


**PP3 Hemorrhagic Disorders**
**PP3.1 Hemophilia**
**PP3.1-1**
**Co-activation of selected knee muscles in haemophilic patients**

 Kurz E<sup>1,2</sup>, Herbsleb M<sup>2</sup>, Anders C<sup>3</sup>, Czepa D<sup>1</sup>, Puta C<sup>2</sup>, Ziezio R<sup>2</sup>, Scholle H<sup>3</sup>, Hilberg T<sup>1</sup>
<sup>1</sup>Department of Sports Medicine, University of Wuppertal, Germany, <sup>2</sup>Department of Sports Medicine, Friedrich-Schiller-University Jena, Germany, <sup>3</sup>Clinic for Trauma Surgery Jena, Div. for Motor Research, Germany

Previous studies of knee muscle activity in haemophilic patients during upright standing proved increased amplitudes of extensors and impaired coordination patterns. The purpose of this investigation was to study the co-activation of knee flexors and extensors during upright standing in haemophilic patients. Co-activation indices of extensors (rectus femoris (RF), vastus medialis (VM), vastus lateralis (VL)) over biceps femoris (BF) were investigated during non-perturbed upright standing in 27 haemophilic patients (H) in comparison to 26 healthy control subjects (C) by surface EMG (SEMG). Prior to calculations of co-activation indices patient's extremities were classified according to major (MA) and minor (MI) affected legs (Gilbert-Score), respectively. Data from both sides of C was pooled. Different SEMG co-activation indices could be detected during bipedal standing in H compared to C. In both H groups, indices of RF over BF [H-MA: 2.14(5.29/1.16), median(upper/lower quartile), H-MI: 2.20(4.29/1.40), C: 1.11(2.37/0.73)] and VM over BF [H-MA: 2.16(5.13/1.37), H-MI: 1.98(4.61/1.17), C: 1.39(2.40/0.95)] exceeded those of C significantly ( $p < 0.05$ ). By application of Holm's adjustment procedure only RF/BF-index of the MA group remained significantly different from C. The comparison of MA vs. MI showed no statistical differences at all. The findings show that MA of H maintain the stability demands during standing by using higher co-activation levels. A higher co-activation indicates higher metabolic costs and thereby a lower metabolic efficiency. In the author's opinion, early treatment by sports therapy is necessary. (This study was supported by Baxter-Deutschland)

**PP3.1-2**
**Bleeding tendency in female carriers of haemophilia A**

 Miesbach W<sup>1</sup>, Krekeler S<sup>1</sup>, Aleksi S<sup>1</sup>
<sup>1</sup>Goethe University Frankfurt, Medical Clinic III, Institute of Transfusionmedicine, Germany

Female carriers of haemophilia might not only have an increased bleeding tendency but the symptoms may be frequent and severe, therefore the assessment of the bleeding risk is very important for improving care. This study documents the occurrence of bleedings in 46 carriers of haemophilia A including spontaneous bleeding of nose or gums, easy bruising, prolonged menstruation, and prolonged bleeding after giving birth or after surgical interventions. The FVIII gene mutation of all 46 carriers was determined and family history of haemophilia was recorded as well as FVIII plasma activity (FVIII:C) of the carrier. For analysis the bleeding tendency of the carriers was differentiated by strength into three groups. A clear correlation was found between the strength of bleeding tendency and the FVIII:C level of the carriers, as well as the type of FVIII gene mutation and the severity of haemophilia in affected male relatives. Results show that even carriers with FVIII:C as high as 50%-60% of normal are already at risk of bleedings in everyday life and are at risk of prolonged bleedings from surgery or after giving birth. The chromogenic assay showed a more sensitive association of FVIII:C and bleeding symptoms than the one stage assay. In conclusion, FVIII:C levels as high as 60% might be considered a risk factor for bleeding of carriers. Further evaluation of correlation between FVIII:C, mutation type, and family history of haemophilia might allow to predict bleeding tendency of carriers and to improve care.

**PP3.1-3**
**Cardiovascular interventions in patients with hemophilia and severe von Willebrand Disease**

 Tiede A<sup>1</sup>, Aleksi S<sup>2</sup>, Klamroth R<sup>3</sup>, Holstein K<sup>4</sup>, Krause M<sup>5</sup>, Fischer R<sup>6</sup>, Scholz U<sup>7</sup>, Horneff S<sup>8</sup>
<sup>1</sup>Medizinische Hochschule Hannover, Germany, <sup>2</sup>Johann-Wolfgang-Goethe-University, Frankfurt, Germany, <sup>3</sup>Vivantes-Klinikum Berlin Friedrichshain, Germany, <sup>4</sup>Universitätsklinikum Eppendorf, Hamburg, Germany, <sup>5</sup>Deutsche Klinik für Diagnostik, Wiesbaden, Germany, <sup>6</sup>Universität Giessen, Germany, <sup>7</sup>Zentrum für Blutgerinnungsstörungen, Leipzig, Germany, <sup>8</sup>Universitätsklinikum Bonn, Germany

**Objectives:** Information on the management of cardiovascular interventions in patients with hemophilia and VWD is limited to few case reports. As prospective studies are unlikely to be feasible in this area, retrospective cohort studies may be useful to improve clinical practice.

**Design:** This is a multicenter cohort study of patients enrolled because of hemophilia or VWD (VWF:RCO  $< 30$  IU/dl), who underwent coronary angiography (CA), percutaneous coronary intervention (PCI), or cardiac surgery with the use of cardiopulmonary bypass (CPB).

**Results:** Thirty-nine interventions were studied in 28 patients with a median factor activity of 3 IU/dl ( $< 1-35$ ). For CA (N=11), patients received replacement therapy for a median of 2 days (1-15). Minor bleeding at the site of puncture occurred in 5 patients. For PCI (N=18), patients received replacement therapy for a median of 4 days (1-41). Most patients experienced minor (N=15) or major (N=1) bleeding at the site of puncture. Of 14 patients receiving bare metal stents, 12 were on aspirin/clopidogrel for 4 to 6 weeks; 7 of these, including all with severe hemophilia, received prophylactic replacement therapy. No thromboembolic or cardiovascular events occurred. For cardiac surgery (N=10), patients received replacement therapy for a median of 23 days (11-46) and unfractionated heparin during CPB. Minor wound bleeding occurred in 8 patients. Four patients received transfusion.

**Conclusion:** Combining replacement therapy with antiplatelet agents or anticoagulants appears to be a safe strategy, despite of frequent minor bleeding. Major bleeding mainly occurred in patients on antiplatelet agents without concomitant replacement therapy.

**PP3.1-4**
**Quantitative expression analysis of the F8 mRNA in haemophilia A patients with no detectable mutations in the F8 gene**

 El-Maarri O<sup>1</sup>, Nüsgen N<sup>1</sup>, Müller J<sup>1</sup>, Oldenburg J<sup>1</sup>
<sup>1</sup>Experimental Hematology and Transfusion Med, Bonn, Germany

**Objectives:** The expression levels of F8 gene in both normal and pathological conditions is not well studied.

**Design and Methods:** In this study we used a TaqMan based assay to analyse the expression levels in a subgroup of 15 haemophilia A patients with undetectable mutations in the F8 coding region; in addition a group of 96 healthy males were also included.

**Results:** Our previous hypothesis of the absence of expression of the F8 mRNA in one severe patient was further confirmed by our quantitative analysis; the rest of the patients did not show severe deficiency in the F8 mRNA. When we group the patients, depending on the severity of the phenotype, we observe that severe patients have higher levels of expression in comparison to mild and moderate patients. Further analysis of the healthy male controls reveals high variability in the expression of the F8 that was not correlated with the measured blood coagulation activity. Further expression analysis of two F8 nested genes: F8a and F8b, are underway.

**Conclusions:** In summary our analysis shows that absence of detectable F8 mRNA may not be the cause of F8 deficiency in all cases of such patients; in addition this would point to the heterogeneity of the molecular cause underlying the F8 deficiency in these patients.

**PP3.1-5**
**Factor V G1691A or prothrombin G20210A independently influence inhibitor development in children with severe hemophilia A – data of a multicenter cohort study**

 Halimeh S<sup>1</sup>, Bidlingmaier C<sup>2</sup>, Escuriola Ettingshausen C<sup>3</sup>, Krümpel A<sup>4</sup>, Kurnik K<sup>2</sup>, Schobess R<sup>5</sup>, Nowak-Goettl U<sup>4</sup>
<sup>1</sup>Ambulanz für Gerinnungs-erkrankung und Hämophilie MVZ Labor Duisburg, Germany, <sup>2</sup>Kinderklinik und Kinderpoliklinik im Dr. von Haunerschen Kinderhospital München, Germany, <sup>3</sup>Klinikum Joh. Wolfgang Goethe-UNI, Zentrum d. Kinderheilkunde, Abt. Kinderheilkunde III, <sup>4</sup>Klinik und Poliklinik für Kinder- und Jugendmedizin- Pädiatrische Hämatologie und Onkologie - Hämostaseologie, <sup>5</sup>Strümpellstr. 4 04289 Leipzig, Germany

It has been recently suggested that the clinical phenotype of severe hemophilia A (HA) is influenced by co-inheritance with the factor V (FV) G1691A mutation. Thus, the present non-concurrent cohort study was performed to investigate the impact of FV and FII mutations on clinical meaningful inhibitor development (outcome variable) in white children with severe HA. 122 patients with HA  $< 1\%$  consecutively ascertained from four German catchment areas were followed over a median (min-max) period of 15 years (1-24) from HA onset. 19 of 122 children (15.6%) additionally carried either the FV or the FII variant. During the follow-up period 28 of 122 children (22.9%) with developed a clinical important inhibitor: 9 out of 19 in carriers of thrombophilia (47.4%) compared with 19 of 103 (18.44%) in the non-thrombophilia affected cohort [ $p=0.01$ ]. Multivariate analysis, adjusted for substitution regimen, dosage ( $< 30$  IU/kgbw versus  $> 30$  IU/kgbw), and first-line use of plasma-derived versus recombinant FVIII concentrations revealed that the presence of thrombophilia independently increases the risk of clinical meaningful inhibitor development to an odds of 3.9 [95%CI: 1.21-12.412;  $p=0.02$ ]. None of the modifiers adjusted for, e.g. substitution regimen, start of prophylaxis, or factor concentrates administered for first-line therapy showed a statistically significant

influence on clinical important inhibitor development. Data presented here support the hypothesis that clinical meaningful inhibitor development is of multifactorial origin and that FV and FII mutations should be included in the aetiology research in future studies.

PP3.1-6

**Austrian Haemophilia Registry: Preliminary results**

Reitter S<sup>1</sup>, Sturm R<sup>1</sup>, Streif W<sup>2</sup>, Schabetsberger T<sup>3</sup>, Male C<sup>4</sup>, Muntean W<sup>5</sup>, Pabinger I<sup>1</sup>

<sup>1</sup>Medizinische Universität Wien, Universitätsklinik für Innere Medizin I, Germany, <sup>2</sup>Medizinische Universität Innsbruck, Universitätsklinik für Kinder- und Jugendheilkunde, Germany, <sup>3</sup>UMIT, The Health & Life Sciences University, <sup>4</sup>Medizinische Universität Wien, Universitätsklinik für Kinder- und Jugendheilkunde, Germany, <sup>5</sup>Medizinische Universität Graz, Universitätsklinik für Kinder- und Jugendheilkunde, Germany

The treatment of haemophilia requires continuous development of knowledge related to various aspects of diagnosis and therapy. It is essential to collect valid and representative data that are comparable on an international level. The Austrian Haemophilia Registry was set up by the Scientific Advisory Panel of the Austrian Haemophilia Society and by the patient organisation. Regarding the design, it was decided to divide the registry into three sections, two concerning quality control and a third concerning scientific questions, the latter requiring written informed consent. A web-based software is used to collect data. Transfer and storage of data are secured and the server is situated in a computer center with video and access control. Data entry was initiated in early 2008. Currently, 309 patients are included in the registry, comprising 267 patients (86.4%) with haemophilia A and 42 patients (13.6%) with haemophilia B. The median patient age is 31.32 years, with 30.7% (n=95) being children or adolescents and 69.3% (n=214) being adults. Regarding severity, 45.6% (n=141) have severe haemophilia. Among these patients, 19.86% (n=28) had an inhibitor in their history, whereas only 5 patients (2.98%) had an inhibitor in the non-severe group. Although the data is only preliminary, the registry is obviously an appropriate instrument for documenting the quality of haemophilia treatment. Our focus is on continued data entry, which will further enable us to provide information on the characteristics of the Austrian haemophilia patient population and the actual treatment modalities.

PP3.1-7

**Factor VIII activity and antigen levels in female carriers of hemophilia A**

Horvath B<sup>1</sup>, Freitag R<sup>1</sup>, Male C<sup>2</sup>, Pabinger I<sup>3</sup>, Thom K<sup>2</sup>, Mannhalter C<sup>1</sup>

<sup>1</sup>Klin. Institut für Medizinische und Chemische Labordiagnostik, Wien, Germany, <sup>2</sup>Universitätsklinik für Kinder- und Jugendheilkunde, Wien, Germany, <sup>3</sup>Universitätsklinik für Innere Medizin I, Klin. Abteilung für Hämatologie und Hämostaseologie, Wien, Germany

**Introduction:** Factor VIII (FVIII) activity levels are highly variable in carriers of hemophilia A due to influences of external factors (age, hormone status, blood group, inflammation). The variability of FVIII antigen (FVIII:Ag) concentrations in carriers and the correlation between activity and antigen levels in this population has not been studied in detail.

**Patients & Methods:** We determined factor VIII activity by a one stage assay (CA-7000, Sysmex, normal range 60–230%) in 45 female carriers of hemophilia A. FVIII:Ag was measured in 44 women by Asserachrom VIII:Ag enzyme immunoassay (normal range 63–199%, Diagnostica Stago).

**Results:** Only in 13 of 45 confirmed carriers FVIII:C levels were below 60% whereas 24 carriers had decreased FVIII:Ag (<63%). FVIII activity and antigen corresponded in 25 females, in 17 subjects FVIII:Ag levels were lower (20 to 65% less antigen than activity), in two women antigen levels were higher than activity (+30, +53%). In all women the mutations were determined. No association between mutation type and activity or antigen level was detected.

**Conclusion:** Only 29% female carriers of hemophilia A could be correctly diagnosed by the one step coagulation test. In contrast, 50% of the carriers were correctly identified by determination of FVIII:Ag. The reasons for the unsatisfactory performance of the coagulation test for detection of carriers are unclear. Possible causes for the high factor VIII activity levels and the differences between antigen and activity will be evaluated in future studies.

PP3.1-8

**Elbow endoprosthesis in patients with hemophilic arthropathy 5 case reports**

Huth-Kühne A<sup>1</sup>, Staritz P<sup>1</sup>, Lages P<sup>1</sup>, Findekle J<sup>1</sup>, Zimmermann R

<sup>1</sup>SRH Kurpfalzkrankenhaus und Hämostasezentrum Heidelberg, Germany

**Introduction:** Hemophilic arthropathy of the elbow is associated with severe pain and decrease in the range of motion. Total or partial joint replacement is not regarded as an established therapy in former reviews. We report our experiences on 5 patients with total (4pts) and partial (1pt) elbow endoprosthesis.

**Material and Methods:** Patient A: 64 years, severe hemophilia A. In 2001 total replacement of the left elbow was performed. Despite sufficient FVIII levels a post-

operative haematoma needed wound revision. Patient B: 53 years, female, severe von Willebrand disease type III. In 2001 total replacement of the right elbow was performed without any periprocedural complications. Patient C: 27 years, severe hemophilia A. In 2007 a synovectomy and implantation of a radial head-prosthesis of the left elbow was performed without perioperative complications. Patient D and E: 49 and 48 years, severe hemophilia A, HIV and HCV coinfecting. Total elbow joint replacement was performed in 2008 without any complications. In all patients pre- and postoperative (p.o.) range of motion, peri- and p.o consumption of concentrates and the grade of pain relief were recorded.

**Results:** All but one patient had complete relief of pain. There were no bleedings after arthroplasty during follow-up. All patients needed complete normalization of the FVIII- or Ristocetin-Co-factor activity during and after surgical procedure and a long term replacement therapy for 10 to 16 weeks until rehabilitation was completed.

**Discussion:** Elbow arthroplasty improves impaired quality of life. Further studies are needed to determine the long-term outcome.

PP3.1-9

**Impressive reduction of bleeds in patients with severe haemophilia B by prophylaxis requires a 3fold higher FIX consumption: A retrospective data analysis**

Siegmund B<sup>1</sup>, Richter H<sup>1</sup>, Pollmann H<sup>1</sup>

<sup>1</sup>Institut für Thrombophilie und Hämostaseologie, Münster, Germany

**Objective:** To treat haemophilia B coagulation factor IX (FIX) is either administered in case of an acute bleed (on demand treatment, OD) or perpetually as continuous prophylaxis (CP). Based on documented bleeds from patients in the home treatment setting OD vs. CP is compared.

**Design and Methods:** Therapy regime, number and localisation of the bleeds, number of substitutions to control a bleed, and consumption of factor IX, of patients with severe haemophilia B (<1% FIX) without further coagulation disorders either treated with Berinin under OD or CP without any change of the FIX concentrate and complete documentation were analysed retrospectively over the year 2006.

**Results:** 6 patients, 2 with OD and 4 under CP could be included into the study. OD treated patients experienced 35 bleeds (14 and 21 bleeds, mean = 17.5), patients under CP experienced 1.5 ± 1.9 (mean ± SD, range 0 - 4) bleeds. To control a bleed under OD 1.1 ± 0.0 and under CP 1.8 ± 0.4 days with a FIX substitution were needed. The mean amount of FIX consumption per year and per body weight (bw) of patient of 48.000 IU ± 13.576 (629 ± 156 IU/kg bw) under OD was faced by 162.900 ± 68.532 IU (2.045 ± 1.042 IU/kg bw) under CP.

**Conclusions:** The ability of CP is demonstrated by an impressive reduction in bleeding episodes. However, CP requires a 3fold higher consumption of FIX.

PP3.1-10

**The German Haemophilia Register (DHR) is starting its work**

Haschberger B<sup>1</sup>, Hesse J<sup>1</sup>, Heiden M<sup>1</sup>, Seitz R<sup>1</sup>, Schramm W<sup>2</sup>

<sup>1</sup>Paul-Ehrlich-Institut, Langen, Germany, <sup>2</sup>Ludwig-Maximilians Universität, München, Germany

The collaboration between the Paul-Ehrlich-Institut (PEI) and the GTH as well as the two patient organisations, DHG and IGH has been official since February 2007 with the signing of the collaboration contract. After the concept had been accepted by the data protection representatives of the Federal Republic of Germany and the Länder (federal states) in May 2007, the programming activities for the database could be started. Additional security requirements made by the data protection representatives, i.e. the independent third party and the so called "Intermediär" caused a delay in the project: Intermediär is an independent software installed on a separate server without hard disk (HD) and with random access memory (RAM). It receives personal data from the treaters over the internet, calculates the pseudonyms, which are then forwarded to the database. The Intermediär needs to be protected from data access by the PEI and its operating system and software has to be started afresh with each start. These facts require the involvement of a trustworthy third party. It shuts Intermediär off from the PEI without itself coming into the possession of personal data. An agreement has been prepared laying down the duties of this third party with the local authority of Langen. In March 2008, the University of Munich was included into the pilot phase. After the test and pilot phases had been completed successfully, necessary extensions of the DHR were projected, ordered, and implemented with regard to user friendliness, temporal and financial feasibility, and sustainability.

PP3.1-11

**Training programme for patients with haemophilia and other coagulation disorders**

Wermes C<sup>1</sup>, Belkaidi R<sup>1</sup>, Sykora K<sup>1</sup>

<sup>1</sup>Medizinische Hochschule Hannover, Germany

**Objectives:** Before starting home treatment, children with haemophilia and their parents have to be instructed to infuse factor preparations. In the past, only technical instructions were given. During these courses we realized that young parents



and even school kids with haemophilia were often not well informed about their disease and that parents were afraid of injecting their children. Since comprehensive care centres for haemophilia (CCCs) are required to conduct educational courses to ensure the careful handling of the expensive factor concentrates we developed the following training programme to address these issues:

**Materials and Methods:** The program consists of three parts: 1. practice of self-infusion, 2. to gain knowledge about haemophilia, and 3. reflection of emotions. Different modules in each category allow individual training of groups. The training materials consist of one trainer's book and different exercise books for children and adults. A CD for the theoretical part, genetic cards to demonstrate the inheritance of haemophilia, practice guidelines for self-infusion, and mandalas to enhance the concentration are included. The work with different materials for reflection and coaching include postcards, stones, feathers, marbles, finger puppets and cloths, and proposals for music and text for fantasy journeys are made. Certificates of attendance, written examinations and lists of participants complete the equipment.

**Conclusions:** Our 10-year experience with this concept demonstrates that haemophilia training should include not only instruction of factor infusion but also theoretical exercises and coaching of patients and their families to increase knowledge about haemophilia and enhance self-confidence.

#### PP3.1-12

##### Quality of Life of healthy siblings of boys with inherited bleeding disorders (SIB-QoL-Study)

Wermes C<sup>1</sup>, Belkaidi R<sup>1</sup>, Bidlingmaier C<sup>2</sup>, Escuriola C<sup>3</sup>, von Alten S<sup>1</sup>, Sykora K<sup>1</sup>, Welte K<sup>1</sup>, Wieland I<sup>1</sup>

<sup>1</sup>Kinderklinik, Medizinische Hochschule Hannover, Germany, <sup>2</sup>von Haunersche Kinderklinik, München, Germany, <sup>3</sup>Kinderklinik, Universität Frankfurt/Main, Germany

**Objectives:** Quality of life (QoL) of children and young adults whose brothers are affected by an inherited bleeding disorder could be impaired.

**Methods:** Healthy siblings and their parents were asked by a questionnaire which included general questions concerning age, sex, education, religion, family background, health situation, and social environment. In addition standardized tests, as KINDL-Test (Ravens-Sieberer, Bullinger) for children and parents, "problem questionnaire" (Westhoff) for children and WHOQoL-Bref-test for parents were performed and questions concerning the relationship between siblings, haemophiliacs and parents were asked. The results were compared to those of other patients.

**Results:** 63 siblings (19 male, 44 female) with a median age of 11 years (4-24) and 62 parents were included. 56 brothers had haemophilia, 7 boys had other bleeding disorders. KINDL-scales (range 0-100) (8-16 years, n=37): Body 77.4(SD14.5), emotion 83.6(SD11.1), self-esteem 58.4(SD18.3), family 82.4(SD17.2), friends 76.9(SD13.6), school 70.0(SD16.8), total score 75.0(SD9.3). Younger siblings had poorer results (self-esteem 48.8) whereas parents estimate these children's QoL higher (self-esteem 76.9). In the "problem questionnaire" siblings showed comparable or less problems than the controls.

**Conclusions:** Especially the self-esteem of younger siblings was not only decreased in comparison to normal children but was also inferior to those of haemophiliac boys (HaemoQoL-, EschQoL-Study) and to children with other chronic diseases as asthma, atopic dermatitis and obesity. The observation that healthy siblings are „ostentatiously inconspicuous“ could be supported by the results of the „problem questionnaire“. Although only a small number of children had been observed, these findings suggest that siblings need more attention.

