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BLOOD TRANSFUSION

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

ABSTRACT BOOK

9th BIC International Conference

Rome (Italy), 15-17 September 2017

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* Some patients who are well-controlled on a once-weekly regimen might be treated with up to 75 IU/kg on an interval of 10 or 14 days.
† In previously treated patients ≥12 years on prophylaxis regimens.
‡ Select dosing regimen based on patient needs.

1. Santagostino et al. *Blood* 2016; 127(14): 1761–1769.
2. IDELVION Summary of Product Characteristics, April 2017.
3. Santagostino et al. *Blood* 2012; 120(12): 2405–2411.
4. Négrier et al. *Haemophilia* 2016; 22(4): e259–266.
5. Kenet et al. *Thromb Haemost* 2016; 116(4): 659–668.

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9TH BIC
INTERNATIONAL CONFERENCE

Rome (Italy), 15-17 September 2017

ORAL COMMUNICATIONS

DESMOPRESSIN (DDAVP): 40 YEARS LATER

OC_32

PHARMACOKINETIC MODELLING TO PREDICT FVIII:C RESPONSE TO DESMOPRESSIN AND ITS REPRODUCIBILITY IN NON-SEVERE HEMOPHILIA A PATIENTS

Schütte L.[1], van Hest R.[2], Stoof S.[1], Leebeek F.[1], Cnossen M.[3], Kruip M.[1], Mathôt R.[2]

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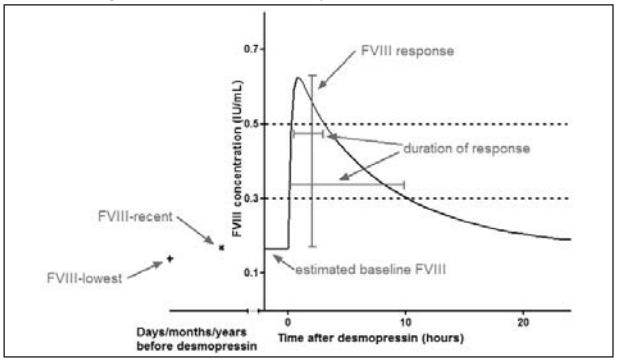
Background/Aims Non-severe hemophilia A (HA) patients can be treated with desmopressin. Response of factor VIII activity (FVIII:C) differs between patients and is difficult to predict. Our aims were to describe FVIII:C response after desmopressin and its reproducibility by population pharmacokinetic modeling.

Materials and methods Retrospective data of 131 non-severe HA patients (age 7-75 years) receiving an intravenous dose of desmopressin, were used. Pharmacokinetic modeling of FVIII:C was performed by non-linear mixed effect modeling. Reproducibility of FVIII:C response was defined as less than 25% difference in peak FVIII:C between infusions.

Results A total of 657 FVIII:C measurements was available from 153 desmopressin administrations; 22 patients had received two administrations on different occasions. The FVIII:C time profile was best described by a two-compartment model with first order absorption and elimination. Inter-individual variability of the estimated baseline FVIII:C, central volume of distribution and clearance were 44, 60 and 59%, respectively. The most recently measured FVIII:C (FVIII-recent) and the presence of a *F8*-gene mutation in the C1-domain were significantly associated with FVIII:C response to desmopressin ($p<0.001$). Desmopressin administration resulted in an absolute FVIII:C increase of 0.49 IU/mL (median, IQR 0.35-0.74 IU/mL, n=153). FVIII:C response was reproducible in only 55% of patients receiving two desmopressin administrations.

Conclusions FVIII:C response to desmopressin in non-severe HA patients was adequately described by a population PK model. Large variability in FVIII:C response was observed, which could only partially be explained by FVIII-recent and a *F8*-gene mutation in the C1-domain. FVIII:C response was not reproducible in slightly less than half of the patients. Therefore repeated test doses of desmopressin or monitoring of treatment around surgical interventions may be recommended.



OC_37

PERIOPERATIVE MANAGEMENT OF VON WILLEBRAND PATIENTS WITH DESMOPRESSIN; TOWARDS A PREDICTIVE POPULATION PK MODEL

Heijdra J.[1,2], Kruip M.[1], Leebeek F.[1], Cnossen M.[1,2]

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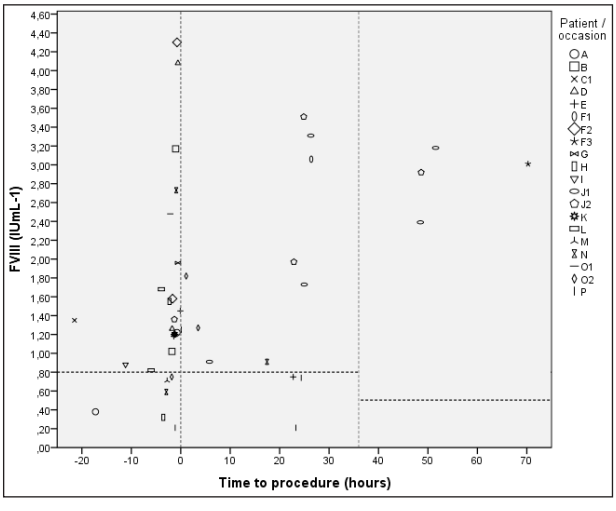
Background Von Willebrand disease (VWD) is the most common inherited bleeding disorder. In low to moderate risk procedures, patients in whom desmopressin (DDAVP) has been proven effective in a test, can be treated with DDAVP, aiming for FVIII/VWF target levels as defined in Dutch National Guidelines.

Aim To evaluate perioperative management with DDAVP in VWD patients in relation to FVIII/VWF target levels, in order to improve prediction of effect by construction of a population pharmacokinetic (PK) model.

Methods In this retrospective observational cohort study, VWD patients (historical VWF:Ag or VWF:RCo levels ≤0.30 IU mL^{-1}) treated in the Hemophilia Treatment Center of Erasmus University Medical Center - Sophia Children's Hospital undergoing a procedure between 2000-2016 were included. DDAVP dosing and achieved perioperative FVIII/VWF levels were compared to target levels.

Results A total of 159 procedures in 79 patients were analyzed. FVIII/VWF levels were available for 20 surgical procedures in 16 patients. During the first 36 hours, 86.4% of FVIII levels (Figure 1) and 63.3% of VWF:RCo levels were above target level (0.80 IU mL^{-1}). In 7 dental procedures, all FVIII/VWF levels were above target level (0.50 IU mL^{-1}) in this period. Bleeding complications occurred in only 3.8% and were unrelated to FVIII/VWF levels. Very high FVIII plasma levels (≥ 2.0 IU mL^{-1}) were reached in 59.1% of patients. DDAVP was administered 2-3 times in 9 procedures.

Conclusion DDAVP treatment is effective in this group, as patients achieve adequate FVIII/VWF plasma levels. Perioperative bleeding complications are rare, although some patients do not reach target levels. However, a large proportion of patients achieve very high FVIII plasma levels, a potential risk factor for thromboembolic complications. Better prediction of DDAVP response, both after first and consecutive dosing, may be realized by construction of population PK models, thus personalizing treatment in VWD patients.



OC_25

THE EFFECT OF *F8* MISSENSE MUTATIONS ON INDIVIDUAL PHARMACOKINETIC PARAMETERS OF DDAVP RESPONSE IN NONSEVERE HEMOPHILIA A

Loomans J.[1], van Hest R.[2], Bagher M.[1], Coppens M.[3], Kruip M.[4], Castaman G.[5,6], Mancuso M.E.[7], Nijziel M.[8], Peerlinck K.[9], Laros-van Gorkom B.[10], Mathôt R.[2], Fijnvandraat K.[1,11]

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Background/aims Desmopressin (DDAVP) increases endogenous factor VIII activity (FVIII:C) in most but not all nonsevere hemophilia A (HA) patients. Currently identified predictors of DDAVP response do not explain all observed inter-patient variability. Insight into individual pharmacokinetic (PK) FVIII parameters may help to clarify, as these are potentially influenced by *F8* missense mutations. We aim to explore the association between genotype and individual PK parameters of DDAVP response in a large cohort of nonsevere HA patients.

Materials and methods The RISE study is an international cohort study including data of 1,474 nonsevere HA patients with DDAVP exposure between 1980 and 2012. The main outcome variable is the incremental response (peak FVIII:C after DDAVP administration divided by pre-DDAVP FVIII:C). PK parameters are estimated based on population PK modeling using nonlinear mixed effects modelling software. Mutations are classified according to the grading system by Sengupta *et al.*[1]. The higher the Sengupta grade, the more impaired the FVIII protein. We used univariate regression techniques.

Results Genotype was known in 791 patients (55%) with 181 different mutations. We classified 59 mutations present in 137 patients by the Sengupta method. Grade four was excluded as there were only three patients in this group. Table I displays the frequency distribution for each group and the corresponding significant PK parameters (incremental response P=0.025, baseline FVIII:C P=0.026 and FVIII clearance P=0.01). Mutations with a higher Sengupta grade showed lower baseline FVIII:C and higher incremental response, which is probably due to the reciprocal effect of baseline FVIII:C on incremental response. Interestingly, especially grade two mutations show higher FVIII clearance. No difference was observed between the mutation groups for volumes of distribution.

Sengupta grade (ranging from 0-4)	N patients	Incremental response (median, IQR)	Baseline FVIII:C in IU/dL (median, IQR)	Clearance FVIII in dL/h (median, IQR)
0	13	2,9 (2,6-3,5)	19 (12-26)	18,6 (17,3-21,5)
1	62	3,1 (2,2-4,2)	20 (13-30)	18 (15,3-18,9)
2	29	3,4 (2,4-3,9)	12 (12-21)	18,8 (17,8-19,3)
3	30	4,2 (3,3-5,3)	16 (11-21)	18,2 (15,8-19,6)

Conclusions The functional effects of *F8* mutation influence incremental DDAVP response, baseline FVIII:C and FVIII clearance. It is important to include these PK parameters as determinants, as they explain inter-patient variability.

Reference

1) Sengupta M, Sarkar D, Ganguly K, *et al.* In silico analyses of missense mutations in coagulation factor VIII: identification of severity determinants of haemophilia A. Haemophilia 2015: 1-8.

OC_15

THE INTRACELLULAR BINDING OF FVIII TO VWF MAY ALLOW A CLINICALLY USEFUL RESPONSE TO DDAVP IN PATIENTS WITH HEMOPHILIA A DUE TO FVIII MUTATIONS IMPAIRING FVIII BINDING TO VWF

Jacquemin M.[1], Feyen L.[1], Lavend'homme R.[1], d'Oiron R.[2], Peerlinck K.[1,3]

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Background/aims Substitutions Arg2150His, Ile2098Ser and Ser2119Tyr in the FVIII C1 domain reduce the affinity of FVIII for VWF by 3, 8 and 80-fold, respectively, and result in mild/moderate hemophilia A with basal FVIII levels of 2 to 15 IU/dl. Despite the reduced affinity of the FVIII mutants for VWF, the administration of DDAVP to patients carrying these mutations results in a 7 to 11-fold FVIII increase relative to the basal levels. Because DDAVP is believed to induce the release of FVIII stored with VWF in endothelial cells, we investigated whether the three FVIII mutants were be able to interact with VWF in conditions representative of these found in the Golgi where the interaction between FVIII and VWF is expected to occur first.

Methods Sepharose beads coated with VWF were incubated with recombinant normal or mutated FVIII. Experiments were performed in buffer at pH 7.2 and at pH 6.0, representative of the trans-Golgi. Intracellular VWF antigen concentrations were determined by ELISA after lysis of different types of endothelial cells.

Results At pH 6, the binding of three recombinant rFVIII mutants to VWF was about 30% of the binding at physiological pH, indicating that the three mutants could still interact with VWF albeit with affinity lower than in plasma.

Besides affinity, the interaction between proteins is determined by concentration. Intracellular VWF concentrations ranged between 1.1 and 2.4 mg/ml, two orders of magnitude higher than in plasma. Accordingly, based on the law of mass action, a significant fraction of the FVIII mutants could be bound to VWF in endothelial cells.

Conclusions These observations suggest that a significant albeit reduced VWF-dependent intracellular storage of the

FVIII mutants is a plausible explanation for the unexpectedly high FVIII increase relative to the basal FVIII levels following DDAVP administration to patients carrying mutations impairing FVIII binding to VWF.

OC_26

DESMOPRESSIN IN MODERATE HEMOPHILIA A PATIENTS: A TREATMENT WORTH CONSIDERING

Loomans J.[1], van Velzen A.[1], Peters M.[1], Kruip M.[2], Platokouki H.[3], Jackson S.[4], Carcao M.[5], Santagostino E.[6], Beckers E.[7], Voorberg J.[8], van der Bom J.[9,10], Fijnvandraat K.[1,8]
(1) *Department of Pediatric Hematology, Immunology and Infectious diseases, Emma Children's Hospital, Amsterdam, the Netherlands;* (2) *Department of Hematology, Erasmus University Medical Center, Rotterdam, the Netherlands;* (3) *Aghia Sofia Children's Hospital, Athens, Greece;* (4) *Division of Hematology, Department of Medicine, St. Paul's Hospital and University of British Columbia, Vancouver, Canada;* (5) *Division of Haematology/Oncology, Department of Paediatrics and Child Health Evaluative Sciences, Research Institute, The Hospital for Sick Children, Toronto, Canada;* (6) *A. Bianchi Bonomi Hemophilia and Thrombosis Center, IRCCS Ca' Granda Foundation, Maggiore Hospital Policlinico, Milan, Italy;* (7) *Maastricht University Medical Centre, Maastricht, the Netherlands;* (8) *Department of Plasma Proteins, Sanquin Research, Amsterdam, the Netherlands;* (9) *Leiden University Hospital, Leiden, the Netherlands;* (10) *Sanquin Research, Leiden, the Netherlands*

Background/aims Desmopressin (DDAVP) increases endogenous factor VIII activity (FVIII:C) in hemophilia A (HA). Large inter-individual variation in the response to DDAVP is observed. Patients with a lower baseline FVIII:C tend to show reduced achieved peak FVIII:C levels. Moderate HA patients (baseline FVIII:C 1-5 IU/dL) are therefore less frequently tested for or treated with DDAVP, even though FVIII:C may rise substantially in some of them. We aim to describe the response to DDAVP in moderate HA patients and to identify predictors.
Materials and methods We selected data on all 169 patients with moderate HA from the multicenter RISE cohort study, consisting of 1,474 nonsevere HA patients. Adequate response to DDAVP was defined as a peak FVIII:C ≥30 IU/dL after DDAVP administration. Excellent response was defined as a peak level ≥50 IU/dL. We used univariate and multiple linear regression techniques to analyze predictors of the peak FVIII:C.
Results Response was adequate for treatment in 68 patients (40%), of whom 25 showed excellent response (15%). Pre-DDAVP FVIII:C, VWF:Ag, intravenous administration, age, peak level VWF:Act and DDAVP induced rise in VWF:Ag were significant predictors of peak FVIII:C and explained 65% of the inter-individual variation.
Conclusions In spite of low endogenous FVIII:C, 40% of moderate hemophilia A patients demonstrate a response to DDAVP that is adequate for treatment of minor bleeding or trauma. Therefore, it is important to assess DDAVP responsiveness in moderate hemophilia A patients. We identified six predictors of which pre-DDAVP FVIII:C and DDAVP induced rise in VWF:Ag influenced peak FVIII:C the most.

OC_83

SAFETY AND EFFECTIVENESS OF DESMOPRESSIN FOR THE MANAGEMENT OF BLEEDS, DELIVERY AND MAJOR SURGERY IN MILD-MODERATE VON WILLEBRAND DISEASE: RESULTS OF THE PRO-DES-WIL STUDY IN A COHORT OF 84 PATIENTS

Federici A.B.[1,2], Castaman G.[3,4], Iorio A.[5,6], Blanchette V.S.[7], Bonduel M.[8], D'Amico E.[9], Lethagen S.[10], Oliovecchio E.[5], Santoro C.[11], Siboni S.M.[1], Zieger B.[12], Peyvandi F.[1], Lillicrap D.[13], Mannucci P.M.[1]
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Even though desmopressin (DDAVP) is considered the treatment of choice for most patients with inherited von Willebrand disease (VWD), no prospective data have been jet reported to correlate biological response with clinical efficacy of DDAVP in VWD. Among 268 patients enrolled in a 24-month prospective study, 225 (85%) met inclusion criteria as VWD1 (n=184), VWD1C (n=14), VWD2A (n=15), VWD2M (n=12). DDAVP biological response was complete, partial and absent in 89%, 10% and 1% of all VWD. 84/225 (37.3%) received DDAVP for bleeds (n=104), oral surgeries (n=33), deliveries (n=12), minor/major surgeries (n=25). Total injections were 652 with median, range/episode during bleeds (2,1-12), oral surgeries (1,1-10), deliveries (3,1-13), minor/major surgeries (3.6,1-16). Clinical efficacy was excellent/good in bleeds (93.3%), oral surgery (100%), deliveries (91.7%), minor/major surgeries (92.3%). Efficacy was rated poor during menorrhagia (n=4) in 2 VWD1 and 2 VWD2A, during nose (n=2) and gastrointestinal (n=2) bleeds in 3 VWD2A and 1 VWD1C. During deliveries poor response was found in only one VWD1C who required VWF concentrates after caesarean section. Among the 14/25 major surgeries [abdominal (n=5), hysterectomy (n=3), tonsillectomy (n=3), orthopaedic and others (n=3)] efficacy was poor in only 2 episodes (partial resection of kidney in VWD2A and tonsillectomy in VWD1). The 16 side effects were mainly minor (flushing, headache, tachycardia) with water retention reported in 2 patients who received >12 doses for delivery or major surgery. Based on these results, DDAVP must be always recommended as first line therapy in responsive VWD not only in bleeds and oral surgery but also in deliveries and major surgeries.

CLINICAL SCIENCE

OC_05

FITUSIRAN, AN INVESTIGATIONAL RNAi THERAPEUTIC TARGETING ANTITHROMBIN FOR THE TREATMENT OF HEMOPHILIA A OR B WITH AND WITHOUT INHIBITORS: INTERIM RESULTS FROM A PHASE 2 EXTENSION STUDY

Chowdary P.[1], Pasi K.[2], Georgiev P.[3], Mant T.[4], Creagh M.[5], Lissitchkov T.[6], Bevan D.[7], Austin S.[8], Hay C.[9], Hegemann I.[10], Kazmi R.[11], Rangarajan S.[12], Soh C.-H.[13], Monpara A.[13], Van Nguyen H.[13], Madigan K.[13], Ragni M.[13]

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Background Hemophilia is a bleeding disorder characterized by an inability to generate sufficient thrombin for effective hemostasis. Fitusiran is a subcutaneously (SC) administered investigational RNA interference (RNAi) therapeutic targeting antithrombin (AT) as a means to improve thrombin generation (TG) and promote hemostasis in patients with hemophilia A or B with and without inhibitors. Interim data from the Phase 1 study showed fitusiran was generally well tolerated and administration of monthly fitusiran led to dose-dependent AT lowering, TG improvement, and decrease in bleeding frequency. We will report interim safety, pharmacodynamics (PD), and clinical activity of fitusiran from the Phase 2 extension study.

Methods The Phase 2 open label extension study (NCT02554773; EudraCT: 2013-003135-29) included patients with hemophilia A or B with and without inhibitors, previously dosed in the Phase 1 study (NCT02035605; EudraCT: 2013-003135-29). Patients received monthly, fixed SC doses of fitusiran, 50 mg or 80 mg.

Results As of May 2017, 33 patients were enrolled in the study and had received continuous dosing of up to 14 months. Previously reported data showed that fitusiran was generally well tolerated, with no serious adverse events related to study drug and no thromboembolic events. Once-monthly subcutaneous dosing achieved dose-dependent AT lowering of ~80% and TG levels approaching the lower end of normal range. Exploratory post-hoc analysis of bleed events showed median ABR=1 in patients without inhibitors and median ABR=0 in patients with inhibitors. Bleed events were successfully managed with either replacement factors or bypassing agents. Updated safety, tolerability and clinical activity, including the management of bleed events, will be presented.

Conclusions Emerging clinical data suggest that fitusiran-mediated lowering of AT to improve TG may be a promising investigational approach for promoting hemostasis in hemophilia.

OC_58

"FEIBA GLOBAL OUTCOME STUDY (FEIBA GO)" FIRST DATA READ-OUT: REAL WORLD BLEEDING FREQUENCY IN INHIBITORS PATIENTS ON PROPHYLAXIS WITH APCC

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Background/aim The "FEIBA GO" study was designed to capture long-term outcomes on effectiveness, safety and quality of life in subjects with haemophilia and inhibitors treated with APCC in routine clinical practice. The primary objective is to describe the hemostatic effectiveness of APCC in different settings such as prophylaxis and on demand, including patients on immune-tolerance induction; most relevant secondary objectives are: joint functionality outcomes, safety, health-related quality of life (HR-QoL), daily activity level, acute and chronic pain associated with haemophilia, health resources used.

Methods FEIBA GO is a prospective, non-interventional, observational multicenter cohort study in patients with hemophilia A or B and high-responding inhibitors treated with APCC prior the decision to enroll in the study. Target for enrollment is 100 subjects. Treatment regimens are at the discretion of the attending physicians according to routine clinical practice, either in prophylaxis or on demand, including immune-tolerance induction. The observation period per subject is planned to be 4 years.

Results Data read-out was carried out on September 15, 2016 on 28 subjects with severe haemophilia A and inhibitors (median titer at screening 10 BU, min-max 1-2,410), recruited in 14 haemophilia centres in 8 countries: median age 23 years (range 3-71). 21 of them were on prophylaxis. Data was available for 18/21 as shown in Table I.

Conclusions These preliminary findings show that prophylaxis with APCC in patients with haemophilia and inhibitor can be effective, as it demonstrably prevents joint bleeding in a proportion of subjects approximating that reported in non-inhibitor patients on replacement prophylaxis. This study will further augment the knowledge of long-term prophylaxis in real world clinical setting by assessing the treatment regimens, effectiveness, HR-QoL and safety of APCC in this rare patient population.

