Blood clotting supports glioma invasion and colonization

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Objective: High-grade gliomas are highly invasive brain tumors that are extremely difficult to treat. High concentrations of pro-angiogenic factors cause glioma cells to induce vascular leakage, which in turn leads to the generation of a fibrin-rich edema. We hypothesize that the blood clot surrounding the glioma cells provides a provisional extracellular matrix that promotes tumor cell infiltration and growth. The objective of this study is to probe high-grade glioma cells for their capacity to infiltrate clotted plasma in comparison to benign meningioma and assess the underlying mechanism.

Methods: Tumor tissue from patients with high-grade glioma (WHO grade III-IV) or benign meningioma (grade I-II) was cultured in DMEM + 10% FBS to allow tumor cells to outgrow. Primary tumor cells were then embedded in clotted plasma, fibrin or Matrigel™ and scored for invadopodia formation as well as proliferation using phase contrast microscopy. Fibronectin mRNA expression was analyzed from tumor cell and tissue extracts using quantitative PCR. In addition, we assessed fibronectin and Slug/Snail2 protein expression by western blot.

Results: Primary tumor cells isolated from patients with high-grade glioma invaded and colonized extensively in clotted plasma and fibrin while lagging behind in Matrigel™. This clot-invasive tumor cell phenotype was specific for malignant brain tumors as tumor cells derived from benign meningioma were considerably less invasive in clotted plasma. Clot invasion correlated positively with fibronectin mRNA expression, which was strong in high-grade glioma, comparably weak in low-grade glioma (WHO grade II) as well as meningioma, and barely detectable in normal brain. High-grade glioma invasion in fibrin was accompanied by the generation of an elaborate fibronectin meshwork that serves to stabilize the adhesive interactions in the 3D environment. This in turn is relevant for the expression of the EMT transcription factor and tumor stem cell marker Slug/Snail 2, which we found to be upregulated in high grade glioma.

Conclusion: Our data show that clotted plasma, which is present in the fibrin-rich edema of the tumor extracellular matrix, strongly and specifically promotes invasion as well as colonization of high-grade glioma. Further research about the role of fibronectin and Slug/Snail2 in glioma invasion and colonization is warranted.
Improved values regarding acquired von Willebrand syndrome in patients with HeartMate III compared to HeartMate II

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Objective: Impaired binding of Von Willebrand factor (VWF) to platelets and to collagen due to an acquired Von Willebrand syndrome (AVWS) has been observed in patients with left ventricular assist device (LVAD) support. In addition, platelet count and function are also affected in patients with LVAD. Due to AVWS, decreased platelet count and impaired platelet function, the bleeding symptoms seem to be enhanced in LVAD patients. The new HeartMate III (HM III) is a left ventricular assist device featuring several design improvements that show promise to ameliorate the severity of AVWS in comparison to its predecessor, the Heartmate II (HM II). In this study, our aim was to analyze AVWS parameters and platelet function in patients with HM III compared to patients with HM II support.

Methods: Data sets of 14 patients under HM III support and 23 patients under HM II support were analyzed pre-surgery as well as 1, 3, 7, and 30 days post-surgery. Collagen binding capacity (VWF:CB), VWF antigen (VWF:Ag) as well as VWF:CB/VWF:Ag-ratios were determined. Presence of high molecular weight multimers of VWF was investigated. Platelet counts were monitored and platelet function was tested using light transmission aggregometry. The number of bleeding events and amount of fresh frozen plasma transfusions after implantation was recorded.

Results: The VWF:CB/VWF:Ag ratios were significantly higher in patients with HM III than in patients with HM II at day 1, 3, and 7 after implantation (p < 0.001, p < 0.05 and p < 0.05). More HM III patients had intact VWF high molecular weight multimers compared to HM II patients at day 1 and 3 after LVAD implantation (p < 0.05). Platelet counts and functions were comparable in both study groups. The HM III cohort exhibited a tendency towards less bleeding events compared to the HM II group (2/14 versus 8/23). In addition, the need for fresh frozen plasma transfusions was significantly lower in HM III patients compared to HM II patients (p < 0.05).

Conclusion: Severity of AVWS was milder in HM III patients compared to HM II patients, especially during the days after surgery when usually most hemorrhagic events occur. The lower severity of AVWS coincided with a trend towards less bleeding symptoms,
Reconstituted whole blood using fresh frozen plasma versus coagulation factor concentrates: an in vitro study

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Objective: Current guidelines suggest the use of a combination of red blood cells (RBC) and either fresh frozen plasma (FFP) or coagulation factor concentrates in the coagulation management of patients with massive hemorrhage. Despite this strong recommendation, data are limited in respect of the actual hemostatic potential of these two different approaches. The aim of this study is to compare qualitative and quantitative parameters of reconstituted whole blood (RWB) aliquots obtained by mixing RBC and platelet concentrate (PC) with either FFP or coagulation factors concentrates i.e. fibrinogen concentrate (FC) and/or prothrombin complex concentrate (PCC).

Methods: Ten healthy volunteers participated in the study to donate RBC, FFP and PC units (manufactured by the blood bank of Red Cross, Linz). FC and PCC were purchased from CSL Behring. Aliquots of RBC (mean unit volume 240mL) and PC (50mL) were mixed with aliquots of FFP (200mL) or FC (1g/50mL) or FC+PCC (1g+250IU/60mL) in a 2:1:1, 1:1:1 and 1:1:2 reconstitution ratio. Blood cell count, endogenous thrombin potential (ETP), single coagulation factors activity, fibrinogen level (Clauss method) and ROTEM® Extem analysis were performed on each reconstitution ratio of the three groups (FFP, FC and FC+PCC).

Results: Hematocrit and fibrinogen were significantly higher in FC and FC+PCC groups at all reconstitution ratios (Figure). ETP values were lowest in FC and highest in FC+PCC, the latter of whom also showed significantly higher activity of coagulation factors II and X (both present in the PCC); however, Extem clotting time was not different among the groups except for a significant prolongation in FC+PCC 1:1:2 versus the corresponding ratio of FFP group.

Conclusion: The use of coagulation factor concentrates to reconstitute whole blood allows keeping higher hematocrit in the final mixture as compared to FFP. Moreover, the mixtures containing FC or FC+PCC result in higher fibrinogen level than FFP based reconstituted whole blood. Noteworthy, thrombin generation in the FC+PCC group was significantly increased in any reconstitution ratios.

Hemostatic potential of reconstituted whole blood using fresh frozen plasma versus increasing concentration of lyoplasma

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Poster Topic
Acquired problems and alterations of coagulation
17.02.2017, 17:15 - 18:15
Poster Number: 04

Objective: Whole blood reconstituted with Red Blood Cell to Platelet Concentrate to Fresh Frozen Plasma (RBC:PC:FFP) ratio of 1:1:1 has lower levels of hematocrit and fibrinogen compared to original fresh whole blood. The aim of this study is to compare reconstituted whole blood (RWB) using FFP versus lyophilized plasma (Lyoplasma) in multiple reconstitution ratios. Two additional groups, in which the dilution volume of Lyoplasma is reduced to half and one quarter respectively, are added to the analysis.

Methods: Ten healthy volunteers donated Blood for the study (RBC, FFP and PC; blood bank Red Cross Linz). Ten Lyoplasma vials were purchased. Aliquots of RBC (mean unit volume 240mL) and PC (50mL) were mixed with either FFP (200mL) or Lyoplasma in a 2:1:1, 1:1:1 and 1:1:2 reconstitution ratio. The Lyoplasma vials were first diluted with 50mL of water (serving for the Lyo50 group) then increasing amounts of water were added for the Lyo100 and Lyo200 groups respectively (200mL is the reconstitution volume suggested by the manufacturer). Blood cell count, endogenous thrombin potential and fibrinogen (Clauss) analysis were performed on each combination of reconstitution ratio and plasma component (total of 12 groups).

Results: Hematocrit, platelet count and fibrinogen level were not different between FFP and Lyo200 in any ratio tested. Decreasing the dilution volume of the Lyoplasma (Lyo50) led to a progressive increase in hematocrit, platelet count and fibrinogen level compared to the Lyo200 and FFP groups. Conversely, thrombin potential did not vary among groups in any ratio tested (Figure).

Conclusion: RWB using Lyoplasma had the same cellular component and hemostatic potential than RWB obtained with corresponding volume of FFP. Increasing the concentration of Lyoplasma offers the advantage to reach higher hematocrit and fibrinogen levels without a relevant increase in thrombin generation, presumably because of a preserved balance between pro and anti-coagulant factor in the concentrated plasma preparations.


Objective: Objective: Acquired hemophilia A (AHA) is a rare bleeding disorder, caused by the development of autoantibodies against human coagulation factor VIII. AHA may lead to spontaneous or trauma induced bleeds that are treated with bypassing agents. The incidence of the disease was estimated to about 1.4 per million per year in the UK. Data from Germany are not available. A specific ICD 10 code for AHA has existed since 2010, allowing epidemiological insights. We examined the frequency of AHA based on these data and compared it to data from the recent GTH-AH 01/2010 study.

Methods: Methods: The reports from German DRG Institute (InEK), Statistical Office (DESTATIS) and the hospital quality reports for 2010-2014 were analyzed for cases of AHA and treatments with high amounts of bypassing agents (APCC > 150.000 units, rFVIIa > 500 mg). Statistical analysis was performed using Microsoft-Excel and Access version 2013.

Results: Results: The number of cases with a main diagnosis of AHA (D68.31, ICD10-GM) increased from 29 (2010), over 40 (2011), 37 (2012), 69 (2013) to 109 (2014, + 275%). The mean age of patients (73.4 +/- 15.9 years) and the gender distribution (58% male) remained stable over time and were very similar to data from the GTH-AH study. The average length of hospital stay of male patients was with 25.2 days significantly longer than for females (18.4 days), possibly reflecting the trend towards a less favorable prognosis of AHA in male patients as seen in the GTH study. The number of cases with a secondary diagnosis of AHA increased moderately from 186 (2010) over 200 (2011), 128 (2012), 134 (2013) to 225 (2014, +21%). The total number of cases in 2014 was 334 (~4 per mio. per year), indicating a higher incidence than suggested from UK data. The increase in cases was not associated with a growth in treatments with high doses of rFVIIa (108 in 2010, 97 in 2014) or APCC (66 in 2010, 56 in 2014).

Conclusion: Conclusion: We found an increase in documented hospital cases with AHA from 2010 to 2014. The overall number exceeds the expected number of patients based on previously reported incidence. This may reflect a growing awareness towards AHA, under-diagnosis in previous studies, or both. Remarkably, the number of patients intensively treated with bypassing agents decreased, suggesting that higher awareness may lead to earlier diagnosis and prevention of high costs due to bleeding.
Procoagulant microparticles and nanoparticles from whole blood reconstituted with age-different blood components.

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Poster Topic
Acquired problems and alterations of coagulation
17.02.2017, 17:15 - 18:15
Poster Number: 06

Objective: Haemostatic potential of blood products depend on storage duration. Microparticles (MP) and exosomes (NP) are released after blood manufacturing process and their concentration increases over time. Furthermore, the procoagulant properties of MP and NP have been previously shown. The aim of this study was to compare the hemostatic capacity of leuko-depleted-whole blood (LD-RWB) versus reconstituted blood using either solvent-detergent treated plasma (SD-RWB) or prethawed plasma (PT-RWB) versus fresh whole blood (WB), with the hypothesis that despite loss of function of platelets and coagulation factors over days of storage, the increase in MP and NP procoagulant action would counteract the former resulting in maintained or increased haemostatic capacity.

Methods: WB was donated from 10 donors at Linz Blood Bank of the red Cross. We produced LD-RWB by adding Platelet Concentrate (PC) to LD blood; the other groups were obtained by mixing RBC, PC and either SD treated plasma or PT plasma. All components were mixed at different shelf age (days 0-1-3-5). These aliquots were tested with ROTEM EXTEM and INTEM assays. We then obtained MP and NP from the RWB aliquots by centrifugation and flow cytometry. Finally we spiked WB of healthy donors (n=10) with MP and NP fraction and tested it with ROTEM NATEM assay.

Results: EXTEM MCF and INTEM MCF were significantly increased over time in the SD-RWB and PT-RWB groups (Figure 1). This was paralleled by an increase in MP and NP with increasing age of blood components (Fig 2). WB of healthy donors spiked with MP and NP consistently showed a reduction in the NATEM clotting time (Figure 3).

Conclusion: Increasing the age of blood component from 0 to 5 days led to an increase in the clot firmness as evaluated by EXTEM and INTEM assay. A contemporary rise in MP and NP was noted in the RWB samples. We finally proved that MP and NP exert procoagulant effects on WB freshly collected from donors. We could then speculate that the increase in clot firmness noted in the RWB aliquots would originate from these small, hemostatically active particles.
Longitudinal analyses of microparticle-associated tissue factor activity and venous thromboembolism (VTE) in cancer patients

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Poster Topic
Acquired problems and alterations of coagulation
17.02.2017, 17:15 - 18:15
Poster Number: 07

Objective: Tissue factor (TF)-bearing microparticles (MP) are discussed to have an impact on VTE occurrence, specifically in pancreatic cancer patients. Therefore, we evaluated changes in this parameter longitudinally to see whether longitudinal determination of TF-MP could lead to an improvement of VTE prediction in cancer patients.

Methods: Of 112 cancer patients (with pancreatic, colorectal, lung cancer or glioblastoma), who were included before receiving chemo- and/or radiotherapy and then followed during the course of disease over a period of six months, 11 developed VTE.

We investigated these 11 patients (five with pancreatic cancer and three each with glioblastoma and lung cancer), as well as 27 patients (ten with pancreatic, five with colorectal and six each with lung cancer and glioblastoma), who did not develop VTE and who served as control group. Of these 38 patients (19 female, 19 male) with a mean age of 60.7 years (± 8.9), venous blood samples for determination of TF-MP activity were drawn on a monthly basis over six months.

The measurement of TF-MP activity was performed according to standardized protocols for a chromogenic kinetic assay.

Study end points were VTE occurrence, death or completion of the study period (max. 250 days).

Results: TF-MP levels did not differ between patients with VTE (n=11) and those without VTE (n=27). The median baseline value was 0.08 pg/ml for both, patients with VTE [interquartile range (IQR) 0.03-0.29], and those without VTE (IQR 0.00-0.46), within the study period. After two months of follow-up, a median value of 0.08 pg/ml (IQR 0.07-0.13) in VTE patients and a median value of 0.07 pg/ml (IQR 0.0-0.15) in those without VTE were found. Three months after inclusion, the median value for patients with VTE was 0.22 pg/ml (IQR 0.14-0.90) and 0.04 pg/ml (IQR 0.00-0.42) for those without VTE. At the last sampling, a median value of 0.13 pg/ml (IQR 0.13-0.13) for the one patient, who developed VTE thereafter, and a median value of 0.06 pg/ml (IQR 0.00-0.38) for patients without VTE, was observed.

No statistically significant prognostic influence of the TF-MP levels on VTE occurrence was observed, neither at baseline (p=0.824), nor at the six monthly sampling time points (p=0.346) accounting for longitudinal measurement.

Conclusion: We conclude that TF-MP activity does not allow prediction of VTE occurrence in cancer patients.
Thrombin formation capacity in patients with two different left-ventricular assist devices and after heart transplantation

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**Poster Topic**
Acquired problems and alterations of coagulation
17.02.2017, 17:15 - 18:15
Poster Number: 08

**Objective:** Implantation of a left ventricular assist device (LVAD) is associated with the risk for thrombotic and bleeding events that are caused by preactivation on artificial surfaces and changes in hemodynamics. Almost all LVAD patients are diagnosed with acquired von Willebrand syndrome. Additionally, substantial contact activation might lead to clotting factor consumption. The new HeartMate III (HMIII) is a left ventricular assist device featuring several design-improvements over its predecessor (HMII) that hopefully ameliorates bleeding symptoms.

We aimed to evaluate the risk of bleeding in patients under HMII and HMIII support compared to patients with heart transplants using Calibrated Automated Thrombography.

**Methods:** Thrombin generation traces were obtained from patients with HM II- (N=14), HM III-implants (N=12), and heart transplants (N=8). Additionally, INR, aPTT, antithrombin, prothrombin fragments 1+2, and D-dimer were recorded.

**Results:** The endogenous thrombin potential and peak thrombin generation was significantly higher in HM III patients compared to HM II patients (P<0.05).

Interestingly, the endogenous thrombin potential and peak thrombin generation did not differ between HM III patients and heart transplant patients, but HM II patients exhibited a significantly lower endogenous thrombin potential and peak thrombin generation than heart transplant patients (P<0.05). D-dimer was significantly higher in all LVAD patients (HM II and HM III) compared to heart transplant patients (P<0.001). INR, aPTT, antithrombin, and prothrombin fragments 1+2 were comparable.

**Conclusion:** Increased D-dimer in HMII as well as in HM III patients hint to a clotting factor consumption after LVAD implantation. However, a consequent impact on thrombin formation capacity was only observed in patients with HM II implants. Patients with HM III implants exhibited less impairment of thrombin formation capacity which may result in reduced bleeding tendency.
Chronic disseminated intravascular coagulation in a patient with antiphospholipid antibodies and acquired protein S deficiency


**Poster Topic**
Acquired problems and alterations of coagulation
17.02.2017, 17:15 - 18:15
Poster Number: 09

**Objective:** We describe the rare case of a 76-year-old woman presenting with a thrombohemorrhagic syndrome due to disseminated intravascular coagulation (DIC) in the context of elevated antiphospholipid antibodies and acquired protein S deficiency.

**Methods:** We measured coagulation inhibitors, activated protein C resistance (APC-R), factor V gene mutation Leiden, lupus anticoagulant (LA), and IgG/IgM antibodies to cardiolipin (aCL) and beta2-glycoprotein I (anti-β2-GPI) by standard laboratory tests. Mixing studies with normal human plasma (NHP) were used to screen for a functional protein S inhibitor. Tissue factor (TF)-specific procoagulant activity (PCA) of plasma microvesicles (MVs) was assessed by single-stage clotting assay.

**Results:** At presentation, a diffuse and painful reticular erythema indicated severely impaired microcirculation. Coagulation tests revealed DIC with consumptive coagulopathy: prothrombin time 45.5% (normal: 80-130%), fibrinogen 0.5 g/L (1.8-4.0 g/L), D-dimer 34 mg/L (< 0.5 mg/L). Platelet count was normal (166 x 10^9/L). Surgical and antibiotic treatment of sigmoid diverticulitis did not resolve DIC, and overt malignancy was excluded by endoscopy and FDG-PET/CT. The coagulopathy was controlled by application of enoxaparin (2 x 40 mg/d), or apixaban (2-3 x 5 mg/d), but reoccurred within 24 hours after cessation of anticoagulation. Titers of IgM-aCL were elevated, ranging from 17 U/mL (< 10 U/mL) at presentation to 90 U/mL during follow-up. Antinuclear antibodies were negative, as were tests for LA and anti-β2GPI. There was APC-R with a ratio of 1.7 (> 2.0) in the absence of factor V gene mutation Leiden. Free and total protein S antigen were normal, but protein S activity was severely reduced to 14% (55-125%) with no appropriate correction upon mixing with NHP. Significant MV-associated TF PCA was detected. No underlying rheumatologic or malignant disease evolved over the next two years. A short-term course of oral glucocorticoids was ineffective.

**Conclusion:** In this patient, generation of MV-associated TF PCA, possibly due to monocyte activation by antiphospholipid antibodies, in combination with acquired APC-R and severe depletion of protein S activity caused a systemic coagulopathy characterized by clotting factor consumption and microvascular thrombosis, which could only be controlled by uninterrupted systemic anticoagulation.
Successful inhibitor eradication with ofatumumab in a patient with acquired hemophilia A

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Poster Topic
Acquired problems and alterations of coagulation
17.02.2017, 17:15 - 18:15
Poster Number: 10

Objective: Acquired hemophilia (AH) is a rare autoimmune disorder characterized by the development of neutralizing autoantibodies against clotting factors, mainly factor VIII, leading to various bleeding patterns up to severe and life-threatening bleedings in patients with a negative personal and family history of hemorrhagic disorders. The management of AH focuses on the goals: control and prevention of bleeding (if present or significant), inhibitor eradication, and treatment of the underlying disease (if applicable). Bleeding control can be achieved with recombinant activated factor VII (rFVIIa or NovoSeven® RT), activated prothrombin complex concentrate (aPCC or FEIBA®), or recombinant porcine factor VIII (OBIZUR).

First-line therapy in inhibitor eradication is prednisolone or cyclophosphamide, second-line is rituximab.

Methods:

Results: Case report: 73-year-old man with Morbus Waldenström and acquired hemophilia (factor VIII < 1%, max. inhibitor titer 36 BU). First-line treatment with prednisolone and cyclophosphamide did not lead to inhibitor reduction and application of mycophenolic acid in exchange for cyclophosphamide was not successful either. Due to an anaphylactic reaction to rituximab in the past which was given for treatment of the M. Waldenström ofatumumab was given alternatively (4 cycles). This lead to a successful inhibitor eradication.

Conclusion: Ofatumumab and rituximab are specific human monoclonal IgG1 antibodies. Both bind to the CD20 antigen which is expressed on almost all B-cells and eliminates B-cells through several mechanisms, including complement-dependent cytotoxicity. The binding sites of ofatumumab and rituximab to the B-lymphocytes differ. Our case shows that ofatumumab can be applied as an alternative therapy for inhibitor eradication in acquired hemophilia.
Is a too low plasminogen level in the plasma of kidney transplant recipients the reason of irreversible rejection reactions?

H. E. Karges (Marburg, Germany)

Poster Topic
Acquired problems and alterations of coagulation
17.02.2017, 17:15 - 18:15
Poster Number: 11

Objective: Introduction: A functioning fibrinolytic system is the prerequisite for the patency of blood vessels. For this, the balance between the central protein of the fibrinolytic system, Plasminogen(Plg) and the main Plasmin inhibitor Alpha2-Antiplasmin(APL) has to be warranted. Under certain circumstances (e.g. severe septicaemia) the APL level of the patients plasma exceeds the Plg level markedly leading to persistent clots in the capillaries and thus to organ failure (e.g. the kidney). In a prior study we could show that by substitution of Glu-Plasminogen to balance the APL-Level, the organ failure could be reversed and the patients survived.

Aim: A similar situation as in septicaemia can also be expected in severe rejection reactions after organ transplantation, which resemble inflammation reactions. We therefore now investigated the levels of APL, Plg and other plasma parameters at rejection reactions after kidney transplantations.

Methods: Methods: The plasma parameters were determined by immunological methods. We report the data of 20 kidney transplantations. As control group served 41 patients with chronic renal diseases.

Results: Results: The patients were assigned to three groups. Group A: patients with only moderate rejection reactions and successful graft survival (7 cases). Group B: patients with severe rejection reactions leading to graft removal (13 cases). Group C: control group (41 cases).

Table: Concentrations and Ratios of APL and Plg

<table>
<thead>
<tr>
<th>Group</th>
<th>Plg (% of norm)</th>
<th>APL (% of norm)</th>
<th>APL/Plg</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>93,7</td>
<td>109,3</td>
<td>1,17</td>
</tr>
<tr>
<td>B</td>
<td>76,5</td>
<td>115,2</td>
<td>1,51</td>
</tr>
<tr>
<td>C</td>
<td>88,2</td>
<td>92,3</td>
<td>1,05</td>
</tr>
</tbody>
</table>

Conclusion: Conclusion: The results show that in patients with severe rejection reactions (group B) the level of APL exceeds markedly the level of Plg; thus, the reactive fibrinolysis is impaired and thrombus formation in the blood vessels of the grafts occurs and persists, leading finally to the loss of the graft.

When clinically applicable Glu-plasminogen concentrate would become again available, it should be tested, if its substitution to balance the level of APL could save the grafts.
Venous thromboembolism and vascular access thrombosis in patients with end-stage renal disease on chronic hemodialysis: results of the VIVALDI study

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**Poster Topic**
Acquired problems and alterations of coagulation
17.02.2017, 17:15 - 18:15
Poster Number: 12

**Objective:** Venous thromboembolism (VTE) and vascular access thrombosis are common complications in hemodialysis (HD) patients. We aimed at investigating the burden of thromboembolic events and antithrombotic practice patterns in a cross-sectional analysis of HD patients.

**Methods:** The Vienna InVestigation of AtriaL fibrillation and thromboembolism in HeModialysiS patients (VIVALDI) is a population-based cohort study to investigate the risk of stroke, venous thromboembolism, bleeding and mortality in HD patients from 7 major dialysis centers, recruited in 2014-2015. At baseline, detailed patient histories were taken and verified with medical records at the HD centers. A history VTE including deep vein thrombosis [DVT] and pulmonary embolism [PE], was differentiated from vascular access thrombosis, including shunt thrombosis and catheter-associated thrombosis.

**Results:** We recruited 626 patients on maintenance HD (median age 66 years, 37 % women). A history of VTE was found in 61 (9.7%) patients (32 PE [5.1%], 44 DVT [7.0%]) and a history of access thrombosis in 178 patients (28.4%), 146 patients with shunt thrombosis and 38 patients with catheter-associated thrombosis. In multivariable age-adjusted logistic regression, VTE was independently associated with atrial fibrillation (AF) (odds ratio [OR] 3.0, 95% confidence interval [CI] 1.2–7.5) and time on hemodialysis treatment (OR 1.1 per 1 year increase, CI 1.0–1.2). A history of access thrombosis was only associated with time on hemodialysis (OR 1.2, CI 1.1–1.2) in a multivariable logistic regression model, but not with a history of VTE.

Fifty-five patients (30.9%) with access thrombosis were receiving long-term anticoagulation at the time of recruitment. In 27 cases low-molecular-weight heparins s.c. on non-HD days and in 28 cases oral anticoagulation with vitamin-K-antagonists was the treatment of choice. Patients with a history of access thrombosis were more likely to receive long-term anticoagulation treatment if they had AF (OR 3.2, CI 1.5-6.9), a history of stroke (OR 2.4, CI 1.1–5.5), or a history of VTE (OR 5.8, CI 2.1-15.9).

**Conclusion:** Histories of VTE and access thrombosis were frequent in hemodialysis patients. Patients were more likely to receive long-term anticoagulation when further risk factors for thromboembolic events, such as a history of VTE, stroke or AF were present, indicating the need for hemodialysis-specific risk evaluation.
Dual mechanical assistance with veno-arterial extracorporeal membrane oxygenation and percutaneous continuous-flow device: double trouble for von Willebrand factor


**Objective:** The addition of the Impella-2.5 (Abiomed®) device is an emerging therapeutic option to control an acute pulmonary edema (APE) occurring under peripheral veno-arterial extracorporeal membrane oxygenation (VA-ECMO) in patients with refractory cardiogenic shock (CS). Both VA-ECMO and Impella-2.5 are high shear continuous-flow pumps known to promote von Willebrand factor (VWF) high molecular weight (HMW) multimers proteolytic degradation. We report here a severe acquired von Willebrand syndrome (AVWS), requiring VWF concentrates infusion, after the onset of dual continuous-flow mechanical circulatory support (CF-MCS).

**Methods:**

**Results:** A peripheral VA-ECMO was implanted in a 62-year-old male with refractory CS. VA-ECMO was associated with the induction of a partial HMW-multimers defect. Impella-2.5 percutaneous implantation was decided after 2 days because of an APE under VA-ECMO support. While rapidly reducing the APE, the addition of Impella-2.5 device was associated with the onset of major bleedings. Recurrent epistaxis and bleedings from all vascular accesses were observed under dual VA-ECMO/Impella-2.5 support. This recurrent bleeding pattern under dual CF-MCS was simultaneously associated with a complete HMW-multimers deficiency. To the opposite, platelet count remained above 80 G.L⁻¹ during the 12 days of dual CF-MCS. In this context, off-label administration of a plasma-derived VWF concentrate almost devoid of FVIII (Wilfactin®) was decided. Bleeding was controlled by high doses of VWF concentrates infused every 24 hours for 3 days. Once VWF administration was stopped, we observed the recurrence of bleedings and transfusion dependency. The patient was further implanted with the LVAD HeartMate-II (HM-II) as a bridge to heart transplantation. The surgery was complicated by massive bleedings controlled by VWF concentrates infusions. After HM-II implantation, there was a partial recovery of HMW-multimers defect (HMW-multimers ratio=0.82). Despite requiring curative anticoagulation, no overt bleedings occurred under HM-II support. The patient was discharged home one month after HM-II implantation and underwent successful heart transplantation 3 months later.

**Conclusion:** We report a severe AVWS-related bleeding pattern under dual CF-MCS. The use of VWF concentrates as “rescue therapy” might be efficient to control severe bleedings under dual CF-MCS.
Direct oral thrombin inhibitor dabigatran etexilate is able to control chronic disseminated intravascular coagulation: a case report

W. Korte, L. Graf (St. Gallen, Switzerland)

Poster Topic
Acquired problems and alterations of coagulation
17.02.2017, 17:15 - 18:15
Poster Number: 14

Objective: The Kasabach Merritt syndrome (KMS) consists of consumptive coagulopathy due to vascular tumors (often giant hemangioma). Consumptive coagulopathy often progresses to disseminated intravascular coagulation (DIC) rendering the patient at risk for both bleeding and thrombotic events.

Methods: We report on a 63-year-old female patient with a known KMS. She had a history of chronic DIC with severe hyperfibrinolysis that was successfully controlled with low molecular weight heparins (LMWH; dalteparin 7500U s.c. qd) and tranexamic acid (500mg bid) for years. Concomitantly, the patient suffered from chronic hepatitis C infection leading to liver cirrhosis (CHILD C) and localized liver cell carcinoma. In early 2016, she was referred to our center with central artery occlusion of the left eye. Tranexamic acid was stopped immediately and anticoagulant therapy was switched to enoxaparin 100mg s.c. qd. However, coagulation parameters deteriorated massively within a few weeks (platelets 36G/l, fibrinogen <0.2g/l, antithrombin 33%, factor XIII 16%, d-dimer 226.5mg/l). In order to control massive bruising and epistaxis, the patient was repeatedly substituted with fibrinogen, concentrate, antithrombin concentrate, and factor XIII concentrate as well as with fresh frozen plasma.

Results: With the idea to control excessive thrombin generation we started a trial with the intravenous direct thrombin inhibitor (DTI) argatroban (subtherapeutic levels). One day after starting this therapy, coagulation parameters improved remarkably with platelets >50G/l, fibrinogen >0.5g/l, factor XIII >30%, antithrombin >30%, d-dimer 50mg/l without the need of further substitution with factor concentrates. In order to discharge the patient in an outpatient setting we decided to continue thrombin inhibition with the oral DTI dabigatran etexilate. Weighing risk for bleeding and for thromboembolic events, a reduced dose of 75mg bid was used. Thereafter, coagulation parameters remained stable and neither new bleeding events nor thromboembolic events occurred.

Conclusion: Dabigatran etexilate in reduced dose might be an attractive compound to control chronic DIC given its higher potential to inhibit thrombin generation than heparins.
Management of epidural bleeding in a patient with acquired hemophilia A masked by phenprocoumon therapy

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**Poster Topic**
Acquired problems and alterations of coagulation
17.02.2017, 17:15 - 18:15
Poster Number: 15

**Objective:** Acquired hemophilia A (AHA) is a rare but potentially life-threatening bleeding disorder, caused by development of autoantibodies (inhibitors) directed against plasma coagulation factor VIII (FVIII). Early diagnosis is important to allow immediate hemostatic treatment and to prevent dangerous bleeding complications. Diagnosis of AHA is confirmed by demonstrating reduced factor VIII activity (FVIII:c) and neutralizing inhibitors. Therapy is aimed at controlling of bleeding episodes and eradication of factor VIII inhibitors.

**Methods:** Case report: A 60-year-old patient was transported to emergency department with a femur fracture subsequent to a traffic accident. He has an atrial fibrillation and therefor takes vitamin K antagonist (phenprocoumon®). He was scheduled for emergency surgical procedure.

**Results:** The patient received 3000 IU prothrombin-complex concentrate (PCC). He bled during the surgery and therefor received further PCC, erythrocytes concentrate (EC) and fresh frozen plasma (FFP). Coagulation analysis showed after surgery a normal prothrombin time (PT) 91 % of normal and prolonged activated partial thromboplastin time (aPTT) 62 seconds (sec) (norm 25-35), clinically, he bled further through surgical wound drainage.

The patient fell out of bed at the evening and an epidural hemorrhage was diagnosed, he was transferred to our hospital to remove the hematoma. At presentation a blood sample was collected for coagulation diagnostics. aPTT was 91 sec, FVIII:c activity was 2.5 % of normal (norm 70-130), FVIII:c / aPTT cross-mixing test was positive and Inhibitor Bethesda was 28 BU/ml. All other parameters were within reference range. An acquired hemophilia A was diagnosed and a bypassing therapy with active recombinant factor VII (rFVIIa) 90 µg/kg body weight / 2 – 3 hours was started. No bleeding complications occurred during as well as after surgery. An immune suppressive therapy with prednisolone was started.

FVIII:c started to increase and one week later was 25 % of normal. rFVIIa was stopped two weeks later due to good clinical status of patient as well as improvement of the FVIII:c activity.

An anticoagulation with low dose of low molecular weight heparin (LMWH) was started as factor VIII:c was > 70 % of normal.

**Conclusion:** Acquired coagulation disorders should not be overlooked as a potential cause of unusual bleeding in patients taking anticoagulants.
How to assess in vivo anticoagulant effect of Rivaroxaban

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Poster Topic
Antithrombotic treatment
17.02.2017, 17:15 - 18:15
Poster Number: 17

Objective: Rivaroxaban (RVX), an orally active direct FXa inhibitor, does not require monitoring. Nevertheless, in some clinical circumstances it is helpful to estimate its in vivo anticoagulant effect, in order to assess individual bleeding risk, e.g. during pharmacological or surgical interventions. Aim of this study was to assess ex vivo and in vivo anticoagulant effect of RVX in patients undergoing bariatric surgery.

Methods: Blood was collected during 24 hours from patients receiving a single dose of 10 mg RVX before surgery. We determined the anticoagulant effect of various RVX concentrations, in vivo by monitoring thrombin-antithrombin complexes (TAT) and prothrombin fragments 1+2 (F1+2), and ex vivo by thrombin generation assay (CAT) in patients’ platelet poor plasma (PPP), using PPP-reagent normal (5pM tissue factor) and high (20 pM tissue factor).

Results: RVX Cmax was observed 1h after ingestion (120 ng/ml). RVX concentration was stable between 2h and 4h (80-90 ng/ml), decreased between 6h and 8h (from 60 to 50 ng/ml), declined to 30 ng/ml at 12h, and was close to baseline at 24h (15 ng/ml). In vivo: TAT and F1-2 values significantly decreased at Cmax. During RVX plateau (80-90 ng/ml), a further significant decrease for both activation markers was observed. At drug concentrations of 60-50 ng/ml the anticoagulant effect reached a steady state and when RVX concentration dropped below 50 ng/ml TAT and F1+2 significantly increased. Ex vivo: CAT parameters (lag time, time to peak, peak, velocity index) were all statistically significantly altered at every time point except for the peak at 24 hours (reagent PPP high).

Conclusion: The pattern of ex vivo inhibition of thrombin generation (TG) observed with PPP reagent normal or high is very similar. Both reagents show that the thrombin generation profile strongly differs between RVX concentrations of 80-120 ng/ml, 50-60 ng/ml, 30 ng/ml, and 15 ng/ml. For the first time, we demonstrate that in obese individuals the degree of in vivo anticoagulation is not the same as assessed ex vivo by CAT: at concentrations <60 ng/ml, RVX is still able to inhibit TG ex vivo but not in vivo. Our data suggest that a relative inhibition of <60% in velocity index with PPP normal and <30 % with PPP high are the best indicators of a RVX concentration which does not exert an in vivo anticoagulant effect in obese patients.
Single-center experience with thrombolysis in high- and intermediate-risk pulmonary embolism

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**Poster Topic**

Antithrombotic treatment
17.02.2017, 17:15 - 18:15
Poster Number: 18

**Objective:** Pulmonary embolism (PE) is a potentially life-threatening acute cardiovascular syndrome. While thrombolysis is the guideline-recommended standard of care treatment for high-risk patients (in shock), it may be considered in a subset of intermediate-risk patients (at risk for shock). The individual bleeding risk must be considered in the decision-making.

**Aims:** We report single center registry data with 30-day mortality to evaluate guideline-recommended treatment algorithms.

**Methods:** We followed 74 patients with pulmonary embolism either at high- or intermediate risk (all-comers, 2013-2016). Patients at intermediate risk were treated with alteplase (rtPA), either full- (100 mg) or low-dose (0.6mg/kg, 50mg max) at the treating physician's discretion when these patients were considered at especially high risk or had already signs of hemodynamic decompensation.

**Results:** Thirty-three (45%) of the 74 patients were at intermediate risk. The average age of the patients at intermediate risk was 65.3 years vs. 64.8 years in the patients at high risk. Fifteen of the patients at intermediate risk (45%) received thrombolysis. 7 were treated at full dose and 8 with low dose. All patients at high risk received full-dose thrombolysis. The survival in the intermediate risk group was 100%, compared to 57% (25 patients) in the high risk group. In the intermediate risk group, relevant bleedings occurred in 2 patients (6%, one case of pulmonary and one case of intraarticular bleeding) while in the high risk group in 19 patients (46%) had relevant bleedings. There was no intracerebral bleeding in patients at intermediate risk compared to 2 (5%) in patients at high risk.

**Conclusion:** The 30-day survival of patients at intermediate risk PE was 100%, after approximately half of these patients had received thrombolysis. The risk of relevant bleedings in patients at intermediate risk receiving thrombolysis was relatively low – possibly because half of them received low-dose alteplase. According to current guideline recommendations, the choice for thrombolysis in intermediate-risk PE patients needs to take into account each individual patient’s risk for bleeding and PE-related death and these data reinforce this approach. Patients with a low bleeding risk and at younger age appeared to benefit from thrombolysis and low-dose alteplase was safe.
Real life efficacy and safety of edoxaban for stroke prevention in atrial fibrillation – Results of the prospective NOAC registry (NCT01588119)

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Poster Topic
Antithrombotic treatment
17.02.2017, 17:15 - 18:15
Poster Number: 19

Objective: The effectiveness and safety of long-term anticoagulation with direct oral anticoagulants (DOACs) such as edoxaban needs to be evaluated in daily care patients.

Methods: In the prospective, non-interventional Dresden NOAC registry a network of more than 230 physicians enroll eligible patients. More than 3200 DOAC patients receive prospective follow up by phone visits by the registry office. All events are centrally adjudicated using standard scientific definitions.

Results: Between 1 January 2016 and 31 July 2016, 217 patients receiving edoxaban for atrial fibrillation were enrolled (54% male, mean age 73.2 years, mean CHA2DS2-VASc-Score 3.5). 20 patients (9.2%) were switched from VKA to edoxaban, mainly due to patient's inconvenience (40.0%) or instable INR (30.0%). 15 patients (6.9%) had a history of stroke or TIA. 11 patients (5.1%) had a history of malignant disease.

177 (81.6%) of all patients received edoxaban 60 mg OD, remaining 40 (18.4%) received 30 mg OD. Reason for dose recommendation was renal impairment (CrCL 15 – 50 mL/min) in 10 cases (25%), low body weight (≤60 kg) in 8 cases (20%) and both in 1 case (2.5%). Reasons for dose reduction were unknown for the remaining 21 cases (52.5%).

During follow-up, mean duration of edoxaban exposure was 130.5±75.3 days (median 98 days; 25th/75th percentile 85/182 days). So far no major cardiovascular events (such as stroke, TIA or systemic embolism) have occurred. One patient suffered a fatal intracranial bleeding on treatment. There were no further major bleeding events.

Up to this point, edoxaban treatment discontinuation occurred in a total of 14 patients. Most common reasons for discontinuation were non-bleeding side effects (7/217, 3.2%), patient’s inconvenience (5/217, 2.3%) and others (2/217, 0.9%).