#### PP3.1-13

##### Tolerability and safety of a highly purified, plasma derived factor IX concentrate in prospective clinical studies

Feddern J<sup>1</sup>, Auerswald G<sup>2</sup>, Jansen M<sup>3</sup>, Klukowska A<sup>4</sup>

<sup>1</sup>Octapharma GmbH, Langenfeld, Germany, <sup>2</sup>Professor Hess-Kinderklinik, Bremen, Germany,

<sup>3</sup>Octapharma PPG, Austria, <sup>4</sup>Medical Academy Warsaw, Poland

Since 2001 Octanine® F, a plasma derived factor IX concentrate is available for haemophilia B patients. With the finalisation of a 5-year post-marketing surveillance study and a clinical trial in children below 6 years including PUPs, the planned programme for clinical validation is complete and a summarised evaluation of tolerability and safety can be done. In total, 71 patients were included in 4 prospective clinical studies. Almost 50 % (33) were children. The patients were followed for a total observation period of 4534 weeks. In that period they have received 3 Million units of Octanine® F with 3396 injections on 3370 exposure days. Tolerability was assessed for each injection using a 4-point rating scale. In all studies, adverse events, independent from causal relationship, were documented. Pooled analysis of tolerability assessments showed the rating "very good"/"good" in 100 % of rated injections. No adverse event had a causal relationship to the treatment. In clinical studies, the high purity factor IX concentrate Octanine® F has demonstrated an excellent safety and tolerability profile in adults as well as in children. No adverse drug reaction occurred.

#### PP3.1-14

##### Comparison of clotting factor stability in lyophilized plasma and S/D-plasma stored for 6 days at 4°C

Keller M<sup>1</sup>, Pruss A<sup>2</sup>, Spiess C<sup>1</sup>, Sander M<sup>1</sup>, Schönfeld H<sup>1</sup>, Kiesewetter H<sup>2</sup>, Rosenthal C<sup>1</sup>, von Heymann C<sup>1</sup>

<sup>1</sup>Dept. of Anesthesiology and Intensive Care Medicine Charité-Universitätsmedizin Berlin, Campus Virchow Klinikum and Campus Charité Mitte, Germany, <sup>2</sup>Institute for Transfusion Medicine, Charité-Universitätsmedizin Berlin, Campus Virchow Klinikum and Campus Charité Mitte, Germany

**Objectives:** Human plasma is frequently used to treat coagulation factor deficits and disseminated intravascular coagulation. Solvent/Detergent (S/D)-treated plasma carries a significantly lower risk of TRALI (Solheim BG, Intensive Care Med (2007); 33 (Supl.1): 1-2). Lyophilized plasma can be stored without a refrigerator for 24 months. The aim of this study was to compare stability of clotting factors in S/D-treated plasma and lyophilized plasma which were thawed, respectively liquefied and stored at 4°C for 6 days.

**Design and Methods:** The activity of Factor II (FII), Factor V (FV), Factor VIII (FVIII) and plasmin inhibitor (PI) were repeatedly determined over 6 days in 20 units of S/D-treated plasma and lyophilized plasma, each. The change of parameters over time was analyzed by means of a nonparametric ANOVA for repeated measurements.

**Results:** Over 6 days after thawing and liquefaction, activities of FII, FV and PI decreased significantly in lyophilized plasma compared to S/D-treated plasma (p < 0.01). FVIII decreased in both plasma types, but was not significantly lower in lyophilized plasma compared to S/D-treated plasma.

**Conclusions:** These results show a preserved quality of thawed S/D-treated plasma and lyophilized plasma after prolonged storage, which may improve rapid availability in emergency situations. With regard to the easier storage, administration of lyophilized plasma may offer a safe and effective option. However, activity of PI was significantly lower in both plasma types and may not be suitable to treat the according deficiency.

#### PP3.1-15

##### Development of a computerized data registry to improve patient care in hemophilia

Holstein K<sup>1</sup>, Langer F<sup>1</sup>, Bokemeyer C<sup>1</sup>, Eifrig B<sup>1</sup>

<sup>1</sup>Universitätsklinikum Hamburg-Eppendorf, Germany

**Objective:** Adequate documentation is critical to patient care in hemophilia and required by the German transfusion law § 21. Furthermore, transparency of hemophilia treatment and adherence to published treatment guidelines become increasingly important in times of limited financial resources. To our knowledge, there is a need for computerized documentation systems in German hemophilia centers.

**Methods:** We have developed the software for a computerized documentation system, which allows for easy extraction of information for other data registries such as the German hemophilia registry (DHR) or the German cause-of-death registry. Accordingly, the data structure of our registry is similar to that of the DHR. Basic patient information (i.e. demographic data, past medical history) is entered as static data, whereas follow-up information (i.e. prescription of factor concentrates, charges) is entered as dynamic data. Additionally, we document the outcome of therapy such as the number and sites of bleeds, joint scores, absence from work, and quality of life as well as treatment-related side effects such as viral infections and inhibitor development. Furthermore, additional treatment strategies such as radiosynoviothrosis, physiotherapy, surgery, or change of factor replacement as well as their respective outcomes are entered.

**Conclusion:** Our computerized data registry is likely to improve both the transparency and the quality of care in a German hemophilia center. In addition, it will help verifying the results of controlled clinical trials in unselected observational patient cohorts, a critical step in the elaboration of obligatory treatment recommendations. Finally, the data registry will facilitate the conduction of clinical research projects.

#### PP3.1-16

##### Inhibitor development and efficacy of recombinant versus plasma-derived factor VIII concentrates in haemophilia A patients, an update

Siegmund B<sup>1</sup>, Richter H<sup>1</sup>, Pollmann H<sup>1</sup>, Orlovic M<sup>2</sup>, Gottstein S<sup>2</sup>, Klamroth R<sup>2</sup>

<sup>1</sup>Institut für Thrombophilie und Hämostaseologie, Münster, Germany, <sup>2</sup>Klinik für Innere Medizin - Angiologie und Hämostaseologie, Vivantes Klinikum in Friedrichshain, Berlin, Germany

**Objective:** Since implementation of recombinant coagulation factors into hemophilia A therapy, concerns exist that switching to a recombinant FVIII (rFVIII) product may lead to a higher inhibitor rate and that rFVIII products are less effective in controlling bleeds than plasma derived factor VIII (pFVIII) concentrates. The present study compares efficacy and immunogenicity with regard to inhibitor development and reports results obtained in an observation period of fourteen years in two German haemophilia centres.

**Method:** Data were evaluated retrospectively from paper-based diaries of patients with severe haemophilia A (FVIII activity <1%) who were switched from pFVIII to rFVIII and from laboratory tests for inhibitors (Bethesda) over the years 1994 to 2007. Above values of 0.6 BU, a patient is defined as having developed an inhibitor. Efficacy of treatment is analysed as numbers of infusions needed to stop a bleed. Data are given as mean  $\pm$  SD.

**Result:** A total of 110 patients (age: 30.1  $\pm$  13.1 years), representing 1068 patient-years (PY), were included in this study. Since 1994 no patient developed a clinically relevant inhibitor, neither against pFVIII (322 PY) nor against rFVIII (746 PY). 24,203,500 IU pFVIII and 87,053,250 IU rFVIII were administered by 18,988 and 71,475 documented injections, respectively. To control a bleed 1.72  $\pm$  0.86 and 1.60  $\pm$  0.55 (p = 0.4116) injections per bleed of pFVIII or rFVIII were needed.

**Conclusions:** In respect of inhibitor development the use of rFVIII is as safe as pFVIII. The efficacy of both product types in controlling bleeds is equal.

### PP3.1-17

#### Treatment of haemophilia patients in the elderly

Miesbach W<sup>1</sup>, Alesci S<sup>1</sup>, Krekeler S<sup>1</sup>

<sup>1</sup>Goethe University, Medical Clinic III/Institute of Transfusionmedicine, Frankfurt, Germany

We included patients with haemophilia A older than 60 years of age, who visited our haemophilia centre over the last two years. We conducted a retrospective study focussing on the patients' co-morbidities as well as changes in their bleeding patterns over the last four years.

**Results:** Twenty-nine patients were included with a median age of 64 years (60 – 85 years). 8/29 patients suffered from severe haemophilia, 7/29 from moderate haemophilia and 14/29 patients from mild haemophilia. 19/29 patients had a chronic hepatitis C infection and 2/29 suffered from chronic hepatitis C and HIV. Two patients had haemophilia A and a factor VIII inhibitor. 9/29 patients suffered from cardiac disease, primarily coronary heart disease and myocardial infarction. 8/29 patients suffered from malignancies, primarily hepatocellular carcinoma and prostate cancer. 16/29 patients were treated for hypertension and 4/29 patients for diabetes mellitus. Four patients received ASS and two patients Marcumar. 7/29 patients died during the observation time. In 8/29 patients (median age: 73.5 years, 61 – 85 years) a change of bleeding patterns was noted, with a subsequent change of the substitution regimen and an increase of factor concentrates. This was mainly caused by underlying malignant disease, increasing frequency of joint bleeds, or the continuous treatment with Marcumar or ASS.

**Conclusion:** In the older haemophilia patients an adjustment of replacement therapy is necessary to compensate for frequently occurring bleeding problems. Coronary heart disease and malignancies are the most frequently occurring co-morbidities. Anticoagulant or anti-platelet therapy may complicate the treatment.

### PP3.1-18

#### Inhibitor-Immunology-Study: Toll-like-receptor (TLR)-polymorphisms in the inhibitor development of haemophilia A

Wieland I<sup>1</sup>, Wermes C<sup>1</sup>, Eifrig B<sup>2</sup>, Bidlingmaier C<sup>3</sup>, Pollmann H<sup>4</sup>, Nimtz-Talaska A<sup>5</sup>, Niekrens C<sup>6</sup>, Sykora K<sup>1</sup>

<sup>1</sup>Medizinische Hochschule Hannover, Germany, <sup>2</sup>UKE Hamburg, Germany, <sup>3</sup>Universitätskinderklinik München, Germany, <sup>4</sup>Praxis Münster, Germany, <sup>5</sup>Praxis Frankfurt/Oder, Germany, <sup>6</sup>Kinderklinik Delmenhorst, Germany

We are presenting an update of our study in which risk factors for the development of inhibitors in patients with haemophilia are to be explored. The ultimate goal is to find out why some children suffering from severe or moderate haemophilia develop inhibitory antibodies during replacement therapy and others do not and to define genetic and immunological risk factors. The development of inhibitors is one of the most important complications of replacement therapy in haemophilia, affecting both mortality and morbidity. Inhibitor development is based on complex immunological factors, and to date, only little is known about its underlying mechanisms. Cytokines and their receptors, T-cell receptors, the Major Histocompatibility Complex, as well as polymorphisms in genes associated with immune response may play important roles. So far we have analysed 81 samples, 66 patients with haemophilia A and 15 patients with haemophilia B. 35 out of 66 patients with haemophilia A did not develop any inhibitory antibody, whereas 31 did (11 low titre, 20 high titre). We have evaluated 4 SNPs in 3 different TLR-genes (TLR 2 (G2408A), TLR4 (Asp299Gly (A->G)), TLR9 (T-1237C), TLR9 (T-1486C)). Analyses were performed by restriction length fragment polymorphism. Due to a limited number of samples statistical analyses were only done for haemophilia A. We could find no association between any TLR-polymorphism and the development of inhibitory antibodies. Interestingly we could find the C-allele of the T-1486C-TLR9-polymorphism significantly more often in patients with haemophilia A than in the normal population. Sponsored by CSL Behring

### PP3.1-19

#### Compliance with long-term prophylaxis in children, adolescents and young adults with haemophilia A

Escuriola Ettingshausen C<sup>1</sup>, Heller C<sup>1</sup>, Ingrid G<sup>1</sup>, Martinez Saguer I<sup>1</sup>, Aygoeren E<sup>1</sup>, Rusicke E<sup>1</sup>, Kreuz W<sup>1</sup>  
<sup>1</sup>Klinikum der JW-Goethe-Universität, Zentrum der Kinder- und Jugendmedizin, Klinik <sup>3</sup>, Frankfurt, Germany

Primary prophylaxis of factor (F) VIII /IX in severe haemophiliacs results in the reduction of bleeds and the prevention of haemophilic arthropathy. This prospective study evaluates compliance with primary prophylaxis and its influence on bleeding frequency and joint outcome in severe haemophilia A patients. Since 1/2006, 84 haemophilia A patients (F VIII <2%) undergoing prophylaxis up to 25 years have been included. In order to evaluate therapy adherence all patients have to document each factor infusion, reason for infusion, bleeding frequency/localisation. The prescribed as well as the documented amount of concentrate used for prophylaxis is compared to the prescribed prophylactic therapy regimen. As of December, 2006, 58/84 patients (69%) documented >95% of the prescribed amount of concentrate (full compliance with documentation). Incomplete documentation (<95–50%): 4/84 (5%) and no documentation 22/84 patients (26%). Full compliance with documentation was highest in young children <12 years (81%) and decreased in older patients (12–18 years: 75%; >18 years: 53%). Patients fully compliant with documentation (n=58) are evaluated for therapy adherence: Full compliance to prophylaxis (>90% of prescribed prophylactic infusions/year) was observed in 42/58 patients (72%). Moderate compliance (75–90%) was assessed in 12/58 (21%) and weak compliance (50–75%) in 4/58 (7%) patients. Loss of compliance increased with increasing age: Full compliance was 83% in patients <12 and 47% in those >18 years. Therapy adherence decreased when patients started to inject themselves. Joint bleeds were prevented (0–1 bleeds/year) in 90% of patients with full therapy compliance whereas patients with weak compliance reported significantly more joint bleeds/year.

### PP3.1-20

#### Potency testing of recombinant FVIII products: Impact of pre-dilution on potency assignment

Dodt J<sup>1</sup>, Nawrot R<sup>1</sup>, Rosenkranz S<sup>1</sup>, Schroda A<sup>1</sup>, Hunfeld A<sup>1</sup>

<sup>1</sup>Paul-Ehrlich-Institut, Langen, Germany

Several critical parameters which may impact the result of a potency assay for FVIII-products have already been identified.

**Objective:** This contribution focuses on the impact of the pre-dilution step for potency assays for rFVIII-products. Design and method: A FVIII chromogenic assay validated for plasma-derived FVIII-concentrates was applied to a rFVIII product. After reconstitution, samples were pre-diluted in two steps using first the test kit buffer (Tris/BSA) to achieve the concentration of the standard and second using factor VIII deficient plasma to obtain a concentration of 1 IU/ml.

**Results:** FVIII-potencies estimated with the OMCL method were approx. 20% lower than the manufacturer's results. Calculations relative to the EP Batch No 3, to the 7th International Standard for FVIII-concentrate and to the manufacturer's in-house standard revealed the same trend (72%, 76% and 82%, respectively). A method comparison showed that neither test kits nor deficient plasmas or incubation time are responsible for the discrepancies. The composition of the buffer used for the pre-dilution step 1 appears to be responsible for the observation, as the potency increased from 83% to 102% (n = 5) when using Tris/Tween buffer instead of Tris/BSA. Using deficient plasma for pre-dilution step 1 and 2, as described by Ph. Eur. Method 2.7.4, confirmed the results obtained with Tris/Tween buffer in predilution step 1.

**Conclusion:** Since minor modifications may impact FVIII potency estimates, it is advisable for OMCLs to use the Ph. Eur. method for recombinant FVIII-product testing rather than to use the in-house method validated for plasma-derived FVIII-products.

### PP3.1-21

#### Activity of selected ankle muscles in haemophilic patients during bipedal standing depending on orthopaedic joint score

Kurz E<sup>1,2</sup>, Herbsleb M<sup>2</sup>, Anders C<sup>3</sup>, Czepa D<sup>1</sup>, Puta C<sup>2</sup>, Ziezio R<sup>2</sup>, Scholle H<sup>3</sup>, Hillberg T<sup>1</sup>

<sup>1</sup>Department of Sports Medicine, University of Wuppertal, Germany, <sup>2</sup>Department of Sports Medicine, Friedrich-Schiller-University Jena, Germany, <sup>3</sup>Clinic for Trauma Surgery Jena, Div. for Motor Research, Germany

Due to frequent bleedings haemophilic patients suffer from functional deficits in daily life. Adequate activity of shank muscles might be one key for appropriate postural control. The aim of this study was to determine whether differences exist in shank muscle activity between haemophilic (H) and healthy subjects (C) during upright standing. Five ankle joint muscles [tibialis anterior, fibularis longus, medial (GCM), and lateral head of gastrocnemius, and soleus (SOL)] were investigated in



25 haemophilic patients and 25 healthy control subjects by surface EMG (SEMG). According to the Gilbert-Score SEMG data of H was separated as belonging to the major (MA) and minor (MI) affected joints, respectively. Both sides of C were pooled. Nonparametric two-sample tests compared results and were further adjusted using Holm's procedure. Reduced SEMG muscle activation amplitudes could be detected during bipedal standing in H compared to C. For the muscles in MA GCM [H: 3.0(4.0/1.6), median(upper/lower quartile) in  $\mu\text{V}$ , C: 4.8(7.2/2.7)] and SOL [H: 4.4(6.4/2.7), C: 6.3(9.8/4.2)] reached significantly lower amplitudes in the H group ( $p < 0.05$ ). In MI only GCM [H: 2.6(3.8/2.0)] activation level was significantly lower in H compared to C. After the application of Holm's adjustment procedure only GCM remained at significant levels. The comparisons of the MA vs. MI showed no statistical differences. The decreased calf muscle activity of H is either due to restricted motion of the ankle or a result of increased activity of knee extensors. However, joint integrity seems to be reduced in H. (This study was supported by Baxter-Deutschland)

#### PP3.1-22

##### Surgical treatment of the hemophilic pseudotumor

Panotopoulos J<sup>1</sup>, Trieb K<sup>2</sup>, Wanivenhaus A<sup>1</sup>

<sup>1</sup>Dept of Orthopaedics Medical University Vienna, Austria, <sup>2</sup>Dept of Orthopaedics KH Wels, Austria

**Objectives:** Hemophilic pseudotumor was defined by Fernandez de Valderrama and Matthews as a progressive cystic swelling involving muscle, produced by recurrent hemorrhage and accompanied by roentgenographic evidence of bone involvement. The radiographic changes resemble that of malignant bone tumors, and this is why this rare but severe complication of haemophilia is called pseudotumor. The most common site for the hemophilic pseudotumor is the proximal skeleton around the femur and pelvis.

**Methods:** We performed a resection of a hemophilic pseudotumor of the iliac bone in 3, of the distal tibia in one and of the ulna in one patient. The mean age at surgery was 45.2 (37-60) years.

**Results:** At the latest follow up normal healing with no recurrence were observed. Only in one case the postoperative course was complicated by deep infection. In this patient revision surgery was necessary.

**Discussion:** The hemophilic pseudotumor is a rare but severe complication of hereditary bleeding disorders. In the international literature the resection and postoperative course is described as demanding and difficult and requires detailed preoperative planning. Operation should be done in specialised centers with close cooperation between surgeons and hematologists. Keywords: Hemophilic pseudotumor

#### PP3.1-23

##### Age-dependent consumption of Factor VIII concentrates in patients with severe haemophilia A in the Eastern part of Germany

Klamroth R<sup>1</sup>, Koscielny J<sup>1,2</sup>, Knöfler R<sup>1,3</sup>, Franke D<sup>1,4</sup>, Scholz U<sup>1,5</sup>, Steiner B<sup>1,6</sup>, Syrbe G<sup>1,7</sup>

<sup>1</sup>Kompetenznetz hämorrhagische Diathesen Ost, Berlin, Germany, <sup>2</sup>Charité Berlin, Germany, <sup>3</sup>Universitätsklinikum Dresden, Germany, <sup>4</sup>Praxis für Blutgerinnungsstörungen Magdeburg, Germany, <sup>5</sup>Praxis für Blutgerinnungsstörungen Leipzig, Germany, <sup>6</sup>Klinikum Süd Rostock, Germany, <sup>7</sup>Klinikum Stadtroda, Germany

**Background:** Diagnostic and treatment of severe forms of haemorrhagic diatheses (HD), such as haemophilia A (HA) need high standards of care in haemophilia treatment centers. Due to their generally low incidence, a close cooperation of haemophilia treaters is necessary to optimize treatment strategies. For this purpose, the Kompetenznetz Hämorrhagische Diathesen Ost (KHDO) was founded.

**Method:** The actual project of the KHDO was the conduction of a survey of epidemiological data of patients with severe HA and the consumption of Factor VIII concentrates in the year 2007. The data was documented in age-dependent 10-year cohorts.

**Results:** 249 patients with severe HA without an inhibitor were included. The general treatment regimen in children (age <20 years) with severe HA (n=65) was prophylaxis (85%) and in adults (n=184) about 65% were treated with an intermittent or prolonged secondary prophylaxis. A quite similar amount of factor VIII consumption was found over 10-years age-dependent cohorts in the adult patients with a maximum of 185,000 IU/year in the group of 40 to 49 years (n=58) and a minimum of 155,000 IU/year in the group of 30 to 39 years (n=41).

**Conclusions:** The data shows age-dependent consumption of factor VIII concentrates in patients with severe HA in the eastern part of Germany. In contrast to our data the centers in Great Britain (UKHCDO) found a age-dependent decrease of factor VIII-concentrates consumption in the adult patients ( $\geq 20$  years) with HA. Different explanations have to be discussed.

#### PP3.1-24

##### Consumption of recombinant FIX compared to plasma derived FIX is increased in on demand and prophylactic treated patients with severe haemophilia B

Siegmund B<sup>1</sup>, Richter H<sup>1</sup>, Pollmann H<sup>1</sup>

<sup>1</sup>Institut für Thrombophilie und Hämostaseologie, Münster, Germany

**Objective:** Haemophilia B therapy in principle consists in a sufficient supply of either plasma derived (pFIX) or recombinant factor IX (rFIX). The efficacy of rFIX (Berinin) vs. pFIX (Benefix) based on documentation by patients in home treatment setting is compared.

**Design and Methods:** Data of two patients with severe haemophilia B (FIX <1%) treated with continuous prophylaxis and of two patients treated on demand were collected over two 1-year periods before and after switch from pFIX to rFIX, so that data to be compared derived from the same patient. Data of body weight (bw), number of bleeds and substitutions, and consumption of FIX, were taken from the patients' paper diaries. Additionally, recoveries of FIX concentrates after various time periods were evaluated from 17 different patients.

**Results:** No difference was observed in the recovery of rFIX compared to pFIX. Nevertheless, total consumption increased from 459,400 pFIX IU to 577,500 IU rFIX. An increase, expressed in consumption per kg bw, was observed in all four patients after being switched from pFIX to rFIX, namely from 713.2 to 866.7 (+21%) and from 3,508.4 to 4,457.1 (+27%) under on demand treatment, and from 1,613.8 to 2,166.7 (+34%) and 2,143.2 to 2,264.6 (+6%) during prophylaxis.

**Conclusions:** Possibly, the observed increase in rFIX versus pFIX consumption can be explained by differences between packaging size and actual content of the vials. Further investigations are needed in a larger study population to verify the observation.

