PP3 - Hemorrhagic Disorders

PP3.1 - Hemophilia

PP3.1.1 - Co-activation of selected knee muscles in haemophilic patients

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Previous studies of knee muscle activity in haemophilic patients during upright standing showed 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 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 calculation of co-activation indices patients' extremities were classified according to major (MA) and minor (MI) affected legs (Gilbert-Score). 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; C, 1.11–3.70) and VM over BF (H: MA: 2.16–3.13; C, 1.98–4.61) were 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-Dutschland).

PP3.1.2 - Bleeding tendency in female carriers of haemophilia A

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Female carriers of haemophilia might not only have an increased bleeding tendency but the symptoms may be frequent and severe, thereby 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 A was recorded as well as FVIII plasma activity (FVIII:C) of the carrier. For analysing 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

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The modifiers adjusted for, e.g. substitution regimen, start of prophylaxis or factor concentrates administered for first-line therapy showed a statistically significant

Design: This is a multicenter cohort study of patients enrolled because of hemophilia or VWD (VWF:F.R.C< 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 (1-35). M inor bleeding at the site of puncture occurred in 5 patients. For PCI (N=38), patients received replacement therapy for a median of 1 day (1-3). Most patients experienced minor (N=15) or major (N=13) bleeding at the site of puncture. Of 14 patients bearing 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. M inor 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 patients with no detectable mutations in the F8 gene

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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 mR NA in one severe patient was further confirmed by our quantitative analysis; the rest of the patients did not show severe deficiency in the F8 mR NA. 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 mR NA 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

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It has been recently suggested that the clinical phenotype of severe hemophilia A would point to the heterogeneity of the molecular cause underlying the F8 deficiency in these patients.

PP3.1.6 - Hemostaseologie 1/2009

Hämostaseologie 1/2009

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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
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Material and Methods: Registration of the Austrian Haemophilia Registry started in 1993. Data have been collected from patients treated in Austria. From 1995 to 2008, 2,625 patients were included of which 2,378 (90.7%) had haemophilia A and 247 (9.3%) had haemophilia B.

Objective: To provide information on the characteristics of the Austrian haemophilia patient population and the actual treatment modalities.

Results: 45.6% of patients had severe haemophilia (30.7% of all patients). In 13.6% of patients, FVIII activity was below 1%, whereas 24 carriers had decreased FVIII:Ag (<63%). FVIII activity and antigen will be evaluated in future studies.

Conclusions: The Austrian haemophilia patient population is larger than previously thought. This could be of great importance for the ongoing EPIR study.

PP3.1-8
Patient A: 64 years, severe hemophilia A. In 2004 total elbow replacement was performed without peroperative complications. Patient D: 49 and 48 years, severe hemophilia A, H IV and HCV coinfected. 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-Ca-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 3-fold higher FIX consumption: A retrospective data analysis
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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 (<3% FIX) without further coagulation disorders either treated with BTermin under OD or CP. With 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 3-fold higher consumption of FIX.}

PP3.1-10
The German Haemophilia Register (DHR) is starting its work
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The German Haemophilia Register (DHR) is starting its work. 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. A fter 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. A dditional 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 (H D) and with random access memory (R A M). 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 worthy third party. It shuts Intermediär off from the PEI without itself coming into
Posters

proposals for music and text for fantasy journeys are made. Certificates of atten-
of haemophilia, practice guidelines for self-infusion, and mandalas to enhance the
materials consist of one trainer’s book and different exercise books for children and
adults. 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
programs for music and text for fantasy journeys are made. Certificates of atten-
dance, written examinations and lists of participants complete the equipment.

Conclusions: Our 10-year experience with this concept demonstrates that haemo-
ophilia training should include not only instruction of factor infusion but also theo-
retical 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)

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Objectives: Quality of life (QoL) of children and young adults whose brothers are
affected by a 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 back-
ground, 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, haemophi-
liacs and parents were asked. The results were compared to those of other pa-
tients.

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) (6–16 years m=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 compara-
tible 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 haemophiliacs
who were treated 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

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Since 2001 Octacine® F, a plasma derived factor IX concentrate is available for
haemophilia B patients. With the finalisation of a 5-year-post-marketing surveil-
lance study and a clinical trial in children below 6 years including PU Ps the planned
programme for clinical validation is complete and a summarised evaluation of tol-
erability and safety can be done. In total, 71 patients were included in 4 prospective
clinical studies A (most 50%), 13) were children. The patients were followed for a
total observation period of 4534 weeks. In that period they have received 3 Mil-
lion units of Octacine® 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 Octacine® 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

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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 refrigera-
tor 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 lique-
fied 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 param-
ters over time was analyzed by means of a nonparametric ANOVA for repeated
measurements.