Conclusion: In comparison to our other DOAC-cohorts, patients receiving edoxaban are younger and healthier than previously enrolled patients receiving dabigatran or rivaroxaban, possibly because older and higher-risk patients were already treated with a NOAC before edoxaban approval.
Prevention and treatment of venous thromboembolism in patients with solid brain neoplasms: results of a survey among Italian physicians

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Objective: The decision on the introduction of primary and secondary prophylaxis of venous thromboembolism in patients with solid brain neoplasms and brain metastases is often challenging due to the potential risk of an intracranial hemorrhage and to the limitation of existing literature in this setting. A survey on this topic was conducted among the members of the Italian Federation of Internal Medicine Hospital Executives' Associations (FADOI).

Methods: A standardized questionnaire composed of nine multiple-choice questions regarding primary venous thromboembolism prevention in non-surgical patients during high risk conditions and venous thromboembolism secondary prevention in patients with a solid brain neoplasm or cerebral metastases was sent via electronic mail in June 2015.

Results: 352 Italian physicians (14.5%) returned (participants median age 51 years; females 46.9%). The majority of respondents would prescribe primary thromboprophylaxis (usually with heparin) in non-surgical patients with solid brain neoplasms and brain metastases during high risk conditions. Full-dose anticoagulation with either low-molecular- weight heparin or fondaparinux was the preferred option for the acute venous thromboembolism (69.6%), while a reduced dose was chosen by 21.0% of physicians. The presence of a highly vascular brain neoplasm histotype would mandate the prescription of a reduced-dose antithrombotic regimen in a minority of respondents. Vena cava filter placement was considered an option for the treatment of acute venous thromboembolism in more than 6% and for primary thromboprophylaxis in 1.4% of respondents. Most physicians (58.6%) would call for at least one external consultancy done by another specialist before prescribing anticoagulation.

Conclusion: Our survey among a large group of Italian internists indicates that the thrombotic risk linked to the presence of solid brain cancer or brain metastases is perceived greater than the estimated bleeding risk, and anticoagulants are often prescribed for both venous thromboembolism primary prevention and treatment. Physicians’ approach is partially in contrast to the recent evidence of the literature reinforcing the need for educational programs and high quality studies in this setting.
CONKO-011: Rivaroxaban in the treatment of venous thrombembolism (VTE) in cancer patients – a randomized phase III study

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Poster Topic
Antithrombotic treatment
17.02.2017, 17:15 - 18:15
Poster Number: 21

Objective: Venous thrombembolism is a common and severe event in cancer patients, bleeding and recurrent VTE occur more frequently than in non-cancer patients and complicate treatment strategies. The actual standard is anticoagulation for 3 to 6 months with weight-adjusted low molecular weight heparine (LMWH). Direct oral anticoagulants as the Xa-inhibitor Rivaroxaban are considered to be an interesting alternative, but data for the direct comparison to LMWH from prospective studies are still missing.

Methods: The aim of the study is to investigate patient-reported treatment satisfaction (convenience) with Rivaroxaban in the treatment of acute VTE in cancer patients in comparison with LMWH measured by the anti-clot treatment scale (ACTS; Bamber Thromb Haemost 2013) as the primary endpoint of the study. Secondary endpoints are recurrence of VTE, clinically relevant bleeding and overall mortality after 3 and 6 months.

Results: CONKO-011 is a prospective, randomized, open-label, multicenter phase III study with two treatment arms. The trial is conducted in cooperation with the “Working group of medical oncology” (AIO) and the “Working group of Hemostaseology” of the German Society of Hematology and Oncology (DGHO). Main inclusion criteria are a newly diagnosed and objectively confirmed acute VTE, an active treatment-requiring malignancy, life expectancy of at least 6 months and a performance-status according to Karnofsky Performance Scale of ≥ 70%. Main exclusion criteria are therapeutic anticoagulation > 96 hours, relevant bleeding events, severe renal insufficiency with GFR < 30 ml/min, liver disease with coagulation impairment (including Child B and C cirrhosis) and treatment of underlying cancer with experimental therapies. In arm A patients are treated with Rivaroxaban orally 15 mg 2x daily for 21 days, followed by 20 mg 1x daily, in Arm B with LMWH in therapeutic dosage according to standards of the study centers. Anticoagulation treatment is done for at least 3 months or until symptomatic recurrence of VTE, major clinically relevant bleeding, severe side effects or withdrawal of informed consent. Begin of recruitment was March 2016 with an expected recruitment period of 24 months and a follow up of 6 months, end of study is planned for end of 2018. Currently, 37 patients are recruited by 19 study centers, 32 study centers have started active recruitment.

Conclusion:
Long-term clinical outcomes of patients with CYP2C9 and VKORC1 variants treated with vitamin K antagonists: A prospective, multicenter cohort study of elderly patients with venous thromboembolism.

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Poster Topic
Antithrombotic treatment
17.02.2017, 17:15 - 18:15
Poster Number: 22

Objective: The application of individualized medicine based on genetic factors to patients requiring anticoagulant treatment is hampered by a lack of knowledge on their long-term effects on relevant clinical outcomes. To fill this gap, we examined the association between polymorphisms of the vitamin K-epoxide reductase (VKORC1) as well as the cytochrome P450 enzyme gene (CYP2C9) and long-term clinical outcomes in a prospective, multicenter cohort study of elderly patients treated with vitamin K antagonists (VKA) for venous thromboembolism (VTE; SWITCO65+).

Methods: Consecutive patients aged 65 years or older with an acute, objectively confirmed VTE were identified between 2009 and 2013 in all five university hospitals and four high-volume non-university hospitals in Switzerland. The primary outcome was the time to any event, i.e. overall mortality, major- and non-major clinically relevant bleeding, or recurrent VTE.

Results: Overall, 774 patients were followed for a median duration of 30.1 months. The primary outcome occurred in 334 patients (43.2%) and 119 patients died (15.4%). Major bleeding happened in 100 patients (12.9%), clinically relevant non-major bleeding in 167 patients (21.6%), and recurrent venous thromboembolism in 100 patients (12.9%). After adjustment, the presence of CYP2C9 variants was significantly associated with any clinical event (hazard ratio [HR] 1.34; 95% CI 1.08, 1.66), death (HR 1.74; 95% CI 1.19, 2.52) and clinically relevant non-major bleeding (sub hazard ratio [SHR] 1.38; 95% CI 1.01, 1.55). It was not associated with major bleeding (sub hazard ratio [SHR] 1.03; 95% CI: 0.69, 1.55) and recurrent VTE (SHR 0.95; 95% CI 0.62, 1.44). The presence of VKORC1 variant was not associated with any clinical event. No relevant differences in the percentage of time spent within the therapeutic range were observed in patients with and without CYP2C9 variants.

Conclusion: In conclusion, our results demonstrate a significant association between CYP2C9 polymorphisms and deaths, probably because of effects independent from quality of anticoagulation and major bleeding. Future investigations shall confirm this observation in independent cohorts as well as patients with atrial fibrillation and address the causal relationship.
Clinical observations on potential interactions of phenprocoumon, colchicine and rivaroxaban in a patient with compound homozygosity for FV Leiden and familial Mediterranean fever (FMF, M680I).

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**Poster Topic**

Antithrombotic treatment

17.02.2017, 17:15 - 18:15

Poster Number: 23

**Objective:** The patient of armenian descent had been suffering from typical symptoms since childhood. Given his family history, he was clinically diagnosed as having Familial Mediterranean Fever (FMF). He was later diagnosed at our center with homozygosity for FMF M680I and amyloid deposits in colon biopsies, why he was put on regular colchicine therapy. In 2004, recurrent superficial thrombophlebitis occurred; the patient received a shortened course of phenprocoumon before being referred to our center for thrombophilia work-up, which also revealed homozygosity for FV Leiden (FV G1691A). Also, markedly increased FVIII levels were seen during autoinflammatory FMF episodes. After restitution of (indefinite) phenprocoumon therapy, the patient soon experienced deterioration of his FMF symptoms despite continuing his regular colchicine therapy. Stopping phenprocoumon therapy rendered his symptoms of FMF well controlled again. Thereafter, continuous LMWH therapy was suggested, which he decided against: LMWH was only given during additional risk situations such as immobilisation and travel.

In early 2016, the patient was started again on LMWH due to recurring thrombophlebitis. After switching to phenprocoumon therapy, deterioration of his FMF symptoms was again noted despite continuation of his colchicine therapy. Cystatin measurements were in the normal range and confirmed that his renal function had not deteriorated despite the initial finding of amyloid deposits in colon biopsies.

After discussion of potential advantages and disadvantages with the patient and his primary care physician, therapy with Rivaroxaban 20 mg qd was initiated instead of oral anticoagulation with phenprocoumon. Thereafter, the patient remained free of any changes in his FMF symptomatology while continuing his regular colchicine therapy, suggesting that earlier changes in FMF symptomatology were due an interaction between phenprocoumon and colchicine therapy. Trough levels were in the expected range on three different occasions after Rivaroxaban was started.

We conclude that Phenprocoumon might interact with colchicine therapy, while Rivaroxaban (and potentially other NOACs) might not.

**Methods:**

**Results:**

**Conclusion:**
Activation of neutrophils - but not endothelial cells - is regulated by serotonin during myocardial reperfusion injury

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**Poster Topic**
**Arterial thrombosis**
17.02.2017, 17:15 - 18:15
Poster Number: 24

**Objective:** During reperfusion after myocardial infarction neutrophils migrate actively into the affected myocardium and a subset of these neutrophils form platelet neutrophil complexes (PNCs). Peripheral serotonin is synthesized by tryptophan hydroxylase isoform 1 (Tph1) and regulates neutrophil recruitment during acute inflammation.

**Methods:** Myocardial infarction was induced in WT and Tph1-/- mice followed by 24 hours of reperfusion. Cardiac and blood neutrophils, endothelial cells were analyzed by histology and flow cytometry. Heart function and infarct size was determined by echocardiography and histology.

**Results:** Infarct size was reduced by 33% in Tph1-/- mice and fractional shortening was significantly improved (25% vs. 12% in WT). Flow cytometry and histological preparation revealed dampened neutrophil migration into the affected myocardium in Tph1-/- mice compared to WT, whereas macrophage and monocyte populations were similar. We found a 50% decrease in neutrophil integrin alpha M expression on neutrophils of Tph1-/- mice. Interestingly, aortic ICAM expression was not affected by the lack of serotonin. This was also confirmed by in vitro stimulation of neutrophils and endothelial cells with serotonin.

**Conclusion:** Peripheral serotonin regulates CD11b on neutrophils, which is responsible for attachment and subsequent migration. Its counterpart, ICAM-1, on endothelial cells is not affected. This results in a milder inflammatory response during myocardial reperfusion injury in mice lacking peripheral serotonin.
Protein C and factor X activities from patients with ischemic stroke and type II diabetes mellitus.

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**Poster Topic**
**Arterial thrombosis**
17.02.2017, 17:15 - 18:15
Poster Number: 25

**Objective:** Similar to blood coagulation factors II (protrombin) and X, the protein C synthesis in the liver depends on the presence of vitamin K. However, in contrast to the other coagulation factors, protein C, when activated by thrombin, is not a procoagulant but a potent anticoagulant. Abnormalities in the plasmatic coagulation system have been repeatedly described in patients with diabetes mellitus (DM) and have been linked to variety vascular diseases, including atherosclerosis and ischemic stroke (IS), which have been associated with hemostasis disorders too. So the aim of the present study were to determine the activities of protein C (PC), factor X and protrombin content in patient under ischemic stroke and stroke complicated by type II diabetes mellitus.

**Methods:** Coagulation studies were performed on 67 patients shortly after their admission to the hospital. These persons were selected by tomography and magnetic resonance imaging confirmed of IS. The 24 from them had type II diabetes mellitus, which was established by laboratory tests. The activities of protein C (PC) and factor X were analyzed using specific chromogenic substrates. Protrombin content was determined by Elisa. Statistical analysis of the data was performed by computerized analysis.

**Results:** The protein C activity was found to be significantly decreased in the both total groups of IS and IS with diabetic patients compared with the controls. The estimated reduction of PC activity was more marked in case of IS alone. Thus the plasma protrombin contents were increased on 18% from the control for the both investigated groups of patient. The plasma coagulation factor X activity was increased only under IS and accounting for 117% of the controls.

**Conclusion:** Because the levels of coagulation factor II and factor X activities were not reduced neither in ischemic stroke patients nor IS with diabetic patients, the reduction of protein C seems to be caused not by reduced its synthesis in the liver, but more likely by an increased clearance from the blood plasma. The decrease of protein C activity accompanied by increase of factor X activity with high protormbin content in the plasma of ischemic stroke and type II diabetic patients indicates an abnormal, probably hypercoagulable conditions in aforementioned disorders.
Inhibition of coagulation factor Xa attenuates myocardial ischemia reperfusion injury in mice


Objective: Myocardial infarction (MI) accounts for over 10% of all mortalities and is thereby one of the leading causes of death worldwide. Ischemic/reperfusion injury (IRI) has substantial effects on MI outcome. Several processes are involved in IRI, including the complex interaction between coagulation and inflammation. Although there is great progression in reperfusion treatment, long term morbidity in patients with coronary syndrome is still substantial. A limitation of the current treatment options for MI is the lack of prevention regarding IR events. Coagulation proteases such as thrombin or factor Xa (FXa) play central roles in the crosstalk between coagulation and inflammation. In vivo studies demonstrate that inhibition of coagulation proteases can attenuate IRI, mediated by protease activated receptors (PARs). Although in vivo thrombin inhibition (indirect) decreased IRI, FXa’s involvement in IRI is less evident. To elucidate FXa’s role in IRI after a MI, we studied the effect of FXa inhibition on myocardial IRI in an IR mouse model.

Methods: Male WT c57BL/6 mice (age 8-9 weeks) surgically received a ligature around the left anterior descending coronary artery. After seven days of recovery, myocardial ischemia was induced by tightening the ligature for 1h followed by loosening the ligature for 4h or 24h to induce reperfusion. The intervention consisted of one IV-injection of 100μl rivaroxaban (400 ng/ml) or placebo (0.9%NaCl) after 15min ischemia and 5min after reperfusion. Mice were then injected with Evans blue to visualize the area at risk (AAR). AAR is the area exposed to ischemia. Heart tissue was collected and stained with triphenyl tetrazolium chloride to differentiate between the AAR and area of infarction (AOI). AOI is the area with irreversible damage.

Results: Rivaroxaban treated mice showed significant reduced AOI/AAR compared to controls after 4 and 24h reperfusion. A reduction in AOI/AAR of 19.34% ±4.381 (mean+SEM) (n=8, p=0.0007) and 17.06% ±5.262 (mean±SEM) (n=8, p=0.0055) was observed after respectively 4 and 24 hours’ reperfusion.

Conclusion: Direct FXa inhibition by Rivaroxaban significantly reduces IRI in mice. This observation suggests that it might be beneficial to treat myocardial IRI with anti-coagulants. Although it has great clinical potential, future research is needed to elucidate the mechanisms of action.
Idiopathic catastrophic thrombosis with happy end - a case report

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Poster Topic
Arterial thrombosis
17.02.2017, 17:15 - 18:15
Poster Number: 27

Objective: A 59 year old male patient suffered three life threatening stent thromboses after an initial resuscitation due to a STEMI of the anterior cardiac wall. With a high risk profile for a Heparin Induced Thrombocytopenia Typ II (HIT II) he was placed on argatroban after the second reinfarction. Under this apparently appropriate treatment a third reinfarction happened and the patient had to undergo high risk cardiac bypass surgery. Later on a deep vein thrombosis and an intracardiac thrombus formed.

Methods: Case report

Results: Despite a positive screening test for HIT II and a single positive result in the Heparin incuded platelet aggregation (HIPA) test we are not convinced that HIT II was the only underlying cause for this “catastrophic thrombotic syndrome”. We assume that a massive generation of thrombin, reflected in consistently high D-dimers and the need of copious amounts of a direct thrombin inhibitor, triggered the set of events.

Conclusion: With this case report we want to raise awareness for cardiac complications in complex haemostasiological cases and share our experiences in the diagnostic and therapeutic management of these delicate patients.
The molecular etiology and pathophysiology of platelet-mediated aspirin responsive erythromelalgia and microvascular thrombosis in JAK2, CALR and MPL mutated thrombocythemia

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Objective: Introduction. This insight review article describes the sequential steps between 1975 and 2016 in the discovery of aspirin responsive platelet-mediated erythromelalgic inflammation followed by microvascular thrombosis in thrombocythemia patients caused by prostaglandin endoperoxides released from spontaneous activation, release reaction and aggregation of hypersensitive constitutively activated platelets due to gain of function mutations in the JAK2, TPO, MPL and CALR genes (Acquired and Congenital Sticky Platelet Syndrome).

Methods:

Results: Results. The initial stages of platelet-mediated aspirin erythromelalgia in clonal thrombocythemia are featured by livido reticularis of the skin, acroparesthesias of prickling 'needles' sensations and caused by shear stress induced activation of hypersensitive JAK2 mutated platelets in the endarterial microcirculation followed by platelet mediated arteriolar occlusive thrombosis if left untreated. The release of prostaglandin endoperoxides, serotonin, adenosine account for the inflammatory signs, burning pain and red congestion of typical erythromelalgia. In this ongoing process of platelet activation, platelet aggregation and platelet-endothelial interactions, the endothelial cells release thrombomodulin indicating endothelial cell activation and damage. Release of platelet derived growth factor (PDGF) by activated platelets account for the fibromuscular intimal proliferation. The analgetic effect of aspirin immediately and completely relieves the inflammatory, burning painful vasomotor manifestations for a few days by irreversible inhibition of platelet cyclo-oxygenase (COX-1) and subsequently will prevent the erythromelagic microvascular complications through maintained irreversible inhibition of platelet cyclooxygenase (COX-1) by low dose aspirin 50 to 75 mg daily. The analgesic agents sodium salicylate and paracetamol and anticoagulation with coumadin and heparin do not inhibit platelet cyclo-oxygenase and are ineffective to relieve the pain and inflammatory manifestations of erythromelalgia. The high shear rate of blood flow in arterioles as compared to that in arteriolar circulation contributes to the location of erythromelalgia and MIAs in the endarterial circulation in thrombocythemia caused by a gain of function mutation in the JAK2, CALR, MPL or TPO gene. Spontaneous activation and reversible aggregation of hypersensitive JAK2, TPO, CALR or MPL-mutated thrombocythemic platelets in the arteriolar circulation is a pathophysiological process which transforms platelet membrane phospholipid by phospholipase A2 into achidonic acid as the source for cyclooxygenases (COX-1) to produce prostaglandin endoperoxides and the release by platelet of serotonin, platelet factor 4, beta-thrombglobulin, platelet derived growth factor (PDGF), adenosine diphosphonate. The inflammatory mediators, such as prostaglandin, adenosine and serotonin directly affect the afferent (pain-signalling) nociceptive C-fiber neurons of the vasomotor-neurosensory arteriolar-capillary-AVS functional unit in the skin and induce erythromelalgic pain and inflammatory manifestations of red warm and congested skin. This can only be reversed by irreversibly inhibition of platelet COX-1 with aspirin as the explanation of relief of erythromelagic pain and inflammatory manifestation in JAK2, CALR, MPL and TPO mutated thrombocythemia (‘one
Effect of alpha-2 plasmin inhibitor p.Arg6Trp polymorphism and antigen level on the risk of myocardial infarction in young patients


**Poster Topic**
Arterial thrombosis
17.02.2017, 17:15 - 18:15
Poster Number: 29

**Objective:** Alpha2-plasmin inhibitor (a2-PI) is the main physiological inhibitor of plasmin. Increased a2-PI levels have been associated with increased thrombotic risk. Alpha2-PI is a heterogeneous protein, as in the plasma it undergoes both N- and C-terminal cleavages, which significantly modify its activities. In about 70% of circulating a2-PI the N-terminal cleavage by APCE (antiplasmin cleaving enzyme) results in a 12 amino acid residue shorter isoform. This isoform is cross-linked more effectively to fibrin alpha-chain by activated factor XIII; thereby the alteration of the ratio of N-terminal isoforms may influence the fibrinolytic resistance of fibrin clot. The p.Arg6Trp polymorphism of a2-PI may modify the ratio of N-terminal isoforms as APCE cleaves the Arg6 form 8-fold faster than the Trp6 form.

In this case-control study the effect of a2-PI p.Arg6Trp polymorphism and a2-PI antigen concentration on the risk of myocardial infarction (MI) in young patients were investigated.

**Methods:** 109 patients who had coronary sclerosis proven by coronary angiography and suffered MI (MI+) below the age of 40 and two age-matched control groups (n=98 clinical controls without significant coronary stenosis and MI (MI-), and n=139 healthy controls (HC)) were enrolled in the study. Total a2-PI antigen levels were determined by a sandwich type ELISA method, a2-PI Arg6Trp genotype was determined by RT-PCR using LightCycler® 480.

**Results:** Trp allele frequency did not differ significantly among the study groups and were in good agreement with data obtained from the HapMap database. The presence of Trp allele did not influence the risk for MI when patient groups were compared to the MI- or HC groups. Adjusted total a2-PI antigen levels (mean (95% CI)) were significantly elevated in MI+ patients compared to both controls (MI+: 75.4 mg/L (73.6-77.1), MI-: 72.5 mg/L (70.6-74.3), and HC, 64.0 mg/L (62.5-65.5). Elevated a2-PI level (above 73.3 mg/L) increased the risk of MI (OR MI+ vs HC, 7.25, 95%CI, 3.53-14.87).

**Conclusion:** In our study, the a2-PI p.Arg6Trp polymorphism had no effect on the risk of MI in young patients, however a2-PI levels in the upper third resulted in a significant risk enhancement.
rVIII-SingleChain: results of a phase III PK, efficacy and safety study in children less than 12 years of age with severe hemophilia A

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Poster Topic
Bleeding disorders, coagulation and fibrinolytic factors
17.02.2017, 17:15 - 18:15
Poster Number: 30

Objective: rVIII-SingleChain, a novel B-domain truncated recombinant Factor VIII (rFVIII) comprised of covalently bonded FVIII heavy and light chains, was shown to have a higher binding affinity to von Willebrand Factor (VWF) leading to an improved pharmacokinetic (PK) profile. Safety, efficacy and PK of rVIII-SingleChain were investigated in previously treated pediatric patients <12 years of age with severe hemophilia A in a phase III study.

Methods: Patients with severe hemophilia A (endogenous FVIII <1%), <12 years of age, and with more than 50 previous exposure days (EDs) to FVIII products received either on-demand or prophylactic infusions of rVIII-SingleChain.

Results: A total of 84 patients were included in the study (0 to <6 years, n=35; ≥6 to <12 years, n=49). Thirty-nine patients underwent a PK evaluation (0 to <6 years, n=20; ≥6 to <12 years, n=19); younger and older pediatric patients had similar PK and FVIII activity profiles. Mean PK parameters for patients 0 to <6 years and patients ≥6 to <12 years, were; AUCinf: 1080 and 1170 IU*h/dL, clearance: 5.07 and 4.63 mL/h/kg, and half-life: 10.4 and 10.2 h, respectively. Of the 81 patients on prophylaxis, 83% were on twice weekly (n=43, 53%) or three-times weekly (n=25, 31%) regimens. The median starting doses were 35 and 32 IU/mL, respectively. Three patients were assigned to an on-demand regimen. Hemostatic efficacy was rated as excellent or good in 96.3% of the 347 treated bleeds evaluated by the investigator. The median annualized spontaneous bleeding rate (AsBR) was 0.00 (Q1, Q3: 0.00, 2.20), the median annualized bleeding rate (ABR) was 3.69 (Q1, Q3: 0.00, 7.20), and the median joint ABR was 1.62 (Q1, Q3: 0.0, 4.87) across all prophylaxis regimens. The total cumulative exposure during the study was 5239 EDs, with 65 participants reaching >50 ED (0 to <6 years, n=27; ≥6 to <12 years, n=38). Tolerability was excellent, 99.4% of 4774 injections were assessed as producing no reaction. The adverse event profile was in line with the expected background pathology for pediatric patients with severe hemophilia A. No patient had a serious AE related to rVIII-SingleChain, and no patient developed an inhibitor during this study.

Conclusion: The novel rFVIII molecule, rVIII-SingleChain, has demonstrated excellent hemostatic efficacy with a positive safety profile in a clinical study in children <12 years of age with severe hemophilia A.
Comparison of biomarkers and immunological parameters between hemophilia patients, rheumatoid arthritis patients, and control subjects

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Objective: Patients with haemophilia or rheumatoid arthritis (RA) may develop severe joint damage caused by recurrent joint bleeds in haemophilia and by chronic inflammation in RA, respectively. Similarities in clinical presentation and medical imaging have also been shown. Biomarkers are useful diagnostic tools to assess joint damage in RA. However, to date only limited data are available for biomarkers in haemophilia arthropathy.

Methods: A panel of biomarkers was assessed in 129 men older than 30 years (40 haemophilia patients without arthropathy, 23 haemophilia patients with arthropathy, 23 patients with RA and 43 control subjects). The median age was 49.1 years for the haemophilia patients, 62.9 years for the RA patients, and 46.7 years for the control group, respectively. During follow-up examination 61 different biomarkers were analyzed including immunological, inflammation, coagulation, angiogenesis-related parameters and cytokines. Arthropathy was characterized by painful swelling, loss of function, typical radiology images and surgical treatment of joints. The RA patients were classified according to ACR/EULAR criteria.

Results: We identified 24 parameters of angiogenesis and cytokines with significant differences between haemophilia patients, RA patients, and healthy individuals. Most of them (20) were reduced (e.g. VEGFR1 or TNF-alpha) whereas only EGFR, osteopontin, IL6-RA and IL-7 were elevated. Both groups of patients had a significant increase of the acute phase protein ferritin, the angiogenesis parameter HGF and the cytokine MIP-1b. Similar reaction patterns were observed for alpha2-macroglobulin, follistatin, leptin, PECAM-1, IL-10, and VEGFR-2.

Conclusion: We assessed 61 different immunological blood parameter and biomarkers in haemophilia, RA patients, and healthy individuals. Twenty four parameters were different in haemophilia patients. In addition, significant differences could be demonstrated between haemophiliacs and RA patients compared to controls for three parameters (ferritin, HGF, and MIP-1b). Therefore, we could show a specific immunological profile for haemophilia as well as a common biomarker profile for the arthropathy in haemophilia and RA. Further research should be performed to evaluate the potential of established and new biomarkers to follow-up joint damage and chronic arthropathy in haemophilia.
Introduction of a guideline for the treatment of synovitis in patients with hemophilia

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**Poster Topic**

Bleeding disorders, coagulation and fibrinolytic factors

17.02.2017, 17:15 - 18:15
Poster Number: 32

**Objective:** Treatment of patients with hemophilia (PwH) has changed through the last decades. Especially the implementation of a prophylactic treatment with factor concentrates in young patients has reduced the incidence of severe bleedings. Nevertheless, many patients suffer from synovitis due to previous bleedings and there is still a lack of knowledge of how and when to treat a synovitis in those patients. Since a synovitis after a bleeding event is the start point of joint degeneration, it is crucially important to start an adequate treatment at the right moment. The aim of our work was, to develop a guideline based on the evidence in literature to support physicians, medical staff and patients in the treatment of synovitis in PwH.

**Methods:** All member societies of the AWMF were invited to take part in the work of our guideline group. The group consisted of experts in the fields of hemophilia and hematology, nuclear medicine, pediatrics, physiotherapy, orthopedics, radiology, and sports medicine. Based on the regulations of the AWMF, the present and past literature on synovitis and hemophilia was acquired, systematically reviewed and thereafter assessed by the Oxford rating system. All papers and abstracts were scanned and finally reviewed in detail by the working group. The content was evaluated and summarized to a comprehensive paper.

**Results:** We developed the underlying work for a guideline that may help physicians, medical staff and patients to treat a synovitis according to the available evidence in literature. The result is subdivided in diagnostics, treatment and secondary prophylaxis of synovitis. Besides a comprehensive paper we plan to develop short versions for physicians and patients that offer a quick guide in the daily routine work.

**Conclusion:** The guideline may be a valuable tool for physicians, medical staff and patients for the treatment of early and late onset synovitis. With it, treatment of the PwH will be based on what has been published in literature in the near future. However, the review of the literature has shown that many established treatment options can’t be substantiated with sufficient evidence in literature and are only recommended by experts’ opinion. Nevertheless, the content of our guideline can be also a central basis for necessary further research.
The coagulation Factor XIII (FXIII) A subunit activation peptide directs the evolution of FXIII A as a proteolytically activated dimeric molecule

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**Poster Topic**
Bleeding disorders, coagulation and fibrinolytic factors

**Objective:** To trace the evolutionary origins of Factor XIII (FXIII A) subunit activation peptide (FXIII-AP) and its importance in the speciation events leading to generation of FXIII A subunit dimeric molecule.

**Methods:** Nucleotide and amino acid sequences of coagulation FXIII A subunit, the activation peptide itself and of other Transglutaminase paralogues were downloaded from pubmed. Sequences were manually curated to eliminate redundancy and reduce error rates arising from partial/incorrect sequences. After generating high quality multiple alignments phylogenetic analysis was performed for both types of sequences on the GUI platform of MEGAversion7. On a structural level all zymogenic and activated forms of FXIII A and other known TGs were downloaded from protein structure database. The TGs for whom no structures were available, threading based structures were generated on the ITASSER server. Structure alignment and visual comparative analysis was performed on the YASARA platform.

**Results:** The FXIII-AP has evolved from the membrane anchorage region of TGM1 as a result of a loss of function deletion. The evolution of the FXIII-AP has directed the evolution of FXIII A subunit as a functionally similar but structurally distinct (dimer) class of enzyme within the transglutaminases. The N terminal region of the FXIII-AP shows a higher substitution rate than the C-terminal part (which is highly conserved) indicating that while the C-terminal part is structural in function, the N-terminal part is meant for protein-protein interaction. Cross conservation analysis suggests that part of the activation peptide originates from a sequence on the beta sandwich domain of TGM5 and Erythrocyte membrane protein band 4.2 and moves through TGM1 to FXIII A through a series of gain of function and loss of function mutations. 

**Conclusion:** FXIII-AP is not only an important region for the activation of FXIII A subunit; it has also played a very important role in the specialized evolution of FXIII A.
FSAP and altered fibrin clot structure: Is there a link to FXIII?

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Objective: Factor VII activating protease (FSAP) is a plasma protease affecting both coagulation and fibrinolysis, but a physiologic role in haemostasis is still under debate. Fibrinogen (Fbg) is the clot forming protein during coagulation and is composed of 3 pairs of polypeptide chains arranged in two identical subunits (AαBßγ)2. Recently we showed that FSAP cleaves Fbg in the Aα and Bß chains, altering the fibrin clot structure and facilitating fibrinolysis. FXIII is a plasma transglutaminase that forms crosslinks between Fbg α and ß chains, and is an important determinant in clot structure. Here we investigated whether FXIII is involved in the altered clot structure observed with FSAP.

Methods: FXIII as a potential target substrate for FSAP was studied in SDS-PAGE/WB and FXIII activity assays. The fibrin structure of normal plasma or FXIII-depleted plasma upon treatment with FSAP was studied by confocal laser scanning microscopy (LSM). The influence of FSAP on clot pore size was analyzed in permeability experiments.

Results: We found no evidence that FSAP could directly activate or inactivate FXIII. However, when FXIII-depleted plasma (FXIII-DP) was treated with FSAP, no clear change in the clot structure was seen in LSM, in contrast to normal plasma. This effect was partially reversible, when FXIII-DP was supplemented with FXIII. Moreover, FSAP-treated normal plasma -but not FXIII-DP- showed a significantly reduced clot pore size (permeability). Also this effect could be reversed when FXIII-DP was supplemented with FXIII.

Conclusion: FSAP does not directly activate or inactivate FXIII, but there is a link between FSAP, FXIII and fibrin clot structure. Cleavage of Fbg by FSAP apparently involves regions in the Aα chain crucial for FXIIIa function and contributing to overall clot structure. Most likely these are the FXIIIa Gln donor and Lys acceptor sites in the αC-region of Fbg, which are partially released by FSAP. The denser fibrin clot structure with thinner fibers seen in FSAP-treated normal plasma after clotting is obviously not only caused by N-terminal truncation of the Bß chain of Fbg (release of BßN1-53), which destabilizes protofibrils and fiber formation. Also the truncation of the αC-region and, thus, the limited formation of α-α and α-γ crosslinks by FXIIIa seem to contribute to the altered clot structure of fibrin clots of FSAP-treated Fbg.
Amidolytic thrombin activity under the ischemic stroke peptide pool influence

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**Poster Topic**
Bleeding disorders, coagulation and fibrinolytic factors
17.02.2017, 17:15 - 18:15
Poster Number: 35

**Objective:** Group of peptides 3-5 kDa are able to reflect the level of metabolic disorders in the organism (3). Clear and unambiguous answer to the question regarding protein pool (PP) properties is absent. At the same time, stroke is one of the most pressing health problem which require urgent solution (4). Full recovery of patients after stroke was not observed (5). The leading mechanism of ischemic stroke realization correlated with haemostatic profile (6-8). And the thrombin is key factor of haemostasis (1,2).

Investigation of the potential influence of PP formed in the bloodstream after suffering a stroke, on the amidolytic activity of thrombin has utmost importance.

**Methods:** PP fractions were obtained by the NikolaichykV.(9) method from the blood plasma of 35 healthy donors and 56 atherothrombotic (AIS) and cardioembolic (CIS) ischemic stroke patients in acute phase as well as 56 patients one year past acute phase. Isolated PP fractions were dialyzed against vehicle. Experimental mixture preparation and registration of absorption were done like previously (10). An activator of prothrombin derived from the venom Echis multisquamatus (ecamylin) were used (11). Control sample contained the same components but equal volume of vehicle instead of PP. Different concentrated PP fractions were tested.

**Results:** Different concentrations of PP showed various and often opposite effects. Thus PP fraction derived from blood plasma of healthy donors inhibited tested activity of thrombin in concentrations lower then average PP concentration analogical group. The maximum inhibition was observed under influence of healthy donors PP fractions in concentration half (34 mkg/ml) and quote (17 mkg/ml) less than average concentration in analogical group by the 12% and 20% correspondingly. In contrast stroke PP fractions led to opposite effects. Maximum effect of PP fractions on amidolytic thrombin activity was observed by using half less concentrated PP. Thus PP from acute stroke AIS as well as CIS activated study process by 20% and 8% respectively. Opposite effect observed for thrombin activation from the prothrombin in plasma.

**Conclusion:** Therefore results affirm that ischemic stroke accompanied by the formation of the peptide pool in the bloodstream that could take part in recurrence of the disease.

What hemophilia patients expect from the new extended half-life (EHL) products and their willingness to switch - Results from the DACH region

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Objective: Since half-life extended (EHL) products have been recently launched in Europe the National Member Organisations (NMO) of persons with haemophilia (PWH) from the DACH Region (D=Germany, A=Austria, CH=Switzerland) were interested to better understand PWH’s expectations and concerns towards these new EHL products in order to adapt haemophilia treatment to real patients’ needs.

Methods: A systematic postal survey was sent out to 2,644 PWH with haemophilia A or B in Germany (2015), Switzerland (2016) and Austria (2016).

Results: In total 1,007 questionnaires were sent back (38.1%), from whom 743 were adult haemophilia patients and 262 parents of haemophilic children participated, 2 could not be classified. The majority of patients had haemophilia A (84.5%), were severely affected (73.7%), received prophylaxis (57%), mostly three times a week (47.4%) and used recombinant products (60.2%). 14.9% had a history of an inhibitor (past, actual). One quarter did not know the correct half-life of their actual FC [HA/FVIII: 26%, HB/FIX: 31.1%]. Only 4% were unsatisfied with their actual FC, mainly with short half-life of FC and difficult manageability. They expected from the new products to provide less frequent injections, better efficacy and safety/no side effects. 59.5% would be willing to switch to new products if they have a prolonged half-life (87.1%) and the same safety of the actual FC (62.8%). Reasons for not willing to switch were fear of inhibitor development (71.4%) and fear of uncertain safety (60.9%). They wish more information about half-life (84.4%), possible side-effects (81.3%) and efficacy (77%) and would consider changing product if the prolongation of the half-life is at least double as high as the actual FC (40.5%). The majority wanted to receive information about new products from their haemophilia treater (76.3%) and the newsletter of their NMO (74.3%). Significant difference across countries were found regarding treatment regimen (p<.0001), used product category (p<.0001) and willingness to switch to new EHL products (p<.0001).

Conclusion: In this representative survey it could be shown that although PWH were generally satisfied with their actual FC the majority would be willing to switch from their actual FC to the new up-coming EHL products assuming the half-life is prolonged and has the same safety of the actual FC.
Monitoring of perioperative factor VIII treatment in a remote hospital by electronic diary smart medicationTM

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Poster Topic
Bleeding disorders, coagulation and fibrinolytic factors
17.02.2017, 17:15 - 18:15
Poster Number: 37

Objective: Surgical procedures for patients with hemophilia may be required in hospitals far from the hemophilia center. Close surveillance of postoperative bleeding, subsequent factor dosing and documentation of factor consumption is often difficult to achieve.

Methods: A patient with severe hemophilia A decided for ankle joint replacement in a specialized clinic. The patient was asked to document factor VIII treatment and clinical course by his electronic diary smart medicationTM.

Results: Due to intensive care treatment a personal visit was required on the first postoperative day only, but not thereafter. Factor VIII treatment and clinical course, including photographs of the operation sight were thoroughly documented by the patient himself. Total consumption of factor VIII concentrates and batch numbers were in file of the hemophilia center already at the day of discharge.

Conclusion: Electronic diary smart medicationTM improves and facilitates hemophilia surveillance and documentation in remote perioperative hemophilia care. Inclusion of the patient’s responsibility led to a positive feedback.
An Asp94Gly missense mutation in GP1BB causes severe Bernard-Soulier syndrome in an Iraqi family

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Objective: Bernard-Soulier syndrome (BSS) is a rare, autosomal recessive bleeding disorder with abnormalities of the platelet GPIb-IX-V complex. Typical are mucocutaneous bleedings (easy bruising, epistaxis), thrombocytopenia, presence of giant platelets and impaired ristocetin-induced agglutination. Three disease-related genes (GP1BA, GP1BB, GP9) harbor mutations of all types. We investigated a family of middle east origin with two sisters presenting severe bleeding symptoms. Aim of the study was to define the phenotype and reveal its underlying pathogenic mutation.

Methods: Blood count, smear, platelet aggregometry (after stimulation with collagen, ristocetin, ADP, epinephrin) and flow cytometry analyses (CD41 (GPIIb/IIIa), GP42a (GPIX), CD42b (GPIb)) were performed. Sequencing of exons and splice site regions of all genes was done for index patient. Variants were analyzed by ALAMUT®. Occurrence in variant databases (dbSNP, EVS, ExAC), ClinVar, locus-specific mutation database of the BSS international consortium, conservation status and in silico pathogenic prediction (SIFT, MutTaster, PolyPhen2) were investigated. Genotyping was performed for all family members.

Results: Patients showed thrombocytopenia (11 and 7 G/l), giant platelets, impaired ristocetin-induced agglutination and severely reduced expression of GPIb/IX. Normal results were obtained for the other family members. Sequencing revealed a homozygous variant in exon 2 of GP1BB (coding for GPIb beta) in both patients (NM_000407.4: c.281A>G; p.Asp94Gly). This mutation was not listed in any of the above mentioned sequencing databases. Only the BSS consortium report mentioned this mutation, originally found in a Lebanese patient, without further information. Functional prediction is concordant pathogenic in all tools. The heterozygous parents are not affected and the brother shows wildtype sequence.