#### PP3.1-25

##### Beriate® P in the treatment of patients with hemophilia A: Update of a long-term pharmacovigilance

Klamroth R<sup>1</sup>, Zimmermann R<sup>2</sup>, von Stackelberg A<sup>3</sup>, Zacharias C<sup>4</sup>, Broder M<sup>4</sup>, Kurnik K<sup>5</sup>

<sup>1</sup>Vivantes Klinikum in Friedrichshain, Berlin, Germany, <sup>2</sup>SRH Kurpfalzkrankenhaus Heidelberg GmbH, Heidelberg, Germany, <sup>3</sup>Charité University Hospital, Berlin, Germany, <sup>4</sup>CSL Behring GmbH, Marburg, Germany, <sup>5</sup>Dr. von Haunersches Childrens' Hospital, University Hospital, Munich, Germany

Comprehensive large-scale pharmacovigilance surveillances are very effective tools to collect data on products in the post authorization period. Patients at any age suffering from hemophilia A treated with Beriate® P are eligible to be enrolled. Based on the standard schedule preferred at the centers, patients are routinely screened every 3 to 12 months. At these visits, the following parameters were documented (non-interventional design): overall clinical response, occurrence of bleeding, adverse drug reactions including the incidence of inhibitors, laboratory safety parameters, virus safety, relevant concomitant diseases, and relevant concomitant medication. Pharmacokinetic data are also collected. Treatment modalities with Beriate® P, including average factor consumption per month and exposure days, are recorded. Sixty-nine patients have been included into the investigation up to now; data from 441 visits were available for this fourth interim analysis. The median age was 20 years. Two patients suffered from mild, eight from moderate, and 58 from severe hemophilia A; in one patient the information on severity was missing. 70% of patients were treated prophylactically (at least one infusion per week). Median duration of the pharmacovigilance was 30 months per patient (range zero to 55 months). One case of inhibitor development in a PUP was reported. The average number of bleedings documented per year was 3.8. The median number of infusions per bleeding was 1.5. Efficacy of Beriate® P was assessed as good or excellent in 97% of all cases. The results included in this interim analysis confirm the very good tolerability, efficacy and safety of Beriate® P.

#### PP3.1-26

##### Update of a long-term pharmacovigilance surveillance: Helixate® NexGen for the treatment of hemophilia A

Oldenburg J<sup>1</sup>, Zimmermann R<sup>2</sup>, Auerswald G<sup>3</sup>, Niekrens C<sup>4</sup>, Zacharias C<sup>5</sup>, Broder M<sup>5</sup>, Lenk H<sup>6</sup>

<sup>1</sup>University Clinic Bonn, Germany, <sup>2</sup>SRH Kurpfalzkrankenhaus Heidelberg GmbH, Germany, <sup>3</sup>Prof.-Hess-Kinderklinik, Klinikum Bremen-Mitte GmbH, Germany, <sup>4</sup>Klinikum Delmenhorst GmbH, Germany, <sup>5</sup>CSL Behring GmbH, <sup>6</sup>University Hospital Leipzig, Germany

This project is assessing the long-term efficacy and safety of Helixate® NexGen (CSL Behring), a recombinant FVIII concentrate, in the post authorization period in Germany, Austria, Italy, France, Sweden. Hemophilia A-patients at any age treated with Helixate® NexGen are eligible to be enrolled. Based on the standard schedule preferred at the centers, patients are routinely screened every 3 to 12 months (non-interventional design): clinical response, occurrence of bleeding, adverse drug reactions (including inhibitors), laboratory safety parameters, pharmacokinetic data, average factor consumption and exposure days. Data from 194 patients with a total

of 1.375 visits were available for this update. The median age was 24 years (range: 15 days to 68 years). Five patients suffered from mild, 29 from moderate, and 156 from severe hemophilia A; in four patients the information on severity was missing. Most of the patients were treated prophylactically (70 %, at least one infusion per week). Exposure to Helixate®NexGen during the pharmacovigilance ranged between one day and 67 months. Median time between visits to the center was 3.7 months. Two cases of inhibitor development were reported (1 PUP, 1 PTP). The median number of bleedings per year was 2.75 per patient. 16 % of the patients had no bleedings at all and 89 % had no major bleedings. A median number of 1 infusion was administered per bleeding. Efficacy of Helixate®NexGen was assessed as good or excellent in 97% of all documented bleedings. The results included in this interim analysis confirm the very good tolerability and efficacy of Helixate®NexGen.

### PP3.1-27

#### Prevalence of nucleic acid sequences specific for human parvoviruses, hepatitis A and hepatitis E in preparations of blood coagulation factors

Modrow S<sup>1</sup>, Schimanski S<sup>1</sup>, Wenzel J<sup>1</sup>, Meier C<sup>1</sup>, Schwarzbeck J<sup>2</sup>, Rothe U<sup>2</sup>, Jilg W<sup>1</sup>

<sup>1</sup>Institut für Medizinische Mikrobiologie, Universität Regensburg, Germany, <sup>2</sup>Apothek des Universitätsklinikums Regensburg, Germany

Non-enveloped human viruses present a special problem in blood plasma products. Whereas parvovirus B19 (B19V), hepatitis A and E viruses (HAV, HEV) are well-known contaminants of plasma pools, limited data are available for human bocavirus (HBoV) and Parv4. We intended to gain knowledge on the presence of nucleic acid sequences of B19V, HBoV, Parv4, HAV and HEV in currently used commercial coagulation factor products. Three different batches of twelve different recombinant and plasma-derived factor products used for treatment of patients with factor VII, VIII, IX deficiencies were tested for the presence of viral genomes by qPCR. Isolations of viral nucleic acids were performed from each sample using three independent assay runs. Each nucleic acid preparation was submitted for quantitative analysis of viral genomes in two independent test runs. Results were estimated as positive in cases with positive nucleic acid detection either in two of the independent DNA preparations or in both qPCR runs. Whereas all recombinant factor products did not contain nucleic acids derived from either of the viruses tested, significant amounts of B19V-DNA (2x10<sup>1</sup>-1.5x10<sup>3</sup> geq/ml) were detected in one plasmatic factor VIII, one plasmatic factor VIII/vWF, one factor VII, and one activated prothrombin complex concentrate product. All recombinant factor preparations were free from nucleic acid sequences derived from HAV, HEV, HBoV and Parv4. Significant amounts of B19V-DNA were observed in plasma-derived products. This reflects the frequent contamination of human plasma pools with B19V and points to the necessity for efficient methods to inactivate the infectivity of non-enveloped viral pathogens.

### PP3.1-28

#### Management of a patient with severe haemophilia A and acute lymphoblastic leukaemia

Müller-Beisshirtz H<sup>1</sup>, Novotny J<sup>1</sup>, Dührsen U<sup>1</sup>

<sup>1</sup>Klinik für Hämatologie, Universitätsklinikum Essen, Germany

**Introduction:** The coincidence of severe haemophilia and acute leukaemia is extremely rare and poses a challenge to prevent bleeding during long periods with severe thrombocytopenia.

**Case report:** A 20-year old male with severe haemophilia was diagnosed with acute lymphoblastic leukaemia (ALL). On admission the patient suffered from mild to moderate epistaxis. The patient was treated according to the GMALL 07/03-Protocol, no asparaginase was given to prevent further coagulopathies. To prevent bleeding because of additional thrombocytopenia following chemotherapy factor VIII was administered according to the following protocol: Platelets 50 - 100/nl: 15 IU F VIII/kg bodyweight (BW) 3 times weekly; Platelets 30 - 49/nl: 15 IU F VIII/kg BW daily; Platelets 20 - 29/nl: 20 IU F VIII/kg BW daily; Platelets <20/nl: 15 IU IU F VIII/kg BW twice daily. Before and 8 hours after lumbar puncture 40 IU F VIII/kg BW was substituted, before bone marrow aspiration 40IE F VIII/kg BW. Platelet-concentrates were transfused when the platelet count was <20/nl because of the additional bleeding risk. In case of bleeding additional F VIII was administered and in case of mucosal bleeding additional antifibrinolytic therapy was initiated.

**Results:** After receiving 3 cycles of conventional chemotherapy the patient underwent myeloablative chemotherapy and allogeneic blood stemcell transplantation because of a high risk ALL-constellation. Unfortunately he died following transplantation because of a meningococcal meningitis. No relevant bleeding episodes occurred during the treatment period.

**Conclusion:** Bleeding risk during aggressive chemotherapy in a haemophilic patient can be minimized and chemotherapy according to protocol is feasible.

### PP3.1-29

#### Hemostasis management in elderly patients with bleeding disorders and significant comorbidity

Kramer-Steiner B<sup>1</sup>, Bruhn P<sup>2</sup>, Hähling D<sup>3</sup>, Berthold B<sup>4</sup>, Leithäuser M<sup>5</sup>, Suhren H<sup>1</sup>

<sup>1</sup>Klinikum Südstadt Rostock, Germany, <sup>2</sup>Hämatologische Gemeinschaftspraxis Rostock, Germany,

<sup>3</sup>Helios Klinikum Schwerin, Germany, <sup>4</sup>Bonhoeffer Klinikum Neubrandenburg, Germany,

<sup>5</sup>Universitätsklinikum Rostock, Germany

Improved overall treatment in patients with congenital bleeding disorders contributes to their longevity. At the same time, new challenges evolve including increased numbers of medical and surgical conditions commonly associated with advanced age. Except for anecdotal evidence, no recommendations are available with respect to cancer, cardiovascular disease, and renal failure to name but a few. We surveyed a regional Northeast Germany-focused database comprised of 151 patients (121 males and 30 females, median age 38 years, range 12 to 84) suffering from bleeding disorders (79 hemophilia A, 23 hemophilia B, 38 von Willebrand disease) for the presence of significant comorbidity. Among 54 patients affected, challenging diagnoses included malignancy (n=3), cardiovascular disease (n=12), and urogenital disorders (n=8). Hemostasis management was modified according to the predicted bleeding risk. Treatment on demand decreased from 41 to 26 patients. In contrast, prophylactic treatment increased from 13 to 28 patients and home treatment from 28 to 50 patients, respectively. Demanding situations including cancer surgery, heart valve replacement, and hemodialysis treatment could all be successfully managed without the occurrence of significant bleeding events. Networking of all medical staff involved was an essential prerequisite. We conclude that the increasing number of significant medical and surgical comorbidity can be successfully managed if a true interdisciplinary approach is implemented. Sufficient control of hemostasis can be achieved by moving from treatment on demand to prophylactic substitution protocols in high risk patients.

### PP3.1-30

#### Nine years of the ReFacto Pharmacovigilance evaluation – safety and efficacy in daily clinical practice

Pollmann H<sup>1</sup>

<sup>1</sup>ITH Institut für Thrombophilie und Hämostaseologie, Münster, Germany

**Objectives:** Non-interventional trials appear to be appropriate means to monitor treatment of a rare disease like haemophilia. The pharmacovigilance evaluation (PE) of ReFacto has been ongoing in Germany and Austria for more than nine years. Its aim is to continuously monitor safety and efficacy of treatment of haemophilia A with Moroctocog alfa under routine clinical conditions.

**Methods:** The study is a non-interventional trial. Patients with haemophilia A of any severity, treated with Moroctocog alfa can be included in the study. Safety is assessed by documentation of all (serious) adverse events during treatment with ReFacto. Special focus is on the development of inhibitors in PTPs and PUPs. Efficacy assessment is performed e.g. by evaluating the number of exposure days per bleeding episode.

**Results:** Until August 2008, 270 patients were recruited in 45 centers in Germany and Austria. 24 (8.9%) were previously untreated (PUPs) and 246 (91.1%) previously treated patients (PTPs). 225 patients (83.3%) suffered from severe haemophilia A. 26 PTPs had a positive inhibitor history at baseline. De novo inhibitors developed in 4/222 (1.8%) PTPs and 3/24 (12.5%) PUPs. Treatment was effective with a median number of 1.33 exposure days per bleeding episode.

**Conclusions:** The ongoing PE of ReFacto® is the first long-term analysis of a currently marketed FVIII product in Germany and Austria under routine clinical conditions. Data from nine years duration and 270 patients confirm the safety and efficacy of ReFacto® in treatment of haemophilia A in daily clinical practice.

### PP3.1-31

#### 12-years efficacy, safety and inhibitors in patients treated with one recombinant Factor VIII Concentrate

Gottstein S<sup>1</sup>, Heinrichs C<sup>1</sup>, Klamroth R<sup>1</sup>

<sup>1</sup>Vivantes Klinikum im Friedrichshain, Berlin, Germany

**Introduction:** The safety and efficacy of clotting factor concentrates in hemophilia is of continuous interest as substitution is life-long. Many improvements concerning safety had been made in the last 30 years.

**Methods:** We retrospectively investigated efficacy, virus safety, rate of inhibitors, immunological parameters, liver enzymes and immunoglobulins over a 12-year period from 1996 to 2007 in 22 patients with hemophilia A. All patients were treated at least one year with the recombinant factor VIII concentrate (rFVIII, Kogenate Bayer) in this time period.

**Results:** Of 22 patients investigated, 17 patients suffered from severe, 2 patients from moderate and 3 patients from mild hemophilia A. These patients represented a total of 160 patient years and a consumption of 22.752.500 IU of rFVIII (Koge-



nate Bayer). 12 patients had a change of product during the shortage of rFVIII in 2001/2002 (transition from first generation Kogenate to albumin free Kogenate Bayer). All patients but 1 used Kogenate Bayer again when available in 2002. We did not find any seroconversion towards the viruses investigated nor did we identify any inhibitor. There were no immunological changes. In 18 surgeries (11 total joint replacements, 1 cholecystectomy, 1 strumektomy and 5 minor surgeries), efficacy of rFVIII was always rated to be good or excellent and rFVIII was always well tolerated.

**Conclusions:** rFVIII (Kogenate Bayer) has a good to excellent efficacy, safety and inhibitor profile. Switching patients to different factor VIII concentrates (shortage in 2001/2002) had no impact on inhibitor development in our patient group.

#### PP3.1-32

##### Open synovectomy in a 4.5 year old boy with chronic non responding synovitis of the ankle joint

Horneff S<sup>1</sup>, Goldmann G<sup>1</sup>, Berdel P<sup>2</sup>, Schott D<sup>2</sup>, Vidovic N<sup>1</sup>, Niemann B<sup>1</sup>, Wirtz D<sup>2</sup>, Oldenburg J<sup>1</sup>

<sup>1</sup>Institut für Experimentelle Hämatologie und Transfusionsmedizin, Bonn, Germany, <sup>2</sup>Klinik und Poliklinik für Orthopädie und Unfallchirurgie

**Background:** Chronic synovitis is a frequent complication of joint bleeding in hemophilic patients. Conservative therapy regimens including factor replacement, oral non-steroidal anti-inflammatory drugs and physical therapy are not always successful.

**Methods:** We report on a 4.5 year old boy with severe hemophilia A (FVIII<1%, Intron-22- inversion) and traumatic ankle joint bleeding. Initially, twice daily substitution with recombinant FVIII was performed followed by once daily treatment. Regular physical therapy was performed. Despite of consequent FVIII replacement therapy over 6 months the swelling of the ankle persisted. Joint aspiration, intraarticular triamcinolone, ibuprofen and later in the course a short oral prednisolone pulse were performed resulting in only transient improvement. There was no evidence for systemic disease. Marked effusion with pannus and thickening of the synovium were demonstrable by ultrasound and MRI. There was no cartilage destruction so far. An open synovectomy of the ankle joint with biopsy was done without complications. The histological examination showed hemophilic hypertrophic synovitis. Additionally, a radiosynoviorthesis was performed recently. Long term outcome has to be awaited.

**Conclusion:** In rare cases chronic refractory synovitis induced by joint bleeding in hemophilic patients occurs also during early childhood and may necessitate synovectomy and even radiosynoviorthesis.

#### PP3.1-33

##### Haemoassist™ – implementation and challenges of an electronic patient diary in daily practical application

Mondorf W<sup>1</sup>, Klamroth R<sup>2</sup>, Siegmund B<sup>3</sup>, Westfeld M<sup>4</sup>, Pollmann H<sup>5</sup>

<sup>1</sup>InThera - Investigating Advances in Therapy Frankfurt, Germany, <sup>2</sup>Vivantes - Krankenhaus im Friedrichshain, Berlin, Germany, <sup>3</sup>ITH - Institut für Thrombophilie und Hämostaseologie, <sup>4</sup>Wyeth Pharma GmbH, Münster, Germany, <sup>5</sup>ITH - Institut für Thrombophilie und Hämostaseologie, Münster, Germany

**Objectives:** Haemoassist™ was developed as an electronic patient diary in order to improve communication between patients and their physicians and to enhance quality of haemophilia care. Haemoassist™ is a learning system that can be adapted to meet the changing needs in haemophilia. This work presents the recent improvements and future challenges of the tool.

**Methods:** From April 2007 until September 2008 Haemoassist™ has been rolled out in 22 treatment centers throughout Germany. Currently 74 patients use Haemoassist™ regularly for treatment documentation. During scale-up, potential modifications and improvements of the tool occurred and were discussed in a scientific advisory board before implementation.

**Results:** In the first 18 months of roll-out, a number of changes and improvements have been made to the original software. The QoL questionnaire has been removed; changes to the reminder functions have been made. Furthermore, the mobile phone function of the PDA has been activated exclusively for the emergency number of the respective patients' treatment center in order to improve compliance and enable immediate contact in critical situations. A medication management system is currently in development including automated reminders for physicians in case the patient is close to stock-out of factor concentrate.

**Conclusions:** The successful application of Haemoassist™ in daily routine underlines the value of a learning system that is able to react flexibly to changing needs of patients and physicians. As a next step we are planning to open Haemoassist in order to facilitate the inclusion of a significantly larger number of patients.

#### PP3.1-34

##### Evaluation of safety and efficacy of recombinant Factor IX in daily clinical practice: a pharmacovigilance evaluation of BeneFIX

Westfeld M<sup>1</sup>, Pollmann H<sup>2</sup>, Laws H<sup>3</sup>, Huth-Kühne A<sup>4</sup>, Niekrens C<sup>5</sup>, Girisch M<sup>6</sup>, Ries M<sup>7</sup>, Oldenburg J<sup>8</sup>

<sup>1</sup>Wyeth Pharma GmbH, Münster, Germany, <sup>2</sup>ITH - Institut für Thrombophilie und Hämostaseologie Münster, Germany, <sup>3</sup>Universitätsklinikum Düsseldorf, Klinik für Kinder-Onkologie, Hämatologie und klinische Immunologie, Germany, <sup>4</sup>SRH Kurpfalzkrankenhaus und Hämostasezentrum, <sup>5</sup>Klinikum Delmenhorst, Germany, <sup>6</sup>Universitätskinderklinik Tübingen, Germany, <sup>7</sup>Klinikum Memmingen, Germany, <sup>8</sup>Universitätsklinikum Bonn, Germany

**Objectives:** Primary objective of the described study is the long-term evaluation of the safety profile of Nonacog alfa (BeneFIX), indicated for treatment and prophylaxis of haemophilia B (HaemB), in daily clinical practice (pharmacovigilance evaluation, PE). Since HaemB is a very rare disease and only a limited number of patients can be included in clinical trials, a non-interventional post-authorization study with a focus on safety parameters appears to be adequate.

**Methods:** Patients with HaemB of any severity treated with reformulated BeneFIX can be included in the study. Safety of treatment is evaluated by recording all (serious) adverse events of BeneFIX treatment during the study period. The aim is to include approx. 80–100 patients. The data collection period will last for at least 3 years and is very likely to be extended. The study is set up and managed by the medical department of Wyeth Pharma GmbH in collaboration with a scientific advisory board.

**Results:** The PE started in Germany in February 2008. Until September 2008, 12 centers have been initiated and 27 patients included in the study, of which 24 received > 100 infusions of another FIX concentrate prior to inclusion. 23 patients have severe HaemB. Further results will be presented in February 2009.

**Conclusions:** The recruitment data so far show high acceptance and interest in the study from the participating centers. Non-interventional trials appear to be adequate means to assess the safety and efficacy of a treatment in the post-authorization phase, especially in very rare diseases such as HaemB.

#### PP3.2 Von Willebrand Disease

##### PP3.2-1

##### Characterization of two novel large deletions causing von Willebrand disease type 3

Ahmad F<sup>1</sup>, Schneppenheim R<sup>2</sup>, Oyen F<sup>2</sup>, Obser T<sup>2</sup>, Kannan M<sup>1</sup>, Saxena R<sup>1</sup>

<sup>1</sup>All India Institute of Medical Sciences (AIIMS), New Delhi, India, <sup>2</sup>Pediatric Hem. & Onc., University Med. Ctr., Hamburg-Eppendorf, Germany

**Objective:** To characterize the breakpoints of two large novel deletions in two unrelated patients with severe VWD type 3.

**Patients and Methods:** Two unrelated children from India with type 3 VWD were initially diagnosed by ristocetin induced platelet aggregation followed by other VWD specific tests (VWF:Ag, VWF:RCo, FVIII:C and multimer analysis). Initial mutation screening by PCR revealed large deletions in both patients that were further characterized by PCR and primer walking using 37 different sets of primer pairs. Finally a deletion spanning PCR was carried out followed by DNA sequencing to exactly map the deletion breakpoints.

**Results:** Both patients were homozygous for 2 different deletions. Patient 1 had a deletion of approximately 81kb from the centromeric 5' deletion breakpoint in the untranslated region between CD9 and VWF and the telomeric 3' breakpoint located in intron 6 of VWF. A 24bp direct repeat flanking the deleted sequence was present. Patient 2 had a deletion of approximately 2kb including part of intron 13 and exon 14 of VWF. The centromeric 5' breakpoint was located in intron 13 and the telomeric 3' breakpoint in exon 14 of VWF. In this case there was no large homologous sequence flanking the deletion breakpoints.

**Conclusion:** We report two large novel deletions in two Indian unrelated patients probably caused by DNA double strand breaks followed by non homologous end joining. Characterization of the deletion breakpoints allowed to design deletion specific primers which enable the identification of heterozygous carriers as a reliable basis for genetic counseling.

##### PP3.2-2

##### The evaluation of whole blood platelet aggregation in von Willebrand disease

Lison S<sup>1</sup>, Dick A<sup>2</sup>, Giebl A<sup>2</sup>, Kauke T<sup>2</sup>, Spannagl M<sup>2</sup>

<sup>1</sup>Klinik für Anaesthesiologie, Munich, Germany <sup>2</sup>Klinik für Anaesthesiologie, Abteilung für Transfusionsmedizin und Hämostaseologie

**Objectives:** Von Willebrand disease (VWD) is characterized by deficiency or dysfunction of von Willebrand factor (VWF) (VWD type 1–3). The diagnosis of VWD is based on both patient's medical history and extensive laboratory evaluations of VWF antigen, structure and activity. Multiple electrode aggregometry (MEA)

allows for the assessment of platelet function in whole blood and can be performed using ristocetin as the trigger. Ristocetin-induced platelet aggregation is dependent on VWF and GpIb. The aim of this study was to evaluate MEA for diagnosis of VWD.

