Thieme

GTH Congress 2024 – 68th Annual Meeting of the Society of Thrombosis and Haemostasis Research – Building Bridges in Coagulation

Date/Venue:
27.–01. March 2024, Vienna, Austria

Congress Presidents:
Ao. Prof. Dr. Christoph Male, Prof. PD Dr. Cihan Ay

Contents

S2 Topics

S2 T-01. Venous and arterial thrombosis

S10 T-02. Arteriosclerosis and inflammation

S13 T-03. Pathomechanisms of thrombosis

S20 T-04. Coagulation and cardiovascular complications

S23 T-05. Coagulation management in extracorporal circulation and cardiac assist devices

S25 T-06. Cancer and thrombosis

S31 T-07. Haemophilia and von Willebrand disease

S60 T-08. Other congenital bleeding disorders

S62 T-09. Paediatric haemostasis and thrombosis

S67 T-10. Bleeding of unknown origin

S70 T-11. Platelet dysfunction and associated bleeding disorders

S79 T-12. Gene and cell therapy

S83 T-13. Acquired coagulation disorders

S91 T-14. Fibrinolysis

S94 T-15. Perioperative haemostasis

S96 T-16. Haemostasis in women

S101 T-18. Laboratory issues

S111 T-19. Blood components and plasma concentrates

S112 T-20. Vascular wall biology and disorders

S113 T-21. Various Topics

S117 Index

S117 Author Index

S122 Keyword Index

Topics

T-01. Venous and arterial thrombosis

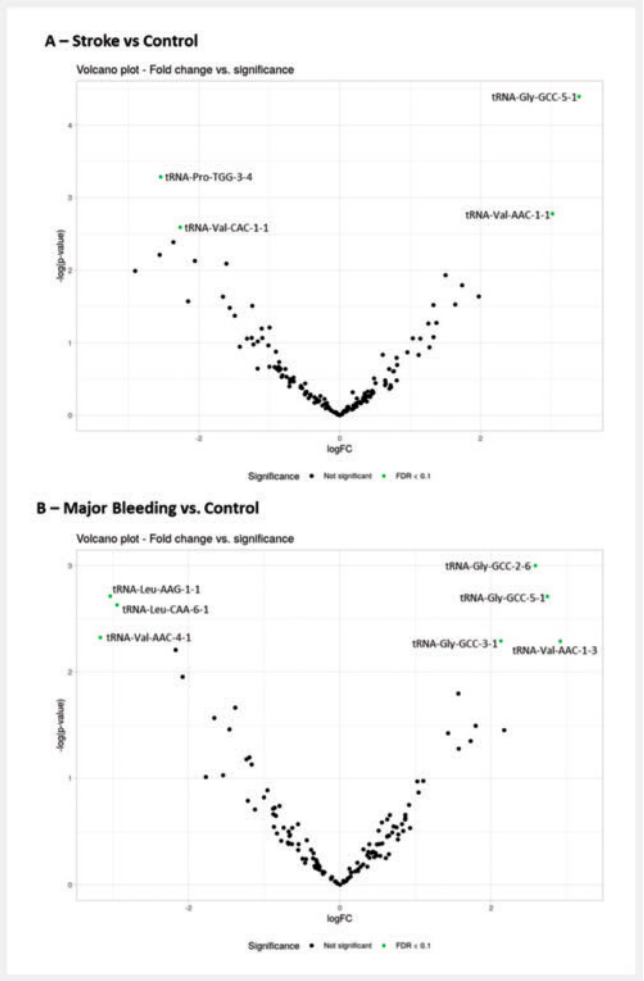
T-01-01 Transfer RNAs, not microRNAs, are linked to ischemic stroke and major bleeding in patients with end-stage kidney disease – a nested case-control study from the VIVALDI cohort

Authors S. Nopp[1], O. Königsbrügge[1], M. Säemann[2], I. Pabinger[1], A. Y. Nossent[3], C. Ay[1]

Institutes **1** Medical University of Vienna, Vienna, Austria; **2** Clinic Ottakring, Vienna, Austria; **3** Exercise and Sports (NEXS), Department for Nutrition, Copenhagen, Denmark

DOI 10.1055/s-0044-1779057

Introduction Patients with end-stage kidney disease (ESKD) are at particularly high risk for thromboembolism and bleeding. This study aimed to identify small non-coding RNAs (sncRNAs), specifically microRNAs and transfer RNA (tRNA)-derived fragments (tRFs), as potential novel biomarkers for thromboembolism and bleeding in this high-risk population.



▶ **Fig. 1** Volcano plot representing differential expression and statistical significance of tRNAs in

Method In this sncRNA discovery research, we leveraged the VIVALDI cohort, consisting of 625 ESKD patients on hemodialysis, to conduct two nested case-control studies, each comprising 18 participants. The primary outcomes were ischemic stroke in the first investigation and major bleeding in the second. Plasma samples were processed using the mIND pipeline for RNA-seq analysis to investigate differential expression of microRNAs and tRNA/tRFs between cases and their respective matched controls, with results stringently adjusted for false discovery rate (FDR).

Results No significant differential expression of microRNAs for either ischemic stroke or major bleeding outcomes was observed in either of the two nested case-control studies. However, we identified four tRNAs significantly differentially expressed in ischemic stroke cases and seven in major bleeding cases, compared to controls (FDR < 0.1, see ▶ **Fig. 1**). Coverage plots indicated that specific tRNA fragments (tRFs), rather than full-length tRNAs, were detected (example provided in ▶ **Fig. 2**). Alternative mapping approaches revealed challenges and technical limitations that precluded in-depth differential expression analyses on these specific tRFs. Yet, they also underscored the potential of tRNAs and tRFs as markers for thromboembolism and bleeding.

▶ **Fig. 2** **Coverage plots of the differential tRNA-derived fragments pattern coding for tRNA-Val-AAC-1**; Highlighted in red is a pattern of fragments present in patients with a major bleeding event compared to their controls.

Conclusion While microRNAs did not show significant differential expression, our study identified specific tRNAs/tRFs as potential novel biomarkers for ischemic stroke and major bleeding in ESKD patients, which need to be further validated externally.

Conflict of Interest The authors declare no conflict of interest.

T-01-02 Cardiovascular and genetic determinants of platelet ADP- and epinephrine-induced hyperreactivity – Results from the Gutenberg Health Study

Authors G. Baidildinova[1, 2], V. ten Cate[1, 3], M. Panova-Noeva[1], B. Dahlen[1, 3], A. Gieswinkel[3], S. Rapp[3], K. Strauch[4], M. Beutel[5], N. Pfeiffer[6], K. L. Lackner[7], T. Münzel[8], H. ten Cate[2, 9], P. S. Wild[1, 3], K. Jurk[1, 3]

Institutes **1** University Medical Center of the Johannes Gutenberg University Mainz, Center for Thrombosis and Hemostasis (CTH), Mainz, Germany; **2** Maastricht University, Department of Biochemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht, Netherlands; **3** University Medical Center of the Johannes Gutenberg University Mainz, Preventive Cardiology and Preventive Medicine, Department of Cardiology, Mainz, Germany; **4** University Medical Center of the Johannes Gutenberg University Mainz, Institute of Medical Biostatistics, Epidemiology and Informatics (IMBEI), Mainz, Germany; **5** University Medical Center of the Johannes Gutenberg University Mainz, Department of Psychosomatic Medicine and Psychotherapy, Mainz, Germany; **6** University Medical Center of the Johannes Gutenberg University Mainz, Department of Ophthalmo-

logy, Mainz, Germany; **7** University Medical Center of the Johannes Gutenberg University Mainz, Institute for Clinical Chemistry and Laboratory Medicine, Mainz, Germany; **8** University Medical Center of the Johannes Gutenberg University Mainz, Department of Cardiology, Cardiology I, Mainz, Germany; **9** Maastricht University Medical Center, Maastricht, Thrombosis Expertise Center, Heart and Vascular Center, Maastricht, Netherlands

DOI 10.1055/s-0044-1779058

Introduction Platelets contribute to the development of cardiovascular diseases with arterial or venous origin. This study aimed to compare platelet ADP and epinephrine hyperaggregability to other platelet function tests and to determine the predictive value of clinical factors and common genetic variants.

Method Normal- vs. high-responsive platelets to 0.5 µM of ADP and epinephrine in the light transmission aggregometry (LTA) were discriminated by the intersection of two distributions in a Gaussian mixture model from 903 population-based Gutenberg Health Study participants. Utilizing robust Poisson regression analysis, prevalence ratios (PR) were estimated between four platelet hyperaggregability comparison groups and 1) additional platelet parameters assessed by LTA, flow cytometry, platelet function analyzer, and calibrated automated thrombinography, 2) clinical characteristics, and 3) platelet-related common genetic variants.