Table 1

Patients on prophylaxis analysed in the data read-out	n=18
Median Annualised Bleeding Rate (ABR)	3.7 (0-19)
Subjects with ABR "0"	16.7%
Subjects with ABR "≤2"	22.3%
Subjects with ABR "≤3"	39.0%
Median Annualised Joint Bleeding Rate (ABJR)	1.6 (0-17)
Subjects with AJBR "0"	38.9%
Subjects with AJBR "<2"	55.6%
Subjects with AJBR "<3"	77.8%

OC_57

A CUMULATIVE REVIEW ON FOUR DECADES OF THROMBO-EMBOLIC EVENTS REPORTED WITH THE USE OF ACTIVATED PROTHROMBIN COMPLEX CONCENTRATE (APCC) IN CONGENITAL HAEMOPHILIA

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Background/aims Bypassing agents have contributed to an improvement in prevention and treatment of bleeding in persons with haemophilia (PWH) and inhibitors. While proven effective, bypassing therapy has introduced a potentially increased risk of thrombo-embolic events (TEEs) associated with the treatment. The small size of clinical trials and post-authorization studies in PWH and inhibitors limits the capability to ascertain risk factors for APCC-associated TEEs. This review provides an overview of all TEE cases that have occurred with the use of APCC in congenital haemophilia as reported spontaneously in literature, and documented in the Company's global safety database (GSD).
Methods The GSD was reviewed for all APCC spontaneous and literature adverse event reports from 1975 to July 2016, addressing patient demographics, dosing regimens, confounding and risk factors considered relevant for the development of TEEs in temporal association with APCC treatment.
Results APCC became commercially available in 1975. More than 7 billion units of APCC (>2 million infusions) were distributed during the review period. 85 reports including ≥1 TEE events were received for PWH, aged 0-76 years (median 22) (Table I) resulting in a reporting rate of approximately 3.6 TEEs/105 infusions (3,000 U/infusion). rFVIIa was reported to be administered in temporal relationship with APCC in 32/85 (37.7%) of TEEs. No thrombotic microangiopathic events were reported. From 2000/02/01 to 2016/07/31, 73 TEEs for all indications were received from spontaneous sources (excluding literature).
Conclusion The reporting rate of TEEs associated with APCC is comparable with published data and confirms its overall 40-year safety profile. A clinically relevant portion of the TEEs occurred in the presence of additional/confounding risk factors such as underlying disease and concomitant medications. The review of all TEEs reported in temporal association with the use of APCC is a valuable resource for the understanding and perhaps the prevention of such events.

Table I

	Number of cases	Median age (min-max)
All reported TEEs in patients with congenital haemophilia	85	22 years (0-76)
Deep ven thrombosis and/or pulmunary embolism	18	11 years (1-22)
Myocardial infarction/ischemia	17	41 years (8-73)
Cerebrovascular accident	18	55 years (2.5-70)
DIC	18	49 years (0-71)
Other	14	30 years (3.57)

OC_22

INFLUENCE OF VARIANT VWF MULTIMER PATTERNS ON THE DIAGNOSIS OF VWD TYPE 2B

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Background/Aims Von Willebrand disease type 2B (VWD2B) is characterized by enhanced binding to platelet GPIBα due to gain of function (GOF) mutations in the VWFA1 domain, subsequent loss of von Willebrand factor high molecular weight multimers (VWF-HMWM) by their enhanced ADAMTS13-proteolysis and variable degrees of thrombocytopenia. The diagnosis of VWD2B by means of Ristocetin-Induced Platelet-Aggregation (RIPA) is not trivial and requires fresh platelet-rich plasma. A VWF:GPIBα-binding ELISA at different ristocetin concentrations has been applied to overcome these obstacles (Caron *et al*., BJH 2006; 133: 655-63). We assessed the diagnostic benefit and possible influencing factors by our own comparable assay.
Materials and methods Sixteen patients with a previous diagnosis of VWD type 2B, carrying 8 different VWD2B mutations were studied by conventional assays, VWF multimer analysis and our VWF:GPIBα-ELISA at different concentrations of ristocetin. In addition, VWF2B mutants were recombinantly expressed and analyzed in the same way as plasma VWF.
Results Recombinant VWF2B mutants displayed normal VWF multimer patterns compared to rwtVWF. Their RIPA unequivocally confirmed the GOF. GPIBα-binding of patients' plasma VWF was diagnostic in all cases, in particular at concentrations of 0.3 and 0.15 mg/ml ristocetin, thereby confirming the diagnosis of VWD2B. However, heterogeneity in the binding curves was present, and lower VWF-GPIBα-binding in individual patients was associated with reduced VWF-HMWM independent of the genotype (Fig. 1).
Conclusion The recombinant GPIBα-binding assay reliably differentiates between normal plasma VWF and all VWF2B plasma samples studied, in particular at concentrations of 0.15 to 0.3 mg/ml ristocetin. The assay only requires patients' plasma. In addition, positive results of this assay exclude, and negative results speak in favor of the alternative diagnosis, "Platelet type- or Pseudo-VWD". We could also show that variably reduced VWF-HMWM in individual patients does influence the diagnostic power, which may obscure particularly the results of the conventional RIPA-assay.
Funding: DFG-Research-Group FOR1543.

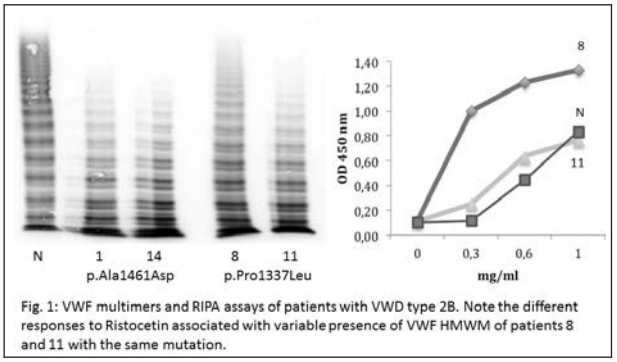

Fig. 1: VWF multimers and RIPA assays of patients with VWD type 2B. Note the different responses to Ristocetin associated with variable presence of VWF HMWM of patients 8 and 11 with the same mutation.

OC_44

COMPARING PLATELET-DEPENDENT VON WILLEBRAND FACTOR ACTIVITY ASSAYS IN 661 PATIENTS WITH VON WILLEBRAND DISEASE - FROM THE WiN STUDY

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Background/aims Measuring platelet-dependent von Willebrand factor (VWF) activity is crucial for the diagnosis and classification of von Willebrand disease (VWD). There are now several widely used VWF activity assays, but it is not well known if differences in assay methods translate to clinically relevant differences. Therefore, we compared three widely used VWF activity assays in a large cohort of VWD patients.
Materials and methods We included 661 VWD patients (historically lowest VWF ≤30 U/dL) from the nationwide "Willebrand in the Netherlands" (WiN) Study. We compared three VWF activity assays based on 1) ristocetin-induced of platelet agglutination (VWF:RCo, BC von Willebrand Reagent, Siemens), 2) binding of a monoclonal antibody directed against the GP1b binding domain of VWF (VWF:Ab, HemosIL® VWF Activity, Instrumentation Laboratory), and 3) binding of a recombinant gain-of-function mutant platelet glycoprotein 1b fragment (VWF: GP1bM, INNOVANCE VWF Ac, Siemens).
Results All assays were highly correlated (r >0.95). Bias between assays was <5 IU/dL. An absolute difference >10 IU/dL between assays was found in 16% to 24% of patients. VWD classification (using VWF activity/antigen ratio 0.6) did not match between assays in ~20% of patients. In 39% of patients, VWF:RCo was below the detection limit of 12 IU/dL; 17% also had VWF:Ag <20 IU/dL and therefore an incalculable VWF:RCo/VWF:Ag ratio. In 8/21 (38%) "true" type 3 VWD patients (VWF:Ag <5 IU/dL and propeptide <4 U/dL), VWF:GP1bM results were between 5 and 21 IU/dL, whereas all results were below the detection limit for both VWF:RCo and VWF:Ab.
Conclusions In 17% of VWD patients VWF:RCo could not differentiate type 1 and 2 VWD. Almost 40% of type 3 VWD cases were missed by VWF:GP1bM. The choice between platelet-dependent VWF activity assays has a significant impact on the classification of VWD. We are currently investigating the correlation between VWF genetics and assay results.

OC_68

IS TYPE 3 VWD AN HOMOGENEOUS GROUP?

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Background Type 3 von Willebrand disease (VWD), inherited as an autosomal recessive trait, is usually defined as a virtually complete deficiency of von Willebrand factor (VWF) resulting in very low FVIII (<5 IU/dL) concentrations. However some type 3 VWD patients have been incidentally reported with no null VWF and FVIII up to 10-15 IU/dL.
Methods The study was designed to evaluate the clinical, phenotypic and molecular features of the French cohort of type 3 VWD patients. A total number of 80 patients (73 families) were enrolled with the criteria of VWF levels <5 IU/dL and of a proven recessive inheritance.
Results We found 84 different mutations (55 novel). After centralized laboratory reevaluation, we were able to clearly distinguish two groups. Group A included 64 patients (80%) exhibiting undetectable VWF:Ag levels, absence of any plasma multimer and FVIII:C levels ≤5 IU/dL. Group B included 16 patients (20%) exhibiting detectable VWF:Ag levels (median 2.8, IQR 2-5 IU/dL), visible low molecular weight multimers and FVIII:C levels >5 IU/dL. There was a significant difference (P=0.0258) between the median bleeding score in group A (17.5, IQR 12-24) and group B (12, IQR 10-17); moreover haemarthroses were more frequent in group A patients (P=0.002) where 50% of patients benefited from long term prophylaxis and 6.25% developed an inhibitor. No patient from group B required long term prophylaxis and none developed an inhibitor. Missense mutations occurred more frequently in the group B (found on 41% of the alleles) than in group A (12.5%).
Conclusions Tiny amounts of VWF:Ag leading to higher FVIII:C levels induce a less severe bleeding profile and no risk of inhibitor; thus the classification of the defect in these patients is questionable.

TRANSLATIONAL SCIENCE

OC_67

TOWARDS A CLINICALLY RELEVANT HYBRID ADENOVIRUS-SLEEPING BEAUTY TRANSPOSON VECTOR FOR GENE THERAPY FOR VON WILLEBRAND DISEASE

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Background Gene therapy offers potential for a long-term treatment of severe von Willebrand disease (VWD). Using non-viral Sleeping Beauty transposons (SBT), we previously

achieved sustained expression of von Willebrand factor (VWF) in VWF-deficient ($Vwf^{-/-}$) mice. To circumvent clinical limitations associated with hydrodynamic delivery, high-capacity adenoviral vectors (HC-AdV) were combined with the SBT technology.

Aims To develop a clinically applicable gene therapy platform for VWD based on HC-AdV that deliver the SBT system to hepatocytes.

Materials and methods Two HC-AdV were constructed, comprising the two-component SBT system. A first HC-AdV contained the VWF transgene under control of a liver-specific promoter, flanked by FRT sites and SBT inverted repeats. A second HC-AdV carried the Flp recombinase and SB100X transposase. After intravenous co-injection of the vectors in $Vwf^{-/-}$ mice, VWF antigen levels and VWF multimer pattern were regularly determined in plasma for up to 1 year. Correction of the bleeding phenotype was evaluated using tail-clip and saphenous vein bleeding models.

Results Functionality of this hybrid adenovirus-SBT vector was assessed in $Vwf^{-/-}$ mice and resulted in very high and stable VWF levels, still 2,072±383% of wild type levels 1 year after gene transfer. Moreover, FVIII activity was restored to physiological levels (102±33%, 1 year after gene transfer). Both tail-clip bleeding at 12 weeks and saphenous vein bleeding at 1 year resulted in small, but not significant reductions of bleeding time. The reduced fraction of high molecular weight multimers observed in hepatocyte-produced VWF might account for the partial corrected bleeding phenotype.

Conclusions The hybrid adenovirus-SBT vectors efficiently delivered VWF transposons into hepatocytes, resulting in very high and sustained VWF transgene expression. Despite no full correction of the bleeding diathesis, this powerful vector system shows great promise towards a clinically applicable gene therapy for VWD, e.g. for targeting endothelial cells.

OC_36

TREATMENT OF HEMOPHILIA A BY INJECTION OF FVIII-ENCODING mRNA

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Background Treatment of patients with hemophilia A with exogenous therapeutic factor VIII (FVIII) is complicated by high cost, low FVIII half-life and need for life-long treatment. Injection of *in vitro* transcribed mRNA (IVT mRNA) encoding erythropoietin or factor IX allows an endogenous production for a few days without innate immune activation[1,2].

Aim Investigate whether the injection of FVIII-encoding IVT mRNA allows the endogenous production of FVIII.

Methods HEK293 cells were transfected with IVT mRNA encoding B-domain deleted FVIII (FVIII-HSQ), codon-optimized FVIII (CoOpSQ) and codon-optimized FVIII 226/N6 (CoOpN6) formulated in TransIT (Mirus Bio LLC). TransIT-formulated IVT mRNA was also injected intravenously to FVIII-deficient mice. FVIII in cell culture supernatant or plasma was detected by ELISA (FVIII:Ag) and chromogenic assay (FVIII:C) for up to 72 hours.

Results Cells transfected with HSQ or CoOpSQ-encoding mRNA produced more FVIII:Ag than cells transfected with CoOpN6 after 48 hours. FVIII:C levels in supernatant were however equivalent for the three molecules. Plasma levels of FVIII 24 hours after injection of HSQ, SQ and N6-encoding mRNA to FVIII-deficient mice represented 69, 94 and 80% of the levels found in normal plasma, respectively. Moreover, FVIII:C levels greater than 10% of normal were maintained for up to 48 hours. Deconvolution of curves fitting evolution of FVIII plasma levels with time allowed estimation of the half-life of the infused mRNA.

Conclusion Intravenous injection of FVIII-encoding mRNA permits the endogenous production of FVIII ≥10% of normal values for up to 48 hours. We will now confirm whether such FVIII levels protect mice in tail clipping assays, and whether the endogenous FVIII production induces a neutralizing immune response upon repeated treatments. At term, our work will allow to compare the efficiency and safety of IVT mRNA with that of alternative therapeutic approaches that are currently under development: gene therapy, bi-specific antibodies and long-lasting FVIII products.

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OC_34

APPLICATION OF COMBINED GENE AND CELL THERAPY WITHIN AN IMPLANTABLE THERAPEUTIC DEVICE FOR THE TREATMENT OF SEVERE HAEMOPHILIA A

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New regenerative medicine approaches to treat/cure haemophilia A require insights into cell compartments capable of producing factor VIII (FVIII). Hemophilia A (HA) is an X-linked bleeding disease due to FVIII deficiency. We and others previously demonstrated that FVIII is produced specifically in endothelial cells. The main objective of our work is to develop the tools and technologies for a novel *ex vivo* cell-based therapy to treat haemophilia A that should ultimately lead to improved patient quality of life.

We isolated blood outgrowth endothelial cells (BOECs) from healthy and patients' blood. BOECs were efficiently transduced by lentiviral vectors containing the B domain deleted form of human FVIII under the Vascular Endothelial Cadherin promoter (VEC). BOECs were characterized by FACS analysis for endothelial phenotype and FVIII evaluated by APTT and ELISA. The number of integrated LV copies/cell was ~3 for LV-VEC. hFVIII transduced cells. We demonstrated by FACS that FVIII was expressed by 67% of LV-VEC.hFVIII transduced cells. These data were also confirmed by immunofluorescence showing that FVIII was expressed at higher levels from LV-VEC.FVIII transduced BOECs compared to non-transduced BOECs.

Ten million LV-VEC.hFVIII-BOECs were transplanted intraperitoneally in association with cytodex® 3 microcarrier beads in NOD/SCID g-null HA mice (n=3). BOECs survived and secreted FVIII at therapeutic levels (with a peak of 15%

FVIII activity) for up to 8 weeks. Mice are ongoing with longer-term FVIII activity monitoring time points. As next steps, LV-transduced haemophilia patient BOECs will be transplanted into an implanted prevascularized, scalable medical device (Cell Pouch™, Sernova Corp.) and optimized for sustained secretion of therapeutic factor VIII in the NOD/SCID g-null HA mice. This is in preparation for future human clinical testing within the device in haemophilic patients by transplantation of GMP produced autologous gene corrected BOECs.

OC_76

PHARMACOKINETIC PROFILE OF rFVIIIFc-VWF-XTEN (BIVV001) PROTEIN IN CYNOMOLGUS MONKEY GENERATED FROM A LARGE-SCALE MANUFACTURING STABLE CELL LINE

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Introduction and objectives BIVV001 (rFVIIIFc-VWF-XTEN) is a novel fusion protein designed to treat hemophilia A. BIVV001 is comprised of a BDD-rFVIII fused to dimeric Fc, the D'D3 domain of VWF (FVIII binding domain) and two XTEN polypeptides. This protein is designed not to bind endogenous von Willebrand factor (VWF). In a hemophilia A (HemA) mouse model, BIVV001 demonstrated >3-fold improvement in half-life, and efficacy comparable to rFVIII. In this study, the pharmacokinetic (PK) of BIVV001 was evaluated in 8 cynomolgus monkeys to confirm the PK profile of the protein generated from a cell line developed for large scale manufacturing.
Material and methods BIVV001 stable cell line was generated using HEK293 cells. The final clone selected for BIVV001 production has FVIII expression level which is suitable to allow large scale manufacturing. PK evaluation of purified protein in cynomolgus monkeys was done at 100 and 300 IU/kg doses. Post IV infusion, plasma FVIII activity and antigen levels were monitored by BIVV001-specific activity and ELISA assays; PK was calculated by Phoenix WinNonlin software using non-compartmental analysis.
Results In cynomolgus monkeys, BIVV001 displayed 3-fold longer half-life, both by activity and antigen compared to rFVIII. In addition to the extended half-life, there was a 3-fold improvement in AUC with incremental recovery comparable to rFVIII. Same PK parameters by antigen and activity assays suggests that *in vivo* integrity of BIVV001 protein is maintained over the course of study.
Conclusions The VWF independence in combination with the Fc domain and XTEN polypeptides enable a 3-fold increase in circulating half-life of BIVV001 compared to the rFVIII. A similar biochemical and PK profile, identical to previous small scale research grade BIVV001 material, confirms similar properties of the protein generated from the final manufacturing clone. It also validates that it is feasible to produce BIVV001 at large scale, which is essential for BIVV001 clinical development.
Disclosures *This study was funded by Bioverativ. ESC, JS, JS, and RP are employees of Bioverativ. BY was an employee of Bioverativ at the time of the study and is currently affiliated with Acerta Pharma.*

OC_56

RECOMBINANT FACTOR VIII Fc FUSION PROTEIN EXHIBITS IMMUNOMODULATORY EFFECTS ON ANTIGEN-PRESENTING CELLS

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Background The main complication of replacement therapy with factor in hemophilia A is the formation of inhibitors (neutralizing anti-factor VIII antibodies) in ~30% of severe hemophilia A patients. Inhibitor development impacts treatment efficacy as well as the quality of life of affected individuals. Further understanding of how the immune system responds to FVIII is an ongoing effort in order to understand how to eradicate inhibitor formation effectively. The extended half-life recombinant factor VIII Fc fusion protein (rFVIIIFc) is an efficacious and well-tolerated therapy to prevent and control bleeding episodes. The Fc region of this molecule is not only responsible for increasing rFVIII half-life, but may also promote antigen-specific tolerance, as shown in a preclinical animal model (Krishnamoorthy, Cell Immunol 2016), and suggested in immune tolerance induction case reports (Groomes, Ped Blood Cancer 2016; Malec, Haemophilia 2016; Ragni, Haemophilia 2016).
Aim The goal of this study is to characterize the interactions of the Fc portion of rFVIIIFc with the immune system, focusing on the Fcg receptor expressing antigen-presenting cells (APCs) and APC-T cell interactions.
Materials and methods Peripheral blood-derived human APCs were used to investigate the effects of rFVIIIFc on Fcg receptor binding and signaling, cytokine production, gene expression changes, as well as subsequent effects on T cells *in vitro*.
Results rFVIIIFc engages Fcg receptors on APCs, as indicated by the loss of cell surface receptor levels monitored by flow cytometry. Further, phosphorylation signals downstream of the receptors were observed. When compared to immune complex treatment, these signals do not induce pro-inflammatory cytokines. Instead, rFVIIIFc initiates alternatively activated macrophage-specific gene expression pattern. rFVIIIFc-educated APCs co-cultured with naïve T cells support differentiation towards a regulatory T cell phenotype.
Conclusion The Fc portion of rFVIIIFc appears to influence the phenotype and function of antigen-presenting cells orientating them towards immunoregulation.

OC_73

SCAVENGER-RECEPTOR STABILIN-2 IS A MAJOR REGULATOR OF MOUSE VWF PROPEPTIDE CLEARANCE

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Background In recent years, the clinical utility of the ratio between the von Willebrand factor propeptide (VWFpp) and von Willebrand factor (VWF) as a marker for increased VWF clearance has become apparent. Despite this however, the mechanisms regulating clearance of the VWF propeptide itself remain unknown.
Aims To investigate the mechanisms underlying mVWFpp clearance.
Materials and methods Mouse VWFpp (mVWFpp) was expressed via hydrodynamic gene transfer of murine VWFpp

cDNA into VWF$^{-/-}$ mice. Murine plasma containing mVWFpp was collected and subsequently infused via tail vein injection (200 U mVWFpp/kg) into VWF$^{-/-}$ or Stab2$^{-/-}$/VWF$^{-/-}$ mice. Mice were retro-orbitally sampled and mVWFpp was quantified by ELISA.

Results Clearance studies in VWF$^{-/-}$ mice demonstrated poor mVWFpp recovery at 5 minutes post-infusion (residual plasma mVWFpp 21.7±0.8% of injected). Moreover, mVWFpp was virtually undetectable at 10 minutes post-infusion suggesting rapid mVWFpp clearance. A role for macrophages in mediating this phenomenon was excluded as no difference in mVWFpp plasma levels was observed in VWF$^{-/-}$ mice treated with clodronate-liposomes *vs* control-liposomes (26.9±4.6% *vs* 28.5±7.2%, p=0.86). Interestingly however, clearance studies carried out in Stab2$^{-/-}$/VWF$^{-/-}$ mice revealed significantly prolonged survival of mVWFpp. mVWFpp recovery values were ~3-fold greater than those observed in VWF$^{-/-}$ mice (63.3±8.6% *vs* 21.7±0.8%, p=0.0063). Furthermore, mVWFpp was detectable in plasma at 60 minutes post-infusion (elimination half-life 11.2 minutes) indicating that stabilin-2 deficiency significantly prolonged mVWFpp survival. Quantitative analysis of endogenous mVWFpp levels in Stab2$^{-/-}$ mice further corroborated this observation. While mVWF:Ag levels were not significantly different in normal *vs* Stab2$^{-/-}$mice, Stab2$^{-/-}$ mice had significantly elevated plasma mVWFpp levels (100±10.7% *vs* 270.6±21.0%, p<0.0001) confirming stabilin-2 as a critical regulator of mVWFpp plasma levels. Importantly, significantly elevated mVWFpp/mVWF:Ag ratios were also observed in these mice. To our knowledge this is the first description of an elevated VWFpp/VWF:Ag ratio resulting from elevated plasma propeptide levels rather than decreased plasma VWF:Ag, an important consideration for the diagnostic application of VWFpp/VWF:Ag ratio particularly with regard to defining VWD subtype.