**Design and Methods:** 20 healthy individuals, 14 patients with VWD type 1 (VWD1) and 13 patients with VWD type 2 (VWD2) were included in this study. Patients were specified by their bleeding history, determination of VWF antigen, gel electrophoresis and activity and most confirmed through genotyping. Aggregation was triggered by TRAP-6 (thrombin receptor activated peptid) (TRAPtest), arachidonic acid (ASPItest), ADP (ADPtest) and ristocetin (RISTOtest) using the Multiplate® analyzer (Dynabyte, Munich). Analyses were performed in hirudin-anticoagulated blood.

**Results:** Area under the curve (AUC) values with TRAPtest were comparable in all groups. Using RISTOtest significantly reduced AUC values were detected in 8/14 patients with VWD1 ( $p < 0.05$ ) and 12/13 patients with VWD2 ( $p < 0.0001$ ). 6 of these 8 patients with VWD1 showed additionally reduced AUC values with ASPItest and 2 with ADPtest. 3/13 patients with VWD2 revealed abnormal AUC values with ADPtest.

**Conclusion:** MEA is a simple whole blood platelet aggregation method. Our results indicate that MEA contributes to the laboratory diagnosis of VWD.

### PP3.2-3

#### Mechanisms underlying acquired von Willebrand syndrome associated with an IgM paraprotein

Mayerhofer M<sup>1</sup>, Haushofer A<sup>2</sup>, Kyrle P<sup>3</sup>, Chott A<sup>4</sup>, Müllner C<sup>1</sup>, Quehenberger P<sup>1</sup>, Traby L<sup>3</sup>, Eichinger S<sup>3</sup>

<sup>1</sup>Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Vienna, Austria, <sup>2</sup>Institute of Laboratory Medicine, Landeskrankenhaus St. Pölten, Austria, <sup>3</sup>Department of Internal Medicine I, Medical University of Vienna, Austria, <sup>4</sup>Clinical Institute of Pathology, Medical University of Vienna, Austria

**Objectives:** Acquired von Willebrand (vW) syndrome is a rare bleeding disorder which is frequently associated with immunologic, malignant or cardiovascular disorders. The underlying pathomechanisms particularly in patients with IgM monoclonal gammopathies often remain unknown.

**Methods and Results:** We report a patient with indolent small B-cell lymphoma and plasmacytic differentiation with an IgM-paraprotein who was admitted with retroperitoneal hematoma. Medical history and coagulation testing were consistent with acquired vW syndrome. vW immunohistochemistry showed normal cytoplasmic labelling of endothelial cells and megakaryocytes, whereas the lymphomatous infiltrate was negative. Acquired vW syndrome due to adsorption of vW factor on malignant cells was thus excluded. In the multimeric analysis all multimers were present similar to type 1 vW syndrome, but the triplet structures were blurred. The bands on serum immunofixation electrophoresis were also atypically broadened which suggested complex formation between the IgM and vW factor. Immunoprecipitation studies showed that the 176 kDa proteolytic fragment of vW factor co-precipitated with the IgM paraprotein in the patient but not in the controls, suggesting a specific interaction between vW factor and the paraprotein in the patient. The patient required surgery and was successfully managed by chemotherapy and plasma exchange.

**Conclusions:** Binding of vW factor to a paraprotein is a rare pathomechanism of acquired vW syndrome. Treatment with vW factor concentrate and desmopressin are less effective, but chemotherapy and plasma exchange to decrease the paraprotein should be considered.

### PP3.2-4

#### Evaluation of VWF activity assays for VWF/FVIII concentrates, employing different collagen types

Stadler M<sup>1</sup>, Gruber G<sup>1</sup>, Janisch S<sup>1</sup>, Brainovic J<sup>1</sup>, Zapfl C<sup>1</sup>, Römisch J<sup>1</sup>

<sup>1</sup>Octapharma PPGmbH, Vienna, Austria

**Objective:** According to Ph. Eur. supplement 5.5, the collagen-binding assay (VWF:CB) can be used to determine human VWF activity as an alternative to the ristocetin cofactor assay (VWF:RCO). An International Standard with a labelled VWF:CB potency is not yet available, while different collagen types and origins can be utilised to measure adhesion of VWF derived from VWF/FVIII concentrates. In particular controversial results derived from the use of equine collagen I versus human collagen type III were reported in the past, whereas human collagen I or VI has not been sufficiently investigated yet.

**Methods:** TECHNOZYM VWF:CBA ELISAs with human collagen type I, III or VI (all Technoclone, Vienna, Austria) were performed using Wilate® and other VWF/FVIII concentrates, pre-diluted to an adequate VWF:Ag (antigen) concentration. The VWF:CB results were related to the respective VWF:Ag contents.

**Results:** Wilate® revealed no significant difference in the respective VWF:CB/VWF:Ag ratios applying different types of human collagen.

**Conclusion:** Although collagen assays are established for the determination of VWF activity in plasma samples used for diagnostic purposes, the source of collagen used for the assessment of VWF/FVIII concentrates deserves further evaluation. Our studies show that the use of different collagen types (I, III and VI) leads to comparable results when using collagen of human origin, representing the in vivo matrixes better than equine collagen.

### PP3.2-5

#### Improved automated VWF:RCO assay for VWF/FVIII concentrates and plasma samples by adjustment of assay reagents, composition and performance parameters

Stadler M<sup>1</sup>, Hillarp A<sup>2</sup>, Haderer C<sup>1</sup>, Zapfl C<sup>1</sup>, Pock K<sup>1</sup>, Weinberger J<sup>1</sup>, Römisch J<sup>1</sup>

<sup>1</sup>Octapharma PPGmbH, Vienna, Austria, <sup>2</sup>Malmö University Hospital, Sweden

**Objective:** The VWF:RCO assay (Ph.Eur.) is based on the visual assessment of platelet agglutination endpoints in dilution series. Automated methods have established for diagnosis. However, limitations in assessing VWF/FVIII concentrates for the VWF:RCO level utilising these assays are reported.

**Methods:** The BCS-XP instrument (Siemens AG) was employed using the International Standard von Willebrand Factor Concentrate (00/514). Variations of assay reagents and performance parameters were investigated and the most promising approach was validated for VWF/FVIII concentrate and plasma sample testing.

**Results:** Automated measurements revealed an influence on VWF:RCO values upon variation of performance parameters. Improved accuracy, precision and a lower limit of quantification could be achieved by a modification of the current method involving increased ristocetin concentration and a two arm calibration mode.

**Conclusion:** Introduction of automated methods for VWF:RCO testing is appreciated, provided results are reliable and devoid of system-dependent artefacts. Our study resulted into an improved method for concentrate testing, achieved by appropriate reference preparations, optimisation and adjustments of critical assay reagents and performance parameters. Cross-validations with the established method justify its global use for VWF:RCO quantification, especially when different VWF/FVIII concentrates and patient samples are tested for adequate dosing and monitoring.

### PP3.2-6

#### Update on efficacy and tolerability of a new generation VWF/FVIII concentrate in von Willebrand's disease from a prospective post-marketing surveillance

Scharrer I<sup>1</sup>, Halimeh S<sup>2</sup>, Kadar J<sup>3</sup>, Nowak-Göttl U<sup>4</sup>, von Depka M<sup>5</sup>, Feddern J<sup>6</sup>

<sup>1</sup>Octapharma GmbH, Langenfeld, Germany

A prospective post-marketing study (SET = Surveillance of Efficacy and Tolerability) with a high-purity, albumin-free, double virus inactivated VWF/FVIII concentrate (Wilate®) was initiated in Germany in 2005. As pre-marketing clinical trials with coagulation factors usually include only a limited number of cases, such post-marketing studies are an excellent tool to enhance safety surveillance and provide evidence on the efficacy and safety in routine clinical use. Results of an interim analysis from October 2008 including 64 patients with von Willebrand's disease (VWD) are presented. Details of all infusions, on bleeding episodes, prophylaxis and surgical procedures were documented. Clinical efficacy and tolerability were rated using a four-point verbal scale. 40 of 64 patients were female. 57 patients had VWD type 1 or 2. The age ranged from 1–82 years (mean and median 36). 11 patients were children below 12 years of age. 39 patients underwent 47 surgeries (55 % major procedures) which were all assessed "excellent/good" in efficacy and tolerability. In total, 83 bleeding episodes were treated with a median dose of 31 IU/kg per day; the efficacy was assessed as "excellent/good" in 97 % of all cases. 5 patients had been receiving sustained prophylactic treatment. The tolerability was assessed with "very good/good" in 98 % of 930 injections for treatment or prevention of haemorrhage.

**Conclusion:** Results of the interim analysis of the ongoing Wilate SET study, reflecting the experience in routine clinical use, further confirm the excellent data on efficacy and tolerability, which had been demonstrated by the pre-marketing clinical trials.

### PP3.2-7

#### Treatment of pediatric von Willebrand's disease patients with high purity double virus inactivated VWF/FVIII concentrate – experience from clinical studies

Nowak-Göttl U<sup>1</sup>, Halimeh S<sup>2</sup>, Russo A<sup>3</sup>, Scharrer I<sup>3</sup>, Sigl-Kraetzig M<sup>4</sup>, Jansen M<sup>5</sup>, Feddern J<sup>6</sup>

<sup>1</sup>Universitätsklinik Münster, Germany, <sup>2</sup>MVZ Labor Duisburg, Germany, <sup>3</sup>Universitätsklinik Mainz, Germany, <sup>4</sup>Pädiatrische Praxis Blaubeuren in Kooperation mit BGU, Germany, <sup>5</sup>Octapharma Wien, Austria, <sup>6</sup>Octapharma Langenfeld, Germany

Efficacy, tolerability and dosing of a VWF/FVIII concentrate in paediatric patients may differ from those of adults. It is therefore reasonable to collect and evaluate clinical data of this patient group. Treatment of 19 children between 1 and 12 years of age with all types of von Willebrand disease (VWD), who were included either



in a pre-marketing clinical trial or a prospective post-marketing surveillance, with a high-purity, albumin-free, double inactivated VWF/FVIII concentrate (Wilate<sup>®</sup>) is presented. Details of all injections, bleeding episodes, prophylaxis and surgical procedures were documented. Efficacy and tolerability were rated using a 4-point verbal scale. 8 children had VWD type 1, 4 patients had type 2, and 7 suffered from type 3. 11 patients experienced in total 216 bleeding episodes; efficacy of the treatment was rated "excellent/good" for all episodes (100%). The average dose per infusion for treatment of bleed was 30 I.U./kg body weight. In 8 patients, 16 surgeries were performed, all with "excellent/good" efficacy (100%). 9 patients were treated prophylactically for at least three months (mean: 1.6 years). Although five patients had been on prophylaxis with another concentrate before, the bleeding frequency could be further reduced in all 9 patients. The median bleeding frequency/months dropped from 4 to 1. The tolerability was assessed "excellent/good" in all injections (100%).

**Conclusion:** At comparable doses, the efficacy and tolerability of Wilate<sup>®</sup> treatment in children was as high as in adults. The experience in VWD prophylaxis with Wilate<sup>®</sup> suggests that this treatment modality is highly beneficial for paediatric patients.

### PP3.2-8

#### Prolongation of in vitro bleeding time (PFA 100 closure time) in a case of Heyde-syndrome – a case report

Rott H<sup>1</sup>, Halimeh S<sup>1</sup>, Kappert G<sup>1</sup>

<sup>1</sup>MVZ Labor Duisburg, Germany

**Objective:** We describe a case of Heyde-syndrome. A 87 year old patient was sent for coagulation testing because of a planned hip replacement surgery. The patient had already 2 bleeding complications in 2007. 1. bleeding after prostate surgery due to a prostate adenoma 2. and a bleeding gastric ulcer. Both times he needed several red blood cell transfusions. In preparation for the planned hip replacement a measurement of in vitro bleeding time (PFA 100) has been carried out with a strong prolongation of closure time with both cartridges (Coll/Epi and Coll/ADP). The only pathologic finding in coagulation workup was an absence of large von Willebrand factor multimers. All other coagulation tests (fibrinogen, prothrombin, factor V, VII, VIII, IX, X, XI, XII, XIII, thrombocyte aggregation testing (Born) with arachidonat, epinephrine, ADP and collagen and the von Willebrand associated parameters (ristocetin cofactor, von Willebrand antigen, von Willebrand collagen binding capacity) were in normal range. The patient was referred to a cardiologist, who found a combined aortic vitium grade III and a mitral valve insufficiency grade II and therefore the diagnosis of Heyde-syndrome (acquired von Willebrand syndrome Type IIa due to aortic stenosis) was confirmed.

**Conclusion:** In elderly patients with bleeding complications and normal global coagulation tests a test for defects primary blood coagulation like PFA 100 closure time should be conducted not to miss an acquired bleeding disorder related to heart valve disease.

### PP3.2-9

#### Management of acquired von Willebrand syndrome in pregnancy: A case report

Lohse J<sup>1</sup>, Gehrisch S<sup>2</sup>, Tiede A<sup>3</sup>, Budde U<sup>4</sup>, Knöfler R<sup>1</sup>

<sup>1</sup>Dept. of Pediatric Hematology and Oncology, University Hospital Dresden, Germany, <sup>2</sup>Institute of Clinical Chemistry, University Hospital Dresden, Germany, <sup>3</sup>Hannover Medical School, Dept. of Haematology, Oncology and Haemostasis, Germany, <sup>4</sup>Aescu Laboratory Hamburg, Germany

**Objectives:** We report on the management of a 29-year-old pregnant woman with acquired von Willebrand syndrome (AVWS) due to monoclonal gammopathy of undetermined significance (MGUS).

**Design and Methods:** The disease was most likely acquired in the course of a prolonged EBV infection. A serious postoperative bleeding after tonsillectomy led to an extensive coagulation diagnostics. The von Willebrand factor antigen (VWF:Ag) and factor VIII activity (FVIII) were diminished (10–20%), the PFA 100<sup>®</sup> closure times were prolonged, serum protein electrophoresis detected the gammopathy. VWF multimer analysis showed no abnormalities and anti-VWF antibodies were weakly positive for IgG and negative for IgM. Besides the surgery-associated bleeding the patient did not display bleeding symptoms.

**Results:** In the 28th gestational week (GW) the patient was hospitalized because of premature labor and cervical insufficiency. Intravenous immunoglobulin g (IgG) at a dosage of 1.0 g/kg was given leading to the elevation of VWF:Ag and FVIII up to 200%. Premature labor disappeared and during the next 14 days the values decreased continuously. IgG application was repeated at a dosage of 0.4 g/kg. Values for VWF:Ag and FVIII raised again to about 80% and 4 days later, in the 30th GW, an emergency cesarean section was performed without bleeding complications. Neither the patient nor the child did show any bleeding symptoms.

**Conclusions:** A pregnant patient with AVWS due to MGUS was successfully treated with repeated IgG infusions and delivered a healthy premature newborn by emergency cesarean section without bleeding complications.

### PP3.2-10

#### Evaluation of prospective criteria for the clinical assessment of efficacy and safety of DDAVP

Miesbach W<sup>1</sup>, Dück O<sup>1</sup>, Krekeler S<sup>1</sup>, Llugallo B<sup>1</sup>, Asmelash G<sup>1</sup>, Schüttrumpf J<sup>2</sup>, Alesci S<sup>1</sup>, Grossmann R<sup>3</sup>

<sup>1</sup>Goethe University Frankfurt, Internal Medicine III/ Institute of Transfusionsmedizin, Germany,

<sup>2</sup>German Red Cross - Blood Donor Service, Baden-Wuerttemberg - Hessen, Institute for

Transfusionsmedizin and Immunohematology, Germany, <sup>3</sup>Gemeinschaftspraxis Laborärzte Schweinfurt, Germany

**Introduction:** Goal of this study is the identification of prospective markers for severe side effects after DDAVP administration.

**Material and Methods:** DDAVP was administered intravenously in a dose of 0.22–0.35 µg/kg b.w. and laboratory routine investigations were done at baseline and after the test. Patients were again interviewed three days after and asked about side effects.

**Results:** Up to now, 28 female and 21 male patients were enrolled into this study (female: 57%, male: 43%, median age: 33 years, range: 18–60 years). Significant differences (up to p<0.0001) towards normalisation were observed for FVIII:C, vWF:Ag, vWF:RiCoF, vWF:CBA, PFA collagen/epinephrine and PTT prior to and after the test. Further significant p-values were seen for reduction in diastolic blood pressure, increase in heart rate, reductions in chloride and calcium, increase in INR, increase in glucose, reduction in erythrocytes, haemoglobin and haematocrit, increase in leucocytes, platelets, reduction in d-dimers and fibrinogen. Although there was a high percentage of side effects (88%), most of them were mild and are already labelled. Only 3/49 (6%) patients reported 3 days later a worsening of their health-state after the test. Severe side effects were headache (3 patients), abdominal cramps (1 patient) and loss of consciousness (1 patient). One patient with severe headache did not present upfront with abnormal values for FVIII and vW-parameters, however, she had the highest CRP value of all patients (3.6 mg/dL). Summary: No single prospective laboratory parameter for side effects after DDAVP administration so far was identified.

### PP3.2-11

#### Perioperative DDAVP-tests in von Willebrand's Syndrome

Wolf H<sup>1</sup>, Tcherkes A<sup>1</sup>, Frühauf A<sup>1</sup>

<sup>1</sup>Klinik für Innere Medizin IV der Universität Halle, Germany

DDAVP does release von Willebrand factor from endothelial cells. Therefore, in patients (pts) with von Willebrand's (vW) syndrome type 1 or 2 intravenous administration of DDAVP may provide perioperative hemostasis. We tested the efficacy of intravenous DDAVP administration in 30 patients (pts) with vW disease type 1 or type 2 and a history of bleeding complications preoperatively. DDAVP was administered intravenously in a reduced dosage: 0.2 micrograms/kg body weight given in NaCl 0.9% solution within 30 minutes. Coagulation parameters were tested before as well as 30 and 60 minutes after infusion, respectively.

**Results:** We found significant increase in mean plasma concentrations of F VIII, vWF-antigen, vWF-ristocetin cofactor and vWF collagen binding activity, respectively. aPTT tests normalized after DDAVP infusion in type 2 pts. There were no surgical bleeding complications after DDAVP administration in our patients after low-dose DDAVP infusion therapy

**Conclusions:** In pts with mild vW syndrome normalisation of coagulation parameters could be achieved by low dose DDAVP administration. Advantage of reduced dosage were minor systemic adverse events like hypertension and fluid restriction.

### PP3.2-12

#### Laboratory testing to survey substitution therapy with VWF/FVIII-Concentrates

Halm-Heinrich I<sup>1</sup>, Parkner A<sup>1</sup>, Hartung K<sup>2</sup>, Heim M<sup>1</sup>

<sup>1</sup>Institut für Transfusionsmedizin und Immunhämatologie, Magdeburg, Germany, <sup>2</sup>Institut für Klinische Chemie und Pathobiochemie

**Objectives:** Von-Willebrand-factor (VWF) plays an important role in primary haemostasis (adhesion of thrombocytes at blood vessel lesion) and in secondary haemostasis (protecting FVIII from early proteolysis). VWS type3 is characterized by a complete absence of VWF and its functions.

**Case report:** We report on a 39-year-old female patient with vWS type 3. She was administered a daily substitution with 900I.E. VWF/FVIII-concentrate (Wilate<sup>®</sup>; contains 900I.E. FVIII, 800I.E. VWF) prophylactically. 24 hours after the last substitution laboratory tests gave the following

**Results:** PTT 36.9s, FVIII:C 22%, VWF:RCo<5%, VWF:Ag<5%. In preparation for a haemorrhoids operation patient received 3600I.E. Wilate<sup>®</sup> (60I.E. FVIII, 53I.E. VWF/kg) immediately before surgery. Laboratory test results one hour after substitution: PTT 29.9s, FVIII:C 98%, VWF:RCo 74%, VWF:Ag 125%. After surgery substitution was continued every 12 hours as follows: 1800–900–1800I.E., then 1800I.E. Wilate<sup>®</sup> twice a day. On day 2 after operation, 12 hours after the last substitution FVIII:C was 72% (normal range: 70–130%) as an emergency surrogate

marker for adequate substitution. Interestingly enough, the wound did not stop bleeding. Therefore additional testing of VWF:RCo and VWF:Ag was done and gave values of 30% and 51%, respectively (< normal range). After further adjustment of the substitution dose (FVIII:C 93%, VWF:RCo 46%, VWF:Ag 73%) the wound healed without any bleeding-complications.

**Conclusions:** Low values of VWF:RCo and VWF:Ag lead to a deficient primary haemostasis and can cause postoperative bleeding-complications. Therefore, emergency laboratory testing of PTT and FVIII:C is not sufficient to survey substitution, so that VWF:RCo and VWF:Ag should be measured additionally.

### PP3.3 Other Congenital Bleeding Disorders

#### PP3.3-1

##### Heterozygous Factor XIII deficiency: Bleeding tendency, Factor XIII activity in plasma and platelets

Dupin A<sup>1</sup>, Lerch L<sup>1</sup>, Kröniger A<sup>1</sup>, Barthels M<sup>1</sup>, Rein K<sup>1</sup>, Egbring R<sup>1</sup>  
<sup>1</sup>Zentrum für Innere Medizin, Philipps-Universität Marburg, Germany

**Objectives:** It was claimed since 1960 that bleeding tendency in heterozygous patients is low and that FXIII levels of 5% are sufficient for normal haemostasis. We therefore documented bleeding complications and determined FXIII activity in plasma and platelets of heterozygotes.

**Methods:** FXIII activity was determined by measuring incorporation of C14-labeled putrescine into casein: 1.) in platelet poor (PPP) and platelet rich plasma (PRP) of 4 heterozygous patients, 4 healthy donors, 1 double heterozygous and 1 homozygous patient before and after substitution with FXIII-concentrate; 2.) in PPP of 10 other heterozygous patients.

**Results:** FXIII activity of the 4 heterozygous patients was 30,0±3,9% in PPP and 97,8±7,8% in PRP. The double heterozygous and the homozygous patient revealed no FXIII activity in plasma and platelets. The healthy donors showed activities of 104,3±6,2% and 195,5±7,3%, respectively. After administration of 2500 I.U. FXIII-concentrate the plasma level of the homozygous patient raised to 32% but no platelet FXIII activity was detectable. Ten other heterozygotes revealed a FXIII plasma activity of 38,9±13,3%; all of them showed bleeding episodes.

**Conclusions:** The proportion of FXIII activity in plasma and platelets of the heterozygous patients was even higher (1:2) than in healthy individuals (1:1). As platelet FXIII is immediately available during clot formation this could play a role in haemostasis of heterozygous patients. The results also show that FXIII does not transude the platelet membrane in either direction. The bleeding tendency in heterozygous patients is higher than formerly assumed.

#### PP3.3-2

##### Genetic analysis and origin of nine F5F8D patients: novel frame shift LMAN1 mutation (Azerbaijan) and first indel MCFD2 mutation (Argentina)

Sittlinger K<sup>1</sup>, Klarmann D<sup>1</sup>, Vega-Ostertag M<sup>2</sup>, Eisert R<sup>3</sup>, Zieger B<sup>4</sup>, Seifried E<sup>1</sup>, Oldenburg J<sup>5</sup>, Geisen C<sup>1</sup>

<sup>1</sup>DRK Blood Donor Service, University Hospital Frankfurt, Germany, <sup>2</sup>Analysis Institute Fares Taie, Buenos Aires, Argentina, <sup>3</sup>University Hospital Hannover, Germany, <sup>4</sup>University Hospital Freiburg, Germany, <sup>5</sup>University Hospital Bonn, Germany

**Objectives:** Combined factor V and factor VIII deficiency is caused by mutations in LMAN1 or MCFD2. To date, a total of 48 different mutations in about 28 different countries are known. Here we describe the first F5F8D patient with an indel-mutation and novel and recurrent variations in eight additional families.