Results Platelet hyperaggregability in response to ADP and/or epinephrine was associated with maximum aggregation for all tested agonists and tissue factor exposure *in vivo* after adjustment for age, sex, platelet count and platelet function-interfering medications (PR > 1, all group comparisons). Increased platelet aggregation induced by ADP and epinephrine was strongly related to atrial fibrillation (PR > 1, all group comparisons), whereas dyslipidemia was associated with ADP high-responsive platelets and venous thromboembolism with epinephrine high-responsive platelets in the LTA ($p < 0.05$). Common variants in *GPVI* (rs1613662, rs1671152) demonstrated predictive impact on platelet ADP hyperaggregability and rs3737224 (*PEAR1*) and rs11575845 (*MPIG6B*) on platelet epinephrine hyperaggregability ($p < 0.05$).

Conclusion This study identifies common and specific cardiovascular and genetic determinants of platelet ADP and epinephrine hyperresponsiveness independent of established confounders. Evaluation of platelet ADP/epinephrine hyperaggregability may provide 1) diagnostic value for platelet hyperactivity in atrial fibrillation, dyslipidemia, and VTE and 2) benefits for personalized antiplatelet treatment.

Conflict of Interest One author has received research funding outside the present study from Boehringer Ingelheim, Sanofi-Aventis, Bayer HealthCare, Daiichi Sankyo Europe, and Novartis, and honoraria for lectures or consulting from Boehringer Ingelheim, Bayer HealthCare, Evonik, AstraZeneca and Sanofi-Aventis.

T-01-03 Therapeutic influence of ACKR3/CXCR7 in inducing Anticoagulant Acylcarnitines: implications for Deep Vein Thrombosis and Coronary Artery Disease

Authors J. A. Bucio García[1], X. Fu[2], M. Lackner[3], L.-S. Menig-Benzig[3], B. Bouzabia[1], S. Beer-Hammer[1], E. Schaeffeler[3], U. Hofmann[3], M. Schwab[3, 4], M. Haag[3], M. Lämmerhofer[2], M. Chatterjee[1]
Institutes **1** University Hospital Tübingen, Department of Pharmacology, Experimental Therapy and Toxicology, Tübingen, Germany; **2** University of Tübingen, Institute of Pharmaceutical Sciences, Tübingen, Germany; **3** Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology (IKP), Stuttgart, Germany; **4** University of Tübingen, Departments of Clinical Pharmacology, and of Pharmacy and Biochemistry, Tübingen, Germany

DOI 10.1055/s-0044-1779059

Introduction Pharmacological targeting of the chemokine receptor ACKR3/CXCR7 regulates arterial thrombosis, platelet reactivity and thrombo-inflammatory response following myocardial infarction, those induced by immunothrombotic IgGs, and in coronary artery disease (CAD) patients *ex vivo*. CXCR7 agonist triggers the generation of anti-platelet lipid 12-HETrE which ligates the prostacyclin receptor and induces the platelet inhibitory cAMP dependent signaling cascade. Current investigation explores the antithrombotic and anticoagulatory implications of targeting CXCR7.

Method Stasis model of DVT in mice;platelet degranulation, integrin activation, procoagulant response, platelet-leukocyte aggregate formation in DVT model by flow cytometry; lipidomics analysis by UHPLC-QTOF-MS/MS; metabolomics analysis by HILIC-QTOF-MS; platelet mitochondrial respiration using Seahorse platform; calibrated automated thrombinography; phosphorylation of adenosine monophosphate-dependent kinase ($AMPK^{Ser\text{-}172}$) and acetyl-CoA carboxylase ($ACC^{Ser\text{-}79}$) by immunoblot analysis.

Results Administration of CXCR7-agonist (VUF11207) significantly decreased thrombus burden in the inferior vena cava of DVT mice 48hrs post stasis; it reduced circulatory platelet degranulation (CD62P), $\alpha_{IIb}\beta_3$-integrin activation (JON/A), thrombogenic phosphatidylserine exposure on procoagulant platelets and thrombo-inflammatory platelet-leukocyte aggregate formation. VUF11207 administration also retarded subsequent enhancement in such functional responses to collagen related peptide *ex vivo*. Long-chain Acylcarnitines (LC-ACars) exert anticoagulant influence by inhibiting Factor-Xa, circulatory levels of which are reduced in venous thromboembolism (VTE) and STEMI patients potentially accounting for their thrombotic disposition. VUF11207 induced the generation of LC-ACars in platelets from healthy subjects and CAD patients *ex vivo*, and prompted their release in the external milieu. Lipidomics and metabolomics profiling of resting and thrombin activated platelets revealed significantly elevated levels of LC- ACars (16:0, 18:1, 18:2), without affecting mitochondrial respiration, anabolic utilization of glucose, amino acid, lipids, also platelet energetic status, assessed by ATP measurements and adenylate energy charge. CXCR7 ligation caused activation of $AMPK^{Ser\text{-}172}$, and subsequently AMPK-mediated phosphorylation and thereby inhibition of $ACC^{Ser\text{-}79}$, thus fostering lipolysis over *de novo* lipogenesis, which could contribute to LC-ACar generation. Inhibitory effect of VUF11207 on platelet dependent thrombin generation was partially counteracted by carnitine palmitoyltransferase-1 inhibitor etomoxir that prevents LC-ACar formation, suggesting its anticoagulatory potential.

Conclusion Therapeutic targeting of CXCR7 may modulate thrombotic and procoagulatory response in DVT and CAD. Validation of CXCR7-agonist induced LC-ACar as an anticoagulant mediator will be made in future clinical investigations.

T-01-04 Patterns of atrial fibrillation anticoagulation with rivaroxaban – 7 years follow-up from the Dresden NOAC Registry

Authors L. Tittl, S. Marten, C. Naue, J. Beyer-Westendorf
Institute University Hospital "Carl Gustav Carus" Dresden, Thrombosis Research Unit, Department of Medicine I, Division Hematology, Dresden, Germany

DOI 10.1055/s-0044-1779060

Introduction Data on long-term effectiveness and safety of rivaroxaban for stroke prevention in atrial fibrillation (SPAF) are scarce and not available from randomized clinical trials.

Method We used data from the prospective, non-interventional *DRESDEN NOAC REGISTRY* to evaluate rates of stroke/transient ischaemic attack (TIA)/systemic embolism (SE), ISTH major bleeding (MB), in general and changes of event patterns over time.

Results Between 1st October 2011 and 28 February 2013, 1204 SPAF patients receiving rivaroxaban were enrolled and followed until 31st December 2022 (▶ **Fig. 1**). The mean duration of follow-up was 6.7 ± 3.4 years (median 7.2 years; 25th/75th percentile 3.8/10.0 years) with a mean duration of rivaroxaban exposure of 4.9 ± 3.5 years (median 4.5 years; 25th/75th percentile 1.7/8.2 years).

	All patients	Patients receiving 20mg OD at baseline	Patients receiving 15mg OD at baseline	*P*-value 20mg vs. 15mg OD
	n=1204	*n*=820	*n*=384	
Male, *n* (%)	631 (52.4)	461 (56.2)	170 (44.3)	0.0001
Age, years (median; IQR)	75 (70; 81)	74 (68;79)	79 (74; 85)	<0.0001
Mean BMI±SD, kg/m²	28.7±5.1	28.8±5.0	28.3±5.4	0.084
Heart failure, *n* (%)	448 (37.2)	267 (32.6)	181 (47.1)	<0.0001
Arterial hypertension, *n* (%)	999 (83.0)	674 (82.2)	325 (84.6)	0.2937
Diabetes, *n* (%)	480 (39.9)	304 (37.1)	176 (45.8)	0.0038
Prior TIA, stroke, or systemic embolism, *n* (%)	181 (15.0)	113 (13.8)	68 (17.7)	0.0755
PAD/CAD, *n* (%)	265 (22.0)	164 (20.0)	101 (26.3)	0.0139
Concomitant antiplatelet therapy, *n* (%)	91 (7.6)	54 6.6)	37 (9.6)	0.0620
Concomitant NSAID, *n* (%)	123 (10.2)	87 (10.6)	36 (9.4)	0.5097
Impaired renal function,[a] *n* (%)	151 (12.5)	37 (4.5)	114 (29.7)	<0.0001
$CHADS_2$ ≥2, *n* (%)	876 (72.8)	549 (67.0)	327 (85.2)	<0.0001
CHA_2DS_2-VASc ≥2, *n* (%)	1115 (92.6)	737 (89.9)	378 (98.4)	<0.0001
HAS-BLED score ≥2, *n* (%)	750 (62.3)	469 (57.2)	281 (73.2)	<0.0001

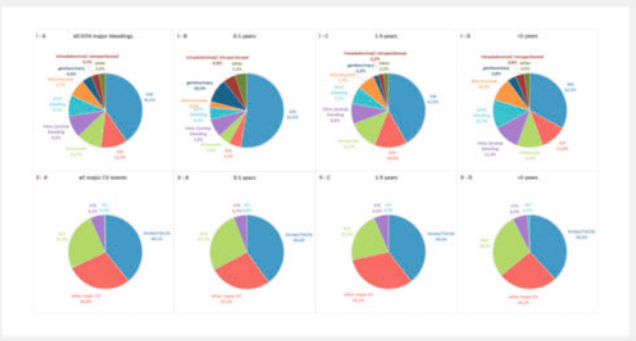
▶ **Fig. 1** Patient characteristics of 1204 SPAF patients and treatment subgroups; BMI, body mass index; IQR, interquartile range; NSAID, non-steroidal anti-inflammatory drug. PAD/CAD, peripheral arterial occlusive disease/coronary artery disease; SD, standard deviation.aImpaired renal function was defined as current or a history of creatinine clearance < 50 mL/min.