Conclusions Stabilin-2 modulates plasma mVWFpp levels by regulating mVWFpp clearance.

OC_61

AN OPEN CONFORMATION OF ADAMTS13 IS A HALLMARK OF ACUTE ACQUIRED THROMBOTIC THROMBOCYTOPENIC PURPURA

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Background Acquired thrombotic thrombocytopenic purpura (aTTP) is characterized by severe thrombocytopenia, microangiopathic haemolytic anaemia, severe ADAMTS13 deficiency (<10%) and presence of anti-ADAMTS13 autoantibodies. The conformation of ADAMTS13 is folded by interaction between its spacer and CUB domains.

Disruption of this spacer-CUB interaction by addition of von Willebrand factor or an activating anti-CUB1 antibody 17G2 changes the ADAMTS13 conformation, resulting in exposure of previously cryptic epitopes.

Aim Determine if the conformation of ADAMTS13 is altered in aTTP patients compared to healthy donors (HD).

Methods Murine monoclonal antibodies recognizing cryptic epitopes in the cysteine/spacer (C/S) domain (hence recognizing ADAMTS13 with an altered conformation) were selected via ELISA. The conformation of ADAMTS13 in HD, aTTP, HUS and sepsis patients was determined via ELISA, in which plasma was added to the antibody that recognizes a cryptic epitope in ADAMTS13.

Results We identified the anti-S domain antibody (1C4) that recognizes a cryptic epitope in ADAMTS13. While the antibody 1C4 readily captured MDTCS, 1C4 only captured full length ADAMTS13 when its conformation was changed by addition of 17G2. When plasma of HD was added to the 1C4 antibody, ADAMTS13 did not bind (n=40). This indicates that ADAMTS13 indeed adopts a folded conformation in circulation where the spacer and CUB domains interact. Similar results were obtained for sepsis (n=63) and HUS (n=12) patients. Intriguingly, the conformation of ADAMTS13 is spontaneously altered in 92% of the patients with acute aTTP (n=63), as the cryptic epitope of 1C4 was readily available. On the other hand, in 78% of the aTTP patients in remission, ADAMTS13 did no longer bind to 1C4 (n=36), showing that this open conformation is specific for the acute phase.

Conclusion Besides a severe ADAMTS13 deficiency (<10%) and presence of anti-ADAMTS13 autoantibodies, also an open ADAMTS13 conformation is a hallmark of an acute aTTP episode.

OC_63

PROFILING OF ANTI-ADAMTS13 ANTIBODIES DERIVED FROM PATIENTS WITH ACQUIRED THROMBOTIC THROMBOCYTOPENIC PURPURA

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Background/aims Acquired Thrombotic Thrombocytopenic Purpura (aTTP) is a thrombotic microangiopathy resulting from

autoantibodies towards ADAMTS13. Most autoantibodies target exosite-3 in the spacer domain, where residues Arg568, Arg660, Tyr661, Tyr665 and Phe592 contribute to binding. Conservative modifications here yielded an autoantibody-resistant gain-of-function ADAMTS13 variant (Jian *et al*., 2012) possessing an open conformation and proteolytic promiscuity (South *et al*., 2016). We created a large set of exosite-3 mutants and evaluated their reactivity against patient-derived antibodies, providing a basis for development of non-promiscuous, autoantibody-resistant ADAMTS13 variants.

Materials and methods Thirty exosite-3 mutants were created including: conservative (F592Y, Y661F, Y665F and all possible combinations thereof); semi-conservative (F or Y→L); non-conservative (F or Y→N; no putative N-glycosylation introduced); and classical mutations (F or Y→A). Variants were expressed in CHO cells employing Icosagen's QMCF technology. Reactivity against human monoclonal antibodies IgG I-9 and II-1 was assessed by ELISA and immunoprecipitation.

Results Alanine mutations resulted in loss of reactivity with II-1 compared to wild-type ADAMTS13; a less pronounced decrease in binding was observed for I-9. Conservative replacements (Y↔F) reduced binding of both antibodies differently. Mutation Y661F strongly reduced II-1 binding whereas binding of I-9 was only modestly reduced. Most semi-conservative mutations resulted in stronger reduction of binding to both antibodies. Non- conservative mutations had a similar outcome as classic alanine mutations. A single mutation F592L or F592N significantly increased binding of I-9 but not of II-1.

Conclusions Our findings document that substitutions within exosite-3 have different effects on the binding of IgG I-9 and II-1. In view of the observed heterogeneity of these antibodies' reactivity, we are currently analyzing patient samples for their binding to these variants. This will provide insight into the heterogeneity of the immune response to the spacer domain in aTTP patients and a basis for the rational design of second-generation autoantibody-resistant ADAMTS13 variants.

OC_54

THE ROLE OF VON WILLEBRAND FACTOR IN EXPERIMENTAL MALARIA-ASSOCIATED ACUTE RESPIRATORY DISTRESS SYNDROME

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Background Malaria is a global health burden resulting in 429,000 deaths in 2015. Recent clinical studies have demonstrated that severe malaria is associated with acute endothelial cell activation, accumulation of highly active von Willebrand factor (VWF) multimers, and a significant reduction in ADAMTS13 activity.

Aims To investigate the role of VWF in malaria using a murine model of malaria-associated acute respiratory distress syndrome.

Materials and methods Wild-type (WT) and VWF knock out ($Vwf^{-/-}$) mice on a C57BL/6J background were inoculated with 104 Plasmodium berghei (Pb) NK65-infected erythrocytes. Blood samples were taken to assess platelet count as well as levels and activities of VWF and ADAMTS13. Giemsa-stained blood smears were made to determine parasitemia. Pulmonary edema was assessed by measuring protein levels in bronchoalveolar lavage fluid.

Results Plasma VWF levels in infected WT mice significantly increased 3 days after infection (2-fold increase; $p<0.0001$), but normalized afterwards. No change in VWF multimer patterns was observed until the end stage (day 8/9) at which high molecular weight VWF multimers were markedly decreased ($p<0.0001$). This was accompanied by a reduction of the ADAMTS13 activity/antigen ratio ($p<0.0001$). Interestingly, severe thrombocytopenia was observed in both WT and $Vwf^{-/-}$ mice, indicating a VWF-independent mechanism. $Vwf^{-/-}$ mice died more rapidly with higher parasitemia compared to WT ($p=0.003$). Alveolar leakage in lungs was significantly lower in $Vwf^{-/-}$ mice ($Vwf^{-/-}$: 1.8±0.4, WT: 3.6±0.5 mg/mL; $p=0.02$).

Conclusion Our data demonstrate that PbNK65-mediated murine malaria infection is associated with early elevated levels of plasma VWF, which is indicative for endothelial cell activation and in accordance with human malaria. Our findings also show that VWF does not contribute to malaria-associated thrombocytopenia. Furthermore, VWF might influence the development of parasitemia and lung pathology, potentially by interfering with the sequestration of infected erythrocytes.

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POSTERS

HEMOPHILIA

P_53

BLEEDING PHENOTYPE AND TARGET JOINTS PREDICT PATIENTS WITH ZERO BLEEDS GIVEN ONCE-WEEKLY PROPHYLAXIS WITH BAY 94-9027

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Background/aims In PROTECT VIII, a partially randomized, open-label, phase 2/3 trial previously treated males aged 12-65 years with severe hemophilia A received BAY 94-9027 for 36 weeks on demand or as prophylaxis at intervals determined after a 10-week run-in period of 25 IU/kg 2×/wk BAY 94-9027 prophylaxis. Patients with ≤1 spontaneous joint or muscle bleed during the run-in were eligible for randomization to every-5th-day (45-60 IU/kg) or every-7th-day (60 IU/kg) prophylaxis for 26 weeks (weeks 11-36). In PROTECT VIII, 60 IU/kg BAY 94-9027 every 7th day was feasible and generally well tolerated in select patients (J Thromb Haemost 2017; 15: 411-9). This analysis aimed to identify clinical predictors for an annualized bleeding rate (ABR) of 0 during every-7th-day prophylaxis with BAY 94-9027 in PROTECT VIII.
Methods In this post hoc analysis, best responders were defined as patients with an ABR of 0 who did not discontinue or change dosing frequency during weeks 11-36.
Results Of the 43 patients randomized to every-7th-day prophylaxis in PROTECT VIII, 11 switched to more frequent dosing after a mean ± SD of 84±38 days (range, 22-131). Sixteen of 32 patients (50%) who remained on every-7th-day prophylaxis had 0 bleeds; 15 patients were responders with an ABR of 0. Responders with an ABR of 0 during weeks 11-36 in the every-7th-day prophylaxis group had fewer total and joint bleeds in the 12-month period before study entry and fewer target joints at baseline compared with patients who switched from every-7th-day prophylaxis to more frequent dosing (Table I). In both groups, most/all patients previously received prophylaxis.
Conclusion The number of bleeds in the previous 12-month period and the number of baseline target joints are clinical indicators for identifying patients who may be suitable for once-weekly prophylaxis with BAY 94-9027.

Table I - Baseline characteristics of patients who received BAY 94-9027 prophylaxis every 7th day.

	Responders with ABR of 0 during every-7th-day prophylaxis (n=15)*	Patients who switched from every-7th-day to more frequent prophylaxis (n=11)	All patients receiving every-7th-day prophylaxis (n=43)
Age, y, median (Q1; Q3)	31.0 (26.0; 52.0)	38.0 (36.0; 47.0)	37.0 (26.0; 50.0)
Number of bleeds in the last 12 months, median (Q1; Q3)	2.0 (0; 11.0)	3.5 (2.0; 6.0)†	3.0 (1.0; 9.0)
Number of joint bleeds in the last 12 months, median (Q1; Q3)	1.0 (0; 11.0)	2.0 (1.0; 5.0)†	2.0 (0; 8.0)
Presence of target joint, n (%)	10 (66.7)	9 (81.8)	31 (72.1)
Number of target joints per patient, median (Q1; Q3)	1.0 (0.0; 3.0)	2.0 (1.0; 4.0)	2.0 (0.0; 3.0)
Previous prophylaxis treatment, n (%)	13 (86.7)	11 (100)	38 (88.4)

*16 patients who remained on every-7th-day prophylaxis had 0 bleeds; 1 patient treated every 7th day discontinued from the study early with 0 bleeds recorded and was not included in the responder group.†Calculated from 10 patients with available data. ABR: annualized bleeding rate.

P_55

BAY 1093884 TARGETS THE KUNITZ DOMAINS 1 AND 2 OF TFPI AND BLOCKS ITS FUNCTION

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**Employed at Bayer at the time this work was performed*

Background/aims BAY 1093884 is a fully human monoclonal antibody against tissue factor pathway inhibitor (TFPI) in development as a bypass agent for patients with hemophilia with or without inhibitors. BAY 1093884 restores a sufficient thrombin burst by blocking TFPI function, which leads to stable clot formation in hemophilic conditions and effectively stops bleeds *in vivo*. In this study, we investigated the mechanism by which BAY 1093884 inhibits TFPI activity.
Materials and methods BAY 1093884-TFPI interactions were studied using x-ray crystallography and surface plasmon resonance (SPR). Functional assessment was performed via thrombin generation assay and diluted prothrombin time (dPT) clotting assay.
Results Co-crystallization of the BAY 1093884-TFPI complex revealed that BAY 1093884 binds at the interface of the Kunitz 1-Kunitz 2 (K1-K2) domains of TFPI. The crystallographic observations were supported by SPR data showing that BAY

1093884 competes with TFPI binding to activated factor VII (FVIIa) bound to soluble tissue factor (TF). In functional assays, BAY 1093884 restored the amidolytic activities of both activated factor X (FXa) and FVIIa bound to soluble TF, corroborating the biophysical studies. BAY 1093884 also improved thrombin generation in a dose-dependent manner in hemophilia A plasma, both in the presence and absence of endothelial cell surfaces.
Peak thrombin with BAY 1093884 was significantly higher than that of BAY 1093889, an antibody that binds only to the K2 domain of TFPI. The faster dPT clotting times of BAY 1093884 were consistent with the thrombin generation assay results.
Conclusions BAY 1093884 binds to the K1-K2 domains of TFPI and inhibits its interactions with both FVIIa and FXa. By targeting both the K1 and K2 domains of TFPI, BAY 1093884 may have an advantage over products that target only the K2 domain of TFPI by increasing the thrombin peak in patients with hemophilia.

P_01

NONACOG BETA PEGOL IN ADULT AND PAEDIATRIC PATIENTS: POOLED DATA FROM THE PARADIGM™ CLINICAL PROGRAMME

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Objective Nonacog beta pegol (N9-GP) is an extended half-life recombinant glycoPEGylated factor IX (FIX). We present pooled N9-GP data from 5 completed trials conducted in previously treated paediatric, adolescent and adult haemophilia B patients (PTPs).
Methods Results from patients who received N9-GP 40 IU/kg (all ages) or 10 IU/kg (adolescent/adult only) once-weekly prophylaxis including treatment of bleeds are summarised.
Results 115 PTPs (FIX ≤2%) were included: 72 adults (18-65 years), 18 adolescents (13-17 years) and 25 children (0-12 years), with a total of 8,801 exposure days to N9-GP. In the phase 3 trials, 30 patients received N9-GP at 10 IU/kg/week, 54 patients received 40 IU/kg/week and 15 were treated on-demand. No inhibitors or thromboembolic events observed. Of 54 (47%) patients treated weekly with 40 IU/kg, 23 (43%) experienced no bleeds. Median overall ABR for all age groups on 40 IU/kg was 1.03 (IQR 0.00-2.89) and median spontaneous ABR was 0.00. ABR was lower in adolescents/adults randomised to 40 IU/kg ($p<0.05$). Overall bleeding success rate was 93%; 87% resolved after a single injection. Adults, adolescents and children showed single-dose (40 IU/kg) half-lives of 83, 89 and 73 hours, respectively, and incremental recoveries of 0.023, 0.020 and 0.016 (IU/mL)/(IU/kg), respectively. Estimated mean steady-state FIX trough levels with weekly 40 IU/kg were ≥0.15 IU/mL in all. 13 adolescent/adult patients receiving 40 IU/kg N9-GP had collectively 20 target joints at study start; by the end of the extension trial, all target joints had resolved. There were significant improvements in quality of life from baseline to end of trial in those receiving 40 IU/kg.
Conclusion N9-GP was well tolerated and effective in preventing bleeding at 40 IU/kg once weekly, maintaining FIX activity levels ≥15% across all age groups. Once weekly prophylaxis resolved existing target joints and improved patient QoL in adults/adolescents.

P_02

NONACOG BETA PEGOL FOR THE PROPHYLACTIC TREATMENT OF CHILDREN WITH HAEMOPHILIA B: INTERIM RESULTS FROM THE PARADIGM™5 CLINICAL TRIAL

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Introduction Nonacog beta pegol (N9-GP) is a recombinant glycoPEGylated factor IX (FIX) developed for the treatment of haemophilia B with an extended half-life compared with conventional FIX products. Here we review new interim findings from ongoing extension of paradigm™5, a non-controlled, phase 3 trial investigating the safety, efficacy and pharmacokinetics of N9-GP for the prophylaxis and treatment of bleeds in previously treated paediatric patients.
Methods The main trial enrolled and treated 25 children (aged ≤12 years) with haemophilia B (FIX ≤2%). Patients were stratified into two age groups: 11 younger (0-6 years) and 11 older (7-12 years) children; and received N9-GP 40 IU/kg once weekly for 52 weeks.
Results No patients developed inhibitors and no unexpected safety concerns were identified (mean treatment period: 2.55 years/patient; total in-trial exposure days: 3,412 [136.5 per patient]). Overall, 69 bleeds were reported in 19 patients, of which 37 bleeds occurred in 15 patients during the main phase (mean treatment period: 0.97 years/patient) and 32 bleeds occurred in 11 patients during the extension phase (1.80 years/patient). A majority of bleeds (60%) occurred >4 days after

the last dose; 2/3 of these (40% of all) were traumatic. Overall proportion of bleeds that showed a successful haemostatic response to treatment was 92.8% (94.4% and 92.2% in younger and older children, respectively) and 85.5% of bleeds resolved after one dose. The estimated mean annualised bleeding rate (ABR) was 1.08 (0.61 and 1.48 in younger and older children, respectively). The estimated mean ABR progressively decreased each 6-month period: 1.60, 1.50, 0.82, 0.82 and 0.33 at 1-6, 7-12, 13-18, 19-24 and >24 months, respectively.
Discussion/conclusion The latest data from the ongoing paradigm™5 extension trial confirm the longer-term safety and efficacy of N9-GP for the prevention and treatment of bleeds in children with haemophilia B.

P_16
MUTATIONAL REPERTOIRE IN THE SIPPET COHORT AND PREDICTION OF FVIII INHIBITOR RISK

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Background/aims *F8* mutation type is the main predictor of inhibitor development in patients with hemophilia A. Mutations expected to allow a residual synthesis of FVIII are likely to play a protective role towards alloantibody formation by inducing immune tolerance. According to the expected full or partial impairment of FVIII synthesis, *F8* mutations are commonly classified as null and non-null; however, there is no consensus in the definition of two groups.
The association existing between mutation type and inhibitor risk was explored in a cohort of 231 patients with severe hemophilia A, enrolled in the SIPPET trial.
Materials and methods Mutational scanning of the *F8* gene was accomplished by long-range PCR, direct sequencing and MLPA. Functional effects of missense and splicing variants were predicted by multiple web-based tools. FVIII antigen levels were measured in patient plasma using the Asserachrom ELISA kit. Kaplan Meier and Cox regression survival analyses were performed to assess the risk of inhibitor development.
Results The genetic defects found in the analyzed patients, consisting of inversions of intron 22 (n=110) and intron 1 (n=6), large deletions (n=16), nonsense (n=38), frameshift (n=28), missense (n=19) and splicing (n=14) mutations, of which 35 previously unreported, were reclassified in null and non-null according to in-silico analyses and FVIII antigen levels.
A 2-fold increase in inhibitor risk development for "in-silico null" mutations compared to "in-silico non-null" mutations (hazard ratio 2.08, 95% confidence interval [CI], 0.84 to 5.17) and a 3.5-fold increase in inhibitor risk development for "antigen negative" mutations compared to "antigen positive" mutations (hazard ratio 3.61, 95% CI, 0.89 to 14.74) were found.
Conclusions Our findings confirm an association between the synthesis of minute amounts of FVIII and inhibitor protection and underline the importance to evaluate plasma expression of FVIII and to investigate *F8* mutations with further in-silico analyses in order to predict the risk of inhibitor development.

P_33
COMPARING THE ONE-STAGE AND CHROMOGENIC ASSAY: FACTOR VIII ACTIVITY ASSAY DISCREPANCY AT BASELINE DOES NOT REFLECT ASSAY DISCREPANCY AFTER DESMOPRESSIN IN NON-SEVERE HEMOPHILIA A PATIENTS

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Background/aims Measurement of factor VIII activity (FVIII) is used to monitor treatment in hemophilia A (HA) patients. Two assays are generally used: the one-stage (OSA) and the chromogenic assay (CSA). Our aim was to investigate discrepancies between the OSA and the CSA after desmopressin administration in non-severe HA patients.
Materials and methods Non-severe HA patients who received a desmopressin test dose (0.3 µg/kg) were included. FVIII was measured with the OSA and the CSA before (T0), 1 (T1) and 4 (T4) hours after desmopressin administration. Assay discrepancy at T0 was defined as a two-fold difference. Desmopressin response was divided into three categories: complete response (CR) (FVIII ≥0.50 IU/mL), partial response (PR) (FVIII 0.30-0.50 IU/mL) and no response (NR) (FVIII <0.30 IU/mL). A difference in response was considered an assay discrepancy.
Results Twenty-nine non-severe HA patients were included with a median age of 41 years (range 6-67). Median FVIII at T0 was 0.20 IU/mL [IQR 0.09-0.27] (OSA) and 0.19 IU/mL [IQR 0.09-0.28] (CSA). At T0 three patients had a discrepancy, all with a higher OSA. Table I shows the desmopressin response for T1 and T4. Discrepancy in response was seen in 24% (n=7) at T1 and in 29% (n=8) at T4. Only one of the discrepant patients of T0 showed a discrepancy in response at T1. Moreover, the median absolute difference between assays increased from 0.03 IU/mL (IQR 0.01-0.07) at T0 to 0.12 IU/mL (IQR 0.03-0.28) at T1. However, the assay resulting in the lowest FVIII at T0 also gave the lowest outcome after desmopressin in all discrepant patients.

Table I - Desmopressin response.

			Chromogenic						
			T1			*T4*			
			NR	*PR*	*CR*	*NR*	*PR*	*CR*	*Total*
One -stage	*T1*	*NR*	2	1*	0				3 (10%)
		PR	1*	3	5*				9 (31%)
		CR	0	0	17				17 (59%)
	T4	*NR*				6	2*	1*	9 (32%)
		PR				1*	3	2*	6 (21%)
		CR				0	2*	11	13 (46%)
		Total	3 (10%)	4 (14)	22 (76%)	7 (25%)	7 (25%)	14 (50%)	

T1: 1 hour after desmopressin administration; T4: 4 hours after desmopressin administration; NR: non-response; PR: partial response; CR: complete response.
* Discrepant patients.

P_62

VON WILLEBRAND FACTOR LEVELS IN PATIENTS WITH HEMOPHILIA A

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Background/aims Given the many different roles of von Willebrand factor (VWF), particularly its possible prothrombotic influence, VWF levels in hemophilia A (HA) patients were measured and correlated to laboratory parameters that assess thrombin generation and to the most important clinical parameters (age at first joint bleed, number of target joints, number of annual joint bleeds and annual FVIII consumption).
Materials and methods Study group comprised 81 patients, 37 severe and 44 non-severe. According to clinical parameters severe group was divided into two subgroups: more severe (N=18) and less severe phenotype (N=18). VWF:RCo was measured by using BCVon-Willebrand Reagent on BCS (Siemens Healthcare, Germany) and VWF:Ag by using VIDAS-VWF (bioMérieux, France). Endogenous Thrombin Potential (ETP-C) was assessed on BCS-XP and prothrombin fragment F1+2 (PF1+2) were measured with Enzygnost/F1+2 (Siemens Healthcare, Germany). ABO blood group was determined for all patients.
Results Significantly higher VWF:Ag (P=0.035) and nearly significantly higher VWF:RCo (P=0.089) were identified in more severe phenotype compared to less severe phenotype subgroup. No correlation between VWF:RCo/VWF:Ag and ETP-C and PF1+2 in severe and non-severe group as well as in patients' subgroups was found. Correlation with clinical parameters revealed weak correlation with number of target joints (r=0.312-0.367, P<0.05). Higher rate of blood group O in HA patients was obtained compared to known distribution in Croatian population (43% *vs* 34%) due to high percentage in non-severe group (52%). Among severe group, blood group O was more frequent in patients with less severe than in more severe phenotype (40% *vs* 25%).
Conclusions Absence of significant correlation with ETP-C, PF1+2 and clinical parameters rule out protective, procoagulant role of VWF in HA patients. Higher VWF levels in patients with more severe phenotype as compared to less severe phenotype can be explained by lower rate of blood group O in more severe subgroup.