Results: Over 6 days after thawing and liquefication, activities of FII, FV and PI
decrease significantly in lyophilized plasma compared to S/D-treated plasma (p<0.01). FVIII decreased in lyophilized plasma and increased in 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 avail-
ability in emergency situations. With regard to the easier storage, administration
of lyophilized plasma may offer a safer 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
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Objective: A dequate documentation is critical to patient care in hemophilia and
required by the German transfusion law § 21. Furthermore, transparency of hemo-
philia treatment and adherence to published treatment guidelines become increas-
ingly 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 sys-
tem, which allows for easy extraction of information for other data registries such
as the German hemophilia registry (DHR) or the German cause-of-death regist-
try. A cordially, 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,
changes in treatment) 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 radioisovnoneorthesis, physiotherapy, surgery, or change of factor replacement as well as
t heir respective outcomes are entered.

Conclusion: Our computerized data registry is likely to improve both the transpar-
ency and the quality of care in a German hemophilia center. In addition, it will help
verifying the results of controlled clinical trials in unsellected 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

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Objective: Since implementation of recombinant coagulation factors into haemo-
philia 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 effec-
tive in controlling bleeds than plasma derived factor VIII (pFVIII) concentrates.
The present study compares efficacy and immunogenicity with regard to inhibi-
tor development and reports results obtained in an observation period of fourteen
years in two German haemophilia centres.

Congress Program: 53rd Annual Meeting - Society of Thrombosis and Haemostasis Research
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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 (B ethedasa) over the years 1994 to 2007. A bove values of 0.6 BU, a patient is defined as having developed an inhibitor. Efficacy of treatment was analysed as numbers of infusions needed to stop a bleed. Data are given as mean ± SD.

Result: A total of 110 patients (age: 30.1 ± 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 a 1.72 ± 0.86 and a 1.60 ± 0.55 (p = 0.416) injections per bleed of pFVIII or rFVIII were needed.

Conclusion: 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
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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 focusing on the patients’ co-morbidities as well as changes in their bleeding patterns over the last four years.

Result: Twenty-nine patients were included with a median age of 64 years (60 – 85 years). 7/29 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 A SS and two patients M acrumar. 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 M acrumar or A SS.

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

PP3.1-18
Inhibitor-Immuno-study: Toll-like receptor (TLR) polymorphisms in the inhibitor development of haemophilia A
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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 M ajor H isocompatibility 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. 85 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, TLR 4 A sp32996 G (A>G), TLR 9 T (1237C), TLR 9 T (1486C)). A nalyses 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-TLR 9 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
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Primary prophylaxis of factor (F) VIII IX in severe haemophiliacs results in the reduction of bleeds and the prevention of haemorrhagic 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. A s of December, 2006, 58/84 patients (69%) documented >95% of the prescribed amount of concentrate (full compliance with documentation). Incomplete documentation (<95-50%): 48/4 (5%) and no documentation 22/84 patients (26%). 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%). Moderately compliant (75-90%) was assessed in 12/58 (21%) and weak compliance (50-75%) in 458 (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
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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 FVIII-products. Design and method: A FVIII chromogenic assay validated for plasma-derived FVIII-concentrates was applied to a rFVIII product. A fer reconstitution, samples were pre-diluted in two steps using first the test buffer (Tris/BSA) to achieve the concentration of the standard and second using factor VIII deficient plasma to obtain a concentration of 3 IU/ml. Results: FVIII-potencies estimated with the OMCL method were approx. 20% lower than the manufacturer’s results. Calculations relative to the E P 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 OMC LS to use the Ph. E ur. 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
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Due to frequent bleedings haemophilic patients suffer from functional deficits in daily life. A degative 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). 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 H. Eight, from moderate, and 58 from severe hemophilia A; in one patient the patient 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


This project is assessing the long-term efficacy and safety of Helixate® NexGen (CSL Behring), a recombinant F VIII concentrate, in the post authorization period in Germany, Austria, Italy, France, Sweden, Hämophilia 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. Patient data were collected over the course of this study and the patients were followed up for a maximum of 10 years. At each visit, the following parameters were documented: 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).

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
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Non-enveloped human viruses present a special problem in blood plasma products. Whereas parvovirus B19 (B IV), 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 were 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 (1x101-1x103 gq/ml) were detected in one plasmatic factor VIII, one plasmatic factor VIII/AWF, one factor VII, and one activated prothrombin complex concentrate product. A II 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 non-enveloped viral pathogens.

PP3.1-28
Management of a patient with severe haemophilia A and acute lymphoblastic leukaemia
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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 GMLL 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 x 10^11 IU F VIII/kg/bodyweight (BW) 3 times weekly; Platelets 30 - 40 x 10^11 IU F VIII/kg BW daily; Platelets 20 - 29 x 10^11 IU F VIII/kg BW daily; Platelets <20 x 10^11 IU F VIII/kg BW twice daily; Eフォール and 8 hours after lumbar puncture 40 IU F VIII/kg BW was substituted, before bone marrow aspiration 40 x 10^11 IU F VIII/kg BW. Platelet-concentrates were transfused when the platelet count was <20 x 10^11 because of the additional bleeding risk. In case of bleeding additional F VIII was administered and in case of severe thrombocytopenia additional antibiotic therapy was initiated.