**Design and Methods:** F5F8D patients were analysed by direct sequencing of the corresponding genes on an automated sequencing system (ABI Prism 3100).

**Results:** In Argentinean ethnicity only the missense mutation Cys475Arg in LMAN1 is known, so far. Genetic testing of patient 1 from Argentina revealed a novel and concurrently the first indel-mutation changing a highly conserved amino acid residue in the first EF hand domain of MCFD2 (Asp81Ile). The first Azerbaijanian Patient 2 did not present mutations originating from adjacent countries (Turkey, Iran and Armenia) but showed the novel frame shift mutation Cys230fs in LMAN1. Almost all Turkish families (3-6) presented the frame shift mutation 249delT in MCFD2, exclusively found in Turkish ethnicity, whereas Patient 7 showed the stop mutation Lys302X. The common splice site mutation in Iranian non-Jewish ethnicity IVS7 DS-1G>A and the Polish frame shift mutation 841delA in LMAN1 could be proven in Patient 8 and 9 from Iran and Poland, respectively.

**Conclusions:** The data suggest apparent correlation between determined mutations and the patients' descent. Founder mutations in the LMAN1 and MCFD2 gene may lead to repeated occurrence of F5F8D in different countries. Therefore a mutation screening strategy based on patients' ethnicity may simplify molecular diagnosis of F5F8D.

#### PP3.3-3

##### Evaluation of desmopressin response in children with inherited thrombocytopathies

Knöfler R<sup>1</sup>, Huhn B<sup>1</sup>, Gneuß A<sup>1</sup>, Kentouche K<sup>2</sup>, Aumann V<sup>3</sup>

<sup>1</sup>Dept. of Pediatric Hematology and Oncology, University Hospital Dresden, Germany, <sup>2</sup>Dept. of Pediatric Hematology and Oncology, University Hospital, Jena, Germany, <sup>3</sup>Dept. of Pediatric Hematology and Oncology, University Hospital Magdeburg, Germany

**Objectives:** The necessity of desmopressin testing prior to the therapeutic use is a matter of controversial discussion. Aim of this retrospective study was to evaluate results of tests carried out between 2001 and 2007 in children with inherited thrombocytopathies (TP).

**Design and Methods:** Data were obtained by personal visits of centers. Complete response to desmopressin was defined as at least 1.5 fold increase of initial values of VWF antigen (VWF:Ag), collagen-binding activity (VWF:CB) or ristocetin cofactor activity (VWF:RCo) and a marked shortening of PFA-100® closure time (CT) reaching normal ranges within 120 min after desmopressin application.

**Results:** Data from 21 children (6 boys, 15 girls, age range: 3.1 to 17.0 years) suffering from aspirin-like defect (n=11), unclassified TP (n=7), hereditary macrothrombocytopenia (n=2) and ADP-receptor defect (n=1) were evaluated. In 20 patients desmopressin was given intravenously (Minirin parenteral®) at a dosage ranging from 0.3 to 0.4 µg/kg (mean: 0.35 µg/kg) and in one intranasally (Octostim®) at 300 µg. Coagulation parameters were determined between 60 min to 4 h after desmopressin application. Complete response was observed in 16 children (76%) and a non-response in 5 (24%). Four out of 5 patients with non-response were identified only by the still prolonged CT after desmopressin application. Side effects were not observed.

**Conclusion:** The non-response in 4 (19%) patients out of 21 children with TP underlines the necessity of DDAVP testing. The test panel in patients with TP should include the PFA-100® CT because this parameter might be able to identify non-responders.

#### PP3.3-4

##### Coagulation parameters and platelet function in whole blood samples of adults with cyanotic congenital cardiac disease

Braun S<sup>1</sup>, Mebus S<sup>1</sup>, Eicken A<sup>1</sup>, Hess J<sup>1</sup>, Vogt W<sup>1</sup>, Kaemmerer H<sup>1</sup>

<sup>1</sup>Deutsches Herzzentrum München, Germany

**Objectives:** In the natural course patients with cyanotic congenital cardiac disease (CCCD) tend to develop both thromboembolic as well as bleeding complications. The bleeding tendency in CCCD-patients may be related to reduced platelet function and additional defects in the coagulation system. We aimed to assess the usefulness of measuring coagulation and platelet function in CCCD-patients by thrombelastometry and platelet aggregometry.

**Methods:** We studied 34 consecutive patients presenting in the outpatient clinic of our department for congenital cardiac disease. One patient took acetylic acid, 5 had oral anticoagulation therapy with vitamin K antagonists. Thrombelastometry was performed in citrated whole blood using the ROTEM™ instrument. Whole blood impedance platelet aggregometry was measured with the Multiplate™ system. Blood cells were counted on a Sysmex XE2100 analyser.

**Results:** The median value of hematocrit (Hct) was 56% (range 43-78%). Negative correlations were found between hematocrit and platelet count (correlation coefficient  $r = -0.5898$ ), maximum clot firmness (MCF) in thrombelastometric analysis activated with tissue thromboplastin (EXTEM) ( $r = -0.5961$ ), alpha angle ( $r = -0.8100$ ) and platelet aggregation after activation with ADP ( $r = -0.5267$ ), arachidonic acid ( $r = -0.6584$ ) and TRAP ( $r = -0.4624$ ). The median MCF value in the FIBTEM test was 8.5mm.

**Conclusions:** Our findings are consistent with publications showing thrombocytopenia and suppressed platelet function in CCCD patients. Thrombelastometry showed that there is a tendency for reduced clot formation dynamics (alpha angle in EXTEM) and decreased fibrinogen or disturbed clot polymerization, but we found no tendency for hypercoagulability. This may be relevant for therapeutic decisions concerning anticoagulation or antiplatelet therapy in CCCD-patients.

#### PP3.3-5

##### Efficient reduction of Prions by the manufacturing process of a VWF/FVIII product

Gröner A<sup>1</sup>, Groschup M<sup>2</sup>, Schäfer W<sup>1</sup>

<sup>1</sup>CSL Behring, Marburg, Germany <sup>2</sup>Institute for Novel and Emerging Infectious Diseases at the Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Germany

**Objective:** Investigational studies were performed to assess the prion reduction capacity of the manufacturing process of a VWF/FVIII product (Haemate® P).

**Design and Methods:** The manufacturing process of Haemate P was divided in two parts which were studied independently twice: Spiked pooled plasma donations were processed from cryoprecipitation to glycine and NaCl precipitations and from spiked dissolved NaCl precipitate to sterile filtration in order to address the impact



of heterogeneous spike fractions regarding the overall reduction factor. The prion spiked starting material and product intermediate were processed according to the manufacturing conditions based on a valid down-scale model. The prion reduction factors were determined as the difference of the prion load in the spiked starting materials and in the respective final samples using a biochemical assay (Conformation-Dependent Immunoassay (CDI)) or a bioassay in hamsters for quantification of PrPSc (dose dependent incubation period measurement).

**Results:** An overall prion reduction factor of 6.1 log<sub>10</sub> and 5.9 log<sub>10</sub> could be demonstrated using the biochemical assay and the bioassay, respectively.

**Conclusion:** These results demonstrate (i) comparable prion reduction factors quantified either by biochemical methods or by a bioassay and (ii) an appropriate overall prion reduction capacity of the manufacturing process of Haemate P. Based on complementary safety procedures, i.e., collection of plasma by stringent donor selection due to geographic donor deferral policy and the overall prion reduction factor, a risk assessment results in an extremely remote risk of prion transmission by the VWF / FVIII product Haemate P.

### PP3.3-6

#### Severe von Willebrand disease with inhibitor: Searching the best way to treat.

Schiavoni M<sup>1</sup>, Coluccia A<sup>2</sup>, Scaraggi F<sup>3</sup>, Ettore C<sup>4</sup>, Ciavarella N<sup>5</sup>

<sup>1</sup>Dep. Internal Medicine - Hemostasis and Thrombosis Centre P.O. „I. Veris delli Ponti“, Scorrano (Lecce), Italy, <sup>2</sup>Thrombosis Centre P.O. „I. Veris delli Ponti“, Scorrano (Lecce), Italy, <sup>3</sup>Dep. Internal Medicine - Hemostasis and Thrombosis Centre „C. Frugoni“, University - Bari - Italy, <sup>4</sup>Coagulation Service - Policlinico, Bari - Italy, <sup>5</sup>Coagulation Service - Policlinico, Bari - Italy

**Background:** patients (pts) with severe von Willebrand disease (vWD) and large deletion within the vWF gene are at high risk of developing precipitating alloantibodies against vWF and severe hemorrhagic complications.

**Experience:** Over a period of 10 years (1996–2006) we experienced 8 critical bleedings, of which 3 lifethreatening events, in pts suffered from severe vWD with inhibitor and critical bleeding.

**Aim:** recombinant activated FVII (rFVIIa), recombinant FVIII (rFVIII), plasma-derived FVIII/vWF (p-d FVIII), antifibrinolytic drugs (tranexamic acid) and the local application of fibrin glue have been used respectively in relation to the severity and type of bleeding with the aim of obtaining the most effective clinical results. The dosage of rFVIIa ranged from 90 to 200 mcg/Kg b.w., rFVIII ranged from 50 to 100 I.U./Kg b.w., p-d FVIII/vWF ranged from 30 to 50 I.U./Kg b.w.

**Results and Conclusions:** rFVIIa provided an effective and safe hemostasis in oral surgery and particularly in treating the lifethreatening hematomas. The continuous infusion of rFVIII proved of great help to maintain the hemostasis after the acute phase of bleeding. In cases of failure of the combined therapy with rFVIIa and rFVIII the most effective treatment was p-dFVIII/vWF strictly monitored by an intensive care unit because of the high risk of immuno-allergic reactions. Tranexamic acid and local application of fibrin glue supported hemostasis in mucosal bleedings. Neither complications nor adverse events in all the pts occurred.

### PP3.3-7

#### A new mutation (Q694X) causing Glanzmann's Thrombasthenia in the Sultanate of Oman

Albalushi T<sup>1</sup>, AlZadjali S<sup>2</sup>, Muralitharan S<sup>3</sup>, AlHaddabi H<sup>2</sup>, Dennison D<sup>4</sup>, AlKindi S<sup>2</sup>, Arinami T<sup>5</sup>, Pathare A<sup>4</sup>

<sup>1</sup>Dept. of Genetics, Sultan Qaboos University, <sup>2</sup>Dept. of Hematology, Sultan Qaboos University, <sup>3</sup>Biology Department, Qatar University, <sup>4</sup>Dept. of Hematology, Sultan Qaboos University, <sup>5</sup>Dept. of Hematology, Sultan Qaboos University Hospital, <sup>6</sup>Dept. of Hematology, Sultan Qaboos University, <sup>7</sup>Medical Genetics Department, Tsukuba University, <sup>8</sup>Dept. of Hematology, Sultan Qaboos University Hospital, Muscat, Oman

**Objectives:** The objective of our study was to identify the underlying mutations responsible for GT in Omani patients, in order to establish a strategy for genetic counseling and carrier detection.

**Design & Methods:** GT was diagnosed in a 17-year old Omani female at the Sultan Qaboos University Hospital based on clinical features, platelet aggregometry and biochemical studies. Platelet surface expression of GPIIb/IIIa was markedly reduced on flowcytometry. Molecular studies performed at Medical Genetics Department, Tsukuba University, Japan, included DNA sequencing of all exons and exon-intron junctions of ITGA2B and ITGB3 of the two genes by the ABI 3100 Genetic Analyzer<sup>®</sup>. [Applied Biosystems, Foster City, CA, USA]. Genomic DNA was also analyzed by Illumina Human-1 Bead Chip Illumina<sup>®</sup> (Illumina Inc., San Diego, CA, USA) to exclude the whole region of the two genes that could produce an apparent homozygous state.

**Results:** We identified a novel nonsense causative mutation (Q694X) by sequencing the ITGB3 gene. In addition, sequencing ITGB3 gene also revealed 2 SNPs (rs 3809863; IVS14+9C/T, rs 3809865; 3383T/A). The Micro-Array assay using Illumina Human-1 Bead chip excluded the possibility of deletion of these genes in chromosome 17 in this patient.

**Conclusion:** A stop codon was found in exon 13 of ITGB3 gene causing the translated protein to be abnormally shortened. It is therefore hypothesized that the altered form of ITGB3 gene is both extremely unstable and rapidly degraded after its biosynthesis, leading to a loss of function of the protein.

### PP3.3-8

#### Hypofibrinogenemia in two families due to missense mutations in the FGB and FGG genes

Meyer M<sup>1</sup>, Helker C<sup>1</sup>, Dietzel H<sup>2</sup>, Kentouche K<sup>3</sup>

<sup>1</sup>Fachhochschule Jena, FB Medizintechnik und Biotechnologie, Germany, <sup>2</sup>Institute of Transfusion Medicine and Clinical Haemostaseology, Klinikum St. Georg Leipzig, Germany, <sup>3</sup>University Hospital Jena, Children's Hospital, Friedrich Schiller University Jena, Germany

**Objectives:** Hereditary hypofibrinogenemia is caused in most cases by molecular defects which result in severe truncation of one of the three fibrinogen polypeptide chains. Missense mutations resulting in the exchange of single amino acids are less common in hypofibrinogenemia. The aim of this study was the elucidation of the molecular defects in patients with low fibrinogen levels in two families.

**Design and Methods:** Mutation screening was performed by direct sequencing of PCR products. Fibrin polymerization was analysed spectrophotometrically. Two-dimensional gel electrophoresis (2-D-PAGE) of purified fibrinogen samples was applied in order to detect abnormal polypeptide chains.

**Results:** Two missense mutations were identified as the underlying molecular defects in the hypofibrinogenemic cases. In family W. mutation FGB G7956A results in the amino acid exchange 414 Gly>Ser in the Bbeta chain. Mild bleeding is observed in these hypofibrinogenemic patients. In the second family Sch. a novel molecular defect was found: FGG G5810A. This missense mutation results in the amino acid exchange 213 Gly>Glu in the gamma chain. In both cases normal fibrin polymerization of purified fibrinogen samples and normal polypeptide patterns in 2-D-PAGE suggest very low levels or absence of abnormal fibrinogen molecules in plasma.

**Conclusions:** Hypofibrinogenemia in two unrelated families is caused by two different missense mutations in the FGB and FGG genes affecting assembly, intracellular processing or secretion of fibrinogen molecules from hepatic cells.

### PP3.3-9

#### Four patients with hypo-dysfibrinogenemia: Clinical and laboratory findings.

Rein K<sup>1</sup>, Kemkes-Mathes B<sup>2</sup>, Kröniger A<sup>1</sup>, Meyer M<sup>1</sup>, Rohrer I<sup>1</sup>, Fuchs G<sup>1</sup>, Roales-Welsch T<sup>1</sup>, Egbring R<sup>1</sup>

<sup>1</sup>Zentrum für Innere Medizin, Philipps-University Marburg, Germany, <sup>2</sup>Center for Hemostaseology, Justus-Liebig-University Giessen, Germany

**Objectives:** We examined if identical gene defects in patients with hypo-dysfibrinogenemia cause similar or different clinical complications and fibrinogen concentrations.

**Methods:** Our population consists of four patients with hypo-dysfibrinogenemia. There is no known relationship between the patients and all live in a close region near Marburg.

**Results:** Three patients, patient 1, 2 and 3 (Fibrinogen Marburg, Giessen and Lixfeld I) have identical gene (A 4713->T) and fibrinogen defects (Aa1-460 Bbg). Patients 1 and 2 were found to be homozygous, whereas patient 3 was classified heterozygous. Fibrinogen activity in all four patients, even in the heterozygote, was < 30mg% or not detectable; fibrinogen concentration was 60–70mg% in the homozygotes, whereas the heterozygote showed only 28mg%. The maximal amplitudes in TEG correlated with this findings. Thrombin and reptilase time were found to be extremely prolonged in all patients. Patient 1 as well as the heterozygous patient 3 showed severe bleeding and thrombotic complications; the homozygote patient 2 however and patient 4 had only mild bleedings.

**Conclusions:** The difference in bleeding and thrombotic complications in the homozygotes may be due to different secondary risk factors as birth, oral contraceptives and surgical procedures. Fibrinogen concentration in the heterozygous patient is even lower than in the homozygotes. We therefore postulate that this patient must have another gene defect on the other allele accounting for afibrinogenemia. We also assume that patient 4 (Fibrinogen Lixfeld II) is homozygous for the defect described above. Genetic investigations for patient 3 and 4 are initiated and will be presented.

### PP3.3-10

#### Management of perioperative bleeding in combined deficiency of Factor V and Factor VIII by the use of DDAVP

Overberg D<sup>1</sup>, Moorthi C<sup>1</sup>, Haubold K<sup>1</sup>, Bade A<sup>1</sup>, Johné J<sup>1</sup>, Auerswald G<sup>1</sup>

<sup>1</sup>Prof.-Hess-Kinderklinik, Klinikum Bremen Mitte, Germany

**Case report:** 16-year old patient with consanguineous parents and a history of secondary bleeding after circumcision, gum bleeds and a posttraumatic hip bleed. Some were treated with FFP due to a diagnosed factor V deficiency. Now FVIII:C

showed a value of 12%. Combined deficiency of factor V and factor VIII (F5F8D) was confirmed by the detection of a homozygote mutation in MCFD2.

**Treatment:** For an extraction of four wisdom teeth a DDAVP test was performed and blood samples before and 60 minutes after administration of DDAVP 300µg intranasal taken. FVIII:C increased from 16% to 37%, ristocetin cofactor from 187% to 335%. Closure time in the PFA100® (collagen/epinephrine) was reduced from 122sec to 79sec. No adverse events were observed. For the dental extraction DDAVP i.v. was applied preoperatively. Postoperative DDAVP administration after 12–24–48 hours was changed to intranasal application. A mouth rinse with tranexamic acid was given. No use of FFP was required.

**Conclusion:** This patient with a history of significant bleedings underwent dental surgery without abnormal hemorrhage. We suppose an efficacy of DDAVP for patients with F5F8D who typically have a FV:C of 5–20%. Even though DDAVP does not increase FV:C, DDAVP seems to be an alternative to FFP in F5F8D for minor surgery since the FV:C often is sufficient for hemostasis. In major surgery DDAVP could be used additionally to FFP to raise the FVIII:C further than normal doses of plasma. The intranasal application of DDAVP enables the patient furthermore to treat minor bleedings independently at home.

### PP3.3-11

#### Peri-interventional control of hemostasis with recombinant Factor VIIa in a patient with combined coagulation Factor VIII- and Factor V-deficiency and anaphylaxis to fresh frozen plasma

Lechner D<sup>1</sup>, Kotz R<sup>2</sup>, Wanivenhaus H<sup>2</sup>, Kyrle P<sup>1</sup>, Eichinger S<sup>1</sup>

<sup>1</sup>Medical University of Vienna, Department of Medicine I – Hematology, Austria, <sup>2</sup>Medical University of Vienna, Department of Orthopedics, Austria

The combined deficiency of factors V and VIII (F5F8D) is a rare, autosomal-recessive disease. Bleeding symptoms are usually mild but may be severe after trauma or invasive procedures. In case of bleeding, factor VIII concentrates and fresh frozen plasma (FFP) to replace factor V are administered. We report a 64 year-old male patient with F5F8D (factor V 15 U/dL, factor VIII 10 U/dL), who required arthrodesis of the hemarthrotic left subtalar joint. Perioperative control of hemostasis included administration of factor VIII concentrates (Helixate®; CSL-Behring, Austria) and solvent detergent FFP (Octaplas®; Octapharma, Austria). The patient developed hypersensitivity to FFP which despite anti-allergic pretreatment resulted in severe anaphylaxis. Several months later, resurgery of the same joint was required and the patient received preoperatively 4000 IU Helixate® and 90 µg/kg of recombinant activated factor FVII (rFVIIa; Novoseven®, NovoNordisk, Denmark), followed by 60 µg/kg rFVIIa every 6 h and 2000 IU Helixate® twice daily. Substitution of rFVIIa was stopped on the fifth postoperative day, while replacement of factor VIII was gradually reduced to 1000 IU once daily and stopped on day 12. Postoperative thromboprophylaxis comprised early mobilization and graduated compression stockings only. The postoperative course showed no bleeding or thromboembolic complications. The same regimen was used for two further orthopedic surgeries also without any complications. This is the first report to show that a treatment regimen consisting of factor VIII and rFVII in preventing perioperative bleeding in a patient with F5F8D. rFVIIa may be considered for perioperative management in patients with F5F8D.

### PP3.3-12

#### Intraosseous hemorrhage and fingertip necrosis: Unusual clinical problems in two brothers with afibrinogenemia

Hainmann I<sup>1</sup>, Erlacher M<sup>1</sup>, Heiss J<sup>1</sup>, Uhl M<sup>2</sup>, Oldenburg J<sup>3</sup>, Pavlova A<sup>3</sup>, Superti-Furga A<sup>1</sup>, Ziegler B<sup>1</sup>

<sup>1</sup>University Hospital Freiburg/ Center of Pediatrics and Adolescent Medicine, Germany, <sup>2</sup>University Hospital Freiburg/ Department of Diagnostic Radiology, Germany, <sup>3</sup>University Hospital Bonn/ Inst. of Exp. Hematology and Transfusion Medicine, Germany

**Introduction:** Congenital afibrinogenemia is a rare bleeding disorder characterized by a heterogenous clinical picture. There are only few reports of afibrinogenemia associated with intraosseous hemorrhages.

**Case reports:** We report on two Iraqi brothers who were admitted to us at the age of 14 and 25 years, respectively. The past medical history of patient 1 (P1) revealed uncontrollable umbilical cord bleeding, cerebral and mucosal hemorrhages and recurrent leg pain. He had been misdiagnosed with hemophilia and treated with different coagulation factor concentrates, before. The prothrombin time and partial thromboplastin time were not measurable. Fibrinogen concentration was below 3 mg/dl (Clauss method) leading to the diagnosis of afibrinogenemia. DNA analysis demonstrated a homozygous mutation in the fibrinogen gene. Magnetic resonance imaging (MRI) (including gradient-recalled echo sequences) showed multiple cystic alterations in the diaphyses of the long bones corresponding to intraosseous medullary hemorrhages. Patient 2 was admitted to us because of a subungual hematoma and necrosis of the fingertip several days after an injury. Fibrinogen level (<3 mg/dl) and DNA analysis verified afibrinogenemia. Amputation of fingertip could be avoided by repeated infusion of purified human fibrinogen concentrate and surgical

release of blood. Comparable with P1, MRI showed cystic lesions within both femoral heads and diaphyses. Iron-containing hemosiderin indicated prior intraosseous and joint hemorrhages.

**Conclusions:** We identified two brothers with congenital afibrinogenemia presenting with unusual clinical problems. Since soft tissue, bone and bone marrow can all be evaluated concurrently, MRI including gradient-recalled echo sequences should be the preferred imaging modality for patients with afibrinogenemia.