During follow up, intention-to treat rates of stroke/TIA/SE were 3.5/100 pt. years (95 % CI 2.5-4.7) in the first year and fell to 1.6/100 pt. years (95 % CI 1.2-2.0) in years 2-5 and 2.1/100 pt. years (95 % CI 1.6-2.7) after 5 years. Similarly, on-treatment event rates fell from 2.4/100 pt. years (95 % CI 1.5-3.5) to 1.1 (95 % CI 0.7-1.5) and 1.6 (95 % CI 1.0-2.3), respectively.

Major bleeding rates on treatment were 3.5/100 pt. years in the first treatment year (95 % CI 2.5-4.8) and 2.7 (95 % CI 2.2-3.4) and 3.5 (95 % CI 2.7-4.6) in the periods 2-5 and > 5 years, respectively. Of note, rates of fatal bleeding were low throughout follow-up (0.2 vs. 0.2 vs. 0.1/100 pt. years).

Of all observed major cardiovascular events, approximately 40 % were related to stroke/TIA/SE, around 25 % related to acute coronary syndrome, approximately 6 % to venous thromboembolism and nearly 30 % to "other", which predominantly was worsening of chronic heart failure. Interestingly, these proportions hardly changed over time and remained consistent from the first treatment year throughout 7 years of follow-up (▶ **Fig. 1**).

In contrast, patterns of MB changed considerably over time. Gastrointestinal bleeding accounted for 40 % of all MB events but this proportion was 53 % in the first treatment year and declined to approximately 42 % (years 1-5) and 33 % (> 5years). Similar declines over time were observed for genito-urinary and intraabdominal/retroperitoneal bleeding. Proportions of intracranial haemorrhage (app. 11 % of all MB) demonstrated a moderate increase (app. 5 % in the first year; 14 % during years 1-5 and 11 % beyond 5 years) but the most relevant increase occurred in skin/mucosal and joint bleedings, which together accounted for 8 % in the first year, 15 % during years 1-5 and 23 % beyond 5 years, respectively (▶ **Fig. 2**).



▶ **Fig. 2** patterns of major bleeding (panel I), major cardiovascular events (CV; panel II); Data are depicted for the entire period (panels A) and changes of patterns depicted over time (panels B-D) in on-treatment analysis; GIB = gastro-intestinal bleeding, ICH = intracranial haemorrhage, VTE = venous thromboembolism, ALI = acute limb ischemia, TIA = transitory ischemic attack, SE = systemic embolism, ACS = acute coronary syndrome

Conclusion Our study demonstrates the long-term effectiveness and safety of rivaroxaban therapy in unselected SPAF patients in daily care. Our data indicate that patterns of cardiovascular events remain constant over many years of SPAF treatment. In contrast, bleeding patterns change over time, possibly due to effects of co-morbidities in an ageing population.

Conflict of Interest J.B.-W.: honoraria and research support from Bayer, Boehringer Ingelheim, Daiichi Sankyo, Pfizer, Alexion, Norgine, DOASENSE and Sanofi. L.T.: honoraria and travel support from Daiichi Sankyo and Bayer. S.M.: honoraria from Daiichi Sankyo and Bayer. C.N.: no conflict of interest with regard to the NOAC registry or this Abstract.

T-01-05 Functional limitations three and twelve months after venous thromboembolism: Results from a prospective cohort study

Authors D. Steiner[1], S. Nopp[1], G. Heinze[2], D. Kraemmer[1], O. Schlager[3], S. Barco[4], F. A. Klok[5], I. Pabinger[1], B. Weber[6], C. Ay[1]

Institutes **1** Medical University of Vienna, Department of Medicine I, Clinical Division of Haematology and Haemostaseology, Vienna, Austria; **2** Medical University of Vienna, Center for Medical Data Science, Institute of Clinical Biometrics, Vienna, Austria; **3** Medical University of Vienna, Department of Medicine II, Division of Angiology, Vienna, Austria; **4** University Hospital Zurich, Department of Angiology, Zurich, Switzerland; **5** Leiden University Medical Center, Department of Medicine – Thrombosis and Hemostasis, Leiden, Netherlands; **6** Medical University of Vienna, Department of Dermatology, Vienna, Austria

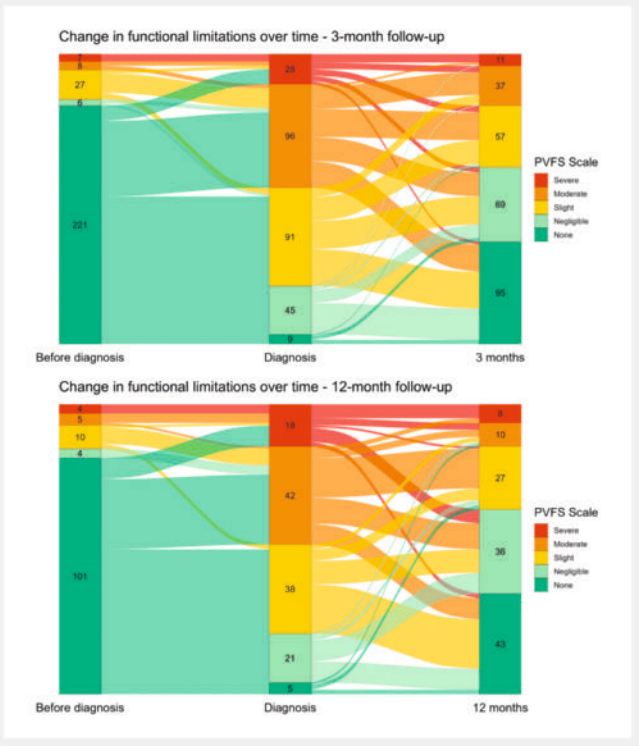
DOI 10.1055/s-0044-1779061

Introduction Venous thromboembolism (VTE) is associated with various long-term complications, including a decline in physical functioning. We aimed to investigate the association of clinical parameters at VTE diagnosis with functional limitations three and twelve months after diagnosis.

Method We conducted a prospective cohort study of VTE patients at a tertiary care center, excluding patients with cancer, pregnant patients, and patients in the postpartum period. Functional limitations were assessed with the post-VTE functional status (PVFS) scale (ranging from 0 to 4, with higher values indicating more limitations) within the first 21 days of diagnosis, after three and twelve months (prospectively), and one month before diagnosis (retrospectively). Twelve-month follow-up was only performed in patients remaining on anticoagulation. We fitted two separate proportional odds logistic regression models for the 3- and 12-month follow-ups and computed odds ratios (ORs) with 95 % bootstrap percentile confidence intervals (CI) to describe the association of

clinical parameters at the time of VTE diagnosis with functional limitations three and twelve months after VTE.

Results We included 307 patients with a median (interquartile range, IQR) age of 55.6 (43.7-65.7) years, of whom 128 (41.7 %) were women. The median (IQR) PVFS scale grade before diagnosis and at baseline were 0 (0-0) and 2 (2-3), respectively. About 49 % of patients had pulmonary embolism, 62 % had unprovoked VTE, 28 % had a history of VTE, 24 % had a history of cardiovascular or pulmonary disease, and 49 % were active or current smokers. After a median (IQR) follow-up time of 13.4 (12.9-16.0) weeks, 269 patients reported a median (IQR) PVFS scale grade of 1 (0-2) (▶ **Fig. 1**). Compared to their pre-VTE functional status, 123 (45.7 %) returned to their status or improved, while 146 (54.3 %) had more functional limitations. Female sex (OR, 2.15, 95 % CI, 1.26-4.14), higher BMI (OR, 1.05, 95 % CI, 1.00-1.10), functional limitations at diagnosis, and older age were associated with increased odds for more limitations at the three-month follow-up (▶ **Fig. 2**). After a median (IQR) follow-up time of 55.9 (53.1-62.6) weeks, 124 patients had a median (IQR) PVFS scale grade of 1 (0-2) (▶ **Fig. 1**). Compared to their pre-VTE functional status, 56 (45.2 %) returned to their status or improved, while 68 (54.8 %) had more functional limitations. Female sex (OR, 4.29, 95 % CI, 1.57-15.83), history of cardiovascular or pulmonary disease (OR, 2.62, 95 % CI, 1.03-7.85), and functional limitations at baseline were associated with increased odds for reporting a higher PVFS scale grade twelve months after VTE (▶ **Fig. 2**).