Results: A liver 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 meningoencephalitis. No relevant bleeding episodes occurred during the treatment period.

Conclusion: Bleeding risk during aggressive chemotherapy in a haemophiliac 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
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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. A mong 54 patients affected, challenging diagnoses included malignancy (n=3), cardiovascular disease (n=12), and urogenital disorders (n=6). 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. D managing situations including cancer surgery, heart valve replacement, and hemodialysis treatment could all be successfully managed without the occurrence of significant bleeding events. Net-working 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 RecFacto Pharmacovigilance evaluation - safety and efficacy in daily clinical practice
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Objectives: Non-interventional trials appear to be appropriate means to monitor treatment of a rare disease like haemophilia. The pharmacovigilance evaluation (PE) of RecFacto 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 Morocotocog alfa under routine clinical conditions.

Methods: The study is a non-interventional trial. Patients with haemophilia A of any severity, treated with Morocotocog alfa can be included in the study. Safety is assessed by documentation of all severe adverse events during treatment with RecFacto. 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 RecFacto® 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 RecFacto® 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
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Introduction: The safety and efficacy of clotting factor concentrates in hemophilia A with Morocotocog alfa under routine clinical conditions.

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. A II patients were treated at least one year with the recombinant factor VIII concentrate (FVIII, Kegonate 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 (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 (12 total joint replacements, 1 cholesteatoma, 1 strumektomie 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

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Background: Chronic synovitis is a frequent complication of joint bleeding in hemophilic patients. Conservative therapy regimes 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 subcutaneous rFVIII 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.

Conclusions: Marked effusion with pannus and thickening of the synovium were demonstrable by ultrasound and M R I. 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. A 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

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Objectives: Patient management in centers taking care of hemophilia patients requires good documentation. Electronic patient diaries (EPD) enable immediate contact in critical situations. A medication management system was developed to minimize errors (the QoL questionnaire has been removed; the pauses for administration can be programmed).

Methods: EPD was implemented in 12 centers. The QoL questionnaire has been removed; decision rules were adapted to the specific requirements of the patients. The 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 online mode to include the data from all participating centers to the electronic patient diary and 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

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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 recombinated 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-authorisation phase, especially in very rare diseases such as HaemB.
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

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**Objective:** Aquired 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 an acute hemorrhage. Retrospective hematoma. Medical history and coagulation testing were consistent with acquired vW syndrome. vW immunohistochemistry showed normal cytoplasmic and nuclear labelling of endothelial cells and megakaryocytes, whereas the lymphomatous infiltrate was negative. A cured vW syndrome due to adsorption of vWF 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 of serum immunoaffinity electrophoresis were also atypically broadened with coagulation factors usually including only a limited number of cases, such as post-transplantation cases.

**Conclusion:** Mechanisms underlying acquired vW syndrome are less effective, and chemotherapy and plasma exchange to decrease the paraprotein concentration. The VWF:CB results were related to the respective VWF:Ag contents.

**PP3.2-4**

Evaluation of VWF activity assays for VWF:FF VIII concentrates, employing different collagen types

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**Objective:** To compare the Ph. Eur. supplement 5.5, the collagen-binding assay (VWF:FCB) can be used to determine human VWF activity as an alternative to the ristocetin cofactor assay (VWF:FRC). An international Standard with a labelled VWF:CB potency is not yet available, while different collagen types and origins can be utilized to measure adhesion of VWF derived from VWF:FF VIII concentrates. In particular, controversies exist concerning the use of equine collagen I versus human collagen type I and which collagen type I or V1 has not been sufficiently investigated yet.

**Methods:** TechnoLBA, Vienna, Austria; E. coli, Vienna, Austria

**Rationale:** A prospective post-marketing study (SET = Surveillance of Efficacy and Tolerability) with a high-purity, albumin-free, double virus inactivated VWF:FF VIII concentrate (Wilate®) was initiated in Germany in 2005. A prospective post-marketing clinical trial with coagulation factors usually includes only a limited number of cases, such as post-transplantation cases.

**Conclusion:** 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:FF VIII concentrate and plasma sample testing.

**PP3.2-6**

Update on efficacy and tolerability of a new generation VWF:FF VIII concentrate in von Willebrand’s disease from a prospective post-marketing surveillance

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**Objective:** A prospective post-marketing study (SET = Surveillance of Efficacy and Tolerability) with a high-purity, albumin-free, double virus inactivated VWF:FF VIII concentrate (Wilate®) was initiated in Germany in 2005. A prospective post-marketing clinical trial with coagulation factors usually includes only a limited number of cases, such as post-transplantation cases.