Conclusion: We report a mutation of the GP1BB gene leading to a clinical phenotype of BSS which so far was only mentioned in the BSS consortium report without any further information. The mutation is located in the C-terminal cysteine rich flanking region next to cysteine at p.93 participating in disulfide bonds. The exchanged amino acids differ in size and polarity and may influence the formation of disulfide bond next to them. This may cause an absent GPIb/IX expression which leads to the severe phenotype.
Impact of maintaining higher FVIII trough levels with BAX 855: Rationale and design of the PROPEL study

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Objective: Despite improvements in prophylaxis for hemophilia A, desired outcomes for patients have not yet been achieved. In the pivotal PROLONG-ATE study with BAX 855 (ADYNOVATE) – a polyethylene glycol (PEG)ylated, full-length, extended half-life (EHL) recombinant factor VIII (FVIII) built on ADVATE – 39.6% of patients achieved zero bleeding and 57.4% experienced zero hemarthroses on prophylaxis intended to maintain a >1% FVIII trough. However, preliminary data suggest that maintaining higher trough levels may further reduce hemarthroses, enable more patients to experience zero bleeding, preserve long-term joint health, and improve quality of life (QoL).

Methods: In the Phase 3 BAX 855 Continuation Study, previously treated patients (PTPs) with severe hemophilia A (FVIII<1%) could remain on a fixed dose prophylaxis regimen, receiving 45 ±5 IU/kg (≥12y) or 50 ±10 IU/kg (<12y) twice weekly. Alternatively, patients could elect to receive prophylaxis based on individual PK (PKP), targeting FVIII trough levels >=3%.

Results: Patients on PKP (n=15) maintaining >=3% FVIII experienced an estimated mean (95% confidence interval [CI]) total annualized bleeding rate (ABR) of 1.56 (0.56, 4.38). In contrast, patients on twice weekly prophylaxis (n=120) in the pivotal study exhibited a mean (95%CI) estimated total ABR of 4.3 (3.4, 5.5). These preliminary data suggest that maintaining higher FVIII trough levels with FVIII replacement therapy may result in lower ABR. To investigate the efficacy of 2 FVIII trough levels (1-3% and 8-12%), a prospective randomized Phase 3 clinical study (PROPEL) has been initiated. PTPs aged 12-65y with FVIII<1% and ABR >=2 are eligible. The dose is based on the patient's PK with infusions twice weekly (1-3%) or every other day (8-12%). The primary objective is to compare the proportion of bleeding-free patients during the second 6-month study period. Key secondary objectives include ABR, consumption, safety & tolerability, and health-related QoL. Correlation of thrombin generation assay with FVIII levels and ABR will also be explored.

Conclusion: The novel design of the PROPEL Study (NCT02585960) evaluates the effects of maintaining higher FVIII trough levels through PK-guided dosing with BAX 855, an EHL FVIII replacement therapy, with the goal of increasing FVIII coverage and bleed protection allowing more patients to be bleeding-free through a personalized treatment approach.
Nonacog beta pegol in adult and paediatric patients: pooled data from the paradigm™ clinical programme

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Poster Topic
Bleeding disorders, coagulation and fibrinolytic factors
17.02.2017, 17:15 - 18:15
Poster Number: 40

Objective: Nonacog beta pegol (N9-GP) is an extended half-life recombinant glycoPEGylated factor IX (FIX). We present pooled N9-GP data from 5 completed trials conducted in previously treated paediatric, adolescent and adult haemophilia B patients (PTPs) (1 phase 1; 4 phase 3 trials, including the pivotal randomised trial).

Methods: Results from patients who received N9-GP 40 IU/kg (all ages) or 10 IU/kg (adolescent/adult only) once-weekly prophylaxis including treatment of bleeds are summarised. This analysis includes pharmacokinetics, safety and haemostatic efficacy.

Results: 115 male PTPs (FIX <=2%) were included: 72 adults (18–65 years), 18 adolescents (13–17 years) and 25 children (0–12 years), with a total of 8801 exposure days to N9-GP. In the phase 3 trials, 30 patients received N9-GP at 10 IU/kg/week, 54 patients received 40 IU/kg/week and 15 were treated on-demand. No inhibitors or thromboembolic events were observed. Of 54 (47%) patients treated weekly with 40 IU/kg, 23 (43%) experienced zero bleeding episodes. Median overall annualised bleeding rate (ABR) for all age groups on 40 IU/kg was 1.03 (IQR 0.00–2.89) and median spontaneous ABR was 0.00 (IQR 0.00–0.80). ABR was lower in adolescents/adults randomised to 40 IU/kg than 10 IU/kg (p<0.05). Overall success rate for the treatment of bleeds was 93%; most bleeds (87%) resolved after a single injection. Adults, adolescents and children showed single-dose (40 IU/kg) half-lives of 83, 89 and 73 hours, respectively, and incremental recoveries of 0.023, 0.020 and 0.016 (IU/mL)/(IU/kg), respectively. Estimated mean steady-state FIX trough levels with weekly 40 IU/kg were >=0.15 IU/mL in all age groups. 13 adolescent/adult patients receiving 40 IU/kg N9-GP once-weekly prophylaxis had collectively 20 target joints at study start; by the end of the extension trial, all target joints had resolved. Two questionnaires (EQ-5D VAS and Haem-A-QoL) in adults/adolescents demonstrated significant improvements in quality of life (QoL) from baseline to end of trial in those receiving 40 IU/kg; by the end of the trial, patient QoL scores approached that of the general population.

Conclusion: N9-GP was well tolerated and effective in preventing bleeding at 40 IU/kg once weekly, maintaining FIX activity levels >=15% across all age groups. Once weekly prophylaxis resolved existing target joints and improved patient QoL in adults/adolescents.
Challenges in immune tolerance induction in an adult patient with non-severe hemophilia A.

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Poster Topic
Bleeding disorders, coagulation and fibrinolytic factors
17.02.2017, 17:15 - 18:15
Poster Number: 41

Objective: Haemophilia A is a bleeding disorder caused by deficiency of clotting factor VIII (factor eight), which may result both in spontaneous haemorrhage or prolonged bleeding following injuries. Therefore, patients with haemophilia A are usually identified in early childhood. Treatment is based on prophylactic or on-demand administration of factor concentrates. Development of inhibitors neutralizes the effect of administrated factor VIII and is one of the most challenging complications in the treatment for haemophilia today.

Methods: We present here the case of a 42-year-old patient with frequent haematuria of initially unknown origin. In another hospital, digital subtraction angiography was performed to determine the cause of the haematuria and resulted in major retroperitoneal bleeding. The developing haematoma compressed the left femoral nerve leading to paraesthesia and to impairment in mobility. Clotting analyses detected a low level of factor VIII activity and initially no inhibitor suggesting a non-severe haemophilia A.

Results: A factor VIII concentrate (turoctocog alfa) was administered twice daily to control bleeding and resorb haematoma. To improve the outcome and to obtain functionality of the femoral nerve the treatment was continued in lower doses at the rehabilitation clinic. On the second day at home after factor VIII administration was stopped a new soft tissue bleeding appeared and treatment was resumed. This time higher doses and more frequent administration were necessary to control bleeding. Pharmacokinetic (PK) analysis revealed diminished recovery and half-life indicating the development of an inhibitor, which was confirmed by a positive Bethesda assay with an inhibitor titre level of 2 BU. According to the latest German Cross-Sectional Guidelines for Therapy with Blood Components and Plasma Derivatives we started immune tolerance induction (ITI) by continuous factor VIII replacement with 50 IE/kg body weight three times per week.

Conclusion: After six months of low dose ITI no breakthrough bleeding occurred, but recovery kept to be diminished with inhibitor titre levels of 6 BU. Therefore we decided to increase frequency and doses of administration adjusted to the Bonn protocol. Despite normalized recovery factor VIII on day 13 half-life kept to be low. High dose ITI was continued for several months till inhibitor did not reappear and half-life normalized.
Prevalence of overweight and obesity in children, adolescents and young adults with severe hemophilia A – results of 3 German pediatric hemophilia treatment centers

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Poster Topic
Bleeding disorders, coagulation and fibrinolytic factors
17.02.2017, 17:15 - 18:15
Poster Number: 42

Objective: The prevalence of obesity among children and young adults in Germany is between 10-20%, depending on age. Data on overweight or obese children with hemophilia in Germany are not available. International data suggest that the usual substitution regimes (IU per kg bodyweight) might lead to overdosing. Aim of this study is to investigate the prevalence of overweight and obesity among patients with severe hemophilia A and the influence of body weight on bleeding tendency, factor consumption per year and possible reduction of economic burden of hemophilia therapy by individualized substitution regimes and weight loss programs. We report preliminary data from 3 large pediatric hemophilia treatment centers.

Methods: After obtaining approval of the institutional review board, cross sectional data on height, bodyweight, bleeds/year, factor consumption/year in patients with severe hemophilia A (Factor VIII <1%) were collected. Age dependent body mass index was calculated according to Krohmaier-Hausschild. Yearly factor VIII consumption and the annual bleeding rate were calculated from the patient documents.

Results: We identified 246 patients with severe (F VIII <1%) hemophilia A. Median Age of patients was 14 years, mean 14 years (range 0-30y). Prevalence of overweight among this patients is 18% and 8% were obese. All patients performed regular prophylaxis 2-3.5 times/week. Further analysis respecting yearly factor VIII consumption, bleeding rate and cost reduction by individualized prophylaxis will follow.

Conclusion: Nearly 26% of patients with severe hemophilia A from the three hemophilia centers suffer from overweight or obesity. The rate appears higher compared to the rate of overweight in the general population in Germany. We suppose that the risk factors for the development of obesity are similar. Reduced physical activity due to overprotection by parents may add to the possibly increased prevalence in this cohort.

In further data analysis we will concentrate on the following questions:
- Is the yearly factor VIII consumption and bleeding tendency influenced by overweight/obesity?
- Can factor consumption be reduced by individualized through level adjusted therapy?
- Is there a possibility to reduce hemophilia therapy costs in obese patients by reducing body weight?

Especially the last point might be helpful in discussions with health insurance to implement weight loss programs.
Pharmacokinetic results of two studies with a novel fibrinogen concentrate in subjects with afibrinogenaemia.

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Objective: A second generation of plasma-derived human fibrinogen concentrate, named FibCLOT®/CLOTTAFACT® (Human Fibrinogen) (1.5 g/100 ml) has been developed. The product recovered from the supernatant fraction of cryoprecipitate using an ethanol fractionation, includes 3 viral safety steps: solvent-detergent treatment, nanofiltration and dry heat (80°C during 72 hours).

Two prospective clinical trials (French and multinational) were conducted consecutively including a total of 26 patients with afibrinogenaemia (6 + 20). Among them, 19 patients, with the average weight of 68 kg, participated in the pharmacokinetic parts of the studies (5 children or adolescents of more than 40 kg and 14 adults). This abstract focuses only on the pharmacokinetic outcome of these two studies.

Methods: In the two studies, pharmacokinetic assessment was performed after a single infusion of 0.06 g/kg of FibCLOT® / CLOTTAFACT®. Concentration of fibrinogen expressed in antigen and activity were followed during 14 days after infusion. These parameters values are presented using geometric mean values.

Results: Pharmacokinetic studies showed that the maximum concentration was observed at the first available time-point (at about 1 hour) and was followed by a slow mono-exponential decrease, reaching the critical haemostatic plasma fibrinogen level of 0.5 g/L in about 3 to 4 days, with no notable differences when the data were stratified by age or by gender. In both studies, incremental recovery was 23.5 g/L per g/kg fibrinogen infused.

In addition the pharmacokinetic profiles of fibrinogen antigen and activity are superimposed showing that the FibCLOT® / CLOTTAFACT® manufacturing process preserves the functional properties of fibrinogen.

Conclusion: CLOTTAFACT® / FibCLOT® shows consistent pharmacokinetic properties in the 19 afibrinogenaemic patients included in two different studies. Our results from two clinical studies conducted separately, demonstrate that an infusion of 3 g of FibCLOT® / CLOTTAFACT® increases plasma fibrinogen level by 1 g/L in a 70 kg person.
Pitfalls in carrier testing of haemophilia A

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Poster Topic
Bleeding disorders, coagulation and fibrinolytic factors
17.02.2017, 17:15 - 18:15
Poster Number: 44

Objective: Background: Females carrier testing in haemophilia families is important for family planning and personal health. Genetic analyses are the golden standard as FVIII activity give not always reliable results. We report a case, where the initial genetic diagnosis failed to identify the second mutation.

Methods: Materials and Methods: All 26 exons and flanking regions of the F8 gene were amplified and directly sequenced from genomic DNA. MLPA was performed following the manufacturer’s instructions. Intron1/22 were analyzed by inverse PCR. X-Inactivation was performed though analyses of HUMARA locus.

Results: Results: A female patient with questionable carrier status showed a reduced FVIII:C value of 30 IU/dl and mild bleeding episodes. No male family member was available for testing at the time. Intron 22 inversion testing revealed an abnormal pattern suggesting, that an additional defect might be combined with the intron inversion. MLPA analysis showed a supplementary large deletion from exon 1 to 22. Thus, our patient carried an intron 22 inversion combined with large deletion of exons 1-22. Subsequently her father was sent for genetic analysis. He was diagnosed as mild haemophilia (FVIII:C 59 IU/dl), rarely experienced bleeding symptoms, which did not correlate with his daughter’s genetic defect. The sequence of the F8 gene revealed a missense mutation in exon 23, described in international register for a mild haemophilia. Reinvestigation of the daughter’s DNA showed that she also carried the paternal mutation – in addition to the other 2 genetic defects. Although both X- chromosomes of our patient have been affected with genetic defects in the F8 gene, the FVIII:C showed relatively high values. Hence we performed skewed X chromosome analysis, which showed complete inactivation of one of the X chromosomes (99:1), most likely the one with the combined intron 22/large deletion. Both, patient’s mother and daughter carry intron 22 inversion/ large deletion defect in heterozygous state. The missense mutation was not detected.

Conclusion: Conclusions: Identification of two genetic defects in females carriers of haemophilia A is of extreme importance for the genetic counselling. In our case haemophilia boy will be born either with severe (intron 22/large deletion) or mild (missense mutation) form of the disease. This will impact the risk of inhibitor development and treatment regimen.
Long-term use of recombinant fusion protein linking coagulation factor IX with albumin (rIX-FP) in previously treated patients with hemophilia B

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Objective: rIX-FP is a fusion protein genetically linking recombinant human coagulation factor IX with recombinant human albumin. It was designed specifically to have an improved pharmacokinetic profile compared with standard factor IX products to allow less frequent dosing.

Methods: The PROLONG-9FP clinical program evaluated the safety and efficacy of rIX-FP both as prophylactic therapy and on-demand treatment (ODT). The 5 clinical studies in the trial program enrolled 100 subjects from 42 hemophilia treatment centers in 12 countries. In this analysis, patients (12–65 years) who had continuously participated in 3 rIX-FP clinical trials over a 4-year period were included. Patients were initially recruited into the Phase II trial and received either ODT or weekly prophylaxis. Patients continued into the Phase III trial, receiving the same treatment for 6 months, after which those receiving ODT switched to weekly prophylaxis, while those receiving weekly prophylaxis were eligible to extend their treatment interval to once every 10 or 14 days. Patients continued into the Phase IIIb extension study where they received prophylaxis every 7, 10, 14 or 21 days.

Results: Of the 15 patients included in the analysis, 4 started with ODT and switched to prophylaxis in the Phase III study; the remaining patients received prophylaxis in all studies. Patients had a median (range) of 4.2 (3.2–4.3) years on rIX-FP. For those on prophylaxis, median (range) number of exposure days was 199 (144–232); for those initiating ODT corresponding figures were 130 (121–137). With 7-day prophylaxis, mean annualized bleeding rate (ABR) fell from 3.10 in Phase II to 1.27 in Phase III. This decrease in ABR over time was achieved with constant monthly consumption of rIX-FP (240–250 IU/kg). With both 7- and 14-day regimens, there was a decrease in spontaneous ABR over time. Monthly consumption of rIX-FP decreased with longer prophylaxis interval: 7-day, 245 IU/kg; 10-day, 220 IU/kg; 14-day, 158 IU/kg. No patients developed inhibitors or antibodies to rIX-FP during the treatment period.

Conclusion: Long-term use of rIX-FP was well tolerated; prophylactic efficacy was maintained throughout the 4-year treatment period and resulted in a decrease in ABR over time. Longer treatment intervals were possible with no increase in consumption.
Resolution of target joints during once-weekly prophylaxis with rIX-FP in patients with hemophilia B

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Poster Topic
Bleeding disorders, coagulation and fibrinolytic factors
17.02.2017, 17:15 - 18:15
Poster Number: 46

Objective: rIX-FP is a fusion protein genetically linking recombinant human coagulation factor IX with recombinant human albumin. It was specifically designed to have an improved pharmacokinetic profile compared with standard factor IX products that allows dosing interval to be increased.

Methods: The PROLONG-9FP clinical program evaluated the safety and efficacy of rIX-FP as prophylactic and on-demand therapy, and comprised five clinical studies enrolling over 100 patients from 42 hemophilia treatment centers in 12 countries. In a Phase II/III study (3001), patients (12–65 years) received either once-weekly prophylaxis for 6 months before switching to 7-, 10- or 14-day prophylaxis, or on-demand treatment for 6 months followed by once-weekly prophylaxis. The total treatment period in both groups was 12–18 months. In a separate Phase III study (3002), pediatric patients (<12 years) received once-weekly prophylaxis for approximately 12 months.

Results: In the adult study, 52.5% (21/40) of patients previously receiving routine prophylaxis and 60.9% (14/23) of those treated on-demand reported target joints prior to study entry. A total of 19 patients switched from on-demand to weekly prophylaxis with rIX-FP. Of these patients, 10 (52.6%) had target joints at the start of the study and 9 (47.3%) patients had confirmed target joints after 6 months of on-demand treatment with rIX-FP. After switching to once-weekly prophylaxis with rIX-FP, 100% of these target joints resolved. Median annualized joint bleeding rate (joint ABR) fell from 15.3 with on-demand therapy (n=23) to 1.19 with weekly prophylaxis (n=19). For patients receiving prophylaxis, median joint ABR was 0.00 with all regimens. In the pediatric study, target joints were reported in three patients on prophylaxis prior to study entry; all target joints resolved with once-weekly rIX-FP prophylaxis treatment. Median joint ABR was 0.5 and 1.13 in patients aged 1–5 years (n=12) and 6–11 years (n=15), respectively, and 0.99 overall (n=27).

Conclusion: In both adult and pediatric patients, weekly prophylaxis with rIX-FP resolved all target joints. rIX-FP is not only effective for the prevention of bleeding episodes, but also for resolving target joints in patients previously on-demand or under-treated with prophylaxis.
Epidemiological insights about hemophilia B by analysis of German hospital quality reports

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Poster Topic
Bleeding disorders, coagulation and fibrinolytic factors
17.02.2017, 17:15 - 18:15
Poster Number: 47

Objective: Hemophilia B (HB), also called factor IX (FIX) deficiency or Christmas disease, is a genetic disorder caused by missing or defective FIX, a clotting protein. Although it is passed down from parents to children, about 1/3 of cases are caused by a spontaneous mutation. The birth prevalence of HB is around one in 20,000 live male births worldwide. The main medication to treat HB is concentrated FIX products of plasmatic or recombinant origin. Both treatments have a specific procedure code in Germany. Since 2008 the Paul-Ehrlich-Institute (PEI) has published annual reports of overall epidemiology and factor use. We have taken this further by examining the frequency of in-hospital HB cases and in-hospital use of FIX.

Methods: Data from the German DRG Institute (InEK), Statistical Office (DESTATIS) and hospital quality reports for 2000-2014 were analyzed for cases of hemophilia B and treatments with FIX. Reference was taken from the corresponding reports of the PEI. Statistical analysis was performed using Microsoft-Excel and Access version 2013.

Results: The number of hospital cases with a main diagnosis of HB (D67, ICD10-GM) decreased from 109 (2000), 75 (2003), 70 (2008), to 58 (2014, -47%). In contrast, the number of patients increased slightly from 537 in 2008 to 596 in 2014. The mean age of hospitalized patients (30.3 +/- 23.9 years) and the gender distribution (95% male) remained stable over time. The average length of stay did not differ between age groups, but was shorter for male (5.4 days) than for female patients (7.7 days). The number of cases with a secondary diagnosis of HB also decreased over time. In 2014, it was 403 patients with an average age of 42.4 years. The decrease in cases was not associated with a decrease in in-hospital FIX usage; it was stable around 5m units annually or 8.6% of all FIX used in Germany. In contrast to overall use, there was a growing preference in-hospital for use recombinant FIX (rFIX in 2014: 37%) over plasmatic.

Conclusion: The rate of hospitalization of patients with Hemophilia B decreased in the last decade. Treatment shifted to the ambulatory sector. But the use of FIX in hospital remained stable due to a trend for much higher use per case. In addition, the in-hospital share of recombinant FIX is increasing versus plasmatic FIX, which is not found in the ambulatory sector.
Efficacy and safety of a VWF/FVIII concentrate in surgical procedures – results from the ongoing study Wilate-STATE

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Objective: wilate® is a double inactivated VWF/FVIII (1:1 ratio) concentrate authorized in Germany since 2005. With the introduction of filling sizes 500 and 1000 IU in 2012, a non-interventional study (NIS) was started in Germany (STATE-Surveillance of tolerability and treatment efficacy). In this study we are aiming to confirm consistency of efficacy and safety data of wilate® used in routine clinical practice with data obtained in previous clinical and post-marketing studies. Probably matching the real-life situation, the predominant reason for administration of wilate® in this study was to prevent bleeding during and after surgery. For this reason, an evaluation of this subgroup was performed of which the results will be presented.

Methods: After obtaining informed consent, patients with hereditary or acquired von Willebrand disease (VWD) of any age requiring replacement therapy are eligible to be included in the study. A thorough documentation of anamnestic data is done before details of all injections in conjunction with the surgeries are documented. Details on the procedures performed and outcome including an efficacy assessment are rated using a 4-point-rating scale according to pre-defined criteria are documented. Depending on the haemostatic challenge, the surgeries are categorized in “minor” or “major” surgical procedures.

Results: Until data lock point, 50 patients underwent surgery. The distribution between the different types of VWD in the surgery group is as follows: 42 (84%) patients have type 1, 7 (14%) type 2 and 1 (2%) acquired VWD. The patients’ age ranges from 8 months to 74 years, including 34 paediatric patients (< 14 years). 13 minor and 37 major surgeries were performed. Adenotomy and tonsillectomy are the most frequently documented surgeries, which are classified as major operations due to their high bleeding risk (58%). In total, 213,000 IU were administered on 125 exposure days (ED) for surgeries. Per ED, a median of 31.2 and 33.3 IU/kg BW respectively for minor and major surgeries were administered (mean 34.0 + 10.9 IU/kg). The efficacy was rated excellent/good in 48 (96%) and moderate in 2 (4%) of cases based on all surgeries. None of the patients experienced an ADR.

Conclusion: The results of this interim analysis confirm the efficacy and safety of the investigated VWF/FVIII concentrate wilate® in managing bleeding prophylaxis during and after surgical procedure.
Combined congenital deficiency of coagulation factors VII and X in two siblings with heterozygous factor V Leiden mutation (R506Q)

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Objective: Introduction: Combined coagulation factor VII (FVII) and factor X (FX) deficiency (combined FVII/FX deficiency) is a rare bleeding disorder, in which both factors show reduced plasma activity. Estimated incidences of isolated FVII and FX deficiencies are 1: 500 000 and 1: 1 000 000. FVII and FX genes are located on the long arm of chromosome 13. Combined FVII/X deficiency can be caused by a single co-inherited clotting defect or by partial deletion within terminal end of chromosome 13. This may be associated with additional clinical features, known as 13q deletion syndrome. Factor V Leiden mutation is a known risk factor for thromboembolism and prevalent in 3 to 7 % of the population.

Methods: Case report: We report about two children presented for thrombophilia screening due to positive family history of factor V Leiden mutation. The boy (12 years old) and the girl (9 years old) never had signs of thromboembolism, but both children suffered from malabsorption syndrome (abdominal distention). Diagnosis of gastrointestinal symptoms was already substantiated by prolonged prothrombin time (PT) (71 % and 61 % of normal) possibly induced by vitamin K deficiency. aPTT was in normal range. Nevertheless, vitamin K substitution showed no effect on PT and celiac disease could not be confirmed.

Results: Further investigations revealed factor VII activity of 31 % and factor X activity of 60 % in the girl. The boy showed factor VII activity of 69 % and factor X of 71 %. Genetic analysis of factor VII and factor X gene were performed. In both children identical gene mutations were found: Factor VII missense mutation F7:c.[589A>G];[=]p.(Lys197Glu) and Factor X missense mutation F10:c.[424G>A];[=]p.(Glu142Lys). Both children did not have any bleeding tendency. Additionally, both children showed heterozygous factor V Leiden mutation.

Conclusion: Conclusion: Combined factor VII and factor X deficiency is a rare coagulation disorder and often misdiagnosed. Single factor activities have to be analysed in all cases with prolonged PT even if aPTT is normal or patient is clinically asymptomatic.
Efficacy and safety of rVIII-SingleChain in 21 major surgeries

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Objective: rVIII-SingleChain is a novel recombinant Factor VIII with a truncated B-domain and a covalent bond between the heavy and light chain, demonstrating a high binding affinity for von Willebrand factor. In the AFFINITY clinical trial program, the safety and efficacy of rVIII-SingleChain was assessed in pediatric, adolescent and adult patients with severe hemophilia A undergoing surgery.

Methods: The studies were approved by the relevant Ethics committee and national authorities and conducted according to GCP and the Declaration of Helsinki. Patients undergoing major surgery (defined as a surgical procedure that required general, spinal or regional anesthesia) were given rVIII-SingleChain as a bolus or continuous infusion. Dosing was guided by WFH recommendations. The efficacy of rVIII-SingleChain in surgical prophylaxis was rated by the investigator based on information by the surgeon on intraoperative hemostasis, using a 4-point rating scale. Treatments assigned an efficacy rating of “excellent” (defined as hemostasis not clinically significant different from normal) or “good” (defined as hemostasis normal or mildly abnormal in terms of quantity and/or quality e.g., slight oozing) were considered successful.

Results: A total of 21 surgeries were performed in 18 patients: extraction of multiple teeth (2), abdominal hernia repair, elbow replacement, ankle arthroplasty, knee replacement (5), knee arthroscopy, cholecystectomy, lengthening of the Achilles tendon combined with straighten up of the right toes, circumcision (5), excision, curettage and bone grafting of radius/ulnar pseudo tumor, open reduction internal fixation right ankle fracture and hardware removal right ankle. Of these 21 surgeries, a significant number carried a high risk of bleeding, among these were 10 orthopedic surgeries. A total of 13 surgeries were performed with a single bolus of rVIII-SingleChain; 8 were performed with continuous infusion. Investigators rated the hemostatic efficacy of rVIII-SingleChain during surgery as “excellent” (n=19) or “good” (n=2) in all cases. No related adverse events or serious adverse events were observed during the surgery period.

Conclusion: rVIII-SingleChain demonstrated effective hemostatic control and a good safety profile during major surgery when dosed either by continuous infusion or by bolus infusion.
A preliminary study to find novel interaction partners for the coagulation factor XIII (FXIII) B subunit free form

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Poster Topic
Bleeding disorders, coagulation and fibrinolytic factors
17.02.2017, 17:15 - 18:15
Poster Number: 51

Objective: To improvise upon an immuno-affinity based purification strategy in order to pull down and detect putative novel interaction partners of the coagulation FXIII subunit B in its free form.

Methods: Full length FXIIIB subunit was derived from two sources: A) Commercial recombinant FXIIIB (Zedira, Darmstadt, Germany) B) Expressed, isolated and purified from HEK293T mammalian cell lines using monoclonal antibodies(Mab) specific for the B subunit. FXIII B from both sources were immobilized on α-FXIIIB monoclonal antibodies (raised in mice) using Pierce co-immunoprecipitation kit (Pierce biotechnology, ThermoScientific, Rockford, USA). Three set of experiments were designed: 1) α-FXIIIB bound rFXIIIB exposed to FXIII deficient plasma (Affinity biological) 2) only α-FXIIIB exposed to FXIII deficient plasma 3) only the immobilization membrane (without α-FXIIIB or rFXIIIB). Eluates from these three set up were run on an SDS PAGE and the resulting band were analyzed by peptide mass fingerprinting

Results: Amongst the bands that were attributed exclusively to the free B subunit two major types of proteins and their individual subunits/chains could be identified: 1) All three fibrinogen chains, i.e. α, β and γ chains were found to co-precipitate with the free form of the B subunit 2) The Complement protein C1qA and C1qb were also found to be co-precipitated with free B.

Conclusion: Recent reports have shown that the fibrinogen γ chain might be interacting with the free B subunit further confirming our findings. The α and β chains might have just co-precipitated along with the γ chain or might in fact also interact with the B subunit. The C1q complex might be interacting with the B subunit as well and this might represent one of the pleiotropic roles of the B subunit in the complement system. However both observations require further studies.
Determining the causality of heterozygous missense mutations detected in F13A1 and F13B genes and reported from patients with mild coagulation factor XIII (FXIII) deficiency

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**Poster Topic**
Bleeding disorders, coagulation and fibrinolytic factors

**Objective:** To evaluate the causality of heterozygous missense mutations reported in F13A1 and F13B genes reported from patients with mild coagulation Factor XIII (FXIII) deficiency by expressing them in heterologous cell lines.

**Methods:** The previously reported 16 F13A1 and 7 F13B missense mutations were cloned in mammalian expression vectors carrying F13A1 and F13B cDNAs and transfected into Cos-1 and HEK293T cell lines respectively. Transfections were also performed in combination with the wild type in order to mimic the heterozygous genotype. The intracellular-lysates/extracellular medium were collected after a transient transfection period and tested with a variety of assays. The B subunit mutations were tested for their secretion pattern (by confocal microscopy) and their ability to bind to A subunit. The A subunit mutations were tested by a variety of activity assays i.e. photometric activity assays, physiological incorporation assay, α-2 antiplasmin incorporation assay, non proteolytic activation (with only high concentration of calcium and no thrombin), fibrin crosslinking and clot thickness using scanning electron microscopy. Structural modeling/docking combined with simulation based analysis were performed on available crystals structures of FXIIIA subunit and homology based models of FXIIIB subunit sushi domains to determine the putative structural impact of the reported mutations.

**Results:** Apart from one FXIIIB subunit mutation i.e. p.Cys5Arg which showed a true secretion related defect, the remaining mutations showed either antigenic instability or lack of interaction with FXIIIA subunit. The effect of FXIIIA subunit mutations could be categorized into mild moderate and severe forms based on their in vitro expression phenotype. Two substitutions at the thrombin cleavage site showed type II FXIIIA deficiency i.e. they showed reduced activities associated with moderate or no change in antigen levels. Majority of the FXIIIA missense mutations showed varied effects on alpha-2-antiplasmin incorporation, clot thickness and fibrinogen cross-linking.

**Conclusion:** Our analysis demonstrates that these mutations influence separate structural and functional aspects of the coagulation FXIII.
Annual Bleeding vs. factor VIII/IX consumption – comparison of result in 2014 and 2015 according to electronic diary smart-medication™

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Poster Topic
Bleeding disorders, coagulation and fibrinolytic factors
17.02.2017, 17:15 - 18:15
Poster Number: 53

Objective: Bleeding frequency and factor VIII/IX consumption (FC) differ widely among patients with the same coagulation defect. However, the individual year to year patterns are often similar.

Methods: FC and Joint bleeds (JB) from 192 patients in 2014 were compared to 213 patients in 2015 from the same centers that completed electronic documentation smart medicationTM.

Results: The average FC was 2,442 IU/kg BW (±2,038 IU/kg BW) in 2014 and 2,701 IU/kg BW (±1,837 IU/kg BW) in 2015. The average number of JB was 2.1 (±3.9) in 2014 and 2.5 (±4.9) in 2015. Four groups were identified and compared between 2014/2015: The majority (group A, 45%/40%) had 2 or less JB with less than average FC, followed by (B, 31%/35%) who also had 2 or less JB but above average FC. A minor group (C, 14%/11%) had more than 2 JB and more than average FC and was similar to a group (D, 10%/14%) who had more than 2 JB but less than average FC.

Conclusion: A majority (76%/73%) of patients documented 2 or less JB per year as a result of optimal home treatment showing no major difference between two consecutive years. Patients with high bleeding frequency in spite of above average FC again revealed a small (14%/11%) but important group requiring intensified attention. The electronic diary smart medicationTM is suitable to focus on groups of patient which may require more or less factor treatment or, in case of group C, need otherwise intensified treatment.
The regulatory profile of the 5’ untranslated region of F13A1 gene of coagulation factor XIII (FXIII) A subunit

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Objective: To in silico screen the 5’ untranslated region of F13A1 gene in order to define putative regulatory genetic parameters governing transcription rates and hence FXIIIA subunit levels.

Methods: The 5’ untranslated region of F13A1 gene upstream of the transcription start site (till 5000 bp) was downloaded from ensemble database (www.ensembl.org; accessed on 01.09.2016) (Ensemble ID: ENSG00000124491). The downloaded sequence was screened on the TRANSFAC platform to generate a list of putative regulatory transcription factor matrices corresponding to this region. Similarly a list of polymorphisms and CpG sequences occurring in this region were extracted by indicating the specific gene locus on the Biomart web tool (Ensemble gene 86; Ensemble Gene ID: ENSG00000124491; Ensemble transcript ID: ENST00000264870.7; http://www.ensembl.org/biomart/; accessed on 25.09.2016). The data was combined and analyzed for relevant overlap.

Results: A total of 246 SNPs were located in this region. Fifty CpG’s were found distributed across this region. One hundred and thirty TF matrices were predicted to bind in different parts of this region. Fifty eight of the 130 TF matrices were located on reported variants many of them deletions and insertions. Twenty seven of CpG’s were also located on reported variants. Only six TF matrices were found to be part of those CpG’s on which two SNPs have been reported (rs760544202 and rs3024306).

Conclusion: A high density of variability in this region is an indicator that it might be associated with the high level (almost 2-fold) of variability in FXIIIA antigen levels that is observed and reported from the general population. This data can be further investigated by screening the general population for these interesting variations or expressing them in an in vitro system.
Long-term immunogenicity, safety and efficacy of human-cl rhFVIII in previously treated children with severe hemophilia A

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Poster Topic
Bleeding disorders, coagulation and fibrinolytic factors
17.02.2017, 17:15 - 18:15
Poster Number: 55

Objective: The study investigated the efficacy and safety of long-term prophylactic treatment with Human-cl rhFVIII (Nuwiq®) in previously treated children with severe haemophilia A.

Methods: This prospective, open-label, uncontrolled, multi-center Phase 3b study was open to children who had completed the predecessor study GENA-03 with at least 50 exposure days (ED) and at least 6 months of treatment. Patients continued prophylaxis with injections every other day or 3 times per week. Inhibitor (Bethesda assay, Nijmegen modification) and non-inhibitory antibody tests (ELISA-based) were performed in a central laboratory at study start, then every 3 months and at study completion. Adverse events were recorded throughout the study.

Results: Informed consent was obtained from the patient/legal guardian(s) prior to any trial-related activity. The study enrolled 49 patients from 10 centers across 6 European countries. Their median (range) age at study start was 6.0 (3.0-13.0) years. They received a total of 27.5 million International Units (IU) with 20,518 injections of Human-cl rhFVIII over a median (range) period of 2.5 (0.8-4.4) years: 19,723 injections were given for prophylaxis, 485 for treatment of bleeding episodes, 261 for surgical prophylaxis and 47 for recovery assessments. The mean (± SD) dose per prophylactic injection was 38.6 ± 6.7 IU/kg. The spontaneous annualized bleeding rate (negative binomial regression) was 0.67 (all bleeds 2.88) compared to 1.36 (all bleeds 3.54) of the same patients in the predecessor study. The majority of bleeding events were traumatic (62.2%). There were 24 surgical procedures (12 minor, 12 major) performed in 14 children. The efficacy was rated as “excellent” for all (100%) documented procedures. Only 2 (<0.01%) injections were connected with adverse drug reactions dyspnea, fever after FVIII injection). No patient developed an inhibitor.

Conclusion: The data suggest that long-term prophylaxis with Human-cl rhFVIII (Nuwiq®) in children is efficacious and has a favourable safety profile.
Individualized prophylaxis with Nuwiq® (Human-cl rhFVIII) in adult PTPs with severe hemophilia A

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**Poster Topic**
Bleeding disorders, coagulation and fibrinolytic factors
17.02.2017, 17:15 - 18:15
Poster Number: 56

**Objective:** Pharmacokinetic (PK)-guided personalized prophylaxis with Nuwiq® was assessed in the GENA-21 study in 66 previously treated adult patients (PTPs) with severe haemophilia who were predominantly treated on-demand prior to study entry. In this study, the median half-life was 15.1 hours, the median dosing interval was extended to 3.5 days, 58% of patients were treated with two or fewer infusions per week and 73% of patients were bleed-free. The prolonged dosing interval was achieved with 7% less FVIII consumption per week when compared to regular prophylaxis. The objective of the present study (GENA-21b) is to confirm the data of GENA-21 and to assess the benefit of PK-guided individualized prophylaxis in patients who were predominantly on regular routine prophylactic treatment prior to study start.

**Methods:** The ongoing prospective, open-label, multicenter phase 3b study will enroll 55 adult PTPs with severe haemophilia A from USA, Canada, Europe, and Japan. Each patient will receive Nuwiq® for PK evaluation (single dose of 60 ± 5 IU/kg) followed by 1-3 months of routine prophylaxis (i.e. 30-40 IU/kg every other day or 3 times per week, Phase I). Individual PK data are analyzed by one-stage clotting assay to determine the dose and injection interval which would theoretically result in a trough FVIII level of ≥1%. Thereafter, prophylaxis will continue for 6 months based on the individually recommended treatment schedule (Phase II).

**Results:** Snap shot data will be presented at the GTH. So far 22 patients underwent PK with a median half-life of 15.2 hours. The median treatment interval in Phase I standard prophylaxis is 2.3 days, with a median single dose of 34.0 IU/kg and a median weekly dose of 105.0 IU/kg. During individualized prophylaxis, the recommended median treatment interval is 3.5 days, the median dose/infusion 36.3 IU/kg resulting in a median weekly dose of 84.9 IU/kg. FVIII half-life relationship with von Willebrand factor antigen and blood group will be analyzed.

**Conclusion:** So far, the available data confirm the favorable results of the GENA-21 study with an even lower weekly consumption of FVIII during personalized compared to routine prophylaxis (84.9 vs. 105.0 IU/kg).
Efficacy and safety of Nuwiq® in clinical trials with previously treated patients with severe hemophilia A

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Poster Topic
Bleeding disorders, coagulation and fibrinolytic factors
17.02.2017, 17:15 - 18:15
Poster Number: 57

Objective: Nuwiq® is a recombinant FVIII concentrate without chemical modification or fusion with any other protein, which is produced in a human cell line that adds only human-specific post-translational modifications. It is approved for the treatment and prophylaxis of bleeding in patients with haemophilia A based on the results of 5 clinical studies in 135 adult and paediatric previously treated patients (PTPs) with severe haemophilia A.

Methods: Data from pre-authorisation studies were combined with those from two new studies, an extension study in 49 children and a pharmacokinetic (PK)-guided individualised prophylaxis study in 66 adults.

Results: The data comprise results on 201 PTPs (59 children, 142 adolescents/adults). 179 PTPs were treated prophylactically and 22 adolescents/adults were treated on-demand only. All were to be treated for >6 months and >50 Exposure Days. The mean half-life (one-stage assay) was 17.1±11.2 hours and 15.1±4.7 hours in two adult PK studies (22 and 66 patients), and 12.5±4.2 in 26 children. In the on-demand study, 986 BEs were treated. Efficacy was rated as excellent or good in 94.4% of cases and 97.4% were managed with 1 or 2 infusions. Standard prophylaxis (every other day in adults, every other day or 3-times per week in children) resulted in an annualized bleeding rate (ABR) of 2.3 and 4.0, respectively. In the PK-guided personalised prophylaxis, the ABR was 1.48. 73% of patients were bleed-free, the median dosing interval was extended to twice per week, and 58% of patients were treated with two or fewer injections per week. Median FVIII consumption decreased by nearly 10% compared to standard prophylaxis. In the children’s extension study the spontaneous ABR decreased from 1.36 to 0.67. Haemostatic efficacy was rated as excellent or good for 52 of the 53 surgeries occurring in all studies. Overall, 43,267 injections and >80 million IU of Nuwiq® were administered. Only 9 (0.02%) injections were connected with adverse drug reactions. No inhibitors were detected in any of the patients.