▶ **Fig. 1** Change in functional limitations over time; *Alluvial plot showing absolute number of patients per category of functional limitations. The upper panel depicts change in functional limitations from one month before venous thromboembolism diagnosis over the time of diagnosis to three months after diagnosis, the lower panel from one month before over the time of diagnosis to twelve months afterwards.*

	Model 1 (3-month follow-up, n=275) - Odds ratio	Model 1 - 95% CI[a]	Model 2 (12-month follow-up, n=124) - Odds ratio	Model 2 - 95% CI[a]
Sex	-	-	-	-
Male (ref.)	1.00	-	1.00	-
Female	2.15	1.26-4.14	4.29	1.57-15.83
Age, years (percentile)[b]	-	-	-	-
31.6 (10th; ref.)	1.00	-	1.00	-
43.4 (25th)	2.83	1.61-6.03	2.24	0.75-9.68
54.9 (50th)	2.34	1.27-4.92	2.01	0.53-10.50
63.6 (75th)	1.79	0.84-3.80	1.28	0.29-7.52
76.3 (90th)	3.22	1.57-7.86	1.88	0.48-8.14
BMI (per 1 kg/m² increase)	1.05	1.00-1.10	1.06	0.96-1.15
Type of VTE	-	-	-	-
DVT (ref.)	1.00	-	1.00	-
PE	0.95	0.56-1.52	0.61	0.22-1.46
Major transient risk factor	0.89	0.37-2.11	-	-
History of VTE	1.39	0.85-2.40	0.46	0.16-1.17
History of cardiovascular or pulmonary disease	1.29	0.64-2.68	2.62	1.03-7.85
Smoking status	-	-	-	-
Non-Smoker (ref.)	1.00	-	1.00	-
Current smoker	0.64	0.36-1.20	0.51	0.11-1.47
Former smoker	0.92	0.46-1.65	0.61	0.18-1.86
PVFS scale grade at diagnosis	-	-	-	-
2 (ref.)	1.00	-	1.00	-
0	0.41	0.10-1.15	3.03	0.29-21.40
1	0.53	0.32-0.74	1.10	0.47-2.10
3	2.81	2.21-4.09	2.29	1.47-4.84
4	11.64	5.43-31.26	13.12	3.35-126.82

▶ **Fig. 2** Odds ratios and 95 % bootstrap confidence intervals (CIs) for model 1 and 2; Model 1 refers to the three-month follow-up, model 2 to the twelve-month follow-up.; [a]95 % bootstrap percentile confidence intervals were calculated with 500 resamples. [b]Age was modelled as a continuous variable using restricted cubic splines with four knots at the 5th, 35th, 65th, and 95th percentile. Odds ratios for age are presented for 10th, 25th, 50th, 75th, and 95th percentile of patients with outcome data available for 3-month follow-up (n = 275), with the 10th percentile as reference.

Conclusion Patients with VTE had a considerable degree of functional limitations three and twelve months after VTE diagnosis and did not return to their pre-VTE functional status. We identified clinical parameters associated with functional limitations which could help in early identification of patients at risk for increased functional limitations after VTE.

Conflict of Interest This project was supported by a GTH Early Career Research Grant. SB reports institutional grants or contracts from Bayer, INARI, Boston Scientific, Medtronic, Bard, Sanofi, and Concept Medical; payment or honoraria from INARI, Boston Scientific, Penumbra, and Concept Medical; and support for attending meetings and/or travel from Bayer and Sanofi. FAK received research support from Bayer, BMS, BSCI, MSD, Leo Pharma, Actelion, The Netherlands Organisation for Health Research and Development, The Dutch Thrombosis Association, The Dutch Heart Foundation, and the Horizon Europe Program, all paid to his institution and outside the current work. CA reports honoraria for lectures from Bayer, Daiichi Sankyo, BMS/Pfizer, and Sanofi, and participation in advisory boards for Bayer, Boehringer Ingelheim, Daiichi Sankyo, and BMS/Pfizer outside the current work. DS, SN, GH, DK, OS, IP, and BW have no conflicts of interest to declare with regard to the present study.

T-01-06 Differences in plasma levels of free thrombin and APC in patients with splanchnic vein thrombosis with and without myeloproliferative neoplasms

Authors S. Reda[1, 2], J. Busse[1], J. Chang[3], N. Schwarz[1], J. Müller[1], C. P. Strassburg[3], J. Oldenburg[1], B. Pötzsch[1], C. Jansen[3], H. Rühl[1]

Institutes **1** University Hospital Bonn, Institute of Experimental Hematology and Transfusion Medicine, Bonn, Germany; **2** University of Cologne, Faculty of Medicine and University Hospital Cologne, Cologne, Germany; **3** University Hospital Bonn, Department of Internal Medicine I, Bonn, Germany

DOI 10.1055/s-0044-1779062

Introduction Splanchnic vein thrombosis (SVT), comprising thromboses in the portal, splenic, and mesenteric vein system, is often associated with myeloproliferative neoplasms (MPN). Affected patients are at high risk of further thrombosis but also of bleeding due to the underlying disease and various accompanying disorders, making antithrombotic therapy challenging. Aim of this study was to characterize the hypercoagulable state in SVT by measuring the key enzymes of coagulation activation and the anticoagulant protein C pathway; thrombin and activated protein C (APC).

Method The study population included 43 patients with SVT, thereof 25 in whom MPN was diagnosed (MPN + , essential thrombocythemia, n = 8; polycythemia vera, n = 6; myelofibrosis, n = 8; unclassifiable, n = 3). In the MPN + cohort JAK2 V617F and CALR mutations were present in 23 (92 %) and 1 (4 %) of cases, respectively, but were not found in any patient in the MPN-negative cohort of patients with SVT (MPN-, n = 18). The control group consisted of 15 healthy individuals. MPN + and MPN- cohorts of SVT patients were similar regarding age (mean, range 56, 36-78 years and 55, 32-72 years, respectively; 47, 30-60 years in the control group). 22/25 (88 %) of MPN + patients and 14/18 (78 %) of MPN- patients were under anticoagulant therapy at the time of analysis. Thrombin and APC in plasma were assessed using oligonucleotide-based enzyme capture assays (OECA). Additionally, prothrombin activation fragment (F1 + 2), thrombin-antithrombin complex (TAT), and D-dimer were measured.
Results Plasma levels of free thrombin were higher in the MPN + than in the MPN- cohort ($P = 0.036$) and in controls ($P = 0.031$), with median (IQR) plasma levels of 0.52 (< 0.46-1.36) pmol/L in the MPN + group versus the upper quartile of thrombin levels < 0.46 pmol/L in both other groups. With 1.39 (0.39-2.42) pmol/L versus 0.54 (0.39-1.00) pmol/L, APC levels were also higher in the MPN + cohort than in the control group ($P = 0.040$), whereas the difference to those in the MPN- group did not reach statistical significance (0.68, 0.42-1.17, pmol/L, $P = 0.278$). Compared with controls (D-dimer of 0.24, 0.18-049 µg/mL), D-dimer levels were higher in the MPN + (0.49, 0.28-0.72 µg/mL, $P = 0.003$) and MPN- group (0.41, 0.21-0.75 µg/mL, $P = 0.022$). In contrast, plasma levels of F1 + 2 and TAT did not differ significantly between MPN + /MPN- patients and controls (median F1 + 2: 0.21, 0.18, 0.15 nmol/L, respectively; median TAT: 23.0, < 21.3, < 21.3 pmol/L, respectively).
Conclusion The increased levels of active coagulation enzymes may be reflective of the increased thrombotic risk in patients with SVT, even while under anticoagulant treatment. In our study population SVT patients with MPN could be better distinguished by thrombin and APC than by conventional coagulation activation markers. Further studies are warranted to assess a potential application of these biomarkers to guide anticoagulant therapy in patients with SVT and to elucidate the role of the PC pathway in MPN-associated hypercoagulability.
Conflict of Interest J.O. has received research funding from Bayer, Biotest, CSL Behring, Octapharma, Pfizer, Swedish Orphan Biovitrum, and Takeda; consultancy, speakers bureau, honoraria, scientific advisory board, and travel expenses from Bayer, Biogen Idec, BioMarin, Biotest, Chugai Pharmaceutical Co., Ltd., CSL Behring, Freeline, Grifols, LFB, Novo Nordisk, Octapharma, Pfizer, F. Hoffmann-La Roche Ltd., Sanofi, Spark Therapeutics, Swedish Orphan Biovitrum, and Takeda. The other authors declare no competing financial interests.