**Conclusion:** Treatment of pediatric von Willebrand’s disease patients with high purity double virus inactivated VWF:FF VIII concentrate - experience from clinical studies Nowak-Göttl U1, Halimeh S1, Kadar J1, Sigg I1, Feddersen J3

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Efficacy, tolerability and dosing of a VWF:FF VIII 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 18 children between 1 and 12 years of age with all types of von Willebrand disease (VWD), who were included either

**PP3.2-7**

Treatment of pediatric von Willebrand’s disease patients with high purity double virus inactivated VWF:FF VIII concentrate - experience from clinical trials

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**Objective:** A prospective post-marketing study (SET = Surveillance of Efficacy and Tolerability) with a high-purity, albumin-free, double virus inactivated VWF:FF VIII concentrate (Wilate®) was initiated in Germany in 2005. A prospective post-marketing clinical trial with coagulation factors usually includes only a limited number of cases, such as post-transplantation cases.

**Conclusion:** 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:FF VIII concentrate and plasma sample testing.

**Result:** A universal measurements revealed an influence on VWF:FCB 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:** Improved automated VWF:FCB assays for VWF:FF VIII concentrates and plasma samples by adjustment of assay reagents, composition and performance parameters.
PP3.2-8
Prolongation of in vitro bleeding time (PFA 100 closure time) in a case of Heyde-syndrome - a case report
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Objective: To describe the case of a 87 year old patient with severe postoperative bleeding complications following an aortic valve replacement requiring emergency surgery.

Methods: Coagulation parameters were performed in the pre- and postoperative phase.

Results: Postoperative coagulation parameters were as follows: PTT 62 s, FVIII:C 5%, vWF:RCo 5%. Intraoperative bleeding times at PFA 100 were prolonged for 90 sec.

Conclusion: The patient was treated with repeated IgG infusions and eventually underwent a successful surgery without further complications.

PP3.2-9
Management of acquired von Willebrand syndrome in pregnancy: A case report
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Objective: To report on the management of a 29-year-old pregnant woman with self-diagnosed GUS.

Methods: Coagulation parameters were monitored throughout pregnancy.

Results: Throughout pregnancy, the patient’s coagulation parameters remained within normal limits, and no additional treatment was necessary.

Conclusion: GUS is a benign condition that does not require specific treatment during pregnancy.

PP3.2-10
Evaluation of prospective criteria for the clinical assessment of efficacy and safety of DDAP
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Introduction: To develop a reliable and simple tool for the assessment of efficacy and safety of DDAP.

Methods: Prospective criteria were developed based on clinical data from various studies.

Results: The criteria showed a high negative predictive value, indicating a low risk of adverse events.

Conclusion: The criteria can be used as a simple tool for the assessment of efficacy and safety of DDAP.

PP3.2-11
Perioperative DDAVP-tests in von Willebrand’s Syndrome
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Objective: To assess the efficacy of DDAVP in preoperative management of von Willebrand’s Syndrome.

Methods: DDAVP was administered perioperatively in 30 patients with VWD type 1 or type 2.

Results: In patients with VWD type 1, DDAVP was effective in reducing bleeding time. However, in patients with VWD type 2, DDAVP was less effective.

Conclusion: DDAVP is an effective preoperative management tool for patients with VWD type 1, but less so for patients with VWD type 2.

PP3.2-12
Laboratory survey to test substitution therapy with Wilate® and FFVIII-Concentrates
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Objective: To assess the efficacy and safety of Wilate® and FFVIII-Concentrates in a large cohort of patients.

Methods: A survey was conducted to assess the efficacy and safety of Wilate® and FFVIII-Concentrates.

Results: The survey showed a high efficacy and safety profile for both treatments.

Conclusion: Wilate® and FFVIII-Concentrates are effective and safe substitution therapies for VWD patients.

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marker for adequate substitution. Interestingly enough, the wound did not stop bleeding. Therefore additional testing of VWF:R:C and VWF:Ag was done and gave values of 30% and 51%, respectively (< normal range). A further adjustment of the substitution dose (VFXI:C 93%, VWF:R:C 46%, VWF:Ag 73%) was the wound was completely healed for bleeding-complications. Therefore, emergency laboratory testing of PT: and FVIII:C is not sufficient to survey substitution, so that VWF:R:C 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

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Objectives: It was claimed since 1960 that bleeding tendency in heterozygous individuals in the few cases that FXIII is missense (case 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 30,4±9.9% 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. A further administration of 2500 I.U. FXIII-concentrate to the plasma level of the homozygous patient raised to 52% but no platelet FXIII activity was detectable. Ten other heterozygotes revealed a FXIII plasma activity of 38,9±13,3% and 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 a 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 F5FD patients: novel frame shift LMAN1 mutation (Azerbaijan) and first indel MCFD2 mutation (Argentina)

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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 acetylsalicylic 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 X E 12000 analyser.

Results: The median value of hematocrit (Ht) was 56% (range 43-78%). Negative correlations were found between hematocrit and platelet count (correlation coefficient r = -0.588), maximum clot firmness (MCF) in thrombelastometric analysis activated with tissue thromboplastin (EXTEM) (r = -0.596), alpha angle (r = 0.8100) and platelet aggregation after activation with ADP (r = 0.9526), 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.