Conclusion: These results show excellent safety and efficacy in treatment of bleeding episodes, a low ABR in particular during personalised prophylaxis, an extended treatment interval under PK-guided personalised prophylaxis and no inhibitors in PTPs.
Interim bleeding data of 522 hemophilia A patients from the international AHEAD Study after 3 years of observation

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Poster Topic
Bleeding disorders, coagulation and fibrinolytic factors
17.02.2017, 17:15 - 18:15
Poster Number: 58

Objective: Continuous long-term real-world data on treatment outcome and safety in a large cohort of hemophilia A patients are still insufficient.

Methods: The AHEAD study is a non-interventional, prospective long-term cohort study including severe and moderate hemophilia A patients treated with ADVATE. Study endpoints include long-term joint health outcomes, annualized (joint) bleeding rates (ABR/AJBR), factor consumption, quality of life and safety data. This report includes data from 3 years of follow up.

Results: Interim report includes data from 522 patients from 21 countries (German sister study is not included), for overall 1,160 patient years. Of these, 334 patients completed year 1, 238 year 2 and 136 year 3 study visits. Median age at screening was 17 years (min-max: 0 – 78) and 57% of patients had severe HA (FVIII<1%); the majority was on prophylaxis.

The median ABRs in year 1, 2 and 3 were 1.2/1.2/1.9 respectively in patients on prophylaxis and 8.4/10.0/7.2, respectively in patients on OD. Median AJBRs were 0.9/0.9/1.0 in the prophylaxis group and 6.4/5.5/5.9 for patients on OD in the first three years of observation. Very similar data were reported taking only severe hemophilia A patients on prophylaxis into account. Moreover, about 44% of patients on on-demand had zero annual joint bleeding rates (AJBR). Effectiveness of prophylaxis assessed by investigators was excellent/good in 93-96% of cases in the three years of observation.

Functionality assessment using the hemophilia activity level (HAL) questionnaire showed a median summary score of 77.3-86.7 for patients on prophylaxis and 67.9-71.3 in patients OD over the 3 year follow up period. Health related quality of life (HRQoL) assessment showed differences in the domain physical functioning (median score of 75-100 in patients on prophylaxis vs. 50-75 on OD) and role physical (median scores of 75 vs. 62.5-75 in patients on prophylaxis and OD, respectively).

Nine patients developed inhibitors that all disappeared spontaneously.

Conclusion: Interim read-out of 3 year follow up of patients enrolled in the AHEAD study show a clinically meaningful difference in ABR/AJBR, HAL, HRQoL of patients on prophylaxis or OD treatment. These data also confirm that the goal of zero bleeds is achievable, although not yet achieved in all patients.
Feiba global outcome study (FEIBA-GO): first demographic data from a real world study on FEIBA in patients with inhibitors

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Poster Topic
Bleeding disorders, coagulation and fibrinolytic factors
17.02.2017, 17:15 - 18:15
Poster Number: 59

Objective: Data on FEIBA`s effectiveness, safety and quality of life are relatively poor. The FEIBA-GO study is designed to capture long-term outcomes on effectiveness, safety and health-related quality of life (HR-QoL) in subjects with severe hemophilia A receiving FEIBA in different settings in routine clinical practice.

Primary objective is to describe the hemostatic effectiveness of FEIBA; most relevant secondary objectives are joint functionality outcomes, safety, HR-QoL, daily activity level, acute and chronic pain associated with hemophilia, healthcare resources used.

Methods: The study is a prospective, non-interventional, multicenter cohort study in patients with hemophilia A or B and high-responding inhibitors treated with FEIBA. A hundred subjects are targeted for enrollment and observed for 4 years; treatment regimens are at the discretion of the attending physicians in accordance with routine clinical practice, either on early, secondary or tertiary prophylaxis or on demand, including patients treated with FEIBA during immune tolerance induction.

Results: As of September 20, 2016, 40 centres have been qualified in 14 countries and 16 initiated. 29 patients have been enrolled at 14 sites in 8 countries.

Data are available for 28 patients: 23 caucasians, 1 african (4 missing). At the enrolment, 21 were on prophylaxis and 7 on demand.

Overall median age is 24.1 years (min-max:3-71), 17 years in patients on prophylaxis (min-max:3-71) and 39 years in those on demand (min-max:5-65).

Overall median inhibitor titer at screening was 10 BU (min-max:1-2410), 7.3 BU for prophylaxis (min-max:1-92) and 15 BU in on demand (min-max:3-2410).

Six subjects (4 on prophylaxis, 2 on demand) have been reported to have overall 7 target joints.

Gilbert Score was assessed in 6 patients at screening.

Assessment for acute pain was performed in 12 patients and for chronic pain in 9. Two of 12 patients assessed reported acute pain (both on demand). Two of the 9 patients in whom the assessment was performed reported chronic pain (treatment regimen missing).
Inhibitor development in previously untreated patients with severe hemophilia A treated with human-cl rhFVIII, a new generation recombinant FVIII of human origin


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Poster Topic
Bleeding disorders, coagulation and fibrinolytic factors
17.02.2017, 17:15 - 18:15
Poster Number: 60

Objective: Studies have shown that the incidence of inhibitor development varies between FVIII concentrates. In the SIPPET study, the cumulative incidence of high-titre inhibitors with hamster-cell derived recombinant FVIII (rFVIII) products was 28.4% vs 18.6% for plasma-derived von Willebrand factor-containing FVIII products (Peyvandi F et al. N Engl J Med 2016;374:2054-2064). However, new generation rFVIII products produced in human cell lines were not included. Human-cl rhFVIII (Nuwiq®) is the first and only rFVIII produced in human cells without chemical modification or protein fusion. No inhibitors were reported in 201 previously treated patients with severe haemophilia A treated with human-cl rhFVIII. The immunogenicity, efficacy and safety of human-cl rhFVIII in PUPs with severe haemophilia A is being assessed in the NuProtect study (initiated 2013).

Methods: NuProtect is ongoing in 17 countries and 38 centres worldwide. One hundred evaluable male PUPs of all ages and ethnics are being studied for 100 exposure days (EDs) or 5 years. No prior treatment with FVIII concentrates or other blood products containing FVIII is permitted. Primary objective is to assess the immunogenicity of human-cl rhFVIII by determining inhibitor activity (≥0.6 BU) using the Nijmegen modified Bethesda assay.

Results: Data for 66 PUPs with ≥20 EDs (the time by which inhibitors are most likely to arise) were analysed in the first pre-planned interim analysis (May 2016). The median age at first treatment was 13 months (range: 3–135). Of 59 patients with available F8 gene mutation analysis, 1 (1.7%) had no identifiable mutation, 44 (74.6%) had high-risk mutations and 47 (81.0%) had null mutations. High-titre inhibitors developed in 8 of 66 patients after a median of 11.5 EDs (range 6–24). Five patients developed a low-titre inhibitor (4 transient). Only 2 patients developed an inhibitor (1 high-titre) after 20 EDs. The cumulative incidence of high-titre inhibitors was 12.8% (95% CI: 4.49–21.15), of low-titre inhibitors it was 8.4% (95% CI: 1.28–15.59) and of all inhibitors it was 20.8% (95% CI: 10.68–30.95). Twelve of 13 inhibitor patients had identifiable F8 gene mutation, all were null, and all but one were high-risk.

Conclusion: These interim data support the low rate of inhibitor development in PUPs treated with human-cl rhFVIII. Final data are expected in 2018.
Autologous serum infusion induces no thrombin formation but generation of activated protein C

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Poster Topic
Bleeding disorders, coagulation and fibrinolytic factors
17.02.2017, 17:15 - 18:15
Poster Number: 61

Objective: We demonstrated previously that infusion of autologous serum does not induce generation of quantifiable amounts of thrombin, even when monitored using a highly-sensitive oligonucleotide-based enzyme capture assay (OECA) which detects thrombin in the low picomolar range. Objective of the present study was to evaluate if the small amounts of free thrombin present in the serum induce generation of activated protein C (APC).

Methods: 15 healthy subjects received infusions of 50 mL of autologous serum over 30 min without accompanying medication, while the thrombin inhibitor argatroban was infused into four volunteers simultaneously with the serum at a dose of 1 µg/kg/min. APC and thrombin were measured before, during and after serum infusion using an OECA platform. In in vitro experiments, APC formation was induced by addition of serum or purified thrombin to buffer containing CaCl2 at physiological concentrations. and protein C as well as thrombomodulin in excess. APC generation was subsequently measured by OECA.

Results: Median (interquartile range) concentrations of thrombin and APC were 185.56 (128.61 - 242.50) pmol/L and 163.75 (136.25 – 248.21) pmol/L in the autologous serum, thus doses of 3.33 (1.94 – 4.17) pmol/L of thrombin and (2.50 – 3.93) pmol/L of APC were infused per mL of the probands’ plasma volume. Peak thrombin levels of 1.11 (0.00 – 2.22) pmol/L were measured in plasma of the subjects without anticoagulation, indicating a rapid inactivation. APC peak levels in plasma exceeded the infused APC doses by a multiple (25.18 (13.57 – 53.04) pmol/L), even in samples from probands that received argatroban (16.79 (14.11 – 21.79) pmol/L). In the in vitro experiments addition of argatroban at the concentrations achieved in the probands completely abolished APC generation up to a thrombin concentration of 138.89 pmol/L. Addition of human serum as a thrombin source in the same purified system consistently induced formation of greater amounts of APC than expected on the basis of the amount of thrombin present in the serum samples.

Conclusion: The data presented here suggests a mechanism of APC generation that does not depend on thrombin. We hypothesize that further experiments with endothelial cells will shed more light on this alternative way of APC generation.
Treatment of acquired hemophilia A with recombinant porcine FVIII in Germany

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Poster Topic
Bleeding disorders, coagulation and fibrinolytic factors
17.02.2017, 17:15 - 18:15
Poster Number: 62

Objective: Here we report on the clinical outcome of four patients from Germany treated for acquired hemophilia A (AHA) with recombinant porcine FVIII (rpFVIII). The patients were treated in various institutions and by different physicians.

Methods: All four cases were referred from external hospitals with a major hemorrhage. Three of four cases were initially treated with bypassing agents however with limited efficacy. Subsequent treatment with rpFVIII was initiated with 100 U/kg bodyweight and 50 U/kg bodyweight, respectively.

Results: The FVIII activity increased from 13 % - 120 % within 30 min after the first rpFVIII dose in three of four cases in which the baseline anti-rpFVIII antibody titers were below 0.6 BU. rpFVIII administration was associated with satisfactory hemostasis within 24 hours. In the fourth case no increase in FVIII activity could be measured due to a baseline anti-rpFVIII antibody titer of approximately 100 BU. Here several cycles of immunoadsorption and plasmapheresis resulted in a decrease of anti-rpFVIII antibody titer (< 20 BU). The following administration of 100 U/kg bodyweight rpFVIII elevated FVIII activity to 114 % with satisfactory hemostasis within 24 hours.

Subsequent doses of rpFVIII were in a range between 25 and 100 U/kg bodyweight. FVIII activity was maintained at levels between 2 % - 136 % during subsequent rpFVIII therapy. No adverse events associated with rpFVIII, including thrombocytopenia or thromboembolism, were observed. Two patients survived with successful inhibitor eradication, one patient died from E-coli sepsis with an inhibitor present and one patient was discharged and died 3 months later from acute liver and kidney failure unrelated to AHA (human inhibitor titer was negative).

Conclusion: rpFVIII administration resulted in rapid bleeding cessation. Insufficient FVIII increase after initial rpFVIII dosage due to baseline cross reactivity in one patient could be altered by immunoadsorption and plasmapheresis. Loading doses and median subsequent doses of rpFVIII were lower than those stated in the SPC. Treatment decisions were based on FVIII activity levels as well as clinical response. The considerable variability in treatment requirements and responses among these cases describe the need of treatment by experts from Hemophilia Treatment centers.
Pulmonary embolism in a patient with severe factor VII deficiency after femoral neck fracture despite thromboprophylaxis with low-molecular-weight heparin

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Poster Topic
Bleeding disorders, coagulation and fibrinolytic factors
17.02.2017, 17:15 - 18:15
Poster Number: 63

Objective: Thromboembolic complications are rare in patients with factor VII deficiency and mostly associated with replacement therapy or other thrombotic risk factors. In addition, it is known that residual factor VII activity does not precisely predict bleeding risk.

Methods: We report the case of a 75-year-old male patient with severe factor VII deficiency and pulmonary embolism.

Results: The patient presented after 4 weeks of conservative treatment of femoral neck fracture. Despite the previously known severe factor VII deficiency (FVII:C, < 1%) he obtained thromboprophylaxis with certoparin 3000 anti-Xa units daily. His previous medical history was uneventful in terms of bleeding complications, but no major surgery had been performed; factor VII deficiency was diagnosed in 2003 due to pathological coagulation tests. On admission, because of dyspnoea, tachycardia and hypotension, bilateral pulmonary embolism (PE) and pneumonia were diagnosed. Anticoagulation was initiated with therapeutic unfractionated Heparin (UFH). Two bleeding episodes (epistaxis and after tracheostoma manipulation) were treated successfully with a single dose of plasmatic factor VII concentrate (Immuseven^TM) at 10-20 IU/kg body weight (BW). Eleven days after diagnosis of PE, surgery with hip joint replacement was performed after a single dose of 20 IU/kg BW factor VII without bleeding complications. Anticoagulation with UFH was soon restarted, and he was discharged to rehabilitation with certoparin 8000 anti-Xa units OD. Six weeks after diagnosis of PE, anticoagulation was reduced to a prophylactic dose due to recurrent epistaxis.

Further laboratory work-up showed no severe thrombophilia apart from elevated factor VIII and fibrinogen levels. Unfortunately, determination of the FVII antigen level and testing for a F7 gene mutation could not be performed. Native thrombelastometry showed a procoagulant state. Interestingly, we observed increased thrombin generation compared to commercially available factor VII deficient and normal human plasma.

Conclusion: Although severe factor VII deficiency is perceived as a bleeding disorder, patients are not always protected against thromboembolic complications. Thorough individual risk evaluation considering bleeding history and thrombotic risk factors should thus be performed.
Genetic therapy for hemophilia: What’s new?

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Poster Topic
Bleeding disorders, coagulation and fibrinolytic factors
17.02.2017, 17:15 - 18:15
Poster Number: 64

Objective: The standard-of-care therapy for haemophilia comprises frequent prophylactic administrations of factor concentrates to maintain factor levels >5%. This prophylaxis is associated with impaired quality of life for patients and high costs for the health care system. Numerous gene therapeutic strategies aimed at introducing functional copies of the mutated FVIII or FIX genes into somatic cells that then in vivo produce the missing factor have been developed. Here we summarize the recent advances in research and outline the clinical trials.

Methods: Review of literature and the clinicaltrials.gov website

Results: In preclinical studies, numerous gene therapy system have been utilized successfully to stably introduce the FVIII or FIX cDNAs into different target cell populations. For in vivo gene transfer, the most important vector systems were AAV, lentiviral, retroviral and nonviral vectors including DNA and the piggyback transposons. So far, only AAV-based vectors have achieved any meaningful efficacy in animal models or in early gene therapy trials. Currently, all ten clinical trials listed at the clinicaltrials.gov website are using a single i.v. injection of high-dose AAV vectors with different serotypes to express the therapeutic transgene(s) in the liver. Predominantly due to the limitations in transgene size (<4.5 kb) that can be efficiently delivered by AAV vectors, nine phase I/II studies are focussing on FIX. Only one study (NCT02576795, Biomarin) uses B-domain deleted FVIII as transgene. Six studies are actively recruiting, three are active but nonrecruiting and one is terminated. One ambitious study (NCT02695160, Sangamo) proposes to use coinjection of three different AAV vectors for delivery of two Zinc finger proteins and a promotorless FIX cDNA that is directed for integration in the highly expressed albumin intron 1. So far, limited data from the ongoing trials has been published, but the intermediate results seem to be quite promising, despite very high numbers of vector genomes/kg body weight (up to 6x10^13 vg/kg) and strong immune reactions directed against the AAV capsids. An interesting approach for haemophilia B uses a FIX variant with a gain-of-function mutation (Padua), thereby addressing the problem of low factor activities.

Conclusion: Despite ongoing challenges, genetic therapy of haemophilia A/B has finally come of age.
In-vitro analysis of siRNA based inhibition of function of activated protein C (APC): Towards an alternative therapeutic option for hemophilia.

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**Poster Topic**
Bleeding disorders, coagulation and fibrinolytic factors

**17.02.2017, 17:15 - 18:15**
Poster Number: 65

**Objective:** To analyze different siRNA sequences directed against different functional domains of protein C gene (PROC) and study their silencing effect on protein C in an in vitro and in vivo system using human hepatic cell lines and mice respectively, which may serve as an alternate therapeutics for hemophilia management.

**Methods:** Human HepG2 hepatic carcinoma cells were cultured and reverse transfected using various concentrations and different combinations of different siRNA's specific to PROC, in presence of lipofectamine, to down regulate the PROC gene expression. After successful transfections of particular siRNA, RNA was isolated from the transfected cells and was converted to cDNA using reverse transcriptase followed by PCR amplification using real time PCR. PROC down regulation was determined using qRT-PCR by calculating fold change.

**Results:** The individual siRNA against;
- Exon 3 of PROC showed 35% of gene silencing at 10pmol concentration and 75% silencing with 12pmol concentration.
- Exon 9 of PROC showed 25% of gene silencing at the concentration of 3pmol and 70% silencing at 6pmol concentration.
- Exon 7 of PROC showed 50% of gene silencing at the concentration of 6pmol and 80% silencing at 10pmol concentration.

The combination of siRNA against;
- Exon 3 and exon 9 of PROC showed 27 % of gene silencing even at 1.5pmol concentration and 67% silencing at 3pmol concentration.
- Exon 9 and exon 7 of PROC showed 36% of gene silencing even at 3pmol concentration and 59% silencing at 4.5pmol concentration.
- Exon 3 and exon 7 of PROC showed 34% of gene silencing even at 1.5pmol concentration and 58% silencing at 3pmol concentration.

This shows that siRNA targeting different exons of protein C gene in combinations gives higher silencing effect at lower concentration than individually.
Novel insights in the molecular etiology of recessive von Willebrand disease 2N due to D’D3 Factor VIII-binding site mutations of von Willebrand factor

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Poster Topic
Bleeding disorders, coagulation and fibrinolytic factors
17.02.2017, 17:15 - 18:15
Poster Number: 66

Objective: Introduction. Von Willebrand disease (VWD) type 2N is caused by a Factor VIII (FVIII) binding site on von Willebrand factor (VWF) located in the D’ region of VWF (766-864). The D’ region consists of 2 domains: trypsin-inhibitor-like (TIL’) and E’. The trypsin-inhibitor like (TIL’) E’ domains form a protrusion from the D3 domain, thereby presenting the TIL’E’ domains to the physiological binding partner FVIII. A detailed characteristic of the VWF-FVIII binding site has remained intractable until recently when the two distinct and conserved domains TIL’(residues 766-827)E’(residues 829-863) were shown to contain a majority of the known type 2N mutations. This has led to better insights in the structure of the FVIII binding domain in the VWF protein and the VWF binding domain in FVIII protein, which prompted to look into the molecular etiology and the clinical and laboratory manifestation on autosomal recessive von Willebrand disease (VWD) type 2N.

Methods: FVIII binding D’ domain of VWF. The TIL’E’ domains are located immediately after the Furin cleavage site and form independently folding domains separating D’ into two distinct domains and all cysteine residues of these domains form disulfide bridges (Cys767-Cys808;Cys776-Cys804;Cys810-Cys821) (Figure 2). The TIL’ and E’ domains tumble as a single entity with only limited inter-domain motion [16]. TIL’ is formed of short beta sheets 12 (residues 772-775;806-809) and 34 (residues 814-817;820-823). The 1-to-2 loop encompass an eight residue insertion between the second and third conserved TIL’ cysteine residues. The second antiparallel sheet, formed of strands 3 and 4, is connected by a reversed turn forming a small hairpin structure. TIL’ and E’ are connected at 827-829. The greater proportion of E’ is formed of a triple-stranded antiparallel sheet formed of strands 3 (residues 839-844), 4 (residues 847-852) and 5 (residues 855-858). The N-terminus of E’ contains a short double strand 12 sheet (residues 829-831:834-836). The relative positions of these two sheets are restrained by the E’ Cys829-Cys851 disulfide bond. The dynamic characteristics of TIL’E’ is of great importance for elucidating the mechanism by which FVIII is stabilized in plasma.

Results: Results. Correct pairing of the 16 cysteine residues and hence correct folding of the TIL’E’ is of critically importance for the binding between TIL’E’ and FVIII. The homozygous Factor VIII binding defects (FVIII-BD) R854W/R854W and R816W/R816W in the D’ domain (VWD 2N in its purity) result in a clinical phenotype of mild/moderate hemophilia A with normal bleeding time and VWF function. The E787K, T791M and R816W mutations have pronounced type 2N VWD with decreased FVIII-BD and normal VWF functions. Type 2N mutations that involve a cysteine (C788R/Y, Y795C, and C804F in TIL’, C858S/F in E’) are associated with aberrant multimerization and poor secretion on top of reduced FVIII-BD. Two mutations, T791M and R816W together with T789P, M800V and H817Q are clustered around a region of positive charged density of TIL’. VWD type 2N patients with the heterozygous mutation near to or at the Furin cleavage site R763G present with VWD type 2 in early childhood featured by a smeary pattern and ultralarge VWF multimers caused by the mixture of circulating R763G mutant Pro-VWF and mature wild type VWF on top of a
Protein S and purpura fulminans: What’s new?

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**Poster Topic**
Bleeding disorders, coagulation and fibrinolytic factors

**Objective:** Complete protein S (PS) deficiency causes purpura fulminans (PF) characterized by disseminated intravascular coagulation (DIC) and skin necrosis. PF is thought to result from the imbalance between pro- and anticoagulants. Beyond its role in coagulation, PS exerts cellular functions through receptor tyrosine kinases Tyro3, Axl and Mer. The aim of this work is to mimic severe PS deficiency and monitor PF development.

**Methods:** Pros1+/- and Pros1+/+ mice were treated with warfarin (0.8mg/day). E15 embryonic dorsal skin vasculature was explored by immunostaining: anti-VE-Cadherin, anti-Ter119, anti-CD31, anti-Lyve1 and F4/80 antibodies. Colony forming assays with fetal liver cells allowed us to study erythropoiesis.

**Results:** Warfarin caused 95% mortality in Pros1+/- and 5% in Pros1+/+ mice, but only a few Pros1+/- mice developed PF. Early PF lesions showed vascular engorgement, intradermal edema and rare thrombosis. In advanced lesions, we noticed massive red blood cell (RBC) extravasation, intra-epidermal hemorrhagic blisters and necrosis. RBC extravasation in mice treated by warfarin pointed to vascular wall damage during PF. Immunostainings of embryonic dorsal skin (VE-Cadherin, Ter119) confirmed RBC extravasation while anti-CD31 showed areas with underdeveloped and less dense vascular networks and less vessels branch points in Pros1-/- than in Pros1+/+ mice. Massively enlarged lymphatic vessels and increased macrophage infiltration were observed in Pros1-/- embryos (Lyve1, F4/80). Blood vessel and liver histology from Pros1-/- embryos revealed numerous circulating immature RBC compatible with increased erythropoiesis because of severe bleeding due to DIC and vascular lesions. In addition, many isolated erythroblast nuclei were found indicating altered phagocytosis. Pros1-/- embryos displayed higher cytokine levels than Pros1+/+ embryos. No difference was found between Pros1-/- and Pros1+/+ embryos for BFU-E colonies, whereas Pros1-/- displayed 50% less CFU-E colonies than Pros1+/+ mice, pointing to a blunted response to erythropoietin (EPO) in the context of inflammation promoted by PF. These results were confirmed by flow cytometry.

**Conclusion:** PF developed under warfarin and in Pros1-/- embryos and was characterized by thrombosis occurring together with vascular damage and inflammatory processes disturbing nuclei phagocytosis, iron metabolism and response to EPO.
Successful factor VIII re-exposure after long-term aPCC prophylaxis in two hemophilia A patients with inhibitors

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Objective: Inhibitor development is still the most severe complication in modern hemophilia A treatment and the eradication of inhibitors during immune tolerance induction (ITI) is a great challenge. After failed ITI, patients are treated on demand or by prophylaxis with bypassing agents including aPCCs. We report on two patients that were successfully re-exposed to factor VIII (FVIII) after failed ITI and aPCC treatment.

Methods: Two patients aged 24 and 66 years with a longstanding history of inhibitors were closely monitored for coagulation, pharmacokinetic and immunological parameters during reexposure to FVIII.

Results: Both patients had been positive for FVIII inhibitors for years. Earlier ITI had failed. The younger patient was on prophylaxis with aPCC (FEIBA®), the older was treated on demand. Thus both patients had a minimal dose FVIII exposure during aPCC treatment over years. Both patients were negative for FVIII inhibitors at the time of re-exposure, but they were discordant for FVIII non-neutralizing antibodies. The patients were re-exposed with either pdFVIII with high vWF-content or rFVIII-Fc. The patient negative for FVIII antibodies before re-exposure never developed any neutralizing or non-neutralizing antibodies during the first 100 re-exposure days. Of note, after 10 days of re-exposure the FVIII recovery and half-life decreased dramatically and only recovered to initial values after another 8 days of rFVIII-Fc treatment. The patient positive for FVIII non-neutralizing antibodies at re-exposure also did not develop any inhibitors so far: non-neutralizing FVIII inhibitors remained detectable. The anti-FVIII IgG titre increased after re-exposure with antibodies mainly directed against the light chain and without any detectable anti-A2 antibodies, but with a change in the IgG-subclass distribution from IgG1 to IgG2 and 4.

Conclusion: After long-term aPCC exposure in patients with inhibitors FVIII re-exposure appears feasible for effective FVIII treatment. Monitoring of immunological and coagulation parameters is essential as anamnestic or d novo immune responses may occur and as the mechanisms of tolerance are poorly understood.
rVIII-SingleChain in hemophilia A patients: A population pharmacokinetic model

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Objective: rVIII-SingleChain, a novel recombinant Factor VIII (FVIII), is a single-chain construct in which a truncated B-domain covalently links the heavy and light chain. A model was developed to characterize the population pharmacokinetics (PPK) of rVIII-SingleChain in adult and pediatric subjects with hemophilia A and to support clinical dosing.

Methods: A total of 130 hemophilia A patients taking part in the AFFINITY clinical trial series (≥12–65 years [n=91]; 0–<12 years [n=39]) were dosed with a 50 IU/kg dose of rVIII-SingleChain and serial blood samples were taken over a 72-hour period. Plasma FVIII activity was determined using a validated chromogenic assay. The data were analyzed using a nonlinear mixed effects modeling approach (NONMEM). The model was evaluated using a visual predictive check (VPC). The effect of various covariates in the PPK model were analyzed, including anti-drug antibody levels, body weight, baseline von Willebrand factor (VWF) and hematocrit. The final FVIII activity PPK model was used to simulate FVIII activity-time profiles for a range of dosing regimens.

Results: A two-compartmental model with first-order elimination adequately described the FVIII plasma activity data following rVIII-SingleChain dosing. For central distribution volume body weight was found to be significant covariate; for clearance body weight and levels of VWF were significant covariates. The model evaluation was deemed adequate and showed good precision by VPC. Using the final PPK model, simulations predicted that 63–93 % patients would maintain FVIII activity levels above 1% with rVIII-SingleChain dosing at 20–50 IU/kg 2- or 3-times weekly.

Conclusion: The PK of rVIII-SingleChain in adult and pediatric hemophilia B patients was well described by the PPK model. The model can be used to simulate FVIII activity-time profiles for various dosing regimens. The PPK simulation supported the dose regimens of 20–50 IU/kg rVIII-SingleChain 2- or 3-times weekly, so that the majority of patients maintain total FVIII activity levels above 1%.
Comparison of data security of the electronic patient diary smart medication™ with manual documentation in a paper diary

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Poster Topic
Bleeding disorders, coagulation and fibrinolytic factors
17.02.2017, 17:15 - 18:15
Poster Number: 70

Objective: Comparison of data security of the electronic patient diary smart medication™ with manual documentation in a paper diary

Methods: The risk assessment method was used to compare risks associated with the electronic documentation platform smart medication™ with the traditional methods of paper documentation. Conceivable scenarios, such as loss of data, incorrect data transmission and possible misuse of data have been analyzed and evaluated using a risk-based approach.

Results: The risk assessment revealed clear advantages for the electronic patient diary in particular in the following areas:

- no irreversible data loss possible
- error minimization through plausibility checks during the patient's entry and later by the physician or the haemophilia nurses
- seamless traceability of all data inputs and amendments
- highly reduced risk of data misuse due to pseudonymization and access rights control to the electronic diary
- sustainable long-term storage reliability

Conclusion: The results show that the electronic patient diary is clearly superior to paper documentation in terms of the confidentiality, security and integrity of the data.

However, the selection and implementation of appropriate measures for minimizing risks is decisive. In doing so, product manufacturers must take into account not only the relevant standards, directives and laws, but also technical frameworks such as the recommendations of the Federal Office for Information Security (Bundesamt für Sicherheit in der Informationstechnik, BSI) or standard ISO 27000 ff. on information security.
Development and evaluation of a novel FVIII domain-specific immunoassay for characterization of anti-FVIII antibodies

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Poster Topic
Laboratory tests
17.02.2017, 17:15 - 18:15
Poster Number: 71

Objective: FVIII neutralizing antibodies develop in response to replacement treatment in 30% of patients with severe hemophilia A. Such inhibitory antibodies represent a serious complication in the treatment of these patients. The aim of the present study was the development and evaluation of a microsphere-based immunoassay on the Luminex™ system for rapid detection and early characterization of anti-FVIII antibodies in patient plasma samples.

Methods: The FVIII protein was structured into several single and multi-domain fragments and introduced to a baculovirus expression system. The domains were purified (>80%) and individually coupled to colored-coded magnetic microspheres. Commercially available full length (FL-FVIII) and B-domain deleted (BDD-FVIII) FVIII proteins were immobilized accordingly. The coupled target proteins were used to establish the Luminex™ based assay, termed LumiTope.

Results: Different FVIII constructs were prepared and verified by western blotting. Later, eight constructs (A1a1, A2a2, A1a1A2, a3A3, C1, C2, C1C2 and Light chain (LC)) were chosen and purified in large scale. All prepared microspheres were tested in singleplex and multiplex assay formats on the Luminex™ system. Then, LumiTope was applied on plasma samples from healthy controls, patients with FVIII inhibitors. The first results show that LumiTope is a sensitive test for detection of anti-FVIII antibodies and can run in a routinely based multiplex-configuration covering the whole FVIII protein. LumiTope revealed positive results against FL-FVIII beads for all patients with antibody titers> 0.6 BU/ml and positive results on FVIII-ELISA antibody test. Moreover, we were able to identify several monoclonal and polyclonal antibodies against A2a2, a3A3, LC, C1, C2 and C1C2 domains. Detected antibodies were predominantly directed against the A2a2 and C2 domains of FVIII.

Conclusion: Our new immunoassay, LumiTope, provides a sensitive and fast method for early characterization of inhibitory anti-FVIII antibodies in hemophilia A patients. The characterization of the binding regions of these antibodies may provide the basis for better understanding of inhibitory mechanisms and help for the eradication of FVIII inhibitors.
Activation and inactivation of rVIII-SingleChain

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Poster Topic
Laboratory tests
17.02.2017, 17:15 - 18:15
Poster Number: 72

Objective: Activation and inactivation of FVIII is an essential pathway in the regulation of coagulation. Thrombin activates FVIII leading to the formation of active FVIII (FVIIIa), and is also able to inactivate FVIIIa by further proteolytic degradation. Activated protein C (APC)/protein S also mediates the proteolytic degradation of FVIIIa. The dissociation of the A2 subunit from the FVIIIa trimer also results in inactivation. The present study assessed the activation and inactivation of rVIII-SingleChain, a novel recombinant FVIII product with covalently linked heavy and light chain.

Methods: rVIII-SingleChain and various rFVIII products were investigated. The formation of FVIII fragments was visualized by SDS-PAGE / CBB stain and/or Western Blot using anti FVIII antibodies recognizing the FVIIIa components (light chain, A1 and A2 domains). The activity was measured by a one-stage clotting FVIII activity assay using FVIII deficient plasma (Siemens Healthcare) and Pathromtin SL® (Siemens Healthcare) as activator reagent.

Results: The incubation of rVIII-SingleChain and rFVIII comparator products with thrombin resulted in proteolytic activation and formation of the expected FVIIIa band pattern. In particular, SDS-PAGE analysis demonstrated the cumulative appearance of the light chain and the A1 and A2 domain. Further incubation led to the formation of smaller degradation fragment(s). The incubation of FVIIIa with APC/protein S also resulted in the degradation of the A2 domain followed by the A1 domain into smaller fragments. The FVIII activity under these conditions decreased to values of below 1 % of the initial activities. Without APC/Protein S, A2 dissociation was comparable for rVIII-SingleChain and the rFVIII products.

Conclusion: The data of this study demonstrate functional comparability of rVIII-SingleChain and the FVIII comparator products analyzed with respect to the kinetics of FVIIIa inactivation by A2 dissociation and proteolytic degradation via APC/protein S or thrombin. A similar thrombin activation profile of rVIII-SingleChain and rFVIII products was shown. Slight differences in the activation intermediates are most likely due to the different molecular design of rVIII-SingleChain compared to two-chain FVIII molecules.
Protein Z - Does its analyzation make any clinical sense?

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**Poster Topic**

**Laboratory tests**

17.02.2017, 17:15 - 18:15

Poster Number: 73

**Objective:** Protein Z (PZ) is a vitamin K-dependent (VKD) plasma glykoprotein belonging to different natural anticoagulant systems involved in the regulation of blood coagulation. The literature is mentioning an association between a low level of Protein Z and haemorrhage, thromboembolic events and miscarriage. But the role of PZ plasma level and PZ gene polymorphisms remains debated with conflicting results.

For that reason, we decided to collect the data of patients in our centre, who got measured PZ.

**Methods:** PZ-concentration was measured by an ELISA. The datas of 707 patients (552 females and 155 males) were collected including age, sex, bleeding score, body mass index BMI, VWF-Activity, number of venous and of arterial thromboembolism, drug intake and number of miscarriages. Patients receiving Vit. K-Antagonists were excluded. The differences of medians of the PZ-level of different categories were examined with Mann-Whitney-U-Test or ANOVA.

**Results:** PZ does not differ between sexes but is raising with the age significantly (p=0.011). We did not see any difference in the PZ-level between the woman suffering from Hypermenorrhea or not. Also there was no difference between women suffering from miscarriage or not.

No significant difference was also seen between patients suffering from venous thromboembolism or from arterial thromboembolism. We also get no significant difference between over- and underweight patients, so PZ does not seem to be BMI-dependent. Furthermore we could not see any association of PZ with the bleeding score of the patients or with the activity of the Von Willebrand factor. Women taking contraceptives with ethinyloestradiol had a 28% higher PZ-concentration than women not taking these contraceptives. (p=0.011)

**Conclusion:** In this overview over our patients, we did not find any evidence for a reasonable use of PZ in clinical routine.
Platelet aggregation testing on a routine coagulation analyzer

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Poster Topic
Laboratory tests
17.02.2017, 17:15 - 18:15
Poster Number: 74

Objective: Measurement of thrombocyte function is time consuming and technical challenging influenced by many pre-analytical and analytical variables as well as the lack of standardization and automation.

Methods: Two different aggregometers were compared: the fully-automated routine coagulation analyzer Sysmex CS-system (Siemens) and the semi-automated APACT aggregometer (Haemochrom), a specific instrument for platelet aggregation analysis. On both analyzers platelet aggregation agonists from Hyphen© were applied, including adenosine diphosphate (ADP), arachidonic acid (AA), ristocetin (RI), collagen (CO) and epinephrine (EPI). In total, 123 patient samples were investigated: 22 patients taking ASS, 20 patients taking clopidogrel (17 plus ASS), 20 patients with different known platelet dysfunctions and 61 healthy controls. Aim of this study was to test the accuracy and comparability of the two systems for platelet function testing.

Results: Overall, there was good correlation between both systems for maximum aggregation and maximum velocity with correlation coefficients in the range of 0.83-0.97 and 0.77-0.95, respectively.

For patients with ASS intake the maximum aggregation of AA was reduced at median 5.5 (95 %SI 0.4 – 18.7) at CS vs. 14.1 (95 %SI 3.8 – 32.4) at APACT.

Conclusion: Platelet function measurements performed with the CS system and APACT show comparable results and a strong positive correlation. The automated processing of platelet aggregation testing on the Sysmex CS analyzer reduced the hands-on time and provides a step forward to a more standardized processing.
Unambiguos and sensitive measurement of active coagulation proteases

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**Poster Topic**
Laboratory tests
17.02.2017, 17:15 - 18:15
Poster Number: 75

**Objective:** The advent of chromogenic substrate has revolutionized protease measurements in terms of sensitivity, but has not fully resolved issues related to selectivity. When measuring closely related proteases usually the sum of activity rather than the activity of a single protease is determined. Adding specific inhibitors is a valid approach, but increases the complexity of the assay. Here we describe the use of solid phase-bound protease inhibitors to measure active proteases by formation of the immobilized inhibitor-protease complex and its immunological detection by zymogen-specific antibodies. We demonstrate the applicability of this concept for activated coagulation factors XII (FXIIa), factor XI (FXIa), kallikrein (KK) and factor Xa (FXa).

**Methods:** The protease inhibitors corn trypsin inhibitor (CTI), C1-inhibitor (C1-inh), and tissue factor pathway inhibitor (TFPI), diluted in PBS and coated to polystyrene plates, were used to set up specific assays for measurement of FXIIa, FXIa and FXa. Detection of the formed inhibitor-protease complexes was achieved by the corresponding peroxidase-labelled polyclonal anti-zymogen antibodies, namely, anti-human FXII, anti-human FXI, and anti-human FX. Calibration curves were obtained using purified, active protease preparations.

**Results:** The effect of related proteases was determined by measuring of FXIIa in the presence of FXI and kallikrein, FXIa in the presence of KK and FXIIa, and FXa in the presence of plasmin, FVIIa, FXIIa, thrombin, and KK. The selectivity of the FXa measurement was confirmed by competition with the synthetic FXa inhibitor rivaroxaban. Furthermore, the FXa determination also worked in the complex matrix of an activated prothrombin complex concentrate. Sensitive calibration curves were obtained to measure the four proteases used as model systems. All mixing experiments demonstrated the assay selectivity as even high excesses of related proteases demonstrated no effect.

**Conclusion:** The high selectivity depends on the specificity (i) of the inhibitor and (ii) of the detection antibody used to measure the amount of complex formed on the solid phase.
Quantification of TAFI in human plasma by LC-MS/MS

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Objective: Thrombin activatable fibrinolysis inhibitor (TAFI) is supposed to play a role in thrombotic disorders as well as in inflammatory diseases. Further, a defective activation of TAFI might be related to the severity of bleeding disorders like hemophilia A. To measure plasma TAFI concentrations, different methods have been developed based on antigen determination and measurement of TAFI activity after quantitative conversion of the proenzyme. These assays have major drawbacks. They are lacking standardization and show different analytical sensitivity to the TAFI cleavage products. Mass spectrometry has the potential to overcome some of observed assay limitations. In the case of proteins, a specific tryptic peptide can be selected as a stoichiometric representative of the protein from which it is cleaved.

Methods: Our aim was to use LC-MS/MS to quantify TAFI activity by Liquid Chromatography/Electrospray Ionization Mass Spectrometry. We investigated different sample pretreatment strategies and developed a standardized sample pretreatment protocol for absolute quantification of TAFI in human plasma by LC-MS/MS using proteotypic peptides and corresponding stable isotope-labeled peptides as internal standards.

