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Cancer-associated Thrombophilia

OC01-01 Development and validation of machine learning predictive models applying for cancer-associated deep vein thrombosis: A 1035-sample retrospective cohort study

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Objective This study aims to develop machine learning(ML) models for cancer-associated deep vein thrombosis (DVT), and compare the performance of these models with the currently widely-applied predictive model, the Khorana Score, with or without using D-Dimer.

Material and Methods We consecutively and retrospectively extracted data of 1035 cancer patients from a tertiary hospital. Both uni-variable analysis and the Lasso regression were applied to select the important predictors. Model training (training set, 652/725) and hyper-parameter tuning (testing set, 73/725) were implemented on 70 % (725/1035) of the data using a ten-fold cross-validation method. The remaining 30 % (310/1035) data was used to compare the performance with six indicators, the area under the receiver operating characteristic curve(AUC), sensitivity, specificity, accuracy, the Brier Score, and the calibration curve, among all five ML models, linear discriminant analysis (LDA), logistic regression (LR), classification tree (CT), random forest (RF, an assemble algorithm), and support vector machine (SVM), and the Khorana Score, with or without using D-Dimer.

Results The percentage of cancer-associated DVT in this study was 22.3 % (231/1035). The top five important predictors were D-Dimer, age, Charlson Comorbidity Index (CCI), length of stay (LOS), and previously VTE history. Five ML models were developed and validated. In validation set, LDA (AUC: 0.756 & 0.773, none-D-Dimer model & D-Dimer model, respectively), and LR (AUC: 0.752 & 0.772) performed best, followed by RF (AUC: 0.638 & 0.660), Khorana Score (AUC: 0.604 & 0.642), CT (AUC: 0.604 & 0.638), and SVM (AUC: 0.593 & 0.665).

Conclusion This study developed and validated ML predictive models for cancer-related DVT. The combination with D-Dimer showed improved performance of all models. LDA and LR out-performed Khorana Score, but CT, RF, and SVM did not surpass it. A nomogram and a web calculator were used to visualize the best recommended model, the D-Dimer LR model, which largely narrowed the gap between model development and clinical application. The web calculator can be found at https://webcalculatorofcancerassociateddvt.shinyapps.io/dynnomapp/. In the future, the performance and clinical application of ML models for cancer-associated DVT might be improved further.

OC01-02 Modelization the impact of antithrombotic agents on pancreatic tumoral micro-environment.

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Objective Interactions between cancer cells and their micro-environment with antithrombotic agents is an emerging field of research. In the present study we investigated the impact of apixaban, fondaparinux, enoxaparin and tinzaparin on the procoagulant properties of pancreatic cancer cells BXPC3. Reciprocally, we investigated the impact of BXPC3 on the potency of these antithrombotic agents.

Material and Methods BXPC3 (400 cells/ml) were exposed to apixaban (2 microgram/ml), fondaparinux (2 microgram/ml), enoxaparin, or tinzaparin (2 anti-Xa IU/ml) for 48h. Then, cells and supernatants were separated and added in normal platelet poor plasma (PPP) for thrombin generation (TG) experiments. Viability of cancer cells (assessed with the MTT assay), gene expression for TF, VEGF, THSB1 (assessed with RT-qPCR) and expression of TF protein and activity were also examined. Microparticles (MP) were tested for TF with specific ELISA. Residual anti-Xa activity was measured in the supernatant using specific amidolytic assays.

Results BXPC3 enhanced TG. Incubation of the BXPC3 cells with all antithrombotic agents did not significantly modify their TG capacity. Apixaban resulted in significant TF mRNA expression decrease by BXPC3 cells. None of the antithrombotic agents significantly modified the amount of BXPC3 cells -TF protein. Fondaparinux and enoxaparin significantly decreased VEGF mRNA expression and apixaban significantly increased the expression of THBS1. The viability of BXPC3 cells was significantly reduced following exposure to apixaban (25 %), fondaparinux (12 %), enoxaparin (14 %) or tinzaparin (11 %). Exposure of BXPC3 to antithrombotic agents did not significantly modify the release of MP. Apixaban, fondaparinux, enoxaparin and tinzaparin decreased TG induced by the supernatant by 70 %, 30 %, 40 %, 90 % respectively. After exposure to BXPC3 cells the concentration of fondaparinux, enoxaparin and tinzaparin in the supernatant reduced by 27 %, 48 % and 26 % respectively. In contrast the concentration of apixaban did not significantly change.

Conclusion Antithrombotic agents do not alter cancer cells' TF expression or procoagulant MP release, but inhibit the procoagulant potency of microenvironment. Nevertheless, a LMWHs and fondaparinux degradation occurs following two days of exposure to cancer cells. Antithrombotic agents reduced

▶**Tab 1.** Impact of antithrombotic agents on BXPC3 cells, on their MP, and on the capacity of trigger thrombin generation of their culture supernatants. Cells were treated with 2 UI/ml of Fondaparinux/Apixaban, or 2 micro gram/ml of Tinzaparin/Enoxaparin for 48h. mRNA expression were normalized using the 2^(-delta(delta Cq)) method. * $p < 0.05$ as compare to Control experiment.

	Cells				
	Control	Fondaparinux	Enoxaparin	Tinzaparin	Apixaban
Peak of Thrombin Generation (nM)	120,57 ± 26,36	136,95 ± 35,96	135,71 ± 28,87	135,70 ± 32,98	126,71 ± 29,52
TF normalized mRNA expression rate	1,00 ± 0,35	0,64 ± 0,10	1,41 ± 0,13	0,62 ± 0,77	0,50 ± 0,19 *
TF protein expression (pg/ml)	0,71 ± 0,36	0,79 ± 0,60	1,02 ± 0,58	0,99 ± 0,45	0,74 ± 0,41
Viability rate	1,00 ± 0,00	0,88 ± 0,03 *	0,86 ± 0,06 *	0,89 ± 0,03 *	0,75 ± 0,05 *
VEGF normalized mRNA expression rate	1,00 ± 0,12	0,27 ± 0,00 *	0,69 ± 0,08 *	0,55 ± 0,83	0,88 ± 0,01
THBS1 normalized mRNA expression rate	1,00 ± 0,21	0,65 ± 0,25	0,57 ± 1,17	0,51 ± 0,45	1,40 ± 0,03 *
	MP				
PS (nM)	0,71 ± 0,36	0,79 ± 0,60	1,02 ± 0,58	0,99 ± 0,45	0,74 ± 0,41
TF protein expression (pg/ml)	1,42 ± 0,40	1,53 ± 0,60	2,87 ± 1,35	2,85 ± 0,70	1,10 ± 0,63
	Supernatant				
Lagtime (min)	3,23 ± 0,56	4,45 ± 1,07 *	5,04 ± 1,57 *	3,73 ± 1,75	5,23 ± 1,10 *
ETP (nM.min)	1472,05 ± 269,96	1174,79 ± 265,83	471,15 ± 207,88 *	154,63 ± 19,63 *	508,37 ± 258,51 *
Peak (nM)	194,18 ± 29,22	145,34 ± 24,36 *	42,80 ± 11,96 *	9,48 ± 2,00 *	49,67 ± 16,76 *
ttPeak (min)	6,26 ± 0,80	7,73 ± 1,32 *	8,37 ± 2,13	14,30 ± 5,16 *	7,99 ± 1,55 *
Vellocity Index (nM/min)	63,92 ± 5,25	44,22 ± 5,31 *	14,12 ± 7,01 *	1,29 ± 0,69 *	19,22 ± 8,26 *
MRI (nM/min)	63,87 ± 5,26	44,10 ± 5,05 *	14,03 ± 7,07 *	1,24 ± 0,72 *	19,15 ± 8,27 *

tumour cells' viability and impaired mRNA expression of pro- and antiangiogenic factors.

OC01-03 Aggressiveness of breast cancer cells is related with tissue factor expression and procoagulant activity.

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Objective The procoagulant potency of cancer cells may vary according to the histological type and their aggressiveness. We identified the procoagulant « fingerprint » of breast cancer cells lines of various degrees of aggressiveness.

Material and Methods Breast adenocarcinoma cell lines MCF7, MCF7-sh-WISP2, BT-20 and MDAMB-321 were used. Flow cytometry and western blot analysis for TF expression were performed using an anti-human TF murine IgG1 monoclonal antibody. Cancer cells were added in platelet poor plasma (PPP) from healthy volunteers and thrombin generation (TG) was assessed using Calibrated Automated Thrombogram.

Results Incubation of MCF7, MCF7-sh-WISP2, MDAMB-231, BT-20 and HUVEC cells with PPP resulted in acceleration of the initiation phase of thrombin generation. BT-20 manifested higher procoagulant potential than MCF7-sh-WISP2 than MCF7wt than MDAMB-231;

Flow cytometry analysis of TF expression showed a significantly higher expression on the membrane of MCF7-sh-WISP2 and BT-20. Western blot analysis showed that TF was present in eluted proteins after immunoprecipitation of cell lysates with anti-TF and in cell lysates. Two different major bands were observed at levels of 47 kDa and 25 kDa. In MCF7 a significant 47 KDa band was observed though its intensity was lower compared to the corresponding band of the lysate from MCF7-sh-WISP2; MDAMB-231; BT-20 cells.

Conclusion We demonstrate that procoagulant phenotype of pancreatic and breast cancer, is related to the expression of functional TF. The procoagulant fingerprint of breast cancer cells varies in function of the degree of aggressiveness. MCF7-ShWisp2 and BT-20 were the more aggressive cells and present higher procoagulant potential which is correlated with TF expression on the cell membrane. The present experimental model will allow the characterization of the procoagulant fingerprint of cell lines from the same or different histological types of cancer. It will also allow toevaluate the efficiency of antithrombotic agents to downregulate hypercoagulability induced by cancer cells.

OC01-04 LMWH or DOACs for cancer associated thrombosis (CAT) in daily clinical practice ? – Insights from the GECAT registry

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Objective National and international guidelines for the diagnosis and treatment of venous thromboembolism (VTE) recommend anticoagulation treatment for 3 to 6 months with preference for direct oral anticoagulants DOACs and a re-evaluation for resumption depending on the individual risk of every patient. In cancer associated VTE (CAT) however, low molecular weight heparin (LMWH) was the guideline recommended treatment until recently. The prospective German Evaluation of CAT (GECAT) registry investigated the anticoagulation practice within the Berlin region.

Material and Methods The GECAT registry was set up for Berlin´s two main hospital companies Charité-Universitaetsmedizin Berlin and Vivantes (covering about 50 % of the hospital beds in Berlin) to document prospectively in-hospital newly diagnosed VTE in patients (pts) with active cancer. A follow-up of these pts was scheduled after 3 and 6 months per telephone interview. Major points of interest were the kind of initial and follow-up anticoagulation as well as the treatment-leading physicians.

Results Between May 2015 and May 2017, 382 pts with active cancer and newly diagnosed VTE were identified. Initially, the majority of pts,n = 334 (87,4 %) was treated with LMWH and 22 pts (5,8 %) with DOACs. Close to discharge from hospital data were available from 364 pts, 281 pts (77.2 %) remained on LMWH and 47 pts (12,9 %) received DOACs. After 3 months out of 251 evaluable pts, 154 pts (61.4 %) received LMWH and 60 pts (23.9 %) DOACs, 29 pts (11.6 %) stopped anticoagulation. From 208 evaluable pts after 6 months 81 pts (38.9 %) received LMWH, 72 pts (34.6 %) DOACs and 48 pts (23.1 %) were without anticoagulation.

LMWH administered once daily was prescribed more often than LMWH administered twice daily in the ambulant setting (at discharge 28.8 % of LMWH-prescriptions,after 6 months 51.9 %). In most cases (71.1 %) the oncologist was responsible for the treatment followed by the general practitioner (32 %) and other physicians (3.5 %). Oncologists more often prescribed LMWH.

Conclusion In compliance with the guidelines most pts with CAT were anticoagulated for 3 to 6 months. They were mainly followed by oncologists who determined the kind and length of anticoagulation treatment without strict adherence to guideline recommendations. LMWH was prescribed more often by oncologists in comparison to general practitioners and the once daily administration was preferred.

OC01-05 Anticoagulation practice of cancer-associated thrombosis in the outpatient sector of Germany

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Objective Venous thromboses involving deep or superficial veins are bothrelevant complications in cancer patients with serious consequences on quality of life and prognosis. In difference to common venous thromboses, low molecular weight heparins (LMWH) until very recently were the recommended treatment for at least 3 to 6 months in cancer patients. But little is known about the reality of care for these patients in the outpatient sector of Germany with regard to compliance with contemporary guidelines and drug label.

Material and Methods We analysed anonymized data from 4.1 million statutory insured patients during a four year period (2012-2015). Cancer patients with newly diagnosed thrombosis and anticoagulation in the outpatient sector were identified and descriptively evaluated in the course of the three following quarters with the main focus on anticoagulant drug use.

Results 7,313 cancer patients with newly coded thrombosis (ICD-10:I80*) and an outpatient prescription of an anticoagulant were evaluated. More than 80 % of the anticoagulant prescriptions were made by general practitioners, close to 10 % by oncologists and less than 5 % by other physicians. 57 % of patients were anticoagulated dominantly (>50 % of the time) with different LMWH, 24 % with vitamin K antagonists (VKA) and 17 % with direct oral anticoagulants (DOAC). Within the LMWHs the relative frequency of the different brands reflects the overall market distribution of that time, with less than 3 % of patients treated dominantly with dalteparin, the only LMWH explicitly approved for longterm use in cancer patients during that period. Anticoagulant drugs were prescribed for an average of 4.5 months. LMWH had a significantly longer prescription period (90 - 135 days) than VKA (53 days) or DOAK (47 days). Gastrointestinal bleeding in conjunction with hospitalization as an indicator of bleeding frequency durung anticoagulation therapy was documented in 1.76 % of patients with a range of 1.3%-3 % for the different LMWHs.

Conclusion The prescription practice documented with this representative and comprehensive evaluation demonstrates an anticoagulation duration in adherence to the guidelines. But more than 40 % of the patients were dominantly treated with oral anticoagulant drugs. Thus the prescription choice of the respective anticoagulant drug was largely not in compliance with the approved label or contemporary guidelines.

Cardiology & Neurology

OC02-01 Current status of anti-thrombotic therapy in patients with atrial fibrillation undergoing PCI. Results of the prospective RIVA-PCI Registry.

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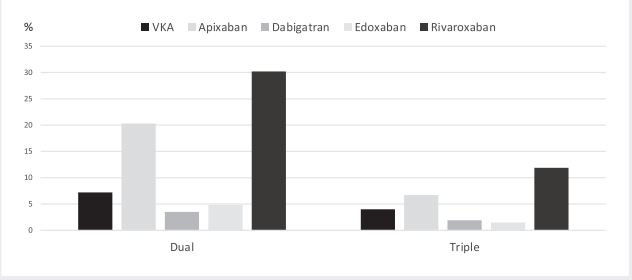
Objective A combination therapy with oral anticoagulation and antiplatelet therapy is recommended in patients with atrial fibrillation undergoing percutaneous coronary intervention. Little is known about the current status of antithrombotic regimens in clinical practice in Germany.

Material and Methods We performed a prospective registry in 51 hospital in Germany and included patients with atrial fibrillation treated with PCI with coronary stent implantation. Baseline characteristics, procedural informations, in-hospital events and antithrombotic regimens at discharge were collected and centrally analysed.

Results Between 01/2018 and 01/2020 a total of 1636 patients with atrial fibrillation were included in the registry. The indications for PCI were: elective 52 %, unstable Angina 18 %, NSTEMI 21 % and STEMI 9 %. At discharge aspirin was prescribed in 32 %, clopidogrel in 94 %, prasugrel in 1 % and ticagrelor in 3 %. A dual antithrombotic regimen consisting of oral anticoagulation and a P2Y12 inhibitor was given 72 %, triple therapy in 26 % and a monotherapy in 2 % of patients. The figure shows the distribution of the different antithrombotic regimens, with rivaroxaban as the most often used NOAC. Until discharge bleeding occurred in 2.9 %, while stent thrombosis, stroke and MI were reported in 0,2 %, 0.2 % and 0.1 %, respectvely.

Conclusion Dual therapy with a NOAC and clopidogrel is the preferred antithrombotic strategy in patients with atrial fibrillation undergoing PCI. However, recommended treatment regimens for 12-month follow-up vary with dual therapy for 6 months given most often. The in-hospital complication rate after PCI in patients with atrial fibrillation rate is low.



▶**Abb 1.**

OC02-02 Anticoagulation for stroke prevention in patients with atrial fibrillation on hemodialysis is associated with net-clinical harm

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Objective Evidence supporting the use of anticoagulation for the prevention of stroke and thromboembolism in patients with kidney failure on hemodialysis (HD) and atrial fibrillation (AF) is limited. Patients on HD patients are at high risk of stroke, but also particularly prone to bleeding. We prospectively assessed the incidences of stroke and major bleeding, as well as anticoagulation strategies in HD patients with AF.

Material and Methods We recruited 625 prevalent HD patients into a population-based cohort study. Patients were prospectively followed for the occurrence of thromboembolic events (stroke, TIA, systemic embolism), and for major bleeding. Secondary outcomes were 3P-MACE (myocardial infarction, stroke, and cardiovascular death), and a composite outcome of stroke, TIA, systemic embolism, major bleeding and cardiovascular death to determine net-clinical harm.

Results Of the 625 patients, 238 (38.1 %) had AF, 165 (26.4 %) already at baseline and 73 (15.9 %) developed AF during follow of up (median follow up 870 days). 40 (6.4 %) thromboembolic events, 89 (14.2 %) major bleedings, and 154 (24.6 %) 3P-MACE events occurred. Overall, 256 patients died (41.0 %), including 105 patients (16.8 %) from a cardiovascular cause. The incidence and case-fatality rates of all outcomes was higher in AF patients compared to non-AF patients.

In AF patients, use of vitamin K antagonists (VKA) in 61 patients (25.6 %) and low-molecular weight heparin (LMWH) on non-HD days in 38 patients (16.0 %) was not associated with significantly reduced risk of the primary outcome (SHR adjusted for CHA2DS2-VASc score and antiplatelet co-medication 1.41, 95 % confidence interval [CI] 0.49–4.07, and SHR 0.49, 95 % CI 0.11–2.18, respectively), but VKA was associated with significantly increased risk of major bleeding compared to AF patients not receiving anticoagulation (N = 139, 58.4 %). Use of VKA was associated with net-clinical harm (adjusted SHR 2.07 95%CI 1.25–3.42).

Conclusion Stroke and major bleeding are common complications in HD patients, especially in those with AF. Anticoagulation with VKA was not associated with decreased thromboembolic risk, but increased major bleeding and may be net-harmful to patients with AF on HD.

OC02-03 Red Cell Omega-3 FA Correlate with Reduced DDimer and Beta-Thromboglobulin as Markers of Coagulation Activation in Atrial Fibrillation

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Objective DDimer (DD) and beta-thromboglobulin (BTG) reflect activation of coagulation and platelets, respectively. DD play an important role in the diagnosis of venous thromboembolism (VTE). In previous studies, we and others have found that high nutritional intake of Omega-3 fatty acids (n-3 FA) are associated with a lower risk of cardiovascular events. We therefore hypothesized that n-3 FA are correlated with decreased plasma levels of DD and BTG in diseases such as atrial fibrillation with an increased risk of thromboembolic complications including stroke.

Material and Methods We analyzed the cohort of 2,415 well characterized patients with atrial fibrillation (the Swiss AF trial). Red cell n-3 FA content was measured using gas chromatography; DD and BTG were analyzed by standard ELISA from citrated plasma. Only detectable levels of DD were included. Levels of n-3 FA were correlated with DD and BTG concentrations in multivariate analyses adjusted for age at baseline and sex as well as additional potential confounders such as family history or comorbidities (coronary artery disease, diabetes mellitus, kidney failure, stroke and transient ischemic attack, major bleeding and heart failure).

Results 1096 patients with detectable values of DD and 2371 patients with BTG could be analyzed. In our multivariate analysis, both BTG and DD levels correlated negatively and significantly with n-3 FA. Pearson's correlation coefficient (r) for BTG was – 0.06 (95 % CI -0.10 to -0.02) and for DD -0.08 (95 % CI -0.14 to -0.02).

Conclusion

1. Red cell n-3 FA content correlates with reduced BTG as marker of platelet activation as well as with reduced DD reflecting activation of coagulation.
2. N-3 FA may reduce platelet activation and coagulation by their anti-inflammatory (reduction of TNF and tissue factor), anti-platelet (reduced activatability and aggregation, reduced expression of platelet adhesion receptors) and antioxydative effects (including reduced TMAO).
3. High red cell content of n-3 FA may reduce thromboembolic events in atrial fibrillation.

OC02-04 Growth differentiation factor-15 predicts major cardiac adverse events and all-cause mortality in patients with atrial fibrillation

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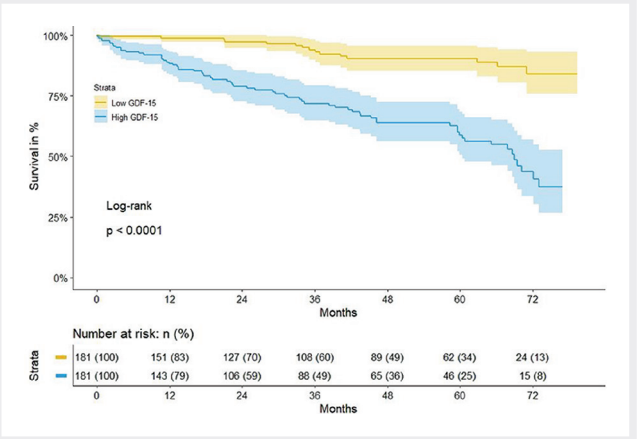
Objective Growth differentiation factor-15 (GDF-15) has recently been introduced as a potential biomarker for predicting risk of cardiovascular events and mortality in patients with atrial fibrillation (AF) and is awaiting evaluation in clinical practice.

Material and Methods We prospectively included 362 patients with non-valvular AF (mean age: 71 years, 37 % female) into an all-comers cohort study. Relationship of GDF-15 with all-cause mortality, major cardiac adverse events (MACE), and bleeding events was analyzed using Cox regression. Survival analysis for all-cause death was based on the national death records. MACE and bleeding events were recorded at personal follow-up at a 6 to 12-month interval. Further, we evaluated the ABC-death risk score, a recently developed GDF-15-based prognostic score, regarding its predictive ability for all-cause mortality.

Results Over a median observation period of 4.3 years, we recorded 81 (23.3 %) deaths. Furthermore, we observed 45 MACE and 34 clinically relevant bleeding events during a median follow-up of 316 days. GDF-15 was independently associated with all-cause mortality (adjusted hazard ratio [HR] per double increase 2.33, 95 % confidence interval [CI] 1.74-3.13) and MACE (adjusted HR per double increase 2.33, 95%CI 1.60-3.39) but showed no association with bleeding events. Six-year survival probability of patients above and below the median GDF-15 serum level (1428.5 ng/L) was 44 % (95%CI 34-57) and 84 % (95%CI 76-93), respectively. The ABC-death risk score, which includes GDF-15, demonstrated good predictive ability for death in our cohort (c-statistic 0.80).

Conclusion GDF-15 predicts risk of all-cause mortality and MACE in patients with non-valvular AF. Further, we externally validated the ABC-death risk score in a "real-world" cohort. Our data suggests that introduction of GDF-15 in clinical practice could enhance risk prediction of morbidity and mortality in AF patients.



▶ **Fig. 1** Patients were stratified into low and high serum level groups according to levels below or above the median growth differentiation factor-15 (GDF-15) level (median 1428.5, range 343-43218 ng/L). After 1, 3 and 6 years, survival probability for patients with low GDF-15 levels was 99% (95%CI 97-100), 93% (95%CI 90-98), 84% (95%CI 76-93), and 88% (95%CI 84-93), 72% (95%CI 65-80), 44% (95%CI 34-57) for patients in the high level group.

OC02-05 Intracranial involvement and neurologic manifestations in Lemierre syndrome: analysis of 712 patients.

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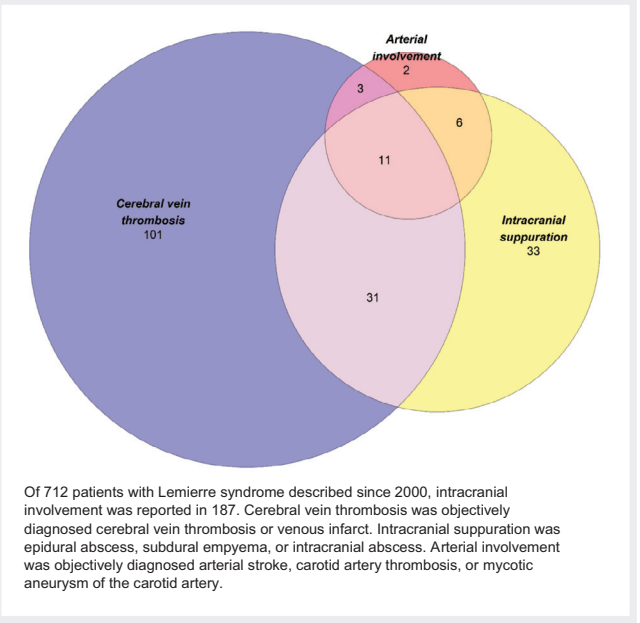
Objective Lemierre syndrome is a rare septic thrombophlebitis following a head-neck infection in the adolescence and young adulthood. Intracranial involvement in these patient is frequent, a major prognostic factor for early complications when observed at presentation, and among the main causes of death and long-term sequelae. However, with available information limited to case reports and small series, the pattern of intracranial involvement and neurological symptoms in Lemierre syndrome has never been studied systematically.

Material and Methods We analyzed 712 patients described between 2000 and 2017 and identified through systematic search of the grey and published literature. We distinguished three types of intracranial involvement: cerebral vein thrombosis, intracranial suppuration, and arterial stroke or carotid damage. In these patients, we characterized the neurological manifestations at presentation, the clinical and demographic characteristics, and the rate of death or persisting clinical sequelae at discharge.

Results Among 712 patients, 187 (26.3 %) had intracranial involvement at any time during their clinical course. These included 146 (78.1 %) with cerebral veins thrombosis, most often in the lateral sinus (N = 93), the jugular bulb (N = 76) or the cavernous sinus (N = 51); 81 (43.3 %) with intracranial suppurations; and 22 (11.8 %) with arterial involvement. At least two forms of involvement were found in 57 patients (27%; Figure). The most common neurological symptoms at presentation were focal neurologic signs (57/187, 30 %) followed by headache (47/187, 25 %) and decreased level of consciousness (37/187, 19 %). Compared with patients without intracranial involvement, those with it had a similar sex distribution, but were slightly younger, more likely to report a previous otomastoiditis as primary infection and less likely to have consulted a general practitioner before the presentation that led to diagnosis (Table). Among patients with a complete clinical follow-up, those with intracranial involvement were more likely to die (8.0 % vs 1.5 %) and, among survivors, to have sequelae at discharge (8.6 % vs 1.7 %).

Conclusion Systematic neurologic assessment at presentation and in-hospital neurologic monitoring may be indicated in patients with Lemierre syndrome. As different intracranial involvements frequently co-occur, the demonstration of any of them should prompt the search of others.



►**Abb 1.** Patterns of intracranial involvement in 141 patients with Lemierre syndrome.

Progress in Hemophilia Treatment

OC03-01 AMT-060 Gene Therapy in Adults with Severe or Moderate-Severe Hemophilia B Confirms Stable FIX Expression and Sustained Reductions in Bleeding for up to 5 Years

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Objective Gene therapy aims to provide long-term therapeutic benefit from a single administration. AMT-060, an adeno-associated virus serotype 5 (AAV5) vector with a codon-optimized wildtype human factor IX (FIX) gene and liver-specific promoter, is being evaluated in 10 adults with severe/moderate-severe hemophilia B. over 5 years. Aim: To describe efficacy and safety outcomes from an analysis up to 5-years post-AMT-060 (Phase 1/2 study, NCT02396342).

Material and Methods Adult males with FIX activity ≤2 % and a severe bleeding phenotype received a single intravenous infusion of AMT-060 (5x10^12 gc/kg, Cohort 1, n = 5) or (2×10^13 gc/kg, Cohort 2, n = 5). Assessments include FIX activity, FIX replacement use, annualized bleeding rate (ABR) and treatment-related adverse events (TRAE) up to 5 years (Cohort 1) and 4.5 years (Cohort 2).

Results As of November 2019, mean FIX activity was 5.1 % at 4.0 years for Cohort 1 versus 4.4 % in Year 1; 6.8 % in Year 2; 7.3 % in Year 3 and 7.0 % in Year 4. Mean FIX activity for Cohort 2 was 7.5 % versus 7.1 % in Year 1; 8.4 % in Year 2; 7.9 % in Year 3; and 7.4 % in Year 4. Mean ABR during the last 12, and 6 months of observation, was 3.3 for Cohort 1 and 0.0 for Cohort 2, respectively, representing a 77 % and 100 % reduction in the year prior to treatment. During the same period, FIX replacement therapy consumption declined 90 % (Cohort 1) and 100 % (Cohort 2). Eight of 9 participants using prophylaxis at baseline were able to discontinue.

No participants developed FIX inhibitors or signs of sustained AAV5 capsid-specific T-cell activation. As previously reported, TRAE were mainly reported in the first 3.5 months after treatment, including three participants who experienced transient mild elevations in alanine aminotransferase. One additional TRAE (joint swelling post-exercise) was observed during the last 12 months of observation post-treatment. Updated data, up to 5-years of observation, will be presented.

Conclusion Durable, stable endogenous FIX activity and reductions in ABR and FIX replacement use were maintained over several years following a single treatment with AMT-060. There were no additional safety concerns with longer term follow-up. This data supports the ongoing Phase 3 study of the enhanced construct etranacogene dezaparvovec (AMT-061), which encodes the highly active Padua FIX variant.

OC03-02 Gene Transfer with Etranacogene dezaparvovec (AAV5-Padua hFIX variant) in Adults with Severe or Moderate-Severe Hemophilia B: Two Year Data from a Phase 2b Trial

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DOI 10.1055/s-0041-1728090

Objective Gene therapy for hemophilia may improve disease severity to a mild or functionally curative state through a single administration. Etranacogene dezaparvovec (AMT-061) is an investigational gene therapy for hemophilia B comprising an adeno associated virus serotype 5 (AAV5) vector containing a codon-optimized Padua variant human factor IX (FIX) gene with liver specific promoter. We have previously shown a single dose of etranacogene dezaparvovec provides sustained FIX activity into the mild-to normal range up to 52 weeks in adults with severe or moderate-severe hemophilia B. Two years of follow-up data will be presented.

Material and Methods A Phase 2b, open-label, single-dose, single-arm, multicenter trial (NCT03489291) in adult hemophilia B subjects, who were not excluded based on neutralizing antibodies to AAV5. All subjects received a single intravenous dose of etranacogene dezaparvovec (2x10^13 gc/kg) and will be followed for 5-years. The primary endpoint was FIX activity at Week 6.

Results All participants had FIX ≤1 %, required routine FIX prophylaxis, and had neutralizing activity to AAV5 at baseline. Following treatment, FIX activity increased rapidly to a mean of 31 % at Week 6 and 41 % by Week 52, with FIX activity levels of 50 %, 31 % and 41 % in participants 1-3 respectively. There was no relationship between the presence of anti-AAV5 NAbs and response to treatment. As of 52 weeks, there were no bleeds post-treatment and no requirement for FIX replacement other than protocol specified use in participant 3. There were no clinically significant elevations in liver enzymes and no participants required steroids related to the treatment. One participant experienced 2 mild AEs possibly related to treatment shortly after dosing (self-limiting headache and slightly elevated CRP). Participant 3 underwent hip surgery deemed unrelated to treatment and received FIX per protocol according to standard clinical practice. No participant developed inhibitors to FIX.

Conclusion Patients with AAV5 NAbs were included in the Phase 2b etranacogene dezaparvovec trial and have shown sustained FIX activity into the mild-to normal range. All participants were able to discontinue routine prophylaxis, and there have been no bleeds post-treatment with etranacogene dezaparvovec.

OC03-03 The Innovative Factor VIII Molecules HAT and HAT RI Overcome Impairments of Hemophilia A Therapy

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Objective Current Factor VIII (FVIII) substitution therapies are limited by a short plasma half-life, the lack of subcutaneous bioavailability, and a high immunogenic potential resulting in the development of inhibitory antibodies. Thus, we aimed for novel recombinant FVIII molecules addressing all major limitations of current FVIII substitution therapies to provide a safe and convenient treatment option raising the patient's quality of life.

Material and Methods For the generation of the Hemophilia A Therapeutic (HAT) molecule, four albumin-binding domains were incorporated into a B-domain deleted, single chain FVIII sequence. Additionally, 19 deimmunizing amino acid substitutions were integrated into HAT's FVIII core sequences resulting in HAT RI (Reduced Immunogenicity). Both molecules were produced in a human cell line, purified and extensively tested in vitro. Albumin-mediated half-life extending effects were investigated after i. v. injection in hemophilia A mice, Göttingen minipigs and albumin-deficient mice expressing the human FcRn a-chain. Pharmacokinetic properties and bioavailability for subcutaneous administration were evaluated in hemophilia A mice and in Göttingen minipigs. Pharmacodynamic investigations were performed using the tail vein transection assay in hemophilia A mice to assess in vivo efficacy. As FVIII-bound albumin may shield HAT and HAT RI from present FVIII inhibitors, the bypassing activity of both molecules was determined by a modified Bethesda assay. Further immunological aspects were investigated by LC-MS to identify HLA-presented peptides of HAT RI on dendritic cells.

Results Both FVIII molecules HAT and HAT RI demonstrated full in vitro and in vivo coagulating activity with a great efficacy. Pharmacokinetic assessment revealed a 4-fold half-life extension compared to Moroctocog alfa in the albumin-deficient mouse model. Bioavailability of 50 % after subcutaneous administration was observed in the Göttingen minipig model. A high activity despite the presence of inhibitors was observed in combination with reduced HLA-presentation showing improved immunological compatibility.

Conclusion HAT and HAT RI address the major challenges of current FVIII substitution therapy as both molecules provide a superior half-life, show a high subcutaneous bioavailability in mice and pig models, and reduce FVIII immunogenicity according to utilized in vitro models.

OC03-04 Long-term Efficacy and Safety of Fitusiran in Participants with Hemophilia A and B: An Interim Analysis of the Phase 1/2 Open-Label Extension Study

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Objective Hemophilia A (HA) and hemophilia B (HB) are caused by a deficiency in factors VIII and IX, respectively, leading to insufficient thrombin generation (TG) for clot formation. Fitusiran is a once-monthly subcutaneous (SC) prophylactic investigational siRNA therapeutic targeting antithrombin (AT) designed to restore sufficient TG and rebalance hemostasis in people with HA or HB, with or without inhibitors. The objective of this abstract is to present interim results on the safety and efficacy of fitusiran from the ongoing Phase 1/2 open-label extension (OLE) study (Sept 1, 2020 data cut).

Material and Methods The fitusiran Phase 1 study (NCT02035605) followed by the Phase 2 OLE study (NCT02554773) included male participants, ≥18 years of age, with moderate or severe HA or HB, with or without inhibitors. Participants received monthly SC prophylaxis with fixed doses of fitusiran, 50 mg or 80 mg. The observed annualized bleed rate (ABR) for participants with HA or HB, with or without inhibitors was calculated.

Results 34 participants were enrolled in the Phase 2 OLE study and treated with fitusiran for a median duration of 3.1 years and a maximum duration of 5 years. Serious treatment emergent adverse events were reported in 13 (38.2 %) participants and resulted in study drug discontinuation in 2 (5.9 %) participants (events of cerebral venous sinus thrombosis and increased liver transaminases). Once-monthly SC dosing of fitusiran prophylaxis resulted in sustained AT lowering in the participants (HA, n = 27 [13 with inhibitors and 14 without inhibitors]; HB, n = 7 [2 with inhibitors and 5 without inhibitors]), leading to thrombin generation levels approaching the lower end of the range seen in healthy volunteers. Overall, the observed median [interquartile range] ABR for treated bleeds in the efficacy period was 0.0 [0.0–3.8]. Factor replacement or bypassing agents were used during breakthrough bleeds, which were managed with the revised bleed management guidelines (BMG, 2017).

Conclusion Over a median treatment duration of 3.1 years, fitusiran prophylaxis was generally well tolerated and provided sustained AT lowering in people with hemophilia, resulting in a milder bleeding phenotype over an extended period. These data suggest that fitusiran has the potential to be used as a prophylactic treatment for people with HA or HB, regardless of inhibitor status.

OC03-05 Treatment with Eptacog Beta (Factor VIIa, Recombinant) Results in Early Bleed Resolution for Persons with Hemophilia A or B with Inhibitors: Results of the randomized cross-over PERSEPT 1 phase 3 study

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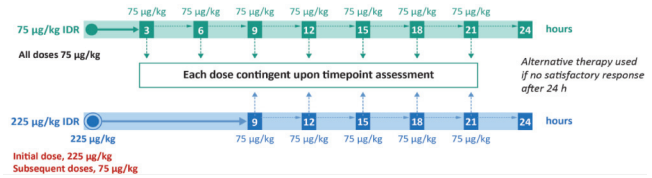
Objective Bleeding events (BEs) in persons with hemophilia A or B with inhibitors (PwHABI) impair musculoskeletal structure and function with consequent poor quality of life. An early bleed treatment and resolution is a primary goal in optimal haemophilia management. Eptacog beta (EB - HEMA Biologics and LFB) is a new bypassing agent approved in the USA for the treatment and control of BEs in adult and adolescent PwHABI. In the PERSEPT 1 clinical trial (NCT#02020369), we evaluated efficacy and safety of EB in home treatment in adults and adolescent (age ≥12 years) in different dosages. The aim was to assess the efficacy of 2 initial dose regimens of EB in the treatment of BE in the home setting in PwHABI.

Material and Methods This was a global, prospective, multicenter, controlled, randomized, cross-over phase 3 study, approved by local ethics committees and all participants gave informed consent/assent. 27 male PwHABI were randomized to either 75 µg/kg or 225 µg/kg kg initial dose regimen (IDR); patients were crossed over to the alternate IDR every 3 months. Success was defined as bleed resolution at 12 hours following the IDR, where the lower bound of the 95 % confidence interval was required to be greater than 55 %.

Results EB demonstrated dose-dependent successful clinical response in the first 24 hours with 2 IDRs (75 µg/kg IDR: q3h; and 225 µg/kg IDR: 225 µg/kg then 75 µg/kg q3h after 9 hours if necessary). The success rate at 3 hours was 84 % in the 225 µg/kg IDR compared to 29 % in the 75 µg/kg IDR. At 9 hours, sustained hemostatic efficacy from a single 225 µg/kg dose was observed (84 %) demonstrating a dose-dependent onset of action. After 12h hours 91 % [95 % CI: 84 %, 98 %, p<0.001] of all mild or moderate BEs were treated efficiently in the 225 µg/kg IDR, as did 82 % [95 % CI: 72 %, 91 %, p<0.001] in the 75 µg/kg IDR. At 9 hours, the proportion of success from the first dose in the 225 µg/kg IDR was 84.4 %.

Conclusion Both IDRs showed successful resolution of BEs. However a higher and earlier success rate was seen with the 225 µg/kg dose. These data support the concept that a larger initial thrombin burst may result in an earlier effective clot formation and earlier bleed resolution. These findings addresses the challenge of poor or limited venous access in home treatment.

		75 µg/kg IDR (N=25, BE=252)	225 µg/kg IDR (N=25, BE=213)
3 hours	Number of successes, n (%)	72 (28.6%)	**133 (62.4%)**
	Post-infusion number of failures, n (%)	174 (69.0%)	25 (11.7%)
	Missing, n (%)	6 (2.4%)	55 (25.8%)
	Success proportion [95% CI]	**29.3% [19.3%, 39.3%]**	**84.2% [73.2%, 95.2%]**
24 hours	Number of successes, n (%)	235 (93.3%)	199 (93.4%)
	Number of failures, n (%)	8 (3.2%)	1 (0.5%)
	Missing, n (%)	9 (3.6%)	13 (6.1%)
	Success proportion [95% CI]	**96.7% [93.3%, 100.0%]**	**99.5% [98.5%, 100.0%]**



▶**Fig 1.** All mild/moderate bleeds at 3 and 24 hours following the initial dose of eptacog beta. Below: 75 µg/kg and 225 µg/kg IDR for treatment of bleeding. IDR: Initial Dosing Regimen

OC03-06 Second interim analysis results from the STASEY trial: A single-arm, multicentre, open-label, phase III clinical trial to evaluate the safety and tolerability of emicizumab prophylaxis in persons with haemophilia A (PwHA) with FVIII inhibitors

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Objective Emicizumab, a subcutaneously administered, bispecific monoclonal antibody, bridges activated factor (F)IX and FX, replacing the function of missing activated FVIII in persons with haemophilia A (PwHA), thereby restoring haemostasis. Here we report updated interim results from STASEY (NCT03191799), a study of emicizumab prophylaxis in PwHA with FVIII inhibitors.

Material and Methods Following ethics committee approval and informed consent, PwHA aged ≥12 years with FVIII inhibitors (N = 195; intent-to-treat population) received emicizumab 3 mg/kg per week for 4 weeks, followed by 1.5 mg/kg per week. Study duration was 2 years. The primary objective was safety (adverse events [AEs], including thromboembolic events [TEs] and hypersensitivity); secondary objectives included efficacy (annualised bleed rates [ABRs]).