T-01-07 Influence of gut commensals on murine deep vein thrombosis and platelet function

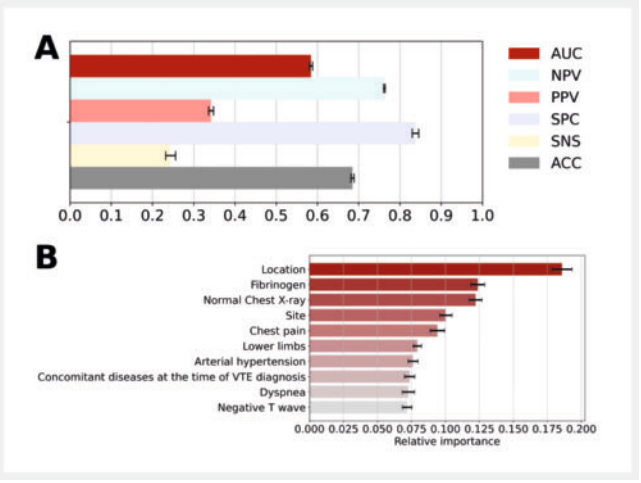
Authors K. Kiouptsi[1, 2], Z. Gao[1], K. Jurk[1], C. Reinhardt[1, 2]
Institutes **1** University Medical Center Mainz, Center for Thrombosis and Hemostasis (CTH), Mainz, Germany; **2** German Center for Cardiovascular Research (DZHK), Mainz, Germany
DOI 10.1055/s-0044-1779063
Introduction The commensal microbiota represents a chronic environmental challenge affecting many inflammatory processes. Although the microbiota's role on arterial thrombosis and atherosclerosis has been increasingly recognized, not much is known about the influence of microbiota on venous thrombosis. The most common type of venous thrombosis is the deep vein thrombosis (DVT). Endothelial activation has a key role in triggering DVT resulting in a procoagulant state. Defects on the gut barrier result in an increase of bacterial products in the circulation via the portal vein, inducing activation of the vascular endothelium and increased adhesion of leukocytes and platelets.
Method A standardized mouse model to study venous thrombosis in vivo is the inferior vena cava (IVC) stenosis model. In this study, we applied the IVC model, restricting the blood flow for 48 hours, to gnotobiotic mice and quantified the thrombus weight of thrombi from germ-free (GF) and conventionally raised (CONV-R) mice. We performed thromboelastometry to study whether the blood coagulation capacities were influenced by microbiota. The effect of microbiota on platelet function was studied ex vivo by functional assays, such as aggregometry and flow cytometry. In addition, we used calibrated automated thrombography to study thrombin generation.
Results The absence of microbiota did not result in changed thrombus weight in the stenosed IVC. Unchanged blood clotting was also confirmed by the ex vivo thromboelastometry analyses, after analyzing blood from GF and CONV-R mice. However, GF mice showed decreased aggregation capacities when stimulated by thrombin which was in line with a reduced P-selectin exposure in flow cytometry following convulxin activation. Interestingly, tissue factor-induced thrombin generation was also slightly reduced in platelets of GFmice.
Conclusion Venous thrombosis, primarily initiated by blood stasis and endothelial cell activation, was not influenced by the gut microbiota in a 48h murine IVC stenosis model. Although the microbiota influenced platelet activation and thrombin generation ex vivo, this did not impact venous thrombus weight in our settings.
Conflict of Interest none

T-01-08 A Machine Learning Approach to Identify Patients at Risk for Long-Term Consequences after Pulmonary Embolism

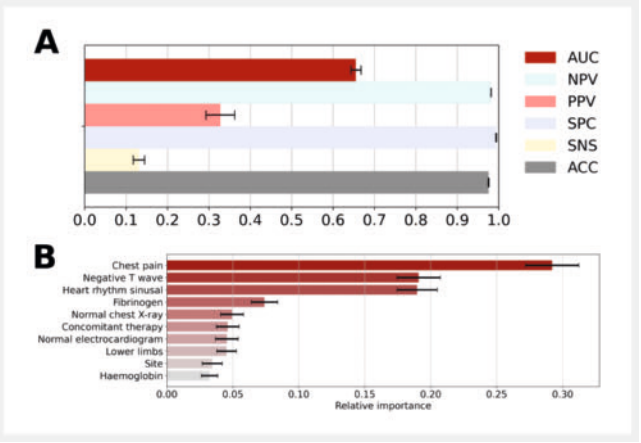
Authors S. Nopp[1], C. Spielvogel[1], B. Bikdeli[2], I. Francisco[3], L. Hernández-Blasco[4], L. Peris[5], R. Otero[6], D. Jiménez[7, 8], M. Monreal[8, 9], C. Ay[1]
Institutes **1** Medical University of Vienna, Vienna, Austria; **2** Harvard Medical School, Boston, USA; **3** Hospital Universitari de Girona Dr. Josep Trueta, Gerona, Spain; **4** Miguel Hernandez University, Alicante, Spain; **5** Universidad Cardenal Herrera-CEU, Castellón, Spain; **6** Hospital Universitario Virgen del Rocío, Seville, Spain; **7** Hospital Ramón y Cajal (IRYCIS), Madrid, Spain; **8** Instituto de Salud Carlos III, Madrid, Spain; **9** Universidad Católica de Murcia, Murcia, Spain
DOI 10.1055/s-0044-1779064
Introduction Pulmonary embolism (PE) can result in long-term sequelae, such as the post-PE syndrome or chronic thromboembolic pulmonary hypertension (CTEPH) in survivors. The underlying factors contributing to cardiac and functional decline in affected patients remain unclear, and existing tools for identifying high-risk patients are insufficiently sensitive and specific. This study aimed to develop a machine learning model to identify patients at risk for long-term consequences, potentially improving diagnosis and understanding of post-PE syndrome.
Method This study utilized data from the RIETE registry, the world's largest prospective international registry for patients with PE, to develop supervised machine learning models for identifying patients at increased risk for post-PE syndrome (according to modified ISTH criteria) and for CTEPH (secondary outcome). All patients with an index event PE and at least six months of follow-up were included. Relevant predictor variables were defined a priori in an analysis plan. Missing values were imputed using multiple imputation by chained equations (MICE). Our approach involved a three-step methodology consisting of data preprocessing, model training via a random forest algorithm, and model validation through 50-fold Monte Carlo cross-validation with patients being randomly sampled into training and test sets at an 80:20 ratio. Additionally, the performance of the CTEPH prediction model was benchmarked against an existing score by Klok et al.
Results Machine learning was based on 113 predictor variables from 8301 PE patients, of whom 2133 (25.7 %) were classified with post-PE syndrome at six-month follow-up. The model developed to predict post-PE syndrome yielded an AUC of 0.58 (95 % CI: 0.58-0.59) in cross-validation (▶ **Fig. 1**). A subset of

Hämostaseologie 2024; 44: S1–S125 | © 2024. Thieme. All rights reserved.

173 (2.1 %) patients developed CTEPH. The validated CTEPH model had an AUC of 0.66 (95 % CI: 0.64-0.67, ▶ **Fig. 2**). Sensitivity analysis for predicting CTEPH was even better with a score of 0.74 (95 % CI: 0.73-0.75), both outperforming the existing CTEPH prediction score (0.54; 95 %CI 0.51-0.57). Key variables consistent across all models included PE location on CT, fibrinogen levels, chest pain at presentation, T-wave inversion in electrocardiogram, absence of electrocardiographic abnormalities, clot site on CT, and co-occurrence of DVT in the lower limbs.



▶ **Fig. 1 Machine learning performances for post-PE syndrome and relative importance of model parame;** (**a**) Performance metrics for the prediction of post-PE syndrome. Error bars indicate the 95 % confidence intervals. (**b**) Relative feature importance of the ten most predictive parameters in the post-PE prediction model.



▶ **Fig. 2 Machine learning performances for CTEPH and relative importance of model parameters;** (A) Performance metrics for the prediction of CTEPH. Error bars indicate the 95 % confidence intervals. (B) Relative feature importance of the ten most predictive parameters in the CTEPH prediction model.

Conclusion Our machine learning approach underscored the complexity and limitations of using post-PE syndrome as a clinical endpoint, while demonstrating promising potential in predicting CTEPH. A more nuanced approach to defining post-PE syndrome seems advisable. Moreover, future research on long-term consequences after PE should build upon the identified predictor variables in our study.

Conflict of Interest The authors declare no conflict of interest.

T-01-09 Multisite thrombosis in a patient with paroxysmal nocturnal hemoglobinuria

Authors L. Beckmann[1], T. D. Faizy[2], F. Flottmann[2], J. Fiehler[2], L. Well[3], C. Bokemeyer[1], A. Beitzen-Heineke[1], F. Langer[1]

Institutes **1** Universitätsklinikum Eppendorf, Zentrum für Onkologie, II. Medizinische Klinik und Poliklinik, Hamburg, Germany; **2** Universitätsklinikum Eppendorf, Zentrum für Radiologie und Endoskopie, Klinik und Poliklinik für Neuroradiologische Diagnostik und Intervention, Hamburg, Germany; **3** Universitätsklinikum Eppendorf, Zentrum für Radiologie und Endoskopie, Klinik und Poliklinik für Diagnostische und Interventionelle Radiologie und Nuklearmedizin, Hamburg, Germany

DOI 10.1055/s-0044-1779065

Introduction Paroxysmal nocturnal hemoglobinuria (PNH) is an extremely rare bone marrow disorder [1]. In addition to complement-mediated hemolysis and cytopenia, venous and arterial thromboses at multiple and/or unusual sites are a common complication [1, 2]. Here, we describe the case of a 58-year-old woman with catastrophic PNH-associated thrombosis.