Results: With isotopic labeled peptides as matched internal standards, we established a quantitative assay for the proteotypic peptide DTGTYGFLLPER and compared it to a commercially available activity assay (r = 0.95) and proTAFI immunoassay (r= 0.96). We have tested several methods of sample processing and established a fast and sensitive chromatographic assay. The validated preanalytical protocol enables a reliable analysis of TAFI antigen in human plasma without depletion.

Conclusion: The assay can be very useful in future research into the identification of pathological conditions where TAFI is generated and would be helpful for standardization.
Diagnostic challenges of von Willebrand disease

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Objective: Von Willebrand disease is caused by mutations of von Willebrand factor, a complex protein, which harbours different structural and functional domains. A normal high molecular weight multimer (HMWM) structure of VWF is essential for its proper function under high shear conditions in the microcirculation and arteries. Due to its complexity, the function of VWF in primary haemostasis and in coagulation cannot be assessed by a single test. Besides quantification of VWF:Ag by ELISA methods, a number of assays have been developed, which measure particular functions of VWF like VWF:GPIb binding, VWF:collagen binding and VWF:FVIII binding. VWF:clearance from the circulation can be estimated by the ratio of VWF:Ag to the VWF:propeptide. However, none of these parameters describes the essential VWF shear-dependent functions, which are definitely dependent on VWF HMWMs under high shear conditions. We assessed the diagnostic power of VWF multimer analysis on the correct diagnosis of VWD.

Methods: From 2002 until 2015, a cohort of 3,669 patients were diagnosed with VWD type 2 by assessing the ratio of VWF:CB/VWF:Ag, and by multimer analysis.

Results: By multimer analysis, patients were diagnosed with either VWD type 2A/2B and enhanced ADAMTS13 proteolysis, patients with VWD type 2A/IIE with reduced proteolysis, patients with VWD type 2M and patients with type 2A/IB (s. Table). Among them, we identified patients with normal functional parameters and normal VWF:Ag, displaying an abnormal electrophoretic migration due to cysteine mutations in the carboxy-terminal region of VWF, implicated in VWF lateral self-association in a high shear environment. The percentages of the correct diagnosis by using the VWF:CB/VWF:Ag ratio compared to VWF multimers are given in Table 1.

Conclusion: Dependent on the type, a significant number of patients with VWD would remain undiagnosed by conventional quantitative and functional assays but without multimer analysis. A normal structure of VWF HMWM in plasma is the result of proper synthesis, secretion, clearance, responsiveness to shear and normal size regulation by ADAMTS13. Since all these parameters influence the structure of VWF multimers, their analysis is further on an indispensable tool for a valid diagnosis or exclusion of VWD. In addition, the multimer pattern hints to the site of particular mutations causing VWD.
A rapid and simple assay for the determination of ADAMTS-13 activity

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Poster Topic
Laboratory tests
17.02.2017, 17:15 - 18:15
Poster Number: 78

Objective: In recent years the determination of ADAMTS-13 activity in plasma has become an important diagnostics test in the differentiation of Thrombocytopenic Purpura (TTP) patients from those with other forms of Thrombotic microangiopathies.

The standard laboratory test for ADAMTS-13 Activity is an ELISA assay, which has an assay which requires 4 hours to process. This assay tends to be performed by specialized laboratories, so samples are usually batch tested. Therefore for a physician to receive a patient’s ADAMTS-13 activity result can range from 24 hours to as much as one week. This delay in reporting could have a negative impact on patient care.

Methods: We have developed a rapid and simple assay which would allow the physician to have an initial reportable result for ADAMTS-13 Activity within 1 hour from the blood draw.

The assay is based on flow through technology, which allows for simple, rapid processing. Plasma samples are pre-incubated with a substrate which is cleaved by ADAMTS-13 present in the plasma. This pre-treated sample is applied to the single test unit which contains a membrane with immobilized antibodies specific for the cleaved substrate. As the sample flows through the membrane into an absorbent pad the cleaved substrate binds to capture antibody. A secondary biotinylated antibody is used to detect the membrane bound complex. Using a Streptavidin-gold conjugate a red colour is produced. The colour intensity is proportional to the level of ADAMTS-13 activity in the plasma sample and compared with a colour standard card, allowing semi-quantitative analysis of the patient sample.

The assay can be performed at room temperature without specialized laboratory equipment and minimal training is needed to perform the test. The assay processing time is under 30 minutes. The test is designed for evaluation of a single patient sample, with a positive control run in parallel.

Results: The designed assay cut-off value of 0.1IU/mL ADAMTS-13 Activity is clinically relevant for differentiation of TTP patients. A 100% correlation was observed when TTP patient samples (n=20) were run in the TECHNOZYM ADATMS-13 ELISA and this assay.

Conclusion: This rapid and simple assay is versatile and suitable as a cost effective screening assay enabling quick turn around for ADAMTS-13 activity reporting.
Evaluation and clinical validation of a chromogenic FIX assay for reliable post infusion measurements of recombinant and next generation factor IX products

J. Müller, J. Oldenburg, B. Pötzsche (Bonn, Germany)

Objective: Assay discrepancies have been described for conventional recombinant (r) but also for next generation rFIX products with prolonged half-life (PHL). When compared to the results of one-stage clotting (OSC) assays, conventional rFIX products generally yield lower results when using a chromogenic (CS) assay while PHL FIX products exhibit more inconsistent discrepancy patterns. In order to adequately address the resulting implications for post infusion testing, the objective of the present study was the evaluation (validation) of currently available CE/IVD-marked FIX CS assays.

Methods: The CE/IVD-marked BIOPHEN FIX (HYPHEN BioMed) and ROX Factor IX (Rossix) CS assays were available for initial evaluation on a BCS XP analyzer (Siemens). The BIOPHEN FIX CA was chosen for further clinical validation that included determination of the lower limit of quantification (LLOQ), intra- and inter-assay precision (CV%) and accuracy (RE%), as well as assay robustness. Further analysis comprised the measurement of patient plasma samples and different concentrations of N9-GP (nonacog beta pegol [Novo Nordisk]), a pegylated PHL FIX, that was spiked into FIX-deficient plasma.

Results: Initial evaluation of assays showed comparable precision characteristics (intra-/inter-assay CVs @ plasma FIX:C of 0.99 IU/ml or 0.33 IU/ml ≤ 12.2%) while the accuracy of the BIOPHEN FIX CS assay was found to be inferior, revealing the need for plasma-based calibrators when using this assay, which, however, due to a higher throughput on the applied BCS XP system, was chosen for final validation. The LLOQ was determined as 0.008 IU/ml FIX:C. Using stored standard curves, the intra- and inter-assay precision and accuracy were tested in the high, medium, and low concentration range and did not exceed a CV of 25% and a RE of +/- 25%. Analysis of patient plasma samples yielded a high overall correlation between an established OSC assay (Actin FSL-based) and the CS assay, whereat product-specific differences were observed. Measurement of plasma N9-GP by the CS assay showed high accuracy within +/- 20% of nominal while the OSC assay underestimated N9-GP by a mean of 40% of nominal.

Conclusion: Due to the advent of novel rFIX and PHL FIX products, the availability of a FIX CS assay will become more essential in the future. The BIOPHEN FIX CS assay was established on the BCS XP platform for corresponding routine clinical analysis.
TechnoZym® ADAMTS-13 activity assay for determination of inhibitory antibodies against ADAMTS-13

L. Wagner, F. Nasufi, M. Kafka, N. B. Binder (Vienna, Austria)

**Objective:** Acquired TTP is frequently caused by inhibitory auto-antibodies against ADAMTS-13. Determination of ADAMTS-13 inhibitors therefore provides a valuable tool for diagnosis and patient follow-up in TTP. ADAMTS-13 antibodies can be detected in vitro either as IgG by ELISA, or functionally in a Bethesda based assay due to their inhibitory effect on ADAMTS-13 activity.

Aim of this study was to evaluate the usability of TECHNOZYM® ADAMTS-13 activity ELISA in combination with a Bethesda based assay for detection of inhibitory antibodies against ADAMTS-13.

**Methods:** We determined ADAMTS-13 activity in plasma samples from patients clinically diagnosed with TTP and in normal plasma as negative control. The inhibitory antibodies against ADAMTS-13 were determined by a Bethesda-type method similar to the one used to analyze inhibitory FVIII antibodies. Patient plasma was heated treated at 56°C for 30 min to eliminate endogenous ADAMTS-13 activity. Then, a serial dilution was made before mixing 1:1 with normal human plasma (NHP). A 1:1 mixture of NHP with buffer was used as control mix. ADAMTS-13 activity of all dilutions was determined with TECHNOZYM® ADAMTS-13 activity ELISA.

Residual activity in % was calculated from the results obtained in IU/mL for all patient plasma dilutions and the control mixture. The residual activity of patient plasma between 25% and 75% was used to further calculate the Bethesda Units (BU), where one BU is defined as the amount necessary to reduce ADAMTS13 activity to 50% of control mixture.

The results for inhibitory auto-antibodies were considered to be positive if the result of patient plasma was found to be >0.5BU.

**Results:** All normal plasma samples were found to be below the 0.5BU limit. The inhibitor titer in TTP samples ranged from 0.64 BU/mL to 8.24 BU/mL. When 2 dilutions had a residual activity between 25% and 75% the calculated BU/mL between dilutions correlated very well.

**Conclusion:** In this study we demonstrate that combining TECHNOZYM® ADAMTS-13 activity ELISA with a Bethesda based assay setting provides a good method for measurement of functional inhibitors. That allows differentiation of different forms of TTP and may enable close monitoring of inhibitor titer changes in the course of the disease and in response to treatment.
Measuring direct oral anticoagulants in standardized fully automated thrombin generation on Ceveron® alpha

L. Wagner¹, E. Wimmer², J. Seier², N. B. Binder¹, A. C. Haushofer² (¹Vienna, Austria, ²Wels, Austria)

Objective: The increasing use of the direct oral anticoagulants (DOAC) creates the need of their measurement in clinical routine. A modified thrombin time-based assay can be used for the measurement of thrombin inhibitors and the direct Xa inhibitors can be measured with a chromogenic anti-Xa assay. However, these assays only measure the initiation phase of the coagulation cascade. In the present wanted to see if the thrombin generation assay (TGA), which measures the entire thrombin generation process, could be used to better discriminate the inhibitory profile of the DOACs in patients.

Methods: For this proof of concept study, platelet-poor plasma spiked with Apixaban, Rivaroxaban, Dabigatran, Arixtra or LMWH as well as Phenprocoumon plasma with different INR values was tested in the TGA. As initiators of thrombin generation two triggers differing in phospholipid concentrations were used. Analysis was performed on the coagulation analyzer Ceveron® alpha TGA.

Results: All anticoagulants, except Dabigatran, which only shows a response at higher doses, inhibited thrombin generation in a concentration-dependent manner after activation with both triggers, influencing all TGA parameters. Percent inhibition was calculated for Peak Thrombin (Peak) and Area under the Curve (AUC) values.

For the Xa inhibitors Rivaroxaban and Apixaban the inhibition of Peak values was more pronounced as for AUC, showing a plateau for both parameters at DOAC concentrations above 200ng/mL, were as for high heparin concentrations and INR values at 4.5 both parameters showed almost complete inhibition. Dabigatran showed a 6-fold prolongation of the Lag time, inhibition of Peak and AUC was below that for Xa inhibitors.

Conclusion: Differences in TGA parameters between the anticoagulants reflect their differing inhibiting capacity in human plasma. Thrombin generation measurement in samples with DOACs at clinically relevant plasma levels could therefore serve as a fine-tuned indicator for hemostatic balance (individualized therapy) in patients using the DOACs.
Evaluation of DOAC Interferences on Technoclot® PT Owren

L. Rosenmayr, L. Wagner, N. B. Binder (Vienna, Austria)

Objective: Prothrombin time (PT) is still the leading test for monitoring oral anticoagulation therapy. PT reagents are known to differ in their sensitivities to individual DOACs. In the Owren’s method the sample is assayed in a 1:10 dilution, whereas in Quick based method samples are assayed undiluted. On account of this difference in sample dilution the Owren PT is expected to be less sensitive to DOAC interferences.

Aim of the study was to investigate the rate of interference of different DOACs used in clinical practice, with the PT determined with Technoclot® PT Owren automated.

Methods: PT were determined with Technoclot® PT Owren automated, a combined Prothombin Time reagent based on Owren’s method. To investigate the assay performance in the presence of interfering substances plasma samples were spiked with increasing amounts of indirect and direct oral anticoagulants and clotting time, % of normal and INR values were determined. The relevant concentration ranges of interfering substances were chosen from the calibration ranges for the respective anticoagulants. A deviation of up to 15% from the un-spiked sample was considered as acceptable.

Results: No interferences (deviation of <15%) on INR values were found for Rivaroxaban samples up to 200ng/mL and for Dabigatran samples up to 300ng/mL. Even at 433.3 ng/mL Rivaroxaban and at 503 ng/mL Dabigatran, the INR value was within the normal range. Orgaran and Arixtra showed no interference with the INR result up to 1.3ng/mL and up to 1.61µg/mL, respectively.

The % of normal results showed a somewhat higher sensitivity to Rivaroxaban. Concentrations up to 145ng/mL were within the acceptance criteria of <15% deviation from initial value. No interference was found for LMWH up to 1.8IU/mL and UFH up to 1.2IU/mL.

Conclusion: Our results indicate that Technoclot® PT Owren automated is relatively insensitive to direct and indirect oral anticoagulants. For Rivaroxaban and Dabigatran the concentrations in which no interference was observed were close to the expected peak concentration.
**Difficulties in diagnosing antiphospholipid syndrome in patients undergoing DOAC therapy**

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**Poster Topic**  
**Laboratory tests**

17.02.2017, 17:15 - 18:15  
Poster Number: 83

**Objective:** It is well known that haemostaseologic laboratory assays are affected by the direct oral anticoagulants (DOACs) such as dabigatran, apixaban and rivaroxaban. This results in difficulties in diagnosing thrombophilia in patients who receive those drugs. The aim of our study was to show the effect on two different DRVVT assays and the lupus sensitive PTT in spiked samples from healthy volunteers and samples from patients receiving a DOAC.

**Methods:** Spiking experiments were performed using the original substances of the drugs and we used plasma concentrations of 0, 10, 30, 50 and 100 ng/mL. In order to evaluate the plasma levels of the drug we used ultra-performance liquid chromatography coupled with electrospray ionization-tandem mass spectrometry (Kuhn et al, 2015). The DRVVT assays we performed were from Instrumentation Laboratory (IL) and Stago, while the lupus sensitive PTT was from IL.

**Results:** All the DOACs showed pathological values in the DRVVT screen assays. In samples spiked with apixaban, no effect on the ratio of DRVVT assays could be observed whereas between 7 % and 11 % of samples from patients receiving apixaban showed pathological values. Up to 71 % of dabigatran spiked samples showed ratio values above the cut-off of 100 ng/mL drug concentration. By contrast, there was no effect detected in the patients’ samples. For rivaroxaban, the DRVVT assays were affected in both the spiked and patient samples.

**Conclusion:** We conclude that a LA/APS testing should be performed during a longer DOAC interruption if possible. Otherwise only the combination of DRVVT screen and DRVVT confirm is helpful before the next DOAC intake (trough level). However, positive results could indeed still turn out to be false positive.
Performance of a recombinant fusion protein linking coagulation factor IX with recombinant albumin (rIX-FP) in the one-stage assay

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Objective: A fusion protein genetically linking recombinant human coagulation FIX with recombinant human albumin (rIX-FP) has been developed to have an improved pharmacokinetic profile compared with standard FIX products, allowing less frequent dosing. During the PROLONG-9FP development program for rIX-FP, a reagent-dependent activity determination was observed in the one-stage (OS) clotting assay. The variability of different OS assay reagents for the activity measurement of rIX-FP was assessed in an international, multicenter field study. In addition, samples from pediatric and adult subjects within the clinical trial program were measured in parallel at local and central laboratories.

Methods: Centers participating in the rIX-FP Phase I/II clinical trial as well as five external laboratories were provided with lyophilized rIX-FP reference plasma for reconstitution and dilution to 0.25, 0.5 and 0.9 IU/mL. Centers participating in the rIX-FP Phase II/III trial (n=8) were provided with rIX-FP plasma samples spiked at concentrations of 0.1, 0.25 and 0.625 IU/mL and assayed samples using the local OS assay with the instrumentation and reagents used routinely in their laboratory. These laboratories, where samples were measured locally, were encouraged to send a paired sample to CSLB’s central laboratory for analysis (n=21).

Results: For both the field study and paired sample analysis, plasma samples at all concentrations showed comparable results across different clinical laboratories using a variety of aPTT reagents. However, when Actin FS and CK Prest (a kaolin-based reagent) were used for analysis, rIX-FP activity was underestimated by approximately 50%.

Conclusion: Following infusion of rIX-FP, plasma FIX activity can be monitored using the OS assay to confirm adequate FIX levels have been achieved and maintained. Results obtained from both the field study and paired sample analysis demonstrated that although the majority of commercially available reagents for the one-stage clotting assay will provide accurate measurement of rIX-FP activity, use of a kaolin-based or Actin FS aPTT reagent is likely to result in an underestimation of FIX activity of up to 50%.
Development, validation and application of a polysialylation-dependent FVIII activity assay to measure SHP656 (BAX 0826), a next generation extended half-life recombinant factor VIII product

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Poster Topic
Laboratory tests
17.02.2017, 17:15 - 18:15
Poster Number: 85

Objective: Hemophilia A is a rare genetic bleeding disorder caused by missing or defective factor VIII (FVIII), a crucial factor in blood coagulation. SHP 826 (BAX 826) is currently developed as the next generation extended half-life (EHL) recombinant (r)FVIII replacement for treatment of hemophilia A. SHP 826 is the first EHL rFVIII in which polysialic acid (PSA) is conjugated to ADVATE (rFVIII) to extend the circulatory half-life of FVIII. Here we describe the development, validation and application of a chromogenic FVIII activity assay to exclusively measure polysialylated rFVIII. During nonclinical characterization this selectivity not only enabled unambiguous measurement of SHP 826 in the presence of endogenous animal FVIII, but also provided information on its integrity.

Methods: An anti-PSA antibody was coated to a polystyrene plate and used to selectively capture SHP 826 from the sample. After a washing step, a conventional chromogenic assay was carried out, for which calibration was achieved using a SHP 826 preparation with a defined FVIII activity. Development focused on defining adequate antibody coating conditions, in particular pH and antibody concentration, and demonstrated the assay’s selectivity in plasma samples from various animal species and human plasma. Assay validation was performed in the matrices of citrated rat, cynomolgus monkey and FVIII-deficient mouse plasma in accordance with the EMA guideline for bioanalytical assay validation. Finally, the assay was used in nonclinical studies of SHP 826 in FVIII-deficient mice, rats and cynomolgus monkeys.

Results: Sensitive and reproducible calibration curves of 1.1 to 34 mU/mL were generated. The results of the method validation proved the assay suitable for its intended use. Mean accuracies given as the recovery of SHP 826 activity spiked at five relevant concentrations (0.05 - 15 U/mL) were 95% to 101%. Intra- and inter-run precision, expressed as relative standard deviations, were lower than 10%. These values were also obtained for samples spiked with concentrations at the lower limit of quantification.

Conclusion: Successful application of the assay during the nonclinical SHP 826 studies with no interference by endogenous FVIII confirmed the validation data,
rVIII-SingleChain plasma activity can be measured using both the one-stage and the chromogenic substrate assay: Results from an international field study

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Objective: rVIII-SingleChain is a novel B-domain truncated recombinant Factor VIII (rFVIII) with covalently bonded FVIII heavy and light chains, demonstrating an increased binding affinity to von Willebrand Factor. For rVIII-SingleChain an approximate 50% discrepancy was observed between the one-stage (OS) and chromogenic substrate (ChS) assays; however, the strong linear relationship between assay results enables reliable interpretation of either method. An international, multicenter and blinded field study of simulated post-infusion samples was initiated to determine the intra- and inter-laboratory variability in measurements of rVIII-SingleChain and a full-length rFVIII (octocog alfa) in routinely performed FVIII activity assays.

Methods: Plasma samples were spiked at 0.04, 0.3, 0.6 and 1.0 IU/mL for rVIII-SingleChain and octocog alfa, blinded, and submitted to 23 local laboratories to be assayed by the OS and ChS assays. Laboratories followed their routine practices and used their own in-house FVIII standard, FVIII-deficient plasma and assay reagents.

Results: Inter-laboratory variability in the OS assays were similar for both products (17–29% rVIII-SingleChain; 14–35% octocog alfa). The OS assay values underestimated rVIII-SingleChain by approximately 45-50%, in a highly predictable and consistent manner across the complete range of investigated FVIII plasma concentrations. When comparing within the OS assay format across different activated partial thromboplastin time reagents, there was a similar and reagent-correlated variability in response to different activators for both octocog alfa and rVIII-SingleChain. When using the ChS assays, results were near the target value at all spiked levels. Inter-laboratory % CV for the ChS assay ranged from 4–16% for rVIII-SingleChain and 4–20% for octocog alfa.

Conclusion: In this field study, a consistent relationship between results obtained with the OS assay and with the ChS assay was observed, with comparable variability between rVIII-SingleChain and octocog alfa. Multiplication of results obtained by the OS assay, with a conversion factor, aligned FVIII activity measurements of rVIII-SingleChain with those obtained by ChS assay, with comparable accuracy to octocog alfa activity measurements obtained with the OS assay. This allows both the OS and ChS assay methods to be used for clinical monitoring of patients treated with rVIII-SingleChain.
Platelet-dependent von Willebrand factor activity - an important constituent of the diagnostic repository to classify von Willebrand syndrome.

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Poster Topic
Laboratory tests
17.02.2017, 17:15 - 18:15
Poster Number: 87

Objective: von Willebrand syndrome (vWS) is the most common inherited bleeding disorder. Acquired von Willebrand syndrome (avWS) comprises a deficiency or defect in von Willebrand factor (vWF). Diagnosis of vWS as well as avWS is difficult last but not lost due to limitations in test procedures and suffers from over- as well as under-diagnosis.

The historical assay for evaluating the platelet dependent VWF function (vWF:RCo) is known for a high imprecision and low sensitivity. The new generation assays offer more reliable results. According to the new nomenclature they differentiate between vWF:GPIbR (ristocetin-induced binding of vWF to a recombinant wildtype GPIb fragment) and vWF:GPIbM (spontaneous binding of vWF to a gain-of-function mutant GPIb fragment).

Methods: 45 routine samples vWF-Antigen (vWF:Ag), vWF-Collagen-binding-activity (vWF:CB) and vWF-Ristocetin-induced-binding (vWF:GP1bR) were determined applying HemosIL AcuStar-Assays (Instrumentation Laboratory, Werfen Group, Kirchheim) including the calculation of vWF:GP1bR /Ag- and vWF:CB/Ag-ratio.

The vWF-binding in the vWF: GP1bR-Assay is proportionally compared to the former ristocetin cofactor activity. The assay overcomes the limitations of the vWF:RCo-Assay, based on the agglutination of formalin-fixed normal platelets in presence of Ristocetin.

Results: The samples contained 35 normal, 7 Type I, 1 Type IIa, 1 Type IIb and 1 avWS. In all normal and Type I cases vWF:GPIbR /Ag- as well as vWF:CB/Ag-ratio was <0,7. In the Type IIa and Type IIb case both ratios were <0,7. In the avWS case vWF:CB/Ag-ratio was >0,7 whereas vWF:GPIbR /Ag-ratio <0,7.

Conclusion: The results are in concordance with the algorithmic approach published by Favaloro in 2015. They underline the importance to include a test covering the glycoprotein GP1b-activity within the primary test-panel.

The secondary differentiation of Type IIa and IIb cases with an identical pattern of vWF:CB/Ag- and vWF:Ac/Ag-ratio could be achieved by Light Transmission Aggregometry applying low- and high-dose Ristocetin. Analysis of vWF-multimers than belongs rather on the end of diagnostic strategy.

Reference:
Favaloro, E. J. Recent advances in laboratory-aided diagnosis of von Willebrand disease. Expert Opinion on Orphan Drugs, 2015, 3, 975-995
Rapid immunoassays for diagnosis of heparin-induced thrombocytopenia: comparison of diagnostic accuracy, reproducibility, and costs in clinical practice

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Objective: Immunoassays are crucial in the work-up of patients with suspected heparin-induced thrombocytopenia (HIT) and a number of rapid tests have been recently developed. However, comparative data on diagnostic accuracy, reproducibility, and analytical costs of different immunoassays in clinical practice are limited.

Methods: Samples of 187 consecutive patients evaluated for suspected HIT in clinical practice using a polyspecific enzyme-linked immunoabsorbent assay (GTI diagnostics; ELISA) and a rapid particle gel immunoassay (PaGIA), were additionally analyzed with two fully automatable assays: polyspecific chemiluminescent immunoassay (HIT-Ab), and IgG-specific chemiluminescent immunoassay (HIT-IgG). Presence of HIT was defined as a positive functional heparin-induced platelet aggregation test. Diagnostic accuracy was determined for low, intermediate and high thresholds as previously established (ELISA: optical density 0.4, 1.3, and 2.0 respectively; PaGIA: positive/negative, titre of 2, titre of 4; HIT-Ab/HIT-IgG: 1.0 U/ml, 2.8, 9.4) and reproducibility was assessed by repeated measurements. Costs of test determination were calculated taking reagents, controls, and working time of technicians according to Swiss health care system into account.

Results: Data on PaGIA results were available for 177 patients (94.7%), ELISA for 146 patients (78.1%), HIT-Ab for 54 patients (28.9%), and HIT-IgG for 187 patients (100%). Sensitivity was 100% for all assays at low and intermediate thresholds. Specificity increased with higher thresholds and was above 90% for all assays with intermediate and high thresholds. Specificity of HIT-IgG was higher than PaGIA at all thresholds and higher than ELISA at low threshold. Reproducibility was adequate for all assays. Total costs per test were CHF 51.02 for ELISA, 117.70 for HIT-Ab and HIT-IgG, and 83.13 for PaGIA.

Conclusion: In clinical practice, we observed favourable diagnostic accuracy measures and a high reproducibility for rapid immunoassays PaGIA and chemiluminescent immunoassays. Implementation into 24-hours-service might improve patient care.
Is platelet aggregation testing dependent on fibrinogen level?

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Poster Topic
Laboratory tests
17.02.2017, 17:15 - 18:15
Poster Number: 89

Objective: Bleeding disorder work up usually includes screening for clotting factor deficiencies, von Willebrand disease (vWD), and platelet disorders. Testing for platelet disorders is often done by light transmission aggregometry (LTA). In order to reduce variability, a lot of laboratories perform LTA on platelet rich plasma with adjusted platelet count.

We studied the influence of other parameters of coagulation to LTA-results.

Methods: Patients being referred to our center for bleeding disorder work up were included prospectively in this study. Every patient underwent basic coagulation work up (prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, and factor XIII), testing for vWD (von Willebrand factor activity, von Willebrand factor antigen, and factor VIII) as well as testing for platelet disorders by LTA using platelet rich plasma samples adjusted to a standardized platelet count between 200 and 300G/l. To induce aggregation ADP, collagen, epinephrin, and ristocetin were used in different concentrations.

Dependence of % maximal aggregation (MA) results from other coagulation parameters were investigated using multiple regression analysis.

Results: 48 patients were evaluated prospectively. MAs induced by ADP in high (10μM) and medium (5 μM) concentration were significantly dependent on fibrinogen level (p=0.02 for ADP10μM and p=0.01 for ADP5 μM) but not on all other coagulation parameters. Fibrinogen levels were in the normal range for all patients tested (1.5-3.5g/l).

MAs induced by all other agonists used were not related to any other coagulation parameter.

Conclusion: Our results suggest that platelet aggregation induced by ADP is dependent on fibrinogen concentration in plasma (even if fibrinogen concentration is within the normal range). Given these results, one could speculate that adjustment for a defined fibrinogen level might improve variance of LTA. Furthermore, fibrinogen concentrations might influence efficacy of platelet inhibition with ADP-receptor antagonists.
Replacing the aPTT for monitoring of unfractionated heparin with the Prothrombinase induced Clotting Time (PiCT) – significant influence on dosing.

W. Korte, L. Graf, C. Knöpfel (St. Gallen, Switzerland)

**Objective:** We recently showed in a prospective multicentre trial that the Prothrombinase induced clotting time (PiCT) is a more reliable tool than the aPTT for unfractionated heparin (UFH) monitoring (Brisset et al., JTH 2016), suggesting that its use might also influence and improve the clinical course of UFH therapy. Compared to chromogenic anti-Xa-assays, PiCT has all the advantages of a clotting based assay, but is predominantly sensitive to F. X and F. II inhibition at the same time. After implementing PiCT for routine UFH monitoring, we therefore investigated whether replacing aPTT with PiCT for UFH monitoring was associated with changes in UFH dosing. Rules to be used for UFH monitoring with both, initially aPTT and later PiCT were based on target anti-Xa levels; rules were not changed with the implementation of PiCT monitoring, unless mandated by the clinical course.

**Methods:** We retrospectively compared patients treated with UFH before implementation of PiCT monitoring and thereafter in various departments (internal medicine, neurology and surgery departments, including the respective intensive and critical care units); we compared the daily doses of UFH used as documented in the patient charts. Data were not adjudicated. Data were compared by Mann-Whitney testing for independent samples.

**Results:** Until now, a total of 439 aPTT measurements and 625 PiCT measurements were identified. Median UFH doses documented were 27'000 U / day with aPTT monitoring (IQR 20'000 – 29'500) and 25'000 U / day with PiCT monitoring (IQR 20'000 – 27'000), indicating a significant difference (p = 0.035).

**Conclusion:** In this first comparison of aPTT and PiCT for routine UFH monitoring outside of a prospective trial, PiCT monitoring was associated with a significantly reduced daily dose of UFH. This suggests that UFH monitoring by PiCT not only is analytically more precise (Brisset et al., JTH 2016), but it also suggests that PiCT allows more precise dosing. More research is needed to confirm these findings and to identify other variables possibly indicating a potential change in outcome when using PiCT instead of aPTT for UFH monitoring.
Clauss fibrinogen assay obviously underestimates fibrinogen level not confirmed in thromboelastometry – A case report

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**Objective:** A case report is presented of presumingly false low fibrinogen value obtained with a commercial fibrinogen assay but not confirmed in Thrombelastometry.

**Methods:** A 44 year old male patient was scheduled for hip endoprothesis (hip-TEP). Because of a preoperative pathological quick value the patient was assigned to our haematological ambulance. Bleeding history was unremarkable. Routine coagulation test were performed on ACL Top (Werfen) using RecombiPlasTin (Hemosil®, Werfen), SynthASil (Hemosil®) and Fibrinogen C (Hemosil®). One step assays were used for determination of coagulation factor activities (all deficient plasmas were purchased from Werfen (Hemosil®). Activity/antigen of factor XIII was determined by a chromogenic/antigen assay. For mixing test normal control plasma (Hemosil®) was used and incubated with patient plasma (1:1) at 37°C for 1 hour. Citrated whole blood was used for measurement for EXTEM and FIBTEM assays on ROTEM delta. start-tem and ex-tem reagents were used for EXTEM assay and ex-tem and fib-tem for FIBTEM assay.

**Results:** Quick 70% (75-120 %) and activated partial thromboplastin time 34 sec. (ref. range: 24-35 sec.), were borderline pathological. Fibrinogen according to Clauss 50 mg/dl (150-400 mg/dl) was pathological. Analysis of coagulation factors showed activities of 30 to 70 %, with exception of factor XIII: activity and antigen were clearly in normal range. Inhibiting antibodies were excluded by mixing Test, which increased fibrinogen to 170mg/dl (normal plasma: 334 mg/dl) and normalized Quick. However citrated whole blood of the patient revealed maximum clot firmness (MCF) of 16mm (12-23mm) for FIBTEM and 68mm (50-72mm) for EXTEM.

Fibrinogen assay according to Clauss indicated a severe hypofibrinogenemia and reduced activity of coagulation factors. Dysfibrinogenämia may be an explanation for impaired fibrin formation in vitro coagulation tests. Surprisingly this was not confirmed in thromboelastometry. Considering lack of bleeding in this patient the fibrinogen assay seems to lead an inappropriate diagnosis of hypofibrinogenemia. Hip-TEP was proceeded without substitution of coagulation factors and perioperative bleeding complications.

**Conclusion:** Preoperative unexplained pathological results without bleeding history should be verified by repeated analysis or better by an independent method; in this case Thromboelastometry.
A Flow cytometer-based platelet aggregation assay permits platelet function testing in small blood volumes

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**Poster Topic**  
Laboratory tests  
17.02.2017, 17:15 - 18:15  
Poster Number: 92

**Objective:** Platelet aggregation assays require high volumes of platelet rich plasma or whole blood. Especially for pediatric patients, platelet function testing requiring small sample volumes is desirable. A low volume flow cytometer-based aggregation assay was introduced by De Cuyper et al. (2013). We compared this flow cytometer-based aggregation assay with light transmission aggregometry (LTA).

**Methods:** 200µL citrated whole blood was aliquoted into two 100µL tubes. Platelets were stained with FITC- or PE-conjugated CD31, respectively. Samples were washed twice, resuspended in 100µL hirudinized buffer (PBS with Ca+Mg, containing 20% normal plasma) and mixed. Anti P-selectin antibodies (CD62P) were added and 20 µL of each mix were incubated with increasing concentrations of ADP (0.078-40 µM), TRAP (1-40 µM), arachidonic acid (0.375-5 mM), epinephrine (0.04-20 µM), or collagen (1-10 µg/mL). Platelet activation was stopped using 0.5% paraformaldehyde after 2, 4, 6, 8, 10, 12 and 20 min. FITC- and PE-double positive events, platelet-counts and CD62P expression were analyzed by flow cytometry. LTA was performed with platelet rich plasma.

**Results:** Maximal aggregation and activation signals were observed after 2 min. Optimal agonist concentrations were for ADP: 20 µM; arachidonic acid: 0.75 mM; epinephrine: 2.5 µM; collagen: 5 µg/ml; TRAP: 20 µM. Platelets counts were decreased from 1500±300 (PBS) to 230±33 (ADP), to 440±250 (arachidonic acid), to 173±42 (collagen), to 170±33 (TRAP). Aggregates (double stained events) increased from 5±2 (PBS) to 20.4±6.5 (ADP), to 20.3±4.6 (arachidonic acid), to 18.5±2.7 (epinephrine), to 19.2±6 (collagen), or to 16.25±7.2 (TRAP). CD62P mean fluorescence intensity increased from 5±2.8 (PBS) to 16.9±10.2 (ADP), to 19.8±12.4 (arachidonic acid), to 11.9±8.2 (epinephrine), to 32±21.5 (collagen), to 29±20 (TRAP).

Patients with impaired platelet function in LTA were assessed by flow cytometry. Both tests correlated in 0/10 (TRAP), 2/7 (ADP), 1/9 (arachidonic acid), 4/11 (collagen) and 5/12 (epinephrine) cases so far.

**Conclusion:** The most sensitive agonists were ADP, epinephrine and collagen, but the flow cytometer-based assay is not directly comparable with the LTA. However, it allows small volume analysis of functional aggregation and could be used to interpret the potential of different agonists or stimuli within this assay.
Genetic heterogeneity in patients with von Willebrand disease type I: a regional study from Northeast Germany

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**Objective:** The most prevalent inherited bleeding disorder von Willebrand disease (vWD) type I is characterized by pathogenic variants throughout the entire molecule and missense gene mutations account for the majority of cases. Approximately half of the known point mutations are located in the central region of von Willebrand factor (vWF) encoded by exons 18-28. The present study was undertaken to investigate if a targeted and/or stepwise sequencing approach can be employed to identify mutations in vWD type I patients in a regional setting.

**Methods:** Thirty-two patients of Northeast German origin (29 females, 3 males) with bleeding tendency classified as VWD type I based on phenotypic assays (VWF:Ag, VWF:RCoF) were further investigated by direct sequencing and MLPA analysis of the VWF gene.

**Results:** Twenty patients (63 %) demonstrated gene mutations located in exons 18-28. Interestingly, recurrent mutations included Pro812Argfs*31 in exon 18 (n=5), Arg924Gln in exon 21 (n=7), and Tyr1584Cys in exon 28 (n=4). Therefore, in this regional cohort, mutation testing for the three most common vWD type I mutation would identify the pathogenic variant in half of all patients. Based on these findings, a validation study using a three-step genetic testing approach consisting of initial analysis of the most common three mutations, followed by sequencing of exons 18, 21, and 28 and by full sequencing of the remaining coding region of the gene should be performed.

**Conclusion:** Results of this regional study confirm the genetic heterogeneity of vWD type I. Analysing molecular genetic data of a given geographic/ethnic population may identify a mutational spectrum which may point to a targeted and/or stepwise genetic approach in the molecular diagnostic workup of vWD type I.
Comparison of factor VIII assays using physiological triggers

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Poster Topic
Laboratory tests
17.02.2017, 17:15 - 18:15
Poster Number: 94

Objective: The determination factor VIII activity is a critical method in haemophilia care. It is widely used for diagnosis of patients and for monitoring of their treatment with clotting factor concentrates. Furthermore, the method is used during the manufacturing process and batch release of plasma-derived and recombinant FVIII concentrates. Several assays have been established for the determination of FVIII activity but the one-stage clotting assay and the chromogenic method are predominantly used. For potency measurements of FVIII concentrates, the European Medicines Agency (EMA) requires the chromogenic method in accordance with the European Pharmacopoeia, while the US FDA requires the one-stage clotting assay. However, modified recombinant FVIII products such as B-domain deleted as well as fusion proteins, yield different potencies with these two assays and also with different reagent kits.

Methods: We have developed a fluorogenic assay for the determination of FVIII activity which uses physiological amounts of FIXa as trigger and in which spontaneous activation of the intrinsic pathway is inhibited by addition of corn trypsin inhibitor. Recently, other groups have shown that also FXIa can be used as a trigger and that the addition of small amount of tissue factor enhances the performance under certain conditions.

Results: Here we present a comparison of FIXa and FXIa as triggers for the reaction and also of different inhibitors of the intrinsic pathway. Furthermore, we have simplified our assay by using commercially available FVIII-deficient plasma and optical monitoring of clotting instead of a fluorogenic detection of FXa and thrombin activity. Finally, we used this assays to compare the potencies of different FVIII concentrates.

Conclusion: The principle of using a physiological trigger may help to resolve assay discrepancies between different FVIII concentrates.
The „German Pediatric Hemophilia Research Database“ (GEPHARD) – a project of the `Standing Committee Pediatrics of the Society for Thrombosis and Hemostasis Research´ to improve the quality of hemophilia care for children

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Poster Topic
Pediatric and neonatal thrombosis and hemostasis
17.02.2017, 17:15 - 18:15
Poster Number: 95

Objective: Hemophilia is a rare disease, diagnosed in 40-60 children in Germany per year. As a chronic hemorrhagic disease it leads to recurrent bleeds resulting in severe morbidity. Prophylaxis with clotting factor concentrates is the standard of care for bleeding prevention, but when and how to start is unclear. Strategies to avoid the development of neutralizing antibodies are strongly discussed. Exact documentation of any kind of treatment related factors in a large cohort is necessary to collect outcome data and to improve hemophilia care. Currently two registries are open for German patients: The German Hemophilia Registry (DHR) collects data from all centers focusing on patient numbers and clotting factor use. Naturally, being a registry for all hemophilia patients, the DHR is not specific on pediatric issues. In contrast, the European PedNet registry focuses on children. However, only 5 German hemophilia treatment centers (HTC) are part of the PedNet group and therefore, PedNet data do not reflect the situation in Germany.

Methods: To investigate German treatment modalities and outcome data in newly diagnosed hemophilia patients the Standing Committee Pediatrics of the Society for Thrombosis and Hemostasis Research (GTH) is establishing a multicenter, prospective, observational registry. It will capture all replacement therapy related variables in children with factor VIII or IX levels of < 1 to 25%. Additionally, the registry will serve as a base for future investigational activities e.g. on immunology, outcome and imaging. Participating centers can get support in case of medical questions and documentation, not only for the GEPHARD registry but also the DHR.