Results At data cut-off (20 May 2019), 193 PwHA had received emicizumab and were evaluable for safety; median age (range) was 28.0 (12–80) years; median (range) treatment duration was 50.9 (1.1–88.1) weeks. Emicizumab was well tolerated (Table 1). Emicizumab-related AEs were reported in 33 PwHA; the most common were injection-site reactions, occurring in 22/193 (11.4 %) PwHA. Two AEs were classified as TEs: ST-elevation myocardial infarction (STEMI; n = 1) and hypertrophic clot following tooth extraction caused by multiple doses of antifibrinolytic combined with rFVIIa (n = 1). The 55-year-old PwHA with STEMI had several risk factors, including a history of smo-

▶**Tab 1.** Safety Summary (Safety-Evaluable Population)

AE, n (%)	Emicizumab 1.5 mg/kg/week (N=193)
Total number of AEs	**551**
Number of PwHA with >=1 event	**145 (75.1)**
Fatal AE	1 (0.5)*
Serious AE	19 (9.8)
AE leading to treatment withdrawal	1 (0.5)
AE leading to dose modification or interruption	3 (1.6)
AE leading to study discontinuation	0 (0.0)
Grade >=3 AE	22 (11.4)
Study treatment-related AE	33 (17.1)
Injection-site reaction	22 (11.4)
AEs of interest	
Systemic hypersensitivity/anaphylactic/anaphylactoid reaction	0 (0.0)
TE	2 (1.0)†
TE associated with aPCC and emicizumab	0 (0.0)
TMA	0 (0.0)
TMA associated with aPCC and emicizumab	0 (0.0)
Most common AEs (>=10% of PwHA)	
Nasopharyngitis	24 (12.4)
Headache	23 (11.9)
Injection-site reaction	22 (11.4)

*Polytrauma with fatal head injury, unrelated to emicizumab, previously reported in first interim analysis.
†ST-elevation myocardial infarction due to thrombus in a coronary artery in one PwHA, and hypertrophic clot at site of tooth extraction in one PwHA who was receiving anti-fibrinolytics. The latter is a known complication of tooth extractions.
Coded using Medical Dictionary for Regulatory Activities (MedDRA) Version 21.1.
AE, adverse event; aPCC, activated prothrombin complex concentrate; PwHA, persons with haemophilia A; TE, thromboembolic event; TMA, thrombotic microangiopathy.

▶**Tab 2.** Efficacy Summary (Intent-to-Treat Population)

ABR*	Emicizumab 1.5 mg/kg/week (N=195)
Treated bleeds†	
Mean ABR, model based‡ (95% CI)	0.5 (0.27–0.86)
Median ABR, calculated (IQR)	0.0 (0.00–0.00)
PwHA with zero bleeds, n (%)	167 (85.6)
All bleeds	
Mean ABR, model based‡ (95% CI)	1.3 (0.95–1.80)
Median ABR, calculated (IQR)	0.0 (0.00–1.14)
PwHA with zero bleeds, n (%)	121 (62.1)
Treated spontaneous bleeds†	
Mean ABR, model based‡ (95% CI)	0.3 (0.13–0.67)
Median ABR, calculated (IQR)	0.0 (0.00–0.00)
PwHA with zero bleeds, n (%)	179 (91.8)
Treated joint bleeds†§	
Mean ABR, model based‡ (95% CI)	0.3 (0.12–0.84)
Median ABR, calculated (IQR)	0.0 (0.00–0.00)
PwHA with zero bleeds, n (%)	182 (93.3)
Treated target joint bleeds†	
Mean ABR, model based‡ (95% CI)	0.2 (0.06–0.68)
Median ABR, calculated (IQR)	0.0 (0.00–0.00)
PwHA with zero bleeds, n (%)	186 (95.4)

*The Bleed and Medication Questionnaire was completed by participants/caregivers via an electronic handheld device. Bleed definitions were based on International Society on Thrombosis and Haemostasis criteria (Blanchette VS, et al. J Thromb Haemost 2014;12:1935–39).
†Treated bleeds were defined as a bleed directly followed by a haemophilia medication reported as a treatment for bleed, without an intervening bleed and irrespective of the time between the treatment and the preceding bleed. If multiple bleeds occurred on the same calendar day, the subsequent treatment was considered to apply to each of these multiple bleeds. Bleeds due to surgery/procedure are excluded.
‡Calculated using negative binomial regression method.
§Joint bleeds are defined as bleeds with type reported at 'joint' in combination with >=1 of the following symptoms: increased swelling or warmth of the skin over the joint; increasing pain; decreased range of motion or difficulty in using the joint compared with baseline.
ABR, annualised bleed rate; CI, confidence interval; IQR, interquartile range; PwHA, persons with haemophilia A.

king, hypertension and family history of coronary heart disease. He did not receive bypassing agents and continued emicizumab without dose adjustment; the treating physician assessed the event as unrelated to emicizumab. One previously reported fatality (polytrauma), was assessed as unrelated to emicizumab. Three PwHA received activated prothrombin complex concentrate and 32 received rFVIIa, with no associated thrombotic microangiopathy or arterial/venous TEs. Ten PwHA (5.2 %) developed anti-drug antibodies, none with neutralising potential (by pharmacokinetic/clinical assessment); they continued on 1.5 mg/kg per week emicizumab with no increased bleeding. ABRs remained low (Table 2).

Conclusion No new safety signals were identified; these data confirm the safety results from the HAVEN clinical program.

Clinical Practice

OC04-01 Efficacy and Safety of Human Fibrinogen Concentrate in Patients with Congenital Fibrinogen Deficiency: Combined Results of the FORMA-02 and FORMA-04 Clinical Trials

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Objective Congenital fibrinogen deficiency (CFD) is a rare disorder characterized by a lack of/low levels of functional fibrinogen. Human fibrinogen concentrate (HFC) is administered for bleeding episode (BE) treatment and for preventing blood loss during surgery in patients with CFD. Here we report combined data from two studies of HFC for on-demand BE treatment and as surgical prophylaxis in adult, adolescent and paediatric patients.

Material and Methods FORMA-02 and FORMA-04 were multicentre, prospective, open-label Phase 3 studies of the efficacy and safety of HFC (Fibryga®, Octapharma) in adult/adolescent and paediatric patients with CFD. Efficacy was assessed by the investigator and adjudicated by an Independent Data Monitoring & Endpoint Adjudication Committee (IDMEAC). Adverse events (AEs) were recorded.

Results A total of 39 patients with a median (range) age of 17 years (1–54) received HFC, including 14 paediatric (aged 0–11), 6 adolescent (aged 12–18) and 19 adult patients (aged ≥18).

Thirty-two patients received HFC for treatment of 99 BEs; 97 minor and 4 major. Mean (±SD) total dose per BE was 65.51 mg/kg (±26.47) for adults/adolescents and 93.78 mg/kg (±64.60) for paediatric patients. Overall haemostatic efficacy was rated successful (rating of excellent or good) for 99.0 % of BEs by the IDMEAC (Table 1). Efficacy was comparable between the three age subgroups.

Twelve patients received HFC for 15 surgeries (13 minor, 2 major); 3 paediatric and 12 adults/adolescents. Mean (±SD) loading dose prior to surgery was 77.39 mg/kg (±20.22) in adults/adolescents and 78.50 mg/kg (±27.96) in paediatric patients. The two major surgeries required 5 and 7 maintenance infusions and five of the minor surgeries required a median (range) 3 (1–4) maintenance infusions. Overall haemostatic efficacy was rated successful for all surgeries by the investigator and IDMEAC (Table 1).

A total of 101 AEs occurred in 23 patients (59.0 %), including 16 serious AEs in 6 patients. Five AEs in 4 patients were deemed possibly related to treatment. No allergic/hypersensitivity reactions or deaths were observed.

Conclusion Across two Phase 3 clinical trials HFC was efficacious for on-demand treatment of BEs and perioperative prophylaxis in patients with CFD. Efficacy of HFC was comparable for adult, adolescent and paediatric patients, with a favourable safety profile.

►**Tab 1.** Efficacy assessments after HFC treatment

Efficacy rating	Investigator	IDMEAC
Bleeding events: Haemostatic efficacy		
4-Point Efficacy Scale	N (%)	N (%)
Excellent	77 (77.8)	89 (89.9)
Good	17 (17.2)	9 (9.1)
Moderate	2 (2.0)	1 (1.0)
None	3 (3.0)*	0 (0.0)
Surgical prophylaxis: Intra-operative efficacy		
4-Point Efficacy Scale	N (%)	N (%)
Excellent	14 (93.3)	14 (93.3)
Good	1 (6.7)	1 (6.7)
Moderate	0 (0.0)	0 (0.0)
None	0 (0.0)	0 (0.0)
Surgical prophylaxis: Post-operative efficacy		
4-Point Efficacy Scale	N (%)	N (%)
Excellent	15 (100.0)	14 (93.3)
Good	0 (0.0)	1 (0.0)
Moderate	0 (0.0)	0 (0.0)
None	0 (0.0)	0 (0.0)

* For three patients, haemostatic efficacy assessment was missing. Thus, as per the statistical plan, the rating by the investigator was considered as 'None'.

HFC, human fibrinogen concentrate; IDMEAC, Independent Data Monitoring and Endpoint Adjudication Committee; N, number of bleeds or surgeries.

OC04-02 Treatment of immune thrombocytopenia (ITP) with Eltrombopag – results of the 3 rd interim analysis of the German non-interventional trial RISA. Focussing on steroid-pretreatment and fatigue

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Objective Eltrombopag (EPAG) is a thrombopoietin-receptor agonist, which is proved to be effective and safe in the treatment of immune thrombocytopenia (ITP).

Material and Methods In the planned interim analysis of the study RISA we evaluated data from the routine treatment with EPAG over two years. Patients were included, if they received at least one dose of EPAG and completed one post baseline assessment. Fatigue was assessed at baseline and during the study using the FACIT-F questionnaire.

Results 210 patients were included in the analysis. Mean±SD age was 63.1 ±17.4 years, median (range) duration of ITP was 5.6 (0.0– 44.9) years. Comorbidity was present in 81.4 % of all cases. 47.6 % were male, median platelet count at baseline was 33.5x109/L. In 37.6 %, bleeding complications occurred within 12 months before baseline. In 10 %, splenectomy had been performed. 85.2 % of the patients were pretreated pharmacologically. 46.2 %

received prednisolone, 9 % prednisone and 24.3 % dexamethasone. 14.8 % of all patients received corticosteroids for longer than 6 months as first-line therapy. In another 10.0 %, the total duration of corticosteroid therapy over first and second line of treatment exceeded 6 months. The longest treatment with corticosteroids lasted 108 months. 20.0 % of all patients received immune globulins, 2.9 % Rituximab.

Mean±SD daily dose of EPAG was 45.1±14.4 mg. Within the first month of treatment, 75 % of the patients responded in terms of raising the thrombocyte counts above 50x109/L. After 24 months, response rate was 89 %. Initially mean±SD FACIT-Fatigue score was 36.3±11.1. It did not change significantly during the two-year observation period. 31 % of all evaluable patients had severe fatigue (FACIT-F score ≤ 30). EPAG was generally well tolerated. No new signals concerning the safety or tolerability of the drug occurred.

Conclusion This 3 rd interim analysis confirmed, that treatment of ITP with EPAG is an effective and safe option. We found that severe fatigue is present in ITP patients to a similar extent as in cancer. A clinically significant improvement in fatigue could not be found in our study, but this finding is preliminary. A quarter of patients received corticosteroids for longer than 6 months, which tremendously exceeds the maximum duration of steroid treatment, as being recommended in evidence-based guidelines.

OC04-03 Acquired Hemophilia A (AHA) Limits of the Awareness Push in Germany

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Objective Acquired hemophilia A (AHA) is an extremely rare, but potentially life-threatening bleeding disorder caused by autoantibodies against human coagulation factor VIII. AHA may lead to spontaneous or trauma induced bleeds, treated with bypassing agents. New and successful therapies had been launched in the last decade. Several manufacturers drove awareness campaigns in Germany to push detection of potential patients. Here we examine the change in frequency of AHA in-hospital coding, based German DRG-data.

Material and Methods Reports from German DRG-Institute (InEK), Statistical Office (DESTATIS) and German hospital quality reports 2010-2018 were analyzed for AHA. Analysis with Microsoft-Access 2019.

Results Cases with a main and secondary diagnosis of AHA (D68.31, ICD10-GM) increased from 215 (2010) to 633 (2015) and stay stable around 500 annually ever since (+132 %). Main diagnosis rose from 29 (2010) to 142 (2015) and remains ever since between 139 and 144 (+396 %). Gender distribution (58.9 % male) remained stable over time, extremes 2010: 54.4 % male and 2016: 61.3 % male. The average length of hospitalization decreased slightly from 22.3 days (2010) to 19.2 days (2018; -14 %). All age groups between age of 60 and 90 had average lengths of stay between 20 and 24 days. Average age of patients was constantly around 70 years (2018: 69.3), the median age increased from 73 (2014) to 77 (2018). Analysis of living place of patients revealed a higher hospitalization rate in states with higher rate of hemophilia centers.

Analysis of 2018 data revealed: 39 % of cases were treated in comprehensive care centers (CCC), 34 % in other hemostaseologic centers and 26 % in non-centers. 86 % treatments in departments of internal medicine, 9 % surgery and 5 % ICU only. All cases in 2018 were treated in 51 hospitals. 56 % cases in university hospitals, 31 % major hospitals and 13 % rural hospitals.

Conclusion We observed a steep increase in documented hospital cases with AHA between 2010 and 2015, thereafter the annual figures remain very constant. It may be concluded that the various awareness campaigns worked, but that a steady level was reached in 2015. The stable age, gender, and length of stay distribution support the assumption that more cases of AHA had been detected. National registration of AHA might deepen the insights

OC04-04 Pain status in patients with hemophilia: evaluation of routine pain assessment in an unselected cohort of patients with hemophilia A and B

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Objective Pain is a common co-morbidity in patients with hemophilia (PWH). Most data on prevalence of pain is generated by patient surveys with potential selection bias or within clinical studies. Therefore, we aimed to analyze prevalence of pain in an unselected cohort of PWH visiting a single German hemophilia center for routine annual check-ups during the previous 4 years.

Material and Methods Assessment of pain intensity (numeric rating scale, NRS, 0 [no pain] to 10 [worst imaginable pain]) and location over the previous 3 months was carried out with an ad-hoc questionnaire. Pain interference was rated on a scale from 0 to 10 with higher values indicating more interference. Impact on mood was reported as none, moderate or severe. Demographic and clinical data were extracted from patient charts.

Results 186 male adult PWH A or B visited the center, of whom 165 (88 %) had a routine annual check-up and 161 (98 %) completed at least 1 questionnaire (94 %, 81 %, 71 % of all PWH with severe, moderate, mild hemophilia, respectively) with a median age at first assessment of 39 years (range, 18-77 years). 145/161 PWH (90 %) reported episodes of pain with a median intensity (NRS) of 6 (range, 1-10), 5 (1-10), 3 (1-9) and median number of affected joints of 2, 2, 1 (range, 0-6) in PWH with severe, moderate, mild hemophilia, respectively. Only 10 % of patients reported no pain and 15 patients (9.7 %) had pain only in locations other than joints. 53 % of PWH with mild hemophilia reported pain. Median pain interference in PWH with pain was rated 4 (range, 0-10); 51, 64, 22 PWH reported no, moderate and severe impact on mood, respectively. Pain intensity correlated with orthopedic joint score ($r = 0.426$, $p<0.001$), but was not different between PWH (severe) treated prophylactically or on-demand at time of assessment. Median number of completed questionnaires per patient was 2 (range,1-5) with 93, 48, 13, 2 PWH completing 2, 3, 4, 5 questionnaires, respectively. Median pain intensity was stable over time, indicating that pain is a permanent burden in PWH.

Conclusion This analysis of a routinely used pain assessment tool in an unselected population of PWH A and B reveals a high prevalence of pain episodes, consistent over time, as well as relevant pain interference and impact on mood. The effect of interventions needs to be further analyzed.

OC04-05 Safety and efficacy of a high-purity plasma derived von Willebrand Factor in patients with von Willebrand disease (VWD) undergoing prophylaxis for joint bleeding: results from a post-marketing study

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Objective Long term prophylaxis is the gold standard treatment for severe haemophilia A patients for preventing haemorrhages and limiting the progression of arthropathy. Patients with severe VWD could have also joint bleeding episodes (BE) and they may benefit from prophylactic von Willebrand Factor (VWF) treatment to prevent the risk of target joint occurrence. The objective was to evaluate the safety and efficacy of a prophylactic treatment with a plasma derived Factor VIII-poor von Willebrand Factor (pdVWF) concentrate (Wilfactin®/Willfact® - LFB) in routine clinical use.

Material and Methods Data from prospective non-interventional post-marketing study conducted in 31 centres with pdVWF in France were collected. The study included 155 treated patients with inherited VWD and patients were followed for up to 3 years. Prophylaxis for joint bleeding was evaluated in capturing the occurrence of breakthrough BE within 3 days of pdVWF administration. Safety was evaluated by monitoring adverse drug reactions, serious adverse events and treatment schedule.

Results 14 patients (12 type 3 and 2 type 2) started or continued prophylactic therapy due to joint bleedings. The median age was 19.5 years (range 5–54) and 71 % (10/14) were female. Target joints were ankle and/or elbow (n = 5), knee (n = 3), hip (n = 3) and unknown site (n = 3). The median duration of prophylactic therapy per patient was 2.7 years (6.5 months - 3.7 years). Patients received a median dose of 42.2 IU/kg pdVWF (range 26-76), 2 times per week (range 1.2-3.3). Breakthrough bleedings (spontaneous or post-traumatic, joint or other bleeds requiring additional treatment with pdVWF) occurred following 1.7 % (51/3069) prophylactic infusions. More than half of patients (8) reported 0 or 1 BE only. The median annualized bleeding rate calculated in 9 patients with a duration of prophylaxis >1 year was 0.8 BE per year (range 0.0-5.4). The treatment was well tolerated without report of thrombotic events, neutralising VWF inhibitors or any other related serious adverse events.

Conclusion In this study, the FVIII-poor pdVWF has been effective and well tolerated in joint bleeding prophylaxis. Benefits reported in this real-world patient study confirm results of a phase III study assessing specifically the long-term prophylaxis with this pdVWF.

Critical Care, Surgery & Transfusion medicine

OC05-01 Prevalence and clinical impact of reduced FXII activity in patients receiving extracorporeal membrane oxygenation

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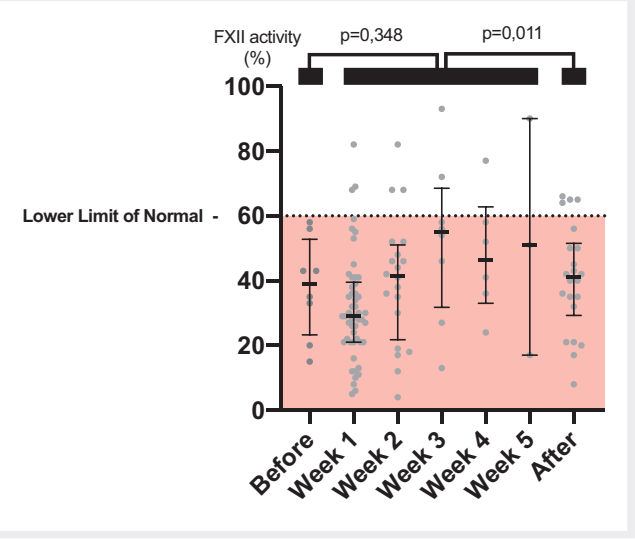
Objective Extracorporeal membrane oxygenation (ECMO) provides large surface exposure to human blood leading to coagulation activation. Only limited clinical data are available on contact activation and FXII activity in ECMO patients.

Material and Methods This observational cohort study included adults receiving ECMO at three medical Intensive Care Units (ICUs) at the Medical University of Vienna. The primary outcome was the prevalence of reduced FXII activity (FXII activity <60 %) among patients receiving ECMO. Secondary outcomes included the time-course of FXII activity before, during and after ECMO as well as associations with clinical endpoints including thromboembolic and bleeding complications, ECMO-, ICU- and hospital mortality. Exploratory endpoints included the association of FXII activity and activated partial thromboplastin time (aPTT) in vitro.

Results Fifty-one patients with a total of 117 samples were prospectively included for analysis. Median FXII activity during ECMO treatment was 35 % (IQR 22-50). Of 51 patients analyzed, 50 (98 %) had a reduced FXII activity at any timepoint during ECMO. FXII activity did not decrease significantly after subjection to ECMO, but increased after discontinuation (p = 0.011; Figure 1). Patients with thromboembolic complications had higher FXII activity during ECMO (p = 0.038). The risk for thromboembolism increased by a crude OR of 2.793 (95 % CI 1.138-6.857) per tertile of FXII activity. No association with bleeding was observed. In the in vitro studies, FXII activity correlated well with aPTT in heparinase-treated samples (R2 = 0.789; p = 0.007).

Conclusion The presented study provides insights into FXII activity during ECMO treatment. Results suggested the following: (i) The majority of patients subjected to ECMO exhibit decreased levels of FXII activity, which may be enhanced by exposure of blood to foreign surfaces; (ii) Reduced FXII activity may impact the validity of aPTT measurements, as this test may be influenced by reduced FXII levels; and (iii) lower endogenous FXII activity may reduce the risk for thromboembolic complications, highlighting the association between contact activation and thrombosis and supporting the efforts to develop contact activation-targeting therapeutics as anticoagulation strategies.



▶ **Abb 1.**

OC05-02 Assessment of the anticoagulant effect of direct oral anticoagulants (DOACs) in patients needing immediate management during emergency procedures

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Objective Assessment of the anticoagulant effect of direct oral anticoagulants (DOACs) still is a challenge for various medical disciplines, especially in patients needing immediate care in a medical emergency. Aside from severe bleeding and thrombotic events, evaluation of the coagulation status is important for urgent indication of fracture care or administration of a specific antidot. An assay that screens for the absence of a DOAC might help accelerate treatment in these situations. The goal of this study is to evaluate the use of a qualitative POC Method (DOAC Dipstick, DOASENSE®) in an emergency setting.

Material and Methods Between 11/2019 and 04/2020 the POC method was available for all clinicians in a level I emergency department. The POC testing was indicated by the physician on duty followed by a standardized questionnaire on basic patients' parameters, indication for the qualitative testing and drawn conclusions based on the tests´ results. Intraindividual reliability blinded to the clinical user (visual testing vs. semiquantitative reader) as well as the interindividual reliability compared with standard anti-factor-Xa (antiXa)- or direct-thrombin-inhibitor (DTI)-tests were investigated.

Results In total, 82 patients were included (30 % Neurology, 50 % Trauma, 10 % Neurosurgery, 10 % Internal Medicine) 28 patients being anticoagulated with antiXa inhibitor and 7 patients with dabigatran. Test results of POC testing could be confirmed using standard anti-factor-Xa (antiXa)- or direct-thrombin-inhibitor (DTI)-tests in all cases. In most cases the POC test was used to identify unknown DOAC status in patients who could not be interviewed concerning their medication. 12 patients received a lysis therapy after exclusion of DOAC anticoagulation, 2 patients were treated with a specific antidot after a major bleeding event. In 6 patients the POC test demonstrated a positive result in patients that stopped using the oral medication several days ago.

Conclusion In an emergency with an immediate consequence under time pressure, the POC test might provide a significant time advantage compared to standard laboratory testing. Due to the cumulative effect within the patients´ urine the test can only be used for the qualitative verification and does not provide any information concerning the actual anticoagulative effect.

OC05-03 Implementation of Direct Oral Anticoagulant (DOAC) Perioperative Interruption and Interval evaluation of the DOASENSE Dipstick performance

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DOI 10.1055/s-0041-1728102

Objective As the use of DOACs increases, so does the need for interruption in patients requiring procedures, reaching up to 20 %. We have addressed this with a standardized institutional interruption policy anchored on emerging data. The urine-based DOAC Dipstick is a novel testing option designed to deliver a real-time qualitative estimate of the presence of DOACs. We designed a trial testing the safety of a standardized interruption protocol and the accuracy of the DOAC Urine Dipstick for determination of DOACs' presence. We present our interim data safety and performance results

Material and Methods A prospective, single cohort study currently undergoing recruitment of patients requiring DOAC interruption. Before-surgery DOAC level was tested with plasma Factor Xa inhibitor assay and urine was tested by DOAC Dipstick to correlate results in the interim analysis. We present continuous variables as median (SD) and categorical as percentages. Independent T-test, Cohen's Kappa, Chi-Square and Fisher exact test used as indicated

Results Thus far, a total of 79 patients have been recruited, 60.8 % male and predominantly Caucasian (87.3 %). Indication for anticoagulation was mostly afib 83.5 %, followed by VTE 24.1 %. Other comorbidities were hypertension 53.2 %, cancer 34.2 %, hyperlipidemia 24.1 %, obstructive sleep apnea 22.8 %, diabetes 19 %, prior bleeding 19 %, coronary artery disease 15.2 % and cardiomyopathy 6.3 %. Anticoagulants used were mostly Apixaban 72.2 %, followed by Rivaroxaban 22.8 % and Dabigatran 2.5 %. Peri-procedural arterial events (stroke) occurred in 1 patient (1.3 %), and VTE (pulmonary embolism) in 1 patient (1.3 %) at 3 month follow-up. Two patients (2.5 %) had major bleeding outcomes as defined by ISTH. Urine samples from 35 patients (44.3 %) were analyzed using DOASENSE. Anti-Xa inhibition for patients with DOASENSE positive urine was significantly higher than those with negative DOASENSE dipstick results (10.90 ± 9.2 vs. 5.05 ± 5.36, p= 0.025) with fair correlation (kappa = 0.342, p= 0.04)

Conclusion Standardized periprocedural interruption of DOACs appears safe, simple to implement and was widely accepted institutionally. In interim analysis, DOASENCE results significantly associate with percentages of anti-Xa inhibition, with fair correlation between effective anticoagulation and dipstick results. Larger numbers are needed to validate this promising strategy

OC05-04 Von Willebrand factor ratio as a risk factor for bleeding in aortic valve stenosis replacement surgery

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Objective Patients with aortic valve stenosis (AVS) often undergo valve replacement by thoracotomy. Since this is a major surgical procedure, sufficient hemostasis is required to avoid postoperative complications. Increased shear stress as a result of stenosis can reduce platelet function and von Willebrand factor (VWF). A VWF ratio (VWF:RCo/VWF antigen) below 0.7 may be associated with the loss of the largest VWF multimers and consequently with reduced VWF functionality. This study focuses on the evaluation of primary hemostasis in patients undergoing AVS and valve replacement surgery.

Material and Methods 60 patients with AVS were included in this study. A categorization of the patients into "more bleeding" group and "less bleeding" group was performed based on the postoperative blood loss. In addition, the severity of stenosis (mild/moderate, severe) was determined by the peak aortic velocity. Clinical characteristics, e. g. hemostasis data and platelet function, were evaluated before and five days after surgery.

Results The "more bleeding" group had twice as much mean postoperative chest tube blood loss as the "less bleeding" group (15.5%; 780.7±220 mL vs. 6.5%; 387.1±190 mL; percent p = 0.016, volume p = 0.035). Furthermore, blood loss was associated with both severity of stenosis (p = 0.05) and VWF ratio (p = 0.033). The risk of higher blood loss increased in patients with a low

VWF ratio (OR = 2.448; 95 % CI:1.1093-9.140; p = 0.064) and in patients with severe stenosis (OR = 7.0; 95 % CI: 1.8391-58.39; p = 0.050). The "more bleeding" group had more cases of severe stenosis (93.3 % vs 66.7 % p = 0.050) and significantly lower VWF ratio (0.6±0.19 vs. 0.41±0.23; p = 0.021). After surgery, a significant reduction in shear stress (102.3±23.6 vs. 31.3±6.0 p<0.0001) and a significant increase in VWF ratio (0.56±0.2 vs. 0.70±0.2 p = 0.001) could be observed. Platelet function measured by light transmission aggregometry (LTA) showed no significant correlation with increased blood loss.

Conclusion Patients with AVS may have an increased risk of postoperative bleeding if their VWF ratio is below 0.7. However, reduced platelet function (assessed by LTA) showed no association with increased bleeding in this collective. Further investigations are necessary to study e. g. platelet-VWF or platelet-collagen interaction.

OC05-05 Safety and efficacy of a high-purity plasma-derived von Willebrand Factor in patients with von Willebrand disease (VWD) undergoing prophylaxis for gastrointestinal bleeding: results from a post-marketing study

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DOI 10.1055/s-0041-1728104

Objective Although prophylaxis is generally accepted as an effective method to prevent bleeding events, little is known about the dosing and the outcome of prophylactic treatment in patients with VWD who experienced recurrent gastrointestinal (GI) bleeding. The objective was to evaluate the safety and efficacy of a plasmatic Factor VIII-poor von Willebrand Factor (pdVWF) concentrate (Wilfactin®/Willfact® - LFB) in routine clinical use.

Material and Methods Patients suffering from all types of VWD undergoing prophylaxis for GI bleeding were enrolled in the prospective non-interventional post-marketing study conducted in 31 centres in France. The frequency of breakthrough bleeding, treatment dosing and tolerability in prophylactic treatment setting for GI bleeding were evaluated.

Results 13 patients (4 type 3 and 9 type 2) who entered or continue prophylaxis for GI bleeding were analysed. All had basal VWF:RCo ≤15 IU/dL. Among them, 8 (62 %) male and 5 (38 %) female. Median age was 57 years (range 33–81 years; 31 % being 65 or older). Most patients had VWD associated angiodysplasia. Patients infused pdVWF concentrate at home (45.2 IU/kg (22 to 55) one to three times per week (median 2.5) for 6.7 to 43.4 months (median 37.1). A total of 4036 infusions were given. Breakthrough bleeds (spontaneous or post-traumatic, GI or other bleeds requiring additional treatment with pdVWF) occurred following 1.4 % prophylactic infusions. During routine prophylaxis, 5 (38 %) patients did not experience any bleeding event within 3 days of administration of concentrate. Overall, the median annual bleeding rate (ABR) in 10 patients treated prophylactically for at least one year was 1.1 (range 0.0-11.0). The highest ABR was due to the recurrence of GI bleedings in one patient with type 2A and multiple angiodysplasia of small intestine (17 events in 18.6 months). There were no notable differences in the frequency of bleeding events when stratified by age or phenotype. During a prolonged exposure, the pdVWF was well tolerated without report of thrombotic events, neutralising VWF inhibitors or any other related serious adverse events.

Conclusion In this study, routine prophylaxis with pdVWF was well tolerated and contributed to an effective management of recurrent GI bleeding.

OC05-06 Removal of citrate from PAS-III additive solution improves functional and biochemical characteristics of buffy-coat platelet concentrates stored for seven days, with or without Intercept pathogen reduction

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DOI 10.1055/s-0041-1728105

Objective Deterioration in the quality of platelet concentrates (PCs) during storage results from the appearance of storage lesions affecting the hemostatic functions and post-transfusion survival of the platelets. These lesions depend on the preparation and pathogen inactivation methods used, the duration of storage and the platelet additive solutions (PAS) present in the storage bags. We investigated the effects of the citrate contained in third-generation PAS (PASIII) on storage lesions in buffy-coat PCs with or without photochemical (amotosalen-UVA) treatment (PCT) over 7 days.

Material and Methods A pool-and-split method was used to obtain four study groups: PCT-PCs stored in commercial PAS-III solution containing 10 mM sodium citrate, untreated PCs stored in PAS-III, PCT-PCs stored in citrate-free PAS-III and untreated PCs stored in citrate-free PAS-III (n = 3 per group). In vitro platelet quality and function were tested over 7 days (D).

Results Platelet counts were conserved in all groups during storage as was platelet swirling without the appearance of macroscopic aggregates. Glycoprotein (GP) IIbIIIa and GPVI expression remained stable whereas GPIbα declined similarly in all groups during storage. Removal of citrate led to a significant decrease in glucose consumption, which largely countered a modest deleterious effect of PCT. Citrate removal also resulted in decreased lactate generation and better maintenance of pH during storage, while PCT had no impact on these parameters. Citrate-free storage moreover significantly reduced expression of the granule secretion marker P-selectin, and exposure of the apoptosis signal phosphatidylserine, thereby abolishing the activating effect of PCT on both parameters. Citrate removal benefited platelet aggregation to various agonists up to day 7, whereas PCT had no impact on these responses.

Conclusion Removal of citrate from PAS-III has a beneficial impact on platelet metabolism, spontaneous activation and apoptosis, and improves platelet aggregation, irrespective of PCT, which should allow the transfusion of platelets with better and longer lasting functional properties.

Reference

New Laboratory Technologies

OC06-01 Diagnosis of inherited platelet disorders: comparison between immunofluorescence analysis on the blood smear and genetic testing

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Objective Inherited platelet disorders (IPD) are rare diseases featured by reduced platelet count and/or impaired platelet function. Diagnostic tools include clinical evaluation, platelet function tests and genetic analysis. We have established a method to assess platelet phenotype on the blood smear by immunofluorescence microscopy as a diagnostic tool for IPDs. The aim of

the study was to compare the outcomes of the immune-morphological analysis with those of genetic testing in a cohort of consecutive patients suspected for IPD.

Material and Methods Three reference Institutes for the diagnosis of IPD from Greifswald, Germany, Murcia, Spain, and Copenhagen, Denmark, participated in the study. The subjects were enrolled from January 2019 to September 2020 by Centers of Copenhagen and Murcia, to whom they had been referred for genetic investigation. The blood smears were centralized in the Greifswald Center, where the immune-morphological assessment was blindly performed. The morphologic changes were reported and the potential diagnoses were formulated. Subsequently, the genetic results were unblinded and the comparison performed. Patients signed written informed consent.

Results 75 consecutive subjects belonging to 56 unrelated pedigrees were enrolled. The microscopic analysis identified alterations suggestive for a form of IPD in 38 cases (51 %). In 33 (44 %), abnormalities not typical for known IPD were reported. In 4 (5 %), no morphologic alterations were found. The genetic testing diagnosed a specific IPD in 54 cases (72 %). In 15 (20 %) and 6 (8 %) variants of uncertain significance (VUS) and no variants were found, respectively. Among 19 forms of IPD with a confirmed genetic mutation, in 11 immunofluorescence correctly predicted the affected gene by the typical morphologic pattern. In further 3 disorders, unreported characteristic morphologic patterns were identified, while in 5 disorders, immunofluorescence showed unclear findings. Among 21 patients without genetic diagnosis, in 20 immunofluorescence found morphologic alterations in platelet structures, which could explain platelet dysfunction.

Conclusion Immunofluorescence analysis on the blood smear is an effective diagnostic tool for a substantial group of IPD. It can guide geneticist to interpret VUS and provide clinicians with relevant information about changes in platelet structure, which can explain the bleeding phenotype of patients.

OC06-02 Next-generation sequencing identified different types of Hermansky-Pudlak syndrome associated with phenotype variability

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Objective Hermansky-Pudlak syndrome (HPS) is a heterogeneous group of 10 rare autosomal recessive multisystem disorders. HPS-associated genes encode components of four proteins complexes: BLOC-1, 2, 3 (biogenesis of lysosome-related organelles complex 1-3) and the transporter complex AP-3 (adaptor protein-3). The main symptoms are bleeding tendency and oculocutaneous albinism. In HPS1 and HPS2 pulmonary fibrosis and granulomatous colitis can occur, in HPS2 and HPS10 immune deficiency is associated. Milder phenotypes have been described for HPS3, HPS5 and HPS6. The symptoms are caused by malfunction of lysosome related organelles: platelet δ-granules, melanosomes, lamellar bodies in lung cells and lytic and azurophil granules in neutrophiles. Identification of the HPS subtype is important for prognosis, clinical management and treatment options. We investigated 5 patients and 8 family members to identify the underlying disease.

Material and Methods Aggregometry and flow cytometry for platelet function studies. Targeting enrichment of a large gene panel followed by sequencing on a MiSeq. SeqPilot (JSI), Alamut® Visual, in silico pathogenicity prediction for data analysis. Sanger sequencing for segregation analysis.

Results Aggregometry revealed impaired platelet function after ADP stimulation. Flow cytometry showed severely reduced δ-granule secretion. Using NGS we diagnosed three young boys with HPS1 who have an increased risk to develop pulmonary fibrosis later on. So far, only very few HPS7 patients have been reported. We identified a 59 year old female with a lifelong history of bleeding symptoms to suffer from HPS7. In a girl who did not show any sign of cutaneous albinism, however, displayed ocular albinism likely pathogenic variants in the HPS3-gene were identified. The HPS1 and HPS3 patients had compound heterozygous pathogenic/likely pathogenic variants (3 novel). The HPS7 patient showed a novel homozygous exon 6 deletion in DTNBP1 (gene associated with HPS7).

Conclusion Depending on the type and location of the mutation in the HPS genes the phenotype can vary significantly. Therefore, it is possible that patients with no apparent sign of albinism may be overseen and underdiagnosed. Furthermore, patients with oculocutaneuos albinism should be investigated for platelet function defect to prevent severe and life-threatening bleedings in case of surgery, especially in mucocutaneous areas.

OC06-03 In-vivo model to assess platelet age in humans: during increased platelet turnover large and small platelets are produced simultaneously

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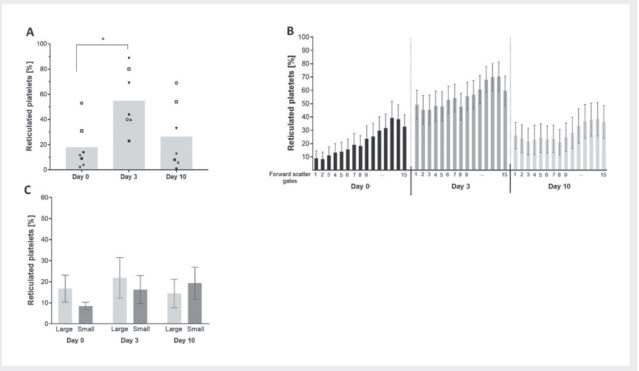
Objective Human platelets vary in size, function, and age. Large platelets are often considered to be young platelets. Two situations have to be distinguished, normal steady state platelet production and increased platelet turnover. Here we focused on large and small platelets in humans during increased platelet turnover. To avoid artefacts by interfering factors (medication, comorbidities), we established a platelet apheresis model to deplete platelets from healthy volunteers with subsequent increased platelet production.

Material and Methods Platelets were isolated from ACD-A anticoagulated blood obtained from healthy donors. Then their platelet count was depleted by platelet apheresis from 227 to 186 Gpt/l median range. At day 3 and 10 after apheresis blood was obtained again. Large and small platelets were assessed by gating using 15 defined forward scatter gates in flow cytometry (gate 1 = smallest; gate 15 = largest) and after separation by differential centrifugation. In flow cytometry platelet activation was measured by CD62P expression and PAC-1 binding and the relative proportion of RNA-positive (reticulated) platelets by Cy5-labeled oligo-dT and oligo-dA.

Results The proportion of reticulated platelets increased on day 3 after platelet apheresis from 18 % to 55 % (p = 0.031, Fig. 1A). Unexpectedly, this was much more pronounced in small platelets. RNA content rose nearly 5 times in smaller platelet fractions (gates 1-5), while the increase in larger platelet fractions (gates 10-15) was only 1.5-2 (Fig. 1B). At day 10 proportion of



▶ **Fig. 1** The proportion of reticulated platelets was increased 3 days after apheresis [A]. The increase was more pronounced and long-lasting in the smaller platelet fractions (gates 1-5) than in the larger platelet fractions (gates 10-15) [B]. Similar effects were observed when large and small platelets were separated by differential centrifugation [C]. Statistical significance was tested by Wilcoxon test (*=p<0.05)

reticulated platelets was still much more increased in small compared to large platelets (Fig. 1B). After separation of large and small platelets by differential centrifugation, the persistence of reticulated platelets in the small platelet fraction until day 10 was confirmed (Fig. 1C). The response to agonists under increased turnover was not different from steady state and differences between large and small platelets remained at the same level.

Conclusion The assumption that in humans large platelets are young platelets has to be revised. During increased platelet turnover the proportion of reticulated platelets increases in small and large platelets, indicating simultaneous synthesis of both subpopulations. Thereby, the RNA-content as marker for young platelets, increases much more pronounced and persists longer in small platelets compared to large platelets.

OC06-04 Assessment of primary hemostasis with an acoustic biosensor using shear dependent kinetics behavior: principle and limitations

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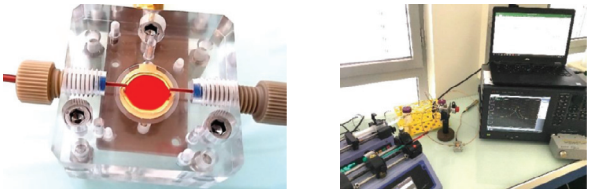
Objective Primary hemostasis involves in-flow interactions between platelets and sub-endothelial matrix at the wall of the damaged vessel. Assessing primary hemostasis defects would benefit from evaluation of the whole sequence of processes involved in platelet plug formation. We propose a novel label-free approach based on characterization of shear-dependent kinetics to evaluate the early stages of primary hemostasis. We developed a quartz crystal microbalance (QCM) biosensor to measure the amount of platelet deposited over time. With experiments and numerical simulations, we investigated the relevance of this approach and its limitations.

Material and Methods We designed and built an acoustic biosensor based on a QCM whose gold surface was functionalized with Horm® collagen and used as the floor of a microfluidic chamber. We recorded with an impedance analyzer the variations of the QCM sensor resonance frequency during a 5-minutes perfusion through the chamber with anticoagulated whole blood from two healthy donors. The real-time QCM measurements performed at 500 - 1500/s range shear rate were supplemented with atomic force microcopy (AFM) observation at the end of the perfusion to evaluate the final morphology of the deposit and the surface coverage. Numerical simulations were used to understand the influence of deposit topology on the acoustic response.