### PP3.3-13

#### A case of sporadic hypodysfibrinogenemia

Ivaskевичius V<sup>1</sup>, Pötzsch B<sup>1</sup>, Harbrecht U<sup>1</sup>, Oldenburg J<sup>1</sup>

<sup>1</sup>Institute of Experimental Haematology and Transfusion Medicine, Bonn, Germany

**Objectives:** The aim of this study was to identify the genetic defect in fibrinogen genes in a two-year-old child with hypodysfibrinogenemia.

**Methods:** FGA, FGB and FGG genes were analysed using an ABI-3130 sequencer.

**Results:** The patient presented at the hospital with diarrhoea, enlarged liver, and elevated liver transaminase levels. Fibrinogen (Clauss method) was reduced (35 to 39 mg/dl, n. 177–376 mg/dl), fibrinogen antigen was at the level of 58 mg/dl (n. 180–400 mg/dl). Thrombin time was 16.6 s (n. 8–16 s) and reptilase time was 33.4 s (n. 14–19 s). Both parents and a sister of the patient showed normal fibrinogen levels. FGG gene analysis revealed a novel heterozygous missense mutation in exon 8 (c.1081T>C gamma Trp335Arg), resulting in a substitution of hydrophobic and neutral tryptophan by hydrophilic and positively charged arginine. In addition, the patient has inherited a common FGG 3'UTR polymorphism (8537C>T) from her mother, and three linked FGB polymorphisms (c.567C>T Ser159Ser, c.1125C>T Tyr345Tyr, c.1433G>A Arg448Lys) from her father. Parents and one sister of the patient were unaffected (335Trp/Trp) suggesting the presence of spontaneous mutation in a patient during early embryogenesis or in the germ cells of the parents.

**Conclusions:** The newly identified mutation might provoke thrombosis, since Mayer et al. (2003) has reported three further mutant variants (Cys326Tyr, Met336Ile, Tyr-354Cys) in the same part of fibrinogen D domain resulting in venous thrombosis. Nevertheless, no thrombosis occurred so far in our index-patient.

### PP3.3-14

#### Cardiac surgery in Factor XI deficiency: A case report

Scholz U<sup>1</sup>, Oppermann J<sup>1</sup>, Kämpfert C<sup>2</sup>, Siegemund A<sup>1</sup>

<sup>1</sup>Zentrum für Blutgerinnungsstörungen Leipzig, Germany, <sup>2</sup>Herzzentrum Leipzig, Universität Leipzig, Germany

Patients with factor XI deficiency show different bleeding symptoms and the factor level is not clear associated with the risk of bleeding. The anticoagulation with heparin is required during the cardiac surgery. The management of this therapy can be monitored with different lab methods. The most used management is influenced by the factor XI deficiency. A 61 year old patient was admitted to cardiac surgery with a factor XI residual activity of 7%. In the medical history he had a bleeding event after a surgical procedure on the Os occygeum 30 years ago. The bleeding symptoms after a dental extraction were mild. A coronary artery disease (single vessel, left coronary artery) with an acute myocardial infarction in 2006 required the cardiac surgery. In a minimal invasive surgical procedure a coronary single bypass were performed. The substitution with Factor XI (Hemoleven®, LFB, Les Ulis, France, 2000 IU) showed a correction of the basic lab results with the possibility of an anticoagulation monitoring intra-operative like normal patients. There were no bleeding symptoms. The following replacement therapy was monitored based on factor XI level (aim > 40% over 10 days). Because of the long half time Hemoleven® was given every day on day 1 and 2 post-operative, later every second day. In summary the patient received 7500 IU of the factor XI concentrate. The anticoagulation with heparin and aspirin was compared to patients without hemostatic disorders. The patient left the hospital 13 days after cardiac surgery with no bleeding or thromboembolic complications.

### PP3.4 ITP

#### PP3.4-1

#### Complement-fixing autoantibodies in immune thrombocytopenic purpura (ITP)

Sachs U<sup>1</sup>, Najaoui A<sup>1</sup>, Giptner A<sup>1</sup>, Bein G<sup>1</sup>, Santoso S<sup>1</sup>

<sup>1</sup>Institut für Klin. Immunologie und Transfusionsmedizin der Justus Liebig-Universität Gießen, Germany

**Objectives:** ITP is characterised by the presence of autoantibodies against platelet-associated antigen(s). Platelet destruction can be mediated by either Fc- or C3b-dependent phagocytosis, or by complement-induced lysis. The aim of this study was to further evaluate the role of complement in chronic ITP.



**Design and Methods:** Sera from ITP patients and controls were analyzed for the presence of platelet- and HLA-antibodies by enzyme-linked immunoassay (MAIPA). All sera were further evaluated in a complement fixation assay and a FACS-based bead assay for C1q immobilization.

**Results:** 10 % of control sera were able to fix complement despite absence of antibodies. All 109 ITP patients had a positive direct MAIPA. 60 sera (55 %) were able to fix complement (60 % of those without free autoantibodies, and 30 % of those with free autoantibodies). If platelets lacking GP IIB/IIIa were used in the complement fixation assay, 60 % of ITP sera lost the ability to fix complement. IgG fractions and PEG-precipitated sera revealed identical results. Complement fixing activity was also demonstrated in a FACS-based assay.

**Discussion:** In a significant number of patients with chronic ITP, autoantibodies are capable of activating the classical complement pathway. Complement fixation can also be seen with sera in which a free autoantibody cannot be demonstrated by MAIPA. GP IIB/IIIa is a major target for complement-fixing autoantibodies. Complement mediated lysis of platelets could be a relevant mechanism *in vivo*; importantly, our data indicate that a GP IIB/IIIa dependent complement fixation assay could add significantly to the diagnosis of ITP.

#### PP3.4-2

##### The Role of platelet antibodies against Glycoprotein V in the diagnosis of immune thrombocytopenic purpura

Rühl H<sup>1</sup>, Giptner A<sup>1</sup>, Bein G<sup>1</sup>, Sachs U<sup>1</sup>

<sup>1</sup>Institute for Clinical Immunology and Transfusion Medicine, Giessen, Germany

**Objectives:** The direct monoclonal antibody-specific immobilization of platelet antigens (MAIPA) assay is commonly applied for the diagnosis of immune thrombocytopenic purpura (ITP). However, it shows a negative result in about 40–50 % of all ITP patients. One possible explanation for this may be the use of platelet glycoproteins (GP) that bear no target for these patients' antibodies. GP IIB/IIIa and GP Ib/IX are well known target antigens in ITP. GP V has also been suggested to be a target of platelet autoantibodies. We studied the prevalence of platelet autoantibodies against GP V in ITP patients in order to analyze if the use of GP V could improve the sensitivity of the MAIPA.

**Design and Methods:** Samples from 4,217 patients with suspected ITP were tested with the direct MAIPA for antibodies against GP V as well as GP IIB/IIIa and GP Ib/IX. Patients without clinical signs for ITP or with nonimmunological explanations for thrombocytopenia were excluded.

**Results:** 657 samples showed positive results in the direct MAIPA. Platelet autoantibodies against GP V were noted in 406 (62 %) of these patients. In 64 (10 %) patients GP V-specific antibodies were the only platelet antibodies the direct MAIPA was able to detect.

**Conclusions:** Our findings suggest that GP V is an important target antigen for platelet antibodies in ITP. Since 10 % of all patients with a positive MAIPA result would have been missed otherwise, additional testing for antibodies against GP V in the direct MAIPA seems to improve its sensitivity significantly.

#### PP3.4-3

##### Apoptosis in platelets from pediatric patients with acute immune thrombocytopenic purpura (ITP) is ameliorated by IVIg

Speer O<sup>1</sup>, Kroiss S<sup>1</sup>, Azzouzi I<sup>1</sup>, Schmutz M<sup>1</sup>

<sup>1</sup>Hämätologie, Kinderspital Zürich, Switzerland

There is evidence that interaction of auto-antibodies with glycoproteins (GP) on the platelet surface leads to accelerated clearance of platelets and cause immune thrombocytopenic purpura (ITP). Mouse models showed that anti- GPIIb injections induce apoptotic like processes in platelets accompanied by the induction of ITP. Apoptotic like processes in platelets are similar to those observed during apoptosis in nucleated cells: activation of caspase-3 (aCASP3), loss of mitochondrial membrane potential and externalisation of phosphatidylserine (PS). Intravenous immunoglobulin (IVIg) ameliorates anti- GPIIb induced ITP in mouse. In a prospective study children with clinical and laboratory diagnosis of ITP were enrolled. At diagnosis and after IVIg therapy blood samples were obtained. The fraction of young reticulated platelets (RP) and apoptotic-like events specifically CASP3 activation and PS externalisation were studied in platelets by flow-cytometry. 10 patients had a platelet count below 10 000/ ul, 2 had platelets counts over 10 000/ ul. ITP patients had increased levels of platelets with aCASP3 and PS exposure and increased RP. 10 Patients with platelet counts below 10 000 / ul were treated with maximal 3 doses IVIg (0.4 – 0.8 g/kg dose). All patients showed a rise in platelet counts above 20 000/ul and amelioration of bleeding symptoms 24–72 hours after IVIg administration. Concomitantly aCASP3, PS exposure and RP decreased. We can demonstrate activation of apoptotic-like processes in paediatric acute ITP such as CASP3 activation and PS exposure similar to the reported mouse model.

#### PP3.4-4

##### Value of recombinant FVIIa in the emergency treatment of autoimmune Thrombocytopenia

Salama A<sup>1</sup>

<sup>1</sup>Charité - Universitätsmedizin Berlin, Germany

**Objectives:** Some patients with autoimmune thrombocytopenia (ITP) suffer from severe and refractory disease and may develop life-threatening bleeding that requires immediate intervention. Recombinant activated FVIIa (rFVIIa) might be used in the emergency treatment of patient with ITP.

**Design and Methods:** A systematic review of all published reports to assess the available evidence on the efficacy and safety of rFVIIa in patients with ITP.

**Results:** To date, 18 patients were found to have been treated with rFVIIa. All these patients had a highly bleeding risk or uncontrolled bleeding, and were defined as being refractory to different therapies. Although the studies show some drawbacks, rFVIIa appears to be useful in the management of uncontrolled bleeding in ITP.

**Conclusion:** rFVIIa may help in the emergency treatment of patients with refractory ITP.

#### PP3.4-5

##### Rare sequence variations of the lymphocyte differentiation transcription factor SOX13 in pediatric idiopathic thrombocytopenic purpura patients

Rischewski J<sup>1</sup>, Johanna W<sup>1</sup>, Stocker S<sup>1</sup>, Hergersberg M<sup>2</sup>, Huber A<sup>2</sup>, Kühne T<sup>1</sup>

<sup>1</sup>University Childrens Hospital Basel, Switzerland, <sup>2</sup>Centre for Laboratory Medicine Aarau, Switzerland

**Objective:** Idiopathic thrombocytopenic purpura (ITP) is an autoimmune mediated process. Cooccurrence with Primary Biliary Cirrhosis (PBC) has been reported. In PBC patients, autoantibodies against SOX13 can be detected. SOX13 is a transcription factor promoting gamma-delta T-cell development while opposing alpha-beta T cell differentiation. As ITP has been linked to the autoimmune bicytopenic Evans syndrome, which in a relevant proportion presents Autoimmune Lymphoproliferative Syndrome (ALPS) with the hallmark of increased alpha-beta double negative T-cells, we investigated a possible role of molecular SOX13 sequence variations in ITP patients.

**Design and method:** SOX13 was amplified by PCR using genomic DNA from 34 ITP patients, and screened by Denaturing High Performance Liquid Chromatography (DHPLC) for sequence variations. All samples with aberrant DHPLC retention patterns were directly sequenced.

**Results:** In a total of 4 patients (12 %) two rare heterozygous sequence variations were detected: c.1603C>T (Pro534Ser) and c.1836 C>T (synonymous).

**Conclusion:** In a healthy cohort the heterozygous c.1603C>T was detected in 1.7 % of individuals. 2.6 % in a healthy cohort carried the heterozygous c.1836C>T. 12 % of pediatric ITP patients carry one of two rare SOX13 sequence variations. The rare heterozygous genotypes are 3.5 (c.1603C>T) respectively 2.3 times (c.1836C>T) more common than in published non-ITP cohorts. Rare alleles of SOX13 could accumulate in pediatric ITP cohorts. Larger case-control sample studies are needed to verify his results. The rare genotypes could influence the alpha-beta versus gamma-delta T-cell balance in the studied pediatric ITP patients, and immunological studies of our cohort concerning this aspect seem worthwhile.

#### PP3.5 Other Acquired Bleeding Disorders

#### PP3.5-1

##### Acquired von Willebrand syndrome in aortic valve stenosis affects platelet function and platelet inflammatory response

Schneller A<sup>1</sup>, Badr Eslam R<sup>1</sup>, Budde U<sup>2</sup>, Kaider A<sup>1</sup>, Lang J<sup>1</sup>, Panzer S<sup>1</sup>

<sup>1</sup>Medizinische Universität Wien, Austria, <sup>2</sup>Aesculabor, Hamburg, Germany

**Objective:** It has been shown that severe aortic valve stenosis (AS) is associated with low levels of the large von Willebrand multimers, rendering patients to an increased risk of bleeding. We were interested to evaluate if aortic valve replacement not only improves platelet function but also the formation of platelet-monocyte heterotypic aggregates, which serve as a marker of platelet participation in inflammation.

**Methods:** We determined large von Willebrand multimers and expression of P-selectin of non-activated and ADP and epinephrine activated platelets, and the PFA-100 ADP closure time (ADPCT) in 36 patients (f/m ratio 19/17) with severe AS before and 6 months after aortic valve replacement. Platelet-monocyte heterotypic aggregates were determined as an indicator of ongoing participation of platelets in inflammation.

**Results:** Large von Willebrand multimers increased and ADPCT decreased significantly ( $p < 0.0001$ ); likewise, P-selectin of resting platelets increased, and platelets

became more susceptible to agonist-inducible activation ( $p=0.0086$ ), Platelet-monocyte formation decreased also significantly ( $p<0.002$ ).

**Conclusion:** Aortic valve replacement not only induces normalization of platelet aggregation but also corrects the increased inflammatory platelet response.

### PP3.5-2

#### Effect of Factor XIII administration in critically ill patients with ongoing bleeding.

Fries D<sup>1</sup>, Vonmetz A<sup>1</sup>, Lorenz I<sup>1</sup>, Friesenecker B<sup>1</sup>, Velik-Salchner C<sup>2</sup>, Oswald E<sup>2</sup>, Martinowitz U<sup>3</sup>, Innerhofer P<sup>1</sup>

<sup>1</sup>University Hospital for General and Surgical Intensive Care Medicine, Medical University Innsbruck, Austria, <sup>2</sup>University Hospital for Anaesthesia and Intensive Care Medicine, Medical University Innsbruck, Austria, <sup>3</sup>Department for Haematology, TelHashomer Hospital, Telaviv, Israel

According to recommendations in the literature, FXIII activities of above 10% have been judged as sufficient in the past, while several clinical studies showed an increased blood loss and blood transfusion requirements in surgical patients with FXIII activities below 60%. The data presented were obtained from 96 surgical critically ill patients with ongoing bleeding and transfusion requirements as a consequence of microvascular bleeding. All patients, who received FXIII concentrate (FibrogamminHS®, CSL Behring, Vienna, Austria) showed FXIII plasma levels below 60% and received a single shot application of about 20 IU/kg bodyweight. A Wilcoxon test for paired samples was applied to assess differences in blood product usage between baseline versus 24 hours measurement and baseline versus 48 hours measurements, respectively. A Mann-Whitney U test was used to compare the effect of FXIII concentrate alone or in combination with other blood products. According to the procedure of Bonferroni to correct for the two multiple comparisons (baseline vs. 24h after administration of FXIII concentrate and baseline vs. 48h after administration of FXIII concentrate)  $p$ -values  $<0.025$  were assumed statistically significant. The need for transfusion of red blood cell concentrates decreased from a median transfusion rate of 4 RBC's (0–22) to 1 RBC (1–9) within 24 hours and 0 RBC (0–4) within 48 hours after FXIII administration ( $p<0.001$ ). Transfusion of FFP and platelet concentrates as well as the administration of fibrinogen and PCC were reduced statistically significant. FXIII concentrate (FibrogamminHS®) was effective to stop microvascular bleeding and to reduce transfusion requirements.

### PP3.5-3

#### Successful low-dose Rituximab treatment in a patient with idiopathic acquired antibodies to Factor VIII

Platzbecker U<sup>1</sup>, Gehrisch S<sup>2</sup>, Ehninger G<sup>1</sup>, Siegert G<sup>2</sup>

<sup>1</sup>Medizinische Klinik und Poliklinik I, Uniklinik Dresden, <sup>2</sup>Institut für Klinische Chemie und Laboratoriumsmedizin, Uniklinik Dresden

Acquired spontaneous antibodies to factor VIII are a rare but serious coagulation disorder. Most of the patients with such antibodies present with severe and life-threatening bleeding episodes. With the availability of activated prothrombin complex concentrates and recombinant factor VIIa acute bleeds can be controlled in the majority of patients. However, long-term eradication of the inhibitor is the major therapeutic goal in these patients. Prednisone is currently the most widely used standard first-line immunosuppressive therapy in these patients. Nevertheless, up to one third of patients are refractory to this therapy. Rituximab, a monoclonal chimeric antibody against the CD20 antigen, is a very effective drug in lymphoma treatment. Recently published data show that "classical dose" rituximab (375 mg/m<sup>2</sup>/d) is also a promising treatment option in acquired antibodies to clotting factors, in particular spontaneous factor VIII antibodies. We here report a 73-year old male patient presenting with retroperitoneal hematoma due to acquired antibodies to factor VIII (5.9 Bethesda Units). Initially, treatment was started with prednisone (2mg/kg/d) and activated F VII and FEIBA in order to allow surgical intervention. On day 5 after first presentation low dose rituximab (50mg/m<sup>2</sup>) was started and repeated every week for a total of 4 courses. Concurrently, prednisone was tapered completely. As a result complete depletion of peripheral B-cells was obtained and F VIII levels normalized over a period of 3 months. We therefore conclude that low-dose rituximab is a safe, effective and inexpensive alternative to the currently used rituximab regimen adapted from lymphoma treatment.

### PP3.5-4

#### Fibrinolytic deficit in chronic kidney disease and end stage renal disease patients contributes to the hemostatic abnormalities

Adiguzal C<sup>1</sup>, Bansal V<sup>2</sup>, Cunanan J<sup>1</sup>, Litinas E<sup>1</sup>, Hoppensteadt D<sup>1</sup>, Fareed J<sup>1</sup>

<sup>1</sup>Dept. of Pathology Loyola University Medical Center, <sup>2</sup>Dept. of Nephrology Loyola University Medical Center

**Introduction:** Increased bleeding is observed in patients with chronic kidney disease (CKD) or end stage renal disease (ESRD) despite a normal coagulation profile and fibrinogen level. The hemostatic deficit in these patients may due to defects in fibrin formation.

**Materials & Methods:** The fibrinolytic profile of CKD (n=50) and ESRD patients on hemodialysis was measured. Citrated plasma was supplemented with thrombin and CaCl<sub>2</sub>. The rate of fibrin formation was measured by monitoring the optical density (OD) at 405 nm. After reaching steady state, urokinase was added to measure the fibrinolytic profile. Forty normal male and female individuals were also analyzed.

**Results:** Fibrinolytic profiles for normals showed strong clot formation (average OD = 1.2±0.3; range 0.7–1.4). In the CKD patients a much weaker clot was formed (average OD of 0.21±0.13; range 0.05–0.41). In the ESRD patients on maintenance hemodialysis, the pre-dialysis sample showed a weaker fibrinolytic profile reaching near normal levels with a clot density of 1.3±0.4 (range 0.8–1.6). In the urokinase induced fibrinolysis assay, CKD patients' plasma exhibited a much stronger fibrinolytic index compared to the normal population (80% vs. 20% lysis). In ESRD patients, clot lysis was weaker compared to the CKD patients.

**Conclusions:** These results are contrary to previous reported observations that dense clot resistant to fibrinolysis is formed in CKD and ESRD patients. Furthermore, the clots observed in these patients were highly susceptible to lysis. Maintenance hemodialysis results in improving the fibrinolytic and fibrinolytic profile in the ESRD patients and may contribute to improved hemostasis in ESRD patients.

### PP3.5-5

#### Acquired haemophilia caused by autoantibodies against Factor V: A case report and review of the literature.

Heisteringer M<sup>1</sup>, Kuen-Kuckenberger B<sup>2</sup>, Sinha P<sup>2</sup>, Geissler D<sup>1</sup>

<sup>1</sup>Med. Abt., LKH Klagenfurt, Austria, <sup>2</sup>Institut für Med. u. Chem. Labordiagnostik, LKH Klagenfurt, Austria

**Objectives:** Acquired haemophilia is a rare disease with an incidence of approximately 0.2–1.3 per million per year. In most cases it is caused by the development of autoantibodies against factor VIII, but also different structures can be antigens for antibody development.

**Case:** A 76-year-old patient was admitted to hospital because of subcutaneous haematoma after minimal trauma and very high INR-values (7–8). He had a medical history of coronary artery disease, arterial occlusive disease and end stage kidney disease. Because of previous thromboembolic events he was on oral anticoagulation. Routine laboratory tests revealed a slightly decreased activity of factor II, VII and X (47, 54 and 45% respectively), but factor V activity was below 1%. A plasma exchange test could clearly prove the presence of an inhibitor. Initial therapy consisted of vitamin K, prothrombin complex preparations and high dose steroids. Because of high co-morbidity there were serious concerns about a more intensive immunosuppressive regimen. The patient developed a spontaneous intracerebral haemorrhage soon after admission. Despite immediate additional administration of rhFVIIa (NovoSeven), the patient died due to this dramatic bleeding complication.

**Conclusions:** 1. Acquired haemophilia is a rare disease, especially, if autoantibodies against different factors than FVIII are involved. 2. Prognosis and outcome depend on a quick diagnosis, efficient removal of the antibody and nature of the underlying or pre-existing disease. 3. Patients with acquired haemophilia due to autoantibodies against different factors than FVIII may have a higher risk for serious bleeding complications.

### PP3.5-6

#### Longterm anticoagulation with Enoxaparin after electrical cardioversion in ESRD: Pilot data of two anuric diabetic outpatients on maintenance hemodialysis with contraindications for coumarin anticoagulants

Hertfelder H<sup>1</sup>, Pöge U<sup>2</sup>, Raab P<sup>2</sup>, Hammerstingl C<sup>3</sup>, Heyder O<sup>3</sup>, Oldenburg J<sup>1</sup>, Brensing K<sup>2</sup>

<sup>1</sup>Institute of Exp. Haematology & Transfusion Medicine, University Hospital Bonn, Germany, <sup>2</sup>Center of Renal and Hypertensive Diseases, Bonn Bad Godesberg, Germany, <sup>3</sup>Department of Internal Medicine, St. Marien-Hospital, Bonn, Germany

**Objectives:** After electrical cardioversion of atrial fibrillation (AF) therapeutic anticoagulation for 4–6 weeks (wks) is needed to avoid thromboembolism. For end-stage renal disease (ESRD) patients on hemodialysis (HD) with contraindications for phenprocoumon enoxaparin s.c. (ENOX) is an alternative option but safety and efficacy data are scarce.