PP3.3-3

Evaluation of desmopressin response in children with inherited thrombocytopathies

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Objectives: The necessity of desmopressin testing prior to the therapeutic use is a matter of controversial discussion. A im of this retrospective study was to evaluate the 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:R:C) and a marked shortening of PFA-100 close time (CT) reaching normal ranges within 120 min after desmopressin application.

Results: 35 tests from 21 children (4 boys, 15 girls, age range: 3.1, 17.0 years) were performed. Among 21 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.

Conclusions: 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 adequate to identify non-responders.

PP3.3-4

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

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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 acetylsalicylic 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 X E12000 analyser.

Results: The median value of hematocrit (Ht) was 56% (range 43-78%). Negative correlations were found between hematocrit and platelet count (correlation coefficient r = -0.588), maximum clot firmness (MCF) in thrombelastometric analysis activated with tissue thromboplastin (EXTEM) (r = -0.596), alpha angle (r = 0.8100) and platelet aggregation after activation with ADP (r = 0.9526), 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

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Objectives: In a previous study we have performed a process risk analysis for the manufacturing of a VWF/FVIII product (Haemate P) to identify the steps which could be modified to optimize the manufacturing process. The production process of Haemate P was divided into two parts which were studied independently twice: Spiked pooled plasma donations were processed from cryoprecipitation to glycerine 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 material 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:** A overall prion reduction factor of 6.1 log10 and 5.9 log10 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 Haemat P B 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 WF / FVIII product Haemat P.

**PP3.3-6**

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

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**Background:** A patient with severe von Willebrand disease (VWD) and a large deletion within the vWF gene were at risk of developing precipitation alloantibodies against WF and severe hemorrhagic complications.

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

**Aim:** Recombinant activated FVII (rFVIIa), recombinant F.VIII (rFVIII), plasma-derived F.VIII/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 in the aim of obtaining the most effective clinical results.

The dosage of rFVIIa ranged from 50 to 100 I.U. b.w., p-d FVIII/VWF ranged from 50 to 100 I.U. b.w., rFVIII/VWF from 30 to 50 I.U. b.w., Tranexamic acid from 2-4 g over 24 hours and cirumcision, gum bleeds and a posttraumatic hip bleed.

**Results and Conclusions:** rFVIIa provided an effective and safe hemostasis in oral surgery and particularly in treating the life-threatening hematoma. The continuous infusion of FVIII helped 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-d FVIII/VWF strictly monitored by an intensive care unit because of the high risk of immune-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 Ganzmann's Thrombathemia in the Sultanate of Oman

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**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:** DT 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 GPIB/IIa was markedly reduced on flowcytometry. Molecular studies performed at Medical Genetics, University of Sohar, Sultan Qaboos University Hospital, based on clinical features, platelet aggregometry and carrier detection.

**Results:** We identified a novel nonsense causative mutation (Q694X) by sequencing the FGB gene. In addition, sequencing ITGB3 gene also revealed 2 SNPs (rs 3808963; rs 3808965; 3383T/A). The M-cro-A ray assay using Illumina H-uman-1 Bead chip excluded the possibility of deletion of these genes in chromo- some 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

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**Objectives:** To identify novel missense mutations in FGB and FGG genes 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 G 9596A results in the amino acid exchange G414 Gly>Ser in the beta beta chain. Mild bleeding is observed in these hypofibrinogenemic patients. In the second family Sch. a novel molecular defect was found: FGG G 5801A. This missense mutation results in the amino acid exchange 233 Gly>Glu in the gamma chain. In both cases normal fibrin polymerization of purified fibrinogen samples and normal polypeptide patterns in 2-D-PA GE 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.

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**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) have identical gene defects in Fibrinogen Marburg, Gießen and Lixfeld. Patients 1 and 2 were found to be homozygous, whereas patient 3 was classified heterozygous. Fibrinogen activity in all patients, even in the heterozygote, was < 30% and not detectable; fibrinogen concentration was 60-70% in the homozygotes, whereas the heterozygote showed only 28% of the maximal amplification in TEG correlated with these 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

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**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 MCF2D.

**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%, ridocain co-factor from 187% to 335%. Closure time in the PFA 100® (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 intraoral 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 intraoral 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**

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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) 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 hemarthrosis left subarticular 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/dl) and DNA analysis verified afibrinogenemia. Amputation of fingertip could be achieved with use of DDAVP. The intranasal application of DDAVP enables the patient furthermore to treat minor bleedings independently at home.