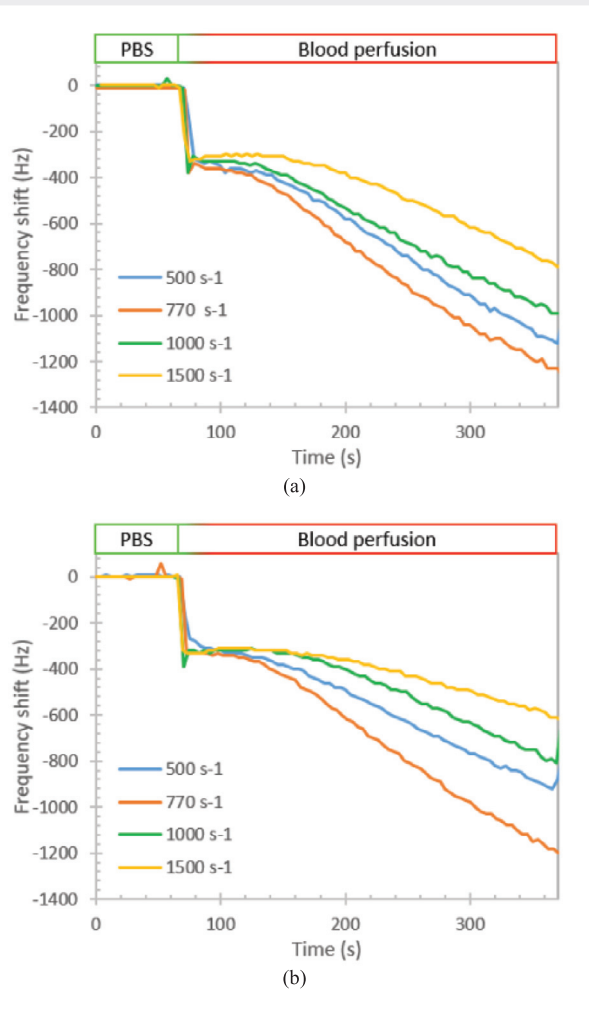
Results For analyzing the complex kinetics profile of the frequency shift, we defined three metrics: total frequency shift, lag time, and growth rate. These metrics enabled the characterization of the kinetics of platelet deposition with good repeatability. We showed that these parameters measured at different shear rates, gave precise indications on the processes involved in the early stage of primary hemostasis, opening the way to analyze abnormal behavior.

However we observed that the frequency shift was not always a direct measure of the platelet amount and depends on the surface topology of the deposit, which varies with the shear rate. The numerical simulation confirmed that if a platelet deposits is modeled as a structured viscoelastic load, the surface coverage affects the frequency shift of the sensor.

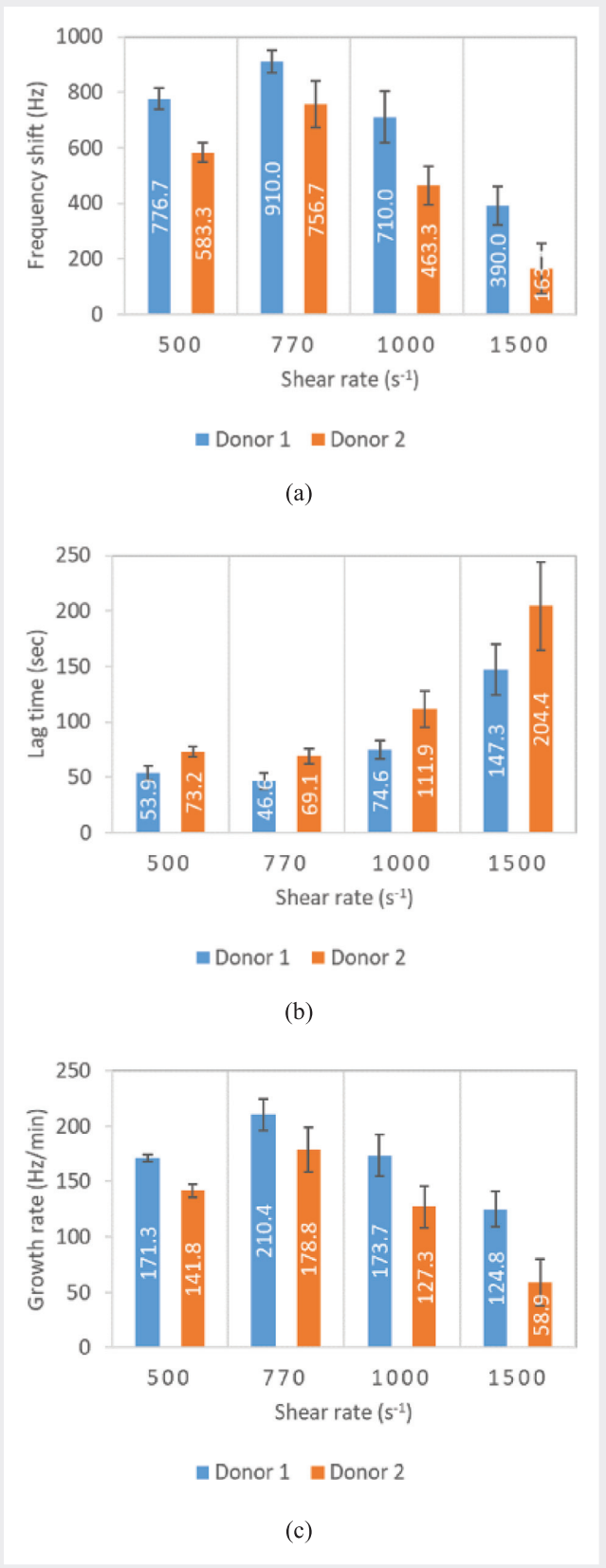
Conclusion Shear-dependent kinetics assays seems to be a promising method for studying primary hemostasis and its defects. We showed that QCM sensor measurements have to be combined with a precise evaluation of deposit topology to be fully usable.



▶**Fig. 1** Photograph of the whole blood perfusion chamber with an installed QCM biosensor (a) and the experimental setup (b)



▶**Fig. 2** QCM sensor admittance frequency shift (Hz) recorded during the blood perfusion during five minutes for two donors (donor1 (a) and donor2 (b)) at a shear rate of 500s-1 (blue), 770s-1 (orange), 1000s-1 (green) and 1500s-1 (yellow).



▶**Fig. 3** Bar charts of the three defined metrics as a function of shear rates for two healthy donors: TFS (a); lag time (b); growth rate (c). Error bars show the absolute deviation from the mean value at the same shear rate the tests were made for the same donor on three different days.

OC06-05 Aptamer-based ex vivo monitoring of thrombin and activated protein C generation on blood outgrowth endothelial cells for individualized assessment of the protein C pathway

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Objective The endothelium plays a crucial role in hemostasis and its regulation, as is demonstrated by the thrombotic risk in disorders affecting the endothelial protein C (PC) pathway, such as PC deficiency and the factor V Leiden (FVL) mutation. Blood outgrowth endothelial cells (BOECs) are endothelial progenitor cells with endothelial character, but a high proliferative capacity. Aim of this study was to develop an assay to monitor the generation of thrombin and activated protein C (APC) in human plasma on BOECs and demonstrate its applicability to assess the PC pathway on an individual basis using cells originating from FVL carriers.

Material and Methods Isolation and characterization of BOECs was performed following standard protocols. All data were obtained at least in triplicate using cells in passage 7 cultured on 24-well plates. First, the APC generation assay was established in a buffer system, to which thrombin, PC, and thrombomodulin (as a substitute for the cells in the control approach) were added. Thrombin formation in defibrinated, recalcified, citrated plasma was induced by 1 pM of tissue factor. During a follow up period of 60 minutes the concentration of thrombin and APC in buffer or plasma on the BOECs was monitored using oligonucleotide-based enzyme capture assays (OECAs).

Results In buffer containing thrombin and PC (1 and 100 nM final concentration, respectively) the peak APC concentration after 60 minutes was 0.034 ±0.001 nM without cells and 0.124±0.001 nM on BOECs from healthy controls. In normal pooled plasma, the APC peak concentration was about 10fold higher on BOECs than without cells (1.02±0.062 versus 0.110±0.004 nM). Conversely, the thrombin peak concentration without cells (47.2±2.8 nM) was higher than on BOECs (27.1±2.8 nM), indicating the endothelial anticoagulant effect. In plasma of three FVL carriers on their individual cells, both thrombin and APC peak levels were higher than in the pooled healthy controls, with 44.6±17.6 and 1.78±0.09 nM, respectively.

Conclusion The observed increased thrombin and APC generation rates on BOECs from FVL carriers, a finding that is consistent with previously obtained in vivo data, demonstrate the applicability of the presented approach to assess the functionality of the PC pathway on an individualized basis. The obtained data warrants further studies in a larger population including patients with other disorders affecting the PC pathway.

OC06-06 Human platelets labeled at two discrete biotin densities are functional in vitro and are detected in vivo in the mouse circulation. A promising approach to monitor in vivo platelet survival in clinical research

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DOI 10.1055/s-0041-1728111

Objective The production of platelet concentrates (PCs) is evolving and their survival capacity needs in vivo evaluation. This requires that the transfused platelets (PLTs) be distinguished from those of the recipient. Labeling at various biotin (Bio) densities allows one to concurrently trace multiple PLT populations, as reported for red blood cells. A method is described to label human PLTs at two discrete densities of biotin for future clinical trials.

Material and Methods Injectable-grade PLTs were prepared in a sterile environment, using injectable-grade buffers and GMP-grade Sulfo-NHS-Biotin. Sulfo-NHS-Biotin concentrations were chosen to maintain PLT integrity and morphology, to

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avoid potential alloimmunization while enabling the detection of circulating BioPLTs in a severe immuno-deficient mouse model, using ex vivo flow cytometry. The impact of biotinylation on human PLT survival was evaluated in vivo in the mouse.
Results BioPLTs labeled with 1.2 or 10 microg/mL Sulfo-NHS-Biotin, displayed normal ultrastructure, retained aggregation and secretion capacity and normal expression of the main glycoproteins. The procedure avoided detrimental PLT activation or apotosis signals. Transfused human BioPLT populations in the mouse circulation could be distinguished from one another and from unlabeled circulating mouse PLTs and their survival was comparable to that of unlabeled human PLTs.
Conclusion Provided low Sulfo-NHS-Biotin concentrations (≤10 microg/mL) are used, injectable-grade BioPLTs are compliant with safety regulations, conserve PLT integrity and permit accurate in vivo detection. This alternative to radioisotopes, which allows one to follow different platelet populations in the same volunteer should be valuable to assess new PC preparations and to monitor platelet survival in clinical research.

Mechanisms of Disease I

OC07-01 Factor Xa and factor IIa inhibitors differentially regulate inflammation in myocardial ischemia reperfusion injury

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DOI 10.1055/s-0041-1728112
Objective While thrombin is the key protease in regard to thrombus formation, other coagulation proteases, such as fXa or activated protein C (aPC), independently modulate intracellular signaling via partially disjunct receptors. Hence, we postulate that inhibition of fXa or fIIa conveys different effects in regard to inflammation in myocardial ischemie-reperfusion injury (IRI), despite comparable anticoagulant efficacy.
Material and Methods Mice were treated with direct fIIa inhibitor (fIIai; dabigatran) or direct fXa inhibitor (fXai; rivaroxaban) at doses conveying a comparable anticoagulant effect in vivo (tail bleeding assay and FeCl3-induced thrombosis). Myocardial IRI was induced via LAD ligation. We determined volumes of infarct size and in vivo aPC generation and conducted gene-expression using an unbiased approach, immunoblotting and ELISA. The signaling-only 3K3A aPC variant and inhibitory antibodies blocking all or only the anticoagulant function of aPC were used to determine the role of aPC for differential effects between fIIai and fXai.
Results Doses of fIIai and fXai conveying comparable an anticoagulant effect in vivo were established and used subsequently in animals challenged with myocardial IRI. fIIai and fXai resulted in a comparable reduction of infarct size. However, unbiased gene-expression analyses revealed marked differences, including pathways related to sterile inflammation and inflammasome regulation. fXai, but not fIIai, conveyed anti-inflammatory effects, hallmarked by reduced expression of proinflammatory cytokines and reduced NF-κB and inflammasome activation. The anti-inflammatory effect observed with fXai reduced myocardial fibrosis 28 days post myocardial IRI. Mechanistically, in vivo generation of cytoprotective aPC was higher with fXai as compared to fIIai. Inhibition of aPC`s anticoagulant and signaling properties abolished the anti-inflammatory effect associated with fXai, while inhibiting only aPC's anticoagulant function spared the fXai-associated anti-inflammatory effect. Concurrently, 3K3A-aPC in addition to fIIai reduced the inflammatory response, mimicking the fXai-associated effect.
Conclusion Here we show that specific inhibition of coagulation via DOACs has differential effects in regard to gene-expression and inflammation, despite a comparable anticoagulant effect and infarct size.

Factor Xa and factor IIa inhibitors differentially regulate inflammation in myocardial ischemia reperfusion injury

Background and aims: While thrombin is the key protease in regard to thrombus formation, other coagulation proteases, such as fXa or activated protein C (aPC), independently modulate intracellular signaling via partially disjunct receptors. Hence, we postulate that inhibition of fXa or fIIa conveys different effects in regard to inflammation in myocardial ischemie-reperfusion injury (IRI), despite comparable anticoagulant efficacy.
Methods: Mice were treated with direct fIIa inhibitor (fIIai; dabigatran) or direct fXa inhibitor (fXai; rivaroxaban) at doses conveying a comparable anticoagulant effect *in vivo* (based on tail bleeding assay and $FeCl_3$-induced thrombosis). Myocardial IRI was induced *via* LAD ligation. We determined volumes of infarct size, atherosclerotic lesion and in vivo aPC generation and conducted gene-expression using an unbiased approach, immunoblotting and ELISA. The signaling-only 3K3A aPC variant and inhibitory antibodies blocking all or only the anticoagulant function of aPC were used to determine the role of aPC for differential effects between fIIai and fXai.
Results: Doses of fIIai and fXai conveying comparable an anticoagulant effect *in vivo* were established and used subsequently in animals challenged with myocardial IRI. fIIai and fXai resulted in a comparable reduction of infarct size. However, unbiased gene-expression analyses revealed marked differences, including pathways related to sterile inflammation and inflammasome regulation. fXai, but not fIIai, conveyed anti-inflammatory effects, hallmarked by reduced expression of proinflammatory cytokines (IL-1β, IL-6, TNFα) and reduced NF-κB and inflammasome activation. The anti-inflammatory effect observed with fXai reduced myocardial fibrosis 28 days post myocardial IRI. Mechanistically, in vivo generation of cytoprotective aPC was higher with fXai as compared to fIIai. Inhibition of aPC`s anticoagulant and signaling properties abolished the anti-inflammatory effect associated with fXai, while inhibiting only aPC's anticoagulant function spared the fXai-associated anti-inflammatory effect. Concurrently, 3K3A-aPC in addition to fIIai reduced the inflammatory response, mimicking the fXai-associated effect.
Conclusion: Here we show that specific inhibition of coagulation via DOACs has differential effects in regard to gene-expression and inflammation, despite a comparable anticoagulant effect and infarct size. aPC generation is enhanced in fXai treated mice, conveying anti-inflammatory and cytoprotective effects. Hence, targeting individual coagulation proteases conveys specific effects related to coagulation protease signaling, but unrelated to the anticoagulant effect per se.

►**Abb 1.**

OC07-02 von Willebrand factor (vWF) significantly modifies the sedimentation profile of FVIII-containing immune complexes (FVIII-IC) based on the type of anti-FVIII antibodies

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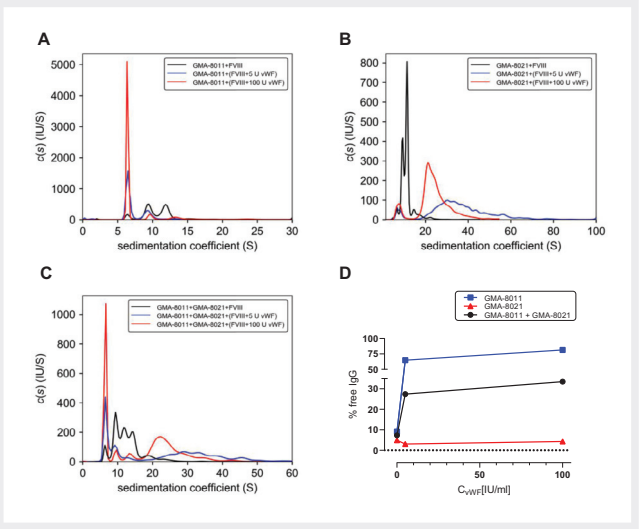
DOI 10.1055/s-0041-1728113
Objective Formation of anti-FVIII antibodies (inhibitors, Inh) is a severe complication of FVIII substitution therapy in haemophilia A. Inh can bind to FVIII thereby forming FVIII-IC with possibly harmful consequences like enhanced FVIII uptake by antigen-presenting cells in ongoing anti-FVIII immune reaction. There is a controversial debate about potential immuno-protective effects of vWF in regard to Inh development, but data about vWF impact on subsequent FVIII-IC formation are not available. This study is focused on the vWF influence on FVIII-IC characteristics using analytical ultracentrifugation (AUC).
Material and Methods Monoclonal IgG antibodies (mAb) GMA-8011 and GMA-8021 recognizing the C1 and A2 domain of FVIII, respectively, were used to generate FVIII-IC. 5 or 100 IU/ml of plasma-derived vWF were added to 100 IU/ml of recombinant human FVIII (rhFVIII) followed by incubation with 10 µg/ml Alexa Fluor 488 labeled mAb in order to investigate the impact of vWF on FVIII-IC formation. Samples were characterized using AUC with fluorescence detection (AU-FDS, Aviv Biomedical; ProteomeLab XL-I, Beckman Coulter) at 30,000 rpm and 20 °C. Sedimentation velocity data were analyzed with a diffusion-deconvoluted differential sedimentation coefficient distribution model, c(s), in the program SEDFIT.
Results rhFVIII incubated with GMA-8011 formed complexes with sedimentation coefficients of 9.5 and 11.9 S (Fig. A, black trace). vWF substantially decreased FVIII-IC generation and increased the amount of free mAb at 5 and 100 IU/ml, consistent with the competitive binding of vWF and mAb to rhFVIII (Fig. A and D). In contrast, vWF did not interfere with FVIII-IC formation in the presence of GMA-8021 (Fig. B and D). Instead, faster sedimenting



▶ **Abb 1.**

complexes (FSC) appeared, suggesting that FVIII-IC increased in size due to additional binding of vWF. GMA-8011 and GMA-8021 together, as a model of more complex immune reactions, resulted in FVIII-IC of heterogeneous size that were also substantially changed by the addition of vWF, as indicated by increased amounts of free mAb and FSC (Fig. C and D).

Conclusion AUC allows studying FVIII-IC formation. vWF can interfere with FVIII-IC formation by preventing the interaction of some antibodies due to competitive binding or by increasing the total immune complex size. Variable effects can be expected in patients depending on anti-FVIII epitope distribution.

OC07-03 Characterization of activity and cleavage of von Willebrand disease type 2B variants

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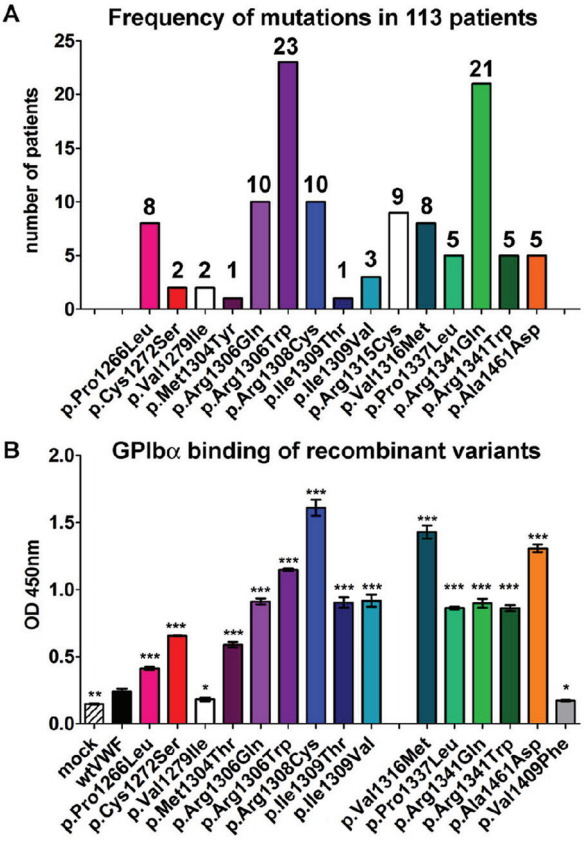
DOI 10.1055/s-0041-1728114

Objective Von Willebrand disease type 2B (VWD2B) is a bleeding disorder of primary hemostasis, characterized by variable lack of von Willebrand factor (VWF) high molecular weight multimers (HMWM) and varying degree of thrombocytopenia. VWD2B is caused by gain-of-function (GOF) mutations in the VWF A1 domain inducing spontaneous binding to platelets. Objective of this study was the identification of the underlying mutations in patients with suspected VWD2B and functional characterization of the respective variants.

Material and Methods VWF exon 28 was sequenced in patient DNA samples. To circumvent the requirement of fresh platelet-rich plasma for the Ristocetin Induced Platelet Aggregation (RIPA), we used a modified VWF:GPIbα binding ELISA employing a recombinant GPIbα peptide as capture component to determine GPIbα binding of plasmatic and recombinant variants. Degradation of the variants by ADAMTS13 was measured employing a modified light transmission aggregometry (LTA) assay.

Results By genetic analysis of 113 patients with suspected VWD2B, we found 15 different mutations (Figure 1A). p.Arg1315Cys could be excluded from the investigation of GPIbα binding as it has previously been characterized as VWD2 M. All variants, except p.Val1279Ile, exhibited significantly increased GPIbα binding (Figure 1B). Thereby, diagnosis of VWD2B was confirmed for 13 mutations. We further characterized those sixteen patients, in more detail, for whom complete clinical data and VWF multimers were available and found a correlation between degree of increased GPIbα binding and loss of HMWM for some mutations. Since the latter could be caused by increased cleavage of the variants in vivo, we employed a modified LTA assay to investigate degradation of 2BVWF-platelet-complexes by ADAMTS13. Counterintuitively, we found that some variants show decreased sensitivity for proteolytic cleavage under flow conditions.

Conclusion Summarizing, we characterized VWD2B variants found in a VWD2B patient cohort. The used ELISA proved to be applicable to differentiate 2B variants from other types of VWD and the absence of patient platelets prevents false positive results due to platelet type-VWD. Additionally, our data indicate that increased proteolysis of some variants does not arise from enhanced degradation of circulating 2BVWF-platelet-complexes. Our data could increase understanding of VWD2B disease phenotypes.



▶ **Fig. 1** (A) Distribution of mutations identified in suspected VWD2B patients and (B) GPIbα binding of the recombinant variants.

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OC07-04 Impact of osteopontin on platelet adhesion and activation in intraluminal thrombus formation and abdominal aortic aneurysm progression

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Objective Abdominal aortic aneurysm (AAA) is frequently accompanied by the formation of an intraluminal thrombus (ILT) that is found in more than 75 % of all AAAs. However, it is still not known how the ILT develops and how it affects the progression of AAA. One protein of interest for developing such a thrombus could be osteopontin (OPN). It is already shown that OPN in serum of AAA patients is increased. Moreover, OPN can be cleaved by enzymes like thrombin which plays a pivotal role in thrombus formation. OPN has an arginine-glycine-aspartic acid (RGD) motif and is able to bind to integrins which are abundant on the platelets surface. Currently, the influence of full length and cleaved OPN in platelet adhesion and activation is only partially understood.

Material and Methods Analysis of platelet activation and adhesion after stimulation with OPN and its fragments were performed. Histological sections of the vessel wall from AAA patients were stained for N - and C - terminal OPN.

Results Platelet activation with different agonist showed no alteration in integrin □IIb□3 (fibrinogen receptor) activation or degranulation after preincubation with full length or cleaved OPN. Furthermore, platelet adhesion was performed on immobilized full length and cleaved OPN coated surfaces. After stimulation with ADP (adenosine diphosphate) or CRP (collagen related peptide) platelets showed an increased adhesion on full length and cleaved OPN. The inhibition of integrin □IIb□3 with tirofiban or eptifibatid resulted in increased platelet adhesion on OPN. However, inhibition of integrin □v□3 (vitronectin receptor) on platelets showed decreased but not fully abrogated platelet adhesion on OPN. Adhesion experiments on collagen coated surfaces were performed to investigate the influence of soluble OPN. After platelets were preincubated with soluble full length and cleaved OPN they showed no alteration in adhesion. In addition, stained histological sections showed more N – than C – terminal OPN in the vessel wall of AAA patients.

Conclusion Taken together, platelet activation is not affected by OPN. However, OPN plays an important role in platelet adhesion mediated –at least in part- by integrin □v□3. This mechanism might be of great relevance for platelet adhesion into the ILT and migration of platelets into the vessel wall that all could affect ILT formation and AAA progression.

Pediatrics & Gynecology-Obstetrics

OC08-01 Differences in the coagulation profile in women with mild and severe eclampsia

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Objective Coagulation abnormalities have been reported in early onset preeclampsia (EOP). However, their association with disease severity is yet unclear. To address this question, we assessed biomarkers of hypercoagulability in women with mild and severe preeclampsia.

Material and Methods This is an observational retrospective case–control study. Plasma samples were collected from 84 women divided into three groups: healthy pregnant (HP; n = 35), mild preeclampsia (MP; n = 34) and severe preeclampsia (SP; n = 15). The study population general characteristics are shown in Table 1. The following biomarkers were assessed: Tissue factor activity (TFa), Procoagulant phospholipid activity (PPL), Protein S, D-Dimers, Antithrombin (AT), thrombomodulin, TFPI levels, aPTT, PT and fibrinogen

Results TFPI, TFa and TMa levels significantly increased in MP and SP as compared to HP. No significant difference of TFPI, TFa was observed between MP and SP. TMa levels were significantly increased in SP as compared to MP. TFa/TFPI ratio was also lower in SP as compared to MP. Women in MP or SP had similarly shorter PPL as compared to HP. D-Dimer levels were significantly higher in SP as compared to MP. The levels of free PS activity in MP was significantly lower than that of the HP or SP. Fibrinogen levels were not significantly different in the three studied groups. PT was increased in MP and SP compared to the HP. The mean PT was found to increase with increasing

►**Tab 1.** General characteristics of study population

	HP	MP	SP
Number,(n)	35	34	15
Age (years)	31.2±3.1	30.2±2.3	32±1.1
Gestational age, (weeks)	32.2±2	31.1±3	30.2±1
BMI (kg/m²)	24.8±4.1	25.2±2.1	26.3±1.1
Blood pressure (mmHg) - Systolic - Diastolic	 133±8 72±7	 148±11** 91±6**	 178±11ᴱᴱ 126±9ᴱᴱ
Hematology - Hemoglobin (g/l) - Hematocrit (%) - Platelet count (x10⁹/l)	 11.8±1.2 35.2±2.6 227±41	 12.4±14* 36.3±3.5 230±52	 11.0±18 34.4±3.6 105±32ᴱᴱ
Nulliparous (n)	30	28	6
Multiparous (n)	5	6	2

*P<0.05 HP versus MP; **P<0.001 HP versus MP
ᴱP<0.05 HP versus SP; ᴱᴱP<0.001 HP versus SP

►**Tab 2.** Plasma markers of coagulation, in normal pregnancy, and in mild and severe preeclampsia

	TFPI (ng/ml)	PS (%)	TFa (pM)	TMa (%)	PPL (sec.)	D-Di (µg/ml)
HP (n=35)	9.0±1.89	56.62±6.31	0.26±0.04	104.46±21.69	58.4±7.34	0.90±0.36
MP (n=34)	12.65±2.48**	49.06±7.93**	0.42±0.08*	212.24±54.87**	51.88±8.65**	1.10±0.31
SP (n=15)	13.80±1.52**	51.07±5.62*ᴱᴱ	0.44±0.06**	261.87±35.58**ᴱ	52.00±5.61*	1.37±0.37**ᴱ

	Ratio TMa/TFa	Ratio TFa/TFPI	Fg (g/l)	PT (s)	aPTT (s)	ATIII (%)
HP (n=35)	422.73±123.24	0.029±0.007	3.71±0.53	12.23±0.59	29.53±1.62	95.4±0.36
MP (n=34)	528.42±179.07**	0.034±0.009*	4.23±0.50	13.24±0.80*	32.64±1.83**	88.06±9.68**
SP (n=15)	604.57±82.21**	0.032±0.006*ᴱ	3.76±0.43ᴱ	14.77±0.96**ᴱᴱ	35.59±1.53**ᴱᴱ	76.33±4.32**ᴱᴱ

** P<0.001 HP versus preeclampsia
* P<0.01 HP versus preeclampsia
£ P<0.05 MP versus SP
ᴱᴱ P<0.001 MP versus SP

severity of disease ($p < 0.001$). The aPTT was increased along with increasing disease severity ($p < 0.001$). The AT levels decreased in severe SP and MP or compared to HP (76.33 ± 4.32 and 88.06±9.68 versus 95.40±0.36 respectively; $p < 0.001$). This decrease was more pronounced in SP compared to MP ($p < 0.001$).

Conclusion Preeclampsia is associated with endothelial cell activation as documented by the increase of TFa, soluble TM levels and TFPI levels in plasma. Release of soluble TM and TFPI rather than TFa by endothelial cells appear to be related with the degree of preeclampsia severity. Women with preeclampsia showed marked decrease of PPL clotting time indicating enhanced platelet activation. In contrast, women with SP showed signs of enhanced hypercoagulability documented by the increase of D-dimer levels consumption of natural coagulation inhibitors and particularly of AT. This phenomenon tended to be reflected on the prolongation of PT and aPTT in severe preeclampsia.

OC08-02 Acquired von Willebrand Syndrom (avWS) Typ 2 in children with severe pulmonary arterial hypertension (PAH)

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Objective In the last few years there has been found some evidence of an increased degradation of von Willebrand factor and a decrease in high molecular weight multimers in patients with pulmonary arterial hypertension (PAH). One of the first reported cases (Sokkary et al. 2011) was that of a young woman with PAH and menorrhagia suffering from avWS type 2. But apart from this case, very little data has been reported so far. In 2018, a small study was published of 8 children with PAH, all of whom had avWS type 1. Meaning, all multimers were found in all cases. (Pelland-Marcotte et al. 2018).

Material and Methods Based on these available publications we decided to analyse von Willebrand parameters (at least: vWF:Ag, vWF:Ak, multimers) in all patients with severe PAH evaluated for lung transplantation starting in spring 2018.

Results From May 2018 to October 2020 we evaluated 10 children with severe PAH for lung transplantation. Von Willebrand parameters were determined in 8 of these patients. 7 of 8 (87.5 %) patients showed a decrease or loss of high molecular weight multimers, which is typical of avWS type 2. Five of these 7 patients (71 %) had an Ak:Ag ratio <0.8. In the remaining 2 patients the Ak:Ag ratio was 1.4 and 1.6 respectively. However, in these patients a sample error may be considered. The patient with normal von Willebrand multimers showed borderline low vWF:Ak and vWF:Ag, an increased PFA-100 of >300 seconds and pathological aggregation. This patient therefore suffered at least from a platelet dysfunction and possibly also from a mild von Willebrand syndrome type 1.

Conclusion Overall, all children with PAH suffered from a coagulation disorder. Most patients had acquired von Willebrand syndrome type 2. Platelet dysfunction could be another possible coagulopathy that occurs in patients with severe PAH. A ratio of vWF:Ak/vWF:Ag <0.8 and a prolongation of PFA-100 could be early indications of avWS type 2. For all these patients, we recommend an analysis of von Willebrand parameters including multimer analysis, PFA-100 and platelet function testing.

OC08-03 Human Fibrinogen Concentrate for Bleeding Prophylaxis During Surgery in Paediatric Patients with Congenital Fibrinogen Deficiency

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Objective Congenital fibrinogen deficiency (CFD) is a rare disorder which predisposes patients to thromboembolic complications, both with and without fibrinogen supplementation. Treatment with human fibrinogen concentrate (HFC) can prevent blood loss in surgical treatment in patients with CFD. Herein we report data from paediatric patients receiving HFC as surgical prophylaxis during two Phase 3 studies.

Material and Methods Patients were enrolled in two multinational, multicentre, prospective, open-label, uncontrolled Phase 3 clinical trials (FORMA-02 and FORMA-04) for the use of HFC in adult and paediatric patients with CFD. Efficacy of HFC (Fibryga® Octapharma) in surgical prophylaxis was assessed at the end of surgery by the surgeon and post-operatively by the haematologist using a four-point objective scale (Excellent, Good, Moderate, None), and by an Independent Data Monitoring & Endpoint Adjudication Committee (IDMEAC).

Results Haemostatic efficacy of HFC in the prevention of bleeding during and after surgery was assessed in four patients (FORMA-02 [n = 1, 12 years old], and FORMA-04 [n = 3, <6 years old]) for a total of four surgeries, one of which was major (splenectomy) and three were minor (circumcision, pulpectomy for two teeth, and extraction of tooth). The median total dose per surgery was 85.8 mg/kg for the FORMA-02 patient and 108 mg/kg for the three FORMA-04 patients. Only one infusion was required for each of the three minor surgeries. The patient undergoing major surgery received a total of six infusions. For all surgeries, overall intra-operative and post-operative haemostatic efficacy was rated 100 % excellent by both the investigator and IDMEAC. In vivo recovery (IVR) for the loading dose for the FORMA-02 patient

Tab 1. Efficacy assessments after HFC treatment

Efficacy rating	Investigator	IDMEAC
Bleeding events: Haemostatic efficacy		
4-Point Efficacy Scale	N (%)	N (%)
Excellent	77 (77.8)	89 (89.9)
Good	17 (17.2)	9 (9.1)
Moderate	2 (2.0)	1 (1.0)
None	3 (3.0)*	0 (0.0)
Surgical prophylaxis: Intra-operative efficacy		
4-Point Efficacy Scale	N (%)	N (%)
Excellent	14 (93.3)	14 (93.3)
Good	1 (6.7)	1 (6.7)
Moderate	0 (0.0)	0 (0.0)
None	0 (0.0)	0 (0.0)
Surgical prophylaxis: Post-operative efficacy		
4-Point Efficacy Scale	N (%)	N (%)
Excellent	15 (100.0)	14 (93.3)
Good	0 (0.0)	1 (0.0)
Moderate	0 (0.0)	0 (0.0)
None	0 (0.0)	0 (0.0)

* For three patients, haemostatic efficacy assessment was missing. Thus, as per the statistical plan, the rating by the investigator was considered as 'None'.
HFC, human fibrinogen concentrate; IDMEAC, Independent Data Monitoring and Endpoint Adjudication Committee; N, number of bleeds or surgeries.

was 0.82 mg/dL/(mg/kg). Mean (±SD) IVR value for the loading dose of the first infusion for each of the three surgeries for the FORMA-04 patients was 1.3 mg/dL/[mg/kg] (±0.22) with a median (range) of 1.3 mg/dL/[mg/kg] (1.0–1.4). No allergic/hypersensitivity reactions or deaths were observed.

Conclusion HFC administration for bleeding prophylaxis during surgery was efficacious for this ultra-rare disease in a paediatric population with congenital afibrinogenemia and showed a favourable safety profile.

OC08-04 The Bacteria-Associated Thrombosis, Thrombophlebitis and LEmierre syndrome (BATTLE) registry: background and rationale.

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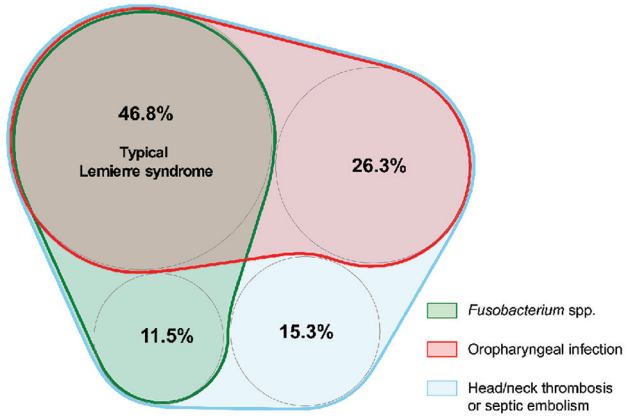
Objective Bacteria-associated septic thrombophlebitis and especially its head/neck form, Lemierre syndrome, have been known for over one century but are still associated with considerable fatality and long-term sequelae in children and young adults. Yet, available evidence is limited to case reports or series and is therefore fragmented, susceptible to bias, and inadequate to guide clinical management. To support the development of guidelines and consensus statements, and ultimately improve the care of patients with bacterial thrombophlebitis, we designed the Bacteria-Associated Thrombosis, Thrombophlebitis and LEmierre syndrome (BATTLE) registry.

Material and Methods The BATTLE registry was specifically planned to (i) fill the gaps in amount and quality of evidence on the continuum of Lemierre syndrome (Figure) and bacterial thrombophlebitis by addressing reporting, detection and case-mix bias, and (ii) comply to current international guidelines on quality and standardization of patient registries, including the FAIR framework and the European Commission Joint Research Centre's recommendations.

Results The BATTLE registry is an independent, investigator-initiated, noninterventionist, multicenter, international, ambispective study. Inclusion criteria are (1) clinically diagnosed or microbiologically documented invasive bacterial infection, (2) history or objective diagnosis of primary infection focus in the head/neck or abdominopelvic district, and (3) thrombosis in location anatomically adjacent to (or embolism consistent with) the primary infection focus (Table). Data entry will be electronic only through the website www.battle-registry.org. The electronic case report form includes the European Commission's set of common data elements for Rare Disease Registration and covers patient history, clinical course until death or discharge, and long-term follow-up including assessment of functional status and quality of life. The registry has an academic sponsor and its establishment is covered by the Marco Brockhaus award, assigned by the Gesellschaft für Thrombose- und Hämostaseforschung. Ethical approval and registration on ClinicalTrials.gov are pending. Data collection will start in early 2021.

Conclusion The innovative features of the BATTLE registry may effectively contribute to supporting clinical management and research priorities for patients with a group of rare and severe acute thromboembolic disorders.



Proportional area chart of the distribution of the traditional criteria for Lemierre syndrome in 712 cases described from 2000 to 2017. All patients had a primary head/neck infection and head/neck thrombosis or septic embolism, but only about half satisfied the typical definition of *Fusobacterium* spp.-associated, post-anginal thrombosis or septic embolism.

Tab 1. General inclusion criteria of the BATTLE registry

Bacteria-associated thrombophlebitis	Lemierre syndrome subgroup
1. Clinically diagnosed or microbiologically documented invasive bacterial infection	1. Clinically diagnosed or microbiologically documented invasive bacterial infection
2. History or objective diagnosis of primary infectious focus in the head/neck or the abdominopelvic district	2. History or objective diagnosis of primary infectious focus in head/neck district
3. Objective diagnosis of thrombosis in location anatomically adjacent to the primary infection focus and/or septic embolism in typical location	2. Objective diagnosis of thrombosis of the head/neck district or embolism in location anatomically consistent with the primary infection focus in the head/neck district

Fig. 1 The Bacteria-Associated Thrombosis, Thrombophlebitis and LEmierre syndrome (BATTLE) registry: background and rationale – tables and graphs. Figure: Spectrum of Lemierre syndrome in cases described from 2000 to 2017. Table: General inclusion criteria of the BATTLE registry.

Megakaryocytes, Platelets & VWF

OC09-01 Light-induced ion influx triggers megakaryocyte polarization

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Objective Bone marrow megakaryocytes (MKs) extend proplatelets into sinusoidal blood vessels where these proplatelets undergo fission to release platelets. The molecular mechanisms that regulate megakaryocyte differentiation, polarization and proplatelet formation are only poorly understood. Here, we expressed an optogenetic construct in primary MKs, which allows us to study these complex processes by spatiotemporally controlling cellular activity using light. A widely used optogenetic construct is Channelrhodopsin2 (ChR2), which is a blue light-activatable, non-selective cation channel from the green alga Chlamydomonas reinhardtii. We modified ChR2 to obtain higher calcium conductance and named the protein ChR2 XXM2.0. We expressed ChR2 XXM2.0 in MKs to manipulate calcium signaling by light in a high spatiotemporal manner in order to better understand the influence of changes in intracellular Ca2+ levels on MK function.

Material and Methods ChR2 XXM2.0 was expressed in bone marrow-derived MKs after virus transduction. Whole cell patch-clamp was used to test the functionality of the channel. MKs were globally or locally illuminated and subsequent MK behavior was analyzed by light and fluorescence microscopy.

Results ChR2 XXM2.0 localized in the plasma membrane and the demarcation membrane system in MKs. Application of blue light induced a significant photocurrent in ChR2 XXM2.0 expressing MKs, which indicates cation influx into MKs. Increased intracellular calcium levels were observed with a Cal 590

sensor in ChR2 XXM2.0 positive MKs after illumination. Global illumination of ChR2 XXM2.0 expressing MKs resulted in a blebbing behavior and exposure of phosphatidylserine on the cell surface. Incubation with the apoptotic inhibitor, Q-VD-OPh, decreased the percentage of MK blebbing from 61 % to 9 %, indicating an apoptotic process. Local illumination of ChR2 XXM2.0 positive MKs spread on fibrinogen triggered increased stress fiber formation, cell polarization and motility towards the direction of light.