P_13

THE ADVANTAGES OF USING PEG-FRACTIONATION IN THE ISOLATION AND PURIFICATION OF FACTOR COAGULATION VIII

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Background The concentrates of coagulation factor VIII (FVIII) are used in the treatment of patients suffering from haemophilia A or von Willebrand disease. The process plasma fractionation is largest industry segment in manufacture of therapeutic concentrate of plasma proteins. Cryoprecipitation is the basic technique most commonly used to recover FVIII from human donor plasma. The modern technologies of purification of factors coagulation consist in combinations of classical methods of protein precipitation and chromatographic steps.
Aims Investigate the effect of using previous methods of protein precipitation in the scheme of obtaining coagulation factor VIII.
Materials and methods Adsorption/precipitation on PEG-4000, ion-exchange chromatography on DEAE-Sepharose, affinity chromatography on the Diasorb-aminopropyl matrix with Active Bright Blue 4K as ligands in combination methods of antiviral treatment (solvent-detergent and chemical method with ammonium thiocyanate).
Results We conducted purification of FVIII from cryoprecipitate with a combination of two chromatographic steps ion exchange chromatography on DEAE-Sepharose and dye-ligand affinity chromatography. In the first case, we conducted additional PEG-precipitation of proteins. We mentioned that in the case of PEG-precipitation the level of purification is the best (the specific activity of FVIII on 219% higher than without PEG). For comparison: 17.33±0.12 IU/mg proteins and 8.44±0.29 IU/mg proteins after ion exchange; 45,45±4.36 IU/mg proteins and 20.82±1.19 IU/mg proteins after affinity chromatography, respectively.
Conclusion Our studies have shown the advantages of using PEG-precipitation in technology of purification of FVIII. In particular: isolation impurity proteins (fibrinogen, fibronectin, lipoproteins, parts denatured proteins, etc.); with ion exchanger not contacted impurity proteins and so specific activity of FVIII in eluate is higher; PEG enhances the antiviral effect of thiocyanate (synergetic effect).
Keywords Coagulation factor VIII, hemophilia A, chromatography, PEG-precipitation.

P_71

APPLICATION OF THE ISTH BLEEDING SCORE IN HEMOPHILIA

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Introduction Hemophilia is an inherited bleeding disorder. With proper treatment and self-care, people with hemophilia can maintain an active, productive lifestyle. Hemophilia can be mild, moderate, or severe, depending on the degree of plasma clotting factor deficiency.
Aim To assess the utility of ISTH-BAT in diagnosis and in determining severity of the bleeding condition in hemophilia patients, compare the bleeding score (BS) in adult and pediatric groups and investigate its association with plasma factor levels.
Methods An observational study. ISTH-BAT was used to calculate BS in a total of 115 patients, 78 with hemophilia A (HA); FVIII deficiency and 37 with hemophilia B (HB); FIX deficiency and in 100 controls.
Results BS was significantly higher in HA and HB patients as compared to controls (P<0.000). However, there was no significant difference in BS between HA and HB patients. BS were significantly lower in pediatric compared to adult groups. BS were significantly higher in severe compared to mild HA patients. Hematomas and hemarthroses were frequent in both HA and HB patients. However, cutaneous, circumcision

bleeding and bleeding after trauma were significantly more frequent in HA patients as compared to HB patients.
Conclusion The ISTH BAT can help diagnose the bleeding condition in hemophilia patients can predict bleeding risk and/or severity. This will ultimately improve the clinical assessment and management of patients. We also believe the tool would be valuable in improving data collection for large studies.
Keywords Hemophilia A and B, ISTH BAT, inherited bleeding disorders.

P_72
HEMOPHILIA CARE IN PAKISTAN
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Introduction Hemophilia is a rare congenital disorder characterized by prolonged bleeding, either spontaneously, or after injury. In the developing world, where majority of the hemophiliacs live, awareness of this disease and its management is poorly done. It is a significant cause of morbidity and mortality and is responsible for psychological, social and economical stress to patients and their families.
Material and methods This is a cross sectional, observational study carried out at the National Institute of Blood Disease Karachi, Pakistan. Adult and pediatric hemophilia A (HA) and hemophilia B (HB) patients of various ages and severity were included. Demographic and management history of patients were recorded and analyzed.
Results A total of 102 male patients diagnosed as HA (n=69) and HB (n=33) were evaluated. Mean age was 15.34±4.75 years. Age at diagnosis ranged from birth to 3 years. History of consanguinity was present in 91% of cases and significant family history of bleeding in 69% of patients. Hemarthrosis and hematoma were more frequent symptoms in these patients. Surgical history including circumcision was done in 55% patients while 4 had major surgeries (hip and femur bones fracture, extensive nasal septum, and head surgery). 29% of patients had transfusion-transmitted infections in which HCV (68%) was most prevalent followed by HBV (11%) and HIV (4%). 14 HA patients (20%) were found to have positive results for inhibitors and none in HB. Treatment included tranexamic acid, fresh frozen plasma, cryoprecipitate, cryosupernatant and factor concentrates on demand basis.
Conclusion Hemophilia A and B are common among congenital bleeding disorders. Availability of poor diagnostic facilities and lack of proper management for this group of patients, often leads to wrong diagnosis and inadequate treatment. Rate of transfusion-transmitted diseases, particularly hepatitis C infection, has gained a huge proportion. Comprehensive haemophilia care centers with multidisciplinary approach needs to be established.

P_78
PRELIMINARY ENROLMENT DATA FROM THE U.S. POST-MARKETING SAFETY (PMS) STUDY OF rpFVIII IN PATIENTS WITH ACQUIRED HEMOPHILIA A
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Background/aim Acquired hemophilia A (AHA) is a rare autoimmune disorder characterized by development of neutralizing autoantibodies to circulating factor VIII (FVIII). Obizur® is a recombinant, B-domain deleted, porcine-sequence FVIII (rpFVIII) for the treatment of AHA with low cross-reactivity human-FVIII. The study will collect data to assess safety and safety-related factors, utilization, and effectiveness of rpFVIII in real-world clinical practice.
Materials and methods This is a multi-center, non-controlled, open-label, non-interventional post-marketing surveillance (PMS) study conducted in the United States on AHA patients treated with rpFVIII: prospective and retrospective data will be collected from approximately 40 patients. Statistical analyses will include, specifically but not exclusively, descriptive statistics.
Results A preliminary, interim data read-out was carried out on Dec 15, 2016, on 7 subjects (4 males and 3 females, 10 bleeds) with AHA recruited in 5 centers: median age 73 years (range 59-75 years). Patient demographics and initial bleeding event characteristics are described in Table I (below). Five of 7 patients were treated with other hemostatic drugs before receiving rpFVIII, while 2 subjects were treated with rpFVIII as first option once diagnosed for the specific bleeding event. The median loading dose was 100.2 IU/kg (range 50-203, mean of 122.3).
Conclusions rpFVIII represents an innovative treatment for AHA subjects and this study, along with the PMS study just started in Europe, will provide data from real world clinical setting on its safety and efficacy profile. Data from real world use of rpFVIII might help with further assessment of dosing regimen and guidance regarding appropriate dosing in order to personalize the treatment for each individual condition.

Table 1

Patient		Description / severity	Location	Specific anatomical location
Pt 1	Male, 64 yrs, 104.3 kg BW	Traumatic, severe	Skin	
Pt 2	Male, 59 yrs, 159.3 kg BW	Traumatic, severe	Deep (musculoskeletal, retroperitoneal)	Retroperitoneal bleed and hematoma on thigh
Pt 3	Female, 74 yrs, 69 kg BW	Traumatic, severe	Deep (musculoskeletal, retroperitoneal)	Left pectoralis major and minor muscles
Pt 4	Male, 75 yrs, 94.9 kg BW	Spontaneous, not severe	Mucosa	Hematuria
Pt 5	Male, 73 yrs, 83 kg BW	Spontaneous, severe	Deep (musculoskeletal, retroperitoneal)	Neck
Pt 6	Female, 73 yrs, 83.7 kg BW	Spontaneous, not severe	Other	Right lower extremity from toes to gluteal region, right upper arm
Pt 7	Female, 75 yrs, 85.1 kg BW	Spontaneous, severe	Deep (musculoskeletal, retroperitoneal)	Left forearm-flexor digitorum profundus muscle and flexor pollicis brevis muscle

P_24

HEPATITIS C VIRAL INFECTION IN PATIENTS WITH HEMOPHILIA: AN EXPERIENCE OF EGE ADULT HAEMOPHILIA CENTER

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Introduction Adults with hemophilia have one of the highest prevalence rates of hepatitis C virus (HCV) among all populations at risk for this disease. HCV management in these population may be complicated by the underlying disease processes. Ribavirin is an essential component of HCV therapy, but new agents may offer a new opportunity of cure.
Methodology Among 294 patients, anti-HCV positivity was found in 39 patients (13.2%). Severity of haemophilia, age, age at the diagnose, comorbidities, smoking history, alcohol consumption, transfusion history of fresh frozen plasma (FFP) and erythrocyte, treatment of HCV infection were recorded.
Results The mean age was found 44 (23-66) and the mean age at the diagnosis was 6 (0.5-52). The patients with severe haemophilia was 77% (n=30) and 87% of them were in treatment of prophylaxis. There were 36 patients (92%) with hemophilia A and 3 (8%) patients with hemophilia B. It was determined that 44% of the patients were diagnosed before 1970 and 49% were diagnosed between 1970-1980. Smoking was positive in 67% of patients and most of the patients (84%) have social level of alcohol consumption. The history of FFP and erythrocyte transfusions were found 95% and 44%. Cirrhosis was found in one patient and he was followed under the treatment of sofosbuvir-ledipasvir. Five patients with HCV RNA positivity were treated with interferon alpha and ribavirin as first-line treatment. Two of these patients did not respond to treatment and switched to sofosbuvir-ledipasvir as a second-line treatment. The level of HCV RNA who received sofosbuvir-ledipasvir were all negative. One patient has recently received a first-line treatment with sofosbuvir-ledipasvir and HCV RNA negativity has not yet been achieved. However one patient was treated as first-line treatment with ritonavir-ombitasvir-paritaprevir and other patient was treated with dasabuvir as second-line treatment. Current HCV RNA levels in these patients are also negative.
Conclusion HCV represents a significant burden for patients with haemophilia. Due to the fact that there is no vaccination to HCV, the disease can only be prevented by taking care of the contamination. The new treatments known as sofosbuvir-ledipasvir and ritonavir-ombitasvir-paritaprevir and dasabuvir are very effective. But clinical trials are needed to assess the efficacy and tolerability of direct-acting antiviral agents in these patient groups.

P_30

CEREBRAL ISCHEMIC INFARCT AS A RARE FIRST PRESENTATION OF HEMOPHILIA A IN A NEWBORN: A CASE REPORT

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Introduction The middle cerebral artery is the most common site of ischemic infarcts, occurring due to a blockage in blood flow through one of its supplying arteries. In the neonatal period, conditions predisposing to ischemic infarcts are thrombotic events, secondary to an underlying congenital or acquired thrombotic state. In newborns with severe hemophilia A, the most frequently reported complication is cerebral hemorrhage; however, intracranial hemorrhages in full term neonates with hemophilia A are uncommon.
Case outline We are reporting the case of a 1 y/o male with severe hemophilia A, presenting during the neonatal period with a left middle cerebral artery ischemic infarct. He was born to an hemophilia A obligate-carrier mother, at term, by spontaneous vaginal delivery. APGAR score was 8 and 9 at 1 and 5 minutes, respectively. Patient presented with right cephalohematoma, despite no history of vacuum or forceps use. At 3 days old, patient developed progressive hypoactivity, severe facial swelling and pallor, requiring admission to NICU. He was found to have severe anemia and PRBC were transfused. A brain MRI showed large MCA ischemic infarct and a large right cephalohematoma. Antihemophilic factor VIII was begun immediately. During hospital course, patient also developed seizures, requiring mechanical ventilation for CNS protection. Pro-thrombotic workup was negative and factor VIII activity was <1%. Due to difficulty weaning from mechanical ventilator, a follow up head CT was done at day 7, showing bilateral subdural hematomas, with left MCA and left PCA territory ischemic infarct and brain edema. A medport catheter was placed, and patient started on prophylaxis with long-acting recombinant factor VIII once a week. Currently, patient is achieving milestones adequately, and without evidence of inhibitors.
Conclusion This case depicts a newborn with severe hemophilia A presenting with cerebral ischemic infarct and intracranial bleeding, both rare presentations and complications of hemophilia.

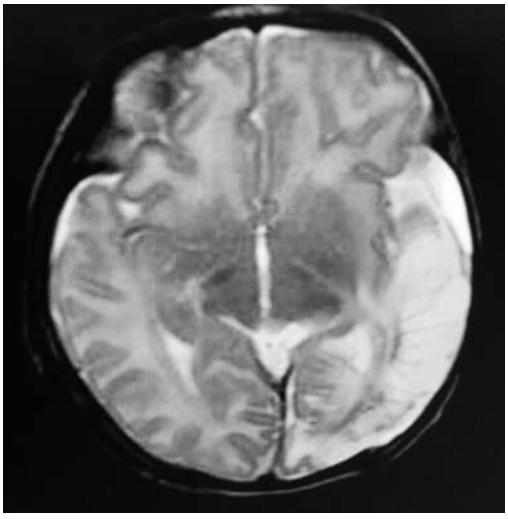

Figure 1

P_60
DEVELOPMENT OF INHIBITORS IN PUPS: DIFFERENT SCENARIO, SAME OUTCOME…
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Background Inhibitors in previously untreated patients (PUPs) are mainly determined by underlying mutations in *F8* gene. However, numerous environmental related risk factors exist that may increase the risk of inhibitor formation.
Aim To present genetic and non-genetic risk factors involved in the development of inhibitors in one family and discuss the outcome.
Case no. 1 A 1-year-old boy presented to ER with a huge hematoma in his right thigh region. Following lab results he was diagnosed with severe hemophilia A (FVIII:C <1%). Family history was negative, however a 3-year-old brother sometimes had bruising. The patient was treated by pdFVIII/VWF* (around 40 IU/kg) and received 6 ED for his bleeding. Following 10 days, the patient presented again to ER because of traumatic bleeding from gums lasting for 3 days. His Hb was 39 g/l and he received RBCs transfusion and 2 additional ED of pdFVIII. Following 2nd episode of bleeding the patient started prophylaxis with pdFVIII 40 IU/kg 1 time/week. He developed high titer inhibitors (62.4 BU/ml) after 10 ED to FVIII.
Case no. 2 A 3-year-old patient's brother following lab results was diagnosed with severe hemophilia A. Until the age of 3 years he has not experienced any severe bleeds except bruising. DNA analysis revealed an intron-22 inversion in both brothers and mother. Prophylaxis was initiated with rFVIII product 20 IU/kg × 1 time/week. Following 11 ED the patient developed high titer inhibitors (92 BU/ml).
Conclusions Despite totally different environmental risk factors for the development of inhibitors in both patients, they resulted in the same outcome: high titer inhibitors. This single-family case report illustrates that despite the progress in understanding of how inhibitors develop along with known risk factors, this knowledge does not uncover complexity of antibody response against FVIII. Therefore, better understanding of pathways involved in this process is needed.

P_87
ERADICATING AN OLD INHIBITOR: ELIMINATION OF A FACTOR VIII INHIBITOR IN A PATIENT WITH CONGENITAL HEMOPHILIA A
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Background Hemophilia A (HA) is a congenital deficiency of clotting factor VIII resulting in spontaneous, life-threatening bleeds. Treatment for patients with severe HA is prophylaxis treatment with FVIII replacement therapy. However, the risk of inhibitor formation (an antibody to exogenous FVIII replacement therapy) in patients started on regular prophylaxis is approximately 30% with increased and prolonged bleeding events resulting in worsened morbidity, mortality and quality of life. Immune tolerance induction (ITI) therapy has traditionally been the only means of eradicating an inhibitor.
Case A 12-month old infant was first diagnosed with severe hemophilia A (FVIII <1%) when he presented with a forehead hematoma; he was started on weekly prophylaxis at this time but developed a low-titer inhibitor shortly thereafter. His family was unwilling to pursue ITI at this time. Over the next decade, he suffered multiple severe bleeds leaving him 50% wheelchair bound. In August 2005, he developed a right forearm compartment syndrome requiring emergent fasciotomy and resultant skin graft. At this time, he agreed to ITI. Despite every other day high dose FVIII infusions for 4 years, his inhibitor persisted. ITI therapy was discontinued and resultant bleeds were managed with on-demand recombinant VIIa infusions. In June 2014, after suffering another severe knee bleed with a prolonged hospitalization, he agreed to a reattempt at eliminating his inhibitor. He was started on weekly rituximab therapy in combination with high-dose FVIII replacement therapy; his FVIII inhibitor titer measured 4 Bethesda Units (BU). He received 5×1,000 mg doses of weekly rituximab and started daily high-dose FVIII replacement therapy at 150 U/kg. After 5 doses of rituximab, his inhibitor titer measured 0 BU. Three months later, his FVIII therapy was titrated down to 100 U/kg daily and continued for 2 months. He was then titrated down to 100 U/kg FVIII therapy every other day for 12 months. In December 2015, while still on high-dose FVIII therapy, he developed a measurable 1 BU titer and was treated with a single dose of 1,000 mg rituximab. To date, no further inhibitor has been measured. He is now on every other day prophylaxis at 50 U/kg.
Discussion Rituximab, an anti-CD20 agent has demonstrated efficacy in the treatment of many B-cell mediated disorders. Yet, in the treatment of inhibitors in patients with congenital hemophilia A, it is still under investigation - both in its efficacy, longevity in effect and applicability depending on titer levels. This example defines a successful example of upfront use of rituximab and, as might be expected with an autoimmune-mediated process, the necessary observation and maintenance treatment needs over time.

VON WILLEBRAND FACTOR (VWF) AND VON WILLEBRAND DISEASE (VWD)

P_42

EFFICACY, SAFETY, AND PRESURGICAL PHARMACOKINETICS OF RECOMBINANT VWF (rVWF) IN PATIENTS WITH SEVERE VWD WHO ARE UNDERGOING ELECTIVE SURGICAL PROCEDURES

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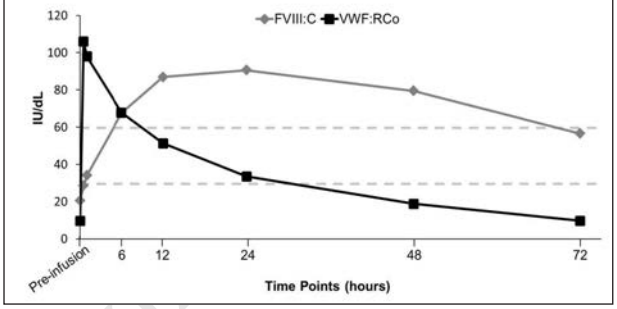
Background/aims rVWF (VONVENDI) may be administered independently of FVIII, potentially minimizing the risk of thrombotic events. This phase 3 study evaluated the hemostatic efficacy and safety of rVWF - with or without coadministered rFVIII (ADVATE) - in patients with severe VWD undergoing elective surgery. Baseline PK analysis was conducted to assess the pharmacodynamic effects of rVWF on endogenous FVIII levels.

Materials and methods rVWF was given 12-24 hours before surgery to raise endogenous FVIII:C; if target FVIII:C levels were not achieved, rFVIII was given with the preoperative rVWF dose within 1 h before the procedure. Hemostatic efficacy was assessed 24 hours after the last rVWF infusion or day 14, whichever was earlier, using a 4-point nominal scale ("none" to "excellent"). Eleven patients also underwent an initial 72-hour presurgical PK assessment.

Results All 15 surgeries (10 major, 4 minor, and 1 oral) managed with rVWF with or without rFVIII had overall and intraoperative hemostatic efficacy rated excellent (73.3%/86.7%, respectively) or good (26.7%/13.3%). In major surgeries, overall and intraoperative hemostatic efficacy were excellent (70%/80%, respectively) or good (30%/20%). Two-thirds of patients (10/15) received rVWF alone, and 11/104 (10.6%) infusions required rFVIII coadministration (intra- and postoperative only). Most major surgeries (7/10) did not require rFVIII coadministration. PK analysis results demonstrated substantial, rapid increases in FVIII:C levels at 6-12 hours (peak, 24 hours) after rVWF infusion for all patients assessed (Figure), with similar results seen among patients with type 3 VWD (n=5). No treatment-related AEs, severe allergic reactions, or neutralizing antibodies to rFVIII or rVWF were reported.

Conclusions These data support the safety and efficacy of rVWF with or without rFVIII in patients with VWD undergoing surgery. The ability to target the primary dysfunction of VWD and adjust VWF:RCo to the desired levels may reduce the risk of FVIII overaccumulation while maintaining excellent/good hemostasis.

Figure. PK analysis results for mean VWF:RCo and FVIII:C levels in response to rVWF 50±5 IU rVWF:RCo/kg in patients with VWD undergoing surgery (n=11). Target endogenous FVIII:C levels (30 IU/dL for minor/oral surgery or 60 IU/dL for major surgery) are indicated by dashed horizontal lines.



P_77

STABILIN-2 DEFICIENCY INCREASES PRO-COAGULANT ACTIVITY AND DEEP VEIN THROMBOSIS IN MICE

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Background/aim Stabilin-2 is an endocytic scavenger receptor expressed on liver sinusoidal endothelial cells. Variants in the human STAB2 gene associate with plasma levels of VWF:Ag, FVIII:C, and an increased incidence of venous thromboembolism. We previously characterized stabilin-2 as a clearance receptor for human but not murine VWF. The aim of this study was to characterize the influence of stabilin-2 on deep vein thrombosis (DVT) in a murine model.

Materials and methods DVT was induced by inferior vena cava ligation in wild-type C57BL/6 (WT) and STAB2 KO mice. Thrombi were weighed and longitudinal sections were analyzed by quantitative IHC 24h post-stenosis. Plasma coagulation factors levels were measured by ELISA, activity assay or thrombin generation assay (TGA). Tail-vein transections (TVTs) were used to characterize murine hemostasis.

Results STAB2 KO mice developed significantly larger thrombi than WT mice (194%, n=19, p=0.003) but did not alter DVT incidence. Thrombi from STAB2 KO mice were comprised of more red thrombus (185%, p=0.004) but comparable white thrombus to WT mice. STAB2 KO thrombi contained significantly more fibrin (130%, p=0.040) and leukocytes (146%, p=0.039) but not platelets than WT thrombi. STAB2 KO mice had significantly elevated baseline leukocyte counts (165%, p<0.0001), including monocytes (152%, p=0.0014) and granulocytes (167%, p=0.0002).