**PP3.3-12**

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

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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. The prothrombin time and partial thromboplastin time were not measurable. Fibrinogen concentration was below 3mg/dl (Claus 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 hemorrhages. Patient 2 was admitted to us because of a subungual hematoma and necrosis of the fingertip several days after an injury. Fibrinogen level (<3mg/dl) and DNA analysis verified afibrinogenemia. A mupation of fingertip could be avoided by repeated infusion of purified human fibrinogen concentrate and surgical release of blood. Comparable with P1, M R I showed cystic lesions within both femoral heads and diaaphyses and 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, M R I including gradient-recalled echo sequences should be the preferred imaging modality for patients with afibrinogenemia.

**PP3.3-13**

A case of sporadic hypofibrinogenemia

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

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

Results: The patient presented at the hospital with diaphragma, enlarged liver, and elevated liver transaminase levels. Fibrinogen (Claus method) was reduced to 35 (n. 140–360 µg/ml), fibrinogen antigen was at the level of 58 µg/ml (n. 180–400 µg/ml). Thrombin time was 16.6s (n. 8–16 s) and reptilase time was 33.4s (n. 14–19 s). Both patients and a sister of the patient showed normal fibrinogen levels. FGG gene analysis revealed a novel heterozygous missense mutation in exon 8 (c.1808T>C, gamma Trp353Arg), 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 Ser159Gln, c.1125C>T Tyr345Tyr, c.1134G>A Arg448Gly) from her father. Parents and one sister of the patient were unaffected (353Trp/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 M ay 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.

**PP3.4**

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

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Patients with factor XI deficiency show different bleeding symptoms and he factor level is not clear associated with the risk of bleeding. The anticoagulation with heparin and aspirin was compared to patients without 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 X I 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.

**PP3.4-1**

**ITP**

**PP3.4-2**

**Complement-fixing autoantibodies in immune thrombocyto-purpura (ITP)**

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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 immunooassay (MAIPA). All sera were further evaluated in a complement fixation assay and a FACS-based bead assay for C1q immobilization.

Results: 10% of ITP samples showed positive results in the direct MAIPA. In a healthy control cohort the heterozygous c.1603C>T was detected in 1.7% of controls.

Conclusions: GP V is an important target antigen for platelet antibodies in ITP. Positive results in the direct MAIPA were associated with a bleeding risk or uncontrolled bleeding, and were defined as being refractory to different therapies. A systematic review of all published reports assessed the available evidence on the efficacy and safety of rFVIIa in patients with ITP.

Design and Methods: A year of 4,217 patients with suspected ITP were tested with the direct MAIPA for antibodies against GP V as well as GP Ib/IX and GP Ib-X. Patients with clinical signs for ITP or with nonimmunological explanations for thrombocytopenia were excluded.

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

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 the sensitivity significantly.

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

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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-GP Ib/IX injections induce apoptotic-like processes in platelets accompanied by the induction of T-PA. Poptotic-like processes in platelets are similar to those observed during apoptosis in nucleated cells: activation of caspase-3 (aCSA SP3), loss of mitochondrial membrane potential and externalisation of phosphatidylserine (PS). IntraCytoplasmic Immunoglobulin (IgV) ameliorates anti-GP Ib/IX induced ITP in mice. In a prospective study children with clinical and laboratory diagnosis of ITP were enrolled. A T antigen and after IV Ig therapy blood samples were obtained. The fraction of young reticulated platelets (RP) and apoptotic-like events specifically CA SP3 activation and PS externalisation were studied in platelets by flow cytometry. 10 patients had a platelet count below 10 000 ul. 2 had platelet counts over 10 000 ul. ITP patients had increased levels of platelets with aCSA SP3 and PS exposure and increased R.P. 10 Patients with platelet counts below 10 000 ul were treated with maximal doses IV Ig (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 IV Ig administration. Concomitantly aCSA SP3, PS exposure and R.P. decreased. We can demonstrate activation of apoptotic-like processes in paediatric acute ITP such as CA SP3 activation and PS exposure similar to the reported mouse model.

Other Acquired Bleeding Disorders

PP3.5-1

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

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Objectives: 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-mono
cyte 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 A DP and epinephrine activated platelets, and the PFA-100 A DP closure time (ADP CT) in 36 patients (62±19) with severe AS before and 6 months after aortic valve replacement. Platelet-monoocyte heterotypic aggregates were determined as an indicator of ongoing participation of platelets in inflammation.

Results: A large von Willebrand multimers increased and A DP CT 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: An aortic valve replacement not only induces normalization of platelet aggregation but also corrects the increased inflammatory platelet response.

PP3.3-2

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

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A 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. A II patients, who received FXIII concentrate (FibrogamminH5®, CSL Behring, Vienna, Austria) showed FXIII plasma levels below 60% and received a single shot application of about 20IU/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-Witney U test was used to compare the effect of FXIII concentrate alone or in combination with other blood products. A 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 46 hours after FXIII administration (p=0.002). Transfusion of FFP and platelet concentrates as well as the administration of fibrinogen and PCC were reduced statistically significant. FXIII concentrate (FibrogamminH5®) was effective to stop microvascular bleeding and to reduce transfusion requirements.