Method Coagulation and flow cytometric analyses and imaging studies were carried out as part of the patient's routine diagnostic workup.

Results The patient was admitted to our emergency department with a 2-day history of rapidly progressive muscle weakness of all extremities and altered behavior. The patient reported severe headache and abdominal pain for about a week. In addition, she had dark-colored urine for about 2 years. Imaging diagnostics revealed extensive thromboses of cerebral sinus and bridging veins with congestive brain infarctions and hemorrhages. In addition, multilocular hepatic vein thrombosis and splenic infarction were identified. Blood count showed mild anemia (Hb 12.1 g/dL) and thrombocytopenia (71 Mrd/L). Bilirubin, LDH and schistocytes were not available at admission. While global coagulation tests were normal, plasma D-dimers were markedly elevated (12.5 mg/L). Deficiencies in antithrombin, protein C or protein S, activated protein C resistance, dysfibrinogenemia, antiphospholipid syndrome, and $JAK2^{V617F}$ mutation were excluded. Flow cytometric analysis of peripheral blood revealed glycosylphosphatidylinositol anchor protein deficiency in up to 65 % of leukocytes and erythrocytes, confirming diagnosis of PNH. Despite immediate initiation of anticoagulation with unfractionated heparin and endovascular mechanical thrombectomy, the patient died few days later. At the time of PNH diagnosis, initiation of complement inhibitory therapy was waived due to the patient's unfavorable clinical prognosis.

Conclusion Although PNH is an orphan disease, it has promising treatment options. PNH should be considered in patients with newly diagnosed thromboses, especially if located at multiple and/or unusual sites.

Conflict of Interest The authors declare no potential conflicts of interest relevant to the content of the abstract.

References

[1] Panse J. Paroxysmal nocturnal hemoglobinuria: Where we stand. Am J Hematol 2023; 98: (Suppl 4): S20–S32

[2] Hill A, Kelly RJ, Hillmen P. Thrombosis in paroxysmal nocturnal hemoglobinuria. Blood 2013; 121 (25): 4985–96.Available from: URL: https://www.sciencedirect.com/science/article/pii/S0006497120572492

T-01-10 Anxiety and quality of life in the acute and post-acute phase after venous thromboembolism

Authors D. Steiner[1], S. Nopp[1], B. Weber[2], C. Ay[1]

Institutes **1** Medical University of Vienna, Department of Medicine I, Clinical Division of Haematology and Haemostaseology, Vienna, Austria; **2** Medical University of Vienna, Department of Dermatology, Vienna, Austria

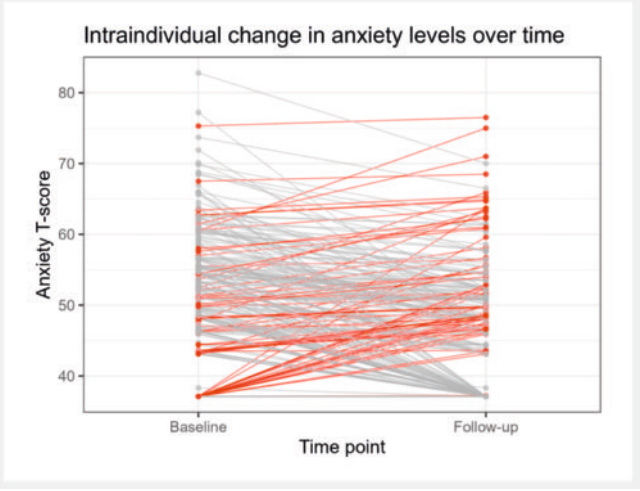
DOI 10.1055/s-0044-1779066

Introduction Venous thromboembolism (VTE) is associated with various short- and long-term complications, including psychological distress and de-

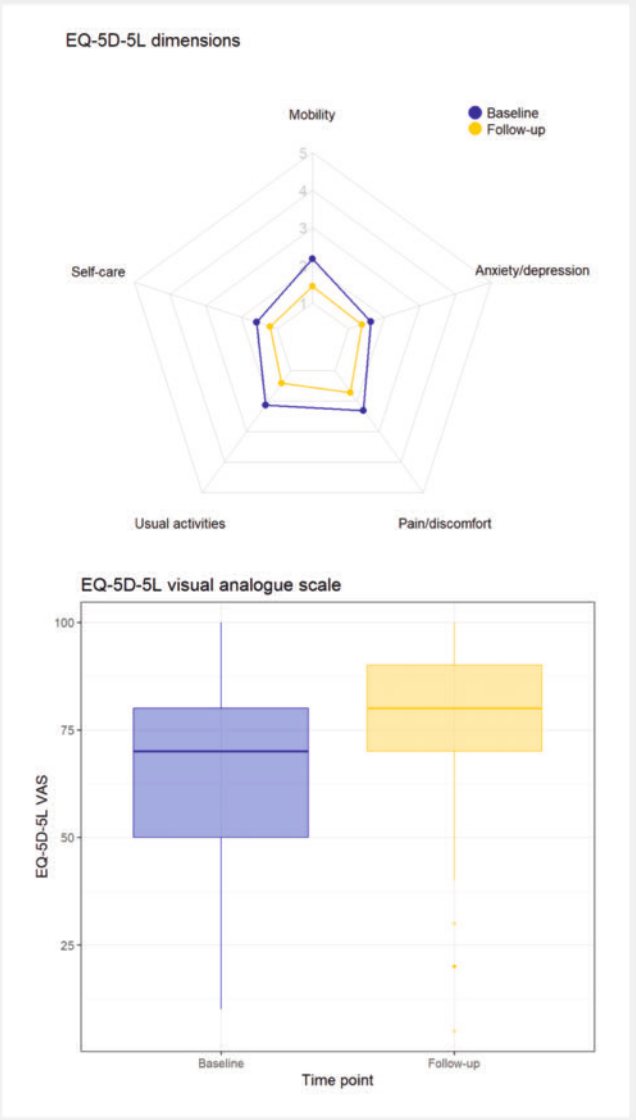
terioration of quality of life. We aimed to describe self-reported anxiety and generic quality of life from diagnosis up to three months after VTE.

Method We conducted a prospective cohort study including unselected VTE patients within 21 days of diagnosis at a tertiary care center. Patients with anticoagulation at the time of diagnosis were excluded. After three months, patients underwent a follow-up visit incorporated into routine clinical care. Anxiety was assessed with the Patient-Reported Outcomes Measurement Information System (PROMIS) short form 8a at baseline and follow-up. Using an online tool, a standardized T-score was calculated from the raw scores, with higher values indicating a higher degree of anxiety. The reference value is derived from a general population, with a mean of 50 and a standard deviation of 10. Quality of life was assessed with the EuroQoL Group 5-Dimension 5-Level (EQ-5D-5L) questionnaire and visual analogue scale (VAS). Based on a reference value set, answers from questions dealing with five dimensions were integrated into an overall index ranging from -0.661 to 1. For both index and visual analogue scale, higher values indicate better quality of life.

Results We included 321 patients with a median (interquartile, IQR) age of 55.6 (43.4-66.1) years; 138 (43.0 %) were women. Nearly 50 % of all patients had pulmonary embolism, median (IQR) BMI was 27.7 (24.4-31.4) kg/m², 91 (28.3 %) reported a history of VTE, and 79 (24.6 %) reported a history of cardiovascular or pulmonary disease. At baseline, 301 patients with available data had a mean (standard deviation) anxiety T-score of 51.1 (9.3). After three months, anxiety T-score improved to 46.8 (9.1), reflecting a mean change of -4.1 from baseline (data available for 254 patients). However, 60 patients (23.6 % of patients with available data) reported increased levels of anxiety at three months (▶ **Fig. 1**). Median (IQR) EQ-5D-5L index for 312 patients at baseline was 0.85 (0.68-0.92). At follow-up, data was available for 263 patients, with a median (IQR) of 0.90 (0.88-1.00). Overall, 17.8 % reported a decrease in quality of life compared to baseline. Regarding VAS, median (IQR) values at baseline and follow-up were 70 (50-80) and 80 (70-90), respectively (▶ **Fig. 2**; data available for 307 and 262 patients, respectively). Similar to the index, 46 patients (17.8 %) reported a decreased quality of life compared to baseline.