Erythrocyte counts were unchanged but platelet levels were decreased (89%, p=0.009) compared to WT mice. STAB2 KO

mice had elevated plasma levels of FVIII:C (118%, p=0.0006), but not VWF:Ag or fibrinogen. Using TGA, STAB2 KO plasma had significantly shorter lagtime (73%, p=0.012) and time to peak thrombin (74%, p=0.006), whereas peak thrombin (144%, p=0.029) and velocity index (167%, p=0.009) were significantly elevated compared to WT plasma. STAB2 KO mice did not demonstrate altered hemostasis in a TVT model (p=0.34).
Conclusions This data suggests that stabilin-2 regulates plasma levels of clotting factors and leukocytes and its deficiency is associated with larger and qualitatively distinct venous thrombi.

P_17
LABORATORY DIAGNOSIS OF VON WILLEBRAND DISEASE TYPE 2A *VS* LVAD-INDUCED ACQUIRED VON WILLEBRAND SYNDROME

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Background Patients suffering from the bleeding disorder von Willebrand disease (VWD) type 2A are diagnosed by severely reduced ratios of VWF:CB/VWF:Ag (<0.7) and VWF:RCo/VWF:Ag (<0.7) and severely decreased high molecular weight (HMW) VWF multimers (0-15%). Patients with implanted left ventricular assist devices (LVAD) have a bleeding diathesis diagnosed as acquired von Willebrand syndrome (aVWS). Laboratory diagnosis of a defect in VWF in these patients is less obvious as their VWF:CB/VWF:Ag, VWF:RCo/VWF:Ag ratios and HMW VWF multimers do not always meet the criteria for clear VWD diagnosis.
Aim Side by side comparison of VWF:CB/VWF:Ag, VWF:RCo/VWF:Ag and HMW VWF multimers of patients with VWD type 2A and LVAD-induced aVWS.
Methods Plasma samples from 9 VWD type 2A and 14 LVAD patients were analysed for VWF:Ag, VWF:CB and VWF:RCo using ELISA and for VWF multimers using SDS agarose gel electrophoresis and compared to plasma of healthy subjects (NHP). Ratios and percentages are represented as median (with interquartile ranges).
Results All VWD type 2A patients had a clear laboratory diagnosis of VWD. VWF:CB/VWF:Ag (0.0 [0.0-0.31]) and VWF:RCo/VWF:Ag (0.15 [0.12-0.64]) ratios were clearly below 0.7 and HMW VWF multimers were severely reduced (0.0% [0.0-12.29] *vs* 32.63% [30.08-33.24] in NHP, p=0.0002). In contrast, VWF defects were less pronounced in LVAD patients. These patients had slightly reduced VWF:CB/VWF:Ag (0.76 [0.71-1.03] *vs* 1.00 [0.98-1.02] in NHP) and normal VWF:RCo/VWF:Ag (1.00 [0.87-1.21] *vs* 1.01 [0.99-1.04] in NHP) ratios. HMW VWF multimers were moderately decreased (20.39% [18.02-22.51] compared to 32.63% [30.08-33.24] in NHP, p=0.0012).
Conclusions Laboratory diagnosis of LVAD-induced aVWS is less obvious than VWD type 2A. Only HMW VWF multimers are significantly reduced making VWF multimer analysis the most sensitive way to diagnose LVAD-induced aVWS. Hence, careful analysis of VWF parameters in LVAD patients is important to recognize aVWS in these patients.

P_49
DIFFERENTIAL DIAGNOSIS BETWEEN TYPE 2A AND 2B VWD IN A 2-YEARS OLD FEMALE CHILD WITH A *DE NOVO* NOVEL MUTATION

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Background A 2-years old female child with a bleeding history of easy bruising, prolonged bleeding after minor wound and recurrent epistaxis was evaluated for a bleeding disorder. Partial biochemical characterization led to type-2A or 2B VWD. The patient showed normal platelet count (540,000/mm^3) and FVIII:C (56 IU/dL) with reduced VWF:Ag, VWF: GPIbR and VWF:CB levels (33, 6 and 3 IU/dL, respectively), whereas her parents and sister had normal VWF levels. Multimeric analysis of patient's plasma at low-resolution showed the lack of high and intermediate molecular weight multimers, while the intermediate-resolution showed increased satellite bands. Although RIPA is able to discriminate type-2B from type-2A, we do not usually perform it in pediatric patients.
Aim To perform VWD differential diagnosis using phenotype and genotype characterization.
Materials and methods Sanger sequencing of exon 28 and short tandem repeats (STR) I and II in intron 41 were carried out on patient and her family members. The VWF:GPIbM assay was performed as previously described using an ELISA method (Stufano *et al.*; JTH 2015).
Results A novel in-frame deletion of six nucleotides (c.4606_4611delCACGTC; p.H1536_V1537del), localized in the A2 domain, was identified at the heterozygous state only in the patient. The proband and her sister shared the same alleles (STR-I/STR-II 101:162 bp; STR-I/STR-II 121:170 bp), identified also in their parents, suggesting a *de novo* origin of the mutation. VWF:GPIbM assay was performed for proband's plasma along with those of type-2A and 2B patients used as controls. VWF:GPIbM results were in line with those of VWF:GPIbR for the proband and type-2A controls, but markedly increased for type-2B controls (Table I).
Conclusions This is a rare case of *de novo* VWF mutation causing type-2A VWD. Both patient's platelet count and the localization of the p.H1536_V1537del mutation (A2 domain) suggested the type-2A VWD diagnosis, although confirmation was obtained using the VWF:GPIbM/VWF:GPIbR ratio.

Table I

Patient	Type	Amino acid change	VWF:Ag (IU/dL)	VWF:GPIbR (IU/dL)	VWF:GPIbM (IU/dL)	VWF:GPIbM/ VWF:GPIbR
Proband	2A or 2B	p.[H1536_V1537del];[=]	33	6	6	1
Control 1	2A	p.[S1506L];[=]	24	8	10	1.3
Control 2	2A	p.[R1597Q];[=]	39	7	9	1.3
Control 3	2B	p.[R1306W];[=]	35	5	42	8.4
Control 4	2B	p.[V1316M];[=]	34	8	34	4.5

VWF:Ag, von Willebrand factor antigen;
VWF:GPIbR, Ristocetin-triggered GPIb binding assay;
VWF:GPIbM, VWF gain-of-function mutant GPIb binding assay;

Mutations are reported following the guidelines of the Human Genome Variation Society (http://varnomen.hgvs.org). Plasma samples of type 2A and 2B VWD patients with VWF levels and multimeric patterns comparable to those of the proband were used as controls.

P_50

PLATELET FUNCTION ANALYZER MEASUREMENT OF CLOSURE TIME AS A BIOMARKER FOR ACTIVITY OF HIGH AND ULTRALARGE MULTIMERS OF RECOMBINANT VON WILLEBRAND FACTOR (rVWF)

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Background/aims Recombinant von Willebrand factor (rVWF), licensed in the US under the brand name VONVENDI™, has the multimeric distribution of freshly secreted VWF with ultralarge (UL) and high molecular weight multimers (HMW) from endothelial cells and megakaryocytes since it has never been in contact with ADAMTS13 or any other proteolytic enzyme. The platelet function analyzer-200 (PFA-200) is highly sensitive in detecting UL-HMW VWF multimers. We hypothesized that (a) von Willebrand patients' whole blood samples, spiked with rVWF shows a normalization in PFA closure time (PFA-CT) and (b) that a dose-response relationship could be demonstrated.
Materials and methods Twelve patients diagnosed with von Willebrand disease (VWD) were selected. A therapeutic dose of rVWF product (1 IU/ml) was spiked in VWD patients' whole blood samples and PFA-CTs were measured. Further, PFA-CTs under incremental doses of rVWF (0.1, 0.2 and 0.5 IU/ml) were investigated.
Results The PFA-CTs were normalized in VWD patients' whole blood samples spiked with rVWF. Also, incremental doses of rVWF resulted in a progressive and dose dependent correction of PFA-CT.
Conclusion Spiking experiments of rVWF in VWD plasma indicate that the platelet function analyzer-200 is a useful tool to detect rVWF activity. As the PFA-CT correction is dose dependent rVWF might be reliably monitored with PFA-200 as a point-of-care analytical method during replacement therapy.

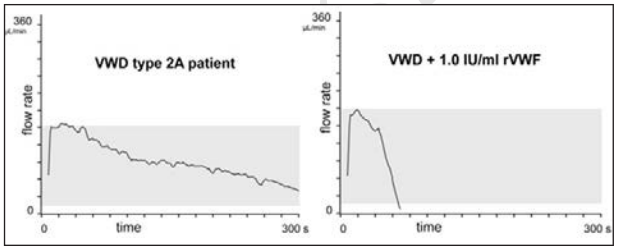

Figure 1 - Example of closure curves from the PFA-200 with cartridges containing collagen and ADP illustrating the comparison of a plasma from a VWD type 2A patient before (left) and after spiking with 1 IU/ml rVWF (right).

P_59

USEFULNESS OF VON WILLEBRAND FACTOR PROPEPTIDE IN THE DIFFERENTIAL DIAGNOSIS BETWEEN VON WILLEBRAND DISEASE AND ACQUIRED VON WILLEBRAND SYNDROME

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An increased von Willebrand factor (VWF) propeptide (VWFpp) to VWF antigen (VWF:Ag) ratio (VWFpp/VWF:Ag) indicates an enhanced clearance of VWF. This finding has been described in both von Willebrand disease (VWD) and acquired von Willebrand syndrome (AVWS). A distinction between these two diseases is primarily based on family and personal history of bleeding, but might be challenging sometimes for the lack of a diagnostic biomarker. In this cross-sectional study we assessed the ability of VWFpp/VWF:Ag in the differential diagnosis between VWD and AVWS. VWFpp/VWF:Ag was measured in a group of 153 patients (125 with VWD and 28 with AVWS). A receiver operating characteristic curve was used to assess the optimal cut-off of VWFpp/VWF:Ag for discrimination of patients with a modest increase (most VWD) *vs* those with a markedly increase (VWD type 1 Vicenza and AVWS) of VWF clearance.
The large majority of VWD and AVWS patients showed an increased VWFpp/VWF:Ag, although at different extent. A markedly increase of VWFpp/VWF:Ag was mainly associated with AVWS and VWD type 1 Vicenza diagnosis.
The best cut-off value of VWFpp/VWF:Ag for the discrimination of patients with modest increase *vs* patients with markedly increase of VWF clearance was 3.9 (sensitivity 0.70, specificity 0.97) (Figure 1). A further molecular evaluation can discriminate VWD type 1 Vicenza from AVWS.
In conclusion, VWFpp/VWF:Ag is helpful to discriminate patients with a markedly increase (AVWS or VWD type 1 Vicenza) from those with a modestly increase of VWF clearance (most VWD).
The ROC curve was obtained from a logistic model containing VWFpp/VWF:Ag, age and sex.
Keywords VWD, von Willebrand disease; AVWS, acquired von Willebrand Syndrome; VWF, von Willebrand factor.

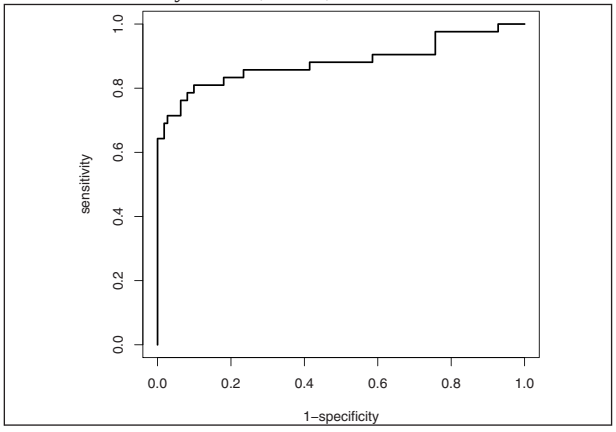

Figure 1 - Receiver operating characteristic (ROC) curve for discrimination of patients with lower VWF clearance (most VWD) from those with higher VWF clearance (VWD type 1 Vicenza and AVWS).

P_69

LARGE IN-FRAME DELETIONS CONTRIBUTE TO TYPE 1 VON WILLEBRAND DISEASE PATHOGENESIS THROUGH DIFFERENT MECHANISMS

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Background The Zimmerman Program on the Molecular and Clinical Biology of von Willebrand disease identified point mutations and large deletions in patients with reduced VWF plasma levels. Multiplex ligation-dependent probe amplification and microarray of the entire VWF locus were used to identify dosage mutations. Of 150 index cases, 3 patients had a previously identified exon 4-5 in-frame deletion, whilst three others had novel in-frame deletions of exons 3, 32-34 and 33-34.
Methods Each novel deletion was prepared using site-directed mutagenesis of full-length VWF plasmids. HEK293 cells were transiently transfected with mutant and/or wild-type (WT) recombinant VWF (rVWF). 72 h post transfection, cells were fixed and stained with fluorescent antibodies for imaging with widefield or structured illumination microscopy (SIM).
Results When transfected, WT rVWF formed numerous pseudo-Weibel-Palade bodies (WPB) close to/emerging from Golgi/trans-Golgi network. These were clearly visible elongated VWF-positive structures. Homozygous rVWF3del produced a diffuse staining pattern co-localised with the ER marker calnexin, indicating endoplasmic reticulum (ER) VWF retention. Homozygous rVWF32-34del also showed ER-localised VWF staining and did not form WPB. Widefield microscopy identified VWF-positive structures that were different to the ordered appearance of pseudo-WPB. SIM confirmed this and revealed formation of VWF-positive puncta, or VWF aggregates. In contrast, homozygous rVWF33-34del produced pseudo-WPB similar to WT, although smaller/rounder pseudo-WPB structures were present in this mutant, similar structures were present in WT. Following co-transfection with WT rVWF, VWF WPB storage was partially restored for rVWF3del and rVWF32-34del, although pseudo-WPB from these co-transfections were fewer, shorter and rounder than WT.
Conclusions The data presented demonstrates how CNV causing VWD1 leads to reduced VWF secretion as a result of defective VWF processing and storage. SIM revealed significant variation in the morphology of pseudo-WPB structures for each of the three in-frame deletion mutants.

P_11

ENHANCED LOCAL DISORDER IN A CLINICALLY ELUSIVE VON WILLEBRAND FACTOR PROVOKES HIGH-AFFINITY PLATELET CLUMPING

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Mutation of the cysteines forming the disulfide loop of the platelet GPIbα adhesive A1 domain of von Willebrand factor causes quantitative VWF deficiencies in the blood and von Willebrand disease.
We report two cases of transient severe thrombocytopenia induced by DDAVP-treatment. Cys1272Trp and Cys1458Tyr mutations identified by genetic sequencing implicate an abnormal gain-of-function phenotype, evidenced by thrombocytopenia, that quickly relapses back to normal platelet counts and deficient plasma VWF. Using surface plasmon resonance, analytical rheology, and hydrogen-deuterium exchange mass spectrometry (HXMS), we decipher mechanisms of A1-GPIbα mediated platelet adhesion and resolve dynamic secondary structure elements that regulate the binding pathway.
Constrained by the disulfide, conformational selection between weak and tight binding states of A1 takes precedence and drives normal platelet adhesion to VWF. Less restrained through mutation, loss of the disulfide preferentially diverts binding through an induced-fit disease pathway enabling high-affinity GPIbα binding and firm platelet adhesion to a partially disordered A1 domain. HXMS reveals a dynamic asymmetry of flexible and ordered regions common to both variants indicating that the partially disordered A1 lacking the disulfide retains native-like structural dynamics.
Both binding mechanisms share common structural and thermodynamic properties, but the enhanced local disorder in the disease state perpetuates high-affinity platelet agglutination, characteristic of type 2B VWD, upon DDAVP-stimulated secretion of VWF leading to transient thrombocytopenia and a subsequent deficiency of plasma VWF, characteristic of type 2A VWD.

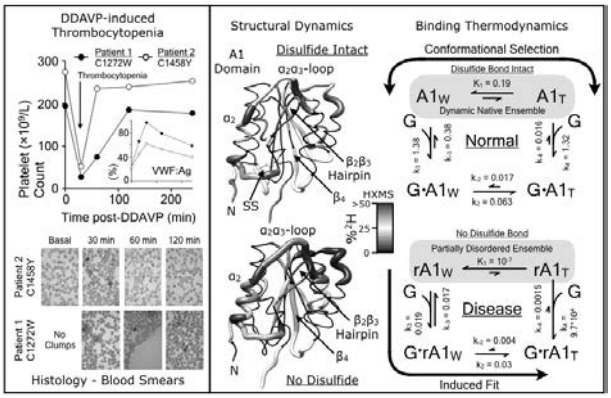

Figure 1

Table I

Time post-DDAVP	Basal	30 min	60 min	120 min	240 min
Patient 1, p.Cys1272Trp, c.3816C→G, 6.3 yrs.					
Platelets (×10⁹/L)	194	27*	74*	185	177
Mean Platelet Volume (fL)	11.6	13.2	13.1	11.1	11
VWF:Ag (%)	24	66	97	79	59
FVIII:C (%)	23	53	52	47	35
VWF:CB (%)	<5	11	10	7	<5
VWF:RCo (%)	<5	<5	<5	<5	<5
aPTT (sec)	47.6	38.1	36.9	37.3	43.8
PT (%)	94	93	89	93	92
Patient 2, p.Cys1458Tyr, c.4373G→A, 9.6 yrs.					
Platelets (×10⁹/L)	274	52*	235	239	253
Mean Platelet Volume (fL)	10	12.7	10.1	9.6	9.5
VWF:Ag (%)	24	45	62	53	40
FVIII:C (%)	34	181	85	64	44
VWF:CB (%)	<5	22	9	<5	<5
VWF:RCo (%)	<5	<5	<5	<5	<5
aPTT (sec)	51.6	43.7	43.3	46.6	50.4
PT (%)	72	68	67	68	69

P_31

PREOPERATIVE VWF IS PREDICTIVE OF PERIOPERATIVE THROMBOSIS IN INFANTS AND NEONATES WITH CONGENITAL HEART DISEASE

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Background Perioperative thrombosis remains a leading source of morbidity and mortality in the management of congenital heart disease (CHD), the etiology of which is multifactorial and incompletely described. An imbalance between von Willebrand Factor (VWF) and ADAMTS13 has been increasingly linked to diverse forms of thrombosis.
Objectives To assess associations between VWF, ADAMTS13 and NETosis and the occurrence of postoperative thrombosis in patients undergoing repair of congenital heart lesions.
Methods Pediatric patients undergoing repair of congenital heart disease were recruited (n=133). The plasma levels of VWF, ADAMTS13 and markers of NETosis were measured pre and postoperatively. Patients were followed for up to 30 days for the occurrence of thrombosis. Logistic regression analyses were conducted to identify variables that associate with the occurrence of thrombosis.
Results Across diverse CHD populations, we found significant postoperative increases in VWF activity, VWF concentration, DNA-histone complexes and cell-free DNA accompanied by an overall decrease in ADAMTS13 activity. Lower intraoperative temperature, higher preoperative lactic acid levels and higher preoperative VWF activity and concentration were found among patients experiencing postoperative thrombotic events (n=12). The level of preoperative VWF activity (OR 9.19, CI: 1.90-43.60) and the transfusion of cryoprecipitate (OR 1.09, CI: 1.02-1.15) were found to be independently associated with thrombosis.
Conclusions Neonates and infants with CHD undergoing surgical repair or palliation are exposed to high levels of VWF in the immediate postoperative period. The level of preoperative VWF appears to carry independent predictive value for the occurrence of postoperative thrombosis.

P_40

A CELL-BASED ASSAY TO QUANTIFY VON WILLEBRAND FACTOR MUTANT BINDING TO INTEGRIN αIIbβ3

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Background/aims Integrin αIIbβ3 is a major constituent of the platelet membrane that critically mediates platelet adhesion and aggregation. It specifically recognizes the arginine-glycine-aspartic acid (RGD) sequence present in several adhesive proteins. Upon platelet activation, the complex undergoes conformational changes that permit ligand binding, e.g. to fibronectin, vitronectin, thrombospondin, fibrinogen, and von Willebrand factor (VWF).
VWF is a large multi-domain plasma glycoprotein essential to primary hemostasis. VWF can directly interact with platelets, as it exhibits binding sites for GPIbα, which is part of the platelet membrane receptor GPIb-IX-V (binding site located in the VWF A1 domain), and for αIIbβ3 (via the RGD sequence in C4 domain).
We strive to investigate the influence of von Willebrand disease-associated mutations in the VWF C-domains on interaction with platelets via αIIbβ3.
Methods We developed a cell-based binding assay by stably co-transfecting HEK293 cells with the two integrins αIIb and β3. To mimic activation of the complex, a constitutively active mutant was generated by introducing a single missense mutation in β3.
Binding was determined by addition of the αIIbβ3-presenting cells to immuno-adsorbed VWF. The complex was detected by anti-αIIbβ3 and an HRP-coupled secondary antibody. After addition of the HRP substrate (3,3',5,5'-tetramethylbenzidine), optical absorbance was measured at 450 nm using an plate reader for quantification of αIIbβ3 binding.
Results We validated our assays by using wildtype (wt) VWF and mutant p.Asp2509Gly, in which the RGD sequence was inactivated, as a negative control. While wtVWF was bound effectively, mutant p.Asp2509Gly exhibited no binding to the αIIbβ3-presenting cells. We further investigated binding of the seven C-domain mutants Cys2257Arg, p.Arg2287Trp, p.Arg2313His, p.Arg2464Cys, p.Gly2518Ser, p.Arg2663Pro and p.Cys2671Tyr. Binding was significantly decreased for p.Gly2518Ser and Cys2257Arg.
Conclusion We successfully established and validated a cell-based assay that allows quantification of binding of RGD-motif containing proteins to plasma membrane incorporated integrin αIIbβ3.

P_43

VARIABILITY IN BLOOD OUTGROWTH ENDOTHELIAL CELL CHARACTERISTICS AND RELATED VON WILLEBRAND FACTOR PARAMETERS

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Background and aim Blood outgrowth endothelial cells (BOECs) are mature endothelial cells derived from peripheral blood. BOECs are a powerful tool to study pathophysiological

mechanisms of vascular diseases, like von Willebrand disease (VWD). In prior research, however, large variations in von Willebrand factor (VWF) expression were observed between BOECs. To confidently use BOECs to study VWD pathophysiology, we need to understand the cause of these variations and their effects on VWF-related parameters. Therefore we aim to understand the relation between the phenotypic characteristics and VWF-related parameters of BOECs from healthy controls.