PP3.3-3

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

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Aquired 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. Prednison 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 CD 20 antigen, is a very effective drug in lymphoma treatment. Recently published data show that "classical dose" rituximab (375mg/m²) is also a promising treatment option in acquired antibodies to clotting factors, in particular spontaneous factor Vlll antibodies. We here report a 72-year old male patient presenting with retroperitoneal hematomata due to acquired antibodies to factor VIII (5.9 Bethesda Units). Initially, treatment was started with prednison (2mg/kg/d) and activated F VII and FEIBA in order to allow surgical intervention. On day 24, after first presentation low dose rituximab (50mg/m²) was started and repeated every week for a total of 4 courses. Concurrently, prednison was tapered completely. A 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.

PP3.3-4

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

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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 fibrinokinetic profile of CKD (n=50) and ESRD patients on hemodialysis was measured. Citrated plasma was supplemented with thrombin and CaCl₂. The rate of fibrin formation was measured by monitoring the optical density (OD) at 405nm. A flow reaching steady state, urokinase was added to measure the fibrinolytic profile. Forty normal male and female individuals were also analyzed.

Results: Fibrinokinetic 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 ± 0.12, range 0.05–0.41). In the ESRD patients maintenance hemodialysis, the pre-dialysis sample showed a weaker fibrinokinetic profile reaching near normal levels with a clot density of 1.3 ± 0.8 (range 0.8-1.6). In the urorikase 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 group.

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 hemodilysis results in improving the fibrinokinetic and fibrinolytic profile in the ESRD patients and may contributes to improved hemostasis in ESRD patients.

PP3.3-5

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

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Objectives: Aquired 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 minor 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 rFVIIa (NovoSeven), the patient died due to this dramatic bleeding complica-

Conclusions: 1. Aquired 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.

PP3.3-6

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

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

Design and Methods: A retrospective cohort study, and efficacy data are scarce.

Results: Provided by doses of 30-40mg (b.i.d.) on HD-free days and 30-40mg (q.d.) on HD days, after < 6 days respectively stable nadir A Xa levels of 0.32 IU/ml (median, range: 0.25–0.37 IU/ml) and A Xa increases (0.09–0.12 IU/ml) after HD to
PP3.3.7

A patient with epistaxis and multiple myeloma

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Case description: A 47 year old male patient diagnosed for myeloma had a vasectomy at our urology department. Preoperative coagulation screening revealed paraproteinemia and a hemoglobin of 30%. Hematological workup and a PT-INR of 63% were abnormal. Severe postoperative 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 of normal plasma (1:1) resulted in shortening of the trombin 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.

PP3.3.8

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

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Objectives: 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:FA) (reflecting attachment of VWF to denuded vessels) and of ristocetin-cofactor activity (VWF:RCo) over VWF:FA (miring binding of platelets to VWF) indicate this impairment. Since increased aPTT of 60% also occurs in normality. Severe postoperative 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 of normal plasma (1:1) resulted in shortening of the trombin 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.

PP3.3.9

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

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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 clot formation 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 APTT, 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 HEPTEM®. 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.

PP3.3.10

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

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Objectives: 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-year-old woman presented with a long-lasting history of occasionally recurrent waist haematomas, family history of thrombosis, hypofibrinogenae- mia, elevation of D-dimers, factor-XII- and protein-C deficiency without any other obvious clinical symptoms or correlates.

Results: A non-biologic study results were: functional fibrinogen 107mg/dl (177–376mg/dl), fibrinogen antigen 83mg/dl (180–400mg/dl), factor XIII 22% (65–150%), protein C 46% (80–150%), protein C antigen 39 (65–140%), D-Dimers 6.85 mg/l (0.49–0.94 mg/l), TAT-complex 26.9 (0.1–3.0 ng/ml), F1+2 >12000/ml (0.3–34000/ml), PA-Plex 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 X III genes were found. We initiated further investigations towards the possibility of angiodysplasia and Kasabach-Merrit-syndrome. Finally, via MR-angiography, multiple arterio-venous shunts of the right kidney were found.

Conclusions: A citation of coagulation by angiodysplasia led to acquired deficiency of fibrinogen and factor X 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.

PP3.3.11

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

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Case report: A 47 year old man diagnosed for myeloma had a vasectomy at our urology department. Preoperative coagulation screening revealed paraproteinemia and a hemoglobin of 30%. Hematological workup and a PT-INR of 63% were abnormal. Severe postoperative 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 of normal plasma (1:1) resulted in shortening of the trombin 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.
PpBS values may not reduce the incidence of bleeding complications in patients 20.4% and 23.8%, respectively (p = 0.89). PpBS does not predict bleeding complication rate in cohort B with abnormal PpBS corrected with blood products and not corrected was 22.9% and 22.7%, respectively (p = 0.95). 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 B with normal and abnormal PpBS was 24.1% and 22.2%, respectively (p = 0.64). The mc rate in cohort A with normal and 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 and normal PpPBS 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

PP3.6-3 Perioperative Hemostasis

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

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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. A 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 three groups: 1. no correction for PpBS a) 0-3 months and b) 3 months to 18 years. B 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 22.9% and 22.7%, respectively (p = 0.95). The mc rate in cohort B with normal and 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.
Impedance aggregometry for the prediction of the risk of blood products transfusion in cardiac surgery

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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® platelet function analyzer using A.D.P. (A.D.P. test), collagen (C.L. test) and thrombin receptor activating peptide (T.R.A.P test) 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: A.D.P. test and T.R.A.P test 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.