**Design and Methods:** Three anticoagulation periods (each 5–6 wks) with ENOX in two anuric ESRD diabetic men (both 80–90 kg b.w.) after electrical cardioversion (2x primary + 1x relapse after 10 wks) for symptomatic AF on out-patient HD (3x/wk, standard heparin) were studied. Both pts had three-vessel CAD requiring aspirin (100 mg/day). Phenprocoumon was contraindicated due to severe bleedings (1x GI, 1x retinal). ENOX was started with 40 mg q.d. and adjusted by anti-factor Xa determination (AXa).

**Results:** Provided by doses of 30–40 mg (b.i.d.) on HD-free days and 30–40 mg (q.d.) on HD days, after < 6 days respectively stable nadir AXa levels of 0.32 IU/ml (median; range: 0.25–0.37 IU/ml) and AXa increases (0.09–0.12 IU/ml) after HD to



peak levels of 0.43 IU/ml (0.39–0.47 IU/ml) were observed. No adverse bleeding or thromboembolic events were seen under this regimen.

**Conclusions:** Compared to standard therapeutic ENOX dose of 1 mg/kg b.w. b.i.d. for pts without renal impairment, both ESRD patients required 60–65% lower doses. Our data suggest that 0.4 mg/kg b.w. ENOX b.i.d. on HD-free and q.d. on HD days, adjusted by AXa levels, provide safe and sufficient anticoagulation. The dose regimen may also be useful for short-term ENOX bridging procedures prior to interventions in ESRD patients under coumarin anticoagulation.

#### PP3.5-7

##### A patient with epistaxis and multiple myeloma

Rechberger E<sup>1</sup>, Aschauer G<sup>1</sup>, Zöchbauer A<sup>1</sup>, Petzer A<sup>1</sup>, Fierlinger F<sup>2</sup>

<sup>1</sup>Med. Abteilung; KH Barmh. Schwestern Linz, Austria, <sup>2</sup>Interne Intensivstation; KH Barmh. Brüder Linz, Austria

**Case description:** A 47 year old male patient diagnosed for myeloma had a vasectomy at our urology department. Preoperative coagulation screening revealed repeated epistaxis, laboratory a PT of 61% as the sole abnormality. Severe post-operative bleeding occurred, necessitating another surgical procedure. At the same time epistaxis worsened and the patient needed substitution of packed RBC. Coagulation testing showed a thrombin time > 180 seconds, fibrinogen could not be measured by clotting assays, an immunologic assay turned out to be normal. Factor V, prothrombin, factor VII and factor X were within normal limits. A correction with normal plasma (1:1) resulted in shortening of the thrombin time to 56 seconds and a fibrinogen concentration of 247mg/dl measured by clotting assay. Fibrinogen was applied with no effect on bleeding, the lab-values did not change. We suspected a defect in fibrinogen polymerization due to the presence of paraproteins as the bleeding cause and began with plasmapheresis simultaneous to myeloma therapy. Bleeding stopped after plasmapheresis and laboratory values gradually returned to normal with chemotherapy. Meanwhile the patient recovered and has no apparent clotting defects.

**Discussion:** Bleeding defects in myeloma patients are common (up to 15% in IgG and 30% in IgA Myeloma). Causes are deposition of perivascular amyloid, secondary factor X defect, acquired Willebrand's disease, thrombocytopenia and interference of paraproteins with fibrinogen polymerization - all generally with rare clinical consequences. Our patient presented is a rare case with severe bleeding which resolved by reduction of paraproteins with plasmapheresis and chemotherapy.

#### PP3.5-8

##### Acquired von Willebrand syndrome could explain bleeding in patients with cardiac assist devices

Heilmann C<sup>1</sup>, Geisen U<sup>2</sup>, Benk C<sup>1</sup>, Berchtold-Herz M<sup>1</sup>, Schlensak C<sup>1</sup>, Budde U<sup>3</sup>, Beyersdorf F<sup>1</sup>, Zieger B<sup>4</sup>

<sup>1</sup>Department of Cardiovascular Surgery, University Cardiovascular Center Freiburg - Bad Krozingen, Germany, <sup>2</sup>Department of Clinical Chemistry, University Medical Center Freiburg, Germany, <sup>3</sup>Labor Lademannbogen, Hamburg, Germany, <sup>4</sup>Department of Pediatrics and Adolescent Medicine, University Medical Center Freiburg, Germany

**Objective:** Unexplained bleeding episodes are associated with ventricular assist-devices (VAD). Interestingly, haemorrhages accompanying aortic stenosis can originate from acquired von Willebrand-syndrome (AVWS) caused by increased shear-stress. AVWS is characterized by loss of high-molecular-weight multimers of von Willebrand-factor (VWF) resulting in functional impaired VWF. Decreased ratios of collagen binding (VWF:CB) over von Willebrand-factor-antigen (VWF:Ag) (reflecting attachment of VWF to denuded vessels) and of ristocetin-cofactor activity (VWF:RCo) over VWF:Ag (mirroring binding of platelets to VWF) indicate this impairment. Since increased shear-stress also occurs in VADs, patients supported by different VAD-types were analyzed for AVWS.

**Methods:** 23 VAD-patients (centrifugal left-ventricular VentrAssist, centrLVAD, n=5, axial left-ventricular HeartMateII, axLVAD, n=10; biventricular assist-device, BVAD, n=4) were assessed for the ratios VWF:CB/VWF:Ag (normal:>0.7) and VWF:RCo/VWF:Ag (>0.65) and for aPTT, INR, HB and HKT 12±9 days after VAD-implantation. Furthermore, VWF-multimers were analyzed.

**Results:** VWF:CB/VWF:Ag was reduced in n=1/3 centrLVAD (0.82±0.28), n=10/10 axLVAD (0.43±0.09, p=0.018 versus centrLVAD), and n=4/5 BVAD-patients (0.49±0.25). VWF:RCo/VWF:Ag was impaired in n=4/5 centrLVAD, n=7/10 axLVAD, and n=5/8 BVAD-patients (0.6±0.1, p=0.824 between groups). Large VWF-multimers were reduced in 12 of 14 examined patients. All patients displayed at least one of the three AVWS-characteristics (n=22 analyzed). INR (1.3±0.6), aPTT (50±13s), hemoglobin (9.1±1.0), and HKT (20.0±3.7) did not correlate to VWF:CB/VWF:Ag and VWF:RCo/VWF:Ag.

**Conclusions:** VAD-patients display functionally impaired VWF despite major constructional differences between VAD-types. These findings indicate that patients with VADs may develop an AVWS which can explain VAD-associated bleeding.

Prospectively, the design of VADs should be improved to decrease increased shear stress and to prevent AVWS.

#### PP3.5-9

##### Monitoring of haemostatic changes during congenital heart surgery in pediatric patients by Thrombelastography (ROTEM®) in combination of other coagulation tests

Mertens R<sup>1</sup>, Clermont A<sup>1</sup>, Dohmen S<sup>1</sup>, Motthaghy K<sup>2</sup>, Oedekoven B<sup>2</sup>, Kiefer P<sup>3</sup>, Vazquez-Jimenez J<sup>4</sup>

<sup>1</sup>Department of Pediatrics, RWTH University Hospital Aachen, Germany, <sup>2</sup>Institutes for Physiology, RWTH University Hospital Aachen, Germany, <sup>3</sup>W&T GmbH, Central Laboratories Berlin, Germany, <sup>4</sup>Department of Cardio Surgery, RWTH University Hospital Aachen, Germany

**Objectives:** Cardiac surgery with cardiopulmonary bypass is accomplished by complex alterations of coagulation abnormalities that result in bleeding diathesis. The study objective was to obtain information about the haemostatic changes and possibilities of efficient monitoring in order to assess predictive parameters for bleeding risk.

**Design and Methods:** The study was performed in 29 pediatric patients with congenital heart disease undergoing elective on-pump cardiac surgery. At four specified points of time (24 hours preoperative, 1 hour after starting the machine, 30 minutes after administration of protamine and 24 hours after surgery) parameters of ROTEM® and standard coagulation assays were investigated. Special attention was focused on thrombelastography parameters like Clotting Time (CT), Clot Formation Time (CFT) and Maximum Clot Firmness (MCF) in comparison to usual clotting times (PT, aPTT, thrombin time) and coagulation activity parameters as there are AT III, D-Dimers and fibrinogen.

**Results:** On the one hand patients with high demand of transfusions (group 1) showed a significant increase of CFT in HEPTEM after donation of protamine in contrast to the group of lower transfusion demand (group 2) which demonstrated a significant decrease of CT in EXTEM. In this group there was also a significant increase of MCF presented compared to group 1 despite higher transfusion demand.

**Conclusion:** The temporary results of the study give references about the possibility of point-of-care-monitoring with ROTEM®. In contrast to standard coagulation parameters which fail mostly during heparinization, the thrombelastography can give special hints to the dynamic of blood coagulation, especially hyperfibrinolysis.

#### PP3.5-10

##### Inherited protein C deficiency combined with an acquired coagulation defect in a young patient with angiodysplasia

Lodemann P<sup>1</sup>, Pavlova A<sup>1</sup>, Ivaskевичius V<sup>1</sup>, Pötzsch B<sup>1</sup>, Oldenburg J<sup>1</sup>, Harbrecht U<sup>1</sup>

<sup>1</sup>Institut für Experimentelle Hämatologie und Transfusionsmedizin, Bonn, Germany

**Objective:** We report about an uncommon combination of inherited and acquired coagulation defect as essential hint for subsequent disclosure of an underlying disease.

**Case report:** A 23-yr-old woman presented with a long-lasting history of occasionally occurring waist haematomas, family history of thrombosis, hypofibrinogenemia, elevation of D-dimers, factor-XIII- and protein-C-deficiency without any other obvious clinical symptoms or correlates.

**Results:** Abnormal laboratory results were: functional fibrinogen 107mg/dl (177–376mg/dl), fibrinogen antigen 83mg/dl (180–400mg/dl), factor-XIII 22% (65–150%), functional protein C 46% (80–150%), protein C antigen 39 (65–140%), D-Dimers 6.85 µg/ml (0–0.49 µg/ml), TAT-complex 26.9 (0.1–3.0ng/ml), F1+2 >1200 nmol/l (0–0.34 nmol/l), PAP-complex 1454 ng/ml (163–606 ng/ml). Family investigations showed Protein-C-deficiency in the mother. Genotyping discovered a novel heterozygous missense mutation in exon 9 of the protein C gene (c.815G>A), whereas no mutations in the fibrinogen and factor XIII genes were found. We initiated further investigations towards the possibility of angiodysplasia and Kasabach-Merritt-syndrome. Finally, via MR-angiography, multiple arterio-venous shunts of the right kidney were found.

**Conclusions:** Activation of coagulation by angiodysplasia led to acquired deficiency of fibrinogen and factor XIII, possibly aggravated by inherited protein C deficiency. Coagulation alterations without obvious clinical correlate may point to so far unrecognized disorders, especially of the vascular system.

#### PP3.5-11

##### Limitations in the therapy of acute bleeding in a patient with acquired FVIII-Inhibitor

Von Auer C<sup>1</sup>, Scharrer I<sup>1</sup>

<sup>1</sup>3. Medizinische Klinik, Hämatologie/ Onkologie, J. Gutenberg Universitätsklinik, Mainz, Germany

Acquired haemophilia is a rare haemorrhagic disorder that often presents life-threatening bleeding situations requiring prompt therapeutic intervention. Recom-

binant activated FVII (rFVIIa) can circumvent the actions of inhibitory autoantibodies. We report on a 72-yr-old patient with acquired FVIII- inhibitor and severe bleeding after accidental lung puncture. He had surgery due to haemothorax and kept bleeding afterwards, therefore a haemostaseologist was consulted. Immediately after diagnosis of the inhibitor he was treated with rFVIIa (90 µg/kg). Global coagulation parameters, thrombocytes, calcium, body temperature and pH- value were kept normal. Over the next few days bleeding symptoms and transfusion requirements reduced, the patient was in stable condition but yet not suitable for immunoadsorption therapy. On the fourth day of rFVIIa therapy the bleeding volume rose again and blood transfusions had to be increased without detectable reason. Due to recurrence of haemothorax three more surgical procedures were conducted. A surgical bleeding was ruled out, tranexamic acid, antithrombin, FXIII and fibrin glue were administered. A cessation of bleeding could not be achieved, after ten days the patient died due to the consequences of uncontrollable bleeding. This case demonstrates that even today the treatment of acquired FVIII- inhibitors can be difficult and with limitations. The reasons for this treatment failure might be the suboptimal administration of rFVIIa by colleagues in charge (every 4 hours instead of every 2 hours as recommended), delayed use of tranexamic acid (from the 8th day of treatment onwards), the tissue involved in trauma (high fibrinolytic activity) or failure of rFVIIa.

### PP3.6 Perioperative Hemostasis

#### PP3.6-1

##### Coagulation laboratory screening is not a good predictor for bleeding complications in pediatric patients requiring peripherally inserted central catheters

Woodley-Cook J<sup>1</sup>, Amaral J<sup>1</sup>, Connolly B<sup>1</sup>, Brandao L<sup>1</sup>

<sup>1</sup>The Hospital for Sick Children, Toronto, Canada

We assessed the incidence of bleeding complications due to peripherally inserted central catheter (PICC) insertion in children and evaluated the predictive value of pre-procedural blood screening (PpBS) for bleeding complications and whether correcting abnormal values of PpBS reduces rates of bleeding complications due to PICC insertion. Retrospective review of 1377 patients submitted for PICC line insertion between 2001 and 2006 was performed. PpBS analyses included pre- and post-PICC hemoglobin (Hgb), platelets, aPTT and INr. Results were analyzed in two cohorts for age appropriate interpretation of PpBS: a) 0-3 months and b) 3 months to 18 years. Bleeding complications comprised any blood loss following 48 hours of PICC insertion. Cohorts A and B had a 28.3% and 22.9% minor bleeding complication rate (mc), respectively. The mc rate in cohort A with normal and abnormal PpBS was 32.1% and 22.8%, respectively ( $p = 0.17$ ). The mc rate in cohort A with abnormal PpBS corrected with blood products and not corrected was 24.1% and 22.2%, respectively ( $p = 0.64$ ). The mc rate in cohort B with normal and abnormal PpBS was 22.9% and 22.7%, respectively ( $p = 0.95$ ). The mc rate in cohort B with abnormal PpBS corrected with blood products and not corrected was 20.4% and 23.8%, respectively ( $p = 0.89$ ). PpBS does not predict bleeding complications in pediatric patients with no bleeding tendency, and correction of abnormal PpBS values may not reduce the incidence of bleeding complications in patients undergoing PICC insertion.

#### PP3.6-2

##### Prothrombin Complex Concentrate but not rec. Factor VIIa reduces haemorrhage in a dilutional coagulopathic rabbit trauma model

Pragst I<sup>1</sup>, Dickneite G<sup>1</sup>

<sup>1</sup>CSL Behring GmbH, Marburg, Germany

Severe traumatic or intra-operative blood loss necessitates massive transfusion. The loss and dilution of coagulation factors results in insufficient haemostasis. Coagulation factor replacement is proposed to restore haemostatic capacity. In a rabbits model of dilutional coagulopathy the effect of PCC (Beriplex(R) P/N) and rF VIIa (NovoSeven(R)) on haemorrhage was compared. Dilutional coagulopathy was induced by phased blood withdraw, salvaged erythrocyte retransfusion and hydroxyethyl starch infusion. Coagulation factors were reduced to less than 50 % of baseline. Bleeding was inflicted by kidney incision. Animals were randomized to groups: 1. Control undiluted, (n=5); 2. Placebo, (n=7); 3. PCC 25 U/kg (n=6); 4. rF VIIa 180 µg/kg (n=6). Thrombin generation (TGA), prothrombin time (PT) and bleeding were assessed. Naive rabbits demonstrated spontaneous haemostasis (blood loss (BL) 4.7±2.4 ml; post injury time to haemostasis (TH) 4.4±2 min). Haemostasis deteriorated after colloid transfusion (BL 60.0±25.9 ml, TH 19.1±2.2 min; placebo group). TGA showed normal lag phase but 50% of normal peak thrombin level. PT was prolonged 1.8 fold. PCC corrected the peak thrombin level and reduced mean BL (21.5±11.1 ml;  $p < 0.01$ ), TH (12.4±1.4 min;  $p < 0.01$ ), as well as PT ( $p < 0.05$ ). Although rF VIIa treatment normalised PT ( $p < 0.01$ ) and decreased TGA lag time, it did not improve haemorrhage (89.2±32.9 ml; 24.4±5.9 min). Substitution

therapy with PCC normalized TGA and reduced bleeding. rF VIIa normalized PT, reduced TGA lag time, but was ineffective in preventing severe haemorrhage. It was concluded, that PCC is superior to F VIIa in preventing severe haemorrhage in dilutional coagulopathy.

#### PP3.6-3

##### The effect of citrate substitution in vitro studies on dilutional coagulopathy

Rosenthal C<sup>1</sup>, Rau J<sup>1</sup>, Volk T<sup>1</sup>, Spies C<sup>1</sup>, Ziemer S<sup>2</sup>, von Heymann C<sup>1</sup>

<sup>1</sup>Department of Anesthesiology with focus on Intensive Care Medicine, Campus Virchow-Klinikum and Campus Charité Mitte, Charité- Universitätsmedizin Berlin, Germany, <sup>2</sup>Institute of Laboratory Medicine and Pathological Biochemistry, Campus Virchow-Klinikum and Campus Charité Mitte, Charité-Universitätsmedizin Berlin, Germany

**Objectives:** The coagulopathic effect of hemodilution with crystalloid or colloid solutions has been extensively studied in vitro and in vivo. Several in vitro studies on citrate-anticoagulated whole blood, using thrombelastometry reported hypercoagulation after hemodilution (Roche et al 2006) and significant differences between electrolyte-balanced and non-balanced diluents (Boldt et al. 2007). However, citrate substitution for compensation of dilution-induced decrease in citrate has usually been neglected. We, therefore, investigated the effect of citrate substitution on coagulation parameters in vitro.

**Design and Methods:** Citrate-anticoagulated blood samples of 10 healthy volunteers were diluted by 60% with normal saline (NaCl) or Hydroxyethylstarch 130/0,42 (HES), each solution with or without adequate additional citrate-substitution. Thrombelastometry (RoTEM®), standard coagulation tests and blood-gas analysis were performed. Statistical inter-group analysis was done using nonparametric analysis of variance (ANOVA) with post-hoc Dunnett T3.  $P < 0,05$  was considered significant.

**Results:** As expected, ionized calcium increased significantly after dilution with citrate-free solution ( $p < 0,01$ ). Citrate substitution normalized InTEM® Clotting-time (CT)® for NaCl dilution (NaCl 278±48sec. vs NaCl+Citrate 209±37sec. ( $p < 0,05$ )) (Undiluted control CT® 174±14sec) but had no effect on HES induced CT prolongation. Citrate substitution partially normalized ExTEM® CT® in HES (HES 188±9sec. vs HES+Citrate 151±13sec. ( $p < 0,01$ )) but had no effect in NaCl dilution. All other coagulation parameters were not influenced by citrate substitution.

**Conclusions:** Dilution studies without citrate substitution lead to increased Ca<sup>2+</sup> with significant effects on RoTEM® not present in standard coagulation tests. If in vitro dilution studies use Ca<sup>2+</sup>-containing solutions, the effect of citrate substitution may even be more pronounced.

#### PP3.6-4

##### Desmopressin (DDAVP) for minimising perioperative allogeneic blood transfusion: A stratified metaanalysis

Zotz R<sup>1</sup>, Araba F<sup>2</sup>, Bux I<sup>2</sup>

<sup>1</sup>Praxis für Hämostaseologie und Transfusionsmedizin, Düsseldorf, Germany, <sup>2</sup>MVZ für Blutgerinnungsstörungen und Transfusionsmedizin, Bonn, Germany

**Background:** DDAVP has been evaluated in post-operative surgery patients without inherited bleeding disorders to determine whether it decreases blood loss and can minimise exposure to allogeneic transfusion. Previous reviews with overall negative results have not discriminated between prophylactic and therapeutic indications for DDAVP (Carless et al. 2006). The aim of this analysis was to create a systematic metaanalysis stratified for prophylactic administration of DDAVP to all patients as compared to a therapeutic indication, defined as blood loss >1l in the control group or prior aspirin medication.

**Methods:** Studies were evaluated in the metaanalysis when they were controlled trials in which adult patients, scheduled for non-urgent surgery, were randomised to DDAVP, or to a control group, who did not receive the intervention.

**Results:** Eighteen trials of DDAVP (1295 patients) reported data on the number of patients transfused with allogeneic RBC transfusion. In subjects treated with DDAVP for therapeutic reasons, the pooled relative risk of exposure to perioperative allogeneic RBC transfusion was 0.36 (95%CI = 0.19 to 0.66). The use of DDAVP did significantly reduce blood loss; weighted mean difference (WMD) = -386 ml: 95% confidence interval (95%CI) = -542 to -231ml per patient) or the volume of RBC transfused (WMD = -340 ml: 95%CI = -547 to -134 ml per patient). In contrast, in subjects treated with DDAVP for prophylactic reasons, no significant benefit from using DDAVP was found.

**Conclusions:** Desmopressin administered for therapeutic indications significantly minimises perioperative allogeneic RBC transfusion in patients who do not have congenital bleeding disorders.



## PP3.6-5

### Impedance aggregometry for the prediction of the risk of blood products transfusion in cardiac surgery

Rahe-Meyer N<sup>1</sup>, Solomon C<sup>1</sup>, Winterhalter M<sup>1</sup>, Calatzis A<sup>2</sup>

<sup>1</sup>Department of Anesthesiology, Hannover Medical School, Hannover, Germany, <sup>2</sup>Department of Hemostasis and Transfusion Medicine, Munich University Hospital, Munich, Germany

**Objectives:** Perioperative bleeding complications in cardiac surgery are associated with abnormal platelet function. Preoperative assessment of platelet function is not performed as standard in most surgical institutions. We compared the usefulness of impedance aggregometry and of standard coagulation analyses in identifying patients at high risk of transfusion of allogeneic blood products during surgery and on the first postoperative day.

**Design and Methods:** The analyses were performed in 60 patients before and after routine cardiac surgery. Impedance aggregometry was assessed on the Multiplate<sup>®</sup> platelet function analyzer using ADP (ADPtest), collagen (COLtest) and thrombin receptor activating peptide (TRAPtest) as triggers for platelet activation. The results of the aggregation tests and of the routine laboratory analyses (hematocrit, PT, aPTT and platelet count) were divided into tertiles and assessed in relation to the amount of platelet concentrates, fresh frozen plasma and red blood cells transfused intraoperatively and in the 24-hour postoperative period and the 24-hour postoperative drainage volume.

**Results:** ADPtest, COLtest, and TRAPtest identified patient groups with significantly higher blood products transfusion, particularly platelet concentrates. This applied for both the preoperative tests concerning the intraoperative period and for the postoperative tests concerning the first postoperative day. Drainage volume was also decreased in the high aggregometry tertile. From the standard laboratory tests, only preoperative PT and postoperative platelet count showed significant association with bleeding parameters.