Results: GEPHARD is patronized by the GTH. A steering committee and a commission consisting of representatives from 18 HTC have been established. The protocol is currently undergoing institutional review for ethical and data security issues. GEPHARD is registered at clinicaltrials.gov (NCT02912143) and the Registry of Patient Registries (RoPR ID: 11758) and will start enrollment in 01/2017.

Conclusion: The new German Pediatric Hemophilia Research Database (GEPHARD), which is open to all German HTCs, will offer a base to investigate any kind of treatment related research questions in children with hemophilia all over Germany. Cooperation with existing registries is intended.
Acquired von Willebrand disease in infants with aortopulmonary shunt

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Poster Topic
Pediatric and neonatal thrombosis and hemostasis
17.02.2017, 17:15 - 18:15
Poster Number: 96

Objective: The acquired von Willebrand disease (avWD) was first described in 1968 by Simone and colleagues in patients with autoimmune diseases. The avWD is very rare in children, most frequently being described in connection with congenital heart defects including aortic stenosis, persistent ductus arteriosus, ventricular septal defect and pulmonary hypertension. The avWD often results in increased bleeding tendency such as mucosal-, gastrointestinal- or surgical bleeding. Until now, there are no reports describing avWD in infants with aortopulmonary shunts.

Methods: Between 07/15-07/16 we evaluated 11 infants younger than 3 months with univentricular hearts and aortopulmonary shunt (9x Blalock-Taussig-Shunt, 1x central aortopulmonary shunt, 1x Sano-Shunt (from the systemic right ventricle to the pulmonary artery)) and tested for avWD. The shunt operation was performed between day 5.-180. (median 8d), the blood samples were collected between days 18-260 after surgery (median 32d).

Results: In all these 11 patients we identified avWD with a reduction/loss of the largest vWF multimers. In 10 patients the collagen binding capacity was reduced, in 3 patients the vWF:Ag was slightly elevated, while in 8 infants it was in the normal range.

Conclusion: Despite the limited number of patients, we can presume that nearly 100% of the patients with aortopulmonary shunt present avWD. Its pathogenesis is explained by the increased activation of the vWF under the influence of the turbulent flow within the shunt. The activated vWF is bound to its specific receptors located on the platelets and on the activated endothelial cells, and undergoes an ADAMTS 13 mediated proteolysis, which leads to the loss of large multimers. First results show that the vWF swiftly normalizes shortly after suppression of the shunt dependent lung perfusion and switching to a cavopulmonary (Glenn) connection.

So far none of our patients demonstrated an increased bleeding tendency in everyday life. However, we must consider this anomaly as a potential cause of increased blood loss during cardiac catheterizations and operations. Knowledge of the existence of an avWD is therefore necessary for introduction of the replacement therapy with FVIII/vWF products.
Late onset of thrombosis in siblings with homozygous protein C deficiency

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Objective: Homozygous protein C (PC) deficiency is an extremely rare life-threatening coagulopathy presenting in the neonatal period with extensive purpura fulminans and very high rates of morbidity and mortality. Prenatal onset of thrombotic events is a common situation that worsens the prognosis of these patients.

Methods: Here we demonstrate a case report of two siblings, 1 year and 14 years old, with a homozygous PC deficiency (PC<10%) with the mutation c.925G>A (p.Ala309Thr, historic Ala267Thr, Exon 9, PROC-Gene). The younger sister had been clinically inconspicuous so far but the older girl suffered from a sinus venous thrombosis at the age of two. Therefore, she was successfully treated with low-molecular-weight heparin (LMWH). Her medical history demonstrated the presence of repeated mild headache and dermal necrosis in recent years. Further laboratory parameters did not show any abnormal values except for the well-known PC deficiency and a mild reduction of protein S (56.5%). Due to the older sister’s medical history and the very high risk of thromboembolic events due to the homozygous PC deficiency we decided to implement a primary prophylaxis with Warfarin in the younger girl although she did not show any thromboembolic events so far. In the older sister we also started a long-term anticoagulation treatment with Warfarin. In both patients a PC replacement therapy and an anticoagulation with a LMWH was given additionally due to the known abnormal thrombotic risk during upitration of Warfarin.

Results: Both girls showed a very good response to Warfarin with a stable INR value of 2-3 after a few days so the concomitant therapy with PC replacement and LMWH was stopped. During therapy no clinical complications or adverse events occurred so far.

Conclusion: In these patients with homozygous PC deficiency a late onset of thromboembolic events was observed which is very uncommon for this type of disease. Anticoagulation treatment should have been given early in childhood before life-threatening complications could occur. A combination of Warfarin therapy and an additional PC replacement therapy as well as an anticoagulation during upitration phase seems to offer an efficacious and safe treatment as secondary prophylaxis as well as primary prophylaxis for recurrent purpura fulminans and thromboembolic events in PC deficient patients.
Adolescent male suffering from thrombosis is not uncommon

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Poster Topic
Pediatric and neonatal thrombosis and hemostasis
17.02.2017, 17:15 - 18:15
Poster Number: 98

Objective: Thromboses in childhood are rare with two peaks: in newborns and from beginning of puberty. Typically, thrombosis occurs in adolescent girls taking estrogen containing oral contraception. Here we report our adolescent patients with thrombosis from the past two years.

Methods: Altogether we treated 13 teenagers with thrombosis, 5 boys (38%) and 8 girls (62%) in our centre. The mean age of boys was 15.8 years (14-17) and of the girl 16.3 years (15-18).

Results: The localization of the thromboses was similar: 2 of the 8 girls (25%) had a cerebral sinus venous and 5 (63%) a lower extremity deep venous thrombosis. Two of these had pulmonary arterial embolism. Among the boys, one suffered from sinus vein thrombosis. The other four (80%) suffered from deep vein thrombosis, all with concomitant pulmonary arterial embolism.

In both groups it lasted from the first symptoms to the diagnosis between 1 day and 2 weeks.

All of the girls took estrogen containing oral contraception. All had additional hereditary or exogenous thrombophilic risk factors: a heterozygote Factor V Leiden mutation (3/6, 50%), one a Prothrombin-mutation (1/6, 17%), two an increased lipoprotein a (2/6, 33%). Two had no hereditary thrombophilic risk factors but one a 6 hour bus ride and one an immobilization with a plaster cast.

In the group of the boys, two had a heterozygote Factor V Leiden mutation (2/5, 40%), two (2/5, 40%) an antiphospholipid syndrome (APS), one of them in the setting of lupus erythematoses. In one case we could not find a hereditary thrombophilic risk factor. Only one of these patients had with the obesity an additional exogenous thrombophilic risk factor. All the others were lean, athletic young men without surgery, long-lasting journey or flight, immobilization or doping.

Conclusion: Adolescent male suffering from thrombosis is not uncommon. Interestingly, APS was reported to concern primarily female patients. In our patient population only adolescent boys were affected. Therefore we recommend screening for APS also in all boys with thrombosis.

Perhaps because thrombosis is rare in childhood, in many cases, it takes a long time from the first symptoms to the correct diagnosis.
Thrombotic events in the neonatal period – a case series of common manifestations

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Objective: Thromboembolic events are rare in childhood, however there are certain manifestations that are well known in the neonatal period. Risk factors include infections, congenital heart disease and central catheter lines, while primary hemostatic disorders are not often diagnosed as underlying cause.

Methods: Here we present four cases and discuss respective treatment recommendations according to Chest Guideline Recommendation (CGR) as published in Chest 2012.

Results: Case 1) Male mature newborn with postnatal seizures. Imaging (ultrasound/MRI) revealed intraventricular hemorrhage grade III and sinus vein thrombosis. Dalteparin s.c. was established for 3 months. Sinus vein was well perfused without signs of persisting thrombosis in a follow-up MRI.

CGR: UFH/LMWH, 6 weeks – 3 months.

Case 2) Male mature newborn with early-onset sepsis and hematuria. Ultrasound showed thrombosis of left renal vein, parts of the right renal vein, the left common iliac vein, subtotal occlusion of the inferior vena cava and a floating tip reaching up to the right atrium. Treatment with tPa and UFH for 48 hours and maintenance with Dalteparin for 6 months. Thrombus was finally dissolved, however the left kidney remained without function.

CGR: UFH/LMWH or initial tPA with UFH/ LMWH, 6 weeks – 3 months

Case 3) In a female fetus an ascites of unknown origin was diagnosed by prenatal ultrasound at 37th week of gestation. On day 9 after caesarian birth the girl developed sudden cardio-respiratory failure. Ultrasound showed a large thrombus in the lower cava inferior. Clinical diagnosis of partial pulmonary embolism was made. Treatment with Enoxaparin s.c. was established. Clinical outcome is to be evaluated.

CGR: UFH/LMWH, 6 weeks – 3 months

Case 4) A male newborn preterm at 34 weeks was diagnosed with VACTERL syndrome. In routine ultrasound a large catheter-associated thrombus in the vena cava inferior with a fluctuating tip in the right atrium was shown. Treatment with UFH and maintenance with Enoxaparin. The fluctuating tip dissolved, however the vena cava inferior part of the thrombus remained unchanged.

CGR: UFH/LMWH, 6 weeks – 3 months.

Conclusion: Presented cases show typical manifestations of thrombotic events during the neonatal period.
Anterior spinal artery syndrome in pediatric patients: diagnostic and therapy

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Objective: Infarction of the anterior spinal artery is a rare but severe disease in children. Symptoms comprise sudden onset of flaccid para- or tetraparesis, dissociated sensory loss off pain and temperature perception and bladder dysfunction. The pathophysiology is often associated with vascular lesions with or without previous surgery. Here we present three patients after trauma. Literature is scarce and not conclusive whether therapeutic heparinization is of benefit regarding the long term outcome of these patients.

Methods: We analyzed clinical and laboratory data of three adolescents who were treated in our center with anterior spinal artery syndrome (ASAS). Patient 1 was a 13-year-old boy who developed ASAS a few days after a taekwondo-fight. Patient 2 was a 14-year-old girl who presented eight hours after experiencing a kink during hair-drying. Patient 3, a 15-year-old boy, was admitted one day following a kink during fitness studio strength training. All three patients were found to be homozygous carriers of methylenetetrahydrofolate reductase (MTHFR) A222V-polymorphism. Accordingly, patient 1 and 2 had slightly elevated homocysteine levels. No other thrombophilic factors were detected. They received anticoagulation therapy with unfractionated or low molecular weight heparin, initially in therapeutic later in prophylactic dose. Patient 1 and 2 received heparin for 16 months and 13 months, respectively. Patient 3 has been treated for 3 weeks so far.

Results: Patient 1 and 2 experienced a slow improvement of their symptoms. Patient 1 has an unrestricted quality of life 7 years after the diagnosis. Patient 2 regained motoric skills without a relevant improvement of sensory loss and neurogenic bladder dysfunction within 7 months. Patient 3 is still in the acute phase of ASAS.

Conclusion: Thromboembolic events represent a rare disease category in children. They are significantly associated with congenital or acquired thrombophilic factors. Our patients were carriers for MTHFR-A222V-polymorphism. However, it remains unclear whether this polymorphism plays a crucial role in the pathogenesis of ASAS. Heparin was promptly administered in all cases. Our patients’ outcome seems to be better than the outcome of patients who have been described in the literature. This supports the hypothesis that an early and adequate therapy with heparin improves the clinical outcome.
Systemic mast cell activation disease during adolescence and heparin levels in blood plasma

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Poster Topic
Pediatric and neonatal thrombosis and hemostasis
17.02.2017, 17:15 - 18:15
Poster Number: 101

Objective: Introduction

Systemic mast cell activation disease (MCAD) presents with a multisystemic spectrum of clinical symptoms induced by the release of mast cell-derived mediators including irritations in the CNS (such as headache, paraesthesia, chronic fatigue syndrome/CFS), irritable bowel syndrome/IBS, musculature, skin reactions, and of other tissues.

Aims

In our previous studies in adult MCAD patients endogenous heparin levels (heparin) was increased in the majority of the patients. In the present study we correlated the medical history, clinical signs and heparin in the adolescent MCAD patients subset of all MCAD patients of our outpatient clinic from 01-2011 until 06-2016.

Methods: 22 MCAD patients (age 10.3 to 20.8 years [y], N=12 female, N=10 male) diagnosed according to the current international criteria were included. CTAD blood (Monovette tubes Sarstedt) was sampled at baseline (BL) and after venous occlusion (VO) as previously described (Seidel, Thromb Haemost 2011). Heparin was determined by anti-factor Xa (AXa) assay (COAMATIC Heparin, Chromogenix/IL).

Results: Increased BL heparin, i.e. > 0.05 AXa IU/ml was detected in 59% of the patients. After VO heparin was increased in 86%. All 6 patients <15 y showed elevated VO heparin. 12/13 patients with CNS signs (92%), 10/12 patients with CFS (83%), 17/19 patients with IBS (89%) and 6/8 patients with skin reactions (75%) had increased VO heparin.

Conclusion: Basal heparin levels, and after VO were increased in >75% of adolescent MCAD patients. Similar to our previous data obtained in adult MCAD patients with CNS signs, CFS, IBS and/or combinations thereof, heparin appeared to be a highly sensitive biomarker with a sensitivity >89%; in patients with skin reactions the sensitivity was >75%. Thus, careful evaluation of clinical signs and medical history in combination with our diagnostic procedure allows to identify adolescent MCAD patients via their heparin levels with a high sensitivity.
Annexin A7 (ANX7) regulates collagen-dependent platelet activation and Ca2+ signaling

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**Poster Topic**
**Platelets - Physiology and Disorders of platelet number and function**
17.02.2017, 17:15 - 18:15
Poster Number: 102

**Objective**: Activation of platelets by subendothelial collagen results in an increase of cytosolic Ca2+ concentration ([Ca2+]i) and is followed by platelet secretion, aggregation and thrombus formation with consecutive vascular occlusion. This study aimed to determine the role of ANX7 in collagen-dependent platelet Ca2+ signaling and function in platelets from ANX7 knockout (anx7-/-) mice.

**Methods**: Fura-2-AM spectrofluorometric Ca2+ measurements, flowchamber and light transmission aggregometry were performed with platelets from ANX7 knockout mice.

**Results**: Stimulation of the collagen receptor glycoprotein VI (GPVI) by collagen or collagen-related peptide (CRP) leads to platelet activation due to increased intracellular calcium concentration ([Ca2+]i). Fura-2-AM spectrofluorometric Ca2+ measurements revealed that ANX7 deficiency strongly blunted Ca2+ mobilisation from intracellular stores and impaired extracellular Ca2+ influx in response to CRP and collagen. As platelet activation is linked with integrin αIIbβ3 activation and subsequent aggregation, light transmission aggregometry was performed uncovering a significant impaired platelet aggregation in anx7-/- platelets compared to anx7+/+ platelets after stimulation with CRP and collagen. Furthermore, GPIV-dependent platelet dense granule secretion (reflected by ATP release) as well as arterial thrombus formation under high shear rates (1700 s-1) on collagen were significantly diminished in anx7-/- platelets as compared to anx7+/+ platelets.

**Conclusion**: In conclusion, ANX7 plays a pivotal role upon collagen-triggered platelet activation and thrombus formation at least due to impaired activation-dependent increase of [Ca2+]i.
The special role of anti-ADAMTS13-IgA in acquired TTP: Production induced by infectious microorganisms?

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Objective: Thrombotic thrombocytopenic purpura (TTP) is a rare syndrome involving the production of thrombi in the microvasculature accompanied by thrombocytopenia and symptoms of organ ischemia. Acquired TTP develops when a patient produces antibodies which react with the protease Adamts13. IgG antibodies have been shown to dominate the immune response in TTP. In order to further assess their clinical implications we conducted a study analyzing the anti-Adamts13 antibody isotypes IgG, IgA and IgM in patients suffering from acquired TTP.

Methods: Plasma samples from 9 patients suffering an acute TTP episode and 27 patients in remission were analyzed. Additionally, medical histories and available documentation of treatment, clinical course and laboratory data were obtained. Patients were recruited at the Department of Hematology at the University Medical Center, Johannes Gutenberg University Mainz, Germany. IgM and IgA anti-Adamts13 antibodies were analyzed by modifications to the Imubind® Elisa kit for anti-Adamts13 IgG.

Results: Three of the 9 patients analyzed during an acute episode were positive for anti-ADAMTS13-IgA. We observed a higher occurrence of severe disease (qualified as number of plasma exchange procedures and duration of inpatient treatment) in these patients. The only two patients suffering an acute episode and showing detectable anti-Adamts13 IgA and anti-Adamts13 IgG were also the only two patients with an infectious disease as trigger factor for the episode. Of the 27 remission patients three were also positive for anti-Adamts13 IgA. An infectious disease had also preceded their last episode.

Conclusion: Eventhough our data is based on a limited number of patients our results indicate that anti-Adamts13 IgA may be of particular significance in TTP. For one, patients with IgA appeared at higher risk for severe disease. Interestingly, Tiede et al. recently found the antibody isotype IgA against factor VIII in acquired hemophilia A to also be associated with poor prognosis (Tiede A, Hofbauer CJ, Werwitzke S, et al. Anti-factor VIII IgA as a potential marker of poor prognosis in acquired hemophilia A: results from the GTH-AH 01/2010 study. Blood 2016;127:2289-2297.). Secondly, we observed a possible link between infectious triggers and production of anti-Adamts13 antibodies of type IgA in acute TTP.
Variable linkage with platelet functions of genetic mutations in ORAI1, STIM1 or FERMT3 in patients with severe immune deficiencies

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Poster Topic
Platelets - Physiology and Disorders of platelet number and function
17.02.2017, 17:15 - 18:15
Poster Number: 104

Objective: In patients with severe immune-deficiency and mutations in stromal interaction molecule 1 (STIM1), calcium release-activated calcium channel protein 1 (ORAI1) or integrin-regulating protein kindlin-3 (FERMT3), platelet functions and hemostatic activity can be impaired. Here, we aimed to assess the overall consequences of homozygous or heterozygous mutations in these genes on overall platelet functionality in whole-blood thrombus formation.

Methods: Platelet function was analyzed for nine patients, including parents, with genetic mutations in ORAI1, STIM1 or FERMT3. Intracellular calcium release was measured by calibrated fluorometry. Whole blood thrombus formation was measured at arterial- or venous-shear conditions using a multiparameter microspot assay.

Results: In platelets from 4 out of 6 patients with ORAI1 or STIM1 mutations, store-operated Ca2+ entry (SOCE) was completely or incompletely diminished in comparison to the signal of healthy control platelets. The SOCE was greatly improved in one patient after bone marrow transplantation.

All patients showed deficiencies in parameters of thrombus formation on collagen microspot due to reduced platelet activation and/or low platelet count. Parameters of thrombus formation on microspots with von Willebrand factor (VWF)/fibrinogen or VWF/rhodocytin were more closely related to the deficiency in SOCE in comparison to those on collagen microspot. However, the extent of the differences was variable within families. For all surfaces, patients platelets with a partial reduction in SOCE showed a marked decrease in phosphatidylserine exposure in comparison to P-selectin expression and integrin activation, both at low and high shear rate. Heat mapped parameters of thrombus formation on all microspots were most severely reduced with blood from a case patient with FERMT3 mutation, and partly diminished with the blood from both parents. A prediction model was built to relate changes in genotype, platelet count and platelet function in thrombus formation.

Conclusion: Within families, mutations in ORAI1, STIM1 or FERMT3 variably link to multiple parameters of altered thrombus formation due to the combination of relatively low platelet counts and impaired platelet deposition and activation.
Phosphorylation of the protein phosphatase 2A inhibitor alpha-endosulfine (ENSA) at two distinct sites, serine 67 and serine 109, in human platelets

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Poster Topic
Platelets - Physiology and Disorders of platelet number and function
17.02.2017, 17:15 - 18:15
Poster Number: 105

Objective: The 13 kDa protein alpha-endosulfine (ENSA; or ARPP-19e), originally identified as sulfonylurea receptor ligand, belongs to the highly conserved cAMP-regulated phosphoprotein (ARPP) family with a conserved PKA phosphorylation site (serine 109). In Drosophila and Xenopus, Greatwall kinase phosphorylated ENSA (at serine 67), is a powerful inhibitor of the protein phosphatase 2A (PP2A). In Xenopus, PKA-evoked ENSA S109 phosphorylation keeps the oocyte in prophase whereas S67 phosphorylation is necessary for M-phase entry (1). Very little is known about ENSA in human platelets.

Methods: Proteomic and phosphoproteomic studies with human platelets and platelet proteins were carried out as described (2). ENSA was cloned from human platelets and expressed in HEK293 cells and E.coli BL21. ENSA phosphorylation was studied in washed human platelets and with recombinant proteins.

Results: In our proteomic studies (2) ENSA was detected in human platelets at significant levels (7800 copies/platelet). By our quantitative platelet phosphoproteomic studies, ENSA S109 was found to be strongly phosphorylated (> 10 fold stimulation) in response to cAMP-(Iloprost) and cGMP-elevating (NO-donors, Riociguat) platelet inhibitors (3, unpublished). ENSA S67 phosphorylation was also detected by phosphoproteomics but down-regulated by these platelet inhibitors. Human platelets have two major ENSA transcripts which allowed its cloning and expression in HEK293 cells and E.coli BL21. Wildtype ENSA as well as various ENSA mutants (at S109 and S67) were then generated, purified and tested as PKA or PKG substrates. Further studies with a phosphosite-specific antibody demonstrated an increase of ENSA S67 phosphorylation in platelets in response to the phosphatase inhibitor okadaic acid.

Conclusion: The PP2A phosphatase inhibitor ENSA of human platelets is an excellent PKA and PKG substrate in intact cells and as purified protein. Platelet ENSA S67 phosphorylation was detected for the first time. Ongoing studies aim to identify the ENSA S67 protein kinase and the functional role of this phosphorylation.

References
Immunoglobulin G subclass distribution of anti-ADAMTS13 antibodies in a cohort of 47 patients with acquired thrombotic thrombocytopenic purpura

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Objective: The common, acquired form of thrombotic thrombocytopenic purpura (TTP) is an autoimmune disease, in which the underlying ADAMTS13 deficiency is caused by inhibitory autoantibodies, predominantly of the IgG isotype. As distinct IgG subclasses can have different immunological properties, the aim of our present study was to determine the subclass distribution of anti-ADAMTS13 autoantibodies in acquired TTP patients, and to investigate its associations with clinical parameters.

Methods: We determined the relative and absolute amount of anti-ADAMTS13 IgG subclasses with an in-house ELISA method based on a commercial ELISA kit. We analysed 52 ADAMTS13 deficient, anti-ADAMTS13 antibody positive samples of 47 acquired TTP patients (mean age 39 years, 38 women). Forty-four samples were drawn during the acute phase of the disease, and eight in remission; four patients had samples from both disease states.

Results: We found that the anti-ADAMTS13 antibodies were mostly of IgG1 and IgG4 subclasses. IgG4 was the most predominant subclass in 69.2% of the samples, and IgG1 in 28.8% of them. The mean proportion of IgG4 was 52.1%, that of IgG1 was 39.2%, followed by IgG3 with only 5.9%. The absolute amount of IgG3 inversely correlated with that of IgG4 (p=0.0001). The percentage of the IgG1 and IgG3 subclasses were lower in relapsing patients (medians: 24% vs. 43% and 0% vs. 5%, respectively), while that of IgG4 was higher (71% vs. 49%). Conversely, the relative amount of IgG3 was higher (14% vs. 1%), and that of IgG4 was lower (10% vs. 65%) in patients, who have deceased during the TTP episode.

Conclusion: Our results are in accord with those of previous studies, which also found that IgG4 and IgG1 are the most predominant subclasses, and that high IgG1 levels are associated with a higher risk of death during the episode. One of the previous studies also found that IgG4 levels are higher in relapsing TTP patients. However, whether the predominance of IgG4 is a risk factor of relapse or rather a consequence of the previous therapy needs to be clarified. Similarly, additional studies are necessary to investigate whether subclass distribution of anti-ADAMTS13 antibodies or its changes during immunosuppressive therapy provide clinically useful information.
Intra- and intercellular fate of iron oxide nanoparticles in platelets

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**Poster Topic**
Platelets - Physiology and Disorders of platelet number and function
17.02.2017, 17:15 - 18:15
Poster Number: 107

**Objective:** Platelet concentrates (PC) are transfused to prevent or treat bleeding in patients with severe thrombocytopenia or platelet function defects. To distinguish between platelet populations in platelet survival studies cell labeling is often required. We established under GMP conditions a non-radioactive platelet labeling method with iron oxide nanoparticles (NP) contained in Resovist® (Bayer AG, Germany). In vitro platelet function is not influenced by the labeling method, in addition survival studies in NOD/SCID mice indicate similar survival behaviour compared to non labeled platelets. Here we report on particle internalization into platelets and the stability of magnetic labeling in vitro and ex vivo.

**Methods:** Platelets from PC and freshly isolated from whole blood were incubated with fluorescent iron oxide nanoparticles (NanoscreenMAG-CMX-R, Chemicell, Germany) with similar structure to Resovist® for 1 hour at 37 °C. After purifying cells from excess nanoparticles, cells were analyzed by electron microscopy and fluorescence microscopy for detailed localization of NP. Labeling rate was determined by flow cytometry, mean intracellular iron content by atomic absorption spectroscopy. Labeling stability was analyzed over 24 hours by flow cytometry. Furthermore magnetic labeled platelets were added to whole blood samples in order to determine labeling stability ex vivo. Samples were taken at certain time points within 24 hours and analyzed by flow cytometry.

**Results:** Platelets internalize nanoparticles mainly in the open canalicular system (OCS), some are found in the alpha-granula. Only a small percentage of nanoparticles is found on the outer cell membranes. Labeling rates differ between platelets from PC (44.2%±21.2%) and platelets freshly prepared from whole blood (78.8% ±9.1%). The mean cellular iron content per platelet is 0.33 pg±0.02 pg for PC and 0.51 pg±0.06 pg for platelets from whole blood. After spiking magnetic labeled platelets into whole blood, the labeling rate of platelets remained stable. We observed no exchange of NP between the labeled platelets and non-labeled platelets in whole blood.

**Conclusion:** We demonstrate that magnetic platelet labeling rate differs between platelet types, but remains stable over 24 hours without any measurable exchange of NP between platelets.
Frequency and analysis of hospital cases of heparin induced thrombocytopenia type 2 (HIT 2) in Germany

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Poster Topic
Platelets - Physiology and Disorders of platelet number and function
17.02.2017, 17:15 - 18:15
Poster Number: 108

Objective: Heparin-induced thrombocytopenia (HIT) is a complication of heparin therapy. There are two types of HIT. Type 2 HIT is an immune-mediated disorder that typically occurs 4-10 days after exposure to heparin and may have life-threatening thrombotic complications. Treatment of HIT 2 consists of discontinuation of heparin and use of alternative anticoagulants. The prevalence of HIT in literature is reported to be 0.2 to 3.0% of heparin exposures. In Germany exist recommendations from the society of medical controllers how to code HIT 2 cases in hospital. Here we researched the frequency of coding of HIT 2 and with which other diagnoses HIT 2 is associated.

Methods: The reports from German DRG Institute (InEK), Statistical Office (DESTATIS) and the hospital quality reports for 2010-2014 were analyzed for cases of HIT 2. Statistical analysis was performed using Microsoft-Excel and Access version 2013.

Results: The annual number of cases with a main diagnosis of HIT 2 (D69.53, ICD10-GM) was in average 59 (±11) from 2005 to 2014 with a low 2014 with 39 cases. The mean age of patients did hardly change over the years (68.2 ±17.0 years in 2014) and the gender distribution (48% male). There were many more cases with a secondary diagnosis of HIT 2: 9.281 cases annually in average between 2005 and 2014, decreasing since 2010 with a low of 8.364 in 2014. Here the share of male patients is higher (58.2%). The female patients are older in average (69.5 y) than male patients (66.9 y).

The HIT 2 cases are grouped with 38.4% into a cardiology DRG, followed by DRGs which include a more extensive ICU stay with 27.1%, thereafter follow urology (8.1%) and pulmonology (6.5%). Prevalence in hospital is 0.05% of all cases, which corresponds to 0.2% cases in the operative partition. HIT 2 cases were found to be relatively frequently ending in the error-group in the G-DRG system.

Conclusion: We found a decrease in documented hospital cases with HIT 2 from 2010 to 2014. The overall number is lower than the expected number of patients based on previously reported prevalence. About the reasons can only be hypothesized e.g. in terms of a better pre-surgery diagnostic. By far most cases of HIT 2 are associated with cardiac interventions, followed by urologic and thoracic surgery. Many cases end up in error-DRG which may be interpreted that HIT 2 is not perfectly reflected in the German DRG system.
Phenotyping platelets from patients with inherited platelet disorders by immunofluorescence microscopy

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Poster Topic
Platelets - Physiology and Disorders of platelet number and function
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Objective: Hereditary platelet disorders are more frequent than previously anticipated. Progress in hematology has identified many hereditary causes for thrombocytopenia and/or platelet function defects, but diagnosis is still challenging. It requires fresh blood and laboratory techniques available only in few specialized centers. Further, required blood volumes are usually prohibitive for investigating young children. Next generation sequencing techniques might allow characterization of the underlying genetic defect. However, it remains challenging to correlate the numerous variants within the genome to the phenotype, especially if the underlying platelet defects are poorly characterized. On the other hand, many hereditary platelet defects are associated with characteristic changes in the distribution of specific protein.

Methods: We developed a method to narrow down or confirm the diagnosis of many hereditary platelet disorders. Standard blood smears are prepared by the treating physician and are then shipped by regular mail. The use of specific fixation and permeabilization methods, followed by staining with specific antibodies, allows us to "phenotype" platelets by immunofluorescence microscopy.

Results: Assessing blood smears of 1150 patients referred to our laboratory with unclear thrombocytopenia or platelet function disorders, we achieved the diagnosis in 260 (23%) patients: MYH9-disorders, 145 patients; Bernard Soulier syndrome (BSS), 25 patients; gray platelet syndrome, 2 patients; GFI-1b mutation, 3 patients; β1-tubulin defects, 10 patients; Wiscott-Aldrich syndrome (WAS), 1 patient; Glanzmann thrombasthenia, 16 patients; alpha storage pool defects, 25 patients; delta storage pool defects, 33 patients. Diagnostic sensitivity and specificity of the method was high for MYH9-disorders/related disease, biallelic Bernard-Soulier syndrome, Glanzmann thrombasthenia, and gray platelet syndrome.

Conclusion: Immunofluorescence microscopy requires minimal blood volumes and allows broad access to specialized laboratories. This morphological technique supports the essential phenotyping of platelets to interpret results of NGS panels for platelet disorders.
Initiation of a prospective observational cohort study of patients with acquired thrombotic thrombocytopenic purpura (TTP)

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**Poster Topic**
Platelets - Physiology and Disorders of platelet number and function
17.02.2017, 17:15 - 18:15
Poster Number: 110

**Objective:** Acquired TTP is an acute life-threatening thrombotic microangiopathy most often caused by autoantibody-mediated severe ADAMTS13 deficiency. The annual incidence is 2-4 cases/1 million and despite intensive daily plasma exchange therapy (PEX) and corticosteroid treatment the mortality is still 10-20%. Survivors have a 40-50% risk of relapse during the ensuing 5-10 years and may be affected by neurocognitive problems, arterial hypertension and increased mortality. Our cohort study aims to answer whether acute relapses in survivors of an acute TTP can be predicted based on clinical and/or laboratory parameters assessed during regular outpatient follow-up visits.

**Methods:** A protocol for all consecutive patients with acute acquired TTP hospitalized at UMC Mainz as well as for survivors of acute TTP referred to the outpatient ward for regular follow-up visits every 3 months has been established and approved by the responsible Ethics Committee of Rhineland Palatine. Clinical and laboratory parameters are assessed daily during the acute disease and at each follow-up visit. In addition, biobanking of whole blood, citrated and EDTA plasma and serum samples is performed. Besides planned Next Generation Sequencing (NGS) of ADAMTS13, von Willebrand factor, complement and complement regulatory genes, stored biomaterial will be available for new assays to be developed. Biodatabanking is supported by an electronic database established for this cohort.

**Results:** The first patient, in remission after surviving acute TTP, was enrolled in July 2016 and by 10th October 2016, a total of 31 patients seen in the outpatient ward in clinical remission and one patient admitted for PEX therapy for acute TTP have been enrolled. One outpatient with earlier recurrent acquired TTP was treated with rituximab because of progressive decrease of ADAMTS13 activity and increasing inhibitor titers according to treating physician’s judgement.

**Conclusion:** This inception cohort study on acquired TTP patients has been successfully started as a single-center study and it is planned to invite other centers to participate. Besides learning more on the natural disease course, we hope to develop new diagnostic and/or prognostic parameters.
Evaluation of platelet parameters in pediatric thrombocytopenic patients

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Poster Topic
Platelets - Physiology and Disorders of platelet number and function
17.02.2017, 17:15 - 18:15
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Objective: The underlying reason of thrombocytopenia in children should be clarified to initiate effective or to avoid unnecessary treatment. For differential diagnostics the measurement of some relatively new platelet parameters (mean platelet volume - MPV, relative and absolute immature platelet fraction – rIPF and aIPF, plateletcrit - PCT, platelet distribution width – PDW, platelet large cell ratio - P-LCR) may be useful but only limited data are available until now. As presented on the GTH congress in 2016, we already determined reference values for these parameters in healthy children of different age groups. In the next step we now evaluated the parameters in pediatric thrombocytopenic patients.

Methods: Data were collected retrospectively in 98 children with the following diagnoses: acute ITP (n=19), chronic ITP (n=17), acute leukemia (n=33; 25 with acute lymphoblastic leukemia - ALL, 4 with ALL relapse and 4 with acute myeloid leukemia), inherited impairment of haematopoiesis (n=23; 8 with thrombocytopenia-absent radius syndrome, 6 with inherited thrombocytopenia of unknown origin, 3 with inherited macrothrombocytopenia, 3 with Wiskott-Aldrich syndrome, 2 with Fanconi anemia and 1 with May-Hegglin anomaly) and acquired impairment of haematopoiesis (n=6; 5 with myelodysplastic syndrome and 1 with severe aplastic anemia). Parameters were determined in 200 µl EDTA-anticoagulated blood using the fully automated haematology analyser Sysmex XE 5000.

Results: Patients with acute ITP showed significantly lower platelet counts and higher rIPF values compared to the groups of acute leukemia and of impaired hematopoiesis. Children with chronic ITP displayed significantly elevated rIPF values in comparison with the acute leukemia group and a significantly increased MPV compared to leukemia patients and children with impaired haematopoiesis. For aIPF and the other parameters no significant differences among the groups were detected.

Conclusion: In the context of the patient's individual history and a careful clinical examination, the determination of rIPF might be helpful in the thrombocytopenic child to distinguish acute ITP from acute leukemia and a disorder with impaired haematopoiesis. This hypothesis should be investigated in a prospective setting. The relevance of other platelet parameters remains unclear yet.
The power of large platelets: Comprehensive functional characterization of large and small platelets from healthy volunteers.

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**Poster Topic**
Platelets - Physiology and Disorders of platelet number and function
17.02.2017, 17:15 - 18:15
Poster Number: 113

**Objective:** An increased mean platelet volume (MPV) is a known risk factor for poorer outcomes in cardiovascular diseases, which is attributed to a higher reactivity of large platelets. However, it is a major challenge to separate functional large and small platelets from the same individual. We developed a protocol that facilitates the separation of large and small human platelets and questioned whether the intensity and the velocity of the response of large and small platelets to different stimuli also differ in healthy volunteers.

**Methods:** Using sequential differential centrifugation steps in defined volumes, large and small platelet fractions were separated. MPV was determined by a cell counter. Generation of platelet derived microparticles and platelet aggregation was assessed after stimulation with collagen and epinephrine using flow cytometry and light transmission aggregometry, respectively. Platelet adhesion and spreading under flow was measured on collagen functionalized defined micropatterned arrays using live imaging and quantitative fluorescence microscopy. All experiments were performed at least n=6.

**Results:** After separation, large platelets had an average MPV of 12.03 fl ± 0.88 and small ones of 7.76 fl ± 0.53 (~1.6 fold difference, p<0.0001). Collagen induced aggregation was faster (lagtime 25s ± 21 vs 49s ± 28, p=0.0086) and the maximum epinephrine induced aggregation was higher (66% ± 23 vs 42% ± 30, p=0.0125) in large platelets. Large platelets secreted 2.5 to 3 times more microparticles compared to small platelets (collagen induced: 15300 ± 7109 vs 5192 ± 1728 microparticles per µl, p=0.0147; epinephrine induced: 13862 ± 8518 vs 5562 ± 2292 microparticles per µl, p=0.0285). On collagen functionalized defined micropatterned arrays, single large platelets covered an area being 1.4 fold higher than the area covered by small platelets (63.76 µm² ± 21.13 in vs. 45.69 µm² ± 17.9, p<0.0001).

**Conclusion:** In healthy volunteers, large platelets respond faster and stronger to different stimuli than small ones. Thus, an increase of platelet size increases the overall functional capacity of circulating platelets, which may be relevant in cardiovascular diseases. Our protocol facilitates the study of large and small human platelets in health and disease.
Evaluation of platelet activation after electric cardioversion

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Poster Topic
Platelets - Physiology and Disorders of platelet number and function
17.02.2017, 17:15 - 18:15
Poster Number: 114

Objective: Atrial fibrillation is known to cause strokes. But also the time after cardioversion bears a risk for thromboembolism, making anticoagulation recommended for several weeks. Therefore anticoagulation is mandatory for these patients even after restoring normal sinus rhythm. This elevated risk of thromboembolism and stroke after intervention is explained with atrial stunning, the delayed return of full function of the atrium myocardium. As an alternative explanation we hypothesized that platelets activated by the electric cardioversion contribute to a higher coagulability and a higher risk of thromboembolism.

Methods: Patients with known atrial fibrillation planned for elective electric cardioversion were included into the study (n=6). Blood was drawn before and 1 h after cardioversion. Flow cytometry was used to detect platelet activation. The platelets were labelled with a CD41 antibody in whole blood. As activation marker CD62P (P-Selectin) and PAC-1 (activated integrin αIIbβ3) antibodies were chosen. Ratios of geometrical mean fluorescence intensities (receptor/isotype control) were used to evaluate changes in respective receptor expression before/after cardioversion.

Results: No significant differences were found in the expression of platelet activation markers before and after cardioversion. The mean changes from before to after cardioversion were -0.6 % (SEM 10.0 %) for CD62P (ratio CD62P/IgG κ) and + 89.9 % (SEM 79.9 %) for PAC-1 (ratio PAC-1/PAC-1 + RGDS).

Conclusion: As electric current has obvious influence on membrane stability of different cells, activation of platelets and a contribution to hypercoagulability is likely to occur. This idea is supported by the observation of thrombosis in the context of electrical accidents. However, these effects appear -if present at all- to be very subtle, so that in this study with small sample size no significant effect could be observed.
Platelet dense granule production and dense granule release defects in pediatric patients with storage pool disorder

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Objective: Storage pool disease (SPD) comprises a group of platelet defects where alpha and/or delta granules are reduced or cannot be secreted in response to agonists. The detection of delta granule release has been hampered as there are no simple assays. We aim to implement a kinetic mepacrine assay to better identify the pathomechanism in children with a dense granule-based bleeding diathesis.

Methods: We developed a flow cytometric mepacrine assay which measures the unstained platelets, determines the mepacrine uptake and finally the decline in fluorescence after agonist stimulation over 5 minutes. The analysis was performed using FlowJo software. We correlated the mean fluorescence intensity of mepacrine uptake and release patterns with CD63 exposure, ATP release and the ADP/ATP ratio. The bleeding score was assessed by the translated ISTH-BAT. We analyzed 56 children with SPD whose initial diagnosis was confirmed in a second site visit.

Results: 90% of children had a positive family history of bleedings, mostly with the mother also suffering from prolonged bleeding episodes. Most children had an ISTH-BAT score of 2, 3 or 4. The delta granule release (ADP and ATP) was significantly reduced in our cohort with 75% having values below the reference threshold value of 10.1 umol/10e12 platelets. The mepacrine release was statistically reduced in patients with SPD compared to controls (p>0.05). The ability to release mepacrine in response to TRAP correlated with the delta-granule release measure by ADP+ATP release. We detected a reduced CD63 expression by flow cytometry in response to ADP or TRAP-6 in 12 children. 6 patients showed defective mepacrine uptake, 7 a selective delta granule release, 13 had a combined uptake and release defect. 10 patients showed a normal uptake and release, when compared to healthy adult controls. The overall response to ADP and TRAP in aggregometry and flow cytometry correlated well, indicating that the underlying defect is a granule-based defect and not due to an agonist receptor.