Conclusion Here, we established for the first time optogenetics in bone marrow-derived MKs. We observed cell polarization after local illumination of ChR2 XXM2.0 positive MKs due to a local increase of ion influx. Currently, we are expressing ChR2 XXM2.0 in genetically modified MKs to identify key proteins and mechanisms involved in calcium regulated MK polarization.

OC09-02 Antibodies protect platelet damage by pneumolysin

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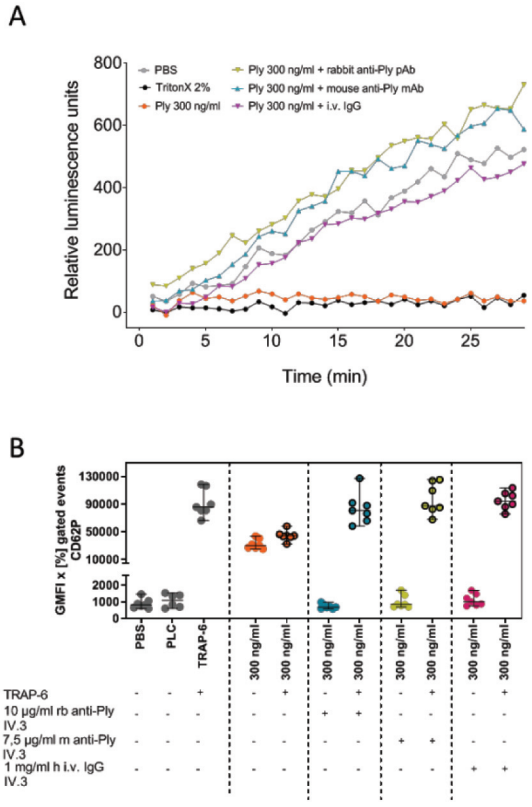
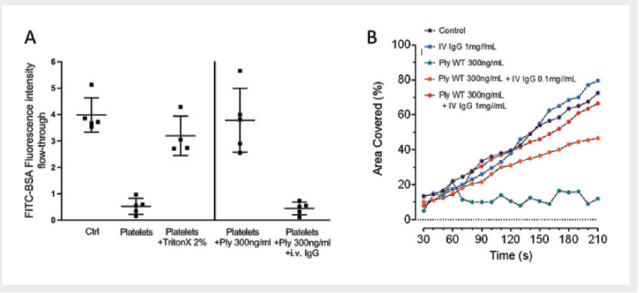
Objective Community-acquired pneumonia is one of the most common acute infections and a leading cause of morbidity and mortality and mostly caused by Streptococcus pneumoniae. The pore-forming pneumococcal toxin pneumolysin renders platelets non-functional and prevents them from covering endothelial cell lesions. This leads to extravasation of fluid and respiratory impairment. We aim to investigate whether antibodies protect platelets from damage by pneumolysin.



▶ **Abb 1.**



▶ **Abb 2.**

Material and Methods Platelets were isolated from healthy donors (n≥3) and incubated with 300 ng/mL pneumolysin in the absence or presence of a polyclonal rabbit-anti-pneumolysin-antibody, a monoclonal mouse-anti-pneumolysin-antibody (abcam) and human derived pharmaceutical i. v.IgG. We assessed the impact of neutralized pneumolysin on platelet viability using RealTime-Glo cell viability kit and on platelet activation by measuring cytosolic Ca2+ and CD62P expression. Subsequent TRAP-6-stimulation (20µM final) was performed to assess remaining platelet functionality. Adhesion and pore-sealing capacity was tested in a Boyden-chamber-assay and thrombus formation on collagen-surfaces was measured in a flow chamber.

Results Both, specific antibodies as well as i. v.IgG, protect platelets from pneumolysin-induced cell death (Fig. 1A). The antibodies also prevent increase of free cytosolic Ca2+ and CD62P-expression induced otherwise by pneumolysin (Fig. 1B). In the presence of pneumolysin and antibodies, platelets remained sensitive to subsequent TRAP-6 stimulation (Fig. 1B). Platelets are able to adhere to Boyden-chamber membranes and seal pores in the presence of antibodies, which is otherwise severely affected by pneumolysin (Fig. 2A). Addition of i. v.IgG also preserved thrombus formation in whole blood (Fig. 2B).

Conclusion Specific antibodies as well as i. v.IgG can protect platelets against pneumolysin-induced perforation and preserve platelet functionality. This indicates that antibody treatment might be able to prevent acute respiratory distress syndrome in pneumococcal infections by preserving platelet function and prevention of capillary leakage.

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OC09-03 Low adhesion and interaction forces of Myh9 mutant platelets lead to impaired clot retraction and unstable thrombus formation

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Objective Once a vessel is injured, platelets adhere and prevent blood loss. The protein non-muscle myosin heavy chain IIA, encoded by the MYH9 gene, is a contractile protein, which binds to filamentous actin and generates forces in cells. Heterozygous defects in the human gene lead to different autosomal dominant syndromes, which are characterized among others by macrothrombocytopenia and a mild to moderate bleeding tendency. We

hypothesized that reduced platelet force generation is responsible for the increased bleeding risk in MYH9 patients.

Material and Methods To investigate this, we analyzed mouse lines each with one point mutation in the Myh9 gene at the positions 702, 1424, or 1841. These mouse lines have been described to recapitulate defects found in patients. We characterized the basic platelet function using flow cytometric, immunoblotting, clot retraction and flow chamber assays. Further, we tested the biophysical properties of the mutant platelets with real-time deformability cytometry, atomic force spectroscopy, and microposts arrays.

Results Myh9 mutant mice displayed a macrothrombocytopenia, but only slightly altered glycoprotein expression. Activation of αIIbβ3 integrin and P-selectin surface exposure of mutant platelets were comparable to controls in response to all tested agonists. Mutant platelets were stiffer, showed more F-actin content, but the G- to F-actin ratio was unaltered. The capacity to assemble actin after activation was reduced in Myh9 mutant platelets. In addition, phosphorylation of the myosin light chain after activation with thrombin was strongly reduced. In line with this, biophysical analysis revealed that Myh9 mutant platelets generate lower adhesion forces to collagen, lower interaction forces between platelets and reduced traction forces when spread on fibrinogen-coated microposts. Clot retraction of mutant samples was delayed and less effective, further reflecting less force generation of mutant platelets. Finally, we observed less contracted and more instable thrombi, when blood of Myh9 mutant mice was perfused ex vivo over collagen fibers.

Conclusion We can show that Myh9 mutant platelets generate and interact at lower forces. These data suggest that reduced platelet-substrate and platelet-platelet forces lead to the increased bleeding tendency found in MYH9 patients. We are currently testing multiple drugs to stabilize the clot in order to prevent bleeding.

OC09-04 Platelets from patients with MYH9 related disorders are mechanically stiffer

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DOI 10.1055/s-0041-1728123

Objective The MYH9 gene encodes for the heavy chain of non-muscle myosin IIA (NMMHC-IIA), which is involved in a variety of cellular processes that require biomechanical contractile force generation. Variants of MYH9 cause autosomal dominant MYH9-related disease (MYH9-RD) and are characterized by thrombocytopenia with giant platelets and mild to moderate bleeding tendency. Although the role of the cytoskeleton for platelet function is well known, the consequences of NMMHC-IIA mutations on platelet biomechanics are only poorly understood. To investigate this, high throughput functional mechanophenotyping was performed by real-time fluorescence and deformability cytometry (RT-FDC).

Material and Methods Using RT-FDC we analysed intrinsic biomechanics of single platelets from MYH9-RD patients with point mutations in the MYH9 gene that lead to following amino acid substitutions in NMMHC-IIA at positions p.D1424N and p.E1841K. For functional mechanophenotyping by RT-FDC, platelets were labelled with the platelet marker integrin β3 (CD61-PE) and with the platelet activation markers integrin αIIbβ3 (PAC1-FITC) and P-selectin (CD62P-AlexaFluor 647) while TRAP-6 was used as PAR1 agonist. Platelet F-actin content was determined by phalloidin-Atto 647 binding in flow cytometry.

Results Platelets from MYH9-RD patients showed two fold lower deformation (i. e. stiffer platelets) and were larger (p.D1424N: median deformation 0.0685, median size 9.63 µm2, n = 790 platelets and p.E1841K: 0.0686, 5.77 µm2, n = 955 platelets) than platelets from healthy individual (median deformation 0.1434, size 3.76 µm2, n = 1334 platelets). Upon TRAP-6 activation, platelets from healthy individuals became highly stiffer (Fold change: 2.87 fold, n = 962 single platelets) while platelets from MYH9-RD patients became only marginally stiffer (p.D1424N: 2.27 fold increase in stiffness compared to non-activated and E1841K: 1.9 fold increase in stiffness compared to non-activated). Quantification of basal F-actin content in non-stimulated MYH9-RD platelets was found to be two-fold higher than in the control platelets. With TRAP-6 activation, the F-actin level in control platelets increased more than in MYH9-RD platelets.

Conclusion We demonstrate that platelets from MYH9-RD, individuals with mutations in the MYH9 gene (p.D1424N and p.E1841K), are biomechanically stiffer with higher F-actin content than platelets from healthy individuals.

OC09-05 Epinephrine enhances platelet aggregation at the expense of procoagulant activity

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DOI 10.1055/s-0041-1728124

Objective Platelet activation is characterized by shape change, granule secretion, activation of fibrinogen receptor (glycoprotein [GP] IIb/IIIa) sustaining platelet aggregation, and externalization of negatively-charged aminophospholipids contributing to platelet procoagulant activity. Epinephrine alone is a weak platelet activator. However, it is able to potentiate platelet activation initiated by other agonists. Here, we investigated the role of epinephrine in the generation of procoagulant platelets.

Material and Methods Human platelets were activated with convulxin (CVX, the agonist of the collagen receptor GPVI), thrombin (THR) or Thrombin Receptor Activator for Peptide 6 (TRAP6, the agonist of the thrombin receptor PAR1), epinephrine (EPI), and combination thereof. Platelet aggregation was assessed by light transmission aggregometry or with PAC-1 binding by flow cytometry. Procoagulant COAT platelets, induced by combined activation with CVX-plus-THR, were visualized by flow cytometry as Annexin-V-positive and PAC-1-negative platelets. Cytosolic calcium fluxes were monitored by flow cytometry using Fluo-3 indicator.

Results EPI increased platelet aggregation induced by low doses of CVX alone, TRAP6 alone, and combined CVX+TRAP6. On the other hand, EPI dose-dependently reduced the formation of procoagulant COAT platelets generated by combined CVX+THR activation. We observed significant decrease (relative -10 %, $p<0.05$) of Annexin-V positivity and increase (relative +10 %, $p<0.05$) of PAC-1 binding with the triple activation (CVX+THR+EPI) compared with CVX+THR. Calcium mobilization with triple activation was decreased with the higher dose (1000 µM) compared with CVX+THR calcium kinetics.

Conclusion While CVX+THR induced the formation of procoagulant COAT platelets (express negatively charged phospholipids at their surface, and lose aggregatory properties), the addition of EPI (triple stimulation) modulated platelet activation reducing cytosolic calcium mobilization, decreasing the procoagulant response and enhancing platelet aggregation.

OC09-06 Phosphorylation and activation of Pannexin-1 upon platelet activation and thrombus formation

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Objective Pannexin-1 (Panx1) is a single membrane channel, which functions as an ion channel for small molecules. Recently, it was shown that platelet activation induces opening of Panx1 channels, amplifies [Ca2+]int and induces platelet aggregation. Panx1-deficient platelets show defects in

hemostasis in in vitro and in vivo. We aim to study Panx1 activation mechanisms in platelets to identify the impact on hemostasis and thrombosis in further detail.

Material and Methods Analysis of activation patterns of Panx1 phosphorylation after platelet activation, Panx1 inhibition by the specific inhibitor Probenecid and ex vivo analysis of thrombus formation on collagen under flow.

Results Western blot analysis demonstrated increased phosphorylation of tyrosine residue 198 (Tyr198) of Panx1 following platelet activation with classical platelet agonists. Tyrosine phosphorylation was shown to be fully dependent on Src - and partially dependent on PKC kinases in human platelets. Moreover, murine Panx1-deficient platelets display reduced Src phosphorylation, which goes ahead with the results in human platelets. Interestingly, ADP does not phosphorylate Panx1 at Tyr198 but at Tyr308; another well-known phosphorylation site of Panx1. Here, Src kinases are only partially involved in phosphorylating Tyr308, which indicates other activation mechanisms of Panx1 channels. Blockage of Panx1 with Probenecid led to reduced [ATP]ex after platelet activation with collagen-related peptide (CRP), Par4 peptide and thromboxane, but not with ADP. Under flow conditions, the inhibition of Panx1 leads to reduced thrombus formation on collagen at low (450 s-1) and moderate (1000 s-1) arterial shear rates ex vivo. Moreover, initial experiments provided evidence that low as well as high agonist activation of platelets lead to extracellular cleavage of Panx1 channels in a time dependent manner, which is known to irreversibly open the channel in inflammatory cells.

Conclusion Panx1 channels function as ATP-release channels in platelets to support arterial thrombus formation. Panx1 activation is regulated by phosphorylation at different tyrosine residues following platelet activation. These results suggest an important role of Panx1 in hemostasis by releasing extracellular ATP to enable cell communication and to support thrombus formation.

COVID-19

OC10-01 Derivation and Validation of a Predictive Score for Disease Worsening in Patients with COVID-19

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Objective The prospective observational study conducted at the COVID-19 center of Tenon University Hospital in Paris aimed to develop a risk assessment model (RAM) for early identification of COVID-19 patients at risk for worsening disease.

Material and Methods Patients with confirmed COVID-19 (n = 420) were devided in derivation (n = 300) and validation (n = 120) cohorts and stratified to those hospitalized at the conventional ward (C-group) and those with worsening disease (W-group). The study end point was disease worsening. All patients were routinely evaluated with full blood count, prothrombin time (PT), fibrinogen, D-Dimers, antithrombin and protein C activity. Data from the first hospitalization day at the conventional ward or the ICU were analyzed.

Results Obesity, hypertention, diabetes and male gender, increased fibrinogen and D-Dimers, thrombocytopenia, AT-deficiency, lymphopenia and compensated DIC-ISTH score ≥5 were significant risk factors for worsening disease. The COMPASS-COVID-19 risk assessment model (RAM) derived from multivariate analysis includes: obesity, gender, compensated DIC, hemoglobin, and lymphocyte count. The score has a very good discriminating capacity to stratify patients at high and low risk for worsening disease, with AUC value at 0.77, sensitivity 81 % and specificity 60 %. Application of the COMPASS-COVID-19 score at the validation cohort showed 96 % sensitivity and 45 % specificity.

Conclusion The COMPASS-COVID-19 score could serve as an accurate clinical decision tool for an easy identification of 80 % COVID-19 patients at risk for disease worsening leading to prompt application of compassionate treatments including antithrombotic agents.

OC10-02 Prothrombotic alterations of von Willebrand factor level and ADAMTS13 activity in hospitalized COVID-19 patients

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Objective Based on our current knowledge, the severity of the COVID-19 disease is associated with the dysregulation of inflammation and haemostasis. Endothelial cells play a central role in regulating both processes.

Our aim was to determine the VWF antigen (Ag) level, the ADAMTS13 activity (Ac) and their ratio in samples of COVID-19 patients, and to analyze their associations with disease severity, thromboembolic complications and laboratory parameters associated with COVID-19 severity.

Material and Methods Between 20th of April and 2nd of June 2020, 128 PCR-positive COVID-19 patients were included in our observational clinical study. Patients were stratified according to the severity at the time of sampling. The following groups were defined: (1) outpatient (n = 26), (2) hospitalized, not requiring oxygen support (n = 31), (3) hospitalized, receiving oxygen therapy (n = 36), (4) critical, requiring intensive care (n = 35). VWF:Ag level was determined by ELISA; ADAMTS13:Ac was determined by a FRET assay. Non-parametric statistical tests were used for statistical analysis.

Results VWF:Ag levels were significantly elevated in all groups of hospitalized patients (median values: 196 %, 270 % and 383 % in groups 2, 3 and 4, respectively). ADAMTS13:Ac was decreased in patients requiring oxygen support or intensive therapy (median 75 % and 50 % in groups 3 and 4, respectively). Consequently, the VWF:Ag/ADAMTS13:Ac ratio was increased in all hospitalized patients, the rate of increase correlated with disease severity (median 1.97, 3.71 and 10.73 in groups 2, 3 and 4, respectively). The VWF:Ag level and VWF:Ag/ADAMTS13:Ac ratio were higher in patients with thromboembolic complications (median 390 % vs. 213 % and 6.0 vs. 2.3, respectively). We found a number of significant correlations between the above parameters and those related to the pathophysiology or severity of the COVID-19 disease: CRP, PCT, IL-6, neutrophil/lymphocyte ratio, RDW, D-dimer and markers of complement activation.

Conclusion Our results show that VWF:Ag, ADAMTS13:Ac and their ratio correlate strongly with disease severity and are associated with an increased risk of thromboembolic complications. Moreover, they are associated with several markers of inflammation, coagulation and complement activation, which may indicate a potential key role of endothelial cells in the pathogenesis of immunothrombosis in COVID-19.

OC10-03 Platelet activation and apoptosis in COVID-19 infection: data from an observational study

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Objective Accumulating evidence indicates an association between SARS-CoV2 pneumonia (COVID-19) and thromboembolic complications. The pathophysiology of the COVID-19 associated-thromboembolic events seem to be complex and multifactorial, involving interplay between cellular and plasmatic elements of the hemostatic system. In this study, we hypothesized that thrombus formation in COVID-19 is accompanied by platelet activation and apoptosis with subsequent alteration of the coagulation system.

Material and Methods 21 COVID-19 positive patients who were admitted to the intensive care unit (ICU) were included. Blood samples were analyzed for platelet activation and apoptosis markers, respectively. Flow cytometer (FC) was used to investigate expression of P-selectin, activated glycoprotein (GP) IIb/IIIa, depolarization of mitochondrial inner transmembrane potential (ΔΨm), intracellular calcium (Ca2+) concentration and phosphatidylserine (PS) externalization.

Results Platelets from COVID-19 positive ICU patients (n = 21) showed significantly higher expression of P-selectin (2.27±0.45 vs. 1.27±0.55, p= 0.0279) compared to healthy donors. In addition, higher ΔΨm depolarization (1.39 ±0.07 vs. 0.99±0.06, p = 0.0005), Ca2+ concentration (2.73±0.31 vs. 1.00 ±0.05, p<0.0001) and PS externalization (2.05±0.48 vs. 0.86±0.11, p = 0.0236) were observed. Most importantly, PS exposure was associated with the SOFA score (sequential organ failure assessment, r = 0.5635, p = 0.0078) and plasma levels of D-Dimer (r = 0.4473, p = 0.0420). Finally, patients with thromboembolic events had higher PS externalization compared to those with no thrombosis (2.85±0.75 vs. 0.99±0.20, p = 0.0340).

Conclusion Our study shows that COVID-19 positive ICU patients had increased platelet activation and signs of apoptosis. The strong correlations between platelet apoptosis and D-Dimer as well as the incidence of thromboembolic complications may indicate that platelet apoptosis potentially contributes to sustained increased thromboembolic risk in COVID-19 positive ICU patients.

Covid-19

OC10-04 Incidence of Thrombosis and Associated Risk Factors in Hospitalized COVID-19 Patients in a New York City Hospital System

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Objective New York City (NYC) became the first epicenter of the 2019 novel coronavirus disease (COVID-19) in the United States. Factors upon admission associated with the development of thrombosis in hospitalized COVID-19 patients are less well defined. Our aim is to characterize the incidence of thrombosis and the associated clinical and

►**Tab 1.** Demographic and clinical characteristics of hospitalized patients by incidence of thrombotic events.

	Total (n=1,352)	Overall Thrombosis* (n=160)	No Thrombosis (n=1,192)	p-value
Age, Years, Median (IQR)	62 (49-72)	62 (52-71)	62 (49-73)	0.701
Female Gender, n (%)	455 (33.7)	36 (22.5)	419 (35.2)	0.001
BMI, kg/m², Median (IQR)	28.7 (25.4-33.7)	28.1 (25.1-32.2)	28.8 (25.5-33.8)	0.174
Race, n (%)				0.026
White	618 (45.7)	71 (44.4)	547 (45.9)	
Black	145 (10.7)	13 (8.1)	132 (11.1)	
Asian or Pacific Islander	118 (8.7)	24 (15)	94 (7.9)	
Other or Mixed race	390 (28.9)	40 (25)	350 (29.3)	
Unknown or not reported	81 (6.0)	12 (7.5)	69 (5.8)	
Ethnicity, n (%)				0.244
Hispanic	301 (22.3)	29 (18.1)	272 (22.8)	
Non-Hispanic	956 (70.7)	116 (72.5)	840 (70.5)	
Unknown or not reported	95 (7.0)	15 (9.4)	80 (6.7)	
Smoking Status, n (%)				0.693
Current	89 (6.6)	8 (5)	81 (6.8)	
Former	271 (20.0)	35 (21.9)	236 (19.8)	
Never	803 (59.4)	92 (57.5)	711 (59.6)	
Unknown	189 (13.4)	25 (15.6)	164 (13.8)	
Admission Labs, Median (IQR)				
D-dimer (ng/mL)	361 (235-600)	476 (330-902)	347 (225-575)	<0.001
ESR (mm/hr)	81 (57-110)	86 (71-108)	79 (53-110)	0.019
CRP (mg/L)	100 (45-160)	141 (76-196)	94 (43-152)	<0.001
Ferritin (ng/mL)	636 (238-1406)	1053 (479-1916)	597 (218-1308)	<0.001
Platelets (1,000/μL)	191 (151-244)	204 (153-259)	190 (150-242)	0.125
Troponin I (ng/mL)	0.03 (0.01-0.06)	0.04 (0.01-0.09)	0.03 (0.01-0.06)	0.072
Comorbidities, n (%)				
Cancer	164 (12.1)	26 (16.3)	138 (11.6)	0.089
Coronary Artery Disease	192 (14.2)	22 (13.8)	170 (14.3)	0.862
Atrial Fibrillation	28 (2.1)	6 (3.8)	22 (1.8)	0.112
COPD	96 (7.1)	6 (3.8)	90 (7.6)	0.079
Asthma	159 (11.8)	18 (11.3)	141 (11.8)	0.831
CVA	29 (2.1)	1 (0.6)	28 (2.3)	0.242
Seizure	31 (2.3)	3 (1.9)	28 (2.3)	1.000
Hypertension	722 (53.4)	86 (53.8)	636 (53.4)	0.925
Diabetes	461 (34.1)	65 (40.6)	396 (33.2)	0.064
Anticoagulation Prior to Admission, n (%)				<0.001
Warfarin	12 (0.9)	2 (1.3)	10 (0.8)	
DOAC	89 (6.6)	28 (17.5)	61 (5.1)	
Mortality, n (%)	357 (26.4)	56 (35)	301 (25.3)	0.009

Abbreviations – BMI: Body Mass Index; COPD – Chronic Obstructive Pulmonary Disease; CVA – Cerebrovascular Accident; ESR – Erythrocyte Sedimentation Rate; CRP – C-reactive Protein; DOAC – Direct Oral Anticoagulant; IQR – Interquartile Range

*Overall Thrombosis includes venous and/or arterial thrombotic events: pulmonary embolism (PE), venous thromboembolism (DVT), CVA, myocardial infarction (MI), acute limb ischemia, and splenic infarct, among others

demographic risk factors of patients hospitalized across a NYC hospital system.

Material and Methods We conducted a retrospective observational study of all patients, age ≥ 18, hospitalized with a reverse transcriptase-polymerase chain reaction confirming severe acute respiratory syndrome coronavirus 2 infection between March 13 and April 4, 2020 in two hospitals in NYC. Clinical demographics, admission labs and medications prior to admission were collected. Thrombotic events were identified manually by chart review and were defined as experiencing arterial and/or venous thrombotic events.

Results 1,352 patients were hospitalized during the study period. Overall median age was 62 years (IQR:49-72), with 455 females (33.7%). There were 160 (11.8%) thrombotic events, including 102 with venous thromboembolism, 45 with pulmonary embolism, 69 with deep vein thrombosis, 32 with cerebrovascular accident and 55 with other thrombotic events (e.g. myocardial infarction, acute limb ischemia, splenic infarct). Females were 46% less likely than males to experience a thrombotic event (OR:0.54[CI:0.36-0.79]). Patients who racially self-identify as Asian or Pacific Islander were observed to have a 2.06 odds compared to other races of having a thrombotic event with COVID-19 (95%[CI:1.27-3.34]). Traditional risk factors including age, admission BMI, ethnicity, smoking status, and comorbidities were not associated with the incidence of thrombosis during hospitalization. Thrombotic events were associated with higher mortality in hospitalized COVID-19 patients (35% vs 25.3%, p=0.009).

Conclusion Traditional risk factors were not associated with an increased risk for thrombotic events in COVID-19 patients, while inflammatory marker values on admission were significantly different, highlighting the impact of the cytokine storm in mediating thrombotic events. Since the incidence of COVID-19-associated thrombosis may vary according to clinical demographics, further investigation to identify high risk patients may enable us to consider the role of adjunctive treatment, such as therapeutic coagulation.

Crosstalks between Hemostasis and other Systems

OC11-01 LMWH prevents platelet and extracellular vesicle mediated thrombo-inflammation

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Objective Low molecular weight heparins (LMWH) are the most commonly used therapeutics for thromboembolic events during pregnancy. However their role in PE remains controversial, but studies suggest an overall protective effect. . It has been proposed that although LMWHs have potent anti-coagulant functions, they may convey their beneficial effects independent of their anti-coagulant activity. We have recently shown that EVs and platelets promote trophoblast inflammasome activation in PE. We therefore hypothesized that LMWH convey anti-inflammatory effects in PE by preventing NLRP3 inflammasome activation in trophoblast cells.

Material and Methods Procoagulant EVs were injected into C57/Bl6 pregnant mice. LWMH was injected s.c. 30 minutes before EV injections and embryonic survival and growth restriction (IUGR) was studied. Trophoblast differentiation and trophoblast proliferation was studied using qRT-PCR and Ki-67 staining respectively. Inflammasome markers NLRP3 and IL-1β were evaluated using immunoblotting. Differentiated mouse trophoblast stem cells were treated with EVs and LWMH to study the protective effect of LWMH on inflammasome activation and proliferation. Analysis of human PE placentae explants treated with LMWH and trophoblast cells established translational relevance.

Results LMWH rescues the EV-injected pregnant mice from embryonic death and IUGR. LWMH prevented EV induced trophoblast inflammasome activation, restored altered trophoblast differentiation and improved proliferation in vivo and in vitro. Mechanistically, LWMH was able to activate HB-EGF signaling pathway resulting in PI3-Kinase-Akt signaling in trophoblast cells in order to prevent inflammasome activation. In human PE placental explants, inflammasome activation and PI3-Kinase-Akt signaling events were restored upon treatment with LMWH.

Conclusion These results establish a protective effect of LMWH in preventing EV induced inflammasome activation and reduced trophoblast function, which contributes to the placental defect and embryonic demise in PE. These effects are mediated by HB-EGF-PI3-Kinase-Akt signaling axis. LWMH is a safe therapy in placental defects associated with excess platelet activation and placental inflammasome activation, as observed in PE.

OC11-02 Platelet-derived chemokines regulating neutrophil activation stages in arterial thrombus formation

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Objective Platelets and neutrophils are among the first cells to appear at the site of thrombus formation and contribute to the pathology of thrombotic events. Platelet-neutrophil interactions are mediated through multiple receptor-ligand interactions and chemokines. However, it remains unknown how these interactions arise and are regulated at the site of a growing thrombus. We aimed to unravel the underlying mechanisms in the setting of arterial thrombus formation.

Material and Methods In vitro thrombus formation was induced by perfusion of human whole blood over collagen at arterial shear in a parallel plate flow chamber. Neutrophil adhesion, movement, Ca^2+-fluxes and surface activation markers were assessed in real-time by fluorescence microscopy, as well as the activation status of the platelets in the thrombi (PS exposure, CD62P and CD63).

Results Platelets in consolidated thrombi attract neutrophils depending on shear conditions. Neutrophil adhesion was mediated through a panel of receptor interactions. Following adhesion, the neutrophils stained positively for activation markers including CD11b and produced reactive oxygen species. Markedly, even after prolonged time, thrombi-interacting neutrophils did not form neutrophil extracellular traps (NETs), despite their activation state. About 65 % of thrombus-interacting neutrophils displayed repetitive intracellular Ca^2+ spiking, which was partly related to their movement at and around thrombi. Suppressing platelet activation by post-treatment of the thrombus with the prostacyclin analogue iloprost severely reduced the Ca^2+ spikes in neutrophils, suggesting a continuous release of neutrophil-activating substances by the platelets. Inhibition of the platelet-derived chemokines CXCL7 (NAP-2), CXCL4 (PF4) or CCL5 (RANTES) resulted in decreased Ca^2+-fluxes in the neutrophils without affecting their adhesive behaviour. In addition, stimulation of isolated neutrophils with CXCL7, CXCL4

and/or CCL5 induced specific patterns of Ca^2+-fluxes. On the other hand, the agonist thrombin only caused Ca^2+ responses in neutrophils in the presence of platelets.

Conclusion Neutrophil-activating effects of platelets in a thrombus are mediated by the continuous release of at least three chemokines. Together, our results suggest a central role of G protein-coupled chemokine receptors in regulating neutrophil activation and Ca^2+ signaling during arterial thrombus formation.

OC11-03 Severity of Transfusion-Related Acute Lung Injury in mice expressing human FcgRIIA is determined by platelet-released serotonin

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Objective Transfusion-related acute lung injury (TRALI) remains a major cause of transfusion-related fatalities. TRALI can occur after transfusion of blood products containing allogeneic antibodies targeting cells of the recipient. The involvement of the human Fcg receptors in this type of TRALI has been poorly assessed. FcgRIIA/CD32A is an activating low affinity receptor for immunoglobulin G (IgG) expressed on human platelets and immune cells, whereas no corresponding ortholog is present in mice. Therefore, since the current murine models incompletely recapitulate the human immunology, our understanding of the pathogenesis of antibody-mediated TRALI is partial. Our study aimed at evaluating the contribution of FcgRIIA/CD32A in a model of antibody-mediated TRALI in transgenic mice expressing this human receptor.

Material and Methods Antibody-mediated TRALI was induced in control mice (WT) and mice transgenic for human CD32A (CD32A+) by lipopolysaccharide priming followed by administration of a recombinant chimeric human/mouse anti-MHC I monoclonal antibody (derived from clone 34-1-2S).

Results TRALI responses were more severe in CD32A+ than in WT mice in terms of pulmonary edema and mortality, the latter reaching 90% in CD32A+ versus 40% in WT animals within 2-hours. Unlike in WT mice, monocytes/macrophages were only accessory for the initiation of TRALI in CD32A+ mice, pointing to the decisive contribution of another cell type. Soon after induction of TRALI, platelet counts dropped more dramatically in CD32A+ than in WT mice. Moreover, massive release of platelet granule contents was observed only in the peripheral blood of CD32A+ animals. Platelet depletion prevented the exacerbation of TRALI observed in CD32A+ mice but did not affect TRALI in the WT. Long-term treatment with the selective serotonin reuptake inhibitor fluoxetine, to deplete the serotonin content of platelet granules, selectively abolished the aggravation of lung edema in CD32A+ mice. This effect could also be achieved by blockade of the 5-hydroxytryptamine 2A serotonin receptor with sarpogrelate when the drug was administered before or after the induction of TRALI.

Conclusion Our findings identify platelet FcgRIIA/CD32A as a critical determinant of the severity of TRALI and provide a rationale for designing prophylactic and therapeutic strategies targeting the serotonin pathway to attenuate antibody-mediated TRALI in patients.

OC11-04 The contribution of bile acid transport and endoplasmatic reticulum stress to tissue factor activation in hepatocytes

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Objective Fibrin deposition in the liver parenchyma contributes to cholestatic liver injury, but the origin of tissue factor activity leading to thrombin generation has not been fully elucidated. We discovered that long-time exposure of viable HepG2 cells to unconjugated bile acids results in tissue factor decryption with a strong correlation to FXR-activation. HepG2 cells do not express sodium taurocholate cotransporting polypeptide (NTCP), which regulates hepatocellular uptake of bile acids. We hypothesized this to be the reason, why no effect was observed with conjugated bile acids. Endoplasmatic reticulum stress (ER-stress) might be the link between prolonged FXR overstimulation and tissue factor decryption. Hence, we wanted to test the effect of conjugated bile acids as well as ER-stress on tissue factor decryption in primary hepatocytes.

Material and Methods Primary hepatocytes from healthy donors exposed to chenodeoxycholic acid (CDCA) and glycochenodeoxycholic acid (GCDCA) in concentrations of 0-200 µM for 24 hours. Furthermore, cells were exposed to the ER-stressor tunicamycin in concentrations of 0-60 µM for 24 hours. Tissue factor activity was measured via conversion of Factor X to Factor Xa in the presence of Factor VIIa and calcium. Generated Factor Xa was determined by measuring the conversion rate of a chromogenic substrate. Cell viability was determined with the MTT assay.

Results Tissue factor activity showed a dose-dependent increase with a lower threshold in primary hepatocytes (>50 µM) than in HepG2 cells (>200 µM). In contrast to HepG2 cells, GCDCA in primary hepatocytes exhibited a higher potential to induce tissue factor activity than CDCA (200µM GCDCA: 31.7 ±6.9 pM FXa/min; 200 µM CDCA: 15.2±4.7 pM FXa/min; P<0.001). Tunicamycin dose-dependently induced tissue factor activity with a threshold of 20 µM (60 µM tunicamycin: 503 ± 62% of vehicle). Viability of cells was unimpaired with all employed concentrations.

Conclusion Expression of NTCP in primary hepatocytes facilitates FXR-overstimulation resulting in tissue factor decryption at lower bile acid concentrations, specifically with conjugated bile acids. ER-stress mediated tissue factor decryption hints to a possible link between prolonged FXR-stimulation and ER-stress. Further studies will have to investigate specifics of this signaling cascade as well as the exact mechanism of tissue factor decryption in hepatocytes.

Diagnostic laboratory: Today & tomorrow

OC12-01 A Comparison of Functional Methods with Absolute Quantification of Heparin Levels in Clinical Samples by Heparin Red Assay

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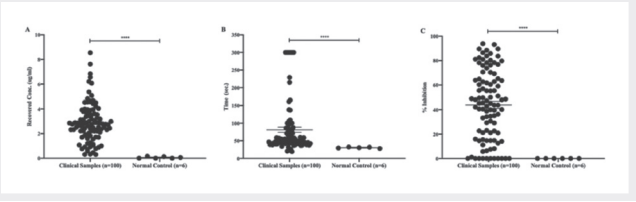
Objective Activated partial thromboplastin time (aPTT) method is widely used as a routine blood test and is commonly used for the monitoring of heparin levels in clinical patient samples. However, many endogenous factors contribute to observed prolongation of this test. Heparin Red assay utilizes fluorescence for the direct and sensitive detection of the absolute level of heparin in plasma. The purpose of this study is to compare functional activities (anticoagulant and anti-Xa levels) in clinical patient samples to the absolute levels of heparin to determine endogenous activity upon the therapeutic administration of this agent.

Material and Methods Plasma samples from patients treated with therapeutic dosage of heparin (n=100) were collected from Loyola University Medical Center. Citrated blood samples were analyzed using aPTT clotting method, anti-Xa chromogenic assay and Heparin Red (Redprobes UG, Deutschland) assay relative to a commercially used heparin (Medefil) calibration curves. Normal controls were comprised of commercially available 25 male and 25 female citrated plasma samples (George King Biomedical, Overland Park,

Kansas City). Results were compiled as mean ± SEM and analyzed for significance and correlation.

Results Marked increases were noted in aPTT (81.11±7.37, normal 30.16 ±0.80 sec.; p<0.001), anti-Xa (43.90±2.83, normal 0±0 % Inhibition; p<0.001), and Heparin Red recovered concentration (2.90±0.16, normal 0.07 ±0.03 ug/ml; p<0.001) in clinical samples compared to normal controls. Although a large scatter in data in all of the assays was noted and shown in Figure 1, significant correlations were observed between Heparin Red and other functional parameters studied.

Conclusion These studies demonstrate that Heparin Red method is a reliable assay for the absolute quantification of circulating heparin level in plasma. Unlike the functional methods, which are also influenced by many endogenous factors, such as AT levels and variations in the clotting proteins, Heparin Red detects absolute amounts of circulating heparin in plasma which are not influenced by endogenous factors and other anticoagulant drugs.



▶**Fig. 1** Observed levels of recovered concentration by Heparin Red (A), aPTT (B) and anti-Xa (C).

OC12-02 Heparinase treatment to remove the inhibitory heparin effect on the thrombin generation assay

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DOI 10.1055/s-0041-1728135

Objective Clinically, reversal of anticoagulation by vitamin K antagonists (VKAs) is obtained by administration of four-factor prothrombin complex concentrates (4F-PCCs). Continuous monitoring of thrombin activity, also known as thrombin generation assay (TGA) is a suitable method for evaluating the hemostatic potency of coagulation factor concentrates in plasma milieu. Commercially available 4F-PCCs contain heparin at different levels, thus TGA results will be biased by the inhibitory effect of heparin and may not fully reflect the clinical efficacy of 4F-PCCs observed. Removal of heparin by anion exchange adsorption has been described but could potentially alter the complex composition of 4F-PCCs, while heparin neutralization with protamine sulphate requires exact titration in order not to alter the hemostatic potency. Here, we describe the use of heparinase I from Flavobacterium heparinum which results in a fast, enzymatic heparin fragmentation associated with the loss of anticoagulant activity.

Material and Methods The heparin-containing 4F-PCC sample (Prothromplex Total, Baxter) was mixed with Heparinase I (Sigma) and incubated for 5 min at room temperature. Then, the hemostatic potential was measured by Technothrombin TGA assay (Technoclone) using the Ceveron® reagents. Normal and VKA anticoagulated plasma was used at a final heparinase I concentration of 1.25 U/mL in the TGA at two trigger levels provided by the TGA reagents RCLow and RCHigh. The optimal heparinase I concentration was determined by investigating a concentration series of heparinase I, ranging from 0.25 to 2.5 U/mL.

Results Heparinase I at a concentration of 1.25 U/mL in the final TGA reaction mix completely removed the inhibitory influence of heparin. At this level, there was only moderate influence on the characteristic TGA readouts lag time, peak thrombin, area under the curve (endogenous thrombin potential) and velocity index for all conditions tested. In contrast, the heparin-containing 4F-PCC concentrate demonstrated dose-dependent TGA response after heparinase I treatment, which was not the case when this treatment was omitted.

Conclusion Enzymatic breakdown of heparin by heparinase I efficiently removed the inhibitory effect of heparin observed during the TGA of 4F-PCCs. Heparinase I treatment can easily introduced in existing TGA test procedures and will increase the significance of data generated with TGA.

OC12-03 Use of a Universal Calibrator for Direct FXa inhibitor DOACs

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Objective Increasing use of direct oral anticoagulants has led to rising numbers of requests for drug level measurement in routine laboratories. Although the same test is used, the anti-Xa assay, individual calibrator and control sets are required for each direct FXa inhibitor (DFXaI). The study was designed to test the usability of a single calibrator set with assigned values for edoxaban, rivaroxaban and apixaban.

Material and Methods A calibrator set composed of normal pooled plasma spiked with different levels of edoxaban was assigned calibrated values for edoxaban, rivaroxaban and apixaban traceable to LC-MS/MS of each DFXaI. On the new Ceveron c100 automated analyzer, TECHNOCHROM® anti-Xa assay was calibrated using the universal calibrator set separately for each DFXaI. For all drugs a high and low range application using different plasma pre-dilutions can be employed. Plasma samples from patients receiving one of the respective drugs were assessed using the appropriate LC-MS/MS method and TECHNOCHROM® anti-Xa assay calibrated for the respective drug.

Results DFXaI-specific calibration curves were established using standard Ceveron c100 applications. As expected, correlation of anti-Xa determined edoxaban to mass spectrometry values for ex-vivo samples was 0.98. For ex-vivo rivaroxaban samples, Passing and Bablok regression between anti-Xa and LC-MS/MS exhibited a slope of 0.95 and intercept of <2 ng/mL (pearson r 0.95). Similarly, the regression for ex-vivo apixaban samples generated a slope of 1.1 and correlation of 0.94.

Conclusion Here we describe a universal calibrator set which can be used to calibrate respective applications for edoxaban, rivaroxaban and apixaban using a chromogenic anti-Xa assay on a routine coagulation analyser. In addition, a similar approach can be used for a universal anti-Xa control set. A universal calibrator and control set for all anti-Xa assays has potential to reduce the cost of DFXaI measurement and improve throughput without compromising quality and traceability.

OC12-04 A universal anti-Xa assay for the determination of rivaroxaban, apixaban, and edoxaban drug levels: development, diagnostic accuracy, and external validation

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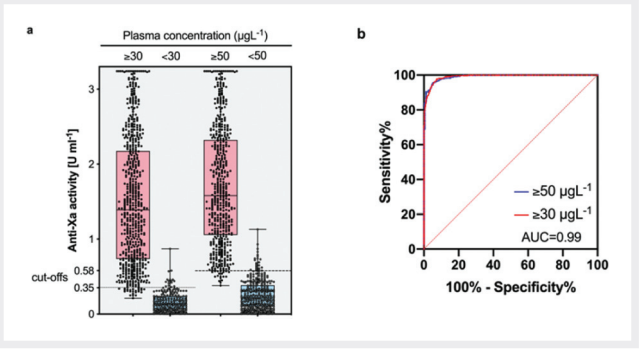
Objective A universal anti-Xa assay for the determination of rivaroxaban, apixaban, and edoxaban drug levels would simplify laboratory procedures and facilitate widespread implementation. We aimed to develop and validate a universal anti-Xa assay to be used in clinical practice.