**Conclusions:** These results suggest that impedance aggregometry may support the identification of groups of patients with enhanced risk of bleeding and blood products transfusion in routine cardiac surgery.

## PP3.6-6

### Reduction of blood transfusion rate by thrombelastometry and impedance aggregometry based point-of-care coagulation management in cardiovascular surgery

Görlinger K<sup>1,2</sup>, Hanke A<sup>3</sup>, Bergmann L<sup>2</sup>, Kamler M<sup>4</sup>, Müller-Beisenhirtz H<sup>1,5</sup>, Hartmann M<sup>2</sup>

<sup>1</sup>Gerinnungskommission, Universitätsklinikum Essen, Germany, <sup>2</sup>Klinik für Anästhesiologie und Intensivmedizin, Universitätsklinikum Essen, Germany, <sup>3</sup>Klinik für Anästhesiologie, Universitätsklinikum Düsseldorf, Germany, <sup>4</sup>Klinik für Thorax- und Kardiovaskuläre Chirurgie, Universitätsklinikum Essen, Germany, <sup>5</sup>Klinik für Hämatologie, Universitätsklinikum Essen, Germany

**Objectives:** In April 2004 respectively in December 2005 we implemented thrombelastometry (ROTEM) and impedance aggregometry (Multiplate) for point-of-care (POC) coagulation management in cardiovascular surgery. Based on our experience in POC coagulation management in liver transplantation and multiple trauma we developed an algorithm for POC coagulation management in cardiovascular surgery.

**Design and Methods:** To evaluate the efficiency of our POC coagulation management we analysed in our retrospective study the transfusion rate of blood products from January 2004 to December 2007.

**Results:** From 2004 to 2007 transfusion rate of red blood cells (RBC) decreased from 3276 to 2840 units per year by 13.3% and fresh frozen plasma (FFP) decreased from 1986 to 358 by 82.0%. Apart from this absolute reduction, the RBC:FFP-ratio was changed from 1.6 to 7.9. On the other hand transfusion rate of pooled platelet concentrates increased from 336 to 619 units per year by 84.2%. This increase is probably a consequence of the increasing number of patients with a dual antiplatelet therapy with acetylsalicylic acid and clopidogrel, particularly in cardiovascular surgery during the last years.

**Conclusions:** ROTEM- and Multiplate-based coagulation management is effective in reducing transfusion rate in cardiovascular surgery. This effect is most pronounced for the reduction of FFP transfusion rate. This may particularly be important for the reduction of FFP-induced morbidity and mortality, such as transfusion-related acute lung injury (TRALI) and transfusion-associated circulatory overload (TACO). Furthermore, the change of RBC:FFP-ratio from 1.6 to 7.9 reflects a more goal-directed therapy of coagulopathies with specific coagulation factor concentrates.

## PP3.6-7

### Reduction of blood transfusion rate and cost-saving by thrombelastometry-based coagulation management in visceral surgery and liver transplantation

Görlinger K<sup>1,2</sup>, Dirkmann D<sup>2</sup>, Hanke A<sup>3</sup>, Saner F<sup>1,4</sup>, Müller-Beisenhirtz H<sup>1,5</sup>, Hartmann M<sup>2</sup>

<sup>1</sup>Gerinnungskommission, Universitätsklinikum Essen, Germany, <sup>2</sup>Klinik für Anästhesiologie und Intensivmedizin, Universitätsklinikum Essen, Germany, <sup>3</sup>Klinik für Anästhesiologie, Universitätsklinikum Düsseldorf, Germany, <sup>4</sup>Klinik für Allgemein-, Viszeral- und Transplantationschirurgie, Universitätsklinikum Essen, Germany, <sup>5</sup>Klinik für Hämatologie, Universitätsklinikum Essen, Germany

**Objectives:** Transfusion of blood products is associated with increased morbidity, mortality and costs in major surgery. Therefore, in January 2000 we implemented thrombelastometry (ROTEM) for point-of-care (POC) coagulation management in visceral surgery and liver transplantation. The goal of our study was to prove if thrombelastometry-based POC coagulation management is effective in reducing transfusion rate and is cost-saving in visceral and transplantation surgery.

**Design and Methods:** In our retrospective study we analysed the intraoperative usage of blood products and coagulation factor concentrates and their respective costs from January 1999 to December 2007 in visceral and transplant surgery. Cost calculation was based on prices in 2008.

**Results:** From 1999 to 2007 transfusion rate of red blood cells decreased from 3454 to 2123 units per year by 38.5%, fresh frozen plasma from 4465 to 975 by 78.2% and pooled platelet concentrates from 433 to 197 by 54.5%. During the same time the usage of fibrinogen concentrate increased from 68 to 590 g per year, prothrombin complex concentrate from 65,500 to 243,000 IU, whereas antithrombin concentrate decreased from 150,500 to 136,500 IU. The reduction of costs for blood products in 2007 compared to 1999 accounted for 431,290 Euro, whereas the increase of costs for coagulation factor concentrates amounted 208,684 Euro. Overall, this resulted in cost-saving of 222,606 Euro per year (-28%).

**Conclusions:** Usage of our coagulation management algorithm based on thrombelastometry and goal-directed therapy with coagulation factor concentrates resulted in reduction of blood transfusion rate and is cost-saving in visceral surgery and liver transplantation.

## PP3.6-8

### Impact of a thrombelastometry-based algorithm for point-of-care coagulation management on blood transfusion rate in trauma patients

Görlinger K<sup>1,2</sup>, Hanke A<sup>3</sup>, Dirkmann D<sup>2</sup>, Müller-Beisenhirtz H<sup>4</sup>, Piepenbrink K<sup>2</sup>

<sup>1</sup>Gerinnungskommission, Universitätsklinikum Essen, Germany, <sup>2</sup>Klinik für Anästhesiologie und Intensivmedizin, Universitätsklinikum Essen, Germany, <sup>3</sup>Klinik für Anästhesiologie, Universitätsklinikum Düsseldorf, Germany, <sup>4</sup>Klinik für Hämatologie, Universitätsklinikum Essen, Germany

**Objectives:** Transfusion of blood products is associated with increased morbidity and mortality in major surgery. Therefore, based on our experience in point-of-care (POC) coagulation management in liver transplantation we developed a thrombelastometry (ROTEM) based algorithm for bedside coagulation management in trauma patients. The goal of our study was to prove if our thrombelastometry-based POC coagulation management is effective in reducing transfusion rate in trauma surgery.

**Design and Methods:** To evaluate the efficiency of our POC coagulation management we analysed in our retrospective study the transfusion rate of blood products from January 2002 to December 2007 during treatment of trauma patients in the emergency room and during surgery.

**Results:** From 2002 to 2007 transfusion rate of red blood cells decreased from 1332 to 1300 units per year by 2.4%, fresh frozen plasma (FFP) decreased from 1221 to 661 units per year by 45.9% and pooled platelet concentrates from 82 to 54 units per year by 34.1%. During the same time the number of operations in trauma surgery increased from 2594 to 2758 per year by 6.3%.

**Conclusions:** Thrombelastometry-based coagulation management is effective in reducing transfusion rate in trauma patients. This effect is most pronounced for the reduction of FFP and platelet transfusion rate. Particularly this is important with regard to the reduction of FFP and platelet transfusion induced morbidity and mortality, such as transfusion-related acute lung injury (TRALI), transfusion-associated circulatory overload (TACO), infection and sepsis.

PP3.6-9

**The effect of 6 % Hydroxyethylstarch 130/0.4 and 4 % gelatin on coagulation and blood transfusion requirements after cardiac surgery.**

Vonmetz A<sup>1</sup>, Löckinger A<sup>2</sup>, Lorenz I<sup>1</sup>, Friesenecker B<sup>1</sup>, Velik-Salchner C<sup>2</sup>, Innerhofer P<sup>2</sup>, Martinowitz U<sup>3</sup>, Fries D<sup>1</sup>

<sup>1</sup>University Hospital for General and Surgical Intensive Care Medicine, Medical University Innsbruck, Austria, <sup>2</sup>University Hospital for Anaesthesia and Intensive Care Medicine, Medical University Innsbruck, Austria., <sup>3</sup>Department for Haematology, TelHashomer Hospital, Tel Aviv, Israel

The question as to the optimal volume replacement is subject of ongoing controversy. Until now, no clinical data are available comparing the effects of 6 % HES 130/0.4 (Voluven®) and gelatin on coagulation parameters and transfusion of blood products in patients following cardiac surgery. 1.050 patients were analysed retrospectively after cardiac surgery between. They either received gelatin (n=633) or gelatine in combination with 6 % HES 130/0.4 (Voluven®, Fresenius). Coagulation parameters and blood transfusion requirements were evaluated immediately after ICU admission and on the first postoperative day. Differences in laboratory parameters were analyzed using a t-test for independent groups. Differences in blood products usage were analyzed using a Mann-Whitney U test, since assumption of normality were not attained. P-values <0.05 were assumed statistically significant. Patients who received 6 % HES 130/0.4 showed a significantly smaller increase in plasma fibrinogen concentrations (60 +/- 61 mg/dl vs. 71 +/- 63 mg/dl; p=0.006) when compared to patients treated with gelatin alone. This effect of 6 % HES 130/0.4 on plasma fibrinogen levels was dose dependent. Furthermore, platelet count in the patients treated with 6 % HES 130/0.4 decreased, while platelets in the patients who received gelatin alone increased after admission at the ICU (p=0.004). 6 % HES 130/0.4 treated patients also showed a higher need for transfusion of packed red blood cell concentrates (median transfusion rate of 0,81 vs. 0,65 unit of packed red cells; p<0.05). Impairment of the coagulation system after 6 % HES 130/0.4 was significantly more pronounced when compared to the use of gelatine alone after cardiac surgery.

PP3.6-10

**Disturbance of fibrin polymerization in patients receiving Methylene blue / light virucidally treated plasma – a randomized, double-blind clinical study**

Wieding J<sup>1</sup>, Taborski U<sup>2</sup>, Teßmann R<sup>3</sup>, Oprean N<sup>4</sup>, Köstering H<sup>5</sup>

<sup>1</sup>Hämostaseologie, Göttingen, Germany, <sup>2</sup>Di. Ges. f. Humanplasma, Langenfeld, Germany, <sup>3</sup>Anästhesie, Klinikum Offenbach, Germany, <sup>4</sup>Anästhesie, Nidderau, Germany, <sup>5</sup>Hämostaseologie, Universitätsklinikum Göttingen, Germany

Quarantine plasma (Q-FFP) still carries a risk of infections. Thus, solvent detergent or methylene blue/light virus-inactivated plasma (MB-/SD-VIP) is also frequently used in Europe. However, applying MB also causes photooxidation of fibrinogen, leading to a disturbance of its polymerization e.g. with 30 % reduced fibrinogen levels by Clauss method. The aim of this study was to examine parameters of fibrin polymerization in patients substituted with MB-VIP. Patients expected to require >4 units of plasma were randomized to receive either MB-VIP or Q-FFP. Blood samples were drawn before and 0–24h after surgery. This examination included n=10 patients having received MB-VIP and 6 pat. receiving Q-FFP within 2 h of extensive bone surgery (polytrauma, spine surgery etc.). Reptilase times increased after MB-VIP infusion and leveled higher than after Q-FFP (20.9±2.8, 21.3±2.6, 21.0±3.4 and 17.2±1.5 vs. 19.8±2.4, 19.5±1.9, 18.8±2.2 and 17.7±3.6s in samples drawn 0, 2, 6 and 24h after surgery). The ratio of immunological and functional fibrinogen was calculated to quantify abnormal fibrin polymerization. In samples taken 0–6h after infusion, this ratio leveled significantly higher after MB-VIP than Q-FFP and corresponded with longer reptilase times. Thromboplastin times, factor V, FVIII, prothrombin fragments and D-dimers did not differ significantly between groups (p>0.05). In conclusion, these results agree with prior in-vitro data. Reptilase time and the ratio of functional and immunological fibrinogen both depicted a disturbance of fibrin polymerization in patients substituted with greater MB-VIP amounts: Since alteration of fibrinogen can possibly contribute to profuse bleeding tendency alternatives like SD-plasma should be kept in consideration.

PP3.6-11

**Factor XIII, fibrinogen and platelet count as predictors of clot firmness during surgical bleeding – implications for the therapeutic approach**

Korte W<sup>1</sup>, Degiacomi P<sup>1</sup>, Jovic R<sup>1</sup>, Graf L<sup>1</sup>

<sup>1</sup>Institut für Klinische Chemie und Hämatologie, Kantonsspital St. Gallen, Switzerland

**Objectives:** We earlier showed in a placebo-controlled trial that early FXIII substitution in high-risk patients maintains clot firmness and significantly reduces intraoperative blood loss. We now evaluated whether FXIII is also an independent predictor of clot firmness in the bleeding patient in both, platelet inhibited and uninhibited whole blood clot firmness assays activated by TF.

**Methods:** Routine assessment of samples submitted from the operating room or the surgical intensive care unit for bleeding episodes included FXIII, fibrinogen, platelet count and (Rotem) Extem and Fitem assays. Frequency of FXIII, fibrinogen and platelets below recommended cut-offs were registered. The influence of these parameters on clot firmness was evaluated by multiple regression analysis. 99 samples were evaluated.

**Results:** FXIII <60 % was seen in 47 %, fibrinogen <1 g/l in 11 % and thrombocytopenia <100 G/l in 24 % of the samples. FXIII deficiency was significantly more frequent than hypofibrinogenemia (p=0.0001) and thrombocytopenia (p=0.0299). FXIII, fibrinogen and platelet count were independent predictors of clot firmness in a TF activated whole blood clot firmness assay with and without platelet inhibition.

**Conclusion:** FXIII deficiency is a) frequent during acute surgical bleeding; b) significantly more prevalent than hypofibrinogenemia or thrombocytopenia; c) an independent predictor of clot firmness. These observations – together with the randomized intervention trial results – suggest that FXIII deficiency might be of great importance as it occurs most frequently. This suggests that FXIII replacement should be used first in the bleeding surgical patient with decreased clot firmness.

PP3.6-12

**Platelet function before and after DDAVP therapy measured bedside by Multiple Electrode Aggregometry (MEA) correlates with blood loss in patients after cardiac surgery**

Weber C<sup>1</sup>, Jámor B<sup>2</sup>

<sup>1</sup>Department of Anesthesiology, Intensive Care and Pain Medicine, Goethe-University Frankfurt am Main, Germany, <sup>2</sup>Clinic for Anesthesiology, University of Munich, Germany

**Objectives:** To evaluate the platelet function before and after DDAVP therapy in postoperative cardiac surgery patients with suspected platelet function impairment using multiple electrode aggregometry (MEA, Dynabyte, Munich) and to correlate it with blood loss.

**Methods:** After IRB approval, ICU-patients after cardiac surgery with CPB and increased blood loss for at least two hours postoperatively were consecutively enrolled to the study. Inclusion criteria: normal thrombelastometry, Quick>50 %, APTT<50sec, fibrinogen>150mg/dl, platelets>80/nl, and hematocrit>25 %; suggesting no requirement for hemostatic therapy. Exclusion criteria: suspected surgical bleeding. MEA was performed at the bedside before and 2–4hours after the infusion of DDAVP, using TRAP-6 (TRAPtest, 32µM), arachidonic acid (ASPItest, 0.5mM) and adenosine-diphosphate (ADPtest, 6.4µM) for platelet activation. Blood loss(ml/h) after DDAVP was recorded for at least two hours. Wilcoxon signed rank test was used to detect differences before and after DDAVP infusion and Spearman rank order correlation to quantify the association between platelet aggregation and blood loss. Statistical significance was set to p<0.05.

**Results:** Eleven patients received 0.32±0.05µg/kg DDAVP after 3.6±1.1hours observation period. Blood loss decreased from 267(223/310)ml/h to 100(75/113)ml/h (median(25/75percentile), p<0.001) and platelet aggregation in MEA increased after DDAVP administration: 85(66/115)U vs. 64(26/88)U in TRAPtest(p=0.007), 49(30/72)U vs. 15(8/21)U in ASPI-test(p<0.001), and 35(24/54)U vs. 14(7/28)U in ADP-test(p=0.002). Significant correlation was observed between blood loss and MEA: r=-0.792, p<0.001 for ASPItest and r=-0.577, p=0.005 for ADPtest, respectively.

**Conclusion:** MEA well detected the improvement of platelet function after DDAVP. Furthermore, arachidonic acid and ADP induced platelet aggregation showed significant correlation with blood loss. Controlled studies should clarify causality between reduction of blood loss and DDAVP therapy.

PP3.6-13

**Cost effectiveness of perioperative point-of-care coagulation management**

Müller-Beilhertz H<sup>1,2</sup>, Moog R<sup>3</sup>, Saner F<sup>1,4</sup>, Dührsen U<sup>2</sup>, Görlinger K<sup>1,5</sup>

<sup>1</sup>Gerinnungskommission, Universitätsklinikum, <sup>2</sup>Klinik für Hämatologie, Universitätsklinikum Essen, Germany, <sup>3</sup>Institut für Transfusionsmedizin, Universitätsklinikum Essen, Germany, <sup>4</sup>Klinik für Allgemein-, Viszeral- und Transplantationschirurgie, Universitätsklinikum Essen, Germany, <sup>5</sup>Klinik für Anästhesiologie und Intensivmedizin, Universitätsklinikum Essen, Germany

**Objectives:** In 2004 we developed algorithms for perioperative point-of-care coagulation management in liver transplantation, multiple trauma and cardiovascular surgery. In our retrospective study we analysed the impact of these algorithms on usage of blood products and coagulation factor concentrates and their respective costs.

**Methods:** We retrospectively analysed the overall usage of blood products and coagulation factor concentrates at University Hospital of Essen from January 2004 to December 2007. To eliminate the effect of price changes during this time period we calculated all costs on the basis of prices in 2008.

**Results:** From 2004 to 2007 the number of transfused units of red blood cells decreased from 40.879 to 35.219 (-14 %), fresh frozen plasma from 28.150 to 10.640



(-62%) and pooled platelet concentrates from 5.299 to 4.801 (-9%). During the same period usage of fibrinogen concentrate increased from 1.035 to 3.040 g (+194%) and prothrombin complex concentrate from 1.215 to 2.277,5 kU (+87%), whereas usage of antithrombin concentrate decreased from 2.019 to 1.278,5 kU (-37%) and F XIII concentrate from 282 to 138,75 kU (-51%). These changes have been connected with a reduction of costs for blood products by 1.874.683 Euro from 2004 to 2007, whereas costs for coagulation factor concentrates increased by 810.610 Euro. In total this resulted in a cost-saving of 1. 64. 073 Euro in 2007 compared to 2004 (-13%).

**Conclusion:** Implementation of algorithms for perioperative point-of-care coagulation management allows a reduction of transfusion rate of blood products and overall costs for blood products and coagulation factor concentrates.

#### PP3.6-14

##### ROTEM-based coagulation management for spinal anesthesia in a patient with congenital Hypofibrinogenaemia and Factor XIII deficiency for Cesarean section

Hanke A<sup>1</sup>, Elsner O<sup>1</sup>, Görlinger K<sup>2</sup>, Kienbaum P<sup>1</sup>

<sup>1</sup>Klinikum der Heinrich-Heine-Universität Düsseldorf, Klinik für Anästhesiologie, Germany,

<sup>2</sup>Universitätsklinikum Essen, Klinik für Anästhesiologie, Germany

**Objectives:** Hypofibrinogenaemia and factor XIII deficiency are known to cause severe bleeding complications and are considered as contraindications for spinal anesthesia which is the recommended anesthetic procedure for caesarean section (CS).

**Case-Report:** A 32 year old woman was scheduled for CS. History included haematoma following minor trauma and prolonged menorrhoea. Laboratory testing revealed: fibrinogen 104 mg/dl, FXIII 48 %, aPTT 27s, INR 1.1, Quick 70 %, platelet count 225 x 109/l, normal platelet function (PFA-100) PFA-EPI 84s, PFA-ADP 64s. Rotational thrombelastometry (ROTEM) showed reduced maximum clot firmness (MCF-EXTEM 47mm, MCF-INTEM 50mm). Fibrinpolymerization was impaired (MCF-FIBTEM 4mm) and clotting times were prolonged (CT-EXTEM 101s, CT-INTEM 169s). Prior to anesthesia and surgery FXIII (1,250 units) and fibrinogen (4g) were administered normalizing ROTEM coagulation variables (MCF-EXTEM 53mm, MCF-INTEM 54mm, CT-EXTEM 79s, CT-INTEM 133s). Spinal anesthesia and CS were performed without increased blood loss or complications due to either anesthesia or surgery.

**Conclusion:** Hypofibrinogenaemia below 1g/l and factor XIII deficiency may cause excessive bleeding during birth so that prior substitution of both factors is recommended. We report on a patient with congenital hypofibrinogenaemia and FXIII deficiency scheduled for CS. On the basis of ROTEM findings coagulation variables were normalized allowing otherwise contraindicated spinal anesthesia and uneventful surgery. This is the first report of ROTEM based management of a patient with congenital bleeding disorders undergoing a regional anesthetic procedure. We encourage systematic evaluation of ROTEM based coagulation management to allow otherwise contraindicated regional anesthesia.

#### PP3.6-15

##### Thickness Shear Mode sensor: A new technique for perioperative bleeding monitoring

Müller L<sup>1</sup>, Sinn S<sup>2</sup>, Wendel H<sup>2</sup>, Northoff H<sup>1</sup>, Gehring F<sup>1</sup>

<sup>1</sup>University Hospital of Tuebingen, Biosensor Research Group, Germany, <sup>2</sup>University Hospital of Tuebingen, Department of Thoracic, Cardiac and Vascular Surgery, Germany

**Objectives:** Besides congenital and acquired cardiovascular diseases, some extracorporeal operations (open heart surgery, dialysis) bear high risks of bleeding and thromboembolic complications. In such situations time plays a critical role. Therefore it is a big advantage to get real-time access to the major hemostatic data, namely prothrombin time, platelet function and fibrinolytic activity. Our developed novel demonstrator based on the technique of Thickness Shear Mode (TSM) sensors allows simultaneous measurements of these different haemostatic tests out of citrated blood and will give sufficient information about complex bleeding complications.

**Methods:** The TSM method permits the detection of any adsorbed masses (cells, proteins, particles, etc.) or changes in viscosity in real-time by changes in resonance frequency. With adequate sensor coatings and reagents, prothrombin time, platelet aggregation and fibrinolytic activity were measured. The measurements were confirmed by comparison with commercially available coagulometers and aggregometers as well as scanning electron microscopy and fluorescence microscopy.

**Results:** Prothrombin times and hyperfibrinolysis were measured in undiluted and diluted whole blood on polyethylene surfaces. Platelet aggregation was carried out in platelet rich plasma on polystyrene and fibrinogen surfaces. The results from these measurements showed good accordance to established methods.

**Conclusion and outlook:** The developed innovative demonstrator based on the TSM method seems to be suitable for real-time investigation of hemostatic pro-

cesses. The next generation of the demonstrator planned by next year shall allow an automated measuring process and shall deliver haemostaseological data to the attending physician to start an adequate therapy as fast as possible.