[(2)], P. Brons[(2,3,4)], W. L. van Heerde[(1,3)]
[(1)]Department of Laboratory Medicine, Laboratory of Hematology, unit Thrombosis Hemostasis - Radboud University Hospital, Nijmegen, the Netherlands; [(2)]Department of Hematology - Radboud University Hospital, Nijmegen, the Netherlands; [(3)]Hemophilia Care Center [(4)] Department of pediatrics - Radboud University Hospital, Nijmegen, the Netherlands.

Background/Aims: The vWF ristocetin assay (VWF:RICOF) is a cumbersome assay and affected by polymorphisms present in the ristocetin binding site of vWF resulting in *in vitro* decreased vWF activity (Flood et al, Blood, 2010). The gain of function Glycoprotein 1b (GP1b) assay is an automated assay that does not require ristocetin. In this study we compared both assays and their ratio's with vWG ag and VWF CBA in a phenotypic and genotypic well defined patient cohort.
Materials and Methods: The GP1b assay uses polystyrene particles coated with an antibody directed against the platelet receptor GPIb. After addition of plasma the two gain-of-function mutation containing recombinant GP1b, is added. vWF induces GPIb-based particle agglutination which can be measured by turbidimetry. 44 VWD patients (genotypically confirmed Type 1: N=9, type 2A: N=9, type 2B: N=6, 2M: N=9, type 2N: N=6, type 1/2N: N=4, type 3: N=1, acquired VWD: N=1), 12 non VWD patients and 156 samples collected from diagnostic testing were analyzed with the GPIb assay and the VWF:RICOF assay.
Results: For routine VWD screening in patients with bleeding tendency we observed a significant difference between the two assays. The overall slope was 1,26 ± 0,09 indicating that the GPIb assay showed overall lower VWF levels as compared to VWF:RICOF assay. 23 samples (15%) were at least 20% higher in the GP1b assay compared to VWF:RICOF. This discrepancy could be due to the p.D1472H polymorphism with a frequency of 19% in a European population.
The GP1B assay is also able to discriminate for VWD subtypes. In combination with VWFag the ratio showed a better sensitivity and specificity for type 1 and type 2. Moreover, the GP1B assay is able to distinguish between the type 2 subclasses based on GP1b activity/AG and GP1b/CBA ratio or GPIb activity /RICOF. The ratio (VWF:RICOF is 100%) against VWF:RICOF of type 1, 2A, 2B, 2M, 2N and non VWD are 0,89, 0,82, 0,48, 1,28, 0,83 and 0,88.
Conclusion: The GP1b assay is able to distinguish between the different types of VWD. Furthermore, the assay allows a complete subtype analysis in combination with RICOF, VWF ag and VWF CBA.

Corresponding Author
C. van Duren, *clint.vanduren@radboudumc.nl*



PO.03.09 - 132
Figure 1

PO.03.10 - 108 PREDICTORS OF VON WILLEBRAND DISEASE DIAGNOSIS IN INDIVIDUALS WITH BORDERLINE VON WILLEBRAND FACTOR PLASMA LEVELS

P. Bucciarelli[(1)], S. M. Siboni[(1)], F. Stufano[(1)], E. Biguzzi[(1)], M. T. Canciani[(1)], M. T. Pagliari[(1)], S. La Marca[(1)], C. Mistretta[(1)], L. Baronciani[(1)], F. R. Rosendaal[(2,3)], F. Peyvandi[(1)]

[(1)]Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico and University of Milan, Milan, Italy; [(2)]Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands; [(3)]Department of Thrombosis and Haemostasis, Leiden University Medical Center, Leiden, The Netherlands.

Background: Individuals with borderline von Willebrand factor (VWF) plasma levels (30 to 60 IU/dL) represent a diagnostic challenge. Second level tests (time-consuming and expensive) are required to confirm or exclude the diagnosis of von Willebrand disease (VWD).
Aim: To assess which individual's parameters can predict VWD diagnosis in individuals with borderline VWF.
Materials and Methods: Between 2007 and 2011, 950 individuals, referred after bleeding episodes or detection of abnormal coagulation tests, were investigated with first screening tests (blood count, PT, APTT, factor VIII:C, VWF:RCo and VWF:Ag), and 95 [64 females and 31 males; median age 28 years (IQR:15-44)] had borderline VWF:RCo levels. All 95 underwent further testing (VWF collagen binding, VWF multimeric analysis, ristocetin-induced platelet agglutination, and VWF intra-platelet analysis) to diagnose or exclude VWD. A multivariable logistic regression model was fitted with individual's characteristics (sex, age, bleeding score, family history, VWF:RCo, ABO blood group) as predictors of VWD diagnosis. The predictive capability of the model was measured as the area under the ROC curve (AUC).
Results: Of the 95 individuals with borderline VWF, VWD was confirmed in 47 (49%, 38 type 1). A negative linear relationship between VWF:RCo levels and risk of VWD diagnosis was found (*Figure1*), most evident for individuals with blood group non-O [adjusted odds ratio: 7.00 (95%CI:1.48-33.11) for every 5 IU/dL decrease in VWF:RCo]. The other variable clearly associated with VWD diagnosis was female sex [adjusted odds ratio: 5.86 (95%CI:1.49-19.37)]. The full logistic model explained about 60% of the outcome variance (R^2: 0.596) and its AUC was 0.89 (95%CI:0.83-0.95).
Conclusions: In individuals with borderline VWF, the two strongest predictors of VWD diagnosis are low VWF:RCo levels (particularly in blood group non-O) and female sex. This predictive model has a promising discriminative capability to identify patients with VWF borderline levels who are likely to have VWD.

Corresponding Author
P. Bucciarelli, *bucciarelli@policlinico.mi.it*

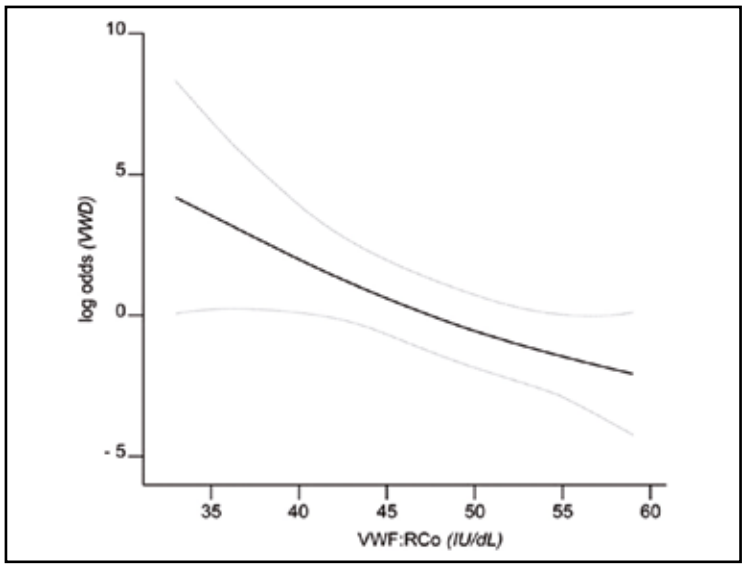
PO.03.11 - 98 VON WILLEBRAND FACTOR IN ESSENTIAL THROMBOCYTHEMIA IS AFFECTED BY RETICULATED PLATELETS AND BY NON-ADAMTS-13-DEPENDENT PROTEOLYTIC PROCESSING

S. Lancellotti[(1)], A. Dragani[(2)], P. Ranalli[(2)], G. Petrucci[(3)], M. Basso[(2)], R. Tartaglione[(4)], B. Rocca[(3)], R. De Cristofaro[(1)]

[(1)]Center for Haemorrhagic and Thrombotic Diseases, Department of Medical Sciences, Catholic University School of Medicine, "A. Gemelli" Hospital, Rome, Italy; [(2)]Center for Haemorrhagic, Thrombotic and Rare Hematologic Diseases, Spirito Santo Hospital, Pescara, Italy; [(3)] Institute of Pharmacology, Catholic University School of Medicine, Rome, Italy; [(4)]Institute of Haematology, Complesso Integrato Columbus, Catholic University School of Medicine, Rome, Italy.

Essential thrombocythemia (ET) is characterized by increased platelet generation and prevalent thrombosis. An acquired von Willebrand factor (VWF) disease has been hypothesized and associated with extreme thrombocythosis or rare bleedings. Whether VWF is modified in ET patients with controlled platelet counts and the mechanisms of VWF structure-function perturbation remain unclear. We measured VWF antigen (Ag), activity (act), multimers (VWFm) and propeptide (VWFpp) in 69 ET patients, 69 matched controls and 10 subjects with reactive thrombocythosis (RT). In ET, VWF:Ag levels were increased by ≈30% vs. controls ($p<0.01$), independently of blood groups. VWF:Ag was increased also in RT by ≈50 %. However, VWF:act in ET was reduced by ≈20% vs. controls and by ≈50% vs. RT (both $p<0.01$). The VWFact/Ag ratio was significantly decreased in ET vs. controls or RT and inversely predicted by immature platelets (beta=-0.34, p=0.007). Larger VWFm were reduced and atypical bands were observed in ET samples. The VWFpp/VWFAg ratio, reflecting VWF clearance, and ADAMTS-13 activity were unchanged. Two platelet metalloproteases, ADAM-10 and ADAM-17, hydrolyzed VWFm *in vitro* with a pattern reminiscent of ET plasmas. In conclusion, in ET VWF:act/Ag ratio is selectively decreased and inversely correlated with reticulated platelets. Platelet's ADAM-10 and ADAM-17 might contribute to VWFm proteolysis. Thus, an adequate antithrombotic therapy might reduce this phenomenon and paradoxically reduce at the same time also the bleeding risk, which might arise from extreme consequences of VWF degradation from unrestrained, activated platelets *in vivo*.

Corresponding Author
R. De Cristofaro, *rdecristofaro@rm.unicatt.it*



PO.03.10 - 108
Figure 1

04. FACTOR VIII - HEMOPHILIA A

PO.04.01 - 87 LATEST RESULTS FROM THE PUP-GCP CLINICAL TRIAL: A LOW INHIBITOR RATE IN PREVIOUSLY UNTREATED PATIENTS WITH SEVERE HEMOPHILIA A TREATED WITH OCTANATE

A. Klukowska[1], S. Lowndes[2], S. Knaub[2], V. Komrska[3], P. Laguna[1], V. Vdovin[4], M. Jansen[5]

[1]Warsaw Medical University, Warsaw, Poland; [2]Octapharma AG, Lachen, Switzerland; [3]University Hospital Motol, Prague; [4]Izmaylovo Children's Hospital Haematological Centre, Moscow, Russia; [5] Octapharma Produktionsgesellschaft mbH, Vienna, Austria.

Background: Octanate is a highly purified, double virus inactivated, human plasma-derived factor VIII (FVIII) concentrate with all coagulation FVIII bound to its natural stabilizer VWF in a VWF:RCo/FVIII:C ratio of approximately 0.4. Five prospective GCP studies with Octanate were conducted in 77 previously treated patients (PTPs) with severe hemophilia A. None of these 77 PTPs developed an inhibitor while treated exclusively with Octanate. A prospective clinical trial has been initiated in 2000 in order to assess the immunogenicity of Octanate in previously untreated patients (PUPs). The goal was to enroll 50 PUPs with severe hemophilia A and to follow them for an observational period of 100 exposure days.

Materials and Methods: Patients with severe hemophilia A without previous exposure to FVIII or FVIII-containing products were enrolled. Efficacy and tolerability were assessed by a 4-point verbal rating scale. Inhibitor assay, according to modified Bethesda method, was tested prior to treatment every 3-4 EDs (ED 1-20), and afterwards every 10 EDs (ED 21-100), but at minimum every three months.

Results: Fifty-one subjects have been enrolled and three of them (5.9%) developed clinically relevant inhibitor titers over the course of the study. Another two displayed transient inhibitors that disappeared spontaneously without change of dose or dosing interval. All inhibitors developed under on-demand treatment and before ED 50. From the 51 subjects, 45 exceeded 50 EDs as of today. Octanate was well-tolerated and the adverse event profile was consistent with the population studied. The haemostatic efficacy in prophylaxis and treatment of bleeding episodes was generally rated as "excellent" and no complication was reported for any surgical treatment.

Conclusion: Despite frequent inhibitor testing and predominant on-demand treatment, the data indicate a low overall inhibitor rate for Octanate in patients who exceeded 50 EDs (5/45) of which only 3 (5.9%) were clinically relevant.

Corresponding Author
S. Lowndes, *shannely.lowndes@octapharma.ch*

PO.04.02 - 83 A CLINICAL STUDY IN PUPS WITH SEVERE HAEMOPHILIA A - IMMUNOGENICITY, EFFICACY AND SAFETY OF TREATMENT WITH HUMAN-CL RHFVIII

R. Liesner[1], M. Jansen[2], S. Knaub[3]

[1]Great Ormond Hospital for Children, NHS Trust Haemophilia Centre, London, United Kingdom; [2]Octapharma Produktionsgesellschaft mbH, Vienna, Austria; [3]Octapharma AG, Lachen, Switzerland.

Background: Human-cl rhFVIII is a human cell-line derived, B-domain deleted recombinant FVIII concentrate for intravenous use. Due to the absence of immunogenic epitopes, as seen in recombinant FVIII concentrates from hamster cell lines, Human-cl rhFVIII is thought to be potentially less immunogenic. So far, five prospective GCP studies with Human-cl rhFVIII were conducted in 135 previously treated adults and children with severe haemophilia A. Pharmacokinetics, efficacy and safety of the product was evaluated. The data indicate that Human-cl rhFVIII is effective in the treatment and prophylaxis of bleeding episodes. There was no product related adverse event and none of the PTPs treated with Human-cl rhFVIII developed an inhibitor.

Objectives and Methods: A prospective, multinational, open-label, non-controlled clinical trial is ongoing in order to assess the immunogenicity, efficacy and safety of Human-cl rhFVIII in previously untreated patients (PUPs). Hundred patients with severe haemophilia A without previous exposure to any FVIII concentrate or FVIII containing products are planned to be enrolled from about 45 centers worldwide. Inhibitor testing according to modified Bethesda method will be performed pre-treatment, every 3-4 exposure days (ED 1-20) and every 10-12 EDs (ED 21-100) but at least every three months after start of treatment. Efficacy and safety of Human-cl rhFVIII during prophylactic treatment, treatment of breakthrough bleeds, and in surgical prophylaxis is assessed. Pharmaco-economic aspects and predictive factors for the development of inhibitors will be evaluated.

Results: The study started in Q1 2013. Until mid of May 2014 32 sites have been initiated, a total of 34 PUPs were screened, and 24 of them have started treatment with Human-cl rhFVIII.

Conclusion: A global GCP-study in PUPs is ongoing in order to investigate whether the reduced immunogenic profile of Human-cl rhFVIII will translate into a lower inhibitor incidence.

Corresponding Author
S. Knaub, *sigurd.knaub@octapharma.ch*

PO.04.03 - 86 OBSERVATIONAL IMMUNE TOLERANCE INDUCTION RESEARCH PROGRAM (OBSITI) – A MULTIFACETED APPROACH TO EXPLORE IMMUNE TOLERANCE INDUCTION

C. Escuriola-Ettingshausen[1], E. Berntorp[2], Y. Dargaud[3], Z. Gutowski-Eckel[1], C. Königs[4], C. Négrier[3], J. Oldenburg[5], A. Pavlova[5], W. Kreuz[4]

[1]HZRM – Haemophilia Centre Rhine Main, Frankfurt-Mörfelden, Germany; [2]University Hospital Malmö, Malmö, Sweden; [3]University Lyon, Lyon, France; [4]University Hospital Frankfurt, Frankfurt, Germany; [5]University Hospital Bonn, Bonn, Germany.

Background: ObsITI is an international, open-label, uncontrolled multicenter observational program initiated in December 2005. The study admits hemophilia A (HA) patients of any age and with any severity, with a confirmed inhibitor titer ≥ 0.6 BU, and reduced FVIII recovery and/or FVIII half-life. Patients with risk factors historically associated with a poor ITI prognosis as well as good prognosis are included. Patients are treated preferably according to the Bonn protocol.

Methods and Materials: The aim of the program is to evaluate patient and therapy related variables on ITI course, outcome and morbidity in HA patients. ObsITI satellite studies additionally look at other factors related to tolerization: the Thrombin generation sub-study to evaluate the correlation between the clinical bleeding phenotype and their thrombin generation capacity before and during ITI, the sub-study on genetic determinants as predictors of the ITI outcome, the concentrate-based thrombin generation assay (TGA), the characterization of specific FVIII epitopes and the immunological sub-study. The results obtained from the sub-studies will be correlated with the ITI success rates.

Results: As of February 2014, a total of 258 patients from 22 countries have been screened for ObsITI. In 146 patients ITI has been documented, and 94 patients completed the study. Preliminary result show a significant correlation between the bleeding rate during ITI, the peak titre during ITI, the inhibitor titre at start of ITI >10 BU and the number of poor prognosis factors with ITI outcome.

Conclusion: ObsITI is a large ongoing study on ITI with the potential to extend our knowledge on ITI.

Corresponding Author
C. Escuriola-Ettingshausen, *carmen.escuriola@hzrm.de*

PO.04.04 - 85 OCTAPLEX® STATE-OF-THE-ART: IMPLEMENTATION OF A NEW NANOFILTER

P. M. Schulz[1], A. Pichotta[2], T. Schmidt[2], K. Pock[1], J. Römisch[1]

[1]Octapharma Produktionsgesellschaft mbH, Vienna, Austria; [2] Octapharma GmbH, Frankfurt, Germany.

Background: The manufacturing process of octaplex®, a state-of-the-art prothrombin complex concentrate, comprises two dedicated orthogonal pathogen reduction steps, solvent detergent (S/D) treatment and nanofiltration to inactivate/remove, respectively, any potentially present lipid enveloped viruses (LEV), non-enveloped viruses (NEV) and infectious prion protein. In order to maintain and further increase the overall pathogen safety, a new nanofilter (Planova 20N, Asahi Kasei Co., Ltd., Japan) was implemented replacing the former pathogen filtration step. Product specifications and biochemical characteristics had to be maintained.

Materials and Methods: Virus and prion safety studies were performed with LEVs, NEVs and hamster adapted scrapie prion preparation (PrPSc), respectively, according to the international pathogen safety guidelines. Beyond extended biochemical characterisation, tests for possible activation of coagulation proteins were performed.

Results: Log reduction factors (LRF) of single steps and global reduction factors (GRF) of the octaplex® manufacturing process are presented as log10. *See table 1*.

Extended biochemical investigations on octaplex® 500 show, that a balanced content of coagulation factors and inhibitors is maintained and coagulation activation markers are present in trace amounts only.

Conclusion: The pathogen safety steps implemented in the octaplex® manufacturing process helped to safeguard patients from infectious transmission for more than a decade, while the implementation of a new nanofilter (20 nm) maintained or even further increased pathogen reduction capacities of the process whilst the product characteristics were maintained.

Corresponding Author
P. M. Schulz, *petra.schulz@octapharma.com*

Step/ Pathogen	HIV-1	PRV	SBV	BVDV	HAV	PPV	PrP^{Sc}
	LEVs				NEVs		
1stIEC	n.d.	n.d.	n.d.	n.d.	2.63	2.16	2.38
S/D	≥ 6.37	≥ 7.19	≥ 7,34	n.d.	n.a	n.a	n.a
2ndIEC	n.d	n.d	n.d	n.d	n.d	n.d	1.80
NF20	**≥ 6.55**	**≥ 7.09**	**≥ 6.61**	**≥ 5.91**	**≥ 6.08**	**5.40**	**≥ 3.58**
GRF	**≥ 12.92**	**≥ 14.28**	**≥ 13.95**	**≥ 5.91**	**≥ 8.71**	**7.56**	**≥ 7.76**

PO.04.04 - 85
Table 1. IEC: ion-exchange chromatography; n.d.: not done; n.a.; not applicable;

PO.04.05 - 105 WHOLE-EXOME SEQUENCING APPROACH TO STUDY INHIBITOR DEVELOPMENT IN SEVERE HEMOPHILIA A PATIENTS

M. M. Gorski[1], K. Blighe[2], I. Garagiola[1], S. Seregni[2], M. E. Mancuso[2], E. Santagostino[2], L. A. Lotta[2], F. Peyvandi[1,2]

[1]Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy; [2]Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico and Fondazione Luigi Villa, Milan, Italy.

Background: Hemophilia A is an inherited bleeding disorder that is predominantly characterized by marked deficiencies in factor VIII (FVIII) due to mutations in the F8 gene. Approximately 40% of severe hemophilia A incidents occur via a single recurrent gene inversion mutation in intron 22. The treatment therapies for patients suffering from hemophilia A come in the form of infused plasma-derived or recombinant FVIII. However, the development of inhibiting antibodies (inhibitors) to therapy is a major complication and represents a serious clinical problem in the treatment of hemophilia A. Several studies have previously identified human leukocyte antigen class II, interleukin 10 and tumor necrosis factor alpha to be associated with an increased risk of inhibitor development.
Aims: To identify novel genetic elements underling the risk of inhibitor development, we performed a whole-exome sequencing analysis of 28 Italian patients suffering from severe hemophilia A, of which 18 patients developed inhibitors to the applied therapy whilst the remainder did not. Exome sequence capture was performed using the SeqCap EZ design library (NimbleGen) and sequencing was conducted on an Illumina HiSeq 2000 in collaboration with the Human Genome Sequencing Centre (HGSC) at Baylor College of Medicine, Houston, USA.
Results: Data analysis revealed approx. 2 million variants, including 1,865,389 single nucleotide variants (SNVs) and 82,537 indels, passed the HGSC quality control with an average read-depth of 59. Fisher's Exact or Cochran-Armitage tests ($p \leq 0.05$) were applied to obtain a list of statistically significant variants. When looking solely at coding variants predicted to be damaging (STOP gain/loss, indels etc.), several putative targets were discovered.
Conclusions: Interestingly, these preliminary results revealed target genes that can be associated with both higher risks for inhibitor development and those that can potentially be protective. Moreover, the pathways affected involve genes implicated in auto-immunity and immune system, indicating that inhibitor development is a complex multifactorial disease.

Corresponding Author
M. M. Gorski, *mgorski@fondazioneluigivilla.org*

PO.04.06 - 110 PATIENTS WITH HEMOPHILIA A (HA) UNDERGOING TOTAL KNEE REPLACEMENT: DOES THROMBIN GENERATION ASSAY (TGA) ADD INFORMATION ON CLOTTING ACTIVATION DURING REPLACEMENT THERAPY

M. E. Mancuso[1], M. R. Fasulo[1], A. Cannavò[1], M. Clerici[1], G. Pasta[2], L. Solimeno[2], F. Peyvandi[1], A. Tripodi[1], E. Santagostino[1]

[1]Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy; [2]Orthopaedic and Traumatology Department, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy.

Background and Aims: FVIII activity measurement is the mainstay of laboratory monitoring during surgery in HA. The aim of this study was to evaluate if TGA may add information on clotting activation during surgery in HA patients treated with FVIII.
Materials and Methods: Adult patients with severe HA undergoing primary knee arthroplasty were eligible. Blood samples to measure FVIII and TGA were drawn daily prior and 30 minutes after FVIII injections on Day 0, 1, 3 and at discharge. TGA was performed on platelet-poor plasma.
Results: Nineteen patients aged 35-56 yrs were included. All patients were treated by boluses. The first bolus was injected 15-30 minutes before surgery at a median dose of 79 IU/kg (IQR: 75-87). From Day 1 onwards all received FVIII at a median dose of 56 IU/kg (IQR: 53-63) twice daily. Pre-operative FVIII levels were <1% in 11 cases (58%) and the median pre-operative FVIII peak was 137% (IQR: 126-159). The median FVIII trough level maintained post-operatively was 65% (IQR: 53-82). FVIII activity was linearly correlated with ETP and peak, and inversely correlated with lagtime and time to peak ($p<0.01$ for all analyses). All TGA parameters were sensitive to FVIII increase after the first bolus. However, from Day 1 when FVIII trough levels were >50% no significant increase of thrombin generation was observed. Two patients bled on Day 1 despite FVIII troughs of 70 and 79%. TGA on Day 1 in these 2 patients was similar to that measured in those who did not bleed.
Conclusions: A linear correlation was found between TGA parameters and FVIII activity during replacement therapy for surgery, however TGA did not provide additional information on global clotting activity even in case of bleeding complications.

Corresponding Author
M. E. Mancuso, *elisamancuso@tiscali.it*

PO.04.07 - 121 DENTAL HEALTH AND ORAL HEALTH-RELATED QUALITY OF LIFE IN HEMOPHILIC CHILDREN TREATED IN AN IRANIAN NATIONAL REFERRAL CENTER OF HEMOPHILIA

K. Salem[1,2], A. Raissi[3], P. Eshghi[4], F. Abdollah Gorji[4]
[1]Guilan University of Medical School Guilan Dental School, Department of Pediatric and Hospital Dentistry, Rasht, Iran; [2] Ebnesina Hospital, Tehran, Iran; [3]Ebnesina Hospital, Tehran, Iran; [4]Shahid Beheshti University of Medical Sciences, Mofid Children Hospital, Tehran, Iran.

Background: Oral health concerns are of great importance among patients with bleeding disorders. The purpose of present study was to investigate different aspects of oral health among young Hemophilia patients and the impact of oral health status on their quality of life (OHR-QoL).
Materials and methods: Oral health parameters including: DMFS-dmfs (decayed, missed, filled tooth surfaces in permanent and primary teeth) index, oral hygiene index, hypoplasia of first permanent molars, and temporomandibular joint dysfunction were evaluated in forty five hemophilic patients aged 2-15 years and their healthy matched controls in a referral Iranian Hemophilia Center (Mofid Children Hospital). Oral health related-quality of life was also investigated using age appropriate questionnaires. Data were analyzed by Chi–square, t-test and Pearson correlation.
Results: In both primary and permanent dentitions hemophilia children were significantly more caries-free than controls. Carious teeth (decayed component of DMFS-dmfs indices) was also significantly lower among hemophiliacs (P=0.03, t= –2.17) than controls but the filling and missing component were similar. The mean scores of OHR-QoL were not significantly different between groups. Bleeding was the main determinant of OHR-QoL in hemophiliacs (r=–0.56, P=0.000), whereas among controls the dmfs index as the indicator of dental health of primary teeth (r=–0.392, P=0.011) and male gender (r=–0.329, P=0.026) were the important parameters. Oral hygiene index, hypoplasia of first permanent molars, and temporomandibular joint dysfunctions were not significantly different between groups, P>0.05 and all were rated in normal range.
Conclusion: Children with hemophilia treated in this referral national center were found to have better oral health situation than their healthy counterparts mainly for appropriate strategies implemented by hemophilia center.

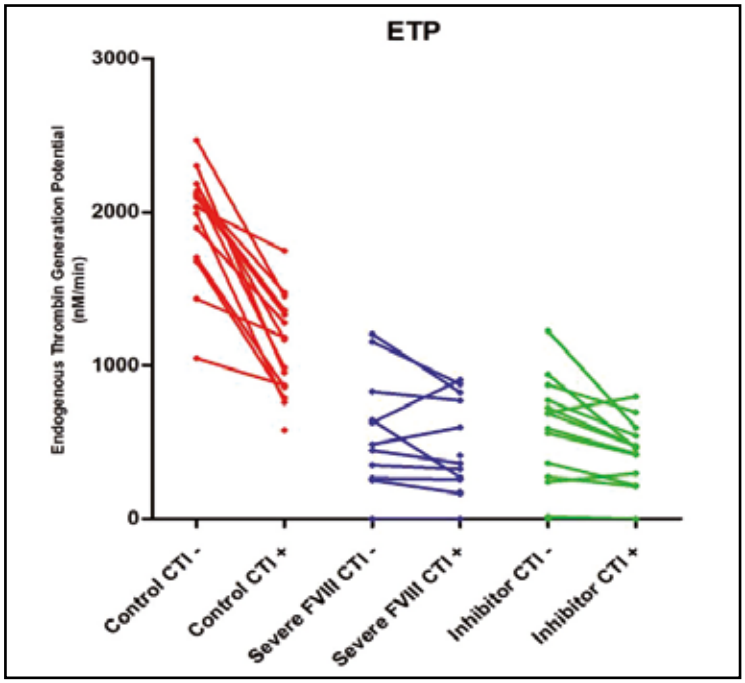
Corresponding Author
K. Salem, *kathy_salem@yahoo.com*

PO.04.08 - 155 FAILURE OF CORN TRYPSIN INHIBITOR TO AFFECT THE THROMBIN GENERATION ASSAY IN HEMOPHILIA PLASMA

R. Carmona[1], B. M. Mohammed[2], V. Salinas[1], E. J. Martin[2], G. A. Young[1], D. F. Brophy[2]
[1]Hemostasis and Thrombosis Center, Division of Hematology/Oncology, Children's Hospital Los Angeles, Los Angeles, CA, USA, University of Southern California Keck School of Medicine; [2]Coagulation Advancement Laboratory, Department of Pharmacotherapy and Outcomes Science, Virginia Commonwealth University, Richmond, VA, USA.

Aim: Thrombin generation assay (TGA) is a global hemostatic assay suffers from the lack of pre-analytical standardization. In this study, we evaluated the use of CTI in platelet poor plasma (PPP).
Methods: Patients with severe hemophilia A (SHA) with and without inhibitors and controls (15 per group) provided a blood sample after factor washout in sodium citrate tubes, pre-loaded with 136 µl of CTI stock solution (1.1 mg/ml). Tissue factor 1 pmol and 80 µl of PPP were pipetted in triplicate into 96-well microtiter plates, and 20µL of fluorogenic substrate and CaCl2 were dispensed into each well immediately before measurement initiation. Thrombogram parameters were reported.
Results: There were few differences in the samples processed with CTI compared to those without CTI. The median lag time between groups remained relatively constant in the healthy volunteers between samples with and without addition of CTI. The CTI resulted in marked and significant reduction in median peak thrombin generation in the healthy volunteers (CTI(-) 280.9 vs CTI(+) 85.2 nmol, p=0.002); however CTI did not significantly affect thrombin generation in the severe FVIII patients (CTI(-) 30.7 vs CTI(+) 21.0 nmol, p=0.568) or in the inhibitor patients (CTI(-) 27.6 vs CTI(+) 15.8 nmol, p=0.111). Similarly, CTI significantly reduced the median thrombin ETP in healthy volunteers (CTI(-) 2063.5 vs CTI(+) 1167.9 nmol/min, p<0.001); but did not significantly affect median ETP in the FVIII deficient patients (CTI(-) 637.0 vs CTI(+) 460.6 nmol/min, p=0.605) or the inhibitor patients (CTI(-) 486.0 vs CTI(+) 343.2 nmol/min, p=0.144, *See figure*).
Conclusions: Our results showed no significant influence of CTI 30 µg/ml on blunting peak thrombin generation or ETP in plasma from patients with severe FVIII deficiency with and without inhibitors. This lack of effect raises additional questions regarding the need for CTI as a pre-analytical addition to blood collection tubes during TGA.

Corresponding Author
G. A. Young, *gyoung@chla.usc.edu*



PO.04.08 - 155
Figure 1

PO.04.11 - 124 ORAL HEALTH STATUS AMONG THE HEMOPHILIA PATIENTS IN NORTH OF IRAN

K. Salem[1,2], B. Darbandi[3], S. Seyyed Khamesi[4], A. Raissi Dehkordi[5]

[1]Guilan University of Medical School, Rasht, Iran; [2]Ebnesina Hospital, Tehran, Iran; [3]Guilan Medical school, 17th Shahrivar Children Hospital, Rasht, Iran; [4]Guilan University of Medical Sciences, Rasht, Iran; [5]Ebnesina Hospital, Tehran, Iran.

Background and aim: Oral health problems are a source of morbidity among hemophilia patients. The purpose of this study was to evaluate the oral health of hemophilia child patients in the North of Iran.

Patients and methods: Fifty three hemophilia children aged 3 to 15 years and their matched controls were evaluated from different oral health aspects including: history of oral bleeding, dental caries, gingival health, dental anomalies, and oral health behaviors.

Results: 92% hemophilia patients were hemophilia type A and 53% were of severe type. 62% of hemophiliacs and 15% of controls reported a history of oral bleeding. In hemophilia patients bleeding was reported mainly during exfoliation and eruption of teeth, and there was no significant difference in oral bleeding in other oral areas between two groups. Among hemophilia children the dental caries indices, including decayed, missed, and filled tooth numbers and tooth surfaces (DMFT and DMFS) and plaque index (the degree of covering of tooth surface by biofilm and oral debris as an index of tooth cleanliness), were significantly higher whereas the frequency of tooth brushing was significantly lower than unaffected children. There was no significant difference in regard to number of dental visits, dental anomalies, fluorosis and hypoplasia of enamel between hemophilia children and their matched controls.

Conclusion: Despite of high quality oral health measures that are reported from main referral centers of hemophilia in Capital city, Tehran, hemophilia children in Guilan province, located in the North of Iran, need more supportive controls regarding their oral health. More investigation about the background variables seems necessary, although lack of a qualified referral center and avoidance of dentists to treat this patients may be important factors.

Corresponding Author
K. Salem, *kathy_salem@yahoo.com*

PO.04.12 - 126 *IN VIVO* FVIII RECOVERY IN HEMOPHILIA A MICE MODEL WITH INHIBITORS TREATED WITH DIFFERENT FVIII CONCENTRATES: IMPACT OF VWF

M. I. Bravo, B. Da Rocha-Souto, S. Grancha, J. I. Jorquera

Research & Development, Instituto Grifols S.A., Parets del Vallès, Spain.

Background/Aims: The role of VWF in the treatment of hemophilia A with inhibitors is being studied extensively. Several authors described the presence of VWF in FVIII concentrates to be beneficial due to its protective effect against inhibitors. This study aims to evaluate the differences of FVIII *in vivo* recovery between FVIII concentrates –containing or not VWF– in the presence of human inhibitors using a mouse model of hemophilia A.

Materials and Methods: Therapeutic concentrates (pdFVIII/VWF, rFVIII and isolated pdFVIII) and *in vitro* preformed complexes (rFVIII+pdVWF and pdFVIII+pdVWF in 1:1 proportion) were used as FVIII source. Inhibitor IgG was purified from a pool of hemophilic plasmas using Protein G Sepharose. Severe hemophilia A mice (FVIIInull E16 KO) previously infused with inhibitor IgG to 2.5 BU/ml (or buffer as control), were administered FVIII concentrates (100 IU/kg). Plasma samples were obtained 5 min post-FVIII administration and FVIII:C was measured (chromogenic assay). FVIII recovery was estimated according to described empirical models.

Results: FVIII recovery in control FVIIInull mice was similar for all FVIII concentrates, with values ranging from 107% to 124%. In contrast, in the presence of inhibitors the FVIII recovery in the same model was higher for VWF containing FVIII concentrates (76.2±15.2% for pdFVIII/VWF) when compared to concentrates composed of isolated FVIII (31.4±9.9% for rFVIII and 25.0±2.7% for pdFVIII). When the isolated FVIII products were premixed with VWF prior to infusion, the FVIII recovery was only partially restored (67.1±15.1% for rFVIII+VWF and 45.2±8.8% for pdFVIII+VWF).

Conclusions: In this model of hemophilia A with inhibitors, VWF-containing FVIII concentrates are more effective to restore FVIII circulating levels. This would support the beneficial role of VWF in this clinical condition. Results also suggest that this protection would be higher in native pdFVIII/VWF complex that in the complex of FVIII+VWF formed from the isolated proteins.

Corresponding Author
M. I. Bravo, *isabel.bravo@grifols.com*

PO.04.13 - 147 SEVERE HAEMOPHILIA A (HA) WITH INHIBITORS AND A NOVEL FVIII SILENT MUTATION: MOLECULAR AND BIOLOGICAL CHARACTERIZATION

A. Borchiellini[1], D. Zanolini[2], F. Valeri[1], A. Valpreda[1], P. Bicocchi[3], M. Acquila[3], E. Dosio[1], P. Schinco[1], A. Follenzi[2]

[1]Haemostasis and Thrombosis Unit-Haemophilia Center, Department of Oncology, S Giovanni Battista Hospital, Turin, Italy; [2]Health Sciences Department; University of Piemonte Orientale, Novara, Italy; [3]Thrombosis and Haemostasis Unit, Department of Haematology and Oncology, Giannina Gaslini Insitute, Genova, Italy.

Background: HA is caused by mutations in factor VIII gene (FVIII). The most serious treatment complication is inhibitors formation; severe gene defects that cause a complete absence of FVIII protein are more frequently involved. We report the case of a 55 year-old man, affected by severe HA, who developed inhibitors against FVIII in adulthood and displayed a novel silent mutation.
Materials and Methods: To investigate FVIII transcript variation total mRNA was isolated from primary macrophages and fibroblasts. Several combinations of primers were used to detect FVIII mRNA and intron retention. Immunofluorescent staining using a FVIII polyclonal antibody assessed FVIII expression.
Results: In silico alignment revealsed that G6273A mutation involved the last nucleotide of exon21 suggesting alteration in splice donor site recognition and intron21 retention. To investigate intron retention several RT-PCR were performed on patient in comparison with a healthy control donor. FVIII transcripts were observed in both patient and control using primers annealing before Exon21, meaning that mutation does not affect mRNA production. Meanwhile using Exon19 and Exon23 primers PCR product was present in control but not in patient cDNA. This may suggest the presence of a large intron between exon19 and 23 in patient cDNA. We assessed intron retention by nested PCR using the forward primer on exon21 and reverse on intron21. As expected, PCR product was obtained only from patient cDNA. Immunofluorescence performed on macrophages showed patient FVIII staining perinuclearly.
Discussion: We describe a novel G6273A mutation involved in C1 domain synthesis, leading to no aminoacidic change, as a putative cause of severe haemophilia. Mutated FVIII visualization by immunofluorescence showed small amount of FVIII localized close to nucleus, in ER -Golgi compartment. Therefore, we hypotize this mutation might influence mRNA and protein maturation, consistent with intracellular retention and accumulation.

Corresponding Author
A. Borchiellini, *alesandre@libero.it*

PO.04.14 - 151 TARGETING FVIII EXPRESSION TO SINUSOIDAL CELLS TO OVERCOME IMMUNOLOGICAL RESPONSES TO GENE THERAPY FOR HEMOPHILIA A

S. Merlin[1], S. E. Cannizzo[1], V. Bruscaggin[1], L. Naldini[2], A. Follenzi[1]

[1]Dept of Health Sciences, University of Piemonte Orientale, "A. Avogadro", Novara, Italia; [2]TIGET, San Raffaele Hospital, University Vita e Salute, Milan, Italy.

Background: Hemophilia A (HA) is an X-linked bleeding disorder due to mutations in clotting factor VIII (FVIII) gene. HA patients are treated with recombinant or plasma-derived FVIII with high probability to develop inhibitors. Lentiviral vectors (LV) were modified to obtain selective targeted expression in sinusoidal cells by transcriptional and post-transcriptional regulation. Liver is known to induce tolerance rather than immunity towards antigens presented to T cells by sinusoidal endothelial cells (LSEC) and Kupffer cells (KC). We investigated the role of LSEC and KC in HA gene transfer using LV-expressing FVIII driven by cell-specific promoters and microRNA target sequences (miRTs).
Methods: We prepared LVs containing GFP or FVIII under the control of the ubiquitous human PGK, the VEC (vascular endothelial cadherin) or CD11b (monocyte/macrophage-specific) promoters containing microRNA target sequences such as miRT142 (silenced in hematopoietic cells), miRT122(silenced in hepatocytes) or miRT126 (silenced in endothelial cells) and injected HA mice.
Results: Immunofluorescence on liver sections of LV.VEC.GFP-injected mice confirmed GFP expression by LSEC and GFP was restricted to them specifically by adding the mirT sequences 142-3p and 122. After LV.CD11b.GFP injection, transgene expression was present in KC and the addition of the miRT126 restricted GFP expression in KC. We then injected HA mice with LV.PGK.FVIII±miRT142, LV VEC. FVIII or LV.VEC.FVIII-miRT142-122 and LV.CD11.FVIII or LV.CD11.FVIII-miRT126. In the PGK mice group anti-FVIII antibodies were detected starting 2w after vector delivery, however the presence of miRT142 alone halved the antibody titer. In LV constructs, the presence of the cell-specific promoters and mirTs assured long-term phenotypic correction demonstrated up to 6m with an average of 5-8% FVIII activity and absence of anti-FVIII antibodies.
Conclusion: LV expressing FVIII under the control of VEC or CD11b promoters combined with miRTs combinations were able to overcome FVIII off-target expression limiting immune responses and providing phenotypic correction in treated HA mice with FVIII expression by sinusoidal cells.

Corresponding Author
A. Follenzi, *antonia.follenzi@med.unipmn.it*

PO.04.15 - 152 PHARMACOKINETIC MODELING OF PROPHYLAXIS WITH RECOMBINANT FULL-LENGTH FVIII AND B DOMAIN-DELETED FC-FUSION FVIII

A. Gringeri, M. Wolfsegger, K. Steinitz, A. J. Reininger
Global Medical Affairs, Baxter Innovations GmbH, Vienna, Austria.

Background: New products with extended half-lives (EHL) may increase patient convenience by reducing the number of infusions due to their distinct pharmacokinetic profiles. However, longer dosing intervals may cause longer time periods at relatively low FVIII levels potentially affecting their efficacy in preventing bleeding.
Aim: To compare the pharmacokinetic profiles of a recombinant full-length FVIII (rAHF-PFM) and a B-domain-deleted FVIII Fc-fusion product (BDD-rFVIIIFc) in severe hemophilia A patients treated prophylactically and the time spent weekly with FVIII below 3% or above 10%.
Materials and Methods: Population PK models of rAHF-PFM and BDD-rFVIIIFc were used to, in a simulated population of 1,000 severe hemophilia A subjects with different dosing scenarios. The population PK models were generated from data of multiple clinical trials using both FVIII products.
Results: The weekly time spent below FVIII 3% is longer in those treated with BDD-rFVIIIFc 30 IU/kg/72 hours, 50 IU/kg/96 hours and 120 hours and 65 IU/Kg/7 days than with rAHF-PFM 30 IU/Kg/48 hours. FVIII never drops below 3% in 57% of patients treated with rAHF-PFM 30 IU/kg/48h as compared to 41.1%, 18.3%, 0.9% and 0% of patients treated with BDD-rFVIIIFc 30 IU/kg/72h, 50 IU/kg/4 or 5 days, and 65 IU/kg/7 days, respectively. Patients on rAHF-PFM 30 IU/kg/48h spend more time weekly with FVIII levels above 10% than those on BDD-rFVIIIFc 50 IU/kg every 4 and 5 days, and 65 IU/kg/7 days.
Conclusion: PK modeling indicates that choice and dosing of FVIII products require careful evaluation of individual PK to allow more time is spent at more protective levels, particularly when an active lifestyle requires it.

Corresponding Author
A. Gringeri, *alessandro_gringeri@baxter.com*

PO.04.16 - 162 DIFFERENCES IN HEMOSTATIC POTENCY OF NATURAL, FULL LENGTH RECOMBINANT FVIII MOLECULE AND B-DOMAIN DELETED RECOMBINANT FVIII

G. Schrenk, H. Gritsch, S. Romeder-Finger, S. Knappe, M. Dockal, P. L. Turecek, F. Scheiflinger
Baxter Innovations GmbH, Vienna, Austria.

Background/Aims: A variety of recombinant FVIII (rFVIII) preparations that provide efficacious replacement therapy in hemophilia A patients are available. These preparations include full-length rFVIII (FL rFVIII), which is equivalent to the naturally occurring FVIII molecule, and genetically engineered B-domain deleted rFVIII (BDD rFVIII). Determination of an accurate FVIII activity is crucial for manufacturing, and especially for product dosing in clinical settings and testing of patient plasma. Several studies have demonstrated that assessing reliable FVIII activity for BDD rFVIII is a significant challenge due to the major discrepancy in results obtained with FVIII chromogenic and 1-stage clotting assay. Moreover, FVIII 1-stage clotting activity depends on the type of aPTT reagent used. The objective of the study was to evaluate differences in potency determination between FL rFVIII and BDD rFVIII with respect to their dependency on the type of aPTT reagent used in the 1-stage clotting assay.

Materials and Methods: Potency of FVIII was measured by a FVIII chromogenic activity assay and 1-stage clotting assay, using different commercial available aPTT reagents. Both the 8th international standard for blood coagulation factor VIII concentrate (NIBSC #07/350) and human normal plasma served as assay reference standards. The performance of the two different types of rFVIII products was compared by calculating the ratios between FVIII 1-stage clotting and chromogenic activities.
Results: Depending on aPTT reagent type, FVIII potencies varied within a relatively broad range, independent of which type of assay reference standard was used. The resulting ratios between FVIII 1-stage clotting and chromogenic activity showed a broader variance for the BDD rFVIII products than for FL rFVIII products.
Conclusion: Although further investigation is required to substantiate our observation, dependence on aPTT reagent type appeared more pronounced for BDD rFVIII than for FL rFVIII. As clinical laboratories use a number of commercial aPTT reagents with different compositions, determination of the correct potency and concentration in plasma post infusion of BDD rFVIII might be more challenging than for the natural, full-length rFVIII molecule.

Corresponding Author
P. L. Turecek, *peter_turecek@baxter.com*

PO.04.17 - 91 POPULATION PHARMACOKINETICS IN HEMOPHILIA A: TOWARDS INDIVIDUALIZATION OF PERI-OPERATIVE REPLACEMENT THERAPY

H. C. A. M. Hazendonk[1], J. Lock[1], K. Fijnvandraat[2], F. J. M. van der Meer[3], K. Meijer[4], P. Brons[5], M. Driessens[6], F. W. G. Leebeek[7], R. A. A. Mathôt[8], M. H. Cnossen[1]

[1]Department of Pediatric Hematology Erasmus Medical Center-Sophia Children's Hospital Rotterdam, Rotterdam, the Netherlands; [2]Department of Pediatric Hematology Academic Medical Center Amsterdam, Amsterdam, the Netherlands; [3]Department of Thrombosis and Hemostasis Leiden University Medical Center, Leiden, the Netherlands; [4]Department of Hematology University Medical Center Groningen, Groningen, the Netherlands; [5]Department of Pediatric Hematology Radboud university medical center, Nijmegen, the Netherlands; [6]Dutch Hemophilia Society; the Netherlands; [7]Department of Hematology Erasmus University Medical Center Rotterdam, Rotterdam, the Netherlands; [8]Department of Hospital Pharmacy-Clinical Pharmacology Academic Medical Center Amsterdam, Amsterdam, the Netherlands.

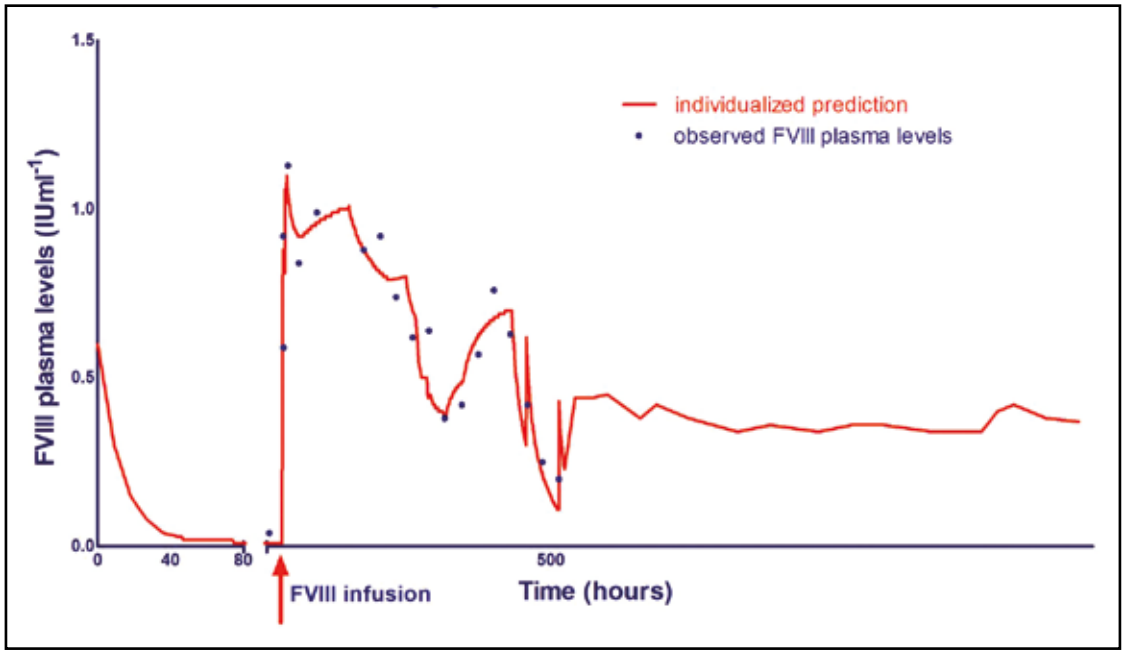
Background: Hemophilia A is an X-linked bleeding disorder caused by a deficiency of clotting factor VIII (FVIII). It is treated by infusion of FVIII clotting factor concentrate with dosing primarily based on bodyweight. Annual cost of treatment in the Netherlands is estimated at 130 million euro, mainly due to costs of clotting factor concentrate. Although it has been shown that FVIII consumption in prophylactic treatment may be reduced with 30% by pharmacokinetic(PK)-guided dosing, few data are available on the possible benefits of peri-operative PK-guided FVIII dosing. As 15% of annual FVIII consumption is used in the surgical setting, peri-operative PK guided dosing may significantly reduce treatment costs. In order to facilitate Bayesian adaptive dosing we conducted a retrospective multicenter study to construct a peri-operative PK population model for severe and moderate severe hemophilia A patients.

Patients and methods: Hemophilia A patients with FVIII levels <0.05 IU/ml and no inhibitor, undergoing low or medium risk surgery between 2000-2012, were included. Data was collected on FVIII treatment and patient characteristics. Population PK modeling was performed using nonlinear mixed-effects modeling (NONMEM).

Results: Population PK parameters were estimated in 75 adults (140 operations) and 44 children (58 operations). PK profiles were best described by a two-compartment model. Typical values for clearance (CL), central (V1) and peripheral volume were 0.20 L/h/70 kg, 3.3 L/70 kg and 1.8 L/70 kg. Inter-patient variability in CL and V1 was 41% and 21%; corresponding intra-patient variability was 29% and 32%. Bayesian analysis allowed precise description of individual FVIII level time profiles (*figure 1*).

Conclusion: A PK population model was constructed, which facilitates iterative peri-operative PK-guided dosing of FVIII in hemophilia A patients. Used for Bayesian adaptive peri-operative dosing of FVIII, the model may greatly improve quality and cost-effectiveness of care in hemophilia as both under- and overdosing is avoided.

Corresponding Author
H. C. A. M. Hazendonk, *h.hazendonk@erasmusmc.nl*



PO.04.17 - 91
Figure 1. Peri-operative FVIII plasma levels and an individualized prediction model based on iterative PK modeling.

PO.04.18 - 84 PERSONALIZED PROPHYLAXIS WITH HUMAN-CL RHFVIII IN ADULT PATIENTS WITH SEVERE HAEMOPHILIA A

A. Tiede[(1)], T. Lissitchkov[(2)], S. Knaub[(3)], O. Walter[(3)], J. Bichler[(3)], G. Yotov[(2)], R. Klamroth[(4)], C. M. Kessler[(5)]

[(1)]Hannover Medical School, Hannover, Germany; [(2)]Specialized Hospital for Active Treatment "Joan Pavel", Sofia, Bulgaria; [(3)]Octapharma AG, Lachen, Switzerland; [(4)]Vivantes Clinic in Friedrichshain, Berlin, Germany; [(5)]Georgetown University Medical Center, USA.

Background: Prophylactic therapy is aimed to maintain sufficient FVIII plasma concentrations between doses to prevent bleeding. Thorough FVIII concentrations and duration of FVIII plasma concentration >0.01 IU/mL are mainly determined by the half-life and the infusion interval. The individualization of prophylactic treatment requires knowledge of individual patient's PK profile in response to replacement factor, which is known to vary considerably between patients. Clinical studies with Human-cl rhFVIII in adult patients with severe haemophilia A also revealed differences between patients with a half-life ranging from 11.1 to 23.8 hours (analyzed by one-stage assay). The current study is designed to investigate the efficacy and safety of individually PK-tailored prophylaxis with Human-cl rhFVIII in previously treated adult patients with severe haemophilia A.

Material and Methods: This prospective, open-label, multicentre phase 3b study plans to enroll around 65 evaluable adult patients. Following a PK evaluation, patients will start with routine prophylaxis every other day or 3x/week for 1 – 3 months with a dose of 30–40 IU/kg body weight until individual PK data have been analyzed. For the subsequent 6-Month Treatment-Phase II, the dose and dosing interval will be individually determined for each patient based on the PK data. The goal is to determine the maximum dosing interval that can be achieved with a dose of not more than 80 IU/kg and that is capable of maintaining a trough level of ≥ 0.01 IU/mL.

Results: So far, 66 patients from 20 study sites in 8 European countries have been enrolled. Around 50 patients have started treatment phase II with a median dosing frequency of 3.5 days without increasing the median weekly dose used in routine prophylaxis. By the time of the 8th Bari International Congress (3-5 October 2014) we will have interim data on at least 30 completed patients allowing us a first evaluation on the validity of this treatment approach.

Conclusion: The results of this study indicate that patients on prophylaxis treated with Human-cl rh FVIII can extend their dosing interval without increasing the weekly dose.

Corresponding Author
S. Knaub, *sigurd.knaub@octapharma.ch*

PO.04.19 - 79 FACTOR VIII ASSESSMENT USING ONE-STAGE CLOT AND CHROMOGENIC ASSAY IN TRIALS INVESTIGATING PHARMACOKINETICS OF DIFFERENT FVIII PRODUCTS

M. Morfini[(1)], A. Tiede[(2)], T. Krogh-Meibom[(3)], M. Lapecorella[(4)], G. Pellegrino[(4)]

[(1)]Department of Hematology, Azienda Ospedaliero Universitaria Careggi, Florence, Italy; [(2)]Department of Haematology, Haemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany; [(3)]Department of Animal Health and Welfare, Danish Institute of Agricultural Sciences, Research Centre Foulum, Tjele, Denmark; [(4)]Haemophilia Department, NovoNordisk S.p.A, Rome, Italy.

Background: Assessment of FVIII:C activity in plasma is conducted with the one-stage clot assay (OS) and the two-stage chromogenic substrate (CS) assay. Discrepancies between them have been reported and attributed to specific products, assay conditions, or the standard used.

Aims: To assess the impact of OS vs. CS assay discrepancy on pharmacokinetic parameters that are typically reported in clinical trials.

Materials and Methods: Plasma of adult PTPs(ED>150) with severe haemophilia A (FVIII <1%) were used after a washout period of at least 4 days. A single injection of different FVIII products was administered at a dose of 25, 50 or 75 IU/kg. Blood samples were collected before dosing, (15 min), 30 min, 1, 4, 8, 12, 24, 30 and 48h post dose. Haemophilia A plasma was spiked with FVIII concentrates (target concentration: 0.03, 0.2, 0.6 and 0.9 IU/mL):3 recombinant (Advate Baxter, Kogenate Bayer, and turoctocog alfa, Novo Nordisk) and three plasma-derived (Hemofil M Baxter, Emoclot Kedrion, and Fandhi Grifols). For OS they used the SynthASil (Instrumentation Laboratory), aPTT reagent and CS assay based on the Coamatic kit (Chromogenix), commercial standard human plasma (Siemens) for measurements on clinical samples and the 6th WHO FVIII standard on spiked plasma.

Results: Recombinant products FVIII activity resulted higher when measured with CS than OS assay (mean CS/OS ratios: Kogenate 1.65, Advate 1.22, turoctocog alfa 1.26) while Plasma-derived products showed no or very low discrepancies (mean CS/OS ratios: Hemophil 1.11, Emoclot 0.97, Fandhi 1.11); the same was for spiked plasma samples. The discrepancy across the whole range of activities remained both in clinical (0.01–1.5 IU/mL) and spiked plasma (0.03–0.9 IU/mL), for most PK parameters (AUC, CL and C30 min).

Conclusion: The discrepancy between the two types of FVIII assay affects parameters in clinical trials as well as routine monitoring. The consistent use of CS assay for labelling and monitoring of products or the use of product specific standards may eliminate discrepancies.

Corresponding Author
M. Morfini, *pellegrinogina@virgilio.it*

PO.04.20 - 69 THE PURIFICATION OF FACTOR VIII BY THE METHOD NEGATIVE AFFINITY CHROMATOGRAPHY

N. Shurko, T. Danysh
Laboratory of Biochemistry, State Institution «Institute of Blood Pathology and Transfusion Medicine NAMS of Ukraine», Lviv, Ukraine.

Background: To correct factor deficiency or to prevent bleeding in patients with hemophilia A the replacement therapy is carried out. This therapy involves administrating plasma or recombinant preparations of factor VIII (FVIII). Coagulation FVIII preparations were obtained mostly using a cold precipitation of whole plasma called cryoprecipitation, followed by polyethylene glycol or glycine precipitation steps to partially remove protein contaminants such as fibrinogen. Additional, affinity chromatography has emerged as an efficient tool to purify FVIII.
Synthetic ligands include dye molecules, which considered as one of the important alternatives to biologic ligands for specific affinity chromatography. Dye affinity chromatography is a protein purification procedure based on the high affinity of immobilized dyes for the binding sites on many proteins. There are three types of dye affinity chromatography: negative, positive and tandem chromatography.
Aim: the method of negative affinity chromatography was investigated as an additional step for purification FVIII.
Methods: Initial raw material was a preparation of cryoprecipitate. We used one-stage clotting method for FVIII activity determination. Chromatographic sorbents are using where the matrix was Diasorb-aminopropyl and triazine dyes as ligands. To 2.0 ml of each of the sorbents (equilibrium 0.05 M Tris-HCl buffer, pH 8.0) was added 2.0 ml of working solution of cryoprecipitate in the same buffer.
Results: Studies have shown that FVIII is not adsorbing any of these sorbents. Many of the undesired proteins (FVIII) are retained by the column while the desired protein as well as some of the undesired proteins flows through the colomn. We received best results when used as ligands Procion blue HB and Procion blue MXR (factor activity increased on the order).
Conclusion: Triazine-dye affinity chromatography on immobilized Cibacron Blue HB and Procion blue MXR may be used on a pilot-scale to purify cryoprecipitate, obtained by a complete chromatographic procedure.

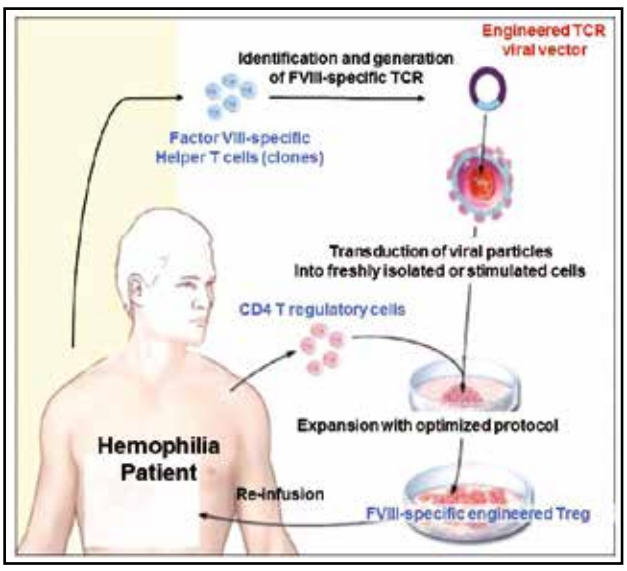
Corresponding Author
N. Shurko, *natalia_shurko@ukr.net*

PO.04.21 - 53 GENERATION AND FUNCTIONAL PROPERTIES OF ENGINEERED ANTIGEN-SPECIFIC HUMAN T REGULATORY CELLS TO TREAT HEMOPHILIA INHIBITOR FORMATION

Y. C. Kim[1], A. Zhang[1], K. P. Pratt[1,2], E. M. Shevach[3], D. W. Scott[1]
[1]Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD, USA; [2]Puget Sound Blood Center Research Institute, Seattle, WA, USA; [3]Laboratory of Immunology, National Institute of Allergy & Infectious Diseases, National Institutes of Health, Bethesda, MD, USA.

Introduction/Background: Undesirable immune responses against human protein therapeutics can be a major impediment for successful therapy. A major example is the antibody response (inhibitors) against human factor VIII (FVIII) replacement therapy in hemophilia A patients. Novel efforts are necessary to overcome this problem: we propose that T regulatory cells (Tregs) can be used to prevent and reverse inhibitor formation. However, therapy with polyclonal Tregs is non-specific and a potential drawback is that it could be generally immunosuppressive. Therapy with antigen-specific Tregs would be highly preferable, as these have proved more potent in animal models of autoimmune diseases. We demonstrate herein the generation and functional properties of human T regulatory cells that specifically recognize FVIII.
Methods: Tregs were generated that expressed the same T cell receptor (TCR) as that found on an HLA-DRB1*01:01-restricted T cell clone isolated from a hemophilia A subject with an inhibitor. Nucleotide/amino acid sequences were identified to design retroviral FVIII-specific TCR constructs. To produce and test FVIII-specific human Foxp3+ Tregs, we transduced T effector cells and Tregs with this TCR; the transduced T cells recognize the same DR0101- restricted epitope as the original clone. Specific reactivity of the transduced cells bearing the designed TCR was validated by staining of the transduced T effector and Treg cells with DR0101 tetramers loaded with the same peptide as that recognized by the original effector T cell clone.
Results and Conclusions: Transduced effector T cells were expanded by stimulation with the same FVIII peptide *in vitro.* Importantly, activation by this peptide in the presence of oligonucleotides mediated functional expansion, stabilization of FoxP3 and demethylation status of transduced Tregs in culture. Finally, the expanded transduced Tregs suppressed effector cell proliferation and cytokine secretion when stimulated by FVIII or the FVIII peptide, even when FVIII-specific effector T cells were present in large excess.
(Supported by NIH grant RO1 HL061883-15 to DWS and unrestricted research funding from Bayer, Pfizer and CSL Behring to KPP, and the intramural program of the NIAID to EMS).

Corresponding Author
D. W. Scott, *david.scott@usuhs.edu*



PO.04.21 - 53
Figure 1. Clinical Application of Engineered Treg Therapy

PO.04.22 - 58 INFLUENCING FACTORS ON THE FUNCTIONAL LEVEL OF HAEMOPHILIC PATIENTS ASSESSED BY FISH

A. R. Kachooei[1,2], Z. Badiei[1], S. Razi[1], M. H. Ebrahimzadeh[1], R. Mahdavian-Naghashzargar[1], B. Shojaie[3], A. Moradi[1,2]
[1]Mashhad University of Medical Sciences, Mashhad, Iran; [2] Massachusetts General Hospital, Harvard Medical School, Boston, USA; [3]Klinikum Bremen Nord, Bremen, Germany.

Introduction: Joint destruction in early adulthood brings the patients to the orthopaedic clinics. If a haemophilic patient becomes disabled, it shows a number of factors such as timely diagnosis, availability of appropriate treatment depending on the country, access and affordability to treatments and equally importantly the responsibility of the patient in managing self care by remaining compliant by prescribed treatment regimen.
Methods: We assessed the functional level by functional independence score in haemophilia (FISH). Overall, 104 patients with haemophilia A and 29 with haemophilia B were evaluated. We assessed the function of the patients by FISH. We divided the sum scores into weak (FISH score 8–16), moderate (17–24), and good (25 32). For evaluating the level of functional deficit in a 2 * 2 table, we categorized the weak and moderate levels into Disordered Group and the good level into Not-Disordered Group.
Results: The average age was 26.9 +_14.24. Each 1 year increase in age can increase 1.07 fold the possibility of being placed in Disordered Function Group. Severe haemophilia can increase 7.34 fold, presence of inhibitor can increase 9.75 fold and home self-care increases 3.89 fold the possibility of being placed in Disordered Function Group.
Conclusion: To decrease the burden of the cost on patient, family and the government, education plays the most important role. We suggest that we send a trained team of physician and nurses to the deprived villages and cities instead of waiting for the patient to refer to our Care Center.

Corresponding Author
A. R. Kachooei, *akachooei@mgh.harvard.edu*

PO.04.23 - 59 VICIOUS CYCLE OF MULTIPLE INVASIVE TREATMENTS IN A HEMOPHILIC INHIBITOR POSITIVE CHILD WITH RESISTANT KNEE FLEXION CONTRACTURE. A CASE REPORT

A. R. Kachooei[1,2], Z. Badiei[1], B. Shojaie[3], A. Moradi[1,2]
[1]Mashhad University of Medical Sciences, Mashhad, Iran; [2] Massachusetts General Hospital, Harvard Medical School, Boston, USA; [3]Klinikum Bremen Nord, Bremen, Germany.

We are reporting an 8-year-old inhibitor-positive boy with hemophilia A affected by left knee flexion contracture after multiple hemarthrosis episodes in spite of recieving rFVII. He underwent knee arthroscopic release for two times and radionuclide synoviorthesis for one time. He also sustained a supracondylar fracture at the time of physiotherapy which we took the advantage to extend the knee. We could extend the knee under general anesthesia and there was no hamstring tightness. However, after all these efforts, he is still positioning his knee in flexion contracture.

1. Inhibitor patients need a kind of "Prophylaxis (rFVIIa and/or aPCCS)" to avoid recurrent bleeding. Sometimes we could not get it in our patient due to patient and parents irresponsibility in managing self-care, lack of access and affordability to treatment and unavailability of proper treatment.
2. This recurrence could have been avoided by the means of orthosis in extension since extension contracture could be better tolerated.
3. Forced knee extension physiotherapy manoeuvres on a boy with hemophilia, inhibitor and recurrent knee bleeds should not be carried out.
4. Perhaps we can hypothesize the boy may have been demonstrating pain-provoked muscle/joint protection spasms to the forced knee-extension manipulations during physiotherapy. Pain management may be a more efficient conservative treatment.
5. Surgery must always be the last resort. Radiosynovectomy might be worthwhile than surgery and we aim the ROM to reach full extension in addition to maximum possible flexion;
6. If surgery is necessary for failed conservative treatment, Arthroscopic synovectomy with "Immobilization Under Anesthesia" must be done to get full extension.
7. If supracondylar fracture occurs, we must take the advantage and fully extend the knee. It is better to walk on extended knee than crutches in flexion contracture.

Corresponding Author
A. R. Kachooei, *akachooei@mgh.harvard.edu*



PO.04.23 - 59
Figure 1

PO.04.24 - 100 IDENTIFICATION OF GALECTIN-1 AND GALECTIN-3 AS NOVEL BINDING PARTNERS FOR FACTOR VIII

J. M. O'Sullivan[(1)], O. Rawley[(1)], P. V. Jenkins[(1,2)], A. Chion[(1)], K. Gegenbauer[(1)], T. M. Brophy[(1)], J. S. O'Donnell[(1,2)]
[(1)]Haemostasis Research Group, Institute of Molecular Medicine, Trinity College Dublin, St James's Hospital, Dublin 8, Ireland; [(2)] National Centre for Hereditary Coagulation Disorders, St James's Hospital, Dublin 8, Ireland.

Background: Recent studies have demonstrated that galectin-1 and galectin-3 can bind Von Willebrand Factor (VWF) in plasma and influence VWF-dependent thrombosis formation. Interestingly, the glycan determinants expressed on human FVIII are similar in structure to those on VWF. Although FVIII glycans are important in regulating its biology, little is known regarding FVIII-lectin interactions. In this study, we report for the first time that galectins also directly interact with FVIII and modulate its activity.
Materials and Methods: Galectin-1/-3 were His-tagged and expressed in E-coli. Different purified commercial concentrates of recombinant FVIII (rFVIII) were utilised. FVIII glycosylation profiles were systematically modified using specific exoglycosidases. Galectin–FVIII interactions were characterised using immunosorbant assays and surface plasmon resonance (SPR).
Results: Galectin-1 and -3 bound to rFVIII in a dose-dependent manner with high affinity. Removal of rFVIII N-linked glycans significantly reduced galectin-1 and galectin-3 binding (8.6 ± 1%, 30.3 ± 3%, respectively). In addition, galectin-3 binding was further attenuated by combined removal of N- and O-glycans.
FVIII concentrates are manufactured in heterologous cell systems and are subsequently characterised by the presence of non-human glyco-epitopes. In keeping with these differential glycosylation profiles, both galectin-1/-3 displayed distinct binding affinities for various FVIII concentrates. The majority of galectin-3 binding is mediated by FVIII B-domain glycans. Interestingly, FVIII high mannose oligosaccharides, which have been previously shown to modulate FVIII immunogenicity *in vitro*, supported a significant proportion of galectin-1.
Galectin-1 was found to directly bind to and precipitate FVIII from plasma. Galectin-1 also served to negatively modulate FVIII functional activity as measured in one-stage clotting assays and reduce intrinsic FXa generation. Conversely, no effect on FVIII function was observed for galectin-3.
Conclusions: Galectin-1 and -3 are novel binding partners for FVIII, binding in a glycan-dependent manner. The ability of differentially glycosylated rFVIII concentrates to interact with these lectins *in vivo* may have implications for understanding the importance of FVIII glycosylation and how it influences function.

Corresponding Author
J. M. O'Sullivan, *josulli1@tcd.ie*

PO.04.25 - 131 ENHANCED FcγR-DEPENDENT UPTAKE OF BLOOD COAGULATION FACTOR VIII CONTAINING IMMUNE COMPLEXES BY ANTIGEN PRESENTING CELLS

R. B. Hartholt[(1)], N. Sorvillo[(1)], A. Wroblewska[(1)], E. Herzenik[(1)], A. ten Brinke[(2)], J. W. C. Claassens[(3)], J. S. Verbeek[(3)], J. Voorberg[(1)]
[(1)]Department of Plasma Proteins, Sanquin Research, Amsterdam, The Netherlands; [(2)]Department of Immunopathology, Sanquin Research, Amsterdam, The Netherlands; [(3)]Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands.

Background/Aims: A major complication in the treatment of hemophilia A is the development of inhibitory antibodies. Eradication of these antibodies can be established by immune tolerance induction (ITI) which consists of repetitive administration of high dosages of FVIII after which FVIII containing immune complexes can be formed. Thus far, the molecular mechanisms contributing to immune tolerance have not been defined.
Materials and Methods: Here we studied endocytosis of FVIII-containing immune complexes by bone marrow derived dendritic cells (BMDCs).
Results: BMDCs were able to efficiently take up FVIII pre-complexed with anti-FVIII antibodies (FVIII-IC) in a dose-dependent manner. Endocytosis of FVIII-IC was 3-6 fold more efficient when compared to equimolar concentrations of non-complexed FVIII. Moreover, enhanced endocytosis led to stronger FVIII-specific T cell proliferation. Uptake of FVIII-IC, but not FVIII alone, could be inhibited with 2.4G2 antibody indicating functional involvement of Fcγ receptors. These results were confirmed using murine DCs isolated from FcγR-deficient mice. The enhanced uptake of FVIII-IC was not observed in murine DCs derived from mice lacking all four FcγRs. Genetic ablation of FcγRI, FcγRII and FcγRIII separately did not affect the ability of anti-FVIII IgG to promote the uptake of FVIII.
Conclusions: FVIII immune complexes are more efficiently endocytosed by BMDCs compared to soluble FVIII, which is mediated via the Fcγ receptor family. The increase in uptake leads to increased FVIII-specific CD4+ T cell proliferation. These findings suggested that multiple FcγRs contribute to the enhanced uptake of FVIII-IC by BMDCs. Collectively, our data provide further insight in to the modulation of FVIII endocytosis by anti-FVIII antibodies.

Corresponding Author
R. B. Hartholt, *r.hartholt@sanquin.nl*

PO.05.01 - 66 INHIBITION OF COMPLEMENT-MEDIATED THROMBOTIC MICROANGIOPATHY WITH ECULIZUMAB IMPROVES HEMATOLOGIC AND RENAL OUTCOMES IN ADULT PATIENTS WITH AHUS

S. R. Cataland[1], C. M. Legendre[2], E. Minetti[3], M. Espinosa[4], J. Menne[5], F. Provot[6], E. Rondeau[7], P. Ruggenenti[8], M. Ogawa[9], F. Fakhouri[10]

[1]Department of Internal Medicine, Ohio State University, Columbus, Ohio, USA; [2]Department of Renal Transplantation, Hospital Necker and University Paris Descartes, Paris France; [3]Department of Nephrology and transplantation, Careggi University Hopital, Florence, Italy; [4]Department of Nephrology, Hospital Universitario Reina Sofia, Cordoba, Spain; [5]Department of Nephrology and Hypertension, Hannover Medical School, Hannover, Germany; [6]Department of Nephrology, University Hospital of Lille, Lille, France; [7]Department of Nephrology, Tenon Hospital, Paris, France; [8]Istituto di Ricerche Farmacologiche Mario Negri, Bergamo, Italy; [9]Clinical Development, Alexion Pharmaceuticals, Cheshire, UK; [10]Department Nephrology and Immunology, University Hospital Nantes, Nantes, France.

Background: Atypical hemolytic uremic syndrome (aHUS) is a rare and serious condition caused by genetic abnormalities in the complement system, leading to chronic, uncontrolled complement activation. This results in systemic thrombotic microangiopathy (TMA), ischemia and severe organ damage. Outcomes are poor; up to 65% of patients have permanent renal damage or die within 1 year of diagnosis despite plasma exchange/plasma infusion (PE/PI). We report on efficacy and safety of eculizumab, a terminal complement inhibitor, in patients with aHUS.

Methods: Open-label, prospective, multicenter, single-arm study in adults (≥18 years) with aHUS. Inclusion criteria included platelet count <150 × 109/L, lactate dehydrogenase (LDH) ≥1.5 × upper limit of normal (ULN) and serum creatinine ≥ULN at screening. Patients with Shiga toxin producing E. coli or ADAMTS-13 activity <5% were excluded, and identification of a complement mutation was not required for admission. Eculizumab was administered intravenously at 900 mg/week for 4 weeks, 1200 mg in Week 5 and every 2 weeks thereafter. The primary endpoint was proportion of patients with complete TMA response at 26 weeks (platelet and LDH normalization and <25% increase in serum creatinine from baseline).

Results: Baseline characteristics and efficacy outcomes at 26 weeks are shown in the table. Of the 24 patients on dialysis at baseline, 20 (83%) were able to stop dialysis. Of the 35 patients receiving PE/PI at baseline, 26 (74%) were able to completely discontinue PE/PI. Most AEs were mild or moderate. Two patients had meningococcal infections; both recovered and one continued with eculizumab.

Conclusion: In adult patients with aHUS, sustained treatment with eculizumab led to clinically meaningful improvements in hematologic and renal outcomes. There were no unexpected safety concerns. These results support the importance of early and accurate differential diagnosis and rapid initiation of eculizumab in adult patients with aHUS.

Corresponding Author
S. Cataland, *spero.cataland@osumc.edu*

TABLE: Baseline characteristics and efficacy outcomes through 26 weeks' treatment with eculizumab in patients with aHUS

Baseline demographics and disease characteristic (N=41)	
Mean (SD) age at first infusion, years	40.3 (15.3)
Female gender, n (%)	28 (68)
Identified complement regulatory protein mutation or autoantibody, n (%)	20 (49)
Median time from diagnosis until screening, Months (range)	0.8 (0.03-311)
Median duration of current clinical manifestation, months (range)	0.5 (0.0-19.2)
Mean (SD) platelet count (×10⁹/L)	119.1 (66)
Mean (SD) LDH level (U/L)	493 (501)
Mean (SD) eGFR (mL/min/1.73m²)	17.3 (12.1)
Mean (SD) serum creatinine level (µmol/L)	411 (265)
Dialysis, n (%)	24 (58)
Prior renal transplant, n (%)	9 (22)
Efficacy outcomes at 26 weeks (N=41)	
Complete TMA response, n (%) [95% CI]	30 (73) [57.1–85.8]
Hematologic normalization, n (%) [95% CI]	36 (88) [73.8–95.9]
Platelet count normalization, n (%) [95% CI]	40 (98) [87.1–99.9]
Mean (SD) increase in platelet count from baseline, × 10⁹/L	135 (114); p<0.0001
Mean (SD) increase in eGFR from baseline	29.3 (23.6); p<0.0001
eGFR increase ≥15 mL/min/1.73 m² from baseline, n (%)	22 (54)

SD, standard deviation; LDH, lactate dehydrogenase; eGFR, estimated glomerular filtration rate; CI, confidence interval; TMA, thrombotic microangiopathy

PO.05.01 - 66
Table 1

PO.05.02 - 50 COMPLEMENT REGULATORY COMPONENTS IN PATIENTS WITH ATYPICAL HEMOLYTIC UREMIC SYNDROME IN IRAN

S. Hosseini[1,2], A. Dorgalaleh[3], E. Kalantar[1], N. Hooman[4], S. Dorgaleleh[5], B. Taregh[6]

[1]Hematology and Flowcytometry Laboratory Ali Asghar Children Hospital, Iran University of Medical Sciences, Tehran, Iran; [2]Gholhak Clinical Laboratory Tehran Iran; [3]Department of Hematology, School of Allied Health Science, Iran University of Medical Sciences, Tehran, Iran; [4]Paediatric Nephrology Ward, Ali Asghar Children Hospital, Iran University of Medical Sciences, Tehran, Iran; [5]Department of Laboratory Sciences, Zabol University of Medical Sciences, Zabol, Iran; [6]Infection Diseases and Tropical Medicine Research Center, Zahedan University of Medical Sciences, Zahedan, Iran.

Background/Aim: Atypical hemolytic uremic syndrome (aHUS), a rare disorder characterized by thrombocytopenia, microangiopathic hemolytic anemia, and acute renal failure, is associated with mutations and polymorphisms in various components and regulators of the complement alternative pathway (AP) including factor H, factor I, membrane cofactor protein (MCP or CD46), factor B, this impaired regulation of the alternative pathway leads to a procoagulant state with micro thrombi formation in the renal vasculature which influences disease onset and progression. The aim of this study is to evaluate the role of complement regulatory factors in occurrence of aHUS we also included evaluation of ADAMTS-13 activity and autoantibody against ADAMTS-13 in our study in order to exclude thrombotic thrombocytopenic cases (TTP) which might have overlapping clinical and laboratory findings.

Methods: This study was conducted on 273 individuals with aHUS. Diagnosis was based on clinical manifestations, kidney function tests, red blood cell count, morphology and reticulocyte count. Then the ADAMTS-13 autoantibody and activity and also complement factor B, complement factor H(CFH) and complement factor-I(CIF) were analyzed. Finally the statistical analysis was performed by SPSS software.

Results: The mean age of our patients was 27.3 years, 55% were female and 45% were male. The mean of urea and creatinine concentration were 92.9 mg/dl and 5.1 mg/dl respectively. The mean level of RBC count, Hb and HCT in these patients were lower than normal but mean percentage of reticulocyte count was higher than normal (2.5%).The assessment of complement regulatory factors revealed that the B and H factors levels were normal except in two cases but the level of factor I was higher than normal.

Conclusion: According to the results of this study it seems that up regulation of factor I had a significant role in occurrence of aHUS in our study group.

Corresponding Author
A. Dorgalaleh, *dorgalaleh.1390@yahoo.com*

PO.05.03 - 163 EARLY VOLUME EXPANSION FOR MITIGATING SHIGATOXIN-ASSOCIATED HEMOLYTIC UREMIC SINDROME. DATA OF THE NORTH ITALIAN HUS NETWORK

G. Ardissino, F. Tel, S. Testa, S. Salardi, R. Colombo, I. Possenti, S. Tedeschi, E. Torresani

Center for HUS prevention, control and management, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy for the North Italian HUS Network.

Background: STEC-HUS is a severe acute illness for which there is no specific treatment. Among supportive therapy, the management of fluids has been traditionally concentrated on avoiding fluid overload because patients often present with oligo/anuric acute kidney injury. Our group has described that hemoconcentration at disease onset is associated with more servere disease. However it is still unclear if early volume expansion can improve disease's outcome.

Methods: Since May 2010, a Network connecting pediatric hospitals in Northern Italy (10 millions gp) was developed aimed at early diagnosis and early referral of STEC-HUS with the working hypothesis that prompt volume expansion with saline solution 0.9% may revert disease severity. All children with STEC-HUS referred to our Center from January 2012 were addressed to intravenous infusion aimed at inducing an overhydration (+10-15% of the working weight within the first 48 hrs). The outcome was compared with an equal and sequential group of historical patients (group A) referred to our Center during 2007-2009 when patients were usually restricted in fluid intake.

Results:

	Group A	Group B
N.	22	22
Gender (M/F)	11/11	9/13
Age (yrs)	4.9	4.8
sCreatinine at diagnosis (mg/dL)	3.6	1.7*
Peak sCreatinine (mg/dL)	5.6	2.9*
RRT (% of total)	64	18*
Days of hospitalization (mean)	14.1	9.4*

*: p<0.01 vs group A

Conclusions: Early diagnosis and early volume expansion of patients with STEC-HUS can substantially improve the outcome. It can be speculated that hypovolemia (due to diarrhea, reduced food intake and reduced onchotic pressure for endothelial leakage of plasma proteins), if uncorrected, favors trombi formation and hipoxic/ischemic tissue damage.

Acknowledgement: thanks to the members of the North Italian HUS Network whose complete list is available at www.centroseu.org. The project has been supported by the " PROGETTO ALICE ONLUS – Associazione per la lotta alla SEU"

Corresponding Author
G. Ardissino, *ardissino@italkid.org*

PO.05.04 - 89 THE ROLE OF ADAMTS-13 IN DVT: *IN VITRO* CHARACTERIZATION OF ADAMTS-13 SINGLE NUCLEOTIDE VARIANTS IDENTIFIED BY NEXT-GENERATION SEQUENCING IN A GROUP OF ITALIAN DVT PATIENTS

M. T. Pagliari[1], L. A. Lotta[1], C. Valsecchi[1], G. Casoli[1], P. Bucciarelli[1], I. Martinelli[1], F. R. Rosendaal[2], F. Peyvandi[1,3]

[1]Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center Milan, Italy; [2]Department of Clinical Epidemiology and Department of Thrombosis and Haemostasis, Leiden University Medical Center, Leiden, Netherlands; [3]Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy.

Background/Aim: The genetic predisposition to venous thrombosis is only partially understood. Rare(minor allele frequency below 1%) and low-frequency(minor allele frequency below 5%) SNVs of the coding area may be responsible for part of the missing heritability of DVT. We developed a tailored next-generation sequencing approach, used to sequence 186 haemostatic genes in 94 DVT patients and 98 controls(Lotta et al, BMC 2012;Lotta et al, JTH 2013). We found few rare variants of the ADAMTS-13 associated with DVT. We proposed to investigate the role of these 11 ADAMTS-13 SNVs in order to assess their functional impact and mechanisms of action.
Materials and Methods: pcDNA3.1ADAMTS-13-WT and mutant expression vectors were transiently transfected in HEK293 cells. The amount and the activity of WT and mutant recombinant ADAMTS-13 proteins were analyzed in the conditioned medium of 3 separate transfections by ELISA and FRET assays respectively. SIFT and PolyPhen algorithms were used to predict the likely damaging effects of these mutations.
Results: We expressed and characterized 6 of these mutations. ADAMTS-13 levels are reported as a percentage of the WT(±SEM). In comparison to WT(100±14.4%), protein secretion was reduced for p.V154I 22.7±2.0%, p.D187H 57.5±9.1% and p.R421C 21.8±3.1% whereas it was normal for p.R925G 146.8±13.6%, p.H1196Q 84.8±10.7% and p.T1249P 110±13.6%.
ADAMTS-13 activity was reduced for p.V154I 14.5±0.5% p.D187H 17.6±1.2% and p.R421C 22.4±1.0% and similar to WT(100±4.0%), for p.R925G 172.7±2.0%,p.H1196Q 89.3±1.7% and p.T1249P 71.3±1.3%.
For p.V154I, p.R421C and p.T1249P, both SIFT and PolyPhen algorithms, agreed on a likely damaging impact, whereas only PolyPhen predicted p.D187H as a damaging mutation.
Conclusions: Expression studies showed a reduction of ADAMTS-13 secretion and activity for p.V154I,p.D187H and p.R421C.These results corroborate the reduction of ADAMTS-13 activity at least for patients with mutations p.D187H and p.R421C. A further replication study, in a large group of DVT patients, is necessary to clarify the role of these variants.

Corresponding Author
M. T. Pagliari, *mtpagliari@gmail.com*

PO.05.05 - 90 ANTI-TAIL MONOCLONAL ANTIBODIES CHANGE THE CONFORMATION OF ADAMTS-13 INTO A HYPERACTIVE STATE

L. Deforche[1], E. Roose[1], A. Vandenbulcke[1], L. Mi[2], H. Rottensteiner[3], T. Springer[2], H. Deckmyn[1], S. De Meyer[1], K. Vanhoorelbeke[1]

[1]Laboratory for Thrombosis Research (LaTrOn), IRF Life Sciences, KU Leuven Kulak, Kortrijk, Belgium; [2]Program in Cellular and Molecular Medicine, Children's Hospital Boston, Harvard Medical School, Boston, MA, USA; [3]Baxter Innovations GmbH, Vienna, Austria.

Background: The multidomain protease ADAMTS-13 only digests its substrate von Willebrand factor (VWF) when VWF undergoes a conformational change. Specific interactions between exosites in the ADAMTS-13 head domains and exosites in the VWF A2 domain guarantee a correct positioning of ADAMTS-13 for VWF cleavage. How the tail domains of ADAMTS-13 contribute to this process, is however not well established.
Aim: Gaining insight into the roles of the tail domains of ADAMTS-13 by developing anti-tail monoclonal antibodies (mAbs).
Methods: Anti-ADAMTS-13 mAbs were generated by the immunization of Balb/C mice with recombinant ADAMTS-13 (rADAMTS-13). Antibodies were purified using protein G Sepharose and ADAMTS-13 mutants were used for epitope mapping. EM studies were performed on rADAMTS-13 in the presence and absence of anti-ADAMTS-13 mAbs. The functional effects of anti-ADAMTS-13 mAbs were tested using FRETS-VWF73 and using an in-house developed VWF binding assay. Changes in accessibility of mAb epitopes in rADAMTS-13 were evaluated using an immuno assay.
Results: A series of different structures of ADAMTS-13 going from folded to elongated forms were identified using EM. EM of rADAMTS-13 in the presence of an anti-head and an anti-tail mAb revealed a folded/closed structure of ADAMTS-13 where tail domains make contact with head domains. Next, epitope mapping of all 30 anti-ADAMTS-13 mAbs identified 24 mAbs with an epitope in the tail domains of ADAMTS-13. Interestingly, half of these anti-tail mAbs stimulated the activity of ADAMTS-13 as demonstrated in the FRETS assay (up to 223%) and 15 of them stimulated the binding of ADAMTS-13 to VWF (up to 373%). In addition, binding of the activating mAbs resulted in the exposure of cryptic epitopes in the head domains of ADAMTS-13.
Summary: ADAMTS-13 tail domains shield the head domains of the enzyme thereby dampening its proteolytic activity. Activating anti-tail mAbs abrogate the shielding effect of the tail domains.

Corresponding Author
L. Deforche, *louis.deforche@kuleuven-kulak.be*

PO.05.06 - 101 THE CHEMICAL CHAPERONE BETAINE LEADS TO INCREASED SECRETION OF AN ADAMTS-13 SECRETION DEFECT MUTANT

M. Underwood[1,2], F. Peyvandi[1,3,4], I. Garagiola[4], S. Machin[3], I. Mackie[1]

[1]Haemostasis Research Unit, University College London, London, England; [2]Department of Pathophysiology and Transplantation, University of Milan, Milan, Italy; [3]Haemostasis Research Unit, University College London, London, England; [4]Angelo Bianchi Bonomi Hemophilia and Thrombosis Centre, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.

Over 130 ADAMTS-13 mutations have been identified in the ADAMTS-13 (a disintegrin-like and metalloprotease with thrombospondin type 1 motif 13) gene in patients with congenital thrombotic thrombocytopenic purpura (TTP). The majority (86%) of these mutations lead to reduced (<50%) secretion *in vitro*. We studied a predicted ADAMTS-13 secretion defect mutant (p.I143T) identified in a patient presenting with TTP during adolescence and investigated whether its secretion could be increased using a chemical chaperone. The mutation was introduced by site directed mutagenesis into a pcDNA 3.1 vector expressing wild type (WT) ADAMTS-13. Mutant and WT ADAMTS-13 cDNA were expressed separately in HEK293T cells to assess secretion levels. Immunofluorescence staining and confocal microscopy were used to study localisation within the endoplasmic reticulum (ER) and cis Golgi. Cell proteasome and lysosomes were inhibited in cells stably expressing WT or mutant ADAMTS-13 to investigate whether this mutant was degraded by either of these organelles. Finally cells stably expressing WT or mutant ADAMTS-13 were incubated with 100mM of the chemical chaperone betaine to investigate whether it could aid the secretion of this mutant protein. After *in vitro* transient transfection no ADAMTS-13 antigen or activity was detectable in the cell supernatant for the mutant, in contrast to WT. The mutant localised within the ER but less extensively in the Golgi compared to WT. Inhibition of the cell proteasome led to increased intracellular levels of mutant, but not WT ADAMTS-13 suggesting degradation by this organelle. The chemical chaperone betaine increased the quantity of the mutant secreted (1.8 fold) with detectable ADAMTS-13 activity in the supernatant. These results may have clinical implications for the future treatment of some congenital TTP patients with similar mechanisms of protein secretion defect.

Corresponding Author
M. Underwood, *m.underwood@ucl.ac.uk*

PO.05.07 - 107 DENSE GENOTYPING OF IMMUNE-RELATED DISEASE REGIONS FOR THE IDENTIFICATION OF GENETIC RISK FACTORS IN ACQUIRED THROMBOTIC THROMBOCYTOPENIC PURPURA

I. Mancini[1], A. Cairo[1], M. M. Gorski[2], B. Ferrari[1], L. A. Lotta[1], I. Ricaño-Ponce[3], C. Wijmenga[3], F. Peyvandi[1,2]

[1]Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico and Luigi Villa Foundation, Milan, Italy; [2]Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy; [3] Genetics Department, University Medical Center and University of Groeningen, Groeningen, The Netherlands.

Background/Aims: Acquired thrombotic thrombocytopenic purpura (TTP) is associated with the development of autoantibodies against the VWF-cleaving protease ADAMTS-13. Evidences of a genetic contribution have been reported, including the association of the human leukocyte antigen (HLA) complex with disease risk. The aim of this study was to identify novel genetic risk factors by high-throughput genetic association studies.
Materials and Methods: A total of 195 Caucasian patients with acquired TTP were genotyped using the Illumina Immunochip, a genotyping array with dense marker coverage across 186 known disease loci from 12 immune-mediated diseases. Cases were selected using the following criteria: (a) diagnosis of TTP (episode of thrombocytopenia, microangiopathic hemolytic anemia without alternative causes); (b) presence of anti-ADAMTS-13 antibodies; (c) availability of DNA. Cases were compared with 1255 previously genotyped Italian controls using logistic regression models. Quality control and association analysis were carried out using PLINK.
Results: After quality control, 186 cases, 1,255 controls and 131,095 variants were available for association testing, with a total genotyping rate of 0.999934. Logistic regression analysis revealed eight statistically significant variants, of which the strongest variant rs6903608 (p-value=1.51x10-14) was identified within HLA locus, with a minor allele frequency (MAF) of 0.498. We also identified a rare variant, rs115265285 (MAF=0.006, p-value=3.20x10-5), positioned 3.5 kb into the 3'UTR of the DNMT3A gene. All identified variants presented strong effects and were associated with either elevated risk of disease, showing odds ratios ranging from 2.13 to 7.52, or protective effect, with odds ratios of 0.39 and 0.59.
Conclusions: The Immunochip genotyping study on patients with acquired autoimmune TTP has confirmed the association of the HLA class II complex with the elevated risk of developing a TTP episode. Furthermore, the identification of novel non-HLA risk factors, if validated, may provide new insights into the etiology of this rare disease.

Corresponding Author
I. Mancini, *ilaria.mancini@guest.unimi.it*

PO.05.08 - 119 THE NATURAL MUTATION ASP173GLY IN THE CATALYTIC SITE OF THE ADAMTS-13 GENE CAUSES A SEVERE UPSHAW-SCHÜLMAN SYNDROME: CLINICAL COURSE, BIOCHEMISTRY

S. Lancellotti[1], F. Peyvandi[2], A. Cairo[2], M. T. Pagliari[2], L. Cavallo[3], I. Lazzareschi[4], S. Mastrangelo[4], R. Oliva[3], R. De Cristofaro[5]

[1]Center for Haemorrhagic and Thrombotic Diseases, Department of Medical Sciences, Catholic University School of Medicine, "A. Gemelli" Hospital, Roma, Italy; [2]Angelo Bianchi Bonomi Hemophilia and Thrombosis Center and Fondazione Luigi Villa, IRCCS Maggiore Hospital, Mangiagalli, Regina Elena Foundation and University of Milan, Milan, Italy; [3]Department of Sciences and Technologies, University; [4]Institute of Pediatrics, Catholic University School of Medicine, Rome, Italy; [5]Haemorrhagic and Thrombotic Disease Service, Department of Medical Sciences, Catholic University School of Medicine, Rome, Italy.

Congenital thrombotic thrombocytopenic purpura (TTP), also referred to as Upshaw-Schülman syndrome, is a rare form of thrombotic microangiopathy, inherited as a dysfunction or severe deficiency of ADAMTS-13 (A Disintegrin And Metalloprotease with ThromboSpondin 1 repeats), a zinc-protease which physiologically is responsible for the proteolytic processing of VWF multimers. The disease is inherited with autosomal recessive mode and causes mutations in the ADAMTS-13 gene. The deficiency of ADAMTS-13 is associated with the presence in plasma of ultralarge VWF multimers, able to adhere and activate platelets in the microcirculation. More than fifty mutations localized throughout the entire sequence of the ADAMTS-13 gene were identified so far, although only a few were characterized by *in vitro* expression studies. A molecular investigation in a family of Romanian origin, living in Italy, was performed in this study. In two male sons an Asp to Gly homozygous mutation at position 173 was identified. This mutation caused a severe (<1%) deficiency of ADAMTS-13 activity and antigen level, associated with periodic thrombocythopenia. Both parents, being cousins, showed the same mutation in heterozygous form. Expression studies were carried out in mammalian cells with a complete biochemical characterization of this mutant enzyme together with a molecular dynamics study. The enzyme was expressed in HEK293 cells, but showed a severe decrease of secretion. Molecular dynamics simulations, performed for 60 ns, showed that in the D173G mutant the orientation between the catalytic and disintegrin-like domains is dramatically changed in the 40.46 ns snapshot as compared to the initial structure. Accordingly, the interface between the catalytic domain (residues 81-286) and the following ≈100 residues is decreased from 1090 to 741 Å^2, likely de-stabilizing the enzyme. We think that the obtained results may help to unravel the role of that site, involved in calcium binding, in the regulation of ADAMTS-13 conformation and secretion.

Corresponding Author
R. De Cristofaro, *rdecristofaro@rm.unicatt.it*

PO.05.09 - 122 CLINICAL RELEVANCE OF ADAMTS-13-SPECIFIC CIRCULATING IMMUNE COMPLEXES IN ACQUIRED THROMBOTIC THROMBOCYTOPENIC PURPURA

B. Ferrari[1], C. Valsecchi[1], S. Pontiggia[1], I. Mancini[1], L. A. Lotta[1], D. Mikovic[2], G. Meloni[3], F. Peyvandi[1,4]

[1]Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico and Luigi Villa Foundation, Milan, Italy; [2]Blood Transfusion Institute of Serbia, Belgrade, Serbia; [3]Dipartimento di Biotecnologie Cellulari ed Ematologia, Università "La Sapienza", Rome, Italy; [4]Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy.

Background/Aims: Acquired thrombotic thrombocytopenic purpura (TTP) is a rare thrombotic microangiopathy due to the development of autoantibodies against the VWF-cleaving protease ADAMTS-13. We recently developed and validated a new ELISA method for the detection of ADAMTS-13-specific circulating immune complexes (CICs) in acquired TTP patients. This study aims at investigating the clinical relevance of ADAMTS-13-specific CICs at disease presentation in a large cohort of acquired TTP patients.

Materials and Methods: We measured ADAMTS-13-specific CICs by ELISA in 51 patients from the Milan TTP Registry, at the first episode of acquired TTP. All patients presented anti-ADAMTS-13 autoantibodies by western blotting and severe ADAMTS-13 deficiency (i.e., <10% of normal) by FRETS-VWF73 or CBA assays. We studied the associations between ADAMTS-13-specific CICs levels and (i) ADAMTS-13-related measurements (i.e., ADAMTS-13 antigen, anti-ADAMTS-13 IgG), (ii) clinical and laboratory markers of disease severity (i.e., muco-cutaneous bleeding, neurological, renal and cardiovascular symptoms, number of platelets, LDH, haemoglobin, creatinine), (iii) short- and long-term clinical outcomes (i.e., number of plasma exchange procedures required to attain remission, recurrence). Statistical analyses were performed using linear, logistic and Cox regression models.

Results: The prevalence of ADAMTS-13-specific CICs in patients experiencing a first episode of acquired TTP was 39% (95% confidence intervals [CI]: 26-52%). ADAMTS-13-specific CICs were only associated with ADAMTS-13 antigen levels at regression analyses (beta, 95%CI: 3.2, 1.1-5.2). The presence of ADAMTS-13-specific CICs was associated with almost a four-fold increase in the risk of recurrence at 2 years after the first TTP episode (hazard ratio, 95%CI: 3.7, 1.3-11.0).

Conclusions: ADAMTS-13-specific CICs are not a biomarker of disease severity, nor a predictor of clinical outcome during acute phase. Conversely, ADAMTS-13-specific CICs seem to have a relevance in predicting the recurrence of acute TTP episodes.

Corresponding Author
B. Ferrari, *barbara.ferrari@policlinico.mi.it*

PO.05.10 - 135 ENDOCYTOTIC MECHANISMS CONTRIBUTING TO THE INTERNALIZATION OF ADAMTS-13 BY MACROPHAGES

F. Verbij[(1)], N. Sorvillo[(1)], P. Kaijen[(1)], A. ten Brinke[(2)], R. Fijnheer[(3)], J. Voorberg[(3)]

[(1)]Departments of Plasma Proteins and Immunopathology, Sanquin-AMC Landsteiner Laboratory, Amsterdam, the Netherlands; [(2)] Department of Immunopathology, Sanquin-AMC Landsteiner Laboratory, Amsterdam, The Netherlands; [(3)]Department of Haematology, University Medical Center Utrecht, Utrecht, the Netherlands.

Background/Aim: Acquired thrombotic thrombocytopenic purpura is a severe disorder characterized by the production of autoantibodies directed against ADAMTS-13, a metalloproteinase that regulates platelet adhesion and aggregation through cleavage of ultra-large von Willebrand factor multimers. At present the cause of antibody formation is unknown. We have previously shown that ADAMTS-13 is efficiently internalized and presented on MHC class II by dendritic cells, suggesting a possible role of CD4+ T-cells in the initiation of the autoimmune reactivity towards ADAMTS-13. Internalization of ADAMTS-13 by macrophages instead may contribute to its clearance from the circulation. Here, we investigated endocytic mechanisms contributing to the uptake of ADAMTS-13 by macrophages.
Methods: Human monocyte-derived macrophages were used to monitor the uptake of fluorescently labelled ADAMTS-13 by flow cytometry and confocal microscopy. To elucidate the mechanism of endocytosis of ADAMTS-13 macrophages were pre-incubated with mannan, D-mannose, GlcNac, D-galactose or with monoclonal antibody directed against the macrophage mannose receptor (MR). In addition, uptake was also analyzed after pre-incubation of the cells with dextran sulphate, heparin, fucoidan, polyinosinic acid and polycytidylic acid.
Results: A time and concentration dependent endocytosis of ADAMTS-13 was observed when MDMs were incubated with ADAMTS-13. Confocal microscopy studies revealed partial colocalization of ADAMTS-13 with early endosomes. Internalization of ADAMTS-13 was partially blocked upon addition of mannan and EDTA suggesting a possible role of C-type lectin receptors (CLRs). However, uptake of ADAMTS-13 by MDMs was not affected by a blocking antibody directed towards the MR. Interestingly, inhibition of ADAMTS-13 endocytosis was observed upon incubation with polyanionic ligands suggesting a role for class A scavenger receptors.
Conclusion: Our data suggest that internalization of ADAMTS-13 by macrophages proceeds via a mechanism that is dissimilar from that previously defined in dendritic cells. We speculate that uptake by macrophages promotes the clearance of ADAMTS-13 from the circulation.

Corresponding Author
F. Verbij, *f.verbij@sanquin.nl*

PO.05.11 - 141 DEMONSTRATION OF DISULFIDE BOND FORMATION-MEDIATED INTERACTION OF ADAMTS-13 WITH VWF UNDER SHEAR STRESS

H. Rottensteiner, S. Skalicky, B. Plaimauer, F. Scheiflinger

Biologics R&D, Baxter Innovations GmbH, Vienna, Austria.

Background/Aims: ADAMTS-13 is the key regulator of VWF activity. In addition to its established proteolytic function involving the metalloprotease domain and adjacent regions up to the spacer domain, a free thiol-dependent interaction of the enzyme's C-terminal part with VWF is hypothesized to regulate the lateral association of VWF multimers under shear stress. Since this latter activity has been associated with the overall antithrombotic function of ADAMTS-13, we attempted to demonstrate such an interaction *in vitro* using purified recombinant (r) components.
Materials and Methods: Recombinant VWF, full-length rADAMTS-13, rMDTCS, a C-terminally truncated version of ADAMTS-13, and rADAMTS-13 C1275S, a mutant described to partially block the thiol-mediated interaction with VWF, were purified from CHO cells. Full-length rADAMTS-13 and variants were incubated with rVWF at room temperature (RT), 37°C and under shear stress. As control, mixtures were incubated in the presence of N-ethylmaleimide. Covalent interaction between ADAMTS-13 and VWF was assayed by agarose gel electrophoresis under non-reducing conditions and immunoblot analysis using VWF- and ADAMTS-13-specific antibodies.
Results: Full-length rADAMTS-13 was shown to time- and concentration-dependently interact with rVWF. The interaction was very weak at RT, but clearly discernible at 37°C, and most pronounced under shear stress. Interaction also occurred in the presence of EDTA, where rVWF is not cleaved by ADAMTS-13. No or only very weak binding was noted for the MDTCS fragment under all conditions tested, whereas binding of the ADAMTS-13 C1275S variant resembled that of the wild-type protein. Addition of NEM prevented the interaction of ADAMTS-13 with VWF.
Conclusions: We demonstrate covalent binding of the C-terminal part of ADAMTS-13 to VWF especially under shear stress. Although the cysteine residues involved remain to be identified, our data support the hypothesis of thiol-dependent regulation of VWF activity by ADAMTS-13.

Corresponding Author
H. Rottensteiner, *hanspeter_rottensteiner@baxter.com*

PO.05.12 - 136 RESTRICTED HLA-DRB1*11 DEPENDENT PEPTIDE PRESENTATION OF ADAMTS-13 BY ANTIGEN PRESENTING CELLS PROVIDES NOVEL INSIGHT INTO THE ETIOLOGY OF ACQUIRED TTP

N. Sorvillo[(1)], F. Verbij[(1)], P. Kaijen[(1)], A. Meijer[(1,2)], R. Fijnheer[(3)], J. Voorberg[(1,4)]

[(1)]Department of Plasma Proteins, Sanquin-AMC Landsteiner Laboratory, Amsterdam, The Netherlands; [(2)]Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands; [(3)]Department of Haematology, University Medical Centre Utrecht, Utrecht, The Netherlands; [(4)]Department of Vascular Medicine, University of Amsterdam, Amsterdam, The Netherlands.

Background/Aim: The mechanisms involved in the onset of the autoimmune response to ADAMTS-13 in TTP patients is unknown. Upon endocytosis ADAMTS-13 is loaded on MHC class II and presented on the cell surface of human dendritic cells. Peptides derived from the CUB2 domain of ADAMTS-13 are presented with higher efficiency compared to peptides derived from other domains, indicating that this domain contains a number of potential immunodominant T-cell epitopes. Interestingly, HLA-DRB1*11 positive donors, previously identified as a risk factor for the development of acquired TTP, present only a single CUB2 domain derived peptide: FINVAPHAR, suggesting its role as a potential immunodominant peptide capable to induce expansion and activation of relevant self reactive T-cells. Activation of T-cells not only requires interaction between peptide-MHC complex and the T-cell receptor but also requires co-stimulatory signals and cytokine stimulation. Several case reports suggest a role for viral or bacterial infections in the etiology of acquired TTP. As yet no single pathogen has been linked with the onset of TTP.

Material and Methods: In silico analysis of the identified ADAMTS-13 CUB2 derived peptide was performed in order to identify homologues microbial peptides (NCBI BLAST program; http://blast.ncbi.nlm.nih.gov/Blast.cgi). Subsequently HLA-DRB1*11 affinity predictions were determined using NetMHCIIpan Server 1.2 and the IEDB analysis resource consensus tool.

Results and Conclusions: The FINVAPHAR peptide revealed considerable homology with peptide sequences derived from several bacterial antigens. Strong binding peptides as predicted by the NetMHCIIpan Server 1.2 derived from Burkholderia pseudomallei and Leishmania donovani. Using IEDB analysis resource consensus tool a peptide from Shewanella sp. was also predicted as a high affinity binder for HLA-DRB1*11. Although further studies are needed, identification of such homologues peptides strengthens the possible role of molecular mimicry in the onset of acquired TTP.

Corresponding Author
N. Sorvillo, *n.sorvillo@sanquin.nl*

PO.05.13 - 142 RECOMBINANT HUMAN ADAMTS-13 (BAX 930) IN HEREDITARY TTP: CHARACTERIZATION OF PRODUCT AND PHASE I STUDY DESIGN

H. Rottensteiner[(1)], B. Plaimauer[(1)], A. Foettinger-Vacha[(2)], M. Kaliwoda[(2)], B. Ewenstein[(3)], F. Scheiflinger[(1)]

[(1)]Biologics R&D, Baxter Innovations GmbH, Vienna, Austria; [(2)] Analytical Science, Baxter Innovations GmbH, Vienna, Austria; [(3)] Clinical Strategy Hemo, Baxter Healthcare, Westlake Village, USA.

Background/Aims: Thrombotic thrombocytopenic purpura is an acquired or hereditary rare disease linked to reduced or missing activity of the metalloprotease ADAMTS-13 in plasma. Patients are treated with frequent plasma exchange using fresh frozen plasma; however, replacement therapy with a recombinant ADAMTS-13 would be more convenient. Baxter has developed a recombinant human ADAMTS-13 (BAX 930) in a CHO expression cell line using a manufacturing process with two dedicated virus inactivation and reduction steps. Here we characterize the clinical material and outline the planned clinical phase I study design.

Materials and Methods: Various physicochemical assays were employed to analyze the structure and post-translational modifications of rADAMTS-13. Functional assays were used to determine the activity of rADAMTS-13 under static conditions (FRETS-VWF73 and CBA assay) and under flow. The latter assay which was established in house using the CellixTM platform measures the extent of platelet aggregate formation upon perfusion of a suspension containing platelets, erythrocytes, and VWF over a surface coated with extracellular matrix components.

Results: Thorough assessment of several clinical BDS and FDP lots revealed their comparability in terms of purity, glycosylation pattern and higher-order structure. Specific catalytic activities measured under static conditions and under flow were similar between lots and comparable to preclinical lots and plasma-derived ADAMTS-13. This material will be used in the upcoming clinical phase I study designed to explore the safety and pharmacokinetics of rADAMTS-13.

Conclusion: The availability of high quality GMP-grade rADAMTS-13 allows a clinical trial to be conducted in patients with hereditary TTP, a disease with a high unmet need for a safer and more efficient therapy. In a first step, the product's safety and pharmacokinetics will be tested in an imminent phase I trial.

Corresponding Author
H. Rottensteiner, *hanspeter_rottensteiner@baxter.com*

PO.05.14 - 157 VISUALIZATION OF VWF CLEAVAGE BY ADAMTS-13 ON A SINGLE MOLECULE LEVEL

H. Rottensteiner[1], K. Bonazza[2], G. Allmaier[2], F. Scheiflinger[1], G. Friedbacher[2], P. L. Turecek[1]

[1]Baxter Innovations GmbH, Vienna, Austria; [2]Vienna University of Technology, Vienna, Austria.

Background/Aims: The plasma metalloprotease ADAMTS-13 is the key regulator of VWF activity as it predominantly cleaves the hemostatically most active ultra-large multimers of VWF. Cleavage of VWF occurs at a single site within the A2 domain, but only upon exposure to shear stress. Here we aimed to visualize the cleavage reaction of multimeric full-length VWF at the single molecule level by comparing the interaction of recombinant (r)VWF and rADAMTS-13 under static conditions and under flow using atomic force microscopy (AFM).
Materials and Methods: Identical VWF molecules before and after reaction with ADAMTS-13 were monitored. The resulting morphological changes were assessed by visual inspection of the micrographs. Stretched VWF was obtained by treating the mica sheet with VWF solution in a custom-made microfluidic device. A binding event was determined to have occurred when the height and size of a VWF globular domain had measurably increased. Cleavage of VWF was easily discernible by the appearance of disrupted VWF chains.
Results: Complex formation between rADAMTS-13 and rVWF under static conditions was discernible by the appearance of several bright spots within a multimeric VWF chain, indicating that several VWF monomers can simultaneously bind ADAMTS-13. No signs of VWF cleavage could be detected. When VWF was maximally stretched by high shear force VWF was rapidly cleaved upon incubation with ADAMTS-13. By reducing the shear force and incubation time, partial cleavage could be demonstrated, with VWF multimeric strings being decomposed into several shorter VWF fragments.
Conclusion: For the first time our approach using AFM allowed visualization of VWF interaction with and cleavage by ADAMTS-13. We demonstrate at the single molecule level binding of ADAMTS-13 to VWF under static conditions and under shear stress. Susceptibility to VWF cleavage was noted already at medium shear conditions where the overall elongation of VWF was not significant. Proteolysis was further enhanced when VWF was maximally elongated.

Corresponding Author
P. L. Turecek, *peter_turecek@baxter.com*

PO.05.15 - 143 PLATELET-ASSOCIATED OXIDATIVE STRESS AND ADAMTS-13 LEVELS ARE INVERSELY ASSOCIATED WITH SURVIVAL IN SEPTIC SHOCK

S. Lancellotti[1], L. Montini[2], M. Pennisi[2], M. Antonelli[2], E. De Candia[3], R. De Cristofaro[3]

[1]Center for Haemorrhagic and Thrombotic Diseases, Department of Medical Sciences, Catholic University School of Medicine, "A. Gemelli" Hospital, Roma, Italy; [2]Department of Anesthesiology and Intensive Care, Catholic University of the Sacred Heart, Agostino Gemelli Hospital, 00168 Rome, Italy; [3]Haemostasis and Thrombosis Center, Catholic University School of Medicine, Rome, Italy.

Introduction: Sepsis causes microvascular thrombosis, responsible for multiple organ failure. Some hemostatic factors mediate the mechanisms involved in sepsis-related organ ischemia. Oxidative stress is also increased in sepsis and reactive oxygen species (ROS) favor secretion of von Willebrand factor (VWF) multimers from endothelium and inhibit the proteolysis of VWF by ADAMTS-13. Moreover, the enzyme indoleamine-2,3-dioxygenase, an important immune regulator, is activated in sepsis and, through generation of kynurenins, promotes antioxidative and anti-infective activities. This study evaluated the relative role of ADAMTS-13 and VWF in the morbidity and mortality of patients with septic shock (SS).
Methods: ADAMTS-13, VWF, kynurenin, plasma protein carbonyls, marker of OS, together with biochemical, hematologic and hemodynamic parameters were measured on days 1 to 4, 7, 14 and 21 in twelve patients with SS, defined using standard criteria, enrolled in the ICU of the 'A. Gemelli' Hospital (Rome,Italy). Twelve age- and gender-matched healthy subjects was used as controls.
Results: Low ADAMTS-13 activity and antigen level was observed in SS patients (268 ± 123 ng/ml vs. 760 ± 80 ng/ml in controls). ADAMTS-13 significantly and inversely correlated with renal function (P=0.016) and the SOFA index (P=0.005). VWF levels (antigen and activity) were increased ~3-fold compared with controls. Likewise, plasma protein carbonyls, a biomarker of OS, and kynurenine were globally increased in patients (2.1 ± 1.5 nmol/mg vs. 0.3 ± 02 nmol/mg and 14.4 ± 9.7 μM vs. 2.3 ± 1.3 μM, respectively). Platelet count and carbonyls were positively associated (P<0.001). Intra-ICU mortality (25%) was inversely correlated with carbonyl levels (P = 0.04) and platelets (P = 0.022).
Conclusion: Based on these findings, we hypothesize that in the SS setting, platelets contribute to OS that counteracts the sepsis-associated mortality. A low platelet count, irrespective of bleedings, may favor mortality in SS patients by generating lower ROS amounts

Corresponding Author
R. De Cristofaro, *rdecristofaro@rm.unicatt.it*

PO.05.16 - 61 RITUXIMAB AS PRE-EMPTIVE THERAPY IN PATIENTS WITH THROMBOTIC THROMBOCYTOPENIC PURPURA AND ANTI-ADAMTS-13 ANTIBODIES: EFFECTIVENESS OF A SINGLE INFUSION

E. Bresin[1], M. Galbusera[2], S. Gastoldi[2], S. Gamba[1], E. Daina[1], M. Alberti[1], E. Sabadini[3], G. Garozzo[4], R. Paolini[5], R. Marcenò[6], G. Remuzzi[2,3]

[1]IRCCS Istituto di Ricerche Farmacologiche Mario Negri, Clinical Research Center for Rare Diseases Aldo e Cele Daccò, Ranica, Bergamo, Italy; [2]IRCCS Istituto di Ricerche Farmacologiche Mario Negri 'Centro Anna Maria Astori' Science and Technology Park Kilometro Rosso, Bergamo, Italy; [3]Unit of Nephrology and Dialysis, Azienda Ospedaliera Papa Giovanni XXIII, Bergamo, Italy; [4]Unit of Immunohematology and Transfusion Medicine, Hospital "Civile M.P. Arezzo", Ragusa Italy; [5]Unit of Oncology, Azienda Ospedaliera of Rovigo, Italy; [6]Unit of Transfusion Medicine.

Background and Aims: Thrombotic thrombocytopenic purpura (TTP) is a severe clinical condition characterized by thrombocytopenia, microangiopathic hemolytic anemia, central nervous system involvement and renal impairment. In the majority of patients deficiency of ADAMTS-13, the von Willebrand factor-cleaving protease, is associated with autoantibodies to ADAMTS-13. These cases take longer to treat and are more likely to relapse because of anti-ADAMTS-13 autoantibodies.
We previously demonstrated that rituximab, a humanized anti-CD20 monoclonal antibody, used pre-emptively at the standard protocol of four-doses in patients with anti-ADAMTS-13 in remission may be effective to prevent relapses of TTP. The aim of this study was to verify if even a single pre-emptive infusion of rituximab induced disappearance of ADAMTS-13 inhibitors from the circulation and maintained a disease-free condition comparably with the standard four-infusions.

Materials and Methods: Ten patients, from the International Registry of HUS/TTP, with anti-ADAMTS-13 autoantibodies and severely reduced/undetectable ADAMTS-13 activity during remission phases of TTP, were treated with rituximab as pre-emptive therapy (Table). Six patients received the standard four-infusions, whereas three received one infusion of rituximab on the basis of CD20 full-depletion. Another patient (***case 1***) received rituximab pre-emptively five times: firstly four infusions, then a single one.

Results and Conclusions: ADAMTS-13 activity ranging from 30% to 105% with disappearance of inhibitors was achieved after 1/3 months in all but one patient (***case 6***), and persisted >40% without inhibitors at 5 months of follow-up. In nine patients disease-free status is still ongoing after a period of time ranging among 5 months and 7.5 years. A relapse was documented in one patient during follow-up (***case 2***), 4 years after prophylaxis with rituximab. Results demonstrate that a single rituximab infusion used as pre-emptive treatment may be as effective as the four-doses standard protocol in maintaining a sustained remission in patients with anti-ADAMTS-13 antibodies.

Corresponding Author
E. Bresin, *elena.bresin@marionegri.it*

Case No	Sex	Age at TTP onset (years)	Disease Duration *(years)	No. of TTP Episodes (before Rituximab prophylaxis)	Rituximab prophylaxis (n. of treatments)	Rituximab prophylaxis (n. of infusions for each treatment)	Length of disease-free/follow-up after Rituximab prophylaxis	ADAMTS-13 activity/inhibitors (at last follow-up)
1	F	32	24	10	5	4,1,1,1,1	7,5 yrs	47%, no
2	M	37	12	3	1	4	4 yrs	114%, no
3	F	20	9	4	1	4	20 mo	55%, no
4	F	41	1,5	2	1	4	5 mo	41%, no
5	F	35	1	2	1	4	7 mo	91%, no
6	F	24	2	2	1	4	18 mo	< 6%, ++
7	F	36	1,5	1	1	4	18 mo	100%, no
8	F	51	3	2	1	1	16 mo	91%, no
9	F	61	4	1	1	1	3 yrs	80%, no
10	F	50	2	1	1	1	2 yrs	60%, no

*** from the onset of the disease until the last follow-up**

PO.05.16 - 61
Table 1. Characteristics of TTP patients treated with rituximab as pre-emptive treatment

PO.05.17 - 140 A NOVEL FLOW-BASED ASSAY REVEALS DISCREPANCIES IN INHIBITOR ASSESSMENT OF TTP PATIENTS COMPARED WITH A CONVENTIONAL CLINICAL STATIC ASSAY

R. Grillberger[(1)], B. Gruber[(1)], S. Skalicky[(1)], G. Schrenk[(1)], P. Knöbl[(2)], B. Plaimauer[(1)], P. L. Turecek[(1)], F. Scheiflinger[(1)], H. Rottensteiner[(1)]

[(1)]Biologics R&D, Baxter Innovations GmbH, Vienna, Austria; [(2)] Department of Medicine, Medical University of Vienna, Vienna, Austria.

Background/Aims: Several static Bethesda-type assays are routinely used to determine ADAMTS-13 neutralizing autoantibodies in acquired thrombotic thrombocytopenic purpura (TTP); however, the inhibitory activity of these antibodies has not been thoroughly evaluated under the more physiological condition of flow. We addressed whether ADAMTS-13 inhibitor assessment by FRETS-VWF73 assay is predictive for results obtained under flow.
Materials and Methods: Anti-ADAMTS-13 autoantibodies were purified from acquired TTP patients by chromatography involving an ADAMTS-13 affinity matrix and/or protein G. ADAMTS-13 activity was measured using FRETS-VWF73 assay and a novel flow assay that determines the ADAMTS-13-mediated decrease in platelet aggregate surface coverage caused by perfusion of a suspension containing platelets, erythrocytes and VWF over a surface coated with extracellular matrix components. Neutralizing activities of ADAMTS-13 inhibitors were compared under static conditions and under flow using these two assays.
Results: The suitability of the flow-based ADAMTS-13 activity assay for quantitation of ADAMTS-13 inhibitors was demonstrated by the reversibility of the ADAMTS-13-dependent decrease in surface coverage upon addition of goat ADAMTS-13 antiserum. Testing the neutralizing activity of purified autoantibodies from six patients according to their FRETS-VWF73-based inhibitor titers in the flow assay gave rise to vastly different inhibitory effects, indicating a discrepancy in inhibitor assessment between static and flow conditions.
Conclusions: Anti-ADAMTS-13 autoantibodies may exhibit inhibitory properties *in vivo* that are inconsistent with the ADAMTS-13 inhibitor levels determined in routine static assays, possibly because certain epitopes are selectively exposed under shear. Consequently, the course of disease and treatment efficacy may vary among TTP patients despite common inhibitor titers.

Corresponding Author
H. Rottensteiner, *hanspeter_rottensteiner@baxter.com*

PO.05.18 - 146 DEVELOPMENT OF A RAT INHIBITOR MODEL TO DEMONSTRATE FEASIBILITY OF RECOMBINANT ADAMTS-13 THERAPY FOR ACQUIRED TTP

C. Tersteeg[(1)], A. Schiviz[(2)], B. Plaimauer[(3)], S. De Meyer[(1)], H. Rottensteiner[(3)], K. Vanhoorelbeke[(1)], F. Scheiflinger[(3)]

[(1)]Laboratory for Thrombosis Research, IRF Life Sciences, KU Leuven Kulak, Kortrijk, Belgium; [(2)]Department of Preclinical Pharmacology & Toxicology, Baxter Innovations GmbH, Vienna, Austria; [(3)]Biologics R&D, Baxter Innovations GmbH, Vienna, Austria.

Background/Aims: Von Willebrand factor (VWF) multimer size is regulated via proteolysis by a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS-13). A deficiency in ADAMTS-13 activity is associated with the pathogenesis of thrombotic thrombocytopenic purpura (TTP), a life-threatening condition in which episodes of thrombotic microangiopathy damage the kidneys, heart and brain. Most TTP patients suffer from the acquired form, where circulating anti-ADAMTS-13 antibodies are responsible for the decreased ADAMTS-13 activity. Current treatment consists of daily plasma exchange, but improved therapies are highly warranted. We have developed a new rat model for acquired TTP involving circulating inhibitory antibodies against ADAMTS-13 to investigate the therapeutic efficacy of recombinant (r) ADAMTS-13.
Materials and Methods: Rats (Sprague-Dawley) were injected with goat anti-ADAMTS-13 IgG to inhibit ADAMTS-13 activity. rADAMTS-13 was injected at different doses and ADAMTS-13 activity and immune complex formation determined over time. Rats injected with anti-ADAMTS-13 antibodies were challenged with 2000 U/kg human rVWF to trigger TTP symptoms; blood and organs were collected to determine the clinical pathology.
Results: Injection of anti-ADAMTS-13 antibodies completely blocked ADAMTS-13 activity. Upon administration of rADAMTS-13, immune complexes were formed with the circulating antibodies, resulting in immediate neutralization. Nevertheless, a dose-dependent increase in ADAMTS-13 activity was observed. When TTP was triggered using human rVWF, the animals displayed thrombocytopenia, hemolytic anemia and the presence of VWF-rich thrombi in the kidneys, heart and brain. Ongoing studies will evaluate the effect of rADAMTS-13 in treating acquired TTP.
Conclusions: We have established a small laboratory animal model for acquired TTP and demonstrated that rADAMTS-13 therapy is able to override circulating anti-ADAMTS-13 inhibitory antibodies. Future studies will demonstrate the feasibility of rADAMTS-13 as a therapy for acquired TTP.

Corresponding Author
H. Rottensteiner, *hanspeter_rottensteiner@baxter.com*

PO.05.22 - 165 ACQUIRED HEMOPHILIA A AND SEVERE SYMPTOMATIC THROMBOCYTOPENIA IN TWO PATIENTS WITH NON-VALVULAR ATRIAL FIBRILLATION TREATED WITH RIVAROXABAN AND DABIGATRAN ETEXILATE RESPECTIVELY

N. Ciavarella[(1)], G. Lucarelli[(2)], F. Mongelli[(2)], A. Coluccia[(3)], M. Schiavoni[(3)]

[(1)]Coordinamento Tavolo Tecnico Trombosi, Agenzia Regionale Sanitaria Puglia (AReS), Italy; [(2)]Centro Sorveglianza Terapia Anticoagulante, U.O.C. Patologia Clinica e Microbiologia Ospedale "Miulli", Acquaviva delle Fonti, Italy; [(3)]Centro Emofilia e Coagulopatie Rare, P.O. "I. Veris delli Ponti", Scorrano-Italy.

We report the severe complications, never described before, occurred in 2 patients out of 184 individuals under NOACs treatment for NVAF experienced respectively over a period of one year by every 3-month follow-up in two regional centres on anticoagulant therapy surveillance in south of Italy. ***Case 1***: a 67-year-old male with a history of myocardial infarction occurred in 2012 and complicated by rising of NVAF was eligible for switching from warfarin to rivaroxaban at the dosage of 15 mg od because of creatinine clearance value of 35 ml/min as measured by Cockroft-Gault formula. Concomitant medication consisted in amyodaron, furosemide, carvedilol and ramipril taken since two years for myocardial infarction and hypertension. After 10 months of treatment with rivaroxaban the patient showed a large, apparently spontaneous, subcutaneous hematoma on his right arm. A prolonged PTT-R (2.96) and a very low level of clotting factor VIII (<1%) associated with the presence of an anti-factor VIII (F.VIII) inhibitor (38 B.U.) were found, so that on the basis of clinical and laboratory pictures the diagnosis of "acquired hemophilia A" was made. Immunosuppressive treatment with prednisone (1 mg/Kg b.w. od) plus oral cyclophosphamide (200 mg od) was started. After one-week treatment the level of F.VIII raised up to 3% while title of inhibitor went down to 19 B.U. respectively. Therapy is still ongoing and patient undergoes a close one-month follow-up in order to observe the clinical outcome. ***Case 2***: a 79-year-old man, under surveillance of anticoagulant therapy with warfarin, affected by chronic NVAF and hypertension, was selected for switching to NOAC, because of instable anticoagulation (TTR <45%). 23 days after the switch, the patient presented a mild epistaxis from the left nostril, that needed only anterior packing and temporary one dose dabigatran discontinuation. However, one week after packing remotion a severe recurrence of nasal bleeding occurred, so that the patient needed hospital admittance. Laboratory control showed a severe thrombocytopenia (8,000/ µ/L) associated with a prolonged PTT-R (2.00) and a slight hypofibrinogenemia (110 mg/ml). Moreover endoscopy evidenced a varicose vessel of nasal mucosa. Dabigatran was discontinued and i.v. continuous infusion of tranexamic acid was given (10 mg/Kg b.w. every 12 hours) until platelets count was above 30,000 µ/L. 120 hours after dabigatran discontinuation a slow but progressive recovery of thrombocytopenia together with the other hemostatic parameters was observed. Patient was discharged with higher platelets count (74,000 µ/L), normal value of PTT-R (1.0) and fibrinogen (325 mg/ml). 2-month-clinical and laboratory follow up showed the complete recovery of platelets count (186,000 µ/L) without any other side effects. Both these adverse events may be life-threatening if not rapidly diagnosed. These experiences reasonably suggest that induction or switch to novel anticoagulants need a clinical and laboratory monitoring after one month and afterwards each three months over a period of at least one year in order to make safer and effective the use of NOACs in clinical practice.

Keywords: acquired hemophilia, thrombocytopenia, NOAC, adverse reactions.

Corresponding Author
M. Schiavoni, *marioschiavoni@gmail.com*

A

F. Abdollah Gorji 28
M. Acquila 30
L. B. Aguilar-López 20
A. Akinc 3
M. Akkerhuis 14
M. Alberti 45
S. Alizadeh 8
G. Allmaier 16, 44
F. Ancona 15
M. Antonelli 44
G. Ardissino 38
A. Artoni 5
M. Auton 23

B

Z. Badiei 35
L. Baronciani 18, 22, 24
S. Barros 3
M. Basso 24
D. Bellissimo 22
A. Bernad Miana 6
E. Berntorp 26
J. Bichler 33
P. Bicocchi 30
R. Bierings 17
E. Biguzzi 21, 24
M. Billwein 13
M. G. Blaizot 10
K. Blighe 27
E. Boersma 14
H. Boijout 10
K. Bonazza 44
A. Borchiellini 30
M. Borhany 9, 10
G. Bou-Assaf 16
M. L. Bowman 4
M. I. Bravo 29
M. A. Brehm 17
E. Bresin 45
P. Brons 23, 32
D. F. Brophy 1, 28
T. M. Brophy 11, 36
V. Bruscaggin 30
P. Bucciarelli 21, 24, 39
U. Budde 21

C

V. Caiani 21
A. Cairo 9, 40, 41
R. Camire 3
M. T. Canciani 24
A. Cannavò 27
S. E. Cannizzo 30
R. Carmona 1, 28
T. Carter 17
A. Cartwright 21
L. Casey 4
G. Casoli 39
S. R. Cataland 37
L. Cavallo 41
V. Chantarangkul 2
J. M. Cheng 14
E. Chhabra 16
A. Chion 11, 36
D. Cianflone 15
N. Ciavarella 47
J. W. C. Claassens 36
M. Clerici 2, 27
M. H. Cnossen 19, 32
R. Colombo 38
A. Coluccia 47
G. Cozzi 18, 22
N. A. Cristell 15

D

E. Daina 45
T. Danysh 34
B. Darbandi 29
Y. Dargaud 3, 26
B. Da Rocha-Souto 29
E. De Candia 44
H. Deckmyn 5, 39
R. De Cristofaro 24, 41, 44
L. Deforche 39
P. G. de Groot 11, 15
B. de Laat 11
M. P. M. de Maat 14
S. De Meyer 4, 5, 39, 46
F. Denorme 4
M. Dockal 12, 13, 31
A. Dorgalaleh 8, 38
S. Dorgaleleh 38
E. Dosio 30
A. Dragani 24
M. Driessens 32
M. M. Dsouza 21
A. Durante 15

E

M. H. Ebrahimzadeh 35
C. Escuriola-Ettingshausen 26
P. Eshghi 8, 28
M. Espinosa 37
B. Ewenstein 43

F

F. Fakhouri 37
M. R. Fasulo 2, 27
H. Fatima 9
N. Fatima 9, 10
B. Ferrari 5, 40, 41
R. Fijnheer 42, 43
K. Fijnvandraat 32
A. Foettinger-Vacha 43
A. Follenzi 2, 30
F. Franchi 18, 22
G. Friedbacher 16, 44
F. Verbij 42, 43

G

M. Galbusera 45
S. Gamba 45
I. Garagiola 27, 40
H. M. Garcia-Garcia 14
I. Garcia-Oya 18, 22
G. Garozzo 45
S. Gastoldi 45
K. Gegenbauer 36
G. Gerstenbauer 13
I. A. Gonzalez Ramos 6
A. C. Goodeve 13, 21, 22
M. M. Gorski 27, 40
S. Grancha 29
R. Grillberger 46
A. Gringeri 31
A. Griset 3
H. Gritsch 7, 12, 31
B. Gruber 46
Z. Gutowski-Eckel 26

H

D. J. Hampshire 13, 21, 22
H. Handrkova 9
R. B. Hartholt 36
R. Hartmann 13
H. C. A. M. Hazendonk 19, 32
J. Heemskerk 11
O. Hegener 9
N. Hellen 17
C. Hemker 11
E. Herzenik 36
W. Hoellriegl 7
N. Hooman 38
S. Hosseini 38
D. Hu 15

I

A. Ibarra-Hernández 20
L. Ivanciu 3
Z. Izsvák 5

J

A. R. Jaloma-Cruz 6, 20
P. James 4
M. Jansen 25
L. F. Jave Suarez 6
P. V. Jenkins 11, 36
J. I. Jorquera 29

K

A. R. Kachooei 35
P. Kaijen 42, 43
E. Kalantar 38
M. Kaliwoda 43
I. Kardys 14
H. Kelchtermans 11
C. M. Kessler 33
Y. C. Kim 34
T. K. Kishimoto 3
R. Klamroth 33
B. Klaus 16
C. Kleinschnitz 4
A. Klukowska 25
S. Knappe 12, 13, 31
S. Knaub 9, 25, 33
P. Knöbl 46
H. Kohler 9
V. Komrska 25
C. Königs 26
D. Kordi-Tamandani 8
W. Kreuz 26
G. Krishnamoorthy 11
T. Krogh-Meibom 33
J. Kulman 16

L

P. Laguna 25
S. La Marca 24
S. Lancellotti 24, 41, 44
F. Langhauser 4
M. Lapecorella 33
I. J. Lara Navarro 6
A. Laricchia 15
B. A. P. Laros 23
I. Lazzareschi 41
F. W. G. Leebeek 14, 19, 32
C. M. Legendre 37
H. Li 15
R. Liesner 25
L. Limite 15
T. Lindhout 11
S. Lippok 17
T. Lissitchkov 33
J. Lock 32
A. Lombardo 2
M. P. López-Montejo 20
L. A. Lotta 5, 27, 39, 40, 41
S. Lowndes 25
G. Lucarelli 47

M

S. Machin 40
I. Mackie 40
P. Madde 23
R. Mahdavian-Naghashzargar 35
A. Maino 5
A. Malato 5
R. Maldonado 3
I. Mancini 40, 41
M. E. Mancuso 2, 27
P. M. Mannucci 15, 21
R. Marcenò 45
J. A. Marchal Corrales 6
E. J. Martin 1, 28
I. Martinelli 39
P. A. Martinez 10
A. Maseri 15
S. Mastrangelo 41
R. A. A. Mathôt 32
A. Meijer 43
K. Meijer 32
G. Meloni 41
M. Menegatti 8
J. Menne 37
S. Merlin 2, 30
M. Messina 2
L. Mi 39
D. Mikovic 41
E. Minetti 37
C. Mistretta 21, 24
A. Miszta 11
B. M. Mohammed 1, 28
F. Mongelli 47
L. Montini 44
L. Moon-Tasson 23
A. Moradi 35
M. Morfini 33
G. Moulis 10
E. Muchitsch 7
A. H. Mufti 13

N

M. Naderi 8
L. Naldini 2, 30
A. Naz 9
C. Négrier 3, 26

O

T. Obser 17
J. S. O'Donnell 11, 36
R. M. Oemrawsingh 14
M. Ogawa 37
J. Oldenburg 26
C. Olgasi 2
R. Oliva 41
N. O'Regan 11
J. M. O'Sullivan 11, 36

P

M. Z. Padilla-Romo 20
L. Padovan 2
M. T. Pagliari 18, 22, 24, 39, 41
M. Palige 13
E. Panholzer 13
R. Paolini 45
I. Pareyn 5
G. Pasta 27
A. Pavlova 26
L. Pelkmans 11
G. Pellegrino 33
J. Pellequer 10
M. Pennisi 44
R. Peters 16
G. Petrucci 24
F. Peyvandi 2, 5, 9, 18, 21, 22, 24, 27, 39, 40, 41
A. Pichotta 26
B. Plaimauer 42, 43, 46
K. Pock 26
S. Pontiggia 5, 41
I. Possenti 38
K. P. Pratt 34
F. Provot 37

Q

J. Quin 3

R

T. Racie 3
J. O. Rädler 17
A. Raissi 28, 29
P. Ranalli 24
O. Rawley 11, 36
A. Raya 2
S. Razi 35
A. J. Reininger 31
G. Remuzzi 45
I. Ricaño-Ponce 40
B. Rocca 24
M. Roest 15
S. Romeder-Finger 7, 31
J. Römisch 26
E. Rondeau 37
E. Roose 39
F. R. Rosendaal 5, 24, 39
R. Rossi 3
H. Rottensteiner 5, 7, 16, 39, 42, 43, 44, 46
P. Ruggenenti 37
B. Rutten 15

S

E. Sabadini 45
S. Salardi 38
J. Salas 16
K. Salem 28, 29
V. Salinas 1, 28
Y. V. Sanders 19
E. Santagostino 2, 27
E. Scalambrino 2
F. Scheiflinger 7, 12, 13, 16, 31, 42, 43, 44, 46
M. Schiavoni 47
P. Schinco 2, 30
A. Schiviz 7, 46
T. Schmidt 26
R. Schneppenheim 17, 22
S. Schoormans 23
J. Schreiner 12
G. Schrenk 7, 12, 13, 16, 31, 46
P. M. Schulz 26
J. Schved 10
B. Schwartz 9
H. Schwarz 7
D. W. Scott 3, 34
A. Sehgal 3
S. Seregni 27
P. W. Serruys 14
B. Seyfried 16
S. Seyyed Khamesi 29
T. Shamsi 9, 10
E. M. Shevach 34
B. Shojaie 35
N. Shurko 34
S. M. Siboni 21, 24
S. Skalicky 42, 46
A. Snijders 17
L. Solimeno 27
M. A. H. Sonneveld 14
B. Sorensen 3
N. Sorvillo 36, 42, 43
J. M. Soto-Padilla 20
M. Spartera 15
T. Springer 39
K. Steinitz 31
F. Stufano 18, 22, 24

T

S. Tabibian 8
M. Talmon 2
B. Taregh 38
R. Tartaglione 24
S. Tedeschi 38
F. Tel 38
A. ten Brinke 36, 42
C. Tersteeg 46
S. Testa 38
B. Theophilus 22
A. Tiede 33
S. Till 12
A. Tischer 23
E. Torresani 38
A. Tripodi 2, 27
S. M. Trisolini 5
P. L. Turecek 7, 12, 13, 16, 31, 44, 46
A. Tuttle 4

U

M. Underwood 40
N. G. Uren 15

V

F. Valeri 2, 30
A. Valpreda 30
C. Valsecchi 39, 41
D. van Breevoort 17
A. Vandenbulcke 39
N. Vandeputte 5
F. J. M. van der Meer 32
C. van Duren 23
R. van Geuns 14
W. L. van Heerde 23
K. Vanhoorelbeke 4, 5, 39, 46
V. Vdovin 25
H. C. Veerman 19
J. S. Verbeek 36
S. Verhenne 5
J. Voorberg 17, 36, 42, 43

W

O. Walter 33
S. J. Webster 21, 22
S. Weckhuysen 17
C. Wijmenga 40
M. Wolfsegger 31
A. Wroblewska 36

Y

G. Yotov 33
G. A. Young 1, 28

Z

D. Zanolini 30
A. Zhang 3, 34

Sponsors

We thank all sponsors for their contribution

Organizing Secretariat

SMC media Srl
Via Giovenale,7 • 20136 • Milan, Italy
p • +39 02 834 194 30.1 • 02 581 028 46
f • +39 02 936 636 65
m • +39 335 653 0304 • +39 339 414 3210
info@smc-media.com • congress@smc-media.com
www.smc-media.com

Italian CME provider

CONGRESS TEAM PROJECT
Viale Brambilla, 60 • 27100 • Pavia, Italy
www.congressteam.com