Material and Methods Following two pilot studies analyzing spiked samples and material from 625 patients, we conducted a prospective multicentre cross-sectional study, including 867 patients treated with rivaroxaban, apixaban, or edoxaban in clinical practice. Anti-Xa activity was measured by a universal assay calibrated to low-molecular weight heparin (LMWH) in addition to ultra-performance liquid chromatography–tandem mass spectrometry (LC-MS/MS). As an external validation, anti-Xa activity was also determined in nine external laboratories.

Results Measurements of the universal anti-Xa assay correlated strongly with rivaroxaban, apixaban, or edoxaban drug levels (rs=0.94, 95% confidence interval, CI, 0.93 to 0.99; area under the receiver operating characteristics curve, AUC, 0.99). The sensitivity with regard to the clinically relevant cut-off levels 30 µg/L and 50 µg/L was 96.2% (95% CI 94.4, 97.4) and 96.4% (94.4, 97.7), respectively (specificity 92.9% and 93.3%). Concordant results were obtained in the external validation study (rs=0.96; AUC 0.98; sensitivity 95.78% and 97.44%; specificity 85.94% and 97.44%).

Conclusion The universal anti-Xa assay based on an LWMH-calibration demonstrated a high accuracy in all phases of the evaluation project. Widespread implementation might simplify laboratory procedures and provide fast and reliable drug measurements in various healthcare settings.



▶**Abb 1.**

OC12-05 Robust and selective chromogenic measurement of factor VIII activity with an antibody-based factor VIII chromogenic assay

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Objective Chromogenic factor VIII (FVIII) activity measurement is essential through the whole life cycle of a coagulation factor concentrate or a gene therapy-based treatment approach. While the presence of endogenous non-human FVIII potentially biases the nonclinical pharmacokinetic study analysis, also certain manufacturing process-related additives can impact the assay performance. In particular, those used for the solvent/detergent viral inactivation process interfere with the assay. Therefore, we developed an antibody-based chromogenic FVIII assay, which facilitates the selective and sensitive activity measurement of human FVIII in the presence of animal plasma. Furthermore, this assay enabled the reliable measurement of FVIII activity even in presence of the solvent/detergent mixture.

Material and Methods Plate-adsorbed murine IgG1 (GMA-8024, Green Mountain Antibodies), binding to the A2 domain of human FVIII, was used. Bound human FVIII was measured with a chromogenic activity assay. A human reference plasma preparation was used to construct the calibration curve. Spike-recovery was carried out in citrated cynomolgus monkey plasma and solvent/detergent mixture. Assay robustness was confirmed by the results of the assay control obtained by three different analysts.

Results The six-point calibration curve ranged from 3.03 to 97.0 mIU/mL with the back-fitted data for a total of 108 curves demonstrating 100 ± 14% agreement to the nominal values. Recovery of spiked human FVIII (about 80 mIU/mL) in citrated cynomolgus monkey plasma was 102.7% and 92.4% for the full length and B domain-deleted preparation, respectively, while native monkey plasma did not show any activity. Relative standard deviations (RSDs) for the mean of the triplicate spikes did not exceed 2.8%. Solvent/detergent solution (1% Triton X-100, 0.3% polysorbate 80 and 0.3% tri-n-butyl phosphate) was shown to have no influence on the assay. Finally, assay robustness was demonstrated by the data obtained for the assay control: 108 tests resulted in an RSD of 10.4% with no statistically significant difference between the results obtained by three analysts.

Conclusion Combining antibody-mediated specific capture of human FVIII and a chromogenic activity assay resulted in the selective and sensitive measurement of human FVIII with no interference by endogenous, non-human FVIII or manufacturing process additives like solvent/detergent solution.

OC12-06 Application of the STG-Bleedscreen assay in clinical routine

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Objective Multiple defects can cause a bleeding diathesis. But often a single factor level is not sufficient to predict the bleeding tendency of the affected patient. Global assays, like the fully automated STG-Bleedscreen, may be better predictors of bleeding events and may help to tailor treatment.

Material and Methods Since January 2020 all patients with suspected or confirmed bleeding diathesis in our center were include in this study (with informed consent). A total of 520 measurements were performed using STG-Bleedscreen on the ST Genesia analyzer (Stago). This includes 64 measurements in samples from patients with hemophilia A, and 19 samples with hemophilia B. Results are expressed as thrombin peak in nanomolar concentration, and normalized with a standard plasma (in %).

Results In average, patients with suspected bleeding diathesis (no hemophilia) showed a normal thrombin generation of 160 ± 100 nM (normalized 107 ± 50 %). Nevertheless, patients with blood type O show significantly reduced thrombin generation accompanied with lower levels of FVIII and VWF.

Patients with hemophilia showed similar reduction of thrombin generation: 51 ± 35 nM or 36 ± 23 % for hemophilia A and 46 ± 13 nM or 31 ± 9 % for hemophilia B. The assay shows a good correlation between factor VIII level and thrombin peak, not enough data could be collected in hemophilia B. In patients receiving products with enhanced half-life, thrombin generation clearly shows the prolonged protective effect.

Conclusion The STG-Bleedscreen assay is useful in monitoring hemophilia and hemophilia treatment. Especially, the assay shows the prolonged activity of enhanced half-life products. In patients with suspected bleeding diathesis, the assay might be able to separate patients with a defect in the plasmatic coagulation system, platelets are not included in the assay.
The STG-Bleedscreen assay is a fully automated assay. It can be easily implemented in daily routine, as it does not require high technical effort. The STG-Bleedscreen shows a similar behavior compared to the manual CAT method.

Mechanisms of Disease II

OC13-01 Upregulated autophagy in antigen-presenting splenic cells hints at promotion of immune-mediated thrombotic thrombocytopenic purpura (iTTP)

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Objective Immune-mediated TTP is a life-threatening autoimmune disorder characterized by abundant occlusion of the microcirculation. iTTP is caused by a severe autoantibody-mediated deficiency of the von Willebrand factor cleaving protease, ADAMTS-13. Emerging evidence points to autophagy as key player driving dysregulated autoimmune responses in various diseases. Considering the crucial role of antigen-presenting cells (APCs) in the presentation of autoantigens, we assessed expression profile of autophagy (ATG)-related genes in iTTP.

Material and Methods We isolated splenic dendritic cells (DC) from one patient carrying the HLA DRB1*11 risk allele and leucocytes from peripheral blood of two healthy donors. Then pan-DCs including plasmacytoid and myeloid DCs were isolated by negative selection followed by FACS sorting. In parallel in vitro differentiated monocyte-derived dendritic cells (Mo-DCs) were cultured from freshly isolated MOs. The expression profile of six essential ATG genes (Beclin-1, Atg5, Atg7, Atg16L1, WIPI1 and LC3B) implicated in autoimmune diseases and the autophagic flux (LC3B expression) were determined by qPCR and detection of LC3B dot formation by immunofluorescence, respectively.

Results Upregulation of 5 ATG genes (Beclin-1, Atg5, Atg7, WIPI1 and LC3B) was observed in patient`s Mo- DCs compared to controls. Basal autophagy of individual pan-DCs versus Mo-DCs revealed a higher autophagic flux than pan-DCs with the highest autophagic flux displayed in Mo-DCs in the iTTP patient.

Conclusion The observed activation of autophagy hints at a contribution to the loss of tolerance in iTTP. A more extensive study to investigate if modulators of the respective aberrantly expressed ATG genes might help improve therapy management is ongoing.

OC13-02 Microvascular immune cell recruitment regulating development of immunothrombosis in systemic bacterial infection

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Objective During systemic bacterial infection coagulation is activated within the microcirculation of infected organs. The immune cells regulating intravascular fibrin formation and immunothrombosis are insufficiently characterized.

Material and Methods Wild type (WT) mice, Jalpha18-/- mice, Valpha14-Jalpha18 mice and bhlhe40/bhlhe41 double knock out (DKO) mice were infected with E.Coli. Kinetic profiles of classical monocytes (Ly6C+,Ly6G-), non-classical monocytes (CX3CR1+,Ly6C-), neutrophils (Ly6G+), B1a cells (CD19+, CD5+), NKT cells (CD1d+) and Th17 cells (CD4+,RORgammat+) were studied specifically at the site of infection. In parallel, association of tissue factor (TF) and uPAR with immune cells was visualized. Immunohistochemistry was also performed to analyze fibrin formation and its association with GFP+ E.Coli in liver microvessels.

Results The earliest recruited cells were identified via tetramer staining as NKT cells. Classical monocytes and neutrophils were recruited rapidly whereas non-classical monocytes were immobilized with a delay. T helper cells and B cells appeared early after infection and increased over time. T helper cells represented largely Th17 cells, while most B cells were B1a cells.
Intravascular fibrin formation and vessel occlusions peaked at 3 and 6h post infection. Bacteria were mostly embedded in fibrin rich areas.
Proteins initiating fibrin formation (TF) or promoting its degradation (uPAR) could be detected on several recruited immune cells. We found TF to be associated at an early time point mainly with NKT cells, as well as with neutrophils and classical monocytes. uPAR was associated with B1a cells, especially during the peak of fibrin formation.
In Jalpha18-/- mice with greatly reduced levels of NKT cells, microcirculatory coagulation activation was reduced. Depletion of classical monocytes decreased fibrin formation to a similar extent. In Valpha14-Jalpha18 mice with raised NKT levels, fibrin deposition and vessel occlusions were augmented. In bhlhe40/41 DKO mice, a model for impaired B1a production, the amount of fibrin formation was unchanged compared to the WT mice.

Conclusion Immunothrombosis development is crucially promoted by the sequential recruitment of NKT cells, classical monocytes and neutrophils. NKT cells immobilized in the liver microcirculation express TF and initiate intravascular coagulation. B1a cells appear to be irrelevant for fibrin homeostasis.

OC13-03 Dietary omega-3 fatty acid reverses age-associated platelet hyperreactivity through modulation of gut microbiota

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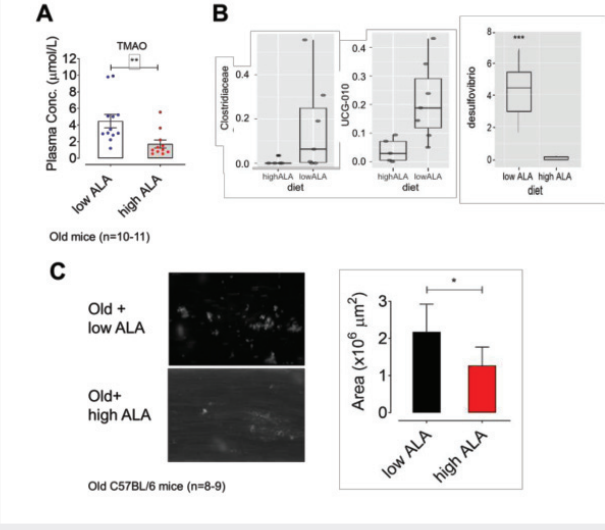
Objective Background: Aging is associated with platelet hyperreactivity, which may contribute to thrombotic events. Aging also affects the gut microbiota and leads to altered metaorganismal metabolites including trimethylamine-N-oxide (TMAO) and short-chain fatty acids (SCFAs), which directly contribute to the platelet hyperresponsiveness. Our previous studies have shown that plant-derived omega-3 α-linolenic acid (ALA) confers beneficial anti-thrombotic effects in human and animal studies.
Objective: We therefore hypothesized that a long-term ALA-rich diet could reverse age-linked platelet function and thrombosis potential via modulation of gut microbiota in our model of aged mice.

Material and Methods Methods: 8-12 week old wild-type C57BL/6J mice were fed a specific low (0.03%) or high (7.3%) ALA containing diet for >16 months. Blood samples were then taken for quantification of plasma TMAO levels, flow cytometric analysis of platelet receptors GPIb, GPIIb/IIIa and P-selection, platelet aggregometry (in response to stimulation with agonists ADP, collagen, and TRAP-6), and clot formation on von Willebrand factor (vWF) under high shear rates. In parallel, faecal samples were collected for 16S rRNA metabarcoding and SCFAs (acetate and propionate) analysis.

Results Results: Our results show that long-term ALA supplementation inhibits aging-linked platelet hyperreactivity, increased platelet aggregation and enhanced clot formation on vWF, likely, through reduction of the abundance of TMAO-producing genera Desulfovibrio and Lachnospiraceae, and inflammation-promoting Clostridiaceae, as well as, restoration of health-promoting Rikenellaceae_RC9_gut group and Intestinimonas. Consistently with our hypothesis on the pathophysiology, ALA-rich diet could also both decline plasma levels of TMAO and increase faecal SCFAs acetate and propionate in aged mice.

Conclusion Conclusion: Our studies reveal that dietary ALA modulates the aged microbial community towards the young enterotype, which leads to the reversal of the platelet hyperresponsiveness and the subsequent thrombosis risk. Hence, ALA-rich diet can be potentially exploited as a nutritional antithrombotic strategy in the aging.



▶ **Abb 1.**

OC13-04 The Glycosylation Nexus Enigma: new clues to the role of the GNE gene in the pathogenesis of inherited thrombocytopenia in both isolated and myopathy-associated forms

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Objective The GNE gene encodes a kinase with a key role in sialic acid biosynthesis and its mutations are classically linked to sialuria and GNE myopathy. Nevertheless, in a few recent cases, GNE variants have been associated with inherited thrombocytopenia (IT) both with and without muscle weakness. Herein, we present two young probands (P1, P2) of unrelated consanguineous couples with significant platelet count reduction and no myopathy signs hitherto, aiming to disclose the molecular causes underlying the disease.

Material and Methods P1 and his family members underwent whole exome and Sanger sequencing, respectively. Variant pathogenicity was assessed by western blot, protein 3D-structure analysis and sialic acid assays to evaluate the mutation effect on protein structure and function. Since P2 is a more recent case, he was investigated only by whole exome sequencing and his variant role was predicted via protein 3D-structure modelling. Further analyses are currently ongoing.

Results P1 and P2 were found to carry two novel homozygous variants, c.1724C>G (p.Thr575Arg) and c.1546_1547delGTinsAG (p.Val516Arg) of the GNE gene, respectively. No candidate variants were identified in the other known ITs-causing genes. In P1, pedigree analysis confirmed segregation within the family in accordance with an autosomal recessive pattern, and western blots of his cell culture unveiled a considerable reduction in protein expression, suggesting mutant protein instability. Moreover, P1 was reported for defective sialylation, which could affect platelet production and/or survival. Both p.Thr575Arg and p.Val516Arg were assumed to alter the three-dimensional structure of the GNE ATP pocket, further supporting their pathogenic effect.

Conclusion Consistently with our cases, severe thrombocytopenia has been found in 5 other families with GNE anomalies in the ATP pocket and no evidence of GNE myopathy, even in adulthood. The same mutations were not reported for any of the 950 known patients with GNE myopathy. Thus, our data claim a possible nexus between the ATP pocket location of the variants, the protein domain disfunction and decreased platelet counts. Although further studies are required, including GNE among the genes associated to ITs could significantly contribute to understanding the role of GNE in these disorders, as well as patients' appropriate molecular diagnosis and clinical management.

OC13-05 Platelet Distribution Width as a marker of platelet reactivity and platelet activation status in men and women of the Moli-family cohort

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Objective Platelets are a key player in (patho)physiological processes as primary haemostasis, thrombosis, tissue inflammation and immune regulation. As a measure of platelet size heterogeneity, the platelet distribution width (PDW) has been explored in several clinical conditions but its value as a marker of platelet function in (sub)clinical disease is still uncertain. Making use of the elaborate set of platelet activation markers available for the Moli-family

cohort, we aimed at validating the strength of PDW as a marker of platelet reactivity and platelet activation status and its gender-specific differences.

Material and Methods Flow cytometric measurements of platelet-bound P-selectin, leukocyte/platelet mixed aggregates of resting and in vitro activated whole blood, PFA-100 Closure Time, soluble P-selectin, pro-coagulant activity in unstimulated and LPS- or TNFalpha-stimulated blood were measured on freshly isolated blood of the Moli-family participants (N=753). Multivariable linear mixed effects regression models (adjusted for age, gender and other platelet indices) were used to evaluate associations between PDW with the above mentioned platelet activation/function markers taking into account the possible effect of Platelet count (Plt) and Mean Platelet Volume (MPV). These analyses were performed on the whole population and in women and men, separately.

Results PDW was associated with platelet/leukocyte aggregates (ß= -0.149, CI(-0.220 – (-0.079), p=3.7E-5,) and with platelet P-selectin expression in unstimulated conditions (ß= 0.149, CI(-0,223 – (-0.075)), p=8.4E-5). These associations remained significant after adjustement for Plt and MPV and were independent on the gender. PDW was positively associated with LPS- and TNFα-stimulated procoagulant activity (ß= 0.720, CI(0.376 - 1.065), p=4.5E-5, and ß= 0.658, CI(0.293 – 1.024), p=4.4E-4, respectively) and negatively with vWF levels (ß= -0.306, p=0.002, CI(-0.502 – (-0.111)), independently from gender differences. A positive association was observed with PFA-100 Closure time in the whole population (ß= 0.005, CI(0.003-0.008), p=3.4E-5) and in women, but not in men.

Conclusion PDW is an overall better predictive marker of platelet activation whose effect is largely independent of both Plt and MPV and is not influenced by gender. PDW might be a useful tool to assess platelet reactivity and activation status in population studies.

Poster

Acquired bleeding disorders

P01-01 Acquired von Willebrand syndrome associated with monoclonal gammopathy of undetermined significance – successful treatment with lenalidomide and dexamethasone

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DOI 10.1055/s-0041-1728145

Objective Management of hemorrhagic complications in acquired von Willebrand syndrome (AVWS) can be challenging. This is particularly true for gastrointestinal (GI) bleeding from AVWS-associated angiodysplasia. In AVWS, secondary to monoclonal gammopathy of undetermined significance (MGUS), therapy with immunomodulatory drugs such as thalidomide or lenalidomide in combination with dexamethasone has been proposed, although not evidence-based. Here, we assessed the effect of this regimen in patients, who failed to respond to intravenous immunoglobulin treatment (IVIG) in a sustained effect.

Material and Methods We report on two males (aged 45 and 54 years) with MGUS-associated AVWS suffering from severe, life-threatening GI and mucocutaneous bleeding. Multimer (MM) analysis of the von Willebrand factor (VWF) displayed loss of large MM, confirming AVWS. Both patients gave informed consent after careful information about off-label use of lenalidomide and the benefit-risk assessment.

Results Patient 1 had experienced recurrent GI bleeding due to angiodysplasia. Over a 12-month period, no remission was achieved by high-dose IVIG treatment (1g/kg/d for 2 days). Therapy with lenalidomide in 28-days-cycles (21 days of treatment, followed by 7 days of pause) was started at low dose (5 mg once daily) due to elevated liver enzymes and combined with weekly administration of dexamethasone (40 mg). Upon stepwise dose increase of lenalidomide (up to 25 mg once daily), bleeding resolved without continued IVIG. During a 2-year follow-up until today, hemorrhage did not recur and VWF parameters restored completely (Table 1). Patient 2 suffered from recurrent spontaneous hemorrhage including severe thoracic hematoma while on IVIG treatment. Upon administration of lenalidomide (20mg once daily) plus dexamethasone (40 mg once weekly) in 28-days cycles, the patient experienced complete remission with regard to any new bleeding episodes and VWF abnormalities during a 2-year follow-up until today (Table 1).

Conclusion Our observations confirm that combined treatment with lenalidomide and dexamethasone can increase VWF plasma levels and resolve bleeding in MGUS-associated AVWS. The findings are compatible with the hypothesis that this effect results from lenalidomide-mediated inhibition of otherwise increased clearance and degradation of VWF in MGUS, as suggested by others.

▶**Tab 1.** Course of laboratory parameters in acquired von Willebrand syndrome associated with monoclonal gammopathy of undetermined significance at time of diagnosis, prior to therapy with immunomodulatory agents (initiated 3-4 weeks after short-lasting response of IVIG treatment) and current outcome after two years of combined treatment with lenalidomide and dexamethasone. Abbreviations: ADP, adenosine diphosphate; APTT, activated partial thromboplastin time; FVIIIc, chromogenic factor VIII activity; PFA, platelet function analyzer; VWF, von Willebrand factor; sec, seconds

	Baseline at time of diagnosis	Prior to therapy with lenalidomide and dexamethasone	Current state after 2 years on lenalidomide and dexamethasone
Patient 1 (blood group: 0 rhesus positive)			
VWF activity (VWF:act) (blood group 0: 44-141%)	7%	4%	138%
VWF antigen (VWF:ag) (blood group 0: 54-149%)	11%	5.8%	129%
VWF:act / VWF:ag ratio (> 0.7)	0.64	0.69	1
APTT (22-29 sec)	50 sec	41 sec	26 sec
FVIIIc (70-150%)	11%	11%	148%
PFA, closure time with ADP (60-100 sec)	> 300 sec	> 300 sec	112 sec
Patient 2 (blood group: 0 rhesus negative)			
VWF activity (VWF:act) (blood group: 0: 44-141%)	27%	42.3%	272.6%
VWF antigen (VWF:ag) (blood group 0 54-149%)	47%	71.2%	225%
VWF:act / VWF:ag ratio (> 0.7)	0.57	0.59	1.2
APTT (22-29 sec)	36 sec	42 sec	20 sec
FVIIIc (70-150%)	39%	109%	336%
PFA closure time, ADP (60-100 sec)	> 300 sec	144 sec	83 sec

P01-02 Case report: Use of recombinant von Willebrand factor in a patient with acquired von Willebrand syndrome due to specific IgM-antibodies directed against vWF

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Objective Acquired von Willebrand Syndrome (avWS) is a rare coagulation disorder which can be associated with IgM-paraproteinaemia. Some patients develop specific antibodies against von Willebrand factor (vWF) that increase its clearance. Recently, recombinant vWF has become available for the treatment of bleedings and prevention of surgical bleedings in patients with inherited von Willebrand`s disease, but experience in patients with avWS is limited.

Material and Methods We report on an 80-year-old patient who presented with recurrent, severe transurethral bleeding and was diagnosed with avWS and underlying IgM-paraproteinaemia with evidence of a specific antibody against vWF (Table 1).

Results Bleeding required several transfusions despite local electric coagulation. Hemostyptic treatment was initiated with tranexamic acid and a plasmatic (p) vWF/factor (f) VIII product. The first administration of pvWF/fVIII (40 IE/kg) resulted in an increase of vWF activity from 6% to 52%, but after 4 hours vWF activity decreased to 26% and after 24 hours to 16%. In parallel vWF specific antibody eradication was started with prednisolone (1 mg/kg/day) followed by Rituximab (375 mg/m^2 body surface area, weekly, 4 cycles). Repetitive application of pvWF/fVIII did not sufficiently elevate vWF activity and bleeding reoccurred. Treatment with recombinant vWF (Vonicog alfa) was initiated. We observed an increased recovery of vWF activity within 2 hours after the first application of Vonicog alfa (42 IE/kg) from 32% to 75%. 12 hours after a second application (42 IE/kg) vWF activity was still 59%. Bleeding stopped after application and did not occur again (even though vWF activity levels were decreasing by time despite higher doses of Vonicog alfa). Due to the insufficient effect of immunosuppressive therapy with Rituximab, we initiated a therapy with dexamethasone (20 mg on day 1, 2, 4, 5, 8, 9, 11, 12) and bortezomib (1,3 mg/m^2 on day 1, 4, 8, 11). After only one cycle of therapy, the IgM-paraproteinaemia disappeared and vWF activity increased to 200%. No adverse events were reported.

Conclusion Use of recombinant vWF was safe and highly effective in a patient with avWS and underlying IgM-paraproteinaemia with a specific antibody against vWF and might be taken into consideration for treatment of acute bleeding in these patients.

▶ **Tab 1.** Patient characteristics and laboratory results at presentation.

	Patient
Age	80
Gender	male
Underlying disease	Monoclonal IgM-paraproteinaemia
Paraprotein	IgM-kappa
Serum concentration of paraprotein (mg/ml)	94 (40-230)
Platelets /nl	288 (163-337)
APTT(sec)	40 (26-37)
FVIII:C (%)	29 (70-150)
vWF:Ag (%)	27 (57-174)
vWF:Ac (%)	6 (47-173)
Thrombocyte aggregometry (Born) (%)	ADP: 89 (>70) Collagen: 82 (>70) Ristocetin: no activation (>70) Arachidonic acid: 85 (>70)
vWF plasma multimers	Type 2
Anti-vWF	positive
Proposed mechanism	Immunologic clearance

Normal values are shown in brackets.

P01-03 Causes of Acquired von Willebrands Disease Type II in Pediatric Patients. Cases and literature review.

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Objective Acquired bleeding disorders in childhood are rare. Acquired von Willebrands disease type two (avWDII) being one example that can cause significant bleeding complications. It may be caused by multiple underlying diseases. Usually patients are quite sick which complicates diagnosis significantly. Willebrand parameters are usually elevated to more than two times of the upper limit (especially after a bleeding episode) as an acute phase reaction. This may lead physicians to discard avWDII as a possible reason for bleeding. However several mechanisms can lead to loss of large and super-large Willebrand multimers causing a significant bleeding phenotype. The ratio of Willebrand factor quantity and activity may point towards avWDII but is not 100% reliable. The diagnosis can be made by Willebrand multimer analysis.
Aim: Raise disease awareness for von avWDII as a treatable cause of bleeding.

Material and Methods We report on patients with mostly severe clinical bleeding phenotypes that have been diagnosed with avWDII. Patients received comprehensive diagnostic coagulation work-up.

Results Patients were aged 4 – 14 years and suffered from complications after allogenic hematopoetic stem cell transplantation (n=4), thrombocytosis (n = 3), liver disease caused by biliary atresia, treated by portocaval shunt (n=1) and congenital heart defects (n=1).
Patients with thrombocytosis had mild bleeding complications that were treated with tranexamic acid. All other patients had prolonged hospital stays due to bleeding and 5/9 patients received multiple blood transfusions to substitute blood loss. In 5/9 patients bleeding was stopped or significantly reduced after iv substitution of Willebrand factor. In 3/9 patients avWDII was resolved after the underlying cause was cured.

Conclusion AvWDII may be a transient cause for bleeding in sick children. Bleeding symptoms can successfully be treated with iv substitution of Willebrand factor.

P01-04 The occurrence of trombocythopathy in CLL patients treated with Ibrutinib

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Objective Ibrutinib is an irreversible inhibitor of Bruton's tyrosine kinase that is an effective therapeutic agent for B-cell neoplasms including CLL. Ibrutinib, however, carries an increased bleeding risk due to its effect on several distinct platelet signaling pathways, resulting to thrombocytopathy. Optical agregometry is used for the identification of platelet dysfunction. Platelet-rich plasma (PRP) optical aggregometry using collagen, ADP, ristocetin and epinephrin as inductors is considered the gold standard for examining platelet function. Patients on ibrutinib have reductions in collagen-mediated and ristocetin-mediated platelet aggregation. The degree of inhibition of either collagen- or ristocetin-mediated platelet aggregation in patients on ibrutinib correlates with bleeding. Ibrutinib does not inhibit ADP-mediated platelet aggregation.

Material and Methods Platelet aggregation using PRP was measured in a series of 103 blood samples from 46 CLL patients treated with ibrutinib. Collagen, arachidonic acid and ristocetin were used to induce platelet aggregation. The aggregometer SD Medical TA was used for the measurement. Cut off value for the definition of platelet dysfunction (maximal platelet aggregation) was 60 %.

Results Collagen-mediated, arachidonic acid-mediated and ristocetin-mediated platelet aggregation was reduced in 8/103 samples (8 %), in 6/103 samples (6 %) and in 5/103 samples (5 %), respectively. Thrombocytopathy was recognized in only a small proportion of CLL patients treated with ibrutinib.
Conclusion In our study, the incidence of platelet dysfunction was 5-8 %. In contrast, the occurrence of bleeding events with ibrutinib is reported 31-61 %. In CLL patients, other factors may explain the variability of bleeding events, including differences in disease type, dosing of the drug, use of other antiplatelet and anticoagulant agents and severity of thrombocytopenia. Individual differences in platelet responsiveness to ibrutinib are also reported. Optical aggregometry represents an objective tool for monitoring of platelet function in ibrutinib treated patiens, especially in cases of bleeding.

Antithrombotic treatment

P02-01 Differences in venous thromboembolism prophylaxis between gastroenterology and cardiology inpatients.

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Objective The multinational cross-sectional study ENDORSE showed in 2008, that most inpatients in Germany treated on internal medicine wards with increased risk of venous thromboembolism (VTE) receive drug-based prophylaxis. The study did not discriminate between different subspecialties. While cardiologists usually treat patients with thrombotic or thromboembolic diseases, their gastroenterology colleagues see more patients with gastrointestinal bleeding complications. Thus, we hypothesized that the prescription of VTE-prophylaxis will be influenced by the field of specialization of treating physicians.
Material and Methods We performed a retrospective chart review of patients on the cardiology and gastroenterology internal medicine wards of our university hospital. A total of 1917 consecutive patients on the cardiology and gastroenterology wards were screened. Patients with clear indication for anticoagulation and contraindication against antithrombotic treatment were excluded. To determine the risk of a VTE and bleeding, quantitative risk assessment models (Padua Score, IMPROVE Score, IMPROVE-Bleeding Score and Has Bled Score) were used. We correlated the risk of VTE and bleeding with the likelihood of receiving VTE-prophylaxis.
Results A total of 450 patients per specialty were included in this study. In the group of low-risk patients for VTE based on Padua Score 55.19 % patients treated on gastroenterology and 82.13 % patients treated on cardiology wards received drug-based thrombosis prophylaxis (OR = 3.73, 95 % CI 2.43-5.73). Stratified by IMPROVE Score 64.18 % patients treated on gastroenterology and 84.52 % treated on cardiology wards received prophylaxis, respectively (OR = 3.05, 95 % CI 2.11-4.39). In the group of high-risk patients, stratified by Padua Score, 85.45 % gastroenterology and 93.26 % cardiology patients for VTE (OR = 2.35, 95 % CI 0.92-6) received thrombosis prophylaxis. Stratified by IMPROVE Score the percentages were 85.45 for gastroenterology and 93.10 for cardiology wards (OR = 2.30, 95 % CI 0.45-11.61). Bleeding risk as calculated by IMPROVE-Bleeding and Has Bled Score did not influence treatment decision.
Conclusion We found that significant more cardiology low-risk patients received drug-based thrombosis prophylaxis compared to gastroenterology patients. No significant difference in thrombosis prophylaxis use between the two specialties was found in high-risk patients for VTE.

P02-02 Efficacy of direct oral anticoagulants in plasma from patients with liver cirrhosis at high thrombotic risk

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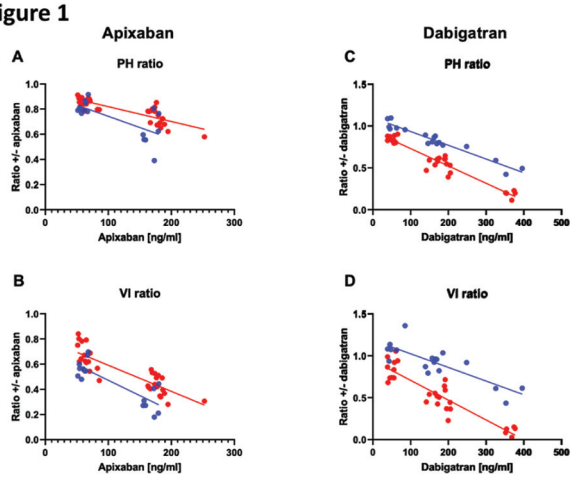
Objective Liver cirrhosis (LC) is a complex pathology which confers a prothrombotic state. The anticoagulation of LC-patients remains challenging because of the unknown efficacy of heparins and direct oral anticoagulants (DOACs), monitoring difficulty (particularly for coumarins), and alterations in drug metabolism. Among DOACs, apixaban and dabigatran seem to have the most adequate metabolic profile for LC-patients. We aimed to analyse the in vitro efficacy of these two anticoagulants in LC-patients at high thrombotic risk.
Material and Methods We included 22 LC-patients identified as prothrombotic using ex vivo thrombin generation (ST Genesia with ThromboScreen reagents, Stago, Asnières-sur-Seine, France) and nine healthy donors. Plasma samples were spiked with either Owren's veronal buffer, apixaban, or dabigatran solutions (final target concentrations: 50, 150 ng/ml; additional concentration of 300 ng/ml for dabigatran). We have chosen these concentrations because they represent peak and through levels observed in clinical studies of a therapeutic anticoagulation with both drugs. After spiking, apixaban and dabigatran concentrations were verified and ex vivo thrombin generation were analysed using ST Genesia with DrugScreen reagents (Stago). Ratios for velocity index and peak height assessed without and with anticoagulants were calculated, and compared between LC-patients and healthy donors for each target concentration using Mann-Whitney test.
Results Ratios for velocity index and peak height according to apixaban and dabigatran concentrations are presented in Fig. 1. At a target concentration of 150 ng/ml (roughly representing peak levels for both DOACs), in apixaban treated samples the peak height ratio was slightly but significantly higher [median 0.74 (LC, red) vs. 0.60 (control, blue),

Figure 1



▶ **Abb 1.** Ratios for velocity index and peak height according to apixaban (A, B) and dabigatran (C, D) concentrations in patients with liver cirrhosis (red) and healthy donors (blue). The lines represent the linear regression lines. PH, peak height; VI, velocity index.

p-value 0.0329] and the velocity index ratio significantly higher (0.42 vs. 0.27, p-value 0.0076) in LC-plasma compared to controls. With dabigatran, both ratios were significantly lower (0.58 vs. 0.80, p-value <0.0001; 0.51 vs. 0.94, p-value <0.0001) in the LC-group compared to controls.

Conclusion We demonstrated a slightly lower anticoagulant efficacy of apixaban and a clearly higher efficacy of dabigatran in LC compared to control plasma. Based on these preliminary data and on DOACs metabolism, apixaban appears to be the safest DOAC for LC-patients.

P02-03 Impact of DOAC Dipstick test and relation to plasma and urine concentrations to exclude the presence of clinically relevant concentrations of DOACs in real-life patient setting – study protocol and case reports

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Objective Rapid and accurate testing for direct oral anticoagulants (DOACs) is required to facilitate medical decision making in specific emergency and other medical situations. The relation of its qualitative results to quantitative plasma concentrators remains to be determined. The objective of the study is to examine whether results of DOAC Dipstick can be used to exclude the presence of clinically relevant concentrations of the three DOACs (dabigatran, rivaroxaban and apixaban).

Material and Methods The one centre study aims to include patients treated with rivaroxaban, apixaban and dabigatran admitted with neurologic and cardiovascular diseases to general wards of Sestre Milosrdnice University Hospital Center at Zagreb. The study is the part of the research project IP-2016-06-8208, LAB-NOAC funded by Croatian Science Foundation. Between 120 and 180 patients will be included treated with rivaroxaban 15 or 20 mg od, apixaban 5 mg bid and dabigatran 110 or 150 mg bid. DOAC Dipstick test is performed by visual colour identification of the pads of DOAC Dipstick and by semi-automatic DOASENSE Reader as described in the respective instructions for use. The visual and reader evaluation of colours of pads of DOAC Dipstick for presence or absence of DOACs is performed independently by two trained medical persons. Concentration of rivaroxaban, apixaban and dabigatran are performed from plasma and urine samples using LC-MS/MS and of plasma samples using specific chromogenic assays and a set of coagulation assays. Data will be analysed at the end of the study.

Results At present 13 patients are included treated with rivaroxaban and apixaban. Methods are easy to perform. Anticoagulation was switched in 3 patients from rivaroxaban to dabigatran for minor bleeding or other minor side effects as decided by the treating physician. The day after switching DOAC Dipstick test revealed presence of both factor Xa and thrombin inhibitor by color identification of the pads with visual and reader evaluation.

Conclusion The case reports indicate that DOAC Dipstick may be positive for rivaroxaban and dabigatran following switching of DOACs. Further assessment of plasma concentrations may be required if the presence of factor Xa and thrombin inhibitors are found by DOAC Dipstick test before further medical decision-making. A relation of plasma and urine tests and DOAC Dipstick results will be analyzed after termination of the study.

P02-04 Sheep Mucosal Heparin as a Substitute for Porcine Mucosal Heparin. A Viable Option to Address the Potential Shortage Crisis.

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Objective Porcine mucosal heparin (PMH) represents the sole anticoagulant for surgical and interventional procedures along with its medical usage. Sheep mucosal tissue have been used to prepare heparin which demonstrates biosimilar characteristics to PMH. The purpose of this study is to compare multiple batches of sheep mucosal heparin (SMH) with PMH to demonstrate their bio-similarities.

Material and Methods Multiple batches of SMH active pharmaceutical ingredient were obtained commercially (Ronnsi Pharma, Suzhou, China). Multiple batches of PMH were obtained from a supplier (Medefil, Inc, Glendale Heights, IL, USA). Both groups of heparin were evaluated for molecular distribution profile using the HPLC methods and the USP potency using the pharmacopeia approved anti-Xa and IIa methods. The anticoagulant activities were profiled in both the whole blood and plasma based assays. The neutralization of these groups of heparin with platelet factor 4 and protamine sulfate were studied in various assays. The comparative effects of these agents were studied in the HIT antibody mediated aggregation. The antithrombotic and bleeding profiles were measured in standard animal models. The pharmacokinetics and pharmacodynamics profile was investigated in non-human primates after intravenous and subcutaneous administration.

Results Both the SMH and PMH produced comparable molecular weight distribution profiles. The USP potency of the SMH was found to be 190±7 U/ml in comparison to PMH at 181±8 U/ml. In the ACT and TEG, both SMH and PMH produced comparable effects (p>0.05). Both the protamine sulfate and platelet factor 4 produced comparable neutralization of SMH and PMH. In a HIT antibody mediated platelet aggregation assay, no differences were seen in the platelet responses. The bleeding and anti-thrombotic profile of SMH and PMH was comparable. The pharmacokinetic parameters in terms of biologic half-life, volume of distribution, and clearance rate were similar.

Conclusion The results demonstrate that SMH and PMH are comparable in producing their anti-coagulant and anti-protease effects. Moreover their antithrombotic and anti-coagulant effects are identical. The PK/PD profile is also comparable for the anti-Xa and anti-IIa effects. These results validate the hypothesis SMH is bio-equivalent to PMH and can be substituted for PMH.

P02-05 Sulodexide as a Parenteral Anticoagulant. A Substitute for Unfractionated Heparin.

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Objective Sulodexide is a widely used glycosaminoglycan derived drug for oral (antithrombotic) and parenteral (anticoagulant) indications and is composed of fast moving heparin (80%) and dermatan sulfate (20%). In view of the current shortage of porcine heparin supply sulodexide may be a potential substitute for heparin for surgical and interventional anticoagulation. The purpose of this study is to compare the anticoagulant activities of sulodexide with heparin in simulated studies to mimic surgical and interventional anticoagulation.

Material and Methods Powder versions of sulodexide were obtained from Alfasigma (Bologna, Italy). Powder versions of porcine mucosal heparin were obtained from Medefil (Glendale Heights, IL, USA). Stock solution of both heparin and sulodexide were prepared in sterile saline at 10mg/ml. Sterile

solutions were made at 1.0 and 0.1mg/ml. USP potency for both drugs were measured, cross referenced against US pharmacopiel standard and expressed as units per milligram. Whole blood activated clotting time was measured in healthy human volunteers at graded concentrations of both drugs. Plasma based anticoagulant assays such as activated partial thromboplastin time (APTT) and thrombin time (TT) were measured. Protamine neutralization studies were also carried out in the different assays used.

Results The USP potency of sulodexide range from 90 – 105 U/mg whereas the porcine heparin exhibited a potency range of 180 – 200 U/mg as measured by the anti-Xa activity. In the ACT assay sulodexide produced concentration dependent prolongation of this test which was comparable to heparin. Both the sulodexide and heparin produce concentration dependent anticoagulant effects in the APTT and TT assays. Protamine sulfate at equigravimetric level produced effective neutralization of the anticoagulant effects of both the sulodexide and heparin.

Conclusion These results suggest that sulodexide is capable of producing similar anticoagulant effects as unfractionated heparin. The USP potency of sulodexide can be adjusted at a comparable potency to porcine heparin to obtain comparable anticoagulant responses in the in vitro setting. Additional in vivo studies are warranted to compare the potency adjusted sulodexide with heparin.

P02-06 Whole blood anticoagulant effects of Sulodexide as measured by activated clotting time and their neutralization by Protamine sulfate

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Objective Porcine mucosal heparin has been conventionally used and still continues to be used in medical and surgical indications. In view of the current shortage of heparin there is a need to develop a suitable alternative. The purpose of this study is to compare the anticoagulant effects of Sulodexide and its protamine neutralization profiles in the activated clotting time (ACT).

Material and Methods Under an IRB approved protocol and following a double-syringe technique, blood was drawn in labeled syringes containing 200 ul of Sulodexide batches 1056,1285,2516.2604,3274,4190 (obtained from Alfa Sigma). In order to study neutralization by protamine sulfate, 200ul of Sulodexide (1056) at a final concentration of 50,25,10 µg/ml, together with 200 ul of protamine sulfate at final concentration of 25ug/ml was placed in a separate set of labeled syringes. After gently mixing the contents of the syringes, ACT was immediately performed and the clotting time recorded in seconds.