▶ **Fig. 1 Intraindividual change in T-score of PROMIS anxiety short form 8a from baseline to follow-up;** Higher values indicate a higher degree of anxiety. Every patient is represented by points and a connecting line. Patients depicted in red reported worsening of anxiety throughout follow-up (T-score change ≥ 1).



▶ **Fig. 2 EQ-5D-5L dimensions (upper panel) and visual analogue scale (lower panel) at baseline (blue)**; For dimensions, higher values indicate more problems. For visual analogue scale, higher values indicate better health. Dimension are presented as means, visual analogue scale values as median (bold line), third and first quartile (upper and lower hinge, respectively), and outliers (points).

Conclusion During the three months following a VTE diagnosis, self-reported anxiety and generic quality of life of patients improved overall. However, a considerable proportion of patients reported increased levels of anxiety and decreased levels of quality of life, necessitating a better understanding and further investigation of this group.

Conflict of Interest DS, SN, and BW report no conflicts of interest. CA received personal fees for lectures and/or participation in advisory boards from Bayer, BMS, Daiichi-Sankyo, Pfizer, and Sanofi.

Hämostaseologie 2024; 44: S1–S125

T-01-11 Gut microbiota does not influence NETosis via Toll-like receptor 2 in a mesenteric ischemia-reperfusion injury mouse model

Authors Z. Gao[1], K. Kiouptsi[1, 2], C. Reinhardt[1, 2]
Institutes 1 University Medical Center of the Johannes Gutenberg-University Mainz, Center for Thrombosis and Hemostasis (CTH), Mainz, Germany; 2 University Medical Center of the Johannes Gutenberg-University Mainz, Partner Site Rhine Main, German Center for Cardiovascular Research (DZHK), Mainz, Germany
DOI 10.1055/s-0044-1779067

Introduction Acute mesenteric ischemia (AMI) is a rare but life-threatening condition. Formation of neutrophil extracellular traps (NETs) contribute to inflammatory damage in acute mesenteric infarction. NETosis is induced by microbial specific molecular patterns recognized by host pattern recognition receptors, such as toll-like receptors (TLRs), specifically TLR4. However, whether additional innate immune receptors such as TLR2 signaling is involved in this inflammatory process, is still not well understood. Therefore, we investigated the adhering leukocytes and NET formation *in vivo* and ex-vivo in conventionally-raised (CONV-R) and germ-free (GF) TLR2$^{-/-}$ mice and wildtype (WT) control mice.

Method In an established ischemia-reperfusion(I/R) injury model, the I/R-induced NETs were stained with SYTOX™ Orange and the adhering leukocytes with acridine orange, subsequently visualized by epifluorescence intravital microscopy (IVM) *in vivo*. For ex-vivo NETosis assays, isolated neutrophils from mouse bone marrow were stimulated with the TLR2/6 agonist MALP-2 and NETosis was measured in a fluorimetric assay.

Results We confirmed that microbiota increases leukocyte adhesion in mesenteric venules post-ischemia. Of note, global TLR2 deficiency does not influence leukocyte adhesion pre- or post-ischemia in the mesenteric venules. Interestingly, TLR2 does not influence *in vivo* NETosis in this model at germ-free conditions. The stimulation of bone marrow neutrophils with a TLR2/6 agonist (MALP-2) did induce NETosis *ex vivo*, indicating that other vascular cell types might contribute.

Conclusion We conclude that conventionally raised TLR2$^{-/-}$ and WT mice have comparable leukocyte adhesion and that the deficiency of TLR2 does not affect NETosis in the germ-free housing state.

Conflict of Interest The authors declare that there is no conflict of interest.

T-01-12 Platelet glycoprotein IIb- and IIIa levels are altered in persistently lupus anticoagulant positive patients with a history of thrombosis

Authors F. Hsu[2], T. Schramm[1], A. Sedighi[1], W. Schrottmaier[2], C. Ay[1], I. Pabinger[1], M. Zellner[2], J. Gebhart[1]
Institutes 1 Medical University of Vienna, Department of Medicine I / Clinical Division of Haematology and Haemostaseology, 1090, Austria; 2 Medical University of Vienna, Centre for Physiology and Pharmacology / 2Institute of Vascular Biology and Thrombosis Research, 1090, Austria
DOI 10.1055/s-0044-1779068

Introduction The glycoprotein IIb/IIIa complex formed by the integrins CD41 and CD61 undergoes a conformational change during platelet activation, enabling fibrinogen binding and leading to platelet aggregation and thrombus formation. Paradoxically, the total amount of these integrins in platelets was reported to be lower in prothrombotic diseases such as lupus anticoagulant (LA), COVID-19 and lung cancer patients. Nevertheless, their role in LA-associated thrombosis development and fibrinogen-binding capacity is still unknown. This study aimed to quantify the basal and ADP-activated platelet surface levels of CD41 and CD61 and their affinity to bind fibrinogen in LA patients with (LA + TE +) and without thrombosis history (LA + TE-) compared to age- and sex-matched healthy control (HC) individuals.

Method Twenty patients with persistent LA + TE + , ten LA + TE- and thirty matched HC from the Vienna Lupus Anticoagulant and Thrombosis study (EC 1268/2014) were included (▶ **Fig. 1**). Patients with antiplatelet drugs were excluded, and the selected controls did not take any antiplatelet medication for at least 10 days before blood sampling. Blood was drawn into 3.5 mL CTAD Vacuette tubes (3.2 % sodium citrate with theophylline, adenosine and dipyridamole). Basal and ADP-activated [c = 15 µM] surface levels of integrin CD41, CD61, CD62P and binding of fibrinogen to the activated CD41/CD61 complex antibody were measured using a PAC-1 antibody from platelet-rich plasma (PRP) by flow cytometry.

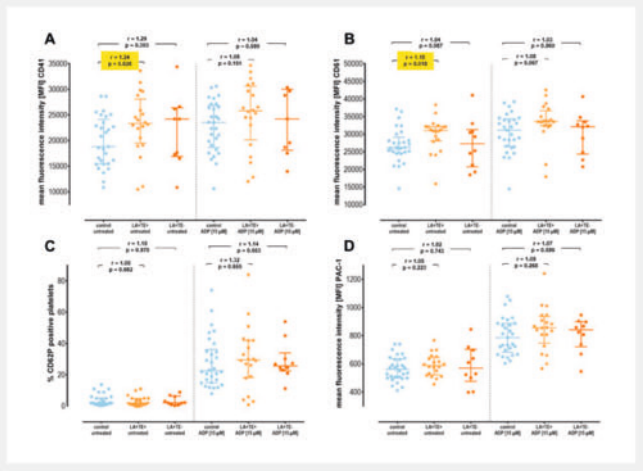
Table 1: Demographic, clinical and laboratory data of the study cohort

		LA+TE+		LA+TE-		Healthy Controls
	n	median (IQR)	n	median (IQR)	n	median (IQR)
Age, years	20	51.0 (45.5-58.0)	10	59 (53.5-67.0)	30	53.5 (46.0-61.5)
Platelet Count, x10^9/L	20	207.5 (178.5-243.5)	10	257 (213.0-280.0)	30	274.5 (245.0-288.0)
Fibrinogen, mg/dL	20	318.5 (296.5-361.5)	10	334.0 (274.0-407.0)	27	306 (275.0-392.0)
aPTT, s	20	68.5 (58.7-84.9)	10	51.5 (48.9-60.6)	27	34.5 (33.2-35.1)
Prothrombin time, %	20	33.5 (28.8-45.3)	10	99.0 (91.0-104.0)	26	94.5 (88.5-100.0)
		n (%)		n (%)		n (%)
Female	20	15 (75.0)	10	8 (80.0)	30	22 (73.3)
aPLAs						
LA alone[a]	20	4 (20.0)	10	4 (40.0)	30	0 (0)
LA+ anti–β2GPI[b]	20	0 (0)	10	0 (0)	30	0 (0)
LA+ aCL[b]	20	1 (5.0)	10	0 (0)	30	0 (0)
LA+ anti-β2GPI + aCL (triple positivity)[b]	20	15 (75.0)	10	3 (30.0)	30	0 (0)
Thrombosis history						
Arterial TE	20	3 (15.0)	10	0 (0)	30	0 (0)
Venous TE (+PE)	20	16 (80.0)	10	0 (0)	30	0 (0)
Arterial + Venous TE	20	1 (5.0)	10	0 (0)	30	0 (0)
Disease specific treatment						
Phenprocoumon	20	14 (70.0)	10	0 (0)	30	0 (0)

Note: LA, lupus anticoagulant, TE, thromboembolism; IQR, interquartile range; n, number; aPTT, activated partial thromboplastin time; aPLA, antiphospholipid antibodies; PE, pulmonary embolism; β2GPI, beta-2 glycoprotein; aCL, anti-cardiolipin antibodies
[a]LA alone defined as the absence of IgG/IgM anti-β2GPI and aCL
[b]Cutoff: anti-β2GPI >8 GPL/MPL U/mL, aCL >40 GPL/MPL U/mL

▶ **Fig. 1** The abundance of these platelet surface receptors is indicated by median fluorescence intensity (MFI) from (A) CD41, (B) CD61, (C) CD62P and (D) the activated GPIIb/IIIa complex by PAC-1 binding from LA + TE + (n = 20), LA + TE- (n = 10) patients and matched HC (n = 30). Median values are indicated with a horizontal line ± 95 % confidence interval. The ratios of changes (r) were calculated by dividing the median value of the diseased group by that of the matched healthy control group. The significance of the change (p values) was calculated between study groups using the Mann-Whitney test.