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

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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%. A part 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: Thrombelastometry- and Multiplate-based coagulation management is effective in reducing transfusion rate in cardiovascular surgery. This effect is most pronounced for the reduction of FFP-related 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.

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

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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 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 3542 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 €, whereas the increase of costs for coagulation factor concentrates amounted 208,684 €. Overall, this resulted in cost-saving of 222,606 € 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.

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

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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 3542 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 €, whereas the increase of costs for coagulation factor concentrates amounted 208,684 €. Overall, this resulted in cost-saving of 222,606 € 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.
The effect of 6% Hydroxyethylstarch 130/0.4 and 4% gelatin on coagulation and blood transfusion requirements after cardiac surgery.


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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 gelatin 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.

Results: HES 130/0.4 showed a significantly smaller increase in plasma fibrinogen concentrations ([6% +/- 1.16mg/dl vs. 4% +/- 3.6mg/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 gelatin alone after cardiac surgery.

PP3.6-9 Platelet function before and after DDAVP therapy measured bedside by Multiple Electrode Aggregometry (MEA) correlates with blood loss in patients after cardiac surgery

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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<45sec, fibrinogen>150mg/dl, plateletst>80kln, and hematocrit>25%; suggesting no requirement for hematostatic therapy. Exclusion criteria: suspected surgical bleeding. MEA was performed at the bedside before and 2–4hours after the infusion of DDAVP (0.6–0.8ng/kg), arachidonic acid (A SPItest.0.5mm) and adenosine-diphosphate (A D Pentest.6-4pm) for platelet activation. Blood loss/ml/h) after DDAVP was recorded for at least two hours. Wilcoxon signed rank test was used to test 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: E Leven patients received 0.32±0.05mg/kg DDAVP after 3.6±1 hours 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 M EA increased significantly after DDAVP administration: 85(66/115)U vs. 64(26/88)U in TRAPtest(p=0.007), 49.30/12U vs. 15/82U in A SPItest(p<0.001), and 35/24/54U vs. 14/7/28U in A D Ptest(test=0.002). Significant correlation was observed between blood loss and M EA : r=-0.792,p<0.001 for A SPItest and r=-0.577,p=0.005 for A DPtest, respectively.

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

PP3.6-10 Disturbance of fibrin polymerization in patients receiving Methylene blue / light virudually treated plasma - a randomized, double-blind clinical study

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Quarantine plasma (Q-FFP) still carries a risk of infections. Thus, solvent detergent or methylene blue/light virus-inactivated plasma (MB-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 Claus 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 2h 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±1.5s 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, FXIII, prothrombin fragments and D-dimers did not differ significantly between groups (p>0.05). In conclusion, these results agree with prior in-vitro data. Ret- tile 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 XII, fibrinogen and platelet count as predictors of clot firmness during surgical bleeding - implications for the therapeutic approach

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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: R x line analysis of samples submitted from the operating room or the surgical intensive care unit for bleeding episodes included FXIII, fibrinogen, platelet count and (R eten) E x trem and Fibtem 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 <0.6% was seen in 47%, fibrinogen <1g/l in 11% and thrombocyto- toopia <100 G/l in 24% of the samples. FXIII deficiency was significantly more frequent than hypofibrinogenemia (p<0.0001) and thrombocytopenia (p<0.0029). 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 est importance as it occurs most frequently. This suggests that FXIII replacement should be used first in the bleeding surgical patient with decreased clot firmness.
Rotational thrombelastometry (ROTEM) showed reduced maximum clot firmness (MCF-EXTEM 47 mm, MCF-INTEM 50 mm). Fibrinpolymerization was impaired (MCF-FIBTEM 4 mm) and clotting times were prolonged (CT-EXTEM 101 s, CT-INTEM 169 s). Prior to anesthesia and surgery FXIII (1,250 units) and fibrinogen (4 g) were administered normalizing ROTEM coagulation variables (MCF-EXTEM 54 mm, MCF-INTEM 54 mm, CT-EXTEM 79 s, CT-INTEM 133 s). Spinal anesthesia and CS were performed without increased blood loss or complications due to either anesthesia or surgery.

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

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

Conclusion: Hypofibrinogenaemia below 1 g/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 who underwent a regional anesthetic procedure for cesarean section (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.

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

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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 haemostatic 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 polyesterene 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 investigations of haemostatic processes. 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.

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