Conclusion: Our data show that implementation of a time-resolved mepacrine allows to distinguish mepacrine uptake, mepacrine release, and combined defects. It is a quick and inexpensive tool that can readily be added to help refining the diagnosis.
Practical limitations of diagnostic algorithms in heparin induced thrombocytopenia: A case report.

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Poster Topic
Platelets - Physiology and Disorders of platelet number and function
17.02.2017, 17:15 - 18:15
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Objective: Heparin-induced thrombocytopenia (HIT) can be potentially associated with devastating complications such as life-threatening thrombosis making it one of the most serious adverse drug reactions. A rapid diagnostic work-up is therefore very important. However, diagnosis of HIT is challenging due to a number of practical issues and methodological limitations. We report on one case of HIT which demonstrates the limited sensitivity of a rapid assay and the 4Ts score, respectively, emphasising the need for a clear diagnostic algorithm in the clinical practice.

Methods: The pretest probability of HIT was determined using the 4Ts scoring system. A Lateral Flow Immunoassay was used as a rapid assay. Serological investigation also included an IgG-specific enzyme-linked immunosorbent assays (ELISAs) for antibodies against PF4/heparin-complexes and the Heparin induced platelet activation (HIPA) as functional assay.

Results: A 61 year old woman with a spinal cord tumour was admitted to our hospital with pain and tenderness in the right leg. Clinical examination and laboratory markers were suggestive for thrombosis. Despite treatment using low molecular weight heparin (LMWH) the patient developed a progressive thrombosis involving calf, popliteal, femoral, and vena cava. The diagnosis of HIT was suspected. First evaluation of the patient by the treating physician revealed a 4Ts score of 3. The rapid immunoassay was negative. Although these results were suggestive against HIT, we initiated further laboratory investigation and decided to switch heparin to argatroban due to the progressive thrombosis. Strong anti-PF4/heparin antibodies were detected using ELISA (OD 2.458) and HIPA (platelet activation 4/4 cells). Critical review of the medical records and direct patient,s interview revealed that the 4Ts score was miscalculated on day 7 because of (1) treatment with LMWH heparin (initially reported as s.c. insulin) before admission was not recognized and rapid-onset HIT was not considered (timing=1 point, correct 2 points), and (2) chemotherapy was considered to be a sufficient reason for thrombocytopenia (other reasons=0 points, correct 1 point). The 4Ts score was re-calculated revealing 6/8 points (high risk).

Conclusion: In clinical practice, we recommend an integrated diagnostic approach combining not only clinical assessment (the 4Ts score) but immunoassays and functional assays as well.
Supra-normal closure time caused by recombinant von Willebrand factor in PFA

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Objective: High molecular weight (HMW) von Willebrand factor (VWF) multimers play a crucial role in primary hemostasis. It was shown that ultralarge multimers (UL) are even more important for VWF-platelet interaction. Especially von Willebrand disease (VWD) patients lacking HMW multimers (type 2+3) show specific bleeding symptoms and have to be treated with factor supplements. In vitro we simulated basic, shear dependent processes of primary hemostasis and showed the influence of VWF supplements on VWD patient’s whole blood.

Methods: Citrate anticoagulated whole blood from healthy donors and severe VWD patients was obtained under standardized conditions and tested. The platelet functional analyzer (PFA-200) measures functional primary hemostasis mimicking the vascular lesion including flow dynamic effects. In the capillary a collagen coated membrane is perfused with increasing shear rates beginning at 5k s^-1. Several platelet agonists (ADP and epinephrine) start the adhesion and aggregation reaction on the surface. Then recombinant VWF containing UL (rVWF/+UL; Baxalta, Vienna, Austria) or plasma derived VWF products were added (1 IU/ml) and the influence on the blood closure time determined. Additionally the effects of high shear (30k s^-1) on VWF spiked whole blood over collagen surface were video documented with flow chamber experiments.

Results: The healthy control group showed PFA results in normal range (ADP 68-120s; EPI 84-170s). Aggregate formation in the flow chamber was 5-25%. Patients lacking large VWF multimers had no closure in the PFA and no aggregation in the flow chamber. Adding rVWF/+UL to patient’s blood supra-normalized the blood closure time and promoted normal to increased aggregate formation on collagen surfaces (ADP 50-70s; EPI 60-90s). On the contrary plasma derived VWF products hardly changed results when added to the blood samples lacking VWF (VWD patients type 2+3).

Conclusion: We demonstrated and visualized that UL are important for primary hemostasis and can establish VWF-platelet interaction in VWD patient blood leading to normal aggregate formation. Independent of the sample’s VWF:Ag value UL decreases blood closure time to supra normal levels. Equimolar doses of plasma derived VWF do not show these effects. Nonetheless all VWF products show good results in patient application.
Prophylactic plasma treatment in patients with hereditary thrombotic thrombocytopenic purpura

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Poster Topic
Platelets - Physiology and Disorders of platelet number and function
17.02.2017, 17:15 - 18:15
Poster Number: 118

Objective: Hereditary TTP is a very rare autosomal recessive disease due to mutations in ADAMTS13 gene resulting in severe deficiency of ADAMTS13, a metalloprotease that cleaves ultra-large von Willebrand factor multimers. There are 7 patients with USS followed and treated at the Blood Centre at the University Hospital in Ostrava who require prophylactic fresh frozen plasma (FFP), and/or solvent detergent plasma (SDP) in empirically determined treatment schedules. ADAMTS13 recovery and pharmacokinetics are affected by treatment modality and reflect associated patient comorbidities as well as their current clinical condition. The aim of this study was to estimate ADAMTS13 recovery upon regular plasma infusion (PI) or plasma exchange (PEX) regimens.

Methods: Six of 7 USS patients, who regularly received prophylactic FFP/SDP therapy between June 2013 and March 2015 took part in this study. During this period FFP was replaced by SDP treatment. ADAMTS13 activity was assessed in patient samples as well as in all individual FFP and SDP bags by two methods – an enzyme-linked immunosorbent assay and FRETS-VWF73 assay.

Results: There was excellent agreement between both ADAMTS13 assays in all samples analyzed and there was no statistically significant difference in ADAMTS13 activity levels between FFP and SDP preparations. All patients had baseline ADAMTS13 activity < 5% and none had a functional inhibitor or anti-ADAMTS13 antibody at any time during this study.

ADAMTS13 recovery in patients after PEX was 54.5±10.6% (mean ± SD) and 25.4±5.2 % after PI.

Conclusion: Usually PI is recommended for prophylactic plasma therapy in USS patients. Based on our experience in patients with a more severe disease course, however, we prefer PEX via peripheral venous access. Thereby, more plasma and thus ADAMTS13 can be administered at a single treatment cycle. In addition the patients benefit from less volume overload and from elimination of degradation products.
Molecular genetic investigations and demonstration of founder effect in Osler-Rendu-Weber disease

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Poster Topic
Vascular wall biology and disorders
17.02.2017, 17:15 - 18:15
Poster Number: 119

Objective: Osler-Rendu-Weber disease (hereditary hemorrhagic telangiectasia; HHT) is a rare autosomal dominant vascular abnormality characterized by mucocutaneous telangiectases and visceral arteriovenous malformations. Many mutations are responsible for the disease, the most commonly involved genes are endoglin (ENG), the activin receptor-like kinase 1 (ACVRL1) and SMAD4, which encode proteins of the transforming growth factor-beta superfamily. Approximately 85 % of HHT cases have heterozygous family-specific mutations either in the ENG or ACVRL1 genes, causing HHT type 1 and 2, respectively. The clinical diagnosis of the disease is based on the Curacao criteria.

Our aims were to identify causative mutations of HHT in North-East Hungary and to examine the possibility of a founder effect.

Methods: HHT patients and their relatives were recruited between 2012 and 2016 (n=82). Mutations within ENG and ACVRL1 were detected by direct fluorescent sequencing. Mutation carriers, their spouses and 50 healthy people were genotyped for 8 polymorphic markers around ACVRL1 gene (D12S1677, D12S85, D12S2196, D12S1712, D12S270 and SNPs rs2071219, rs706815 and rs706816). Haplotype analysis was performed to ascertain the possibility of a founder effect. Founder effect was also demonstrated by genealogical study.

Results: Among the 29 HHT index patients, 59% were identified with ENG (11 known and 6 novel) and 28% with ACVRL1 (2 known and 1 novel) mutations. The novel splicing mutation (ACVRL1 c.625+1 G>C) was identified in 5 families. The mutation was absent in 50 healthy subjects. The ACVRL1 c.625+1 G>C mutation was associated with the same haplotype in all carriers (n=14), while different haplotypes were observed in healthy controls that suggested founder effect. The genealogical analysis revealed that the possible common ancestors were married in 1779.

Conclusion: Genetic background of HHT is heterogeneous in our geographical area, however a founder mutation was found in 5 families with the disease. The demonstration of a founder mutation is helpful, since the clinical presentation of the disease becomes more predictable and it might simplify the molecular genetic diagnosis algorithm.
CLIC1 supports mechanisms related to thrombosis and vascular repair

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Poster Topic
Vascular wall biology and disorders
17.02.2017, 17:15 - 18:15
Poster Number: 120

Objective: Chloride intracellular channel 1 (CLIC1) has been shown to be involved in thrombus formation and angiogenesis but the functional context of CLIC1 action remains largely unexplored. The objective of this study is to determine if CLIC1 supports cell adhesive processes that are relevant for endothelial and platelet function.

Methods: Human umbilical venous endothelial cells (HUVEC) were treated with CLIC1 siRNA or the CLIC1 small molecule inhibitor IAA94 and probed for cell proliferation on plastic (2D) as well as cell invasion/survival after embedding in fibrin (3D). Platelets were treated with IAA94 to assess the effect of CLIC1 on aggregation. Flow cytometry was used to determine integrin alphaIIbbeta3 activation as well as CLIC1 cell surface expression on platelets. The subcellular localization of CLIC1 in HUVEC/platelets was analyzed with fluorescence or confocal microscopy. The effect of CLIC1 on thrombus formation in vivo was assessed by intravital fluorescence microscopy in a mouse dorsal skin fold chamber model.

Results: Treatment of HUVEC with CLIC1 siRNA or IAA94 had a strong anti-proliferative effect in 2D and caused significant reduction of invasion and survival in 3D. At the same time, we detected relocation of CLIC1 into lamellipodia (2D) and invadopodia (3D) in untreated HUVEC. This particular distribution of CLIC1 is functionally significant as we found reduced membrane ruffling in combination with increased stress fiber formation in CLIC1 siRNA-treated HUVEC. Relocation of CLIC1 into the cell periphery was also detectable in platelets, which expressed CLIC1 on the cell surface in an RGD-dependent manner. CLIC1 relocation to the platelet membrane was inhibited after treatment with IAA94, which also reduced integrin alphaIIbbeta3 activation. This in turn led to impaired platelet aggregation in vitro and prolonged vaso-occlusion in a mouse model of photo-chemical thrombus formation in vivo.

Conclusion: Our results show that CLIC1 is regulated by adhesive interactions with integrin ligands that cause CLIC1 to relocate to the cell surface. This process in turn appears to be relevant for integrin-mediated functions involved in platelet thrombus formation, angiogenesis and vascular repair.
Collagen V alpha 2 as a direct target of miR-143 in arteriogenesis

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Objective: Arteriogenesis describes the formation of collateral arteries from preexisting small vessels, that involves the proliferation of endothelial and smooth muscle cells as well as the recruitment of leukocytes. This process is induced by physical forces, most importantly fluid shear stress (FSS).

It was shown that FSS leads to a change in the expression pattern of several microRNAs (miRs) in growing collateral arteries; especially miR-143 is highly expressed. Accordingly, a miR-143 knockdown resulted in the inhibition of the growth of collateral arteries in mice. Collagen V alpha 2 (COLVA2) was identified by us as a candidate target gene of miR-143 carrying the predicted target sequence and being downregulated in growing collaterals.

Methods: To confirm the direct interaction between miR-143 and COLVA2 mRNA, a Dual Luciferase Reporter Assay was performed by cloning the COLVA2-3'UTR-sequence into the miR-specific plasmid vector psiCHECK™-2.

Results: Co-transfection of this plasmid for 48 h together with an artificial miR-143 construct (but not with the scrambled miRNA-construct) into endothelial cells (Ea.hy926; express only low amounts of miR-143) resulted in increased miR-143 expression while luciferase activity decreased. These data indicate that miR-143 modulates COLVA2 expression by directly targeting the 3'UTR-region of the COLVA2 mRNA. Accordingly, 48 h co-transfection of the plasmid with anti-miR-143 (but not with the scrambled anti-miRNA) in murine vascular smooth muscle cells or NIH/3T3-fibroblasts respectively, which both express high amounts of miR-143, induced the knockdown of miR 143 and upregulated the luciferase expression.

Conclusion: These findings demonstrate that miR-143 directly regulates COLVA2 expression, which may play a decisive role in vessel and tissue remodeling during arteriogenesis.
Autophagy status in factor VIII secreting endothelial cells

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**Poster Topic**
Vascular wall biology and disorders
17.02.2017, 17:15 - 18:15
Poster Number: 123

**Objective:** Liver sinusoidal endothelial cells (HHSEC) are considered as major source of F8. It is known that upon synthesis F8 is translocated into endoplasmic reticulum (ER) for cleavage, folding and quality assessment. These processes require constant ER turnover and modulation, in which autophagy plays an important role. ER expansion, ER fragmentation or lysosome degradation may occur because of change in autophagy status. Also Takehiro, et al., states that inhibition or knockdown of autophagy essential genes decrease vWF secretion in endothelial cells; therefore it may be possible to have association between autophagy status and F8 secretion levels in different endothelial cells.

**Methods:** Behrends et. al (2010) proposed a network of six ATG8 orthologues in human interacting with a cohort of 67 proteins, which are playing a significant role in autophagy. To study the status of autophagy in FVIII secreting endothelial cells, we created a network of the 67 proteins interacting with autophagy by using IPA knowledge base. 35 out of 67 proteins were directly interacting with autophagy and some through other proteins from the above mentioned 67 proteins. Thirty out of 35 interacting proteins have direct correlation with autophagy status (activation or inhibition), out of which 28 were positively correlated (activation) to autophagy status and only 2 (inhibiting) were negatively correlated to autophagy status.

We predicted the autophagy status based on above network in 5 endothelial cells; HHSEC (adult and fetal), human cardiac microvascular endothelial cells (HCMEC), human pulmonary microvascular endothelial cells (HPMEC), human pulmonary artery endothelial cells (HPAEC) and human umbilical vein endothelial cells (HUVEC).

**Results:** We calculated the log fold changes of all endothelial cells to each other to rank them based on their autophagy status. Therefore, the endothelial cells could be ranked from highest activated to lowest: HHSEC adult-HUVEC-HPAEC-HPMEC-HCMEC-HHSEC fetal.

**Conclusion:** Our data suggest that higher autophagy-activation status is associated with higher rate of F8 secretion, however the intracellular molecular mechanisms that explains this phenomenon is/are still to be clarified.
Analysis of proteolytic von Willebrand factor (VWF) fragments in patients with VWF-related diseases

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Poster Topic
Vascular wall biology and disorders
17.02.2017, 17:15 - 18:15
Poster Number: 124

Objective: VWF circulates in plasma as a series of multimers of Mr 500 kD up to 20’000 kD, consists of disulfide-linked subunits of 250 kD and physiologically contains small amounts of 140 kD and 176 kD fragments resulting from ADAMTS13-induced cleavage. Some forms of Von Willebrand disease (VWD) type 2A, i.e. subtype IIA of old classification, show increased VWF sensitivity to ADAMTS13 with enhanced proteolysis and loss of large multimers. Thrombotic thrombocytopenic purpura (TTP), caused by severe acquired or hereditary ADAMTS13 deficiency, displays hyperadhesive unusually large VWF multimers which cause microvascular platelet clumping. It is unclear whether some rescue proteases, such as plasmin, may at least partially regulate VWF multimeric size. We aimed to develop a method to analyze and quantitate intact and proteolytically cleaved VWF subunits and to characterize the fragments by immunoblotting.

Methods: Plasma-VWF was immunoadsorbed in the presence of a universal protease inhibitor to CNBr-activated Sepharose 4B-beads coupled with polyclonal rabbit anti-VWF IgG, reduced and alkylated and subjected to SDS-PAGE and immunoblotting using polyclonal HRP-conjugated anti-VWF antibodies. Quantification of intact VWF subunits and ADAMTS13 cleaved bands was achieved using dilutions of rhVWF, before and after ADAMTS13-induced proteolysis. In addition, we studied rhVWF cleavage by plasmin to characterize the generated fragments.

Results: We show that the efficacy of VWF immunoadsorption is independent of the degree of VWF cleavage by ADAMTS13. A plasma sample of an individual patient with VWD type 2A (subtype IIA) displayed increased concentrations of 140 and 176 kD bands reflecting enhanced ADAMTS13-mediated VWF cleavage. Preliminary data in a patient with acquired severely ADAMTS13-deficient TTP and another with hereditary TTP in remission (3-10% residual ADAMTS13 activity) unexpectedly showed similar 140 and 176 kD bands. Using monoclonal antibodies towards the N- and C-terminal domains of VWF no evidence for plasmin-induced cleavage fragments in the patient with hereditary TTP was obtained.

Conclusion: The technique for the analysis of in vivo proteolysis of VWF has been established. Further study of various patient groups with presumably deficient or enhanced ADAMTS13-induced VWF cleavage and the search for different fragments resulting from alternative protease-induced VWF proteolysis is ongoing.
In patients with uncontrolled arterial hypertension platelets and coagulation factor XI are responsible of modifications of thrombin generation.

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**Poster Topic**
Vascular wall biology and disorders
17.02.2017, 17:15 - 18:15
Poster Number: 125

**Objective:** Hypertensive crisis is an extreme phenotype of increased blood pressure that can lead to organ failure and thrombotic complications. We recently found in experimental hypertension an angiotensin II driven factor XI (FXI)-thrombin amplification loop leading to vascular injury. We wanted to evaluate, in patients with uncontrolled hypertension (HT), the role of platelet and factor XI in thrombin generation (TG).

**Methods:** Prospectively, 52 patients with uncontrolled HT (16 grade I and 36 grade II/III) were examined at the outpatient clinic and emergency room of the University Medical Center Mainz, and 16 controls. Calibrated automated thrombography (CAT) was used to measure TG in platelet rich plasma (PRP, adjusted to 150.10^9/L of platelets), platelet free plasma (PFP), with platelets from controls and uncontrolled HT patients resuspended in a pool of healthy controls and in the presence or absence of an antibody blocking FXI activation pathway.

**Results:** We found an increased systolic blood pressure in HT patients, compared to control patients (179±1.9 mmHg for grade II/III patients, 139±1.2 mmHg for grade I patients and 123±2.5 mmHg for controls); age, BMI as well as weight were not different. TG in PRP showed a positive and significant correlation between blood pressure and the other TG parameters as Endogenous thrombin potential (ETP), peak TG and velocity of TG. ETP was significantly increased in grade II/III patients compared to the control group. TG parameters in HT patients and controls were completely blunted by the inhibition of FXI pathway by blocking its apple 3 domain. This result indicates an involvement of the FXI thrombin loop in thrombin generation in PRP of uncontrolled HT patients. Without platelets TG showed no difference between HT patients and controls. Platelets resuspended from HT or controls restored the differences in ETP, peak and velocity between the 2 groups indicating that increased TG in HT patient mostly depends on platelet overreactivity.

**Conclusion:** These results point out the important role of platelet overreactivity as well as FXI-dependent thrombin activation pathway in hypertension. Monitoring the prothrombotic state of platelets might add to risk stratification of patients with HT.
Investigating the interconnection between complement and coagulation in xenotransplantation

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Poster Topic
Vascular wall biology and disorders
17.02.2017, 17:15 - 18:15
Poster Number: 126

Objective: Acute vascular rejection is one of the main issues that hamper xenotransplantation to be clinically feasible. It is characterized by the activation of complement and coagulation systems converging into the onset of thrombotic microangiopathy which leads to the xenograft failure. In this study porcine endothelial cells (pEC), both wild type (wt) and lacking the major xenoantigen Gal-alpha-1,3-Gal (GTKO) were analysed for the activation of complement and coagulation in a xenotransplantation setting. Furthermore, genetically modified pEC overexpressing the human complement regulatory protein CD46 (hCD46) and human Thrombomodulin (hTM) were tested in a whole blood clotting assay.

Methods: Cell ELISA was carried out to analyse immunoglobulin and complement deposition after incubation of pEC with normal human serum (NHS). Activation of pEC was evaluated by immunofluorescence staining of Tissue Factor (TF) and E-Selectin after treatment with TNF or NHS. The anticoagulant properties were analysed by performing a whole blood clotting assay which involves the cultivation of different types of pEC on collagen coated microcarrier beads to increase the endothelial surface-to-blood volume ratio mimicking the in vivo situation in small vessels. After confluency, microcarrier beads were incubated with freshly drawn non anticoagulated human blood and the clotting time was measured. The values obtained with the different types of cells were graphed and compared. Beads were retrieved during the experiment and stained for von Willebrand Factor (vWF) and TF.

Results: A lower deposition of IgM, IgG, C3b/c and C4b/c was observed on GTKO pEC as compared with wildtype, which is due to the GalKO phenotype. NHS incubation led to the activation of pEC as showed by the expression of E-selectin and TF which was lower in GalKO pEC. GalKO/hCD46/hTM transgenic pEC delayed the clotting time of non-anticoagulated human blood in the microcarrier bead assay in a higher extent compared to the other cell lines. At the end of the experiment vWF and TF were strongly upregulated.

Conclusion: Data showed that NHS treatment leads to activation of pEC and genetically modified cells are able to prolong the clotting time of non-anticoagulated human blood much longer than wild type cells. This result suggests that the overexpression of hCD46 and hTM has a beneficial effect on the regulation of coagulation in xenotransplantation.
Platelet-localized FXI promotes a vascular coagulation-inflammatory circuit in arterial hypertension

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**Poster Topic**
Vascular wall biology and disorders
17.02.2017, 17:15 - 18:15
Poster Number: 127

**Objective:** High levels of angiotensin II (ATII) cause hypertension by a complex inflammatory pathway requiring leukocyte recruitment and reactive oxygen species production within the vessel wall coupled to vascular dysfunction.

The aim of this work was to explore the role of thrombin FXI feedback loop in animal models of arterial hypertension.

**Methods:** To induce hypertension mice on a C57BL/6 background were infused with ATII (s.c. 1 mg/kg/d for 7d) and Wistar rats underwent 5/6 nephrectomy (Nx).

**Results:** Here we report an upregulation of tissue factor, thrombin dependent vascular VCAM1 expression and platelet dependent leukocyte adhesion to arterial vessels in ATII-infused mice. ATII-induced vascular dysfunction and Ccl2, VCAM1 and Ly6C mRNA expression were attenuated by thrombin inhibition, platelet depletion as well as in hiL4R/Ibalpha mice missing the extracellular ligand binding domains of GPIbalpha. Blockade of TF during ATII administration also attenuated vascular dysfunction and reduced vascular oxidative stress. PRP of ATII-treated mice showed an increased thrombin evoked endogenous thrombin potential (ETP), whereas PRP from mice with pharmacological inhibition of FXI production by an FXI antisense oligonucleotide (FXI ASO) or PRP of hiL4R/Ibalpha mice failed to amplify ETP following ATII exposure. These data show that a FXI-dependent thrombin generation feedback loop requires GPIbalpha on platelets and suggest that TF-initiated coagulation promotes additional thrombin formation on platelets to cause vascular inflammation.

To further evaluate the therapeutic potential of interrupting FXI synthesis and function on blood pressure we used an additional hypertension model. Nx rats were either preventively injected with FXI ASO or injection was started when blood pressure was already significantly upregulated. Nx rats revealed endothelial dysfunction, vascular oxidative stress, kidney damage as well as an increase in blood pressure. FXI ASO injection in a preventive way or as a treatment significantly improved vascular and renal injury and persistently reduced arterial hypertension.

**Conclusion:** These novel benefits of FXI inhibitors might add to their applicability as antithrombotic agents, especially in cardiovascular disease with an activated RAAS.
Clinical relevance of marginal low Protein S

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Poster Topic
Venous thrombosis
17.02.2017, 17:15 - 18:15
Poster Number: 128

Objective: The laboratory diagnosis of Protein S deficiency is challenging not only due to the fact that Protein S is bound to C4BP but also because there are heterogeneous assays that can be performed. Furthermore, the differentiation into the classical three types of Protein S deficiency is not always as simple as it looks like. We like to discuss the current algorithms of Protein S testing along with presentation of a case, thereby questioning how clinically relevant are marginal low Protein S values.

Methods: We present a case with the c.1501T>C Mutation in the protein S Gene and discuss current algorithms for Protein S deficiency in the context of marginal low cases.

Results: The finding of a marginal low Protein S should be interpreted with caution. Repeated measurements are mandatory to establish a diagnosis. Genetic testing may be helpful in interpreting the data, and find a potential cause, especially if combined with family analysis, but for many mutations derived from one or few cases, clinical data is limited and the causative role has to be scrutinized.

Conclusion: In patients with only marginal low Protein S values, it is difficult to judge whether and to what extend the patient has a thrombophilic diathesis even if the underlying genetic cause can be identified. Interpretations should be made with caution, and balanced with the individual clinical history including the family history if accessible.
Characterisation of previously unknown protein C gene variants

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Poster Topic
Venous thrombosis
17.02.2017, 17:15 - 18:15
Poster Number: 129

Objective: Hereditary deficiencies of natural anticoagulant proteins including protein C are known causes of thrombophilia. Up to now more than 350 different mutations in the Protein C gene (PROC) have been described. Our aim was to analyse the molecular defect underlying Protein C deficiency in a cohort of 314 patients and to predict the potential effect of previously unknown variants detected in this cohort.

Methods: DNA of 314 patients was analysed by Sanger Sequencing of the coding region of PROC including the exon intron boundaries. In cases without mutation MLPA was performed to detect large deletions or duplications. The characterisation of the variants was performed with in silico evaluation tools, including Polyhen-2, SIFT, multiple sequence alignment, splicing prediction and molecular graphic imaging.

Results: 101 different Mutations were detected in 226 patients, correlating with an overall mutation detection rate (MDR) for the PROC gene of 72,0 %. Altogether, 36 (35,6%) unknown and 65 (63,4%) previously described variants of PROC were identified.

Of the unknown variants 23 were potentially missense changes and two were located in the intron, near to the exon intron boundary (potentially splice site changes).

Of the previously unknown missense changes, one was classified as variant of unknown significance and 22 as potential missense mutations with pathogenic effect. The two variants located near the exon intron boundary were predicted as potential splice site mutations by in silico methods.

Conclusion: In silico methods and molecular graphic imaging are useful tools to predict the effect of genetic variants on the protein function or structure and by this to predict the pathogenic effect of certain variants. Nevertheless these methods cannot replace in vitro analysis by laboratory experiments, phenotypic studies of patients and cosegregation analysis within families.
GE-CAT - German Evaluation of Cancer associated Thrombosis A prospective register trial for patients with hematological and oncological diseases and venous thromboembolism in Berlin


Poster Topic
Venous thrombosis
17.02.2017, 17:15 - 18:15
Poster Number: 130

Objective:
Different recommendations for cancer patients with venous thromboembolism (VTE) exist. The recently published german S2-Guideline for diagnosis and treatment of venous thromboembolism recommend anticoagulation for 3 to 6 months preferably with low molecule weight heparin (LMWH). LMWH are actually estimated to be the most effective and safest treatment option in cancer patients with VTE (AWMF 10/2015).

However, it remains unclear how cancer patients with VTE are treated in clinical care in Germany today. A recent published health service trial (Matzdorff A Oncol Res Treat 2016) was done in 2014 by a questionnaire. This survey reveals that practice patterns often do not follow the guideline recommendations. In former register trials, cancer patients were insufficiently characterized and their specific characteristics were inadequately discussed (Marchena PJ Thromb Haemost. 2012). Especially, only insufficient data of patient characteristics and anticoagulation regimens used are available, as well as informations about the mainly treating physicians.

The GE-CAT register was set up for Berlins two main hospital companies Charité and Vivantes. Both together take care for about 375000 hospitalized patients per year, thus covering about 50 % of the in-ward health service of the Berlin region. Primary objective of this health services research is to evaluate the treatment reality of patients with cancer associated VTE in clinical daily practice. It will give results about the relative proportion of VTE patients with cancer and captures cancer-patients course of disease and treatment tracks for the first time.

Methods:
GE-CAT is a prospective register trial with a duration of 3 years. Patient with an age of more than 18 years, a newly diagnosed VTE will be enrolled. Patients without a diagnosis of cancer are anonymously documented captured with a short basis assessment but without further follow-up. After signed written informed consent patients with the diagnosis of any kind of cancer will get a basis documentation by a physician. A follow up is scheduled after 3 and 6 months per telephone interview. Points of interest are: Rate of tumor associated thromboembolism, diagnostics and primary anticoagulation treatment, mortality, relapse of the venous thromboembolism or bleeding within 3 and 6 months, serious adverse events and determination of patients tracks.

Results:

Conclusion:
Study of fibrinolytic parameters in Indian patients with venous thrombosis

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**Poster Topic**

Venous thrombosis
17.02.2017, 17:15 - 18:15
Poster Number: 131

**Objective:** To establish a co-relation between impaired or defective fibrinolytic system and venous thrombosis in Indian patients by comprehensively studying the fibrinolytic pathway factors and detect any possible defects which may be causative for venous thrombosis in Indian patients.

**Methods:** 224 venous thrombosis patients were studied for conventional thrombophilia markers along with Fibrinolytic markers Plasminogen (PLG), Tissue Plasminogen Activator (TPA), Plasminogen Activator Inhibitor (PAI-1), Thrombin activatable fibrinolysis inhibitor (TAFI), Alpha-2-Antiplasmin (A2-AP) along with Thrombomodulin (TM) and Tissue factor pathway inhibitor (TFPI). PAI-1 4G/5G promoter polymorphism was also studied.

**Results:** 17.9% (40 cases) of venous thrombosis were accounted by conventional thrombophilia screening alone. 7.6% (17 cases) of them had Factor V Leiden mutation, 5.8% (13 cases) had Protein C deficiency, 3.1% (7 cases) had Protein S deficiency and 1.3% (3 cases) had Antithrombin III deficiency. However, a comprehensive study including fibrinolytic parameters could explain 35.3% (79 cases) of venous thrombosis. These include 13 cases (5.8%) wherein there was a combined defect i.e. positive for a conventional thrombophilia marker and a defective fibrinolytic parameter. There were also 4 cases (1.8%) wherein at least two fibrinolytic proteins were abnormal. High PAI-1 levels were seen in 16.5% (37 cases) (170.67± 89.89 ng/ml). 17 of these cases also had high CRP level. PAI-1 4G/5G promoter polymorphism is associated with circulating levels of PAI-1. Cases with a 4G/4G genotype had significantly high mean of 96.25 ± 71.19 ng/ml PAI-1 level in comparison with those with 4G/5G genotype (60.84 ± 43.82 ng/ml) and 5G/5G genotype (54.11 ± 41.54 ng/ml). 21.9 % of venous thrombosis cases had a 4G/4G genotype as compared to 13 % in healthy controls. 4.5% (10 cases) had reduced TPA level (0.66 ± 0.08 ng/ml). 2.7% (6 cases) had high A2-AP level (292.52 ± 23.23 ng/ml). 1.3% (3 cases) had reduced PLG level (228.71 ± 40.60 µg/ml). 0.9% (2 cases) had high TAFI level (1120 ± 42.42 ng/ml). 0.9% (2 cases) had low TM level (0.22 ± 0.3 ng/ml). 0.4% (1 case) had low TFPI (7 ng/ml) level.

**Conclusion:** Data shows that testing for markers both in the anti-coagulant system and in the fibrinolytic pathway will facilitate in a comprehensive explanation of the cause of venous thrombosis.
Characterization of antithrombin variants in thrombotic patients with normal and reduced activity.

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Poster Topic
Venous thrombosis
17.02.2017, 17:15 - 18:15
Poster Number: 132

Objective: To characterize mutations in AT deficient and normal patient with arterial and venous thrombosis and correlate mutations with the laboratory and clinical phenotype. To analyse the feasibility of the most suitable technique for an accurate diagnosis of AT deficiency.

Methods: Patients with Arterial and venous thrombosis having low AT activity were screened for the presence of mutations in SERPINC1 gene by direct DNA sequencing. 500 (arterial 125; venous 375) patients with normal AT levels were screened for the presence of mutations in exon 5. AT activity was measured by chromogenic assay based on thrombin (IIa) and Xa inhibition. AT antigen was measured by ELISA and rocket immunoelectrophoresis. 100 normal healthy controls randomly selected to match with the cases by age; sex and geographical area and were sequenced for exon 5. Also 50 patients with borderline AT activity were sequenced for all seven exon and promoter region of SERPINC1 gene.

Results: 2 patients presented with heterozygous type I mutation, p.Ala126Asp and p.Arg164stop; 1 patient presented with type II mutation, a rare case with homozygous p.Met121Val. In Patients with normal AT activity, P305H mutation was seen to be more common and is present in much conserved region on AT gene. 10 out 11 patients with this mutation suffered from cerebral thrombosis and 1 patient had splenic infarct. H351P mutation was found to be deleterious by two of the three prediction softwares. 1 borderline patient presented with splice site variant in promoter region.

Conclusion: In this study, 2 type I and 1 type II mutations are detected. Type I is the most severe type of all AT mutations. Also 4 variants with normal AT activity of which mutations P305H and H351P were found to be deleterious in prediction softwares. Recent evidences show that some SERPINC1 mutations responsible for functional abnormality of AT often show slightly decreased or even normal activity and cannot be detected by currently available laboratory assays. The data strongly suggests that genetic screening for variants in SERPINC1 gene should be performed in all thrombosis cases. Also, genetic counseling and screening of family members is important to prevent further thrombotic episodes.
Application of massive parallel sequencing searching for rare genetic variants associated with thrombophilia - implementation to clinical practice.

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**Poster Topic**  
Venous thrombosis  
17.02.2017, 17:15 - 18:15  
Poster Number: 133

**Objective:** Thromboembolism have multifactorial etiology but one of the main causes is thrombophilia. Really common and severe cause of thrombophilia is deficiency of protein C, S or antithrombin III (PC, PS, AT III). The genetic cause are mutations without mutational hot-spots areas in the studied genes. Therefore it is appropriate to use methodology of massive parallel sequencing (MPS) and expanded molecular genetic analysis for detection of genetic cause of deficiency of those proteins. Selected proteins are encoded by genes: PROS, PROC, SERPINC1. In these genes have been described more than 800 mutations whose clinical manifestation may be a lack or loss of function of the gene product, which are involved in the inhibition of coagulation factors. Pathogenic mutations in these genes exhibit similar risk of thrombosis as routinely tested mutations of FV Leiden and FII Prothrombin (G20210A).

**Methods:** MPS was carried out by Ion Torrent PGM platform. We used Ampliseq Designer for design of multiplex with 100% coverage of coding sequences and exon / intron border areas. The data were processed by Torrent Suite programs - Ion Reporter and Next Gene - available database of clinical variants (ClinVar, HGMD).

**Results:** In the first run there were examined 10 patients. They were selected for repeatedly detected reduced levels of protein C or S, at the same time they were excluded for secondary etiology of that condition. We have revealed six missense mutations in PROS1(2) and PROC (4) so far.

**Conclusion:** MPS offers complex tool for identification genetic causes of severe thrombophilia states applicable to laboratory and clinical praxis. Further it will be useful to add MLPA analysis for patients where MPS doesn’t reveal any genetic cause. MLPA should improve testing for better detection of large rearrangements in examined genes.

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Advances in diagnosis, classification and management of lower extremity deep vein thrombosis (DVT), prevention of DVT recurrence and the post-thrombotic syndrome: personal experiences and appraisal of the literature

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Poster Topic
Venous thrombosis
17.02.2017, 17:15 - 18:15
Poster Number: 134

Objective:

Methods:

Results: Complete compression ultrasonography (CUS) followed by a sensitive D-dimer test and clinical score assessment is safe and cost-effective non-invasive strategy to exclude and diagnose deep vein thrombosis (DVT) and alternative diagnoses (AD) in patients with suspected DVT. Rapid and complete recanalization on CUS within 3 months post-DVT with no residual venous pathology (RVP-) is associated with low risk of DVT recurrence (1.2% patient/years) and PTS on the basis of which both anticoagulation MECS can be withdraw at 4 months post-DVT. Delayed and incomplete recanalization with RVP+ on CUS at 3 months post-DVT is associated with the presence of reflux due to valve destruction, a high risk of DVT recurrence and symptomatic PTS at 6 to 12 months post-DVT indicating the need to wear MECS and to extend anticoagulation for one to several years. Wearing MECS does not prevent DVT recurrence, reflux and outlet obstruction, but only relieves subjective signs in symptomatic PTS patients. Extended anticoagulation with low dose Direct Oral anticoagulants (DOACs) in patients at high risk of DVT recurrence will significantly reduce DVT recurrence and PTS. The Lower Extremity Thrombosis (LET) extension classification identifies patients with CVT LET class I, proximal DVT LET class II and iliofemoral DVT LET class III at time of acute DVT diagnosis. The higher the LET class the higher the risk of DVT recurrence and PTS. LET class II DVT patients do benefit from extended anticoagulation with DOACs. LET class III acute DVT patients do benefit from cathether-directed thrombolysis. A prospective safety efficacy outcome management study bridging the gap between DVT and PTS is warranted to reduce DVT recurrence rates to less than 3% patient/years during life long follow-up.

Conclusion:
The role of an endothelial specific g-protein coupled receptor in thrombosis

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Poster Topic
Venous thrombosis
17.02.2017, 17:15 - 18:15
Poster Number: 135

Objective: Venous thromboembolism (VTE) is the third most common cardiovascular disease and affects roughly 1 in 1000 individuals annually. The role of the endothelium in VTE is understudied. We recently identified an endothelial enriched orphan g-protein coupled receptor (GPCR) that is expressed throughout the adult human vasculature. In a genome wide association study of VTE patients ‘MARTHA’ we found an association between this gene locus and the development of VTE. Using expression quantitative trait loci analysis we found that this VTE associated SNP was linked to reduced transcription of the GPCR in human endothelial cells (EC). The objective of this study was to investigate the possible role of this endothelial enriched orphan GPCR in VTE. Specifically, how this GPCR is regulated and if it modulates the expression of EC proteins important in the regulation of coagulation, cell signalling and blood clotting.

Methods: We used siRNA to deplete the GPCR in human umbilical vein endothelial cells (HUVEC). Effects of the siRNA-mediated depletion on the expression of a panel of EC coagulation and inflammation related proteins were assessed by real-time qPCR, protein immunoblotting and quantitative mass spectrometry. We used a chromogenic activity assay and calibrated automated thrombography (specifically adapted to incorporate EC), to study the effects of the GPCR depletion on tissue factor activity in EC and thrombin generation in plasma respectively.

Results: The siRNA-mediated depletion of the orphan GPCR in HUVEC resulted in a significant increase in tissue factor (F3) transcription. The induction of F3 expression by tumour necrosis factor treatment of HUVEC was also significantly amplified following depletion. A significant increase in tissue factor activity was also observed after siRNA depletion of the GPCR. Using a calibrated automated thrombogram assay we also observed increased thrombin generation in plasma following depletion, an effect that was abolished when an inhibitory antibody against F3 was added.