Results All 6 different Sulodexide batches showed a concentration-dependent anticoagulant response. At a final concentration of 6.2 µg/ml the ACT response observed was Sulodexide-1056 (189 ± 21 seconds), sulodexide-1285(179 ± 13 sec), sulodexide-2516(191± 9 sec), sulodexide-2604(181± 3 sec), sulodexide-3274 (173 ± 7 sec), sulodexide- 4190 (180± 2sec),compared to a saline control value of 138 ±2 seconds. At a final c concentration of 12.5 µg/ml the ACT response observed was Sulodexide-1056 (240 ± 5 seconds), sulodexide-1285(241 ± 8 sec), sulodexide-2516(253 ± 12 sec), sulodexide-2604(202 ± 5 sec), sulodexide-3274 (235 ± 9 sec), sulodexide- 4190 (221 ± 4 sec),compared to a saline control value of 134 ± 4 seconds. At a final concentration of 50 µg/ml, the ACT response observed was Sulodexide-1056 (451 ± 55 seconds), sulodexide-1285 (367 ± 20 sec), sulodexide-2516(373 ± 10 sec), sulodexide-2604(368 ± 17 sec), sulodexide-3274 (377 ± 13 sec), sulodexide- 4190 (375 ± 11 sec),compared to a saline control value of 140 ± 5 seconds.

Conclusion Sulodexide at concentrations of 6.25-50 µg/ml (0.62–5.0 USP/ml) produced comparable anticoagulant effects to heparin which were neutralized by protamine sulfate. These results validate the hypothesis that Sulodexide can be used as a substitute anticoagulant for heparin.

Coagulation and fibrinolysis

P03-01 Changes in Vascular Physiology in Patients with ESRD Compared to Patients with ESRD and Concomitant Cardiovascular Disorder

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Objective Dialysis dependent patients with end-stage renal disease (ESRD) commonly present with a variety of comorbidities, including an increased risk of cardiovascular disease as a result of vascular dysfunction. Biomarkers are useful indicators of endothelial condition and can serve as predictive factors for the estimation of vascular condition in patients with ESRD. Conditions that arise as a result of compromised cardiovascular health such as atrial fibrillation (AFIB) and peripheral arterial disease (PAD), may alter biomarker levels in a way that reflects a worsened state of health for the patient, therefore providing categorical evidence on the pathogenesis of ESRD along with cardiovascular comorbidities. This study profiled biomarkers and laboratory parameters of endothelium dysfunction in patients with ESRD, categorized by additional AFIB and PAD conditions.

Material and Methods Citrated blood samples were collected from 95 patients with ESRD enrolled from Loyola Medical Center under an IRB approved protocol. Biomarker levels were measured from plasma samples using commercially available sandwich ELISAs, including tissue plasminogen activator (tPA), D-dimer, and .nitrotyrosine. Lab parameters, including BUN, calcium, creatinine, parathyroid hormone, phosphate, alkaline phosphatase, ferritin, transferrin, and total iron capacity, and patient comorbidities were obtained through a review of the patient medical records. The comorbidities were determined through provider notes, and evidence of applicable testing.

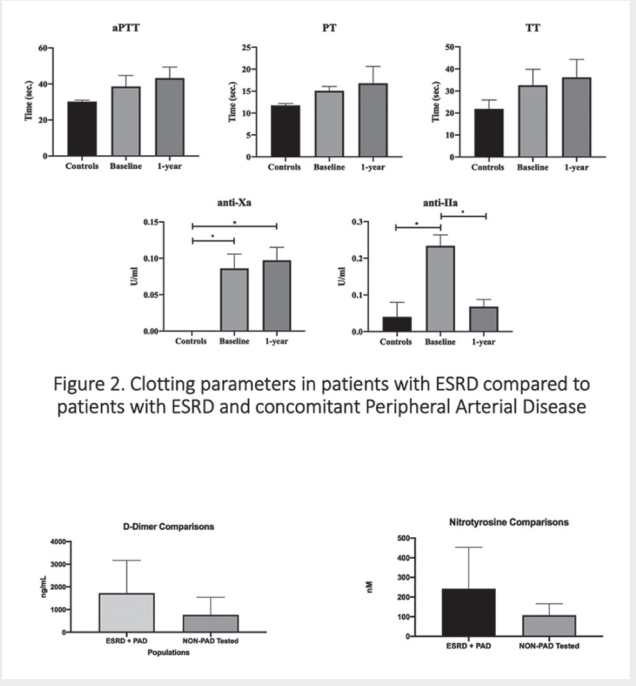

Figure 2. Clotting parameters in patients with ESRD compared to patients with ESRD and concomitant Peripheral Arterial Disease

▶ **Abb 1.** Clotting parameters in controls and ESRD patients at baseline and 1-year. *indicates p<0.05

Results Of the 94 patients with ESRD, 14.89% of patients were found to have atrial fibrillation (n=14), 30.85% of patients were found to have peripheral arterial disease (n=29), and 6.38% of patients were found to have both peripheral arterial disease and atrial fibrillation (n=6). When compared to patients with only ESRD, patients with ESRD and PAD showed elevated levels of D-Dimer (p = .0314) and nitrotyrosine (p = .0330). When compared to patients with only ESRD, patients with atrial fibrillation showed elevated levels of D-Dimer (p=.0372), nitrotyrosine (p=.0322), and tPA (p=.0198).

Conclusion Atrial fibrillation and PAD are frequently encountered in patients with ESRD. When compared to patients with ESRD, patients with concomitant PAD had elevated levels of nitrotyrosine and D-dimer, while patients with atrial fibrillation had elevated levels of nitrotyrosine, D-dimer, as well as tPA.

P03-02 Hepatic Disorders Contribute to Platelet Dysfunction

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Objective The role of platelets in hemostasis involves their adherence to sites of vessel injury, aggregation to form plugs. Agents that physiologically activate platelets in vivo include adenosine diphosphate (ADP), epinephrine, collagen, thromboxane A2 (TxA2) and serotonin. Vascular and platelet dysfunction represent complications of hepatic disease. The purpose of this study is to investigate the effect of various agonists on platelet aggregation profile of patient with hepatic disease.

Material and Methods Icteric Samples from the clinical lab from Loyola University medical center were randomly selected for this study (n=25). Blood samples from the healthy donors were included to prepare platelet rich plasma (PRP) and platelet poor plasma (PPP). PRP and PPP from various donors were separated to make pools. Four different pools were made namely Normal PRP, Icteric PRP, Icteric PRP + Normal PPP 1:1, Normal PRP + Icteric PPP1:1. Agonists such as ADP, arachidonic acid (AA), epinephrine and collagen were used to activate the platelets. The final assay concentration for ADP is 20 uM, AA is 500 ug/ml, collagen is 190 ug/ml and epinephrine is 10 ug/ml.

Results All results were compiled in terms of percent aggregation of platelets and represented as mean ± standard deviation. Normal PRP showed platelet aggregation response reaching a peak aggregation of 93.± 27.70 with ADP agonist. Icteric PRP, and two different pools did not show any significant platelet aggregation with ADP. Varying degrees of the inhibition of platelet aggregation was noted with different agonists.

Conclusion On the basis of the results we concluded that normal PRP showed platelet aggregation response. Icteric PRP and different pools (as shown above) did not show any platelet aggregation response. This data suggests that the icteric plasma contains an endogenous substance which produces the inhibitory effect on agonist induced platelet aggregation.

P03-03 Treatment of acquired thrombotic thrombocytopenic without plasma exchange in selected patients under caplacizumab

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Objective Acquired thrombotic thrombocytopenic purpura (aTTP) is a rare, lifethreatening autoimmune thrombotic microangiopathy. Current standard of care is therapeutic plasma exchange, immunosuppression, and caplacizumab, an anti-von Willebrand factor nanobody, which is effective in treating aTTP episodes.

Material and Methods Here we report on seven episodes of aTTP treated without plasma exchange in six female patients in Germany and Austria. Two episodes were initial presentations of aTTP; in five instances, patients experienced a relapse. In four episodes, moderate to severe organ dysfunction was observed; three cases presented with a mild course. All patients received caplacizumab immediately once aTTP was suspected or diagnosed, and plasma exchange was omitted based on shared decision making between patient and the treating physicians.

Results We observed a rapid and robust increase of platelet counts already after the first dose of caplacizumab, leading to a doubling of platelet counts within 17 hours (median), platelet counts normalized (>150 G/L) after median 84 hours. Lactate dehydrogenase, as a surrogate parameter of organ damage, improved in parallel to the platelet counts, indicating resolving microangiopathy.

Conclusion In conclusion, in selected cases of acute bouts of aTTP, it seems feasible to delay or omit plasma exchange if platelet counts increase and organ function is stable after start of caplacizumab therapy.

COVID-19

P04-01 Evolution of platelet phenotype during SARS-CoV-2 infection

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Objective Arterial thrombosis is common during SARS-CoV-2 infection suggesting possible role of platelet activation.
To determine platelet activation status and procoagulant platelet microvesicles (MVs) in SARSCoV-2 patients.

Material and Methods A prospective longitudinal study was conducted in six SARS-CoV-2 hospitalized patients (four men and two women, ranging in age from 29 to 90 years). During their hospital stay, two patients required oxygen therapy. At study entry, the NEWS2 and SOFA scores were elevated in 3 patients. The median duration of hospital stay was 12 days. Blood was collected on four occasions (Day (D)1, D7, D14 and D28). Surface markers of platelet activation before and after TRAP 50 µM stimulation, and procoagulant MV combining phosphatidylserine (PS) and fibrin (fib) detection were quantified by flow cytometry.

Results The Glycoproteins (GP) IIb/IIIa (CD41+) and Ib (CD42a+) levels were higher at the acute infection phase (D1 and D7) than in the later stages. After stimulation with TRAP, GPIb levels remained higher at D1 than at D28 ($p<0.05$). The same tendency was observed for GPIIb/IIIa and CD62P. Platelet activation resulted in significantly less PAC-1 binding during the acute phase compared with the later stages. The PAC1

binding after TRAP6 was negatively correlated with CRP (r=-0.84, p<10-6). but not with GPIIb/IIIa levels. 54 to 91% of plasma PS+MVs expressed CD41 (PS+CD41+). The % of CD41+PS+MVs did not varied throughout the follow up. At D1, 2.6 to 14% of PS+MVs bind fibrin (7.2+/-0.8%). These levels continue to rise at D7 (21+/-4.7%) or D14 (14+/-1.4%). At D28 all the patients recovered the D1 value or below (5.4+/-0.8%). The same profile was observed for CD41+Fib+MVs

Conclusion During clinical infection with SARS -CoV -2, platelets circulate primed to adhesion, aggregation and leucocyte interaction. Increased levels of procoagulant MVs during SARS -CoV - 2 may participate to the increased risk of thrombosis

P04-02 Retrospective analysis of persons after mild COVID-19 in the context of a convalescent plasma donation

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Objective About a year ago, the first reports from China about the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), were received. At that time, it was not yet foreseeable that this virus would cause a worldwide pandemic. The aim of our study was to characterize persons who had a mild COVID-19 course and to determine the antibody status of the individuals, since most publications focus on patients who need hospitalization.

Material and Methods We have obtained convalescent plasma from individuals in our region after recovery from COVID-19 and included these individuals in our study. From 615 persons who thought they were infected with SARS-CoV-2, we were able to identify 426 who were most likely infected with SARS-CoV-2. We determined this based on the detection of antibodies against SARS-CoV-2 and on virus detection by RT-PCR during the disease.

Results The most common symptoms were fatigue, cough and olfactory and gustatory dysfunction. The median duration of the disease was 12 days (interquartile range: 7-17 In 82.4% of the persons, anti-SARS-CoV-2 IgG was detected, additionally IgA antibodies were found in 73.9% of the persons. Despite a positive RT-PCR result during the disease in 10.8% of our cohort, no antibodies were detectable. Nevertheless, of 24 persons with asymptomatic courses of COVID-19, antibodies against SARS-CoV-2 could be detected in 23 (96%). We also found a correlation between disease duration and IgG antibody detection.

Conclusion In this study, we were able to describe the course and antibody status of mild COVID-19. As already described in the literature, we were able to show that not all persons after recovery from COVID-19 also produced antibodies against SARS-CoV-2 or that these were not detectable. On the other hand, antibodies could be detected in almost all asymptomatic cases. How important the antibodies are for the protection against a new infection with SARS-CoV-2 has to be investigated in further studies. In addition, the role of the cellular immune response with regard to prolonged immunity has to be investigated.

P04-03 Prothrombotic Disturbances of Hemostasis of Patients with Severe COVID-19: a Prospective Longitudinal Observational Study

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Objective COVID-19 is associated with major hemostasis disturbances and a high risk of thrombosis, the management of which remains controversial. Laboratory hemostasis could help to tailor thromboprophylaxis, but available results remain largely incomplete. The aim of this observational cohort study was to describe the longitudinal changes of laboratory hemostasis parameters in severe COVID-19 patients admitted to the intensive care unit (ICU). In addition we explored the association with thrombotic events of the most relevant parameters.

Material and Methods Twenty-one patients admitted to the ICU with confirmed COVID-19 were included. A large panel of hemostasis tests was measured on daily blood samples collected prospectively over four weeks. The association between hemostasis parameters and thrombotic events was explored.

Results We observed a laboratory prothrombotic state in severe COVID-19 patients, characterized by increased von Willebrand factor, fibrinogen, factor VIII, soluble fibrin complexes, D-dimers, thrombin generation endogenous thrombin potential (ETP), and plasminogen activator inhibitor 1 (PAI-1) levels, with decreased fibrinolytic activity. The observed hemostasis disturbances were more pronounced during early ICU stay. The main natural anticoagulant pathways (TFPI, antithrombin, protein C and protein S) were largely unaffected. A large interindividual variability was evidenced both considering maximal values and time-course. Soluble fibrin complexes, D-dimers, ETP, PAI-1 levels and fibrinolytic activity were associated with thrombosis (n=10).

Conclusion Our laboratory data further support and characterize the prothrombotic state observed in this cohort of severe COVID-19 patients. The extent and duration of these changes were, however, variable among patients, adding to the rationale for the individualization of the antithrombotic therapy.

P04-04 Enhanced prothrombotic state in patients with severe COVID-19

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Objective An infection with the novel SARS-CoV-2 virus is accompanied with thrombosis and pulmonary embolism. Comorbidities, like hypertension, diabetes, coronary heart disease, are associated with high mortality. Viral infections as well as above mentioned comorbidity induce an activation of the coagulation system, especially recognizable by increased levels of D-dimer. Here, we present data of COVID-19 patients in comparison to non-COVID-19 patients treated in an intensive care unit.

Material and Methods 24 patients with COVID-19 were included in this study. As a control group, 33 patients treated in the same the intensive care unit with symptoms of upper respiratory infections both negative COVID-19 test were included. Both groups were included between March and May 2020 and show no differences in regard to age and gender. Enzygnost F1+2 (prothrombin fragments), INNOVANCE D-Dimer and INNOVANCE Anti-Xa (all Siemens Healthineers) and Thrombinscope's CAT assay (Calibrated Automated Thrombogram, expressed as thrombin peak) were performed according to manufacturer's instructions.

Results Significant differences between the groups were observed in for the activation marker D-dimer. Non-significant differences were observed for prothrombin fragments and thrombin peak, nevertheless indicating an enhanced prothrombotic state in COVID-19 patients (n=24) compared to non-COVID-19 patients (n=33): prothrombin fragments F1+2 (nM): 405 ±

299 vs. 290 ± 211 (p=0,09); D-dimer (mg/L): 1,9 ± 1,4 vs. 1,2 ± 1,1 (p=0,04); thrombin peak (nM): 324 ± 110 vs. 293 ± 108 (p=0,29).
In samples containing less than 0,25 IU/mL heparin (21 COVID-19, 31 controls), similar results were achieved: prothrombin fragments F1+2 (nM): 426 ± 310 vs. 289 ± 213 (p=0,07); D-dimer (mg/L): 1,9 ± 1,5 vs. 1,2 ± 1,1 (p=0,05); thrombin peak (nM): 339 ± 105 vs. 311 ± 82 (p=0,29).
Conclusion In patients with a severe COVID-19 infection, the coagulation system is strongly activated. The observed imbalance promotes thromboembolism and might result in multi-organ failure. More data are required to strengthen the observed results. Additionally, other methods like fully-automated thrombin generation and clot waveform analysis should be included.

Crosstalks between hemostasis and other systems

P05-01 Cancer cell-derived microparticles expressing tissue factor have pivotal role on the procoagulant shift of endothelial cells

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Objective Endothelium activation is essential in pathogenesis of cancer associated thrombosis (CAT). Endothelial cell (EC) is a potential target of cancer cell derived microparticles (CaCe-dMPs). We recently showed that endothelial cells exposed to CaCe-dMPs acquire a procoagulant phenotype characterized by an enhancement of thrombin generation (TG). This new property is transferable to daughter cells. In this study, we investigated the implication of tissue factor (TF) in the new procoagulant profile acquired by EC exposed to CaCe-dMPs and if TF alone is capable of inducing this change.
Material and Methods CaCe-dMP released in conditioned medium from pancreas adenocarcinoma cells (BXPC3) were isolated with differential centrifugation. Human umbilical vein endothelial cells (HUVEC) were cultured for 72h according to 5 experimental conditions: in presence of (a)BXPC3-dMPs (b)BXPC3 cell conditioned medium depleted in BXPC3-dMPs(c)no TF and 4 µM of phospholipids MP-R (d)5pM of TF and 4 µM of phospholipids PPP-R High(e)1pM of TF and 4 µM of phospholipids PPP-R Low or(f)5nM recombinant TF (rTF Innovin).Subsequently, exposed-EC were washed and re-suspended in platelet poor plasma (PPP).Capacity of exposed-EC to enhance thrombin generation in PPP was assessed with the Calibrated Automated Thrombogram assay (Thrombinoscope b.v, Diagnostica Stago, Asnières, France). TF concentration of exposed cells was determined by using the Zymutest total TF kit (Hyphen Biomed, France).
Results HUVEC exposed to BXPC3-dMPs acquired a procoagulant profile with a significant enhancement of thrombin generation as HUVEC exposed to BXPC3-dMP acquired a procoagulant profile and significantly enhanced TG. HUVEC exposed to BXPC3 conditioned medium, rTF, MP-R, PPP-R high or low were not capable to enhance TG as compared to the control. Only HUVEC exposed to BXPC3-dMPs displayed a high amount of TF (563±47 pg/ml), whereas HUVEC exposed to all other experimental conditions did not express any detectable TF.
Conclusion CaCe-dMPs induce a procoagulant shift of EC characterised by marked expression of TF and enhancement of TG. These properties are transferred to following generations. TF alone is not sufficient to induce the procoagulant shift of EC. The ensemble of CaCe-dMP expressing TF is the vector of the procoagulant transformation of cancer cells. This property of CaCe-dMPs could lead to new therapeutic targets for the prevention of CAT.

►**Tab 1.** Thrombogram parameters in normal PPP of HUVEC cells exposed or not (control) to respectively BXPC3 derived vesicles (BXPC3-dEVs), BXPC3 conditioned medium depleted in vesicles (BXPC3-MC), human recombinant TF Dade, Innovin (5nM TF, phospholipids and calcium), PPP-Reagent High (5pM TF and 4µM phospholipids), PPP-Reagent Low (1 pM TF and 4µM phospholipids) or MP-Reagent (no TF and 4µM of phospholipids). Values are mean ± sd of 3 experiments. Below: Tissue factor concentration of HUVEC cells exposed or not (control) to respectively BXPC3 derived vesicles (BXPC3-dEVs), BXPC3 conditioned medium depleted in vesicles (BXPC3-MC), human recombinant TF Dade, Innovin, PPP-Reagent High, PPP-Reagent Low or MP-Reagent. Values are mean ± sd of 3 experiments.

	HUVEC control	HUVEC + BXPC3-dEVs	HUVEC + BXPC3-MC	HUVEC + Innovin	HUVEC + PPP-R High	HUVEC + PPP-R Low	HUVEC + MP-R
Lagtime (min)	10,92 ± 0,83	3,50 ± 0,24	9,59 ± 2,01	7,92 ± 0,59	9,75 ± 1,06	10,25 ± 2,72	11,09 ± 0,59
ETP (nM.min)	800,98 ± 48,03	698,07 ± 80,49	627,62 ± 183,48	743,73 ± 107,75	621,16 ± 44,80	841,77 ± 38,64	762,31 ± 18,17
Peak (nM)	56,26 ± 15,94	43,12 ± 2,85	52,05 ± 9,69	40,87 ± 4,14	32,91 ± 1,87	64,27 ± 5,90	54,76 ± 1,14
ttPeak (min)	17,92 ± 1,77	12,42 ± 0,12	16,94 ± 2,01	16,34 ± 0,47	18,33 0,71	16,84 ± 2,60	18,50 ± 0,71
MRI (nM/min)	8,26 ± 3,38	3,50 ± 0,24	7,08 ± 1,32	4,85 ± 0,43	3,83 ± 0,06	9,75 ± 0,72	7,39 ± 0,84

	HUVEC control	HUVEC + BXPC3-dEVs	BXPC3-MC	HUVEC + BXPC3-MC	HUVEC + Innovin	HUVEC + PPP-R High	HUVEC + PPP-R Low	HUVEC + MP-R
TF (pg/ml)	0,00	563,84 ± 47,47	361,1 ± 47,49	0,00	0,00	0,00	0,00	0,00

P05-02 Cooperation of platelet beta1 and beta3 integrins in the arrest of inflammatory bleeding in mice

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Objective The main function of platelet is to arrest bleeding at sites of injury. They also ensure the arrest of bleeding under inflammatory conditions through a mechanism proposed to rely on ITAM receptors, with GPVI playing a major role and CLEC-2 a secondary role. However, this role of platelets appears independent of the receptors classically involved in the arrest of bleeding, the GPIb-IX complex and integrin alphaIIbbeta3 in the cutaneous reverse passive Arthus (rpA) model. This raises the question of which platelet receptors support stable platelet adhesion at inflammation site, a role played notably by integrins during a trauma. The aim of this study was to evaluate the role of platelet beta1 and beta3 integrins in the arrest of bleeding under inflammatory conditions.
Material and Methods We used an rpA model based on anti-bovine serum albumin (BSA) antibody intradermal injection and BSA intravenous injection to induce cutaneous inflammation. The intranasal lipopolysaccharide and transient middle cerebral artery models were used to induce lung and cerebral inflammation, respectively.
Results Contrary to mice totally deficient for beta3 integrins, those deficient for beta3 integrins in the platelet lineage (PF4Cre-beta3-/-) and those deficient for alphaIIb presented localized petechial bleedings in the cutaneous rpA model. These results point to a central role of platelet alphaIIbbeta3 integrin in the arrest of inflammatory bleeding. Mice deficient for platelet beta1 integrins did not present bleeding in this model while mice deficient for both

platelet beta1 and beta3 integrins (PF4Cre-beta1-/-/beta3-/-) developed more severe cutaneous bleeding than PF4Cre-beta3-/-, implying a cooperation between both integrin families in the arrest of bleeding under inflammatory conditions in the skin. Similar results were obtained under cerebral inflammatory conditions since PF4Cre-beta1-/-/beta3-/- mice developed cerebral bleeding. Platelet beta3 integrins were also playing a role in the arrest of pulmonary bleeding under inflammatory conditions after lipopolysaccharide challenge.

Conclusion Altogether, these results identify a role of platelet beta1 and beta3 integrins in the arrest of bleeding under inflammatory conditions in several organs.

P05-03 Noonon syndrome as unlikely cause of increased bruising in a case of suspected battered child syndrome

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Objective On a regular basis, children are referred to our hemostaseology center because of increased bruising in order to rule out bleeding disorders. Usually diseases like hemophilia or Willebrand syndrome, as well as thrombozytopathy or thrombozytopenia, or other selective factor deficiencies are examined.

Failing to find a clinical explanation for excessive bleeding cases great concern in all caretakers. This may have great impact on the child's course of life.

Aim: Raise awareness for uncommen causes of bleeding in children with increased bleeding signs.

Material and Methods We report on a 1.5-year-old Asian boy who presented to our center for a coagulation workup prior to planned orchidopexy.

At the time of the presentation, the boy was living with his older brother in a welfare home due to suspicion of child endangerment.

Results Socialmedical history included: Poor bonding between mother and child, failure to thrive, psychomotor developmental delay, calotte fracture with subgaleal hematoma after a fall from the parental bed and constant extensive bruising, especially on the head without known trauma. No further relevant bleeding signs. Transcutaneous heart catheter intervention including balloon valvuloplastie of the pulmonary valve was performed without bleeding complications. The boy was hospitalized several times due to apathy and refusal to eat. After being taken into custody his development picked up. However extensive bruising persisted.

Regular hematological work up revealed Delta Storage Pool disease, prolonged in vitro bleeding time and FIX at the lower limit of age-adjusted reference values (59%). This did not satisfyingly explain his bruises. Considering all his symptoms, a whole genome analysis was performed. It revealed a typical gene variation for Noonan's syndrome. Easy bruising is common for patients with Noonan´s syndrome without any noticeable lab finding. FVII deficiency has been reported in patients with Noonan´s Syndrome but was not found in our patient.

Conclusion Noonan´s Syndrome explains our patient's phenotype. If regular hemostaseological work up fails to provide a reason for bleeding and hints of a syndromic disease are present; extended examinations including genetics should be considered.

Diagnostics and laboratory tests

P06-01 A bedside urine based test strip on identifying anticoagulation with direct oral anticoagulants at a high-volume emergency department – a diagnostic test accuracy study

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Objective Rapid and reliable detection of effective anticoagulation with a direct oral anticoagulant (DOAC) is critical in medical emergency situations. An accurate bedside test could support fast decision-making, help avoiding potentially fatal bleeding complications and may improve patient care and safety. A recently developed point-of-care strip test DOAC Dipstick is intended for detection of the presence of direct oral thrombin (DTI) and factor Xa inhibitors (rivaroxaban, edoxaban, apixaban) (DXI) in urine by visual color identification of pads specific for detecting DTI and DXI providing qualitative test results after 10 minutes.

Material and Methods The aim of the study is to determine the diagnostic test accuracy (DTA) of the qualitative results of the DOAC Dipstick (index test) in patients routinely treated at a high-volume emergency department in relation to cut-off values in plasma and urine samples using liquid chromatography with mass spectrometry (LC-MS/MS) and other coagulation tests

The prospective cohort study enrolls consecutive adult patients with known or presumed DOAC intake at the emergency department at the Vienna General Hospital. The study was approved by the local Ethics Committee in accordance with Helsinki declaration. Patient recruitment started January 2019 and ended August 2020. Patients were recruited 24/7, according to the need for a constantly available test at an emergency department. The DOAC Dipstick test was evaluated using a pocket-guide. The typical DOAC Dipstick test result of a patient on therapy with rivaroxaban 20 mg OD is shown in the figure. Patients' plasma were collected for clotting and chromogenic assays and for LC-MS/MS analysis as well as urine samples for immediate analysis with DOAC Dipstick by visual documentation of pads of DOAC Dipstick, digital photographic documentation and freezing of an aliquot at -80°C for LC-MS/MS. For statistical analysis DTA standard measures will be calculated using dichotomous data of the DOAC Dipstick and of results of LC-MS/MS and coagulation tests.

Results Recruitment was completed (n=320) and results are under evaluation.

Conclusion To our knowledge this is the first study evaluating the accuracy of DOAC Dipstick versus plasma and urine concentrations in patients treated with DOACs and hospitalized for acute diseases to a high-volume emergency department.

P06-02 Abnormal urine is detected by a specific pad of DOAC Dipstick near patient test strip

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Objective A recently developed near patient test strip DOAC Dipstick has shown high sensitivity and specificity for detection of the presence of direct oral thrombin and factor Xa inhibitors in urine and provides qualitative test results after 10 minutes. Its reliability, however, is limited in patients with dark-coloured urine, which may occur in kidney failure, hemoglobin- or urobilinogenuria.

Material and Methods To highlight the impact of a specific pad for identification of dark-coloured urine on the DOAC Dipstick test based on a case example.

Results An 81-year old male was enrolled in an ongoing cohort study on adult patients with chronic DOAC intake at the Emergency Department at the Vienna General Hospital. The patient was treated with rivaroxaban 20mg qd for prevention of stroke due to von-valvular atrial fibrillation. He was diagnosed with acute pre-renal kidney injury due to febrile diarrhea

(plasma creatinine 5mg/dl, BUN 130mg/dl). After obtaining informed consent, a 10ml urine sample was collected and the DOAC Dipstick test was performed. Test results are shown in the figure. While the test was interpreted to be negative for the presence of rivaroxaban (pad#3) and normal creatinine (pad#1) in urine, pad#2 that is designed for identification of abnormal urine colour, turned yellow confirming, that abnormal urine colour precludes a reliable interpretation of the test result for the presence of DOACs and of creatinine.

Conclusion Appropriate caution must be taken to the test pad#2 designed for the detection of abnormal urine colour of DOAC Dipstick near patient test strip in patients with dark-coloured urine to avoid false test results and misinterpretation of the other test pads. The pad for urine colour does not contain reagents and an abnormal colour can immediately be identified by naked eye and further evaluation of the other pads of DOAC Dipstick is not necessary.

P06-03 Accuracy of the STA®-Liquid anti-Xa assay in clinical practice: results from a large cross-sectional study in Switzerland

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Objective Rapid and accurate measurement of direct oral anticoagulant drug level is critical in emergency situations. The diagnostic performance of anti-Xa assays in routine clinical practice is however not well established. We aimed to study the accuracy of the STA®-Liquid anti-Xa assay for the determination of rivaroxaban, apixaban, and edoxban in a large prospective cross-sectional study in clinical practice.

Material and Methods Nine-hundred thirty-two consecutive patients which were referred for the determination of rivaroxaban, apixaban, and edoxaban drug levels to one of nine specialized hemostasis laboratories were included. The STA®-Liquid anti-Xa assay was conducted alongside liquid chromatography-tandem mass spectrometry (LC-MS/MS) as reference standard.

Results Sufficient sample material was available from 758 patients (median age 76 years, inter-quartile range 66-83, 43% female). The overall correlation (rs) of STA®-Liquid anti-Xa assay with LC-MS/MS was 0.98 for rivaroxaban (95% confidence interval 0.97 to 0.98), 0.98 for apixaban (0.97, 0.98), and 0.98 for edoxaban (0.96, 0.99). Bias of Bland & Altman difference plot was < +/- 5 µg/L for all drugs.

Conclusion The diagnostic accuracy of anti-Xa measurements as determined using the STA®-Liquid assay in clinical practice was high among all drugs and consistent throughout the spectrum of measurements. Implementation might facilitate fast and accurate measurement of DOAC levels in the emergency setting.

P06-04 Analysis of adherence to therapy comparing DOAC Dipstick test with plasma concentration of rivaroxaban and apixaban in outpatients with venous thromboembolic disease

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Objective The efficacy and safety of direct oral factor Xa inhibitors rivaroxaban and apixaban (DXI) in patients with venous thromboembolism (VTE) is closely related to patient's adherence to therapy. Objective documentation of drug intake may be a useful tool for patients' education and improvement of adherence to treatment.

We aim to analyze the accuracy of DOAC Dipstick near-patient device compared to plasma concentration for evaluation of the presence of rivaroxaban or apixaban in the urine in outpatients with VTE.

Material and Methods A prospective observational ongoing cohort study is performed including patients on active DXI treatment for secondary prevention of VTE. All participants were routinely assessed for concentration of rivaroxaban or apixaban in plasma using the specific chromogenic assays based on the measurement of the anti-Xa activity. Renal function was assessed by using the Cockroft –Gault equation. DOAC Dipstick test was performed from patients' urine samples and visual evaluation of pads' colors for factor Xa and thrombin inhibitors was performed by trained medical staff according the instructions for use.

Results Interim analysis was performed after enrolment of 41 patients (female/male 15/31, age 55 ± 14 years, mean and standard deviation). Of these, 19.5% (n=8) were treated for deep vein thrombosis (DVT), 12.1% (n=5) for pulmonary embolism (PE), 41.4% (n=17) for recurrent DVT, 19.5% (n=5), for cancer-associated thrombosis, 2% (n=1) for cerebral thrombosis and 2% (n=1) for ocular thrombosis and were treated with rivaroxaban (76%, n=31) and apixaban (24%, n= 10) patients. All patients had normal renal function. The anti Xa levels ranged from 20-418 UI/mL (median value 131.36 ±113.95). The factor Xa inhibitor pad of urine samples was as correct positive in 40/41 cases (almost 98%) and false negative in 1/41 cases (2%) as compared with plasma anti-Xa levels. Thrombin inhibitor pad was negative in all cases.

Conclusion The DOAC Dipstick qualitative test results demonstrated at least 95% correct positive and correct negative results for DXI rivaroxaban and apixaban in urine samples compared to quantitative plasma concentrations. The ongoing study should provide specific data for validation of the device as an easy-to-use and accurate tool assessing adherence to DOAC treatment

P06-05 Clinical usefulness of the novel DOAC Dipstick near patient test strip in emergency settings using semi-automatic DOASENSE Reader

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Objective A recently developed DOAC Dipstick near patient test strip has shown high sensitivity and specificity for detection of the presence of direct oral thrombin (DTI) and factor Xa inhibitors (DXI) in urine and provides qualitative test results after 10 minutes

Material and Methods The aim is to highlight the clinical usefulness of the DOAC Dipstick test for decision making in emergency settings by two case reports

Results Case 1: A 54-year old female presented to the Emergency Department due to acute shortness of breath. Bed-side ultrasound and chest X-ray showed a right-sided pneumothorax with a midline shift that required urgent intervention. Review of her latest discharge report suggested intake of edoxaban due to non-valvular atrial fibrillation but exact recent medication intake could not be obtained due to patient's lack of knowledge. A DOAC Dipstick test was performed to assess the presence of a DXI or a DTI in the patient's urine to guide further clinical management. The semi-automatic DOASENSE Reader showed absence of DTI and DXI on the pads of DOAC Dipstick. Insertion of a pleural chest drain was performed without need for prior administration of prothrombin complex concentrate and no bleeding complications occurred. Post-hoc analysis of factor Xa specific chromogenic test revealed an edoxaban concentration of <20ng/ml in plasma.

Case 2: A 72-year old female was admitted to the emergency department due to aphasia and impaired consciousness. A cerebral CT scan showed a left-sided middle cerebral artery occlusion. Thorough review of her medical history prescription of apixaban for non-valvular atrial fibrillation, but current intake could not be obtained from the patient due to impaired consciousness. A DOAC Dipstick test was performed to assess the presence of FXa inhibitors in the urine to estimate recent intake and guide further clinical management with or without thrombolysis. The semi-automatic DOASENSE Reader showed presence of FXa inhibitor in patient's urine. Since intravenous thrombolysis is not recommended for patients on DOACs a decision was made to directly subject the patient to interventional thrombectomy.

Conclusion These case presentations suggest clinical usefulness of the DOAC Dipstick test in different clinical scenarios, where critical decisions must be made in emergency situations, when thorough anamnesis for recent intake of medication may be not possible or unreliable.

P06-06 Comparing automated FXIII assays

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Objective Plasma transglutaminase FXIII confers mechanical and biochemical stability to blood clots. Congenital or acquired FXIII deficiency occurs rarely but activity levels ≤ 30% may be associated with severe bleeding, therefore careful patient monitoring is recommended. There are several assays for the manual determination of FXIII, however there is still an unmet diagnostic need for a reliable high-throughput assay.

The aim of the study was to demonstrate the characteristics of automated FXIII assays which are suitable for use in routine laboratories.

Material and Methods Next to the well-established ammonia release-based assay, the turbidimetric based FXIII antigen assay was compared to the newly developed fluorogenic FXIII activity assay on Ceveron s100. The assay principle is based on the use of a highly sensitive fluorogenic substrate in combination with a thrombin reagent. FXIII is activated by thrombin. FXIIIa cleaves a dark quenching molecule from the side chain of a modified peptide incorporating glycine methyl ester. Subsequently, the fluorescence intensity of an N-terminal coupled dye increases and is detected by the automated coagulation analyser.

Results For method comparison 110 samples ranging from 0 to 150% factor XIII activity were measured with the respective assay on the companies corresponding analyser platform. In comparison to the chromogenic assay for FXIII activity a Passing and Bablok fit of y=-5.448 + 1.011x and correlation of ≥0.95 could be observed. The comparison to the turbidimetric antigen assay resulted in a Passing and Bablok fit of y=-1.184 + 1.124x and correlation of ≥0.95. When looking into individual samples some known discrepancies can be detected. In samples with high Fibrinogen levels (>5 g/L), the ammonia release assay did result in overestimation of FXIII by 15-25%. More importantly however, was the finding that functional deficiencies of factor FXIII were not detected by the antigen assay, which could be problematic as this assay is commonly used as sole FXIII assay in some laboratories.

Conclusion We have demonstrated in our study that automated FXIII assays work reliable and robust. However, the factor FXIII activity assays have to be seen advantageous as they also detect functional FXIII deficiencies due to inhibitors.

P06-07 Detection thrombogenicity antiphospolipid antibodies with APC modified TGT

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Objective Our objective was to study influence antiphospholid antibodies effect of antiphospholipid antibodies on activated protein C, as the influence of this system may be one of the ways of thrombotic manifestation of this pathology.

The aim of our work is to determine the effect of antiphospholipid antibodies including new potential markers as anti-phosphatidylserin/protrombin antibodies (anti-PS/PT) on thrombin generation based assay modified by activated protein C.

Material and Methods Currently reccomended panel of APS diagnostics (lupus anticoagulants, anticardiolipin antibodies and beta-2-glycoprotein I antibodies by chemiluminiscence antibody assay) was completed with IgG/IgM aPS/PT antibodies, that were assayed using commercial ELISA kit. The determination of thrombogenicity was performed by detection thrombin generation in the absence/in the presence of APC by in-house modified method (Technothrombin TGA, Technoclone, Vienna, Austria) with detection by analyzer Ceveron Alpha (Technoclone, Vienna, Austria).

Results As a marker for thrombogenicity of antibodies was evaluated by the rate of reduction of the overall thrombin generation with/without APC. All individual occurrences of APS markers - i.e. LA and individual antibodies in IgG and IgM classes, were included in the evaluation. From the primary evaluation, the inhibition is highest for LA-type antibodies with an average Inhibition value of 22.8%, significantly higher inhibition for IgM class antibodies (average 10.7%) than for IgG (average 4.9%), which did not differ from the controls.

Conclusion The new in-house method provides a very interesting picture of the effect of antibodies on the C and S protein system, which may be responsible for the manifestation of antibodies in the form of thrombotic complications in APS.

Assessment of antibody thrombogenicity could provide a new parameter for assessing the severity of individual antibody types, including new risks of new potential anti-PT/PS antibody types.

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P06-08 Evaluation of thrombin generation in patients with sickle cell disease in stable disease and sickle cell crises

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Objective Sickle cell disease (SCD) is complicated by repetitive pain crises and coagulation activation. In this study we looked for a possible way to monitor coagulation activation by using thrombin generation assay

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(Calibrated automated thrombogram CAT) as a global coagulation assay. After specifying preanalytical conditions we compared 11 patients suffering from SCD or sickle cell/beta thalassemia in stable disease and pain crises to 6 patients suffering from transfusion dependent thalassemia.

Material and Methods Blood probes were drawn using corn trypsin inhibitor (CTI) or citrate as anticoagulants and fresh probes were compared to frozen. The tests were performed on a CAT Thrombinoscope system using platelet poor plasma (PPP), phospholipids with high/low tissue factor (TF) concentrations.

Results Citrated blood showed higher peak heights with similar lag-times and endogenous thrombin potential (ETP) compared to CTI anticoagulant in 18 normal controls. There was no difference in fresh or frozen samples. In SCD patients and controls PPP with low TF resulted in a more prolonged lag-phase and lower peak while ETP differed only marginally to PPP high TF. SCD patients showed a higher peak and ETP values compared to controls, but ranges were overlapping. ETP was reduced during sickle cell crises. Patients suffering from crises showed higher ETP and peak heights even outside crises compared to patients with stable disease and lacking pain crises during observation period. TGA values were not related to D-dimers.

Conclusion Hypercoagulable states in SCD patients can be monitored by CAT. ETP is lowered during crises reflecting prolonged coagulation activation and factor consumption. ETP and peak heights ranges overlap between SCD patients and thalassemia patients. Including cellular components as platelet rich plasma or whole blood might reflect more physiological conditions.

P06-09 Interaction of direct oral anticoagulants and other anticoagulants on DOAC Dipstick test

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Objective Determination of direct oral anticoagulants (DOACs) in presence of other anticoagulants (ACs) by conventional coagulation assays remains a major challenge in emergency care. In vitro neutralization of ACs improve the validity of coagulation assays but limitations still remain. DOAC Dipstick determines sensitively and specifically direct oral thrombin inhibitor dabigatran (DTI) and direct oral factor Xa (DXI) in urine. We investigated the interaction of DTI and DXI with unfractionated heparin (UFH), low-molecular molecular weight heparins (LMWH), fondaparinux, r-hirudin and argatroban.

Material and Methods Firstly, patient urine samples on treatment with 110 mg or 150 mg bid dabigatran (n=15) and 10 mg rivaroxaban od (n=15) and LMWH subcutaneously (n=30) and controls without AC therapy (n=5) were spiked with each of 0.0, 0.1, and 1.0 IU/mL UFH, LMWH nadroparin, fondaparinux (µg/mL), r-hirudin (µg/mL) and argatroban (mg/mL). Secondly, no or 1 IU/mL antithrombin was added to urine samples of patients treated with UFH, LMWH and fondaparinux. Thirdly, artificial urine samples were spiked with DTI dabigatran and DXIs apixaban, edoxaban, rivaroxaban and at concentrations of 0 ng/mL and 1500 ng/mL in combination with no or with 1 IU/mL UFH or LMWH enoxaparin and 1 µg/mL fondaparinux (n=8 each). Samples were adjudicated by trained observers as negative or positive in absence and presence of a DOAC and an AC by visual evaluation of colours of DTI and DXI pads of DOAC Dipstick.