Results Basal levels of CD41 and CD61 were significantly increased in LA + TE + (CD41: + 24 %, p = 0.03; CD61: + 18 %, p = 0.02) but not in LA + TE- patients compared to HC (▶ **Fig. 2a, b**). Notably, ADP activation significantly increased surface levels of CD41 and CD61 across all study groups. However, LA + TE + patients had a lower ADP- induced increase in platelet surface CD41 levels than in HC (8 % vs. 13 %, p = 0.038), whereas there was no significant difference between LA + TE- patients and HC (11 % vs. 13 %, p = 0.450). A similar trend was observed for CD61, with an ADP-induced increase in LA + TE + patients compared to HC (8 % vs. 12 %, p = 0.070) and in LA + TE- patients compared to HC (11 % vs. 12 %, p = 0.537). There was no difference in classic platelet activation markers CD62P and affinity for fibrinogen (PAC-binding) before and after ADP activation in LA + TE + or LA + TE- patients compared to HC (▶ **Fig. 2c, d**).



▶ **Fig. 2**

Conclusion The increased basal levels of surface integrins CD41 and CD61 indicate a higher platelet activation state in LA + TE + patients, which was not found in LA + patients without prior thrombosis. The attenuated ADP-induced upregulation may be related to the lower total available amount of these glycoproteins in platelets of LA + TE + patients. These observations demonstrate that persistently activated and "exhausted" platelets may contribute to the prothrombotic phenotype in patients with persistent LA, as has already been observed in patients with various cancers.

Conflict of Interest The authors declare no conflict of interest.

T-02. Arteriosclerosis and inflammation

T-02-01 External Validation of the OAC³-PAD Risk Score for Patients Treated with Lower Extremity Endovascular Revascularisation

Authors K. Pelicon[1], K. Petek[1], A. Boc[1, 2], V. Boc[1], N. Kejžar[3], A. Blinc[1, 4]

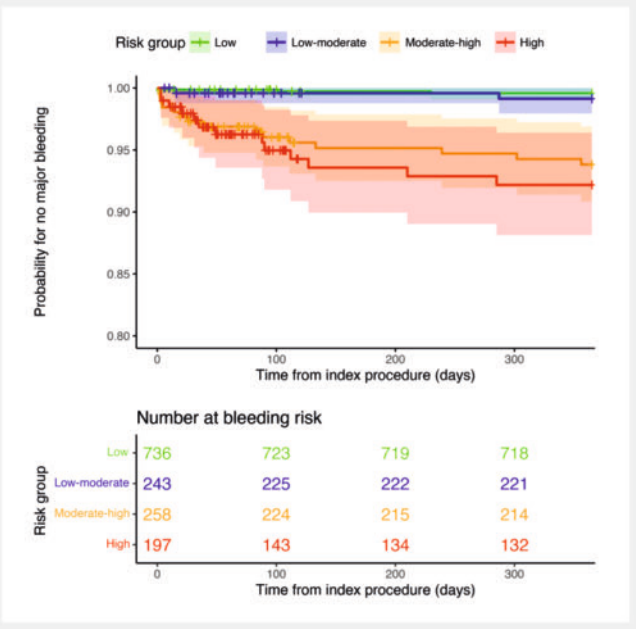
Institutes **1** University Medical Centre Ljubljana, Department of Vascular Diseases, Ljubljana, Slovenia; **2** University of Ljubljana, Faculty of Medicine, Institute of Anatomy, Ljubljana, Slovenia; **3** University of Ljubljana, Faculty of Medicine, Institute of Biostatistics, Ljubljana, Slovenia; **4** University Medical Centre Ljubljana, Department of Internal Medicine, Ljubljana, Slovenia

DOI 10.1055/s-0044-1779069

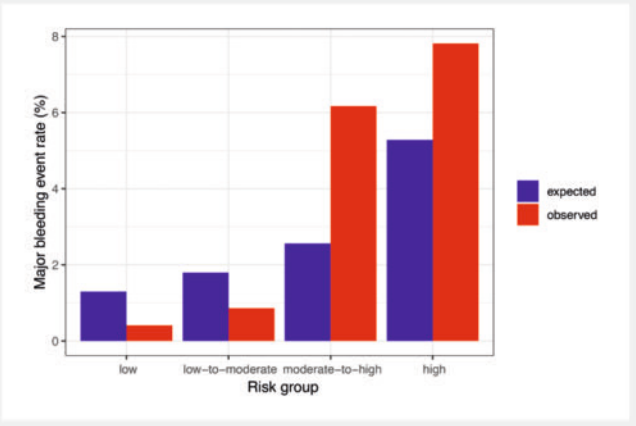
Introduction Various bleeding risk scores have been conceived to assess the bleeding risk in patients with cardiac disease, however the OAC³-PAD bleeding risk score is only the second one specifically designed to assess the bleeding risk in patients with peripheral arterial disease (PAD) [1]. We aimed to externally validate the OAC³-PAD risk score for PAD patients treated exclusively with endovascular revascularisation.

Method We retrospectively reviewed the medical documentation of all 1 612 patients who underwent successful endovascular revascularisation at the Clinical Department of Vascular Diseases at the University Medical Centre Ljubljana in a 5-year period. Performance of the risk score was tested in two steps [2]. We first assessed the Cox proportional hazards (CPH) model upon which the score is based using discrimination, calibration, and a scaled Brier score. Secondly, we tested the risk score itself by calculating the patients' risk scores and grouping patients into the four risk groups. Survival analysis was performed using Kaplan-Meier curves and discrimination was tested using log-rank analysis.

Results Uno's IPCW corrected AUC, a measure of discrimination of the CPH model, was 0.83 (95 % CI 0.76 – 0.90). The scaled Brier score was 3.27 % (95 % CI 0.65 % – 4.40 %), indicating poor overall performance. 1 434 patients were included in the main analysis, of whom 33 (2.3 %) experienced a major bleeding event. Major bleeding rates in the low, low-to-moderate, moderate-to-high, and high risk groups were 0.4 % (3/736 patients), 0.8 % (2/243 patients), 5.8 % (15/258 patients), and 6.6 % (13/197 patients), respectively. The Kaplan-Meier curves presenting the event-free survival of patients for all four groups are shown in ▶ **Fig. 1**. Observed and expected event rates are shown in ▶ **Fig. 2**.



▶ **Fig. 1 Kaplan-Meier curves for the four bleeding risk groups in our patient cohort.**; Kaplan-Meier curves showing the event-free survival of our patients in the low, low-to-moderate, moderate-to-high, and high risk group as determined by the OAC³-PAD risk score.



▶ **Fig. 2 Observed and expected major bleeding rates as obtained from the Kaplan-Meier curves and th**; Observed 1-year major bleeding rates as obtained from the Kaplan-Meier curves for our validation cohort and expected 1-year major bleeding rates as obtained from the CPH model upon which the OAC³-PAD risk score is based.

Log-rank analysis showed successful discrimination between either of the two lower risk groups and one of the higher risk groups: low vs moderate-to-high risk ($p < .001$), low vs high risk ($p < .001$), low-to-moderate vs moderate-to-high risk ($p = .003$), and low-to-moderate vs high risk ($p < .001$). However, the score failed to discriminate between the low and low-to-moderate, as well as between the moderate-to-high and high risk group.

Conclusion While discrimination of the OAC^3-PAD CPH model was adequate, calibration was poor. The score overestimated the bleeding risk in low-risk patients while underestimating the risk in high-risk patients. Although the score failed to stratify PAD patients after percutaneous revascularisation into the four respective risk groups, it can be clinically useful in distinguishing low risk patients from high risk patients.

Conflict of Interest The authors declare no conflicts of interest.

References

[1] Behrendt C-A, Kreutzburg T, Nordanstig J, Twine CP, Marschall U, Kakkos S et al. The OAC3-PAD Risk Score Predicts Major Bleeding Events one Year after Hospitalisation for Peripheral Artery Disease. European Journal of Vascular and Endovascular Surgery 2022; 63 (3): 503–10. doi:10.1016/j.ejvs.2021.12.019

[2] McLernon DJ, Giardiello D, Van Calster B, Wynants L, van Geloven N, van Smeden M et al. Assessing Performance and Clinical Usefulness in Prediction Models With Survival Outcomes: Practical