Conclusion: Our results suggest that this GPCR could play a role in VTE development though the regulation of tissue factor expression.
Angiogenesis in a porcine von Willebrand disease model

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Poster Topic
Women issues in thrombosis and hemostasis
17.02.2017, 17:15 - 18:15
Poster Number: 136

Objective: Women with von Willebrand Disease (VWD) type 3 may develop miscarriage during pregnancy. There is evidence that besides its role in coagulation von Willebrand Factor (VWF) is also involved in angiogenesis and defects of this pathway could play a role in miscarriage. For this reason, VWF as well as proteins with known functions in angiogenesis were analysed by immunohistochemistry of several tissues in a pig model comprising all VWD type 3 genotypes (VWD, heterozygous carriers, wildtype).

Methods: We collected uterus tissue samples including the endometrium of two pigs affected by VWD type 3, two heterozygous carriers, and two wildtype individuals. None of these pigs was pregnant. Hematoxylin-eosin-staining was implemented for morphological evaluation of the tissues, especially with regard to possible differences in structure and pattern of blood vessels. In a second step immunohistochemical analyses comparing the expression of angiogenic factors including VWF were performed in VWD-animals, genetic carriers and wildtype pigs.

Results: The initial hematoxylin-eosin-staining on histological sections of the porcine uteri comprising the different genotypes revealed differences of vessel conformation within the lamina propria. Blood vessels of this tissue were small and less numerous in wildtype and heterozygous pigs. In individuals homozygous for VWD, however, they were partly dilated and thin-walled. The immunohistochemical analysis for VWF showed almost no VWF expression in the pigs affected by VWD. In wildtype and heterozygous pigs, expression was obvious in the endothelium. A narrow band of VWF expression apically on the epithel cells as well as partial expression in glands was seen only in wildtype pigs, but not in heterozygous pigs. For some, but not all further angiogenic factors analysed, expression differences were present among the porcine VWD genotypes.

Conclusion: The results of our study confirm effects of VWF on vessel conformation and structure and also on the expression of specific angiogenic factors. Pigs affected by VWD showed almost no expression of VWF and their blood vessels within the uterine lamina propria presented dilated and thin-walled. Expression differences among the genotypes were obvious for specific angiogenic factors.
Successful pregnancy in patient with homozygous sickle cell disease and prior pulmonary embolism – a case report

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Poster Topic
Women issues in thrombosis and hemostasis
17.02.2017, 17:15 - 18:15
Poster Number: 137

Objective: Pregnancy in patients with sickle cell disease (SCD) is associated with high risk of maternal and fetal morbidity and mortality. SCD seems to be an important thrombophilic condition, which is not fully understood yet, with higher risk of deep vein thrombosis and pulmonary embolism (PE), especially during pregnancy and post-partum. There are still unresolved issues regarding the optimal management of pregnancy in SCD. No recommendations were found concerning the management of anticoagulation in patients with prior PE, pregnancy and SCD, a particular high risk combination. To our knowledge, this is the first case report describing this combination. The objective of our paper is to describe one possible approach to patients with SCD, pregnancy and prior PE.

Methods: Case report.

Results: We describe the case and clinical management of a successful pregnancy of a 30-year-old patient with homozygous SCD and status after two miscarriages and PE. A screening for thrombophilia was negative. The patient was prior to the planned pregnancy under therapeutic dosage of low molecular weight heparin (LMWH) due to a PE 8 months prior and Hydroxyurea, which was stopped two months before conception. The LMWH was reduced to a prophylactic dosage during the pregnancy and Aspirin 100mg was added to reduce the risk of preeclampsia. The pregnancy was monitored closely with regular obstetric and hematological checks. The patient suffered several sickle-cell crises, which were managed with analgesics and adequate hydration. Echocardiography, lung function test and abdominal ultrasound were unremarkable. The Aspirin was stopped at the beginning of the third trimester to reduce birth-related bleeding. Before delivery one exchange transfusion was conducted to minimize bleeding during the C-section and a generous hydration was maintained through the C-section to minimize the chance of a sickle-cell-crisis. A healthy baby girl was delivered full term. The LMWH was discontinued 3 months after delivery. The patient and baby are in a good condition 6 months after the delivery.

Conclusion: A successful pregnancy in a high-risk patient with SCD and prior PE was possible with the aid of LMWH and Aspirin. Interdisciplinary collaboration is recommended to achieve a successful outcome. Further prospective studies are needed to optimize management of pregnancy and PE in patients with SCD.
Highly procoagulant extracellular vesicles in amniotic fluid

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Poster Topic
Women issues in thrombosis and hemostasis
17.02.2017, 17:15 - 18:15
Poster Number: 138

Objective: The pathomechanisms underlying disseminated intravascular coagulation (DIC) following amniotic fluid (AF) embolism remain to be fully elucidated. Highly procoagulant phosphatidylserine (PS)- and tissue factor (TF) exposing extracellular vesicles (EVs) might play a central role. It was the objective of the study to perform analyses of the procoagulant properties of AF with a panel of functional coagulation assays and flow cytometry to investigate the pathogenesis of AF induced DIC.

Methods: A prothrombinase assay, an EV-TF dependent factor Xa (FXa) generation assay, a modified thrombin- and fibrin-generation assay, a whole blood clotting model

and flow cytometry were applied in AF and control plasma.

Results: Phosphatidylserine expression was 21-fold increased in AF compared to plasma. Factor Xa generation was extremely high when TF-exposing EVs from AF were co-incubated with recombinant FVIIa. In the thrombin- and fibrin generation assay AF-derived EVs strongly activated the blood coagulation cascade via PS and TF. In a whole blood clotting model AF-derived TF-exposing EVs significantly shortened the clotting time from 734 ± 139 seconds in the presence- to 232 ± 139 seconds in the absence of an anti-TF antibody. The contact activation pathway via factor FXII was not affected. Applying flow cytometry, a sub-population of PS- and TF co-exposing EVs was clearly identified in AF.

Conclusion: We investigated the effect of AF on blood coagulation and found that PS+ and TF+ EVs determine its procoagulant potential. Taken together our data further delineate the pathomechanisms underlying AF-induced coagulopathy, which could improve diagnostic- and treatment modalities.
Dead fetus syndrome (DFS) in 2017 - a no more existing entity? From individual patients to pathophysiological insights

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Poster Topic
Women issues in thrombosis and hemostasis
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Objective: Multifetal pregnancy reduction and selective termination increased dramatically after widespread use of assisted reproductive technology. Usually the procedure is performed early in pregnancy – however we report on a case, in which the procedure (cord-occlusion) had to be performed in gestational week 22 to rescue one twin. The second twin had developed severe hydrops fetalis due to a complex congenital heart defect. However, no information is published regarding a rare, very high risk pregnancy with monochoriotic-diamnotic twins in case of DFS. Those twins share the same placenta, therefore necrotic fetal tissue will be washed into the second twin and the mother, continuously.

Methods: In case of DFS: Established recommendations are to check platelet count, fibrinogen, antithrombin to detect DIC. If levels are changing to remove the dead fetus immediately from the mother to avoid the so called dead fetus syndrome (DFS): the retention will cause low grade DIC with fibrinogen <1.5 g/l, platelet count <100/nl. DIC is anticipated when the dead fetus remains more than 5 weeks in utero. Nowadays an extremely rare complication which is usually avoided by early detection and intervention. It is caused by washed in necrotic fetal tissue into maternal circulation and induction of inflammatory cytokines.

Results: In gestational week 26 we were asked by the obstetricians to recommend screening tests to detect possible DIC/DFS in the woman described (see tab.1).

Unfortunately worsening of lab data challenged us to discuss an intervention. Reading older recommendation the obstetrician and the coagulation specialists were afraid of what might happen. We assumed low grade DIC and decided to start low dose heparin. At 34 weeks coagulation tests normalized. The surviving twin developed normally, regular uterine artery perfusion and mother’s health remained stabile. At 37 week elective c-section was performed.

Conclusion: In Germany there is no longer enough experience with coagulation abnormalities resulting from DFS. Even in our practice we have not seen a single case for more than 20 years! Due to the further uneventful course of the pregnancy, we decided from week to week not to treat dramatic "lab results". In retrospect, the observational approach in such a rare case was correct. It is necessary to collect again experience on DFS in 2017 when a high risk pregnancy benefits from modern technology.
Risk of pregnancy-associated recurrent venous thromboembolism (VTE) in women with a history of first venous thrombosis

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Poster Topic
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Objective: To evaluate the risk of recurrent pregnancy-associated VTE in women with a single previous episode of VTE and a subsequent pregnancy without heparin prophylaxis.

Methods: We included in the study 142 women with at least one pregnancy without heparin prophylaxis after a first VTE. Evaluation of thrombophilia comprised factor V Leiden, prothrombin G20210A mutation, and deficiencies of antithrombin, protein C or protein S.

Results: Nine of 142 women without heparin prophylaxis (6.3%) had an antepartum recurrence of VTE. Among the 66 women with evidence of thrombophilia and a first episode of VTE associated with a temporary risk factor, 4 (6.06%) had an antepartum recurrence of VTE (first trimenon n=1, second trimenon n=2, third trimenon n=1) (first VTE on oral contraceptives (OC) n=3, during surgery n=1). There was no recurrence in the women with thrombophilia and a first episode of idiopathic VTE (n=8). Among the 61 women who had no evidence of thrombophilia and a first episode of VTE associated with a temporary risk factor, 4 women (6.56%) (first trimenon n=2, second trimenon n=1, third trimenon n=1) (first VTE on OC n=1, during immobilization n=1, during surgery n=2), and among 7 women with no evidence of thrombophilia and a first idiopathic VTE, 1 woman (14.3%) had an antepartum recurrence (first trimenon).

In the postpartum period, 11 VTE occurred after live birth in 142 women (7.75%). The first episode of VTE in all these women was associated with a temporary risk factor. 7 of these 11 women showed evidence of thrombophilia.

Three of 68 women with heparin prophylaxis (4.4%) had an antepartum recurrence of VTE. Among the 32 women with evidence of thrombophilia and a first episode of VTE associated with a temporary risk factor, 3 (6.06%) had an antepartum recurrence of VTE (all factor V Leiden heterozygous) (first trimenon n=1, third trimenon n=2) (first VTE on OC n=2, pregnancy n=1). There was no recurrence in the women without thrombophilia and a first VTE associated with a temporary risk factor (n=36).

Conclusion: In addition to guideline-recommended postpartum heparin prophylaxis, our data also support routine antepartum prophylaxis in women whose previous episode of thrombosis was associated with a transient risk factor not related to pregnancy or use of estrogen, regardless of the presence of thrombophilia and starting in the first trimenon.
Comparison of two laboratory methods for thrombin generation in pregnancy with high risk for thrombosis

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Poster Topic
Women issues in thrombosis and hemostasis
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Poster Number: 141

Objective: Pregnancy and delivery are high-risk conditions for thrombotic complications. Women with heritable thrombophilia with or without thromboembolism in their medical history are at additional higher risk to develop thrombosis and are normally followed-up during pregnancy for evaluation of the prothrombotic state. Follow-up is done by clinical evaluation and sometimes it includes biological markers of activation of coagulation. A disproportional increase would theoretically predict high risk for thrombotic events but usefulness of these markers has not been validated in an evidence-based setting. Aims of this study were: a) to describe changes of biological markers of coagulation activation and thrombin generation in each trimester of pregnancy, b) to compare two different methods for assessment of thrombin generation, c) to check whether heparin prophylaxis in the third trimester affects thrombin generation.

Methods: Data on file of 102 consecutive pregnancies with hereditary thrombophilia or thrombosis were retrospectively analyzed. Pregnant women were routinely followed every 4-6 weeks for thrombotic complications during pregnancy. Any thrombotic event or preeclampsia or premature delivery were recorded. Thrombin-Antithrombin-Complex (TAT), D-Dimers (DD) and endogenous thrombin potential (ETP) by a functional chromogenic assay (CAT, calibrated automated thrombogram) were assayed routinely once in the middle of each trimester using standard methods.

Results: TAT (4.3+2.9, 6.4+5.2, 7.7+4.3 microg/L) and DD (0.9+1.5, 1.6+2.1, 2.0+2.3 microg/ml) were increased slightly but continuously and statistically significantly from each trimester to the other. Women receiving heparin prophylaxis showed the same pattern and did not differ from the whole cohort. CAT did not show any differences between trimesters. Women in third trimester with or without heparin prophylaxis did not differ with respect to DD (2.0+2.6 vs. 2.0+1.7 microg/ml), TAT (7.5+2.9 vs. 8.2+5.9 microg/L), CAT-ETP (1696+962 vs. 1715+804 AU).

Conclusion: TAT and DD were elevated as pregnancy progressed, as expected. CAT-ETP in addition to TAT does not add any value of information. TAT and DD do not add any safe value of information with respect to the effect of heparin prophylaxis and they might not be needed as isolated observation tools.
Bernard Soulier syndrome in pregnancy and delivery – a case report

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Poster Topic
Women issues in thrombosis and hemostasis
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Objective: Background: The Bernard Soulier syndrome (BSS) is a rare bleeding disorder, inherited as an autosomal recessive trait. The main characteristics are thrombocytope尼亚, giant platelets and a quantitative defect of platelet membrane glycoprotein Ib-IX-V complex. BSS is associated with varying bleeding symptoms (hypermenorrhoea, epistaxis and hematoma). We report our experience about the management of a pregnancy and delivery of a patient with Bernard Soulier syndrome.

Methods:

Results: Case report: A 27-year-old woman with known BSS was referred due to first pregnancy. Despite the patient received platelet concentrates in its medical history, no HLA-antibodies or HPA-antibodies could be proved until now. Ex-vivo testing of recombinant factor VIIa (spiked plasma) showed a sufficient effect in coagulation. During pregnancy two bleeding episodes (gestation week 9: retroplacental hematoma, gestation week 11: epistaxis and hematoma) appeared with administration of HLA-matched platelet concentrates. Primary caesarean was at gestation week 37 due to a former laparotomic and inherited bleeding disorder with transfusion of two HLA-matched platelet concentrates without clinical complications. Monitoring of the therapy was possible by platelet count and thrombelastography.

Conclusion: This case shows that BSS can be managed in a cross-functional cooperation of obstetric, hemostaseology and transfusion medicine to minimize the bleeding risk of patient with rare bleeding disorders.
Successful pregnancy outcome in a case of JAK2 positive essential thrombocytopenia after treatment with peginterferon alpha-2b

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\textbf{Poster Topic}

Women issues in thrombosis and hemostasis

17.02.2017, 17:15 - 18:15
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\textbf{Objective:} Diagnosis of myeloproliferative neoplasms (MPN) puts women in childbearing age at high risk for pregnancy complications. Here we present the case of a pregnant woman with Essential Thrombocytemia (ET).

\textbf{Methods:} A 33-year-old woman presented in our clinic in 12/2015 for counseling and treatment of ET during pregnancy. She was at her 3rd pregnancy, 10 weeks of gestation. Her 1st pregnancy (in 2011) was complicated by intrauterine fetal death at 28 weeks of gestation. At her 2nd pregnancy (in 05/2012) due to intrauterine growth restriction, cesarean section was performed at 29 weeks of gestation and she delivered a female newborn weighing 720 g. In 08/2015 she was diagnosed with ET with JAK2 V617F mutation. Her platelet count was 660 G/L. Additionally type 2A von Willebrand disease (VWD) was diagnosed. At a retrospective analysis of blood tests, elevated platelet counts up to 600 G/L were traced back to 2012. The patient was considered at high risk for pregnancy complications and we started cytoreductive treatment with Peginterferon alpha-2b (PEG-IFNα-2b) at a dose of 35 µg weekly and low dose acetylsalicylic acid (ASA), 100 mg daily.

\textbf{Results:} After one month, platelet counts dropped within normal ranges and remained stable during the course of the pregnancy. No bleeding complications occurred in relation to ASA or VWD. Treatment with PEG-IFNα-2b was stopped 2 weeks and ASA 1 week prior to delivery. In 07/2016 cesarean section was performed at 36 weeks of gestation and she delivered a healthy male baby. Prophylactic treatment with LMWH was given for 3 weeks after delivery. The patient presented in our ambulatory in 10/2016 and she and the baby were in good clinical condition. Her platelet counts rose again to 610 G/L. Until now, the patient is being monitored and we did not restart the cytoreductive treatment.

\textbf{Conclusion:} This case emphasizes the need for carefully investigation in patients with pregnancy complications. Treatment for ET with PEG-IFNα-2b and ASA resulted in successful pregnancy outcome. Another challenge was the associated VWD that could prone the patient to bleeding complications. A clear relation between platelet counts and VWF parameters has not been defined. Yet, an inverse relationship between platelet counts and VWF:RCo and VWF:CB has been suggested, underlining the importance of maintaining platelets within normal ranges.
P2Y6 depletion enhances metabolic activity and ameliorates outcome of high fat diet induced obesity in mice

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Objective: With 17.3 million deaths per year cardiovascular diseases are the major cause of death worldwide. A prolonged obese body composition is strongly related with a higher risk of cardiovascular disease (CVD) incidence and mortality. Extracellular nucleotides are known to modulate metabolic pathways such as glucose homeostasis, cholesterol homeostasis, and adipocyte differentiation. These extracellular nucleotides signal through purinergic receptors, e.g. P2Y6, which fine-tune metabolic processes providing a potential target in the treatment of obesity induced metabolic syndrome. We hypothesized a role of P2Y6 in the outcome of high-fat diet induced obesity.

Methods: P2Y6 deficient (ko) 8 week old male mice and C57Bl6/N wildtype (wt) mice were fed for 20 weeks a high-fat or normal diet. Metabolic activity was assessed by metabolic caging at week 17. After 20 weeks mice were euthanized and whole body composition analyzed by necropsy. Blood plasma was analyzed by enzyme-linked immunosorbent assay.

Results: We observed a decelerated gain of body weight in P2Y6 deficient animals (after 12 weeks wt: 45.4 ± 0.9g (n=13) and P2Y6 ko: 40.6 ± 1.0g (n=13), p=0.0022). After necropsy P2Y6 ko mice revealed reduced fat depictions (e.g. pericardial adipose tissue of P2Y6 ko mice: 58.3 ± 5.4mg (n=13) versus wt animals: 75.2 ± 4.8mg (n=9), p < 0.05). Brown adipose tissue was elevated in P2Y6 ko animals under HFD (P2Y6 ko: 380 ± 27.9mg (n=8) versus wt: 303.3 ± 23.2mg (n=10), p < 0.05). P2Y6 deficient mice have a higher O2 consumption (P2Y6 ko: 2666 ± 79ml/h/kg (n=6) versus wt: 2315 ± 62ml/h/kg (n=6), p=0.0059), increased CO2 exhalation (P2Y6 ko: 2006 ± 66ml/h/kg (n=6) versus wt: 1724 ± 44ml/h/kg (n=6), p=0.0051) and increased heat (P2Y6 ko: 12.64 ± 0.38kcal/h/kg (n=6) versus wt: 11.16 ± 0.42kcal/h/kg (n=6), p<0.05) but no difference in movement or food intake. Plasma cholesterol levels were decreased in P2Y6 ko animals (100.2 ± 28.6mg/dl (n=5)) compared to wt animals under HFD (219.5 ± 41.4mg/dl (n=3), p<0.05).

Conclusion: In conclusion our data showed a reduction of HFD induced fat deposition in P2Y6 ko mice due to an increased metabolic activity. These effects make P2Y6 a promising target for the treatment of obesity induced metabolic syndrome and prevention of CVDs.
Development and application of methods for the selective measurement of the human single amino acid exchange variant factor IX Padua

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**Poster Topic**

Innovation and Novelty  
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**Objective:** The naturally occurring single amino exchange (R338L) variant factor IX (FIX) Padua is used in the BAX 335 gene therapy Phase 1/2 clinical trial since this gain-of-function mutation shows a 5 – 10 fold higher activity than that of FIX wild type. Assessment of the gene therapy’s success would benefit from a method that enables specific detection of the transgene product, particularly in the presence of inactive FIX as in cross-reactive material positive patients. Therefore, a Fab2 mini antibody, selectively binding to FIX Padua, was developed and applied for the development of FIX Padua-specific methods.

**Methods:** A Fab2 mini antibody, specifically binding to FIX Padua and isolated by phage display, was applied to capture FIX Padua. This Fab2 mini antibody was used in combination with a polyclonal anti FIX antibody to establish a specific ELISA for analysis of subject samples in clinical trials. The performance of this ELISA was checked in terms of accuracy, precision and parallelism. In addition, this Fab2 mini antibody could also be used to set up a FIX Padua-specific chromogenic FIX activity assay.

**Results:** The Fab2 mini antibody selected showed exclusive binding to FIX Padua with no cross-reactivity to wild-type FIX. The ELISA, using this Fab2 mini antibody to selectively capture FIX Padua in combination with a biotynlated polyclonal anti-FIX antibody and streptavidin peroxidase, covered a FIX Padua protein concentration range of 0.9 to 27.1 ng/mL. Spike-recovery carried out with representative patients’ samples showed acceptable recoveries and there was no influence of the citrated plasma matrix on the assay performance. Furthermore, there was clear correlation between FIX Padua protein concentration and FIX activity, while the presence of functionally inactive FIX had no impact on the assay. The calibration curve of the FIX Padua-specific chromogenic activity assay, carried out after selective capture of FIX Padua with the Fab2 mini antibody, ranged from 0.1 to 3.3 mU FIX Padua/mL, while a normal reference plasma pool showed no response.

**Conclusion:** The FIX Padua ELISA can be used in clinical settings to selectively quantify FIX Padua antigen levels. In addition, the Fab2 mini antibody offers the possibility to set up a FIX Padua-specific chromogenic activity assay.
Fucoidans inhibit cellular responses to the chemokine IL-8 and the anaphylatoxin C5a better than heparin

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Objective: Heparins are known to exhibit anti-inflammatory and antimetastatic activities, however their application in inflammatory diseases and cancer is limited due their bleeding risk. Fucose-containing sulfated polysaccharides (fucoidans) from brown algae, which are currently considered promising candidates for health-supporting and medicinal applications, showed anti-inflammatory and antimetastatic activity in vivo as well, but considerably reduced anticoagulant effects. However, there is only limited knowledge about their mode of actions. Potential targets may be the chemokine interleukin 8 (IL-8) and the anaphylatoxin C5a, as they are closely linked to both inflammatory processes and tumor progression. In the present study, two structurally distinct fucoidans, SLSP from Saccharina latissimi and FVSP from Fucus vesiculosus, and UFH as reference were examined for their effects on IL-8- and C5a-induced reactions of polymorphonuclear neutrophils (PMN) as cell type critically involved in many inflammatory diseases.

Methods: The affinity of the test compounds to IL-8 and C5a was examined by competitive SPC-ELISA. PMN activation was determined by means of intracellular calcium release and Erk1/Erk2 phosphorylation. The IL-8- and C5a-induced PMN chemotaxis was assessed using modified Boyden chambers.

Results: Both fucoidans (SLSP > FVSP), but not UFH, displayed high affinity to IL-8 as well as C5a. Whereas UFH showed only moderate effects, the fucoidans (FVSP > SLSP) conc.-dependently reduced the IL-8- and C5a-induced intracellular calcium release. The conc.-dependent inhibition of Erk phosphorylation was more pronounced in IL-8- than in C5a-stimulated PMN. Similar to this discrepancy, the IL-8-induced chemotaxis was effectively inhibited by the fucoidans and up to 30% by UFH, whereas migration towards C5a was only reduced to 50% by the fucoidans and not at all by UFH. Strikingly, SLSP and FVSP differed in their inhibitory profile on the IL-8-induced chemotaxis with stronger inhibition by SLSP at <10µg/ml and a superior effect of FVSP at higher concentrations.

Conclusion: In conclusion, the two fucoidans showed to interfere with IL-8- and C5a-induced stimulation of PMN by binding to these activators. They proved to be considerably more active than UFH, however, their activity profiles differed. This suggests that further mechanisms may be involved in their inhibitory effects on PMN.
The effect of storage on the platelet hemostatic activity

E. V. Roitman, I. M. Kolesnikova (Moscow, Russia)

**Objective:** Apheresis and storage of platelet concentrates (PCs) affected by the platelets activation and total functional capacity of these cells. We assume that after transfusion the prevalence of platelets with changed activity lead to worse quality of blood clot in vivo. The aim was the in vitro study of platelet-dependent clot properties as a function of storage time.

**Methods:** Fifty single-donor apheresis PCs were divided in two groups: group 1 - platelets were remained in autologous plasma (PCs-P; n=26); group 2 – platelets were resuspended in PAS (SSP+; Macopharma, France) which substituted up to 70 vol% of autoplasm (PCs-PAS; n=24). Storage conditions were equal. PCs samples were analyzed by modified thromboelastography, and by aggregometry, and for platelets count, pH, lactate, glucose, and other platelets parameters. The testing were carried out in the day of proceeding, after 24 hours, and at 3rd and 5th days of storage.

**Results:** Platelets count had not significantly different between PCs-P and PCs-PAS. Between PCs-P and PCs-PAS no significantly differences had for platelets count, glucose consumption and lactate production. Moreover pH was almost unchanged that indicated buffer conditions were good. During the storage platelets aggregability and adhesion had worsened independently PCs type. But in PCs-P such decline was more expressed a little bit. We found that clot demonstrated gradual reduction of elasticity and deformability in both PCs groups. In PCs-P platelets lost their meaning for clot properties from the third storage day. In PCs-PAS activated platelets had no impact to clot properties during full storage time.

**Conclusion:** Irrespective of the proceeding method platelets viability was saved during the first five days of storage. It can be assumed that using platelet additive solution for PCs preparation does not reduce this cells viability. Platelets apheresis and storage are accompanied by aggregation-and-adhesion activity depression. Total decline of clot quality including low elasticity and impaired deformability was found of during period in stored PCs. We assume that clot properties are forming at the day of proceeding. Therefore we suppose that effect PCs transfusion is related to successful of platelets activity recovery in vivo.
Evaluation of platelet hemostatic capacity using new thromboelastography-based assay

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**Poster Topic**
Late-breaking posters
17.02.2017, 17:15 - 18:15
Poster Number: 148

**Objective:** Platelets play a key role in performing hemostasis, including the formation of a good quality clot. This is a main condition to stop bleeding and to the success of platelet transfusion. The aim was to develop a method that allows to determine the hemostatic platelet activity and to evaluate the contribution of platelets to clot formation.

**Methods:** The method is based on two parallel thromboelastogramms recording. Test sample is platelets in any media containing a minimum of blood plasma (e.g., platelet concentrate). The reagents are 1) diluted calcium solution, and 2) calcium solution with ADP in standard concentration for platelet aggregometry. For both cases, calcium concentration is the minimal required for blood clotting. Parallel thromboelastogramms are recorded followed by a calculation of the ratios for clot elastic modulus shear (G) and for clot maximum amplitude (MA). The first ratio reflects quantitatively the degree of involvement of activated platelets in hemostasis. The second ratio shows what are clot properties expected in a result of present platelet functional activity.

**Results:** MA’s-ratio correlates closely to ADP-induced platelet aggregation, spontaneous platelet aggregation and platelet apoptosis according to the percentage of P-selectin (CD62P) and phosphatidyl serine (annexin V) positive platelets. G-module's ratio showed high correlations to bleeding stop or to reducing of blood loss rate within short time after platelet concentrate transfusion. According pilot data, after transfusion in patient blood samples MA’s-ratio demonstrates the capability of circulating platelets to perform hemostasis, and it also shows the degree of functional activity recovery of stored platelets after transfusion. G-module's ratio has predictive value for cases in which repeated transfusions of platelet concentrate might be required.

**Conclusion:** We have developed a simple, available and low-cost assay for platelet hemostatic capacity. The method is based on generally accepted assessment methods: platelet aggregation and coagulation pattern. This assay has predictive value to clinical result of single units transfused platelets concentrates. Also the method allows to predict respect to total requirement for platelet concentrate transfusions. The method has been patented.
Blood rheology factors for thrombus formation in some oncohematological diseases

E. V. Roitman, I. M. Kolesnikova (Moscow, Russia)

Objective: High incidence of venous thromboembolic events (VTE) in cancer patients despite standard antithrombotic prevention suggests the presence of non-hemocoagulation conditions for thrombosis development in cancer patients. The aim was to study the blood rheological features and their role for thrombosis in patients with some oncohematologic diseases.

Methods: Study’s population consisted and 48 children with acute lymphoblastic leukemia (ALL), 26 adults with polycythemia vera (PV), 14 adults with chronic myeloid leukemia (CML), 14 adults with acute myeloid leukemia (AML) and 67 volunteers as the control group. All patients had not any symptomatic organs failures. Hematocrit (Hct), erythrocytes count, leukocyte count and fibrinogen were analyzed. Moreover in all patients we investigated B-type natriuretic peptide concentration (BNP). Whole blood viscosity (WBV) under shear rates range 5-300s⁻¹, plasma viscosity by shear rate 250s⁻¹ were measured with using «cylinder - cylinder» rotational viscometer (AKR-2, Russia). Measurement was performed as decreasing of shear stress (γ̇) (from 300 to 5s⁻¹) followed by an increasing γ̇ (from 5 to 300s⁻¹). No sample extracting from device till the analysis is performed. WBV values were adjusted to Hct=40%.

Results: Elevated BNP had 18-20% of patients assuming subclinical cardiac dysfunction which is an independent VTE risk factor. In all patients WBV differed from normal. The highest WBV was found in patients with PV, the lowest - in patients with AML. The main differences of the blood rheological behavior were due to the ratio of erythrocyte aggregation/disaggregation processes and the difference in the composition and hydrodynamic resistance cell conglomerates at high shear rates. In donors differences of WBV value depending on the direction of measurement (decreasing/increasing γ̇) at the same shear rate were found by low and middle shear rates. In patients WBV differences showed up at all shear rates implying reversible blood structuring process is not full finished.

Conclusion: Thus these patients are with acquired non-hemocoagulation conditions for thrombosis development. We assume that the revealed hemorheological features in combination with the high concentration of BNP could be one trigger to start of VTE despite standard antithrombotic prevention.
Identification of novel mutations in FGA fibrinogen gene in Pakistani congenital afibrinogenemia patients

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Poster Topic
Late-breaking posters
17.02.2017, 17:15 - 18:15
Poster Number: 150

Objective: This study focuses on the detection of mutations in FGA fibrinogen gene by DNA sequencing

Methods: This descriptive and cross sectional study was conducted in Karachi and Lahore and fully complied with the Declaration of Helsinki. Patients with fibrinogen deficiency (tested by Fibrinogen functional assay from Laboratoire Stago, Asnieres, France) were screened for mutations in the Fibrinogen gene alpha (FGA) by direct sequencing.

Results: Total No. of patients of congenital afibrinogenemia were six including two males and four females. Prothrombin Time(PT) in these patients was >120seconds and APTT was >180seconds. Fibrinogen levels were 0.0g/l in all patients. In genetic analysis of six probands; two patients have novel mutations in FGA gene. One is novel nonsense mutation and the other identified novel mutation is a frame shift mutation.

Conclusion: Congenital afibrinogenemia is a rapid growing problem in countries such as Pakistan where consanguinity is frequently practiced. This study illustrates the fact that mutations in FGA are relatively more common in our population than in FGB where as FGG mutations
Quantification of the antimetastatic effects of heparins by Single-cell force spectroscopy

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Poster Topic
Late-breaking posters
17.02.2017, 17:15 - 18:15
Poster Number: 152

Objective: It is well known that platelets support cancer cells in nearly every step of metastasis. Heparin exhibits antimetastatic effects as shown in animal models and clinical studies. Unfractionated heparin consists of a broad range of polysaccharides with varying weights (3-30kDa) whereas low molecular weight heparins (LMWH) contain shorter chain lengths (up to 6kDa).

Goal of this project was to test the efficiency of unfractionated heparin and LMWH to reduce platelet-tumor cell adhesion on the single cell level and to elucidate the fate of platelets upon tumor cell adhesion.

Methods: We used human non-small cell lung cancer cells (A549) and freshly isolated platelets, forming a dense layer on collagen coated dish. Single cell force spectroscopy (SCFS) was used to obtain the max. adhesion force (FA) and the detachment work (WD) between A549 cells and activated platelets. Heparin-Na 25000 (Ratiopharm) and Tinzaparin (Innohep®, Leo Pharma) was used in concentrations between 0.001 – 100 U/mL. In another approach, A549 cells were stained with LysoTracker and incubated with FITC-CD42a-labeled platelets. Confocal laser scanning microscopy was used to visualize localization of platelet CD42a protein within A549 cells.

Results: Adhesion between platelets and A549 cells show values of 1060pN for FA and 4 fJ for WD. The maximum decrease of adhesion is induced by 10 U/mL of unfractionated heparin (FA 38%, WD 29%) and 0.01 U/mL Tinzaparin (FA 38%, WD 50%) respectively. A sigmoidal dose-response fit revealed IC50 values of 8.4 U/ml for unfractionated heparin and 0.01 U/mL for Tinzaparin. Confocal microscopy exhibited clear colocalization of the platelet specific protein CD42a in acidic compartiments of A549 cells already after 30min of incubation. Frequently, the A549 cells exhibited formation of phagocytotic protrusions and invaginations around platelets. Internalized CD42a followed a gradient of size and density originating from these invaginations.

Conclusion: We established a method to quantify the effect of heparins on platelet-A549 cell adhesion on single-cell level and demined EC50 values. Furthermore, we could visualize the uptake of platelets by A549 cells. Since platelet-tumor cell adhesion is a crucial step for metastasis, our approach represents a valuable method to investigate early steps of metastasis and to test the efficiency of substances to block platelet-tumor cell interactions.
Gene polymorphism of plasminogen activator inhibitor-1 is a risk factor of thrombosis in patients with antiphospholipid syndrome

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Objective: Antiphospholipid syndrome (APS) is an autoimmune disease, characterized by thrombotic disorders and pregnancy loss in the presence of antiphospholipid antibodies. Impairment of fibrinolysis is associated with increased level of plasminogen activator inhibitor-1 (PAI-1). The aim of this work was to study the relationship between the 4G/5G polymorphism of PAI-1 gene and thrombotic manifestation in patients with APS.

Methods: It was studied 45 patients with APS (32 women, 13 man, mean ± SD age 35 ± 12 years, duration of disease 0.5 - 40 years), 18 had systemic lupus erythematosus and 27 - APS without autoimmune diseases. Anti-cardiolipin and anti-beta2-glycoprotein I antibodies were measured. All the thrombotic events were assessed clinically and confirmed by objective methods. PAI-1 4G/5G polymorphism genotyping was conducted by PCR method. Activity of PAI-1 was determined from residual activity of tPA added to plasma in excess, using conjugated method.

Results: 17 patients were high, 7 – moderate, 8 – low positive and 13 - negative by aCL, corresponding levels of aβ2GPI had 21, 4, 4 and 16 patients. The PAI-1 genotype 4G/4G had 21 (46.6%), 4G/5G - 18 (40%) and 5G/5G - 6 (13.4%) patients. Thrombosis had 39 (86.6%) patients: 9 (20%) - arterial, 21 (46.6%) – venous, 9 (20%) - combined thrombosis. Venous thrombosis had 14 (66.7%) with 4G/4G, 14 (77.7%) with 4G/5G and 2 (33.3%) with 5G/5G genotype, arterial thrombosis had 7 (33.3%), 6 (33.3%) и 5 (83.3%) patients, respectively. Increased level of active PAI-1 had 34 (75.5%) patients: 16 with 4G/4G, 13 – 4G/5G and 5 – 5G/5G genotype, normal PAI-1 activity had 11 (24.4%) patients: 5, 5 and 1, respectively. Relation between anti-cardiolipin and anti-beta2-glycoprotein I levels and thrombosis, level and polymorphism PAI-1 was not found. Combination of thrombosis, 4G/4G or 4G/5G genotype and increased activity of PAI-1 were observed in 34 (75.5%) patients with APS.

Conclusion: The results indicate on the relationship between 4G/5G polymorphism PAI-1 gene and thrombosis in patients with APS.
Combinations of recombinant staphylokinase (STA) and single-chain urokinase-type plasminogen activator (scuPA) induce synergistic thrombolysis in vitro

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Objective: STA binds to fibrin-bound plasminogen (Pg). Conversion of inactive equimolar Pg*STA complex to active plasmin(Pm)*STA complex is a rate-limited step that is accelerated by the product. ScuPA has affinity only for molecules of Pg which are bound to the inner COOH-terminal lysines of partially degraded fibrin and it activates Pg directly. Since STA and scuPA are distinguished by mechanism of Pg activation and fibrin-selectivity of thrombolytic action, their combinations can induce a synergistic thrombolysis. In this work, thrombolytic and side effects of combinations of STA and scuPA were studied in vitro.

Methods: Kinetics of plasma clots lysis and depletion of plasma proteins (Pg, fibrinogen and alpha2-antiplasmin) levels under the action of different concentrations of STA, scuPA and their combinations were monitored.

Results: Equieffective doses which caused 50% clot lysis in 4 h were found to be 30 nM STA and 75 nM scuPA. To reveal a potential synergistic effect, simultaneous and sequential combinations of various doses of STA and scuPA were studied. Equieffective doses of STA, scuPA and their combinations which caused the same specified effects in 2 or 4 h were analyzed by the algebraic fractional method. Synergism of STA and scuPA was found at total concentrations of the agents of 25 to 65 nM. The synergistic of thrombolysis was more pronounced at simultaneous combinations of the agents than at subsequent addition of STA followed by scuPA (in 30 min). The maximal 3.5-4-fold increase in thrombolysis was observed for combinations of 10-15 nM STA and 15-35 nM scuPA as compared with the expected thrombolysis, obtained by summarizing of the effects of the individual agents. The synergistic combinations of STA and scuPA caused the significant less depletion of Pg, fibrinogen and alpha2-antiplasmin levels in plasma, as compared with the expected effects.

Conclusion: The synergy of STA and scuPA is connected with an initial formation of COOH-terminal lysine residues on fibrin surface under the action of STA, that leads to the increase in Pg activation by scuPA and formation of tcuPA. As a result of Pg activation by tcuPA, concentration of plasmin and Pm*STA complex increases. Because of all these reactions proceed on fibrin surface, the combinations of STA and scuPA cause the enhanced thrombolytic effect at the reduced side effects.
Positive and negative role of oxidative stress in the prognosis of patients with gastric cancer.

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Poster Topic
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Objective: Gastric cancer is the second leading cause of cancer-related deaths worldwide. Reactive oxygen species (ROS) production in cancer cells is one of the mechanisms underlying synergetic cytotoxicity seen with combination anti-tumor treatments. Compared to normal cells, cancer cells have intrinsically higher levels of ROS and are under oxidative stress due to an imbalanced redox status. Targeting ROS is an important therapeutic strategy for cancer as exemplified by cancer drugs, which induce ROS-dependent synergistic cytotoxicity in gastric cancer cells. The present study was designed to assess the level of selected oxidative stress biomarkers in blood plasma derived from gastric cancer patients.

Methods: Detection of nitrotyrosine-containing blood plasma was performed by a competitive ELISA method, using the OxiSelect™ Nitrotyrosine ELISA Kit. The non-enzymatic antioxidant capacity (NEAC) of blood plasma samples was estimated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) reduction assay. The amount of free thiol groups was estimated with 5,5′-dithiobis(2-nitro-benzoic acid). Blood samples were taken from 32 healthy volunteers and 50 patients with gastric cancer who were hospitalized in the Department of Oncological Surgery, Medical University of Lodz, Poland.

Results: Our other studies have demonstrated that the concentration of thiol groups in plasma proteins from metastatic gastric cancer patients differs from the concentration of thiol groups in plasma proteins obtained from healthy volunteers (p<0.05). The concentration of 3-nitrotyrosine of plasma proteins from invasive gastric cancer was significantly higher than in plasma proteins obtained from healthy volunteers (p<0.001). The concentration NEAC of blood plasma from patients with gastric breast cancer was decreased compared with the healthy subject group (p<0.005).

Conclusion: The present investigation was designed to evaluate oxidative stress markers in patients with confirmed gastric cancer. Oxidative stress was monitored in blood serum by determination of protein 3-nitrotyrosine. The intensity of oxidative stress may undergo many fluctuations both during the progression of malignancy and in cancer therapy. Low levels of ROS play an important role in proliferation of tumor cells, angiogenesis and metastasis, however the generation of high levels of various oxidants, results in oxidative stress that may induce cell death.