Results DTI pads were all negative in the absence of dabigatran and absence or presence of UFH, LMWH nadroparin and enoxaparin and fondaparinux. The positivity of all DTI pads in presence of dabigatran did not change in the presence of UFH, LMWH and fondaparinux. The same results were observed on all DXI pads for any DXI, UFH, LMWH and fondaparinux alone and their combinations. DTI and DXI pads were positive in the presence of UFH, LMWH nadroparin and fondaparinux following addition of antithrombin. Results of DTI pads were positive in the presence of high concentrations of r-hirudin and argatroban.

Conclusion The data show a lack of interaction of DOACs with different types of ACs of the heparin classes. DOAC Dipstick determines on a pad creatinine in urine to prevent incorrect determination of by these types of ACs. Detection of ACs in urine such as other direct thrombin inhibitors by DOAC Dipstick warrants further investigation.

P06-10 Non-criteria antiphospholipid antibodies anti-PT/PS, anti-Anexin V: benefit in diagnostics of antiphospholipid syndrome?

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Objective Background: Antiphospholipid syndrome (APS) is a hypercoagulable state accompanied by the occurrence of antiphospholipid antibodies (APA), which non-specifically affect the process of hemostasis. Clinically, APS is manifested by arterial or venous thrombosis and reproductive loss. The classification of APS is very precisely defined, and therefore at least one clinical and at least one laboratory criteria must be met for a definitive diagnosis of APS. Laboratory criteria include positivity of at least one anti-cardiolipin, anti-β2-glycoprotein-I and lupus anticoagulant antibodies.

Aims: Determination of the frequency of anti-phosphatidylserine/prothrombin (aPS/PT) and anti-Annexin V antibodies as new laboratory markers of APS in patients with suspected suspicion of APS.

Material and Methods Methods: Determination of aPS/PT, anti-Annexin V was performed by ELISA using QUANTA Lite aPS/PT IgM and QUANTA Lite aPS/PT IgG and anti-Annexin V IgG/IgM kits.

Results Results: In a total of 395 patients, APA positivity was found in 67 (17%) patients. In terms of the severity of the clinical manifestation of APS, it is important to assess the severity of the combination of single antibody combinations, where single positivity was found in 47 (11.9%) patients, double positivity in 17 (4.3%), triple positivity in 3 (0.8%). There were 328 (83%) seronegative patients (SN-APS), of which 161 (49.5%) were patients who met the clinical criteria for APS. In the group of SN-APS patients with clinical manifestations of APS, non-criteria positivity of anti-Annexin V antibodies was found in 7 (4.3%), aPS/PT positivity in 17 (10.5%), clinically significant aPS/PT level was found in 5 (3.1%) patients.

Conclusion Conclusions: In our cohort, we detected 17% (67) of patients according to current APS laboratory criteria. Using the detection of anti PS/PT and anti-annexin V, the proportion increased by another 24 patients in the group of seronegative patients with fulfilled clinical criteria of APS. For this reason, we consider it beneficial to extend the diagnosis of APS with other non-criteria antiphospholipid antibodies aPS/PT and anti-Annexin V. The question remains whether to investigate the whole group or only specifically.

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P06-11 Performance evaluation of the BIOPHEN chromogenic test for Factor XIII activity on the Cobas c502 System

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Objective Factor XIII (FXIII) is a protransglutaminase. Upon activation by thrombin and calcium, it acts in the last step of the coagulation cascade leading to fibrin crosslinking and clot stiffness. Acquired FXIII deficiency in critically illed patients, e.g. with trauma, surgery and under ECMO therapy, is not

seldom, which needs a prompt laboratory diagnostic. The routine coagulation tests are unable to identify FXIII deficiency. The specific FXIII activity (FXIII:Ac) tests are only available in special coagulation laboratories. This study was aimed to evaluate analytical performance of the BIOPHEN chromogenic test on the Roche Cobas c502 System.

Material and Methods Precision was performed with normal and pathological controls following CLSI EP5-A3. Limit of Blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) were examined following CLSI EP17-A3. Linearity was performed according to CLSI EP6-A. In addition, patient citrated plasma from routine laboratory were collected and frozen at -80 □C until analysis. Each sample was measured with both Berichrom FXIII assay on the Siemens BCS XP and BIOPHEN FXIII assay on Roche Cobas c502. The evaluation of linearity and comparison of patient samples was performed using Validation Manager software (Finbiosoft, Espoo Finland).

Results The intra-assay, inter-assay and between-day coefficient of variation (CV%) were 1.7%, 1.8% and 1.4%, respectively, at the activity of 93% and 3.2%, 1.4% and 2.5%, respectively, at the activity of 39%. LoB, LoB and LoQ values were 2.4%, 2.7% and LoQ 7.0%, respectively. The linear regression equation in the range of 5,5 - 151% was: measured FXIII:Ac = 1.01*expected FXIII:Ac + 0.264 (r = 1). For method comparison, the regression equation according to Passing-Bablok was: FXIII-BIOPHEN Cobas c502 = 1.993*FXIII-Berichrom BCS XP – 3.56 (r = 0.95). Significant discrepant results between two methods were observed in 5 samples with Lipaemia-index ranged between 64 - 160, implying an interference susceptibility of the test on the Cobas C502 to lipaemia. This should be further clarified with more samples.

Conclusion Our results demonstrated acceptable analytical performance of the BIOPHEN chromogenic FXIII:Ac test on the Roche Cobas c502 system, which makes the 24 hour a day available measurement of FXIII:Ac in an emergency laboratory possible.

P06-12 Quantification of Bovine and Porcine Heparin Red Assay Utilizing the Heparin Red Assay. Applications in the Study of Pharmacokinetics and Pharmacodynamics.

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Objective Primate studies have shown that bovine and porcine heparins dosed at equivalent anti-Xa units produce equivalent anti-IIa and anti-Xa responses in vivo. Heparin's pharmacokinetic behavior is typically based on antithrombin (AT)-dependent anti-Xa and anti-IIa activities. Heparin Red is a polycationic substance whose intrinsic fluorescence is quenched upon binding to heparin. Assays utilizing Heparin Red detect heparin oligosaccharides that bind to AT and those that do not. Non-AT binding heparin oligosaccharides can impact coagulation through their interaction with heparin cofactor II, TFPI and platelets. Thus, the pharmacokinetic behavior of heparin as determined using a Heparin Red assay may more closely reflect the clinical behavior of heparin than pharmacodynamic measures where heparin levels are determined based only on AT-dependent activities.

Material and Methods Primates were administered PMH (Medefil, Glendale Heights, IL) or BMH (KinMaster, Passo Fundo, Brazil) at a dose of 100 anti-Xa U/kg IV. Blood samples were collected prior to and at 15, 30, 60 and 120 minutes post-heparin administration. Heparin levels were assessed using a chromogenic anti-Xa assay and a Heparin Red assay relative to product-specific calibration curves. Pharmacokinetic parameters were assessed using a non-compartmental model.

Results Circulating drug levels based on anti-Xa activity were the same in PMH and BMH-treated primates. Peak levels of 1.45 ± 0.11 and 1.48 ± 0.08 U/ml were observed in PMH and BMH-treated primates, respectively. Using drug levels determined by anti-Xa assay, AUCs for bovine and porcine heparin treated animals were calculated to be 111.5 ± 11.0 and 108.8 ± 26.7 U*min/ml, respectively. By Heparin Red assay, peak heparin levels were higher following BMH administration (10.7 ± 0.5 vs. 9.0 ± 0.2 ug/ml; t-test p<0.001) and the AUC for BMH-treated primates was approximately 22% larger than for PMH-treated primates (728.2 ± 35.3 vs. 594.9 ± 5.4 ug*min/ml; Mann-Whitney test p=0.029).

Conclusion At equivalent anti-Xa unit doses, BMH produces comparable pharmacodynamic effects as PMH despite the presence of higher circulating GAG levels as measured by the Heparin Red assay. Measurement of BMH levels using the Heparin Red assay may be useful for identifying the appropriate dose of protamine to completely neutralize BMH.

P06-13 Reversal of DOAC in RVV-test on the ClotPro analyzer

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DOI 10.1055/s-0041-1728177

Objective The ClotPro is a novel viscoelastometry analyzer. In addition to the established viscoelastometry assay portfolio ClotPro also provides an assay which uses a direct FXa activation by a snake venom (FXa activator from Daboia Russelli/Russel Viper, RVV-test), which is sensitive to DOAC. We examined the reversal of DOACs in the RVV-test using activated carbon.

Material and Methods 39 citrated blood samples collected from patients admitted in our general praxis for routine laboratory analysis. Citrated whole blood was used for duplicate measurements on ClotPro using RVV-test (enicor GmbH, Munich) and . LMWH effects were quantified by the clotting time (CT) (reference range: 48-77 sec). 1 carbon pallet (DOAC-Remove, 5-Diagnostics) was added to 150µl NaCl. 50µl of carbon solution was added to 300µl sample and RVV-test performed. Citrated Plasma was used for determination of anti-Xa activity using a chromogenic one step test (Innovance® Heparin, Siemens) calibrated against apixaban. Analyses were performed on BCS XP.

Results Apixaban concentrations ranged from 11 to 322 ng/ml. There was a good correlation beween the CT in RVV-test and apixaban concentrations (r=0.81). Mean value of CT in RVV-test was 167 sec. (±52 SD) and 82 sec (±22 SD) in sample with carbon. CT in was in mead reduced by 85 sec (±49 SD) by adding carbon. There was no correlation between apixaban concentration and CT in samples with carbon.

Conclusion RVV-test is capable to detect apixaban at concentrations higher than 50 ng anti-Xa U/ml. Addition von carbon to sample is able to remove the effect of apixaban on CT. This may allow to differentiate between coagulopathy by apixaban and deficiency of clotting factors.

P06-14 Thrombopoietin levels in sepsis and septic shock - a meta-analysis.

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Objective Homeostasis of platelet numbers is crucial to ensure adequate hemostatic and inflammatory functionality within the body. One major regulator of platelet production is thrombopoietin (TPO), which was shown to not only influence platelet production, but also to modulate platelet activation. Sepsis is associated with platelet turnover and changes in thrombopoietin levels in systemic inflammation have been reported. Nonetheless, thrombopoietin-levels in sepsis and septic-shock have not yet been systematically evaluated. We thus set out to systematically determine changes in thrombopoietin levels in patients with sepsis and septic shock.

Material and Methods We conducted a meta-analysis of TPO-levels in patients with sepsis. Scientific databases were searched for studies examining

thrombopoietin levels in adult sepsis and septic-shock patients until April 10th, 2020. Two independent reviewers screened records and full-text articles for inclusion.

Results Of 81 items screened, 4 studies met the inclusion criteria, including 295 subjects. Sepsis and severe sepsis were both associated with increased levels of thrombopoietin. TPO-levels did not show significant differences between severe sepsis and septic shock patients. Analysis of platelet counts showed high heterogeneity.

Conclusion Increased levels of thrombopoietin are present both in sepsis and severe sepsis and can be utilized to assess sepsis patients. Thrombopoietin cannot be used to differentiate between severe sepsis and septic-shock but might hint towards different stages of platelet production and –consumption being present during sepsis.

Hereditary bleeding disorders

P07-01 A comparison of prophylaxis dosing frequency and associated clinical outcomes between rVIII-SingleChain and other rFVIII products commonly used in Italian patients with haemophilia A

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Objective Several recombinant factor VIII (rFVIII) products are available in Italy to treat haemophilia A, including long-acting products with improved pharmacokinetic properties enabling extended dosing intervals. In the absence of head-to-head comparisons, an evaluation of their real-world utilisation may help to optimise treatment choice. The aim of this study was to determine factor consumption and annual bleed rates (ABR) following the prophylactic use of rVIII-SingleChain compared with other commonly used rFVIII products.

Material and Methods Haemophilia Treatment Centres provided de-identified chart information for 290 patients treated with one of the following four rFVIII products for a minimum of 8 weeks: rVIII-SingleChain (n=60), rFVIIIFc (n=73), BAY 81-8973 (n=74), and octocog alfa (n=83). Where possible, patients were matched by age and disease severity in order to ensure heterogeneity of the groups. To calculate mean ABR, the number of reported bleeds were annualised, and prophylactic factor consumption was calculated using the dose and infusion frequency on the patient's latest prescription.

Results A data summary is shown in Table 1. Most patients treated with long-acting products were dosed ≤2 x weekly (rVIII-SingleChain, 56.7%/53%; rFVIIIFc, 76.7%/75.4% for all patients and those with severe disease, respectively). In contrast, most patients treated with standard acting products infused >2 x weekly (octocog alfa, 83.1%; BAY 81 8973, 63,5%). Mean ABR was 0.4 with rFVIIIFc, 0.7 with rVIII-SingleChain, 0.8 with BAY 81 8973 and 1.2 with octocog alfa. All products reported a median annual spontaneous bleed rate of 0.0; zero spontaneous bleeds were reported in 88.3%, 87.7%, 82.4%, and 71.1% of patients treated with rVIII-SingleChain, rFVIIIFc, BAY 81-8973 and octocog alfa, respectively. Overall, mean weekly consumption was lowest with rVIII-SingleChain (92.7 IU/kg), which was 10.7%, 17.7% and 24.3% less than rFVIIIFc, BAY 81-8973 and octocog alfa, respectively.

Conclusion Similar to other long-acting products, rVIII-SingleChain shows reduced dosing frequency and factor consumption whilst maintaining comparable efficacy to standard-acting rFVIII products. This retrospective patient chart review provides further evidence that the excellent outcomes observed during rVIII-SingleChain clinical trials can also be achieved in real-world practice.

▶**Tab 1.** Patient characteristics, bleeding rates, infusion frequency and factor consumption with selected long-acting and standard-acting rFVIII products.

Product (patients, n)	Age (years), mean (SD)	Disease severity	Treatment duration (weeks), median (IQR)	ABR, median (IQR)	ABR, mean (SD)	Frequency of infusion	Consumption (IU/kg/week), median (IQR)	Consumption (IU/kg/week), mean (SD)
Long-acting								
rVIII-SingleChain (60)	33.1 (18.5)	Severe (81.7%) Mild/Moderate (18.3%)	40.6 (17.6–52)	0.0 (0.0–0.5)	0.7 (1.4)	>2×/week (43.3%) ≤2×/week (56.7%)	91.6 (62.7–108.9)	92.7 (36.0)
rFVIIIFc (73)	28.4 (19.9)	Severe (89.0%) Mild/Moderate (11.0%)	52 (52–52)	0.0 (0.0–1.0)	0.4 (0.8)	>2×/week (23.3%) ≤2×/week (76.7%)	92.3 (80.0–109.9)	103.8 (70.5)
Standard-acting								
Octocog alfa (83)	27.1 (17.2)	Severe (85.5%) Mild/Moderate (14.5%)	52 (52–52)	1.0 (0.0–2.0)	1.2 (1.6)	>2×/week (83.1%) ≤2×/week (16.9%)	113.2 (78.9–140.0)	122.4 (66.3)
BAY 81-8973 (74)	27.5 (19.2)	Severe (82.4%) Mild/Moderate (17.6%)	52 (52–52)	0.0 (0.0–1.0)	0.8 (1.3)	>2×/week (63.5%) ≤2×/week (36.5%)	100.0 (80.0–121.7)	112.7 (68.6)

P07-02 A matching-adjusted indirect comparison in patients with severe haemophilia A: Comparing the efficacy and consumption of rVIII-SingleChain vs two recombinant FVIII

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Objective Head-to-head comparisons of recombinant factor VIII (FVIII) products in randomised controlled clinical trials are difficult to conduct due to the rarity of haemophilia. The objective of this matching-adjusted indirect comparison was to compare the prophylactic efficacy and factor consumption of rVIII-SingleChain (lonoctocog alfa, AFSTYLA) versus rAHF-PFM (octocog alfa, Advate) and rFVIIIFc (efmoroctocog alfa, Elocta), for the treatment of bleeding episodes in previously treated adults/adolescents with severe haemophilia A.

Material and Methods Published clinical trials of rAHF-PFM and rFVIIIFc in the target population were identified from a systematic literature search. Individual patient data from the rVIII-SingleChain pivotal study were used to match baseline patient characteristics to those from published trials, using an approach similar to propensity score weighting. After matching, treatment outcomes were compared across trial populations. For prophylaxis, annualised bleeding rates (ABR), percentage of patients with zero bleeds, and weekly FVIII consumption were analysed. Relative treatment effects are presented as risk ratios (RR), odds ratios (OR) and median differences (MD) with 95% confidence intervals.

Results For rVIII-SingleChain, the adult/adolescent patient population receiving prophylaxis in the phase I/III trial was selected (n=145). Three published clinical trials were identified: two for rAHF-PFM (from 2004 [n=111] and 2012 [n=32]) and one for rFVIIIFc (from 2014, [n=118]). After matching, rVIII-SingleChain had similar ABR (RR: 0.74 [0.16; 3.48]; RR: 1.18 [0.85; 1.65]) and percentage of patients with zero bleeds (OR: 1.34 [0.56; 3.22]; OR: 0.78 [0.47; 1.31]) vs rAHF-PFM-2004 and rFVIIIFc, respectively. Median annualised rVIII-SingleChain consumption was significantly lower than rAHF-PFM (MD:

−1507.66 IU/kg/year [−2011.71; −1003.61]) and equivalent to rFVIIIFc (RR: 0.96 [0.62; 1.49]).

Conclusion This study suggests that routine prophylaxis with rVIII-SingleChain in the conditions observed in the study results in consumption comparable to rFVIIIFc and significantly lower than rAHF-PFM, while maintaining a similar ABR and percentage of patients with zero bleeds, attesting to the long-acting nature of rVIII-SingleChain.

P07-03 Annual bleeding rate and factor consumption – comparison between extended and short half-life factor VIII in real life according to electronic documentation smart medication

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Objective With the introduction of extended half-life factor VIII products (EHL) bleeding rate (BR) and factor consumption (FC) may change in comparison to previous treatment with short half-life factor VIII (SHL).

Material and Methods Joint bleeds and factor VIII consumption was compared between patients receiving EHL and SHL. Included were patients who received at least 12 weeks EHL concentrates within 12 months before August 2020.

Results 108 patients were treated with EHL with a total number of 12203 entries in their electronic diary. 67 received only EHL (EHL-group), 41 mainly SHL followed by EHL (SHL-group). The schedule of prophylaxis was 2.26 in the EHL and 3.21 in the SHL group ($p < 0.05$). Weekly factor consumption (IE/kg BW) was 73 in the EHL and the SHL group. Relation of FC for prophylaxis vs. bleeding + follow-up was 94/6 in the EHL and 89/11 in the SHL group. Calculated annual joint bleeding rate was 0,81 in the EHL and 2.48 in the SHL group ($p=0.059$).

Conclusion Patients on EHL documented an approximately twice weekly prophylaxis, compared to nearly three times weekly with SHL. Lower BR and lower FC for bleeding episodes were documented in the EHL group, which was not significant most likely due to small patient numbers. Ongoing real life analysis comparing SHL vs. EHL are required.

P07-04 A retrospective patient chart review of real-world clinical outcomes and prophylactic factor VIII consumption in Italian patients with haemophilia A switching to extended dosing intervals with rVIII SingleChain

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Objective The introduction of long-acting recombinant factor VIII (rFVIII) therapies, with improved pharmacokinetic properties compared with standard-acting FVIII therapies, has reduced treatment burden and improved clinical outcomes for patients with haemophilia A. Clinical trials have shown that rVIII-SingleChain, a long-acting rFVIII, can be used effectively for the prophylactic treatment of haemophilia A. This study aimed to determine real-world dosing intervals, factor utilisation and bleed rates in a cohort of Italian patients with haemophilia A who switched to rVIII-SingleChain from a previous FVIII product.

Material and Methods De-identified patient chart information was provided for 60 patients currently treated with rVIII-SingleChain. Data collected included age, sex, weight, current and prior treatment regimen (prophylaxis/episodic, infusion frequency, dosing), and number of reported bleeds. Prophylactic factor consumption for rVIII-SingleChain and previous product were calculated using the dosing and infusion frequency on the patient's most recent prescription. The number of reported bleeds was annualised to calculate mean annual bleed rate (ABR) for rVIII-SingleChain and previous product.

▶**Tab 1.** Dosing frequency, consumption and bleeding rates with rVIII-SingleChain and prior FVIII product for all prophylaxis-to-prophylaxis patients and prophylaxis-to-prophylaxis patients with severe disease only.

	All patients (N=53)		Severe disease only (N=45)	
	rVIII-SingleChain (n=53)	Prior FVIII (n=53)	rVIII-SingleChain (n=45)	Prior FVIII (n=45)
Age (years), mean (SD)	32.5 ± 18.8*		33.2 ± 18.3*	
Dose, IU/kg/week ± SD	95.0 ± 37.1	94.6 ± 38.5	96.6 ± 38.1	98.3 ± 40.1
Prophylaxis dosing interval, %				
>2×/week	49.1	56.6	51.1	62.2
≤2×/week	50.9	43.4	48.9	37.8
ABR, mean ± SD	0.5 ± 1.2	1.8 ± 1.7	0.6 ± 1.2	1.8 ± 1.5
AsBR, mean ± SD (zero spontaneous bleeds, %)	0.3 ± 1.1 (90.6)	1.1 ± 1.6 (45.3)	0.3 ± 1.1 (91.1)	1.1 ± 1.3 (42.2)

*Age only available for patients on current product; date of birth information not collected to ensure patient anonymity

Results In total, 60 patients (49 with severe disease, 11 with moderate or mild disease) treated with rVIII-SingleChain prophylaxis were included in the study with 50.9% dosed ≤2 x weekly. Of these, 88% (n=53) were also treated prophylactically with their prior drug, with 54.7% of patients dosing at least 3 x weekly. For the 53 patients switching from prophylaxis with previous product to prophylaxis with rVIII-SingleChain (Table 1), mean +/- standard deviation ABR decreased from 1.8 +/- 1.7 (median, 1.0) with previous drug to 0.5 +/- 1.2 (median, 0.0) with rVIII-SingleChain. The percentage of patients with zero spontaneous bleeds increased from 45.3% to 90.6% of patients. Weekly mean prophylactic factor consumption (IU/kg) was similar with previous drug or rVIII-SingleChain (94.6 +/- 38.5 and 95.0 +/- 37.1, respectively).

Conclusion This study shows that patients switching to rVIII-SingleChain can be well managed when treated with extended prophylactic dosing intervals, with effective bleed prevention and no increase in factor utilisation as compared with their previous FVIII product.

P07-05 Characterization of rFIX fusion proteins enabling subcutaneous administration

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Objective The recessive X-linked bleeding disorder Hemophilia B is caused by a mutation in the coagulation factor (F) IX gene leading to partial or total loss of its function. Although new recombinant FIX therapeutics like the albumin-fusion protein rIX-FP (IDELVION®) enable longer half-life in circulation and thus less frequent administration, the complexity of intravenous (IV) injection affects patients' quality of life. Therefore, this project focused on the characterization of rIX-FP-variants with anticipated enhanced specific activity, that would combine rIX-FP's superior pharmacokinetic (PK) profile with that of subcutaneous (SC) administration.

Material and Methods Three different rIX-FP-variants were generated via mutation on the respective amino acid positions: R338L ("Padua mutant"), R338L + E410K or R318Y + T343R + R338E. The resulting rIX-FP-variants were subsequently recombinantly produced, purified and further characterized in vitro. PK profiles of selected variants were evaluated in FIX-deficient mice after SC administration based on antigen levels. Efficacy of the most promising variant was finally tested after activity-based IV dosing in a tail-clip bleeding assay. Data were compared to the marketed wildtype (WT) rIX-FP product.

Results All three rIX-FP-variants showed increased specific activity in vitro compared to WT rIX-FP (4 to 5-fold), whilst FXIa-mediated activation was the fastest for rIX-FP(R338L) and WT rIX-FP. rIX-FP(R338L) furthermore exhibited a favorable PK profile compared to WT rIX-FP (i.e. comparable area under the curve (AUC0-inf) based on antigen values but 7.2-fold higher AUC0-inf based on activity values). Compared to untreated FIX-deficient mice, it moreover demonstrated significantly reduced bleeding time and total blood loss, whilst efficacy was comparable to the marketed WT rIX-FP. The double and triple rIX-FP-variants were excluded from complete in vivo testing due to an inferior PK profile and internal depriorization, respectively.

Conclusion rIX-FP(R338L) demonstrated an increased specific activity combined with a favorable activity-based PK profile. Subsequently, comparable efficacy to the marketed rIX-FP product could be achieved at one sixth of the antigen dose. Hence, rIX-FP(R338L) could be a promising option for SC administration in hemophilia B patients, reducing the burden of IV dosing.

P07-06 Development of annual joint bleeds and factor consumption during the last 6 years in 14 haemophilia centers - real life data from electronic documentation smart medication

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Objective Electronic diary has shown to be a valuable tool for analysis of annual joint bleeding rate (AJBR) and annual factor VIII/IX consumption (AFC) in real life setting. What has changed over a six-year period?

Material and Methods AFC and AJBR among 391 patients with haemophilia A/B from 14 haemophilia centers in 2019 were compared to data to prior years (277 patients from 9 centers in 2017, both gradually increasing since then) according to electronic documentation smart medication.

Results Looking at five consecutive years, the average AFC (IU/kg BW) was 2442, 2701, 2575, 2670, 2924 and 2847 the average AJBR 2.1, 2.5, 2.3, 2.2, 1.9 and 1.7 between 2014 and 2019, respectively. Four groups, comparing above or below average AFC and AJBR, were compared between 2014 -2019: The majority (45%/40%/44%/45%/40%/42%) had an AJBR of ≤ 2 with less than average AFC, followed by a group (31%/35%/32%/32%/29%/28%) with ≤ 2 AJBR but above average AFC. A minor group (14%/11%/15%/15%/14%/14%) had an AJBR > 2 and more than average AFC. Only few (10%/14%/8%/8%/17%/15%) had an AJBR > 2 but less than average AFC.

Conclusion In 2019 the AJBR was slightly lower, the AFC slightly higher compared to all prior years. Whether this reflects changes in treatment or is due to an increasing number of participating patients and centers needs to be further analyzed. With the introduction of extended half-life products, the future bleeding and factor consumption may change, demonstrating the necessity for ongoing electronic surveillance in haemophilia care.

P07-07 Functional assays to unravell the pathogenetic role of variants found in GFI1B in piastrinopenic patients

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Objective GFI1B is an important transcription factor for megakaryopoiesis, known as causative of an Inherited thrombocytopenia (IT) form named platelet-type bleeding disorder 17. During screening analysis of IT patients, we have found five new missense variants and a splicing one. Unravelling the functional impact of non sense or frameshift variants is usually easy, while assessing the role of missense or splicing variants requires gene-specific functional studies.

Material and Methods Our patients have low platelet count and NGS analysis with our Ion Torrent panel showed no other variants in other ITs genes.

All the variants were analysed with bioinformatics tools and for the segregation in the family. We firstly analysed the splicing variant, which is predicted to cause skipping of exon 9. From patient RNA we performed RT-PCR to verify the presence of exon skipping and Real time PCR on genes involved in oncogenic pathways. Finally, we perform a luciferase reporter assay on some known target gene promoters.For the missense variant luciferase assays are in progress.

Results We have firstly confirmed the skipping of exon 9, and we found overexpressed a shorter form of the transcript, previously described in literature because is increased in chronic and acute leukemia. Then, we evaluated the transcriptional activity with a luciferase gene reporter assay: the wild type form act as a transcription repressor, while the variant loses the repression activity and acts as a dominant negative. We repeated this experiment with CD34 promoter, because the increased expression of CD34 on platelet surface is the major character in common between GFI1B patients. Also here, there is a loss of repression. To further confirm that the splicing variant has a role in malignancies development too, we performed Real time PCR on oncogenic genes, which we found dysregulated (overexpressed) in the patient when compared to healthy control.

Conclusion Our findings suggest that the splicing variant has probably a role in thrombocytopenia, because shows a dysregulation of the target genes. Moreover, the dysregulation of some oncogenic genes could be seen as a starting point for malignancies development, thus making patients more

susceptible to develop these diseases. It is therefore clear how also try to evaluate the role of the missense variant found is very important for patient management and for a better understanding of the disease.

P07-08 Increased soluble thrombomodulin influences fibrin clot formation in patients with mild to moderate bleeding tendency

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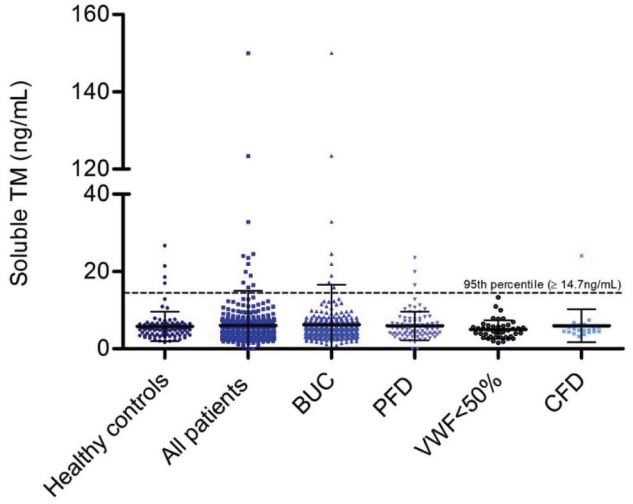
Objective A majority of patients with a mild bleeding disorder (MBD) remains without a diagnosis, despite thorough hemostatic investigations (bleeding of unknown cause, BUC). Recently, a coagulopathy with posttraumatic bleeding caused by 100-fold enhanced levels of soluble thrombomodulin (TM) was reported in several cases.

We investigated TM levels in a large and well-characterized cohort of patients with MBDs and healthy controls and how high TM levels affect thrombin generation and plasma clot formation.

Material and Methods TM and thrombin generation (TG) were measured using commercially available kits (ab46508 – TM (CD141) Human ELISA Kit – Abcam UK; TG Technothrombin, Technoclone, Austria). Plasma clot formation/lysis was assessed according to SCC recommendations of the ISTH.

Results TM was not altered in 507 patients, including 358 BUC patients, with MBD when comparing to 90 sex- and age-matched healthy controls (median [IQR] 5.0 [3.8-6.3] vs. 5.1 [3.7-6.4] ng/mL, multiple regression with adjustment for sex p=0.801; Figure 1). Also in the BUC patients no difference was found (TM: 5.05 [3.8-6.3] ng/mL, multiple regression after adjustment for sex p= 0.695). To identify outliers of TM levels in our patients, a cut-off according to the 95th percentile of TM in healthy controls (≥ 14.7 ng/mL) was defined. No increased number of patients above the predefined cut-off was identified (OR [95% CI]: 1.9 [0.6-6.1]). Nevertheless, 2 BUC patients had clearly elevated TM levels (150.1 and 123.4 ng/mL, reference range: 2.9-7.6), but TG and plasma clot properties were not significantly altered.

We identified a prolonged time to peak (TTP) in fibrin clot analysis between patients with TM levels above vs. below the cut-off (mean (SD): 28.7 (31.7) vs. 20.0 (7.4) minutes, p<0.001), which was also significant in subgroup analysis of patients with BUC (Table 1). In the thrombin generation assay no differences could be found. Levels of TM did not correlate with both the Vicenza (rs=-0.052, p=0.239) and the ISTH bleeding score (rs=-0.057, p=0.368).



▶**Abb 1.** Scatter plot (including mean and SD) of TM levels in patients and healthy controls. BUC: bleeding of unknown cause; PFD: platelet function defects; VWF: Von Willebrand factor; CFD: clotting factor deficiency

▶**Tab 1.** Parameters of fibrin clot formation assay according to TM values (<95th vs. ≥ 95th percentile of the healthy controls).

	TM <95th percentile (< 14.7 ng/mL)	TM ≥ 95th percentile (≥ 14.7 ng/mL)	p	BHC
All patients	***n= 495***	***n= 12***		
Lag time, min, median (IQR)	10.7 (7.7-24.3)	9.7 (6.6-18.2)	*.898*	*ns*
ΔAbs, OD 405nm, mean (SD)	0.72 (0.18)	0.64 (0.15)	*.114*	*ns*
TTP, min, mean (SD)	20.0 (7.4)	28.7 (31.7)	***.001***	***<.05***
Vmax, OD/min, mean (SD)	0.13 (0.05)	0.11 (0.05)	*.175*	*ns*
CLT, min, median (IQR)	15.6 (13.1-18.9)	13.9 (12.6-19.9)	*.798*	*ns*
BUC	***n=350***	***n=8***		
Lag time, min, median (IQR)	10.3 (7.2-13.7)	10.9 (7.5-18.2)	*.397*	*ns*
ΔAbs, OD 405nm, mean (SD)	0.73 (0.17)	0.61 (0.15)	***.046***	*ns*
TTP, min, mean (SD)	19.4 (6.9)	32.2 (37.1)	***<.001***	***<.05***
Vmax, OD/min, mean (SD)	0.14 (0.05)	0.10 (0.04)	*.039*	*ns*
CLT, min, median (IQR)	15.7 (13.3-19.3)	16.5 (13.3-19.9)	*.871*	*ns*

BUC, bleeding of unknown cause; SD, standard deviation; ns, not significant; IQR, interquartile range; BHC, Bonferroni-Holm correction; TTP, time to peak; CLT, clot lysis time

Conclusion Soluble TM was not increased in patients with MBD or patients with BUC in comparison to healthy controls. Two patients with high TM levels were identified, though levels were not as high as in reported cases of TM-associated coagulopathy. Thus, TM appears not to have an impact on bleeding in patients with MBD and/or BUC.

P07-09 In vitro characterization of K5A and K5R variants of Factor IX

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DOI 10.1055/s-0041-1728194

Objective Recent studies indicate that binding of coagulation factor IX (FIX) to extracellular matrix (ECM) contributes to its extravascular (EV) storage and its hemostatic efficacy. It is postulated that the binding affinity of FIX to ECM, more specifically to Collagen IV (Col-IV), increases when Lysine at position 5 is mutated to Arginine (K5R) and decreases when Lysine is mutated to Alanine (K5A). To understand the physiological role of FIX in EV space we aimed to characterize recombinantly generated K5 variants in comparison to wild-type rFIX molecules. We used albumin fused (FP) rFIX variants (rFIX-FP, K5A-FP, K5R-FP) for this purpose.

Material and Methods A functional characterization of the K5 variants was conducted in a FIX one stage clotting assay and in a modified FIX chromogenic activity assay, mimicking the tenase complex in vitro. In addition, enzymatic activity of rFIX variants was characterized using a FIXa-specific chromogenic substrate. Both characterizations were analyzed on a Michaelis-Menten kinetic based model, and the kinetic parameter Km was used as key comparator. Binding of rFIX molecules to Col-IV was measured by SPR.

Results All K5 variants decreased the clot formation time substantially from 76 to 37-38 seconds (≈1IU/mL FIX clotting activity). In the tenase complex both mutants K5A-FP and K5R-FP showed an identical Km (0.20 nM). These results are consistent with the observation that the specific activity (FIX clotting activity per total protein amount) of K5R-FP and K5A-FP displayed similar specific activities, 60 IU/mg and 59 IU/mg, respectively. For the FIXa specific substrate, K5 variants showed comparable rates of enzymatic activity relative to wildtype. Finally, no detectable interaction between immobilized Col-IV and rFIX or rFIX-FP or K5 variants at 0.2 µM was observed.

Conclusion Our experiments showed the structural integrity of the K5 variants and their comparable enzymatic characteristics to wildtype. In-

house data on binding properties of these rFIX to Col-IV contradicts published results that posit Col-IV as the binding partner of FIX in the EVS.

P07-10 Joint bleeds and pain related treatment – comparison of data between 2017 and 2019 according to electronic diary smart-medication

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DOI 10.1055/s-0041-1728195

Objective Joint bleeds in patients with haemophilia A/B may cause severe pain leading to immediate or delayed factor treatment, as well as different dosing in home settings.

Material and Methods Result from 391 patients from 14 haemophilia centers during 2019 were analyzed according to electronic data from smart medication and compared to results of the prior year (277 patients, 9 centers). Severity of pain (SP) on a scale of 1 (very mild) to 10 (very severe) was related to the respective initial treatment dose as well as time gap between joint bleed (JB) and treatment

Results The annual rate of JBs (AJBR) was 2,20 in 2017 and 1.68 in 2019. The initial treatment dose (IU/kg BW) following JBs was 23.54 – 62.55 (2017) and 25.45 – 34.29 (2019). Severe pain (SP 8-10) was followed by treatment doses of 23.54- 27,94 (2017) and 33.11 - 34,29 (2019). The time between bleeding symptom and treatment ranged from <1 hour in 36% (2017) and 25% (2019) to >4 hours 13% (2017) and 18% (2019).

Conclusion In 2019 a lower AJBR and slightly higher dosing following severe pain compared to 2017 was seen. Initial treatment following bleeding was slightly later in 2019 compared to 2017.

P07-11 Long-term efficacy and safety of rIX-FP prophylaxis in adult patients with haemophilia B on a 21-day dosing regimen

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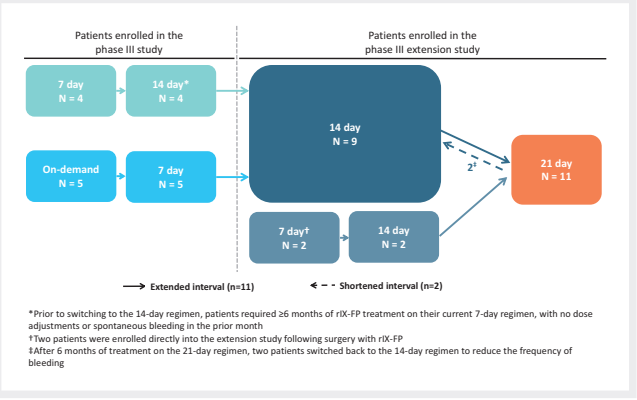
DOI 10.1055/s-0041-1728196

Objective The introduction of factor replacement products with enhanced half-life has helped patients with haemophilia B to overcome one of the limitations of standard factor IX (FIX) products by allowing less frequent infusions. rIX-FP is one such FIX product, that provides a prolonged dosing interval in patients with haemophilia B. A phase III extension study evaluated the long-term efficacy and safety of rIX-FP prophylaxis in adult patients with haemophilia B, including a subset of patients treated on a 21-day dosing regimen.

Material and Methods During the phase III clinical trial, patients with severe haemophilia B (FIX ≤2%) received episodic treatment or routine prophylaxis with rIX-FP. Patients who initially received episodic treatment were switched to a 7-day regimen with rIX-FP (35–50 IU/kg); dosing intervals could then be changed at any 6-month follow up. After 6 months, patients could extend to a 10- or 14-day interval (50–75 IU/kg), if well controlled on the 7-day regimen. During the extension study, patients ≥18 years could extend to a 21-day regimen (100 IU/kg) if well controlled on a 14-day regimen for ≥6 months.

Results A total of 11 patients switched to a 21-day regimen (Figure 1). The mean (SD) monthly consumption of rIX-FP was lower on the 21-day regimen (146.9 [5.53] IU/kg) compared with the 7-day (178.7 [24.63] IU/kg) and 14-day regimens (166.6 [6.21] IU/kg). These 11 patients had a median annualised spontaneous bleeding rate (AsBR) of 0.0 while on prophylaxis throughout the study program; during their time on the 21-day dosing regimen, 64% (7/11) of patients reported zero spontaneous bleeding episodes and the median AsBR was 0.0 (range: 0.0–4.7). After 6 months of treatment on the 21-day regimen, two patients switched back to the 14-day regimen due to breakthrough bleeding. Ten patients reported a total of 29 adverse events over four years; the majority (89.7%) were reported as mild/moderate in intensity with one (peritonsillar abscess) considered severe, and all were unrelated to rIX-FP treatment. No inhibitors, anaphylactic reactions or thromboembolic events were reported.

Conclusion Prophylaxis with rIX-FP on a 21-day dosing regimen demonstrated good efficacy and safety in a selected subset of patients with haemophilia B.



▶ **Abb 1.**

P07-12 Monitoring pain and joint health in patients with haemophilia

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DOI 10.1055/s-0041-1728197

Objective Patients with haemophilia experience joint bleeds and develop chronic joint disease and pain leading to reduced mobility. Patient reported outcome is crucial to optimize treatment. We developed a simple questionnaire to assess pain and joint health to investigate the current status of our patients and the possibility for treatment optimization.

Material and Methods All patients with haemophilia received the questionnaire during a personal visit at our haemophilia treatment center. The following items were asked: presence and location of pain and pain intensity in the last four weeks, triggering factors, physical activity and therapeutic interventions. The patient charts were evaluated for type and severity of haemophilia and treatment regimen.

Results 159 patients (pts) returned at least one questionnaire including 139 (87%) pts with haemophilia A (HA), 20 (13%) with haemophilia B (HB), 127 (80%) pts with severe haemophilia. The average age at the time of the first questionnaire was 38 years. 127 (80%) pts were treated with prophylaxis, 96 (60%) patients reported regular physical activity. Pain in the last four weeks before the visit date was stated by 119 (75%) pts. The ankle joint (n = 74, 47%), knee joint (n = 46, 29%), elbow joint (n = 40, 25%), followed by shoulder joint (n = 27, 17%) and hip joint (n = 14, 9%) were the reported joints with decreasing frequency. 30 (19%) pts reported pain in other parts of the body. As a trigger for pain 93 (58%) pts mentioned extended physical activity and 44 (28%) pts a bleed. 57 (36%) pts reported pain at rest and 26 (16%) pts reported constant pain. The mean severity of pain on the numeric analog scale (NAS) was 3.26. For the treatment of joint pain 86 (54%) pts used factor replacement, 93 (58%) pts pain medication and 62 (39%) pts used conservative measures like immobilization and cooling. 40 (25%) pts performed physiotherapy regularly and 10 (6%) pts were treated by pain specialist. 104 (65%) pts reported that the used treatment was successful.

Conclusion In addition to the medical visit the questionnaire detected relevant information about pain and joint health in pts with haemophilia. Despite significant improvements in the treatment of haemophilia and 80% of pts on prophylaxis pain and joint health are of major importance and need further treatment optimization. Standardized patient reported outcome might add relevant input for this challenge.

P07-13 Newly diagnosed children and adolescents with haemophilia A and B in Germany – the GEPHARD study of the `Standing Commission Paediatrics of the Society for Thrombosis and Haemostasis Research

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DOI 10.1055/s-0041-1728198

Objective In Germany, 40-60 newborns are expected to be diagnosed with haemophilia per year. Haemophilia leads to recurrent bleeds and increased morbidity and mortality. Prophylaxis is the standard of care to prevent bleeds and sequela. The optimal timing or regimen to start prophylaxis to avoid the development of joint disease or neutralizing antibodies to clotting factors are still being discussed. Except for the German haemophilia registry documenting a limited set of data, no national data are available on incidence, treatment, clinical course or outcome of newly diagnosed haemophilias in Germany.

Material and Methods The German Paediatric Haemophilia Research Database (GEPHARD) enrols children and adolescents (<18 years) that have been diagnosed with haemophilia (FVIII or FIX levels <25%) since January 2017. This prospective registry is open to all centres and documents variables related to diagnosis, therapy and outcome including but not limited to inhibitor development, offers quality assurance and serves as a base for future studies. GEPHARD works closely together with the German Haemophilia registry and PedNet.

Results The database has been established and longitudinal documentation has been started. Funding from most companies offering FVIII or FIX products in Germany has been secured for initial five years. Since January 1st 2017 216 children and adolescents have been enrolled from 36 participating centres. For those children and adolescents with information available, 178 children were diagnosed with haemophilia A including 106, 18 and 54 with a severe, moderate or mild phenotype, respectively. Thirty children were diagnosed with haemophilia B including 17, 7 and 6 with a severe, moderate or mild phenotype, respectively. The median age of diagnosis for severe (n = 106), moderate (n = 18) or mild (n = 54) haemophilia A was 0.42, 0.04 and 1.75 years. For haemophilia B, the median age of diagnosis was 0.17, 2.75 and 3.21 for severe (n = 17), moderate (n = 7) and mild (n = 6). Since 2017, inbetween 58 and 66 patient have been recruited every year.

Conclusion The GEPHARD community has included 216 children from January 2017 to June 2020. Following administrative issues which have been solved, the current pandemic poses a further burden on a central and longitudinal documentation. Nevertheless, longitudinal documentation has been started and will provide additional data in the near future.

P07-14 Real-world experience on the use of rIX-FP in patients with haemophilia B: Interim results from a prospective, non-interventional, post-market surveillance study in Germany

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DOI 10.1055/s-0041-1728199

Objective The phase 3 extension study with rIX-FP, a long-acting fusion protein, has demonstrated low annualised bleeding rates in patients with haemophilia B treated with rIX-FP prophylaxis. rIX-FP enables treatment to be tailored to the needs of individual patients, with dosing flexibility allowing selected patients to be treated with prophylaxis intervals of 7, 10, 14 or 21 days. Data on the use of rIX-FP in routine clinical practice are required. Prospective, non interventional, multicentre studies are ongoing to gather data on efficacy, safety and health-related quality of life outcomes in patients treated with rIX FP during routine clinical practice in Europe.

Material and Methods A non-interventional study in Germany was initiated in March 2018; all patients with haemophilia B were eligible for enrolment. Patients are treated prophylactically with rIX-FP with dosing regimens up to every 14 days and undergo routine monitoring every 3–12 months. Patients are followed for 2–3 years or until 100 exposure days. Patient data is collected, stored and pseudonymised in accordance with General Data Protection Regulations.

Results As of May 2020, 52 patients across Germany were enrolled in the study; the majority of patients have moderate or severe haemophilia B. Patients range in age from 1–80 years and have between 0 and 343 exposure days to rIX-FP. At this point, 22 adverse events were recorded in this cohort, none of which were considered related to rIX-FP, and no patients have developed inhibitors. Two patients discontinued from the study, one patient was removed due to lack of treatment satisfaction and one patient requested to withdraw but remained on rIX-FP prophylaxis. The interim data cut-off is due to occur later in 2020, and further data collected will be reported.

Conclusion Initial data from this study indicates that rIX-FP is well tolerated, with low bleed rates and few adverse events in both adults and paediatrics in

routine clinical practice. Further data will be collected to assess the effectiveness and safety profile of rIX-FP in the real-world clinical setting.

P07-15 Usefulness of global assays to monitor treatment of a patient with Hemophilia A switching from factor to non-factor replacement therapy

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DOI 10.1055/s-0041-1728200

Objective Hemophilia A is a rare bleeding disorder characterized by the deficiency of coagulation factor VIII (FVIII). Emicizumab is a humanized bispecific antibody which acts as a bridge between activated factor IX (FIXa) and factor X (FX), thus replacing the hemostatic function of the missing FVIII. However, emicizumab interferes with conventional coagulation assays, precluding the possibility to evaluate the degree of correction of the hemostatic competence of the patient in response to treatment. Global coagulation assays (GCA) could be an interesting alternative. Here we studied thrombin generation (TG) and fibrin clot formation (FCF) profiles in a 18 years old patient switching from factor replacement to emicizumab treatment. Our aim was to investigate whether GCA could be used to successfully monitor non-factor replacement therapy with emicizumab.

Material and Methods TG was measured with the reference method Calibrated Automated Thrombogram (CAT) and with the new fully automated ST Genesia system (Stago, Asnières-sur-Seine, France). FCF was measured with the innovative Thrombodynamics Analyzer (Hemacore, Russia) which monitors the spatio-temporal (tissue factor [TF]-dependent and -independent) dynamics of coagulation. The patient received subcutaneously a weekly dose of Hemlibra® (3 mg/kg per body weight W1-4; 1.5 mg/kg from W5 onwards). Response to treatment was monitored weekly during a two months period. Emicizumab was measured with a modified aPTT-based assay. Analyses of TG and FCF were performed in platelet poor plasma in presence of TF (1 pM or 100 pmoles/m2 respectively) and phospholipids (4uM).

Results Treatment with emicizumab improved TG and FCF compared to baseline. FCF normalized already after one week of treatment, reaching a plateau that lasted until the end of the monitoring two months later. TG, which normalized after two weeks of treatment, and FCF in presence of emicizumab were in the lower normal range and much lower that the values observed after replacement with rFVIII. Of note, increasing emicizumab concentrations observed after W1 did not further improve TG or FCF parameters.

Conclusion According to this limited experience, emicizumab seems to improve the hemostatic potential in an "all-or-nothing" manner. TG assays are a promising tool to evaluate the hemostatic status of patients receiving non-factor therapy. Further investigations are needed to confirm this observation.

Innovation and Novelties

P08-01 An analysis of fatalities in persons with congenital hemophilia A (PwcHA) reported in the FDA Adverse Event Reporting System (FAERS) database

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Objective Disease- and treatment-associated mortality is of great interest. The US FAERS database catalogs adverse events reported to the FDA by industry, healthcare providers and patients/carers. We report the results of an analysis of the causes of death in PwcHA over the last 2 decades treated with FDA-approved coagulation products reported to FEARS and compare them across conventional therapies and emicizumab.

Material and Methods The FAERS dashboard was searched for all FDA-approved Factor VIII products and bypass agents covering the period from 1 January 2000 to 31 December 2019. In cases of multi therapy, the first therapy reported was used for classification. Duplications, events outside of congenital HA were excluded; results were categorized according to the HA mortality framework (NHF-abstract#). It was assumed that known limitations of FAERS apply to all products. The analysis was performed without correction of known biases in the reporting of adverse events.

Results Contemporaneous to coagulation product use 723 fatalities (409 cHA, 223 acquired HA, 91 unknown) were identified in FAERS. Excluding acquired HA: North America (31.6%), Asia/Pacific (29.4%), Europe (23.4%), other/unspecified (15.6%). In 39.8% the age was unknown; most patients were ≥41 years (23.4% in 41–65, 18.2% in >65); deaths in patients <18 years (9.4%) and ages 19–40 were reported (9.2%). In 25.8% the cause of death was unknown. Reports from infection/sepsis (10.6%), malignancy (6.8%), cardiac dysfunction (4.0%) were found for all products; fatalities were reported for trauma (4.6%), HIV/HCV (2%), thrombosis (11.0%) and 'other' (13.4%). The majority of fatalities were due to hemorrhage (21.8%); nearly 50% of them were intracranial. As of December 31, 2019, 10 fatalities in PwcHA on emicizumab have been reported to FAERS, with causes of death consistent with other coagulation products.

Conclusion This analysis demonstrates a generally consistent pattern of reported mortality in PwcHA across treatment regimens and the utility of a unified approach to cross-examining mortality for all hemostatic agents. Underreporting, variability in reporting, limited case information, and small overall numbers in FAERS hamper classification of cases, highlighting the need for detailed, timely reports for evaluation of mortality risk in PwcHA.

P08-02 Evaluation of different processing methods to label platelets for in vivo studies

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Objective The goal of this study is to evaluate, based on in vitro parameters, different methods of platelet (PLT) labeling (using biotin) in platelet concentrates (PCs) and their impact on the cell phenotype and functions. This is the first step to determine the appropriate processing to label PLTs for in vitro and in vivo studies.

Material and Methods In order to screen three different methods, 45mL from pooled buffy-coat PCs (39% plasma + additive solution) were transferred into 150-mL bags as preparation for the biotinylation process. Methods (1) and (2) were prepared as follows: PCs were washed (by centrifugation) once for condition (1) or twice for condition (2) to remove plasma before the biotinylation and to remove biotin reaction products afterwards. No washing step was applied for method (3): 10mL of the 45mL of PCs were biotinylated and added to the remaining 35mL of non-biotinylated PLTs. All previous conditions were compared with control PLTs (C; no processing and storage in small bags), analyzing the relative expressions of P-selectin, CD42b, and phosphatidylserine expression by FACS during 8 days of storage. All conditions shared

the same biotinylation reactant: 1mg [for (1) and (2)] or 1.5 mg [for (3)] of Sulfo-NHS-SS-Biotin (A39258) were diluted in DMSO and SSP+ at 1mg/mL, prior to incubation for 30min at 22°C under agitation. Experiments were done in duplicate.

Results All different conditions tested showed a stable surface expression of biotin with 93% positive PLTs with 1898 median fluorescence intensity (MFI) for (1), 95%/2018 MFI for (2), and 26%/2171 MFI for (3) after 8 days. As expected, processed PLTs (1) and (2) showed an increase of activation (P-selectin), reaching 88% and 83% of positivity after the process vs (3) and (C) with 27% and 21%. This difference was still observed after 8 days with 87%, 62%, 56% and 55% for (1), (2), (3), and (C), respectively.

Conclusion The three conditions of PLTs biotinylation were shown to have different benefits. PLTs in methods (1) and (2) are entirely biotinylated but they are highly activated. In contrast with method (3) only one fraction of PLTs is biotinylated exhibiting the same level of activation as control PLTs (C). This demonstrates that new methods of biotinylation are exploitable. Method 3 will be tested in conventional PC bags where lower levels of lesions are expected due to better storage conditions.

P08-03 smart medication DocuScan: GSAV-compliant documentation of hemophilia preparations for pharmacies, doctors, treatment centers and patients

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DOI 10.1055/s-0041-1728203

Objective With the German Act for Improved Safety in the Supply of Medicines (GSAV), the previous exemption from the pharmacy distribution channel (direct delivery of the manufacturer to doctors and hospitals) for medicines to treat patients with hemophilia was withdrawn in Germany with effect from September 1, 2020. The factor preparations are no longer dispensed to patients by doctors, but by pharmacies. The new regulation of the distribution channel results in extensive requirements for the documentation of the dispensed preparations by the pharmacist and immediate reporting to the prescribing physician.

Material and Methods The DocuScan platform was developed to support pharmacies and physicians in meeting the legal requirements and to optimize the necessary processes. The new platform simplifies data entry and documentation processes on all sides. Doctors and pharmacies that have access to DocuScan benefit from highly optimized processes. Patients can transfer relevant information about the preparations dispensed directly into the electronic diaries where they can record their consumption. The consumption documented in the electronic patient diaries is in turn available to the doctor/treatment center for therapy monitoring and reporting to the German Hemophilia Registry.

Results In a 3-month pilot phase DocuScan was tested and validated with 7 selected pharmacies before the GSAV came into force. Leading haemophilia centers and also patient organizations were involved in the development of DocuScan as well. Currently, more than 100 pharmacies specialized in hemophilia care already use DocuScan.

Conclusion DocuScan enables pharmacies to efficiently implement the legal requirements for the documentation of hemophilia preparations. The combination of DocuScan with the electronic diary ensures complete and consistent documentation from the dispensing in the pharmacy, the consumption by the patient, the therapy monitoring by the physician to the reporting to the German Hemophilia Register in high quality.

Platelets - Disorders of platelet function and numbers

P09-01 A Phase II study to investigate the efficacy and safety of eltrombopag in combination with dexamethasone as first-line treatment in adult patients with newly diagnosed primary ITP (XPAG-ITP)

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Objective Eltrombopag is an oral thrombopoietin receptor agonist (TPO-RA) that increases platelet production (Erickson-Miller et al., 2010, Sun et al., 2012). Eltrombopag is approved in Europe for the treatment of thrombocytopenia, from 6 months following diagnosis, in patients with primary ITP who are refractory to other treatments.

The aim of this trial (NCT04346654) is to compare the ability of eltrombopag in combination with a short course of high-dose dexamethasone to induce a sustained response off treatment in comparison to dexamethasone monotherapy in newly diagnosed primary ITP patients.

Material and Methods This is a Phase II, multicenter, randomized (1:1), open-label study (see Figure 1).

■ Treatment/Tapering Period:

Arm A: Patients who have platelet counts < 30.000/µl will receive eltrombopag (50 mg QD) in combination with a short course of high-dose dexamethasone (40 mg QD for 4 consecutive days) beginning at day 1.

Arm B: Treatment in the control arm consists of 1-3 cycles of high-dose dexamethasone administered orally at a dose of 40 mg QD for 4 consecutive days at 4 weeks intervals.

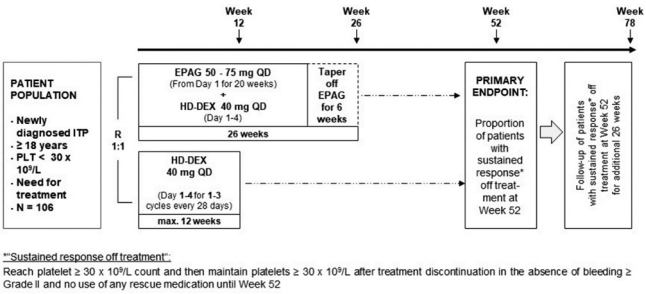
Patients who reach platelet counts ≥ 30.000/µl and maintain them during the tapering phase (week 20 – week 26) will be eligible for treatment discontinuation starting from week 26. During the tapering phase, eltrombopag will be decreased by 25 mg every 2 weeks to a minimum dose of 25 mg every other day for all patients.

■ Observation period:

Sustained response off treatment is defined as:

o maintain platelet counts ≥ 30.000/µl after treatment discontinuation and

o no bleeding events ≥ Grade II and



▶ **Fig. 1** Study design. Abbreviations: ITP: Immune thrombocytopenia; EPAG: Eltrombopag; HD-DEX: High-dose dexamethasone; PLT: Platelets; QD: Daily (quaque die); R: Randomization; TPO-RA: Thrombopoietin receptor agonist.

o without the use of any rescue therapy until week 52 and week 78 respectively, after study start

Results The primary objective is the rate of sustained responses off treatment at 52 weeks. Key secondary objectives include the duration of sustained response off treatment, the rate of sustained response off treatment at 78 weeks as well as patient-oriented outcomes for health-related quality of life.

Currently, the study is recruiting patients in Germany, expected to be completed by 2021.

Conclusion This trial will evaluate the potential of eltrombopag in combination with steroids to increase the rate of sustained response off treatment in comparison to steroids alone in patients with previously untreated primary ITP.

P09-02 Factors influencing bleeding severity in adult patients with primary immune thrombocytopenia

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Objective Immune thrombocytopenia (ITP) is characterized by low platelet counts and associated with an increased bleeding risk. Still, bleeding severity in ITP patients differs individually and is not only determined by the platelet count.

We investigated bleeding severity and bleeding manifestations in a cohort of adult patients with primary ITP.

Material and Methods Patients with primary ITP were included in two haematological centers after written informed consent (EC 1843/2016). Bleeding severity was assessed with the ISTH ITP BAT (BS).1

Results Eighty-four patients (66.7% female) were included in the study (Table 1). The median BS [IQR] was 1 [0-3], with the highest score in the category skin. The most common bleeding manifestations were petechiae (26.8%), bleeding from small wounds (25.6%), and menorrhagia in women (25.9% of women). The median BS [IQR] was higher in patients with a platelet count ≤50x109 versus those with >50x109 (2 [1-7] and 1 [0-2]), in both, the categories skin (1 [0-3] and 0 [0-1]) and mucosal bleeding (1 [0-2] and 0 [0-1]). There was no difference in the BS according to sex, blood group O, chronic ITP, splenectomy status, or current ITP treatment. Of note, patients with bleeding symptoms at ITP onset had a higher BS compared to those without (2 [0-5] and 0 [0-1]). In a multivariable linear regression analysis, duration of disease (ß 0.009, 95%CI 0.001-0.016), and the immature platelet fraction (IPF, ß 0.185, 95%CI 0.043-0.327) were independent predictors of the BS, whereas the platelet count, sex, age, BMI and blood group O, and sP-selectin were not associated with bleeding severity in our cohort of primary ITP patients.

Conclusion Bleeding severity in our cohort of ITP patients was generally low and predicted by IPF and the disease duration of ITP. This might indicate that ITP caused by high platelet destruction is more prone to bleeding.

▶ **Tab 1.** Paients' clinical and laboratory characteristics.

	n	n1	%
Female	84	56	66.7
Duration of ITP			
Acute ITP	75	13	17.3
Persistent ITP	75	9	12.0
Chronic ITP	75	53	70.7
Current ITP treatment	80	33	41.3
Previous ITP treatment	80	58	72.5
Splenectomy	80	13	16.3
ITP BAT BS >0	82	52	63.4
Bleeding at diagnosis	80	52	65.0
BGO	77	25	32.5
		median	**25-75 percentile**
Age, years	84	40	30-55
BMI, kg/m^2	79	25.4	22.9-29.7
Disease duration, months	75	60	9-130
Number of previous ITP treatments	80	1	0-2
ITP BAT BS total	82	1	0-3
Skin	82	1	0-2
Mucosa	82	0	0-1
Organ	82	0	0-0
Hemoglobin, g/dL	82	13.7	12.7-14.6
Platelet count, x10^9/L	82	61.5	28.8-118.5
IPF, %	67	12.7	6-17.2

n, number of patients of whom data is available; n1, number of patients with the respective characteristics; ITP, primary immune thrombocytopenia; BAT, bleeding assessment tool; BS, bleeding score; BGO, blood type O; BMI, body mass index; IPF, immature platelet fraction

P09-03 GATA1 pathogenic variants disrupt MYH10 silencing during megakaryopoiesis

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Objective GATA1 is an essential transcription factor for both polyploidization and megakaryocyte (MK) differentiation. The polyploidization defect observed in GATA1 variant carriers is not well understood.

Material and Methods 146 unrelated propositi with constitutional thrombocytopenia were screened on a multigene panel. Two novel GATA1 variants were identified in three boys from two unrelated families. We described the genotype-phenotype correlation, and investigated the effect of GATA1 variants on transcription assays using MYH10 luciferase constructs

Results The clinical profile associated with the p.L268M variant localized in the C terminal Zinc finger was unusual in that the patient displayed bleeding and severe platelet aggregation defects without early-onset thrombocytopenia. High MYH10 levels were evidenced in platelets of GATA1 variant carriers. Analysis of MKs anti-GATA1 ChIP-sequencing data revealed two GATA1 binding sites (BS), located in the 3′ untranslated region and in intron 8 of the MYH10 gene. Luciferase reporter assays showed their role in the regulation of MYH10 gene expression. Significantly decreased transcriptional activation was shown for the two GATA1 variants.

Conclusion The discovery of an association between MYH10 and GATA1 is a novel one. Overall, this study suggests that impaired MYH10 silencing via an intronic regulatory element is the most likely cause of GATA1-related thrombocytopenia.

P09-04 Insight into the role of miR-223-3p in regulating platelet reactivity

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Objective Platelets have a major role in thrombus formation through their ability to aggregate and to support thrombin generation. During these last years circulating microRNAs (miRNAs), including miR-223-3p, have been suggested as biomarkers to predict clinical outcome in cardiovascular patients. However, the impact of miR-223-3p on the regulation of platelet reactivity is still unclear. This study aims to investigate the relationship between circulating miR-223-3p level, platelet aggregation and in vivo thrombin generation markers, and to identify the putative genes regulated by this miRNA.

Material and Methods MiR-223-3p together with another platelet-derived miRNA (miR-150-5p) was quantified in 191 plasma samples of stable cardiovascular patients. Both miRNAs were normalized against 3 stably expressed endogenous miRNAs previously validated using geNorm algorithm. MiRNAs

level was then compared to both in vivo thrombin generation markers and light transmission aggregometry using various agonists including arachidonic acid, ADP and collagen. Finally, a miRNA's targets network was built based on the gene ontology of pathways implicated in platelet reactivity.

Results MiR-223-3p level was positively associated to both in vivo thrombin generation markers and to ADP and collagen-induced platelet aggregation, while miR-150-5p was associated with platelet aggregation only. The in silico network pointed putative targets that could explain the association between the miRNAs studied and platelet function. This included STMN1, a validated target of miR-223-3p, which could mediate the effect of miR-223-3p on platelet pro-coagulant activity and GLP1R, a predicted target of both miR-223-3p and miR-150-5p known to regulate platelet aggregation.

Conclusion Platelet-derived miRNAs regulate different aspects of platelet reactivity including platelet-supported thrombin generation and platelet aggregation, through regulation of different genes. This study supports the use of a miRNA profile to tailor antithrombotic strategy in cardiovascular patients.

P09-05 The role of platelet mitochondrial function for platelet mediated Aβ40 aggregation

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DOI 10.1055/s-0041-1728208

Objective Alzheimer's disease (AD) is a neurodegenerative disease with Aβ deposits in the brain parenchyma and in the walls of cerebral blood vessels, strongly associated with Reactive Oxygen Species (ROS) production and oxidative stress. Mitochondrial abnormalities act as major producers of ROS. Recently, we have shown that platelets directly contribute to the formation of Aβ fibrils in vitro and in vivo, including integrin outside-in and ADP-P2Y12 signaling. However, the role of mitochondrial damage for platelet-mediated Aβ fibril formation is completely unknown

Material and Methods Cell culture experiments, flow chamber experiments and FACS analysis.

Results Defects in the respiratory chain by treatment of platelets with Antimycin A (inhibition of mitochondrial complex III) and Rotenone (inhibition of mitochondrial complex I) in the presence of soluble Aβ40 increased Aβ fibril formation in a dose-dependent manner in vitro. However, the addition of the antioxidant vitamin C prevents platelet mediated Aβ40 fibril formation suggesting increased ROS production upon mitochondrial complex inhibition. In contrast, blocking of mitochondrial complex I in platelets reduced Aβ40-mediated aggregation and ATP release of platelets. In addition, incubation of platelets with Aβ40 enhances the generation of ROS and superoxide, which leads to the reduction of the mitochondrial membrane potential in platelets. Moreover, the mitochondrial membrane potential of platelets from the AD transgenic APP23 mouse was reduced already under resting conditions, as well as upon Aβ40 and GPVI stimulation compared to controls. Accordingly, superoxide generation was enhanced in platelets from APP23 mice in the presence of Aβ40

Conclusion Platelet mitochondrial dysfunction contributes to platelet mediated Aβ fibril formation.

Platelets - Physiology

P10-01 Examination of the Rac-modulator ArhGAP15 in platelet activation.

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DOI 10.1055/s-0041-1728209

Objective Platelet activation is triggered by various stimuli engaging with surface expressed receptors. Intracellular signaling then leads to the shape change, granule secretion and integrin activation in platelets. One key component of integrin activation in various hematopoietic cells is Rac and Rac-modulating proteins. The de-activating Rac-modulator ArhGAP15 has been shown to control leukocyte functionality in vivo, but little is known about its role in platelets. We thus set out to determine whether ArhGAP15 is expressed in platelets and how deficiency of ArhGAP15 affects platelet functionality.

Material and Methods For this purpose, we removed and washed citrate-anticoagulated whole blood from wildtype and ArhGAP15 deficient mice. Following, samples were incubated with different stimuli (thrombin, ADP, collagen), labelled with antibodies against GpIb in order to determine the platelet population, P-selectin and the activated conformation for the integrin GpIIb/IIIa and activation measured by flow cytometry. A model of mesenteric ferric chloride induced thrombus formation was used to assess in vivo relevance of ArhGAP15-deficiency.

Results Expression of ArhGAP15 in murine platelets was verified by western blot. Flow cytometry-based platelet activation assays revealed no significant difference for thrombin, ADP and collagen stimulation of washed murine wildtype and ArhGAP15 deficient platelets in vitro. In a model of ferric chloride induced rapid thrombus formation, no significant difference was observed between wildtype and ArhGAP15 deficient animals.

Conclusion Lack of the Rac-modulator ArhGAP15 does not impair the classical inside-out activation responses of murine platelets.

P10-02 Reelin supports Glycoprotein VI and integrin outside-in signaling of platelets

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Objective Reelin is known as an extracellular matrix protein mediating cell migration upon brain development. Recent studies provide evidence for reelin to be expressed in platelets, to co-localize to F-actin and to bind to amyloid precursor protein (APP) and apolipoprotein E receptor 2 (ApoER2) at the platelet surface. Furthermore, reelin is important for glycoprotein Ib (GPIb) mediated platelet adhesion and thrombus formation under high shear. Consequently, defective thrombus formation protects reelin-deficient mice (reeler) against arterial thrombosis.

We aim to define the role and signaling mechanisms of reelin in platelet cytoskeletal reorganization and glycoprotein VI (GPVI) mediated platelet activation and aggregation.

Material and Methods In vitro and in vivo analysis of reeler mice.

Results Reelin activated the small GTPases RhoA and Rac1 in platelets and was important for PAK1/2 phosphorylation thereby supporting lamellipodia formation upon spreading on fibrinogen. Experiments revealed strongly reduced clot retraction using platelet rich plasma (PRP) from reeler mice, which could be rescued by the addition of extracellular reelin, emphasizing the role of reelin for integrin outside-in signaling. Furthermore, adhesion and cytoskeletal reorganization of reeler platelets were reduced on immobilized collagen-related peptide (CRP) and collagen. Likewise significantly reduced phosphorylation of PLCγ2 and Syk as well as platelet aggregation and ATP release were detected using reeler platelets that have been stimulated with GPVI agonists. A significant reduction of platelet adhesion to immobilized reelin was observed when GPVI has been inhibited on platelets. Additionally, immunoprecipitation of reelin using recombinant GPVI clearly showed a direct interaction of GPVI and reelin. A direct interaction was confirmed with bio layer interferometry showing an interaction between reelin and GPVI

with subnanomolar affinity. Consequently, in vivo thrombus formation was completely abrogated in reeler mice treated with a GPVI depleting antibody.
Conclusion Taken together these data provide first evidence for reelin to support GPVI signaling and integrin outside-in signaling of platelets. Thus, reelin affects platelet activation via different signaling pathways involving GPIb, GPVI and the classical reelin receptors APP and ApoER2 pointing to a promising role of reelin as therapeutic target for antithrombotic therapy.

Vascular wall biology and disorders

P11-01 Prolonged chemotherapeutic stress is accompanied by an aggressive and singular vascularization process with a high thrombogenic potential in Colorectal Cancer

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DOI 10.1055/s-0041-1728211
Objective Cancer mortality is closely associated with the presence of drug-resistant, invasive subpopulations of tumor cells. However, the functional and mechanistic interactions between the two phenotypes are incompletely understood.In the present study we set up an experimental system that allowed the investigation of the relationship between chemotherapeutic stress and the aggressivity of cancer cells. The acquisition of characteristics related with cancer aggressivenes were studied on chemoresistant cells, such as migration, invasion, tumoral vascularization and procoagulant status.
Material and Methods For Tube Formation Assay, CRC cells were seeded onto 3D matrigel and incubated at 37°, then followed by 24-hour video microscopy. For thrombin generation assay, experiments are realized with FluCa-Kit and run with Thrombinoscope Software (Diagnostica Stago, France)
Results We have developed a panel of 4 isogenic CRC cell lines comprised of the parental HCT-116 cells and three independently derived sublines resistant to 5-fluorouracil, oxaliplatin and SN-38. Tumor vascularization is needed for tumor growth as well as for dissemination of tumor cells. Tumor blood flow is usually provided by capillary endothelial cells. However, increasing evidence suggest that some tumor cells are able to form vascular structures that are connected with the endothelial cells and are able to sustain blood flow. This process is called vasculogenic mimicry and has been associated with a highly aggressive cancer phenotype. Tube Formation Assays revealed that two of the resistant cell lines had acquired the capacity to form cellular networks in vitro in contrast to the parental cells. Further work will include antibody arrays and ELISA assays to identify key players in the tumor cell signaling network. Moreover, the 5-fluorouracil chemoresistant tumor cells enhanced thrombin generation as compared to the parental HCT-116. In contrast, HCT-116/OXA and HCT-116/SN38 showed lower potency to trigger thrombin generation as compared to the parental HCT-116.
Conclusion Taken together, our results indicate that chemotherapeutic stress can be accompanied by an aggressive phenotype. This thrombogenic behavior suggest that tissue factor could be implicated especially for resistant cells. We further suggest that this may not be limited to colorectal cancer cells but is likely applicable to a wide range of other tumor types

Venous thromboembolism

P12-01 International Myeloma Working Group (IMWG) score accuracy in multiple myeloma patients: need for action

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DOI 10.1055/s-0041-1728212
Objective Venous thromboembolism (VTE) remains a common complication in patients with multiple myeloma (MM) occurring at around 10% of cases. This challenging evolution can be optimized with a better identification of patients at risk and a more regular and tailored application of thromboprophylaxis strategy. IMWG guidelines recently proposed a risk-assessment model in order to establish these specific approaches in all IMID-receiving patients .
The aim was to evaluate the predictive strength of the current IMWG score in MM patients.
Material and Methods This is a monocentric, prospective, non-interventional study on a cohort of 93 consecutive MM patients referred to the Hemostasis Center at Tenon University Hospital. The IMWG score was calculated during the 1st visit and thrombotic prevention data were collected. The follow-up period was one year.
Results Among 93 patients with a male/female ratio of 40/53 and a median age of 65.7 years old, 57 patients (61%) were at low risk, (Group A) receiving aspirin in only 18 of cases. The other patients (39%) were stratified at high risk (Group B) receiving heparin thromboprophylaxis in 6 of cases and aspirin in 9 cases. Risk stratification was as follows: 22 patients with a score at 2, 8 patients at 3 and 2 patients at 4. Thrombosis occurred in 11 patients (12%): 7 in group A and 4 in group B. In Group A, 5 presented a symptomatic pulmonary embolism (PE), 1 with distal lower limb thrombosis and 1 with proximal lower limb thrombosis. In group B, 3 patients presented with a symptomatic PE and 1 with distal lower limb thrombophlebitis. These thrombotic events were not correlated with MM staging, treatment response and IMIDs treatment.
Conclusion Despite a systematic application of IMWG scoring and thromboprophylaxis strategy implementation, VTE occurrence remains significantly high in MM patients considered at low risk and heparin thromboprophylaxis regimen was not optimal with a remained incidence around 11%. These disappointing results require the development of more accurate risk assessment models based on a more selected clinical parameters in order to define more adapted thromboprophylaxis strategy based on more pertinent biological markers.

P12-02 Thrombophilic Phaenotype in a Family with High Frequency of Homozygous Antithrombin Deficiency Typ IIb Budapest 3 and Mild Factor VII Deficiency due to Homozygous Polymorphisms in the Factor 7 Gene and Antithrombotic Treatment

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Objective Antithrombin (AT) type IIb Budapest 3 (AT Bp3) deficiency affects the heparin binding site of AT. Homozygosity for ATBp3 deficiency in contrast to a homozygous AT type I deficiency may not be fatal. A family with 6 homozygous AT Bp3 deficient siblings is reported. 3 of 6 had developed thrombotic events. Aim: To analyze triggers for thromboses, to stabilise the therapy to prevent from further recurrence.

Material and Methods Patients: 1st patient (pat), male: deep vein thromboses (DVT) after birth. 2nd pat, female, with 11 years (Y) arterial thromboses of the right arm after influenza, with 13 Y proximal DVT of the right leg under apixaban. 3rd pat, older brother: with 15½ Y proximal DVT of the right leg after a cold with fever. With 17 ½ Y recurrent DVT at the same localisation despite apixaban.

Analytics: AT: factor Xa-based chromogenic assay. D-dimers: automated LIA, prothrombin fragments F1+2: LOCI technology, apixaban: chromogenic assay, apixaban calibrator.

Results All pat had AT between 14-18 %. 1st pat: stable for 4 years under phenprocoumon, elevated D-dimers 0.7 to 1.1 mg/l (ref. <0.5), F1+2 200 pmol/ml (ref <307). 2nd pat (previously reported, GTH 2019, P07): after the 1st arterial thrombosis treatment with apixaban/clopidogrel. New DVT: D-dimers strongly increased: 4.4 mg/l. Checking drug intake: discontinuation of apixaban due to teasing by classmates about her drug intake. AT was supplemented, enoxaparin 2 mg/kg bw. x 24 h given for 7 d, Apixaban 5 mg b.i.d restarded. Then, D-dimers declined from 2.2 to 1.0 mg/l within 3 months. 3rd pat: 1st event, admission: D-dimers 4 to 4.1 mg/l, by AT and enoxaparin 2 mg/kg bw. x 24 h for 10 d declining to 1.3 mg/l. After changing to apixaban 5 mg b.i.d., D-dimers declined to 0.4-0.5 mg/l 6 months later. One month before the recurrence of DVT an apixaban level of 0 ng/ml an D-dimers of 0.7 mg/l was seen. For the recurrent DVT his medical history revealed an idiosyncratic adherence to the schedule of apixaban intake forgetting and irregularly combining doses from b.i.d. to partially q.d. Since reuptake of regular apixaban the D-dimers (0.3 mg/l) and F1+2 (224 pmol/ml) are low.

Conclusion The risk of recurrence in homozygous AT Bp3 deficiency and previous thrombotic events is high already during childhood and puberty. Strict adherence to the anticoagulation treatment is urgently required. A frequent monitoring of the therapy is recommend.

von Willebrand factor and ADAMTS13

P13-01 Laboratory phenotype variability in genetically proven von Willebrand disease type 2B

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Objective von Willebrand disease (vWD) type 2B is a rare bleeding disorder accounting for about 5% of all cases of vWD. An increased response to stimulation with low concentrations of ristocetin in platelet agglutination assay (LD-RIPA) is considered the unifying laboratory hallmark. Here we describe bleeding symptoms and varying laboratory phenotypes of three members of a family with genetically proven vWD type 2B.

Material and Methods vWF antigen (vWF:Ag), vWF collagen-binding activity (vWF:CB) and vWF ristocetin-induced binding as glycoprotein Ib receptor-binding (vWF:GP1bR) were determined applying HemosIL AcuStar-Assays (Werfen GmbH, Germany) including the calculation of vWF:GP1bR/Ag- and vWF:CB/Ag-ratio. LD-RIPA was performed with a final concentration of 0.5 mg/ml ristocetin.

Results Index patient is a 2 years old boy who was diagnosed with vWD type 2B before undergoing adenotomy. The boy did not disclose any bleeding history. The investigation was initiated based on the bleeding history of his mother who had suffered from postoperative and postpartum hemorrhage. Laboratory tests showed a type 2A VWD-pattern including a normal platelet count and LD-RIPA. However, genetic analysis revealed a VWD type 2B by detection of a heterozygous c.4021C>T mutation.

Second case is the 36-year-old father of the boy, who also denied any significant bleeding history. An appendectomy had been performed without any complication. In his case, laboratory tests showed a pathologic vWF:GP1bR/Ag-ratio and a relative reduction of high molecular vWF-multimers. Platelet count and LD-RIPA were normal again. Molecular diagnostics revealed the same heterozygous c.4021C>T mutation as found in his son compatible with type 2B VWD.

Third case is the 40-year-old uncle of the index patient. Again, his bleeding history did not reveal any abnormality. Laboratory tests disclosed a LD-RIPA slightly above the cut-off as well as a vWF:GP1bR/Ag-ratio close to but still above the cut-off of 0.7 and a normal platelet count. Genetically the heterozygous c.4021C>T mutation consistent with type 2B VWD was found again.

Conclusion vWD type 2B patients may not suffer from severe bleeding and show normal LD-RIPA results. Phenotypical variability exists even in family members with identical genetic constellations.

P13-02 Type 2B von Willebrand disease – a rare cause of neonatal thrombocytopenia

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Objective Von Willebrand disease (VWD) type 2B is a rare hereditary disorder characterized by activating mutations in the von Willebrand factor (VWF) gene leading to spontaneous platelet aggregation and variably pronounced thrombocytopenia and coagulopathy. Treatment is primarily based on VWF substitution. Management of VWD 2B in the neonatal period is not standardized and only few case reports exist. Here, we present the case of a newborn with a prolonged course of thrombocytopenia caused by VWD 2B.

Material and Methods Standard laboratory assays for the assessment of VWF activity and antigen were used. VWF multimers were analyzed using agarose gel electrophoresis. Ristocetin-induced platelet agglutination (RIPA) was performed using low-dose ristocetin concentration (≤ 0.6 mg/ml).

Results The female newborn presented with hematomas and thrombocytopenia (11/nl) on the 4th day of life. Family history was notable for a suspected VWD 2B of the patient's father. Initial laboratory exams revealed a low VWF activity to antigen ratio of 0.39. VWF therapy was started because of high suspicion for a VWD 2B. Platelets were administered to treat severe thrombocytopenia. Multimer analysis showed loss of high molecular weight VWF multimers. RIPA with half of the ristocetin concentration (reaching 70% of maximal aggregation) found in this patient is typical for VWD 2B. The diagnosis was confirmed by detection of a known disease-causing heterozygous missense mutation (V1316M) in the VWF gene which is known to be related to severe thrombocytopenia and bleeding diathesis, especially in stressful situations (infections, surgery). Close-meshed clinical and ultrasound exams ruled out new bleeding episodes. Laboratory controls showed increasing platelet counts under VWF substitution and also when substitution was stopped. Capillary blood withdrawals were preferred to venipuncture to reduce stressful events. After discharge from hospital, no new bleeding episodes occurred so far.

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Conclusion VWD 2B is a rare cause of neonatal thrombocytopenia. VWD 2B is strongly suggested by RIPA and confirmed by molecular genetic analysis. The mutation of this patient is related to a severe course. Further bleeding episodes were prevented by prompt initiation of VWF substitution. As stressful events can enhance the degree of thrombocytopenia, capillary blood withdrawals should be performed if the patient carries this variant of the VWF gene.

P13-03 A novel approach to laboratory assessment of platelet von Willebrand factor

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Objective The interaction of platelets with von Willebrand factor (VWF) is essential for primary haemostasis. Concentration and activity of plasma VWF are routine parameters in the assessment of haemostasis disorders. In addition to plasma VWF, platelet VWF, synthesized in megakaryocytes and stored in alpha-granules of circulating platelets, is known to contribute to primary haemostasis and the microenvironment of thrombus formation. The laboratory assessment of platelet VWF however is cumbersome and not widely established as a routine parameter. We here propose a method for laboratory assessment and reporting of platelet VWF potentially useful for laboratory routines in specialised laboratories.

Material and Methods To improve the preparation of platelet VWF we obtained 7.5 ml of EDTA anticoagulated whole blood and added PPACK, apyrase and prostaglandin E1 for platelet inhibition. Platelet separation was conducted with 1 separation and 2 washing steps. The following lysing step with CHAPS as detergent isolated the platelet VWF that was analysed with automated certified test systems. We propose 3 calculation models for a standardised presentation of platelet VWF.

Results Our models allow to describe the concentration of platelet VWF as 1., the proportion of platelet VWF in total VWF activity in whole blood, 2., the amount of platelet VWF in a platelet sample with a defined concentration of 100 platelets/nl, and 3. the concentration of platelet VWF in one platelet. According to our results in healthy individuals, the proportion of platelet VWF activity is estimated to be more than 10 % of total VWF in human plasma under physiological circumstances. The amount of platelet VWF is estimated to be 0.4 IU/ml in a sample with a defined concentration of 100 platelets/nl and to be about 42 IU/ml in one platelet (both expressed as VWF:Ag).

Conclusion We describe a standardized and practical approach for measurement and reporting of platelet VWF concentration and activity. Due to the simplified methodology up to three samples can be reliably processed concurrently, further advantages are small sample volumes, high yields of extracted protein, use of standard certified diagnostic and comprehensible parameters for both clinical and scientific purposes. We conclude that our proposed method may be suitable for routine application in specialized coagulation laboratories.

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