Methods Cultures of separate colonies (n=16) of BOECs derived from six donors were established. All experiments were performed on passage four BOECs confluent for four days. VWF:Ag secretion was measured by ELISA and cell density was determined by ImageJ (ITCN plugin). The angiogenic potential was evaluated using the matrigel angiogenesis assay. Surface marker expression of endothelial, angiogenic and stem cell markers was determined by FACS. Gene expression was determined by qPCR for VWF-related genes, and genes related to angiogenesis, ageing and endothelial to mesenchymal transition.

Results Endothelial lineage of all BOECs was confirmed by FACS. High variability was observed in cell density, VWF:Ag levels and percentage of CD34, CD133 and VEGFR2 positive cells. Interestingly, cell density significantly correlated with VWF:Ag levels (R^2=0.90, P<0.0001), but also with total tube length and percentage CD133 positive cells.

Gene expression levels of mesenchymal marker αSMA and anti-coagulant marker thrombomodulin showed significant correlations with cell density.

Conclusion VWF:Ag secretion in confluent BOECs significantly correlate with cell density, with higher VWF:Ag levels measured in cultures of smaller cells. And although all cell lines proved to be true endothelial cells, the results suggest more mesenchymal phenotypes for cell lines with lower cell densities. This study provides mechanistical insight in the variations of BOECs from healthy controls.

P_51

RECOMBINANT HUMAN VON WILLEBRAND FACTOR HAS A UNIQUE PATTERN OF ULTRA LARGE MULTIMERS: RESULTS FROM PHYSICO-, BIOCHEMICAL AND *IN VIVO* STUDIES

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Background/aims Ultra large multimers (ULM) of VWF are the most active and therefore are of critical importance for the function of VWF in stabilizing the primary hemostatic plug. In contrast to plasma-derived FVIII-VWF concentrates, human rVWF obtained from mammalian cell culture retains the full-spectrum of intact multimers, including ULM, as physiologically stored in platelet α-granules and endothelial cell Weibel-Palade bodies.

Materials and methods VWF is a highly purified human VWF produced in CHO cells. Tapping mode atomic force microscopy (AFM) was used for imaging the structure of rVWF and rVWF-rFVIII molecular interactions as well as the visualization of cleavage by ADAMTS13. Contribution of multimer size was investigated by fractions containing distinct portions of VWF multimers that were generated from rVWF and analyzed for their ability to mediate platelet adhesion under shear stress. Pharmacokinetics and efficacy of rVWF compared to that of a pdVWF-FVIII preparation was evaluated in VWF knock-out mice.

Results In AFM large multimeric filaments appeared to be more prominent in the images of rVWF than in pdVWF. Spiking blood with rVWF resulted in an increased platelet adhesion to collagen type I. When different rVWF fractions were used to promote platelet adhesion it was seen that multimer size of rVWF contributed to platelet adhesion. Terminal half-life of rVWF was longer than that of pdVWF. rVWF stabilized endogenous FVIII in VWD mice as seen by a secondary rise in murine FVIII which lasted longer in mice treated with rVWF than with pdVWF. Only rVWF in combination with rFVIII was able to reduce blood loss upon tail cutting while pdFVIII-VWF did not show any effect on bleeding.

Conclusion Physico- and biochemical, functional and animal studies exhibited superiority of rVWF in structure and function compared to pdVWF. Most of these effects correlated with the multimer size and therefore could be attributed to the presence of ULM in rVWF preparations.

P_27

LABORATORY TESTS FOR EVALUATION OF VON WILLEBRAND DISEASES

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Background Many people with von Willebrand disease (vWD) have mild signs and symptoms and this condition can be difficult to diagnose. Timely laboratory diagnosis of vWD is very important for successful treatment and prevention of excessive bleeding episodes. It is necessary to use combination of specific laboratory tests in order to diagnose or rule out vWD.

Aim To evaluate the prevalence of vWD in our population.

Materials and methods 150 patients with anamnesis of epistaxis, bleeding after tooth extraction or menorrhagia. The several specific blood tests are used: platelet count (MEDONIC counter), vWF antigen level (vWF:Ag), ristocetin cofactor activity (vWF:RCo) and FVIII clotting activity (fVIII:C) performed with automated BCS XP Siemens coagulometer and ristocetin-induced platelet aggregation (RIPA, Chrono-Log 700).

Results Platelet count: 293.64±70.17×10^9/L. vWF:Ag was decreased (29.72%±16.7 IJ/dl) in 32/150 (21%) patients and 118 patients had normal values (106.40%±47.33). vWF:RCo was decreased (35.49%±13.71IJ/dl) in 39/150 (26%) patients while normal vWF:RCo activity (83.65%±31.55 IJ/dl) was present in 111 patients. Six out of 150 patients (4%) had decreased FVIII:C (31.83+11.22 IJ/dl), normal FVIII:C (117.36±30.79 IJ/dl) was present in 144 patients. Decreased RIPA (28.72±22.62%) was detected in 32/150 (21%) patients while normal values (83.35±25.72%) were measured in 118 patients. Eleven patients (7%) had simultaneously decreased vWF:Ag (30.09±21 IJ/dl), vWF:RCo (33.18±14.49 IJ/dl) and RIPA (24.36±19.30%). Three patients (2%) had simultaneously decreased FVIII:C (42.67%±2.22), vWF:RCo (13.33%±1.11), vWF:Ag (12%±2.67) and RIPA (10%±8). Only one patient had

decreased FVIII:C=27 IJ/dl, vWF:Ag=10 IJ/dl, vWF:RCo=3IJ/dl and normal RIPA=106%.
Conclusion Current tools for the accurate diagnosis of VWD and recent developments that may improve its diagnosis in the future are discussed in this review. When to suspect the VWD it is very important to know how to evaluate the results from mentioned laboratory assays and to understand the principles of management according algorithm to determine the type of VWD.

P_75
QUANTITATIVE ELISA ASSAY FOR *IN VIVO* PROTEOLYSIS OF VON WILLEBRAND FACTOR AND BLEEDING: A PILOT STUDY IN TYPE 2A(IIA) VON WILLEBRAND DISEASE

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Background Mutations within the A2 domain of VWF inducing type 2A(IIA) VWD, are classified according to their mechanism: abnormal assembly and secretion (group 1), excessive proteolysis (group 2) or unspecified (group 3). The characterization of the mechanism for type 2A(IIA) VWD could help evaluating the bleeding tendency.
Aim To investigate the relation between the bleeding tendency and VWF proteolysis in patients with 2A(IIA) VWD.
Methods We have developed an ELISA assay to measure VWF-proteolysis. Results are expressed as the proportion of proteolysed-VWF (%). VWF-proteolysis in normal plasma was 62 (%) (meanSD). This quantification was performed in 87 patients of the French cohort of VWD identified with a molecular defect in the VWF-A2 domain resulting in type 2A(IIA) VWD (group 1, n=14; group 2, n=39; group 3, n=34). The bleeding score (BS), a history of epistaxis or gastro-intestinal (GI) bleeding was available for 71 of them. Results are expressed as median [± interquartile].
Results There was a significant difference in VWF-proteolysis between groups (106% [79-155] group 1, *vs* 80% [66-98] group 2 *vs* 48% [32-83] group 3, ANOVA: p<0.01). The higher the proteolysis was, the higher the BS was (*t*-test: p=0.03). No difference in BS was observed between groups. GI-bleeds (Item >3 of the BS) were reported in 17/71 patients (24%) patients with no difference between groups. A history of epistaxis was found in 33/71 patients (46%) and appears to be more frequent in group 1 patients (9/10-90%) compared to groups 2 (17/30, 56%) and 3 (13/26, 50%), respectively.
Conclusion This study underlines the phenotypic heterogeneity of the proteolysis associated with the mutations of VWF-A2 domain in type 2A(IIA)VWD. These results confirm the data previously obtained with mutant recombinant VWF pointing the combination of several mechanisms. Further studies involving more patients, especially patients of group 1 in whom the bleeding tendency appear the most severe, are needed to confirm these preliminary results.

P_84
A NOVEL MACROPHAGE-MEDIATED PATHWAY REGULATES ENHANCED CLEARANCE OF HYPOSIALYLATED VON WILLEBRAND FACTOR *IN VIVO*

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Background Von Willebrand Factor (VWF) is a plasma sialoglycoprotein which plays a critical role in haemostasis. While the biosynthesis and function of VWF are well characterized, the mechanisms underlying VWF clearance remain poorly understood. However, increased clearance is important in the etiology of von Willebrand disease. Previous studies have suggested that loss of terminal sialic acid causes enhanced VWF clearance through the asialoglycoprotein receptor (ASGPR) on hepatocytes.
Aim In this study we investigated whether additional lectin receptors and/or cell types contribute to the reduced half-life of hyposialylated VWF.
Methods To define the mechanisms involved in the clearance of hyposialylated VWF, $VWF^{-/-}$ and $Asgr1^{-/-}$ mice were crossed creating a novel dual $VWF^{-/-}/Asgr1^{-/-}$ knockout model. Human VWF (pdVWF) was modified using specific neuraminidases creating two glycoforms; α2-3-NeuVWF and α2-3,6,8,9-NeuVWF. *In vivo* clearance of these glycoforms was studied in $VWF^{-/-}/Asgr1^{-/-}$ mice.
Results We observed that the markedly enhanced clearance of α2-3-NeuVWF and α2-3,6,8,9-NeuVWF are still observed in $VWF^{-/-}/Asgr1^{-/-}$ mice. ($T½$= 8.2±0.6 and 3.2±0.4 *vs* 50.6±2 mins for pdVWF, respectively). The short half-life of α2-3-NeuVWF is of particular interest given that α2-3-linked sialic acid is predominantly O-linked and accounts for less than 20% of total sialic acid expression. Importantly, the enhanced clearance of hyposialylated VWF variants in $VWF^{-/-}/Asgr1^{-/-}$ mice was reduced in the presence of asialoorsomucoid. Furthermore, immunohistochemistry demonstrated localization of asialo-VWF within hepatic macrophages. In keeping with this asialo-VWF demonstrated increased binding to THP1macrophages. Finally, macrophage depletion with liposomal clodronate significantly attenuated the enhanced clearance of hyposialylated VWF in $VWF^{-/-}/ASGPR1^{-/-}$mice.
Conclusion These data demonstrate that additional previously unrecognized ASGPR-independent asialo-receptors contribute to enhanced clearance of hyposiaylated VWF. Additionally, this study demonstrates that a novel macrophage-dependent pathway is important in the clearance of hyposialylated VWF. Given that quantitative variations in N- and O-linked sialylation has been described in specific patient cohorts, these findings are of direct clinical importance.

P_45
THE FIRST MOLECULAR STUDY OF VWD IN TUNISIA
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Background VWD type 2N is a rare qualitative VW type disease. It is due to a hyperaffinity of VWFF to FVIII. We suspected a type 2N VWD in a woman with FVIII deficiency, normal level of VWF Ag and VWDCO with menorrhagia.
Aims Confirmation of a type 2N VWD with the molecular study.
Material and methods Recovering was performed with rFVIII and plasmatic derived FVIII+VWF. Study of VWF affinity was not available. In order to confirm diagnosis a molecular study was initiated and a PCR-sequencing procedure was used for the concerned exons.
Results Result of recoveries show no response with rFVIII while observed increase of level of FVIII was remarked with plasma-derived product with VW. Sequencing result showed a substitution of C by T (c.3414C>T) located in exon 26. Our patient was homozygous for this substitution which is a reported synonymous mutation (p.1138N/N).
Conclusions Since the identified mutation is reported as a benign mutation, further molecular investigations are needed in order to confirm the type 2N in our patient.

RARE BLEEDING DISORDERS

P_20
CHEMICAL CHAPERONES IMPROVE BIOSYNTHESIS AND SECRETION OF TWO FACTOR VII MUTANTS
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Background The *F7* gene mutations p.Q160R (FVII-160R) and p.A354V-p.P464Hfs (FVII-354V-464Hfs) are associated with very low circulating factor VII (FVII) levels and a bleeding phenotype. We have previously demonstrated *in vitro* reduced secretion of the recombinant (r) variants rFVII-160R and rFVII-354V-464Hfs due to intracellular retention and increased association with endoplasmic reticulum (ER) chaperones. Additionally, we observed increased ER stress and activation of the unfolded protein response (UPR). Chemical chaperones are compounds that can stabilize misfolded proteins and improve the protein-folding capacity of cells, thereby increasing protein secretion and reducing ER stress.
Aims To explore whether chemical chaperones can improve secretion of rFVII-160R and rFVII-354V-464Hfs and alleviate ER stress in an *in vitro* model of FVII deficiency.
Materials and methods Chinese hamster ovary (CHO-K1) cells stably expressing rFVII-160R or rFVII-354V-464Hfs were treated with the chemical chaperones sodium phenylbutyrate (4-PBA), betaine, taurine, taurourosdeoxycholic acid (TUDCA), trimethylamine N-oxide (TMAO) or Lumacaftor (VX-809). Intracellular and secreted levels of FVII antigen were measured by ELISA. FVII activity will be tested in a functional assay based on a fluorogenic substrate. Intracellular localization of the mutant FVII will be assessed by confocal immunofluorescence microscopy. ER stress and UPR will be investigated by Western analysis and qRT-PCR.
Results Treatment of rFVII-160R expressing cells with 4-PBA for 48 hours, but not VX-809, strongly increased the levels of FVII in the culture medium. Similar studies with cells expressing the rFVII-354V-464Hfs mutant and also studies on potential decrease of ER stress are currently in progress.
Conclusions As of today, our preliminary results indicate that chemical chaperones might represent a highly relevant therapeutic approach to recover normal folding and biological function of the studied mutant FVII proteins.

P_39
SIMULTANEOUS MEASUREMENT OF THROMBIN AND PLASMIN GENERATION IN PATIENTS WITH FACTOR XI DEFICIENCY
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Background/aim Factor XI (FXI) deficiency is a rare bleeding disorder with an unexplained heterogeneous bleeding tendency. In the past both thrombin generation and fibrinolysis assays have been performed and correlated to the bleeding tendency with conflicting results. The aim of this study was to establish the capacity of a haemostasis assay, measuring thrombin and plasmin simultaneously, to predict the bleeding risk in patients with FXI deficiency.
Methods The Nijmegen Haemostasis assay (NHA), a simultaneous thrombin/plasmin generation assay, was performed in a group of seven patients with severe FXI deficiency (≤2%). The parameters to assess thrombin and plasmin generation were: initiation (expressed as lag-time, thrombin peak time) propagation (thrombin peak height, area under the curve), and termination (expressed as fibrin lysis time (FLT), plasmin peak height and plasmin potential). The parameters are expressed as a percentage compared to normal pooled plasma of 40 healthy individuals.
Results Figure 1 shows normal results and an example from one of the patients. Initiation was reduced the lag-time ratio was reduced in the patient group to 60% ±34%. In contrast to propagation parameters, all within the normal range, fibrinolysis parameters were diminished. FLT ratio of 57% ±14% ($p<0.001$) and plasmin potential of 54% ±30% ($p<0.01$). This indicates changes in initiation of thrombin generation in patients with FXI deficiency as well as increased fibrinolysis. Four patients who experienced spontaneous bleeding showed a trend towards accelerated fibrinolysis compared to the 3 patients with no spontaneous bleeding, indicated by lower FLT ratio (53% *vs* 63%) and higher plasmin peak height of 127% and 87% respectively. As expected, there was no correlation

between FXI activity and the incidence of spontaneous bleeding symptoms.

Conclusion Simultaneous measurement of thrombin and plasmin generation has the potential to determine the bleeding tendency in patients with FXI deficiency. Further measurements in a larger cohort are warranted.

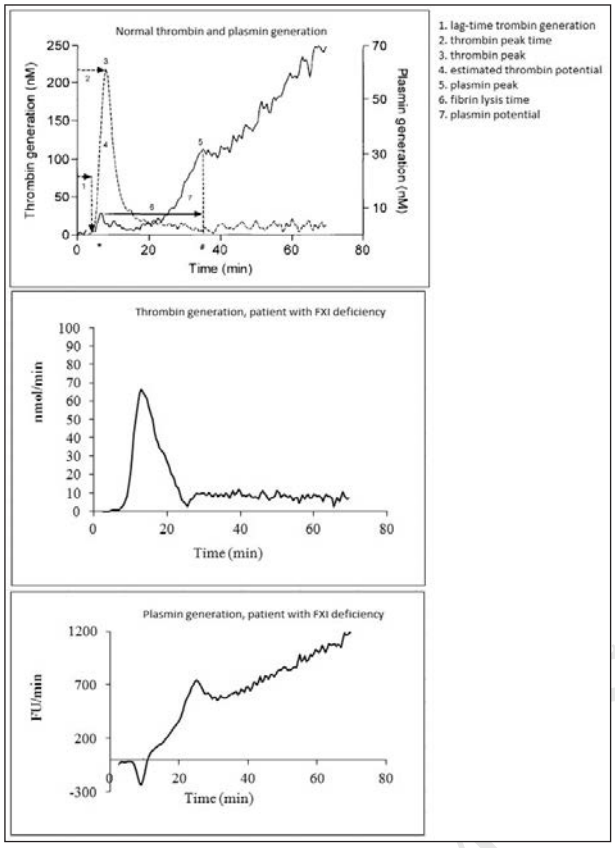

Figure 1

P_06

EFFECTIVENESS AND SAFETY OF rFVIIA IN PAEDIATRIC GLANZMANN THROMBASTHENIA PATIENTS: DATA FROM THE INTERNATIONAL GLANZMANN THROMBASTHENIA REGISTRY

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Introduction Platelet transfusion is standard treatment for Glanzmann Thrombasthenia (GT); recombinant activated factor VII (rFVIIa; NovoSeven®) is known to be effective in GT patients with platelet antibodies and refractoriness to platelet transfusions. We report data from the GT Registry (GTR) on the effectiveness and safety of rFVIIa when used to treat and prevent surgical and non-surgical bleeds in those children with or without platelet antibodies and/or refractoriness.

Methods Data for children aged <18 years were prospectively collected in the GTR, an international, multicentre, observational, post-marketing study of rFVIIa. Effectiveness analyses were based on all patients and treatment-allocated bleeds (n=634) for which efficacy outcomes were known; all patients and bleeds (n=643) were included in safety analyses.

Results Between 2007 and 2011, 27 children were treated for 44 surgical procedures (minor 36, major 8); 104 treated for 599 non-surgical bleeds (severe 145, moderate 454; spontaneous 423, post-traumatic 176). Half of all minor procedures (18/36) were treated with rFVIIa, either alone or in combination with antifibrinolytics (AF) or platelets (P)±AF (other 50.0% received P±AF or AF), while major procedures were treated most frequently with rFVIIa+P±AF (3/8; 37.5%). Of 590 non-surgical bleeds evaluated for effectiveness, 205 (34.7%) were treated with rFVIIa alone or in combination with AF or P±AF (other 65.3% received P±AF or AF); effectiveness for minor procedures/major procedures/non-surgical bleeds was 100.0%/100.0%/89.3% for rFVIIa alone, 100.0%/100.0%/84.2% for rFVIIa+AF, 91.7%/100.0%/75.7% for P±AF, and 83.3%/0.0%/73.3% for rFVIIa+P±AF. Of 25 adverse events (AEs) reported from the 643 admissions included in the safety analysis, nine occurred in rFVIIa-treated patients, but all unlikely to be related to rFVIIa. There were no thromboembolic events.

Discussion/conclusion Regardless of platelet antibody or refractoriness status, rFVIIa (with or without P±AF) provided effective haemostasis with a low frequency of AEs when used for surgery and to treat non-surgical bleeds in paediatric GT patients.

P_38

RARE BLEEDING DISORDERS IN THE NETHERLANDS

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Background/aims Rare bleeding disorders (deficiencies of fibrinogen, factor II, V, V & VIII, VII, X, XI, XIII, α2-antiplasmin or plasminogen activator inhibitor 1) have a diverse clinical presentation, varying bleeding scores, bleeding episodes, health-related quality of life and laboratory parameters. Therefore, correlations between genotype and phenotype

are difficult to establish. The aim of RBIN is to describe the epidemiology, bleeding tendency, laboratory parameters, quality of life and molecular genetic spectrum of all known patients in the Netherlands with rare bleeding disorders (RBD). In addition, the study aims to further explore the relationship between clinical and laboratory phenotype and genotype.

Methods Cross-sectional multicentre observational study in all patients registered in Dutch Haemophilia Treatment Centers (HTC) with known RBD, aged 1-99 years. After informed consent patients are asked to fill out questionnaires on bleeding, socio-demographic characteristics, clinical characteristics (bleeding tendency, treatment, development of antibodies), medical history (hospital admissions, medication used), needle phobia, sports and physical activity, quality of life and functional limitations. ISTH-BAT and other bleeding assessment tools will be performed and validated for RBD. Blood and saliva samples will be collected for laboratory testing, including the Nijmegen Haemostasis Assay. Whole exome sequencing will be performed to unravel the patients genotype.

Currently our WES strategy includes 136 bleeding related OMIM-proved genes, which will be further expanded when indicated.

Results Inclusion of patients for the Rare Bleeding disorders in the Netherlands (RBIN) study will start in 2017. So far, 342 patients have been identified in all HTC in the Netherlands.

Conclusion The RBIN study will provide new insight in the relationship between the clinical and laboratory phenotype and the genotype in rare bleeding disorders.

P_48

WHOLE EXOME SEQUENCING AS A FIRST TIER TEST IN DIAGNOSING PRIMARY BLEEDING DISORDERS: WES FIRST

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Background/aims Inherited disorders of the hemostatic system are characterized by increased bleeding or thrombosis because of genetic defects in one or more components of the hemostatic balance. Currently at least 136 genes are described as causative for hemostatic disorders (OMIM database) and whole exome sequencing (WES) allows for the analysis of all these genes in one single test. Particularly in complex, heterogeneous or rare diseases in which the traditional diagnostic pathway is cumbersome, expensive and often inconclusive, WES promises to speed up and improve diagnosis. The aim of WES First is to assess the diagnostic relevance of upfront WES with a pre-defined 136-gene panel in patients with a suspected inherited bleeding disorder.

Materials and methods WES First is a multicenter randomised controlled trial in 103 adult patients with a suspected inherited bleeding disorder, and includes a cost-minimisation study. Randomisation will be stratified according to ISTH-BAT score (see patient flow in Figure 1). The primary outcome is the difference in the proportion of patients with a definitive diagnosis six months after inclusion. Secondary outcomes are: costs; quality of life; time to diagnosis; number of venipunctures; number of visits; the proportion of patients without a diagnosis after WES up-front; the proportion of patients in whom a diagnosis can be established through the conventional diagnostic pathway after a negative WES; the proportion of patients without a diagnosis after the conventional diagnostic pathway; and the proportion of patients in whom a diagnosis can be established by WES if no diagnosis was made through the conventional diagnostic pathway.

Results and conclusion Inclusion will start early 2018 in three Dutch Hemophilia Treatment Centers. Additional participating centers are welcomed. First results are expected in 2020. WES First will evaluate whether WES can be used upfront in the diagnostic workup of people with a suspected inherited bleeding disorder.

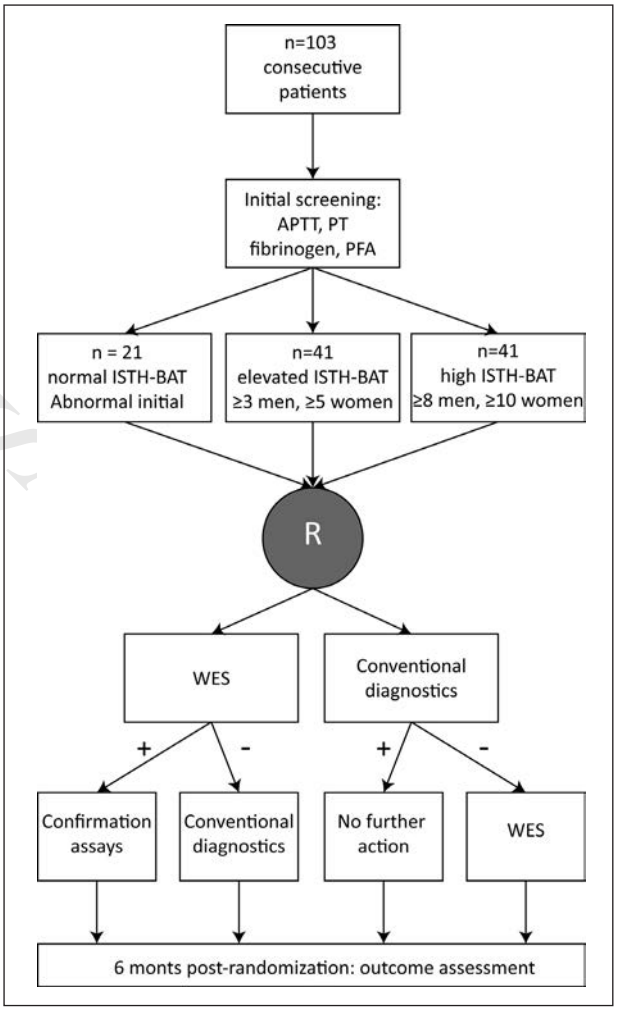

Figure 1

P_70
PREVALENCE OF MENORRHAGIA IN WOMEN DIAGNOSED WITH CONGENITAL BLEEDING DISORDERS
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Introduction Menorrhagia is defined as abnormally heavy or prolonged bleeding during menstrual cycle and blood loss exceeding 80 ml per cycle. It is the most common symptom in women of reproductive age with congenital bleeding disorders. However, there is paucity of data on the incidence, diagnosis and treatment of bleeding in women with these disorders in developing countries.
Objective Here we report the frequency, clinical picture and types of congenital bleeding disorders among women with menorrhagia.
Material and methods An observational study was carried out amongst females diagnosed with congenital bleeding disorders. Frequencies of bleeding disorders with respect to menorrhagia were calculated and compared.
Results A total of 116 females with congenital bleeding disorders, including factor deficiencies (FI, FV, FVII, FXI, FXIII), platelet function disorders such as Glanzmann's Thrombasthenia (GT), Bernard Soulier Syndrome (BSS), and Storage pool disorder (SPD), and Von Willebrand disease (VWD) were studied. The mean age was 12.69±10.13 years. Of the entire cohort, 65 (56%) females were found to be in the reproductive age and out of them 41 (63%) had menorrhagia with mean age of 22.2±7.4 years. Menorrhagia was more commonly observed in BSS, FI, FV, FXIII and SPD patients followed by VWD, FVII and GT. Pictorial blood assessment chart (PBAC) > 100 were observed in all subjects. We intended to observe the association of frequency of menorrhagia amongst all bleeding disorders and it was found that menorrhagia was evenly prevalent in all bleeding disorders.
Conclusion These results demonstrate that menorrhagia is prevalent among all congenital bleeding disorders. Therefore, women, especially in the reproductive age with menorrhagia, should be further evaluated for hemostatic disorders.

P_03
ACQUIRED FACTOR V INHIBITION DUE TO BACTRIM THERAPY - A PREVIOUSLY UNREPORTED CAUSE OF A RARE COAGULOPATHY
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A seventy-year old male with a background of end-stage kidney disease requiring chronic haemodialysis and essential thrombocythaemia for which he was receiving anagrelide was admitted to hospital for an elective surgical procedure. Prior to surgery the patient developed a productive cough, fevers and dyspnoea and was initiated on bactrim antibiotic therapy due to the growth of *Stenotrophomonas spp*. from a sputum culture. The patient's infective symptoms improved however blood testing revealed a markedly abnormal coagulation profile with peak prothrombin time and activated partial thromboplastin time of 40 and 96 seconds respectively. A coagulation profile taken prior to the initiation of antibiotics had been normal. Mixing studies done at the time showed only partial correction and there was no improvement of the coagulopathy with high dose intravenous vitamin K. Lupus anticoagulant testing was negative. Testing of coagulation factors revealed normal activity levels of factor II, VII and X however factor V levels were markedly depressed at 6% (50-150). The presence of a factor V inhibitor was confirmed using the Bethesda assay (5 BU/mL). A thorough review of medications revealed that bactrim had been the only new medication initiated during that admission and there had been no change to the patient's regular haemodialysis prescription.
Coagulation studies began to improve the day after cessation of bactrim with normalisation of PT, APTT and factor V levels within thirteen days of drug cessation. The patient remained well throughout their admission without any overt evidence of significant bleeding and they required no specific treatment beyond removal of the offending agent. Acquired factor V inhibitors are rare causes of coagulopathy with the majority of cases being reported in association with products containing bovine factor V. To our knowledge there has been no previously reported association between bactrim and the development of factor V inhibitors.

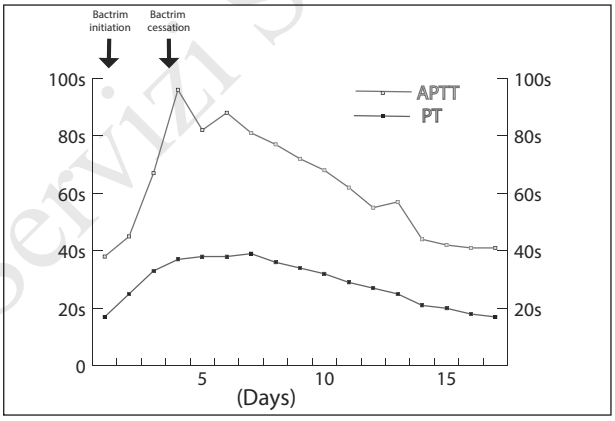

Figure 1 - Graph representing the rise in PT and APTT after the initiation of bactrim on day 0.

P_28
THE RARE COAGULATION FACTOR DEFICIENCIES: SINGLE CENTRE EXPERIENCE
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Introduction The rare coagulation factor deficiencies (RCFDs) are defined as monogenic bleeding disorders caused by deficiency of a soluble coagulation factor or factors, other than von Willebrand disease, haemophilia A or haemophilia B. RCFDs are usually caused by recessive inheritance. The overall frequency of these disorders in the general population is low (with the exception of factor XI deficiency). Homozygous deficiency varies from 1 in 500,000 for factor VII deficiency to 1 in 2 million for prothrombin. RCFDs are more common in ethnic groups in which consanguineous partnerships are common such as Turkey. Registration of the RCFDs data is very important to reveal the real prevalence of the regions.
Objective The aim of the current work is to study the demographic characteristics, clinical presentations and management of RCFDs in Ege Adult Haemophilia Centre adult patients.

Method In this study, 50 patients with RCFD were evaluated retrospectively. Age, diagnosis, deficient factor levels, general coagulation parameters, family history, bleeding symptoms and severity were recorded for each patient.
Results The median age was 37 (18-70); 28 were female (56%) and 22 were male (44%). Factor VII deficiency was the most frequent factor deficiency (42%) with 21 of the 50 patients. Factor XI deficiency was found in 9 patients (18%) and afibrinogenemia/ dysfibrinogenemia in 6 patients (12%), factor V deficiency in 4 patients (8%), factor XII deficiency in 3 patients (6%), factor XIII deficiency in 3 patients (6%), factor X deficiency in 2 patients (4%) and vitamin K dependent multiple factor deficiency in 2 patients (4%) respectively. According to factor activity levels almost half of the patients have (46%) mild factor deficiency. Severe deficiencies were found 20% and moderate was 34%. Fifteen patients had positive family history (30%). The clinical spectrum varied from mild mucocutaneous bleeding to serious sight-threatening haemorrhage. But most of the patients (90%) have only mild mucocutaneous bleeding. These patients were diagnosed during routine or preoperative examinations and a minority (14%) were diagnosed due to family history.
Conclusion RCFDs should be considered in patients with bleeding history. Due to their rarity, there is currently a limited database of these diseases and the treatment. Establishing a national/regional registry of RCFDs in collaboration with other centres is very important to improve the knowledge of RCFDs.

THROMBOTIC MICROANGIOPATHIES (TTP-HUS)

P_52

IMMUNOGLOBULIN G SUBCLASS DISTRIBUTION OF ANTI-ADAMTS13 ANTIBODIES AND ITS ASSOCIATION WITH HLA-DR-DQ HAPLOTYPES AND CLINICAL COURSE IN ACQUIRED THROMBOTICTHROMBOCYTOPENIC PURPURA

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Background The acquired form of thrombotic thrombocytopenic purpura (TTP) is an autoimmune disease, in which ADAMTS13 deficiency is caused by autoantibodies. The genetic background (various HLA-DR-DQ risk and protective haplotypes) influences the risk of developing TTP. The anti-ADAMTS13 autoantibodies are predominantly of the IgG isotype, the subtype distribution of these autoantibodies may affect the pathomechanism and clinical course of the disease.
Our aim was to investigate the associations of the amount and subclass distribution of the anti-ADAMTS13 autoantibodies with the risk and protective HLA-DR-DQ haplotypes and with the clinical course of TTP.
Patients and methods We determined the amount of anti-ADAMTS13 IgG antibodies with the Technozym® ADAMTS-13 INH kit and the subclass distribution with an in-house ELISA method in 104 ADAMTS13-deficient samples of 82 acquired TTP patients. HLA-DR-DQ haplotypes were determined in 70 of the above patients.
Results We observed that the anti-ADAMTS13 IgG levels were higher in patients carrying protective haplotypes, compared to those not carrying protective haplotypes (medians: 280.4 U/mL *vs* 65.3 U/mL). IgG4-predominance was observed in all relapse samples, while samples from the first episode were either IgG1- or IgG4 dominant. The percentage of IgG1 and IgG3 subclasses were lower in relapsing patients, while that of IgG4 was higher. Conversely, the relative amount of IgG3 was higher, and that of IgG4 was lower in patients, who deceased during the episode.
Conclusions We found an association between the genetic background of TTP and the amount of anti-ADAMTS13 autoantibodies. We hypothesise that higher antibody levels might be needed in patients carrying protective haplotypes to evoke the disease. Our results regarding the associations between IgG subclass distribution and the clinical course are in line with those of previous studies. The identification of the mechanism of switch from IgG1-dominance to IgG4-dominance would be an interesting topic of further research.

P_23

CONGENITAL (HEREDITARY) THROMBOTIC THROMBOCYTOPENIC PURPURA (cTTP [hTTP], UPSHAW-SCHULMAN SYNDROME): PATIENT EXPERIENCE, CONCEPTUAL FRAMEWORK, AND PATIENT-REPORTED OUTCOME (PRO) INSTRUMENT DEVELOPMENT

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Background/aims Patients with congenital thrombotic thrombocytopenic purpura (cTTP) (severe ADAMTS13 deficiency) exhibit microangiopathic hemolytic anemia, thrombocytopenia, and diverse signs and symptoms. Prophylactic plasma infusions or factor VIII/von Willebrand factor concentrates may reduce the incidence of acute events, but no cTTP-specific patient-reported outcome (PRO) instrument exists to measure other outcomes important to patients. Our aim was to examine the patient perspective, create a conceptual model of disease burden, and develop a cTTP-specific PRO instrument.
Materials and methods Literature was reviewed to formulate a preliminary conceptual model of cTTP signs, symptoms, and impacts. This model was revised following interviews with five cTTP-treating hematologists (US, UK, Austria) and 11 adult patients (US). Currently available PRO instruments were evaluated for coverage of the most salient symptoms and impacts, and to inform item generation. Two waves of cognitive debriefing patient interviews (n=10) were conducted to revise the instrument, which then underwent linguistic validation.
Results The new 26-item instrument assesses the concepts considered most salient by patients: intensity of fatigue, pain (joint, muscle, abdominal, chest), and bruising; frequency of cognitive impairment, vision problems, and headache; impact on daily activities; frequency of depression, anger, irritability, frustration, anxiety, and mood swings; severity of treatment-related effects; and treatment-related worry, travel burden, and

time missed from work/school. Many of these concepts are not captured by comparator instruments. Linguistic validation demonstrated accuracy of translation and clear understanding of the meaning and intent of the instrument in 10 languages; validation in other countries is continuing.

Conclusions Patients with cTTP experience high disease burden with the current standard of care. They confirmed that the newly developed PRO instrument assesses the salient symptoms and impacts, and is appropriate, comprehensive, and understandable. Further research is underway to assess the psychometric properties of this instrument and its sensitivity to treatment effects.

P_46

EVALUATION OF A FULLY-AUTOMATED CHEMILUMINESCENT IMMUNOASSAY FOR THE RAPID QUANTIFICATION OF ADAMTS13 ACTIVITY AND THE DETECTION OF ADAMTS13 INHIBITORS

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Background Severe deficiency of ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, 13) activity is associated with thrombotic thrombocytopenic purpura (TTP). In acquired TTP, severe deficiency is caused by ADAMTS13 inhibitory autoantibodies. Functional assays that measure ADAMTS13 activity can also be used to detect ADAMTS13 inhibitors and are useful for diagnosis, management, and prognosis of patients with TTP. The aim of the study was to evaluate the performance of an ADAMTS13 activity assay currently in development (Instrumentation Laboratory, Bedford, MA, USA).

Materials and methods The chemiluminescent ADAMTS13 activity assay is a fully-automated, 2-step immunoassay standardized against the WHO 1st International Standard ADAMTS13 Plasma with a run time of 33 minutes. Magnetic particles are coated with the VWF73 peptide containing the ADAMTS13 cleavage site and chemiluminescent detection is based on an isoluminol-labeled monoclonal antibody that reacts specifically with the cleaved peptide. The assay can be used for detection of inhibitors after pre-analytical mixing of the sample with normal plasma in 3:1 ratio. A clinical study with 76 samples including normal donors and patients being evaluated for TTP was performed in comparison to in-house FRET assay and Technozym® ADAMTS-13 Activity ELISA. Agreement between methods was assessed with focus on the severe ADAMTS13 activity deficiency (≤10%), relevant for diagnosis of TTP.

Results The study revealed high agreement with the in-house FRET assay (κ=0.97) and the ELISA (κ=1.00) in classifying TTP patients with severe ADAMTS13 activity deficiency. In addition, there was high agreement in inhibitor detection (κ=1.00; further investigation using the 3:1 ratio mixing procedure is ongoing).

Conclusion The ADAMTS13 activity assay showed excellent clinical performance and high agreement in the severe ADAMTS13 deficiency (≤10% activity) with other laboratory methods. It is a fully automated and rapid assay that would be suitable for diagnosis of acute TTP in emergency rooms.

P_D 65

UNRAVELING ANTI-SPACER IMMUNOPROFILES OF ACQUIRED TTP PATIENTS USING ANTI-IDIOTYPIC ANTIBODIES

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Acquired TTP (aTTP) patients present with a polyclonal anti-ADAMTS13 autoantibody response with clear immunodominant epitopes in the ADAMTS13 spacer domain. A detailed analysis of immunoprofiles in aTTP patients is not available yet. However, insight into immunoprofiles of patients with other autoimmune disorders such as myasthenia gravis, provided crucial diagnostic and prognostic information. By using anti-idiotypic Abs that recognize anti-spacer autoantibodies, we aim at getting insight into the anti-spacer immunoprofiles in aTTP patients. Cloned human anti-spacer autoAbs, autoAb1, 2 and 3 with a strong, weak or no inhibitory effect on ADAMTS13 function respectively, were each injected in mice to generate monoclonal anti-idiotypic Abs. Next, anti-idiotypic Abs that specifically block the binding of autoAb 1, 2 or 3 to ADAMTS13 were selected and used to screen plasma of 96 acute idiopathic aTTP patients. Anti-spacer immunoprofiles were determined since the three anti-idiotypic Abs could capture their respective group of anti-ADAMTS13 autoAbs.

Screening patient plasma on the anti-idiotypic Abs revealed that AutoAb1 group Abs were present in 43%, autoAb2 group Abs in 52% and autoAb3 group Abs in 28% of the patients. Next, immunoprofiles were established based on the presence of different combinations of AutoAb 1, 2 or 3 group Abs in the plasma of the patients. Immunoprofile 1 (autoAb1) was present in 6.3%, profile 2 (autoAb2) in 15.6%, profile 3 (autoAb3) in 3.1%, profile 4 (autoAb1 and 2) in 16.7%, profile 5 (autoAb1 and 3) in 5.2%, profile 6 (autoAb2 and 3) in 5.2%, profile 7 (autoAb1, 2 and 3) in 14.6% and profile 8 (none of the autoAbs) in 33.3% of the patients.

We have developed a powerful tool to determine anti-spacer immunoprofiles in aTTP patients. We now will determine whether specific immunoprofiles present in aTTP patients allow the identification of prognostic factors to predict death and disease recurrence in these patients.

P_66

MODELING-GUIDED IDENTIFICATION OF STRUCTURAL DETERMINANTS CONTRIBUTING TO CONFORMATIONAL CHANGES WITHIN ADAMTS13

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Background/aims In the majority of patients with acquired TTP, antibodies targeting a cryptic epitope in the spacer domain of ADAMTS13 have been identified. Based on these findings an auto-antibody resistant, gain-of-function variant (GoF) of ADAMTS13 was designed containing the following amino acid substitutions: R568K / F592Y / R660K / Y661F / Y665F (Jian *et al*., 2012). Structural studies revealed that GoF-ADAMTS13 exists in an open (active), while wt-ADAMTS13 is found in closed (inactive) conformation, with the C-terminal CUB domains bound to the spacer domain. Our aim is to employ modeling to identify structural determinants in the CUB domains contributing to these conformational changes.

Materials and methods The experimental structure of ADAMTS13 CUB domains are not available in Protein Data Bank. The YASARA Structure tool was used for homology modeling of the C-terminal CUB1-CUB2 domains. Domain interactions between the proximal domains and CUB1-CUB2 domains were studied by HADDOCK protein-protein docking where the structures were constrained according to major epitope residues in ADAMTS13 Spacer domain. GoF-ADAMTS13 mutations were introduced in silico to final poses. Next both WT- and GoF-ADAMTS13 were subjected to binding free energy calculation with AMBER16 over a 100 ns molecular dynamics simulation. Subsequently, these poses were investigated to reveal which residues are contributing to conformational changes of ADAMTS13.

Results A pose with relatively higher binding affinity against WT-ADAMTS13 and lower binding affinity against GoF-ADAMTS13 at the same time was found to be informative to predict which residues are important for conformational changes. These residue predictions are subjected to *in vitro* mutation studies in order to test changes on conformation, proteolytic activity and resistance against autoantibodies.

Conclusion We have used the available structural bioinformatics tools to predict the nature of conformational changes that switch the human ADAMTS13 protein between active and inactive states.

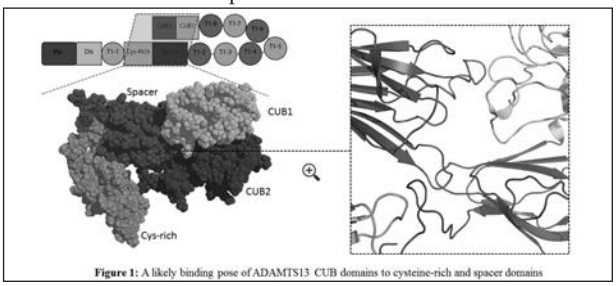

Figure 1: A likely binding pose of ADAMTS13 CUB domains to cysteine-rich and spacer domains

P_74

DIAGNOSIS OF UPSHAW-SCHULMAN SYNDROME IN ADULTHOOD

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Background Thrombotic thrombocytopenic purpura (TTP) is an acute life-threatening disorder characterized by multiple organ ischemia due to disseminated thrombi formation in the microvasculature. Uncontrolled platelet aggregation due to the excess of ultralarge von Willebrand factor (ULVWF) multimers is caused by the deficiency of its proteolytic metalloprotease, ADAMTS13. The congenital form of the disease (Upshaw-Schulman syndrome), is related to ADAMTS13 gene mutations, and is difficult to diagnose, both for its rarity and for its phenotype heterogeneity. Adulthood-onset of TTP does not exclude the congenital form of the disease and a diagnostic delay may account for a great morbidity burden in these patients.

Materials and methods We describe the case of a middle-aged woman, who presented to our attention with a clinical diagnosis of chronic relapsing form of thrombotic thrombocytopenic purpura. Phenotype and genotype tests were performed in the patient.

Results A detailed clinical history revealed multiple past unrecognized episodes of thrombocytopenia and hemolytic anemia. The severe ADAMTS13 deficiency detected in the patient was caused by a homozygous missense point mutation on ADAMTS13 gene, p.Ile143Phe (c.427A>T), located in the metalloprotease domain, which causes a secretion defect as confirmed by *in vitro* expression.

Discussion Upshaw-Schulman syndrome is a rare congenital disease with a great phenotype heterogeneity that can be diagnosed also in adulthood. Accurate clinical history is crucial to identify such cases. In particular, USS must be always considered in women who develop a thrombotic microangiopathy during pregnancy. Early diagnosis and appropriate treatment can avoid recurrences and chronic neurologic damage.

P_86

PREVENTION OF RELAPSES IN PATIENTS AFFECTED BY ACQUIRED TTP UNDERGOING ELECTIVE SURGERY

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Background Thrombotic thrombocytopenic purpura (TTP) is a microangiopathy caused by ADAMTS13 deficiency, leading to inappropriate cleavage of VWF ultralarge multimers, with subsequent platelet aggregation and widespread microthrombosis. Acquired TTP is due to anti-ADAMTS13 autoantibodies, and daily therapeutic plasma exchange (PEX) represents the gold standard of therapy in the acute phase.

Nonetheless, almost one third of patients will experience TTP relapses after achieving remission. Severe ADAMTS13 deficiency (i.e., activity levels <10%) has been recognized as the main risk factor for TTP recurrence, and several conditions, including surgery, may trigger an acute episode. Thus, a prophylactic approach may be advised to prevent relapses before exposure to such triggers, in the presence of severe ADAMTS13 deficiency. Herein, we report three clinical cases of patients followed in our Center for recurrent acquired TTP, candidates to elective major surgery. All of them had severe ADAMTS13 deficiency in remission phase, despite multiple previous treatments (including steroids and rituximab). Therefore, they were offered prophylactic therapies with the aim of increasing ADAMTS13 levels before surgery to prevent TTP relapse.

Materials and methods The first patient is a 67-year-old man, candidate to left indirect inguinal hernioplasty. One PEX was performed immediately before surgery, with an increase in ADAMTS13 levels from <3% to 25% after surgery; no complications occurred. The second patient is a 64-year-old woman, treated with 3 PEX before cholecystectomy, with a rise in ADAMTS13 activity from <3% to 38%, and no complications. She also underwent left total hip arthroplasty; after four weekly prophylactic rituximab infusions, with only a partial correction of ADAMTS13 deficiency (from <3% to 24%), she underwent one PEX immediately before surgery, bringing ADAMTS13 activity to 45%, with a subsequent safe surgical procedure. The third patient is a 58-year-old woman, candidate to total laparoscopic hysterectomy for atypical endometrial hyperplasia. One PEX was performed, with a rise in ADAMTS13 levels from <3% to more than 50% after surgery; no complications occurred.

Results and conclusions We reported three cases of successful surgical procedures in patients affected by recurrent acquired TTP, with undetectable ADAMTS13 activity levels during remission phase. Prophylactic PEX immediately before surgery could be a reasonable choice to increase ADAMTS13 levels and prevent relapses in this setting.

P_04

LIFE-THREATENING PREGNANCY-ASSOCIATED ATYPICAL HAEMOLYTIC URAEMIC SYNDROME AND ITS RESPONSE TO ECULIZUMAB

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A 32-year-old lady presented to hospital 40 weeks through her first pregnancy with abdominal discomfort. Until that point, the pregnancy had been uncomplicated. The patient was otherwise well without medical history or regular medications. Upon arrival she was found to be in labour and underwent spontaneous vaginal delivery that night which was complicated by massive postpartum haemorrhage requiring emergent surgery. Postoperatively a rapid increase in serum creatinine and a markedly elevated lactate dehydrogenase (LDH) were noted in addition to worsening anaemia and thrombocytopaenia that could not be explained by external bleeding. Low haptoglobin levels confirmed haemolysis. A review of the blood film revealed red cell fragmentation. Given the severity of the renal injury, it was felt that haemolytic uraemic syndrome (HUS) was the most likely diagnosis. A targeted history did not reveal any recent gastrointestinal illnesses and there was no family history of renal disease. ADAMTS-13 testing revealed normal activity confirming HUS. Daily plasma exchange was initiated resulting in rapid haematological improvement with normalisation of platelet counts within three days. This was not matched by concomitant improvement in renal function. The patient progressed to anuric renal failure requiring haemodialysis on day 5 postpartum. The patient was urgently vaccinated against meningococcus before receiving her first dose of eculizumab 6 days postpartum. Renal function began to improve with increasing urine output over the following days. Haemodialysis was required for 9 days after initiation of eculizumab; subsequent to this, the patient remained dialysis independent and the remainder of the inpatient stay was uneventful. At the most recent outpatient follow up, the patient remained symptomatically well and had demonstrated no adverse reactions to eculizumab. Pregnancy-associated HUS is a life-threatening condition that rarely complicates pregnancy, but with prompt diagnosis and treatment successful outcomes are possible.

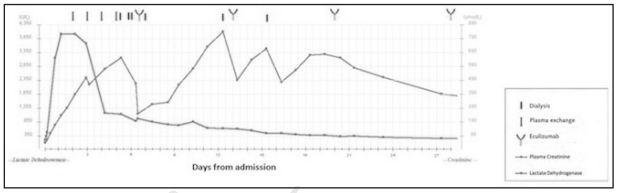

Figure 1

P_21

AN IMMEDIATE aHUS DIAGNOSIS WOULD CHANGE THE PROGNOSIS

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Background Atypical hemolytic uremic syndrome (aHUS) is an inherited disorder due to decreased activity of complement-inhibiting or increased function of coagulation proteins. Many factors trigger aHUS by activating the complement or damaging the endothelium. Prognosis is poor. A 62-year-old man was admitted to the emergency department with fever and drowsiness. Thrombocytopenia and microangiopathic hemolytic anemia were diagnosed (platelets=13,000, hemoglobin=8 g/dL; lactate dehydrogenase=3,543U/L; haptoglobin <0.310 g/L, normal PT, PTT and fibrinogen, INR 1.26, negative direct Coombs test, schistocytes 2.5%), along with renal failure and proteinuria.

Aims Microangiopathic hemolytic anemia, thrombocytopenia and kidney injury combination suggested a thrombotic microangiopathy syndrome (TMA). Hence, we aimed to differentiate among thrombotic thrombocytopenic purpura (TPP), HUS o aHUS.

Material and methods We performed main biochemical, biomolecular and microbiology tests, ADAMTS-13 activity and Ab anti-ADAMTS-13 tests (to discriminate between TTP and aHUS).

Results All microbiology tests were negative, except for E. cloacae positive septiFAST. Blood cultures were negative. No Shiga toxin-producing *E. coli* was found, excluding HUS diagnosis (in any case the patient did not present diarrhea). Patient initiated therapy with metilprednisolone 80 mg/day. Two days later he was admitted to the intensive care unit (ICU) due to worsening anemia (Hb=5.9 mg/dL) and thrombocytopenia persistence, and was treated with three sessions of plasma

exchange (PEx) plus a wide spectrum antibiotic therapy, to manage a possible aHUS trigger. However, platelet count and renal function worsened, requiring hemodialysis. The patient died days later.
Conclusions PEx is used while waiting for the results of ADAMTS-13 discriminating test, which is not rapidly available, particularly in cases of critically ill patients. In our case, the patient died after 6 days in ICU and 3 PEx. Some days later the results of the test (ADAMTS-13 activity 42%; Ab-anti-ADAMTS-13 <12) confirmed aHUS diagnosis. Eculizumab would represent the treatment of choice in a confirmed aHUS, but it was not immediately available.

P_85
SOLUBLE GLYCOPROTEIN VI (SGPVI) MEASUREMENT IS A USEFUL BIOMARKER OF PLATELET ACTIVATION IN HEPARIN-INDUCED THROMBOCYTOPENIA (HIT) AND CORRELATES WITH THROMBOTIC EVENTS
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Background The receptor FcgRIIA plays a key role in the pathogenesis of HIT, a disease with similarities to TTP. The platelet-specific collagen receptor GPVI releases a soluble ectodomain fragment (sGPVI) in hypercoagulable states and by FcgRIIA-mediated signaling upon engagement by HIT (H-PF4) antibodies. sGPVI could serve as a surrogate marker of pathological HIT antibodies and as a unique readout of patient platelet activation *in vivo*, in contrast to using donor platelets.
Aim To assess the utility of measuring sGPVI in concert with standard and new HIT testing methods, and to correlate sGPVI with thrombotic events.
Method sGPVI measurements by ELISA were obtained from stored plasma of 65 patients with clinical suspicion of HIT from 2008-2014 who underwent HIT assessment, including 4T score, ELISA for HIT H-PF4 antibodies (GTI IgG), serotonin release assay (SRA), platelet aggregation (PAT) and a new test, HemosIL HIT-Ab assay (AcuStar, Werfen).
Results Patients with positivity for HIT ELISA and for PAT had higher sGPVI than patients with negative results ($p<0.05$). Negative sGPVI values showed more than 80% agreement with SRA, PAT and AcuStar with ELISA. With SRA as the gold standard, NPV was 100% and PPV 77% with a combination of sGPVI and ELISA GTI IgG. Patients with thrombosis (scored 2 on the 4T score "thrombosis" parameter) had higher levels of both sGPVI and HIT antibody than patients with a score of 0 ($p<0.05$).
Conclusion sGPVI testing is easy to perform, compares well with existing HIT testing, and is a useful tool in assessing patients with clinical suspicion of HIT. Patients negative for both sGPVI and ELISA are unlikely to have HIT and further testing can be avoided. Importantly, sGPVI levels are associated with thrombosis. These findings support measuring sGPVI in HIT, and provides novel insights into the mechanism of pathological platelet response to HIT antibodies *in vivo*.

LATE BREAK

P_257
CLUSTERED *F8* MISSENSE MUTATIONS CAUSE HEMOPHILIA A PHENOTYPIC HETEROGENEITY BY COMBINATION OF ALTERED SPLICING, PROTEIN SECRETION AND ACTIVITY
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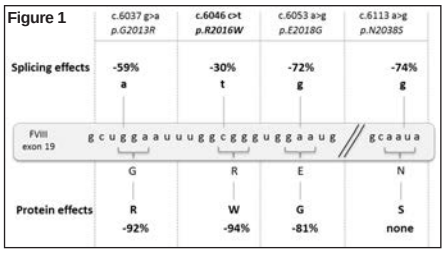
Background Pleiotropic effects of mutations, scarcely investigated in hemophilia A (HA), hamper the elucidation of genotype-phenotype relationships. Missense mutations, frequent in HA, might impair FVIII mRNA processing, protein biosynthesis, activity and/or half-life.
Aims To quantitatively evaluate the pleiotropic effects of the *F8* p.Arg2016Trp/c.6046C>T (exon 19, A3 domain) mutation, one of the most prevalent FVIII amino acid substitutions (>60 cases).
Methods HO cells were transduced with lentiviral expression vectors for recombinant (r)FVIII variants to evaluate secreted FVIII antigen (FVIII:Ag) and activity (FVIII:C) by ELISA and chromogenic assays. FVIII mRNA splicing was evaluated by RT-PCR on RNA from: 1) patients' leukocytes, and 2) HepG2 cells transfected with *F8* minigene variants.
Results The rFVIII-2016W displayed reduced secretion (FVIII:Ag 11.0±0.4% of wt) and activity (FVIII:C 6.0±2.9%). *F8* mRNA studies demonstrated that the c.6046C>T change also decreases correct splicing to 70±5%, predicted to lower further FVIII:C (4.3±0.5%), consistent with FVIII:C levels observed in patients with the mutation (1-5%). Through an antisense U7snRNA targeting the mutated exon 19 region we identified an exonic splicing enhancer, potentially affected by other HA-missense mutations. Strikingly, the c.6037G>A (p.Gly2013Arg) reduced exon inclusion to 41±3% and the c.6053A>G (p.Glu2018Gly) to 28±2% of wt, similarly (26±2%) to the c.6113A>G, (p.Asn2038Ser), a variant affecting the 5'splice splice. At protein level, the p.Gly2013Arg displayed reduced FVIII:Ag (7.0±0.9%) and FVIII:C (8.4±0.8%), while the p.Glu2018Gly produced a dysfunctional molecule (FVIII:Ag, 69.0±18.1%; FVIII:C, 19.4±2.3%). However, the rFVIII-2038Ser displayed normal FVIII:Ag and FVIII:C.
Conclusions These data highlight a *F8* exonic region that has evolved in relation to both mRNA maturation and protein constraints, a feature potentially shared with other exons. The integrated approach of analyzing mRNA and protein levels highlights the combination of both pathogenic mechanisms triggered by clustered mutations (see Figure 1), which accounts for a gradient of residual FVIII:C recapitulating the HA coagulation phenotypes.



Among 35 congenital TTP, 26 developed TTP symptoms at birth and the first acute TTP episode occurred later but before 1 y.o. in 13 patients; 28 patients required prophylactic plasmatherapy and no patient died. All patients had undetectable ADAMTS13 antigen (<30 ng/mL) and exhibited bi-allelic mutations of ADAMTS13 gene.
Conclusion Child-onset TTP exhibits different clinical presentations as a function of the mechanism for ADAMTS13 severe deficiency. International guidelines are needed to improve TTP diagnosis and treatment in children.

P_258
CHILD-ONSET THROMBOTIC THROMBOCYTOPENIC PURPURA: THE FRENCH REFERENCE CENTER FOR THROMBOTIC MICROANGIOPATHIES EXPERIENCE

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Background/aims Thrombotic thrombocytopenic purpura (TTP) is a life-threatening thrombotic microangiopathy (TMA) related to a severe ADAMTS13 deficiency. TTP is either acquired (ADAMTS13 autoantibodies) or congenital (mutations of ADAMTS13 gene). Child-onset TTP is very rare when compared to adult-onset TTP. Based on the experience of the French TMAs Reference Center, our study aimed to provide a picture of child-onset TTP in France.
Material and methods A cross-sectional analysis of the French TMA Registry was performed from Jan 1st, 1999 to March 1st, 2017 to identify, among child-onset TMA patients, those exhibiting an ADAMTS13 activity <10% (TTP patients). We studied ADAMTS13 activity (FRETS-VWF73, full-length VWF ELISA and CBA), anti-ADAMTS13 IgG (Technozym ADAMTS13-INH ELISA®) and ADAMTS13 antigen (IMUBIND ADAMTS13 ELISA®). Genomic DNA was screened for mutations by direct sequencing of ADAMTS13 gene. An exhaustive analysis of clinical records was assessed.
Results Eighty child-onset TTP patients were enrolled in the French TMA Registry: 45 acquired TTP and 35 congenital TTP (sex ratio 2.5F/1M and 1.2F/1M, respectively).
Among 45 acquired TTP, the median age at diagnosis was of 13 years, 29 patients had visceral ischemia and anti-ADAMTS13 IgG were positive in 37 patients. TTP presentation was idiopathic in 25 patients while it was associated with a clinical context in 20 patients. The treatment consisted in plasmatherapy, steroids, and sometimes rituximab (n=21). The mortality rate was of 9%.

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DEVELOPMENT OF A GENE THERAPY STRATEGY FOR VON WILLEBRAND DISEASE BASED ON DUAL ADENO-ASSOCIATED VIRUS VECTORS

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Background Von Willebrand disease (VWD) is the most common inherited bleeding disorder in humans, caused by quantitative or qualitative defects in von Willebrand factor (VWF). Current therapies have still limitations mostly related to their short-term efficacy. In this context, VWD represents a potential target for gene therapy approaches, as a single treatment could results in a long-term correction of the disease.
Aims Develop an innovative gene therapy strategy for VWD based on dual overlapping adeno-associated virus vectors (AAV).
Methods In our approach the large 8.4 kb VWF coding sequence is split in two AAV vectors, thus permitting to overcome the AAV size limitation (5 kb). In this system each AAV delivers one half of the VWF cDNA with an overlapping region, which mediates the reconstitution of the entire genome by homologous recombination. We injected each vector in the tail vein of VWF KO mice, at a dose of 2^{13} vg/kg, and assessed the circulating VWF levels by ELISA assay 4 and 6 weeks post-administration.
Results We generated a dual AAV8 vector expressing murine VWF under the control of the liver specific human alpha-1 antitripsin promoter (hAAT). The two halves of the transgene expression cassette (5' and 3' cassette) contained in the dual AAV share a homologous region of 400 base pairs. Delivery of the two vectors resulted in detectable levels of VWF expressed by the liver (up to 3% of normal) at 6 weeks post-administration. Conversely, circulating murine VWF levels were undetectable in control mice injected with the 5' or the 3'-cassette alone.
Conclusion Results obtained in VWF KO mice demonstrate that we were able to express the large gene of VWF from the liver. Future experiments will be aimed at optimizing the dual AAV in order to increase expression levels and to subsequently evaluate biochemically the effect of VWF transgene expression on the hemorrhagic phenotype.
Keywords Von Willebrand Factor, von Willebrand disease, AAV gene therapy, dual AAV.

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Proven long-lasting bleed protection with a unique single-chain design[1–7]

3x higher binding affinity to VWF*

ZERO AsBR† Low ABR‡ in prophylaxis

2x/3x weekly dosing with no differences in AsBR†#

Increased binding affinity[3]*

Long-lasting bleed protection[6,7]

Allows for individualised treatment[1,6,7]

- **Zero inhibitors observed in PTPs**[1,6,7§Δ]
  – Low incidence of adverse reactions in completed clinical trials
- **High purity and molecular stability**[8]
- **Good safety and tolerability profile**[1,6,7]

* Based on in-vitro comparison of AFSTYLA with octocog alfa (Advate). † Median annualised spontaneous bleeding rate.
‡ Annualised bleeding rate. § Previously treated patients. # Select dosing regimen based on patient needs.
Δ The safety and efficacy of AFSTYLA in previously untreated patients have not been fully established.

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AFSTYLA Essential Information

AFSTYLA® 250/500/1000/1500/2000/2500/3000 IU. Powder and solvent for solution for injection.

Qualitative and quantitative composition: Each vial contains nominally 250/500/1000/1500/2000/2500/3000 IU recombinant, single-chain coagulation factor VIII (rVIII-SingleChain, INN = lonoctocog alfa).

Other ingredients: L-Histidine, Polysorbate 80, Calcium chloride dihydrate, Sodium chloride, Sucrose.

Solvent: Water for injection.

Therapeutic indications: Treatment and prophylaxis of bleeding in patients with haemophilia A (congenital factor VIII deficiency). AFSTYLA® can be used for all age groups.

Contraindications: Hypersensitivity to the active substance or to any of its excipients. Known allergic reaction to hamster proteins.

Warnings and precautions for use: Keep out of the sight and reach of children.

Undesirable effects: Patients with haemophilia A may develop neutralizing antibodies (inhibitors) to factor VIII. If such inhibitors occur, the condition will manifest itself as an insufficient clinical response. In such cases, contacting a specialised haemophilia centre is recommended. The most frequent adverse reactions in clinical trials were hypersensitivity, dizziness, paraesthesia, rash and pyrexia (common, ≥ 1/100 to < 1/10). Erythema, pruritus, injection site pain, chills and feeling hot were uncommon (≥ 1/1,000 to < 1/100). Rarely (≥1/10,000 to <1/1,000) hypersensitivity or allergic reactions which may in some cases progress to severe anaphylaxis (including shock) have been observed.

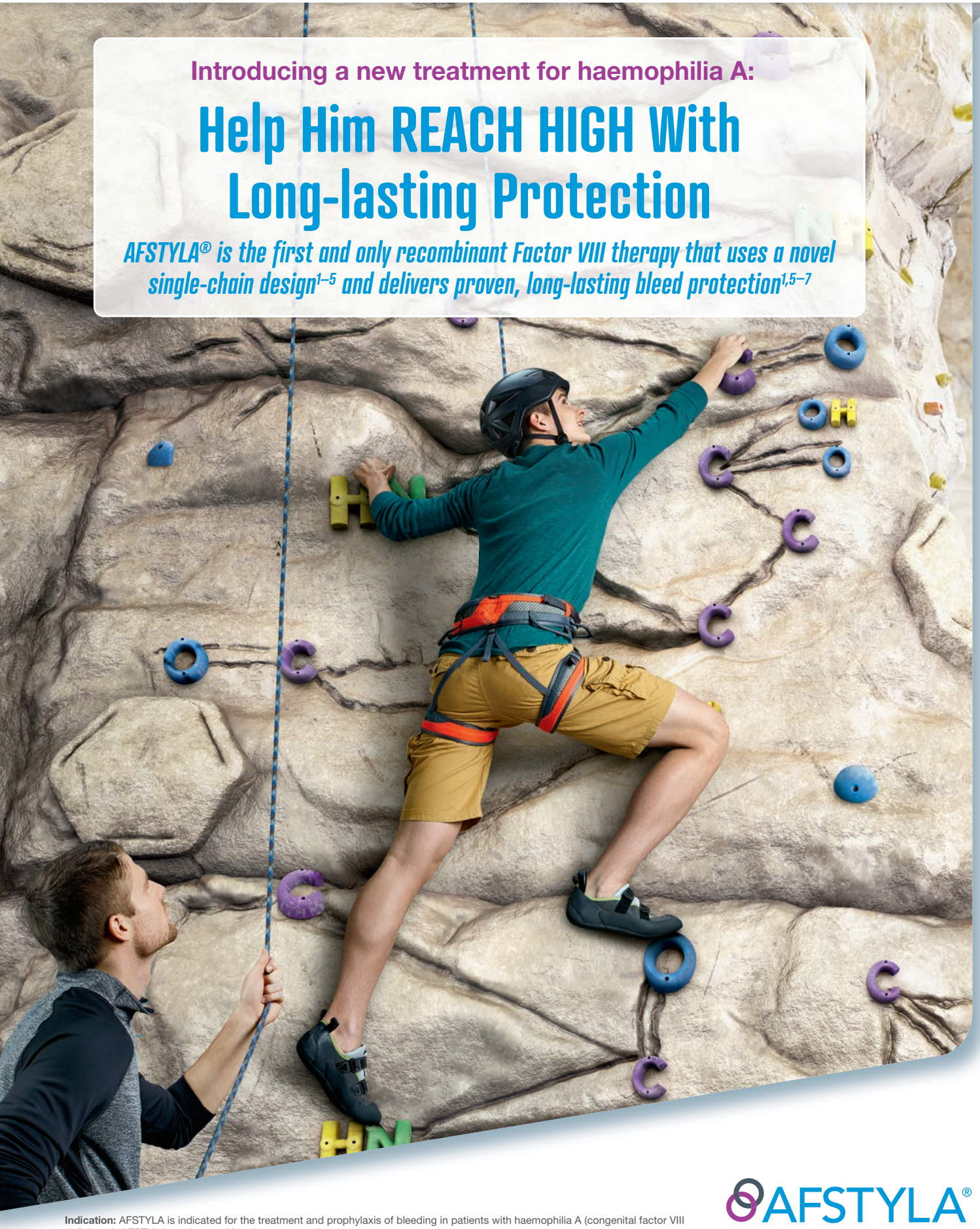
Prescription status: Prescription-only drug.

Manufacturer: CSL Behring GmbH, Emil-von-Behring-Str. 76, 35041 Marburg, Germany.

Date of information: January 2017

Depositato in AIFA in data 14/08/2017 Cod. ZINC: IT/ISN/17-0015

AFSTYLA®
Recombinant Blood Clotting Factor VIII, Lonoctocog Alfa



Introducing a new treatment for haemophilia A:

Help Him REACH HIGH With Long-lasting Protection

AFSTYLA® is the first and only recombinant Factor VIII therapy that uses a novel single-chain design[1–5] and delivers proven, long-lasting bleed protection[1,5–7]

Indication: AFSTYLA is indicated for the treatment and prophylaxis of bleeding in patients with haemophilia A (congenital factor VIII deficiency). AFSTYLA can be used for all age groups.[1]

The safety and efficacy of AFSTYLA in previously untreated patients have not been established.

AFSTYLA Essential Information and references can be found overleaf. Depositato in AIFA in data 14/08/2017. Cod. ZINC: IT/ISN/17-0015

AFSTYLA®
Recombinant Blood Clotting Factor VIII, Lonoctocog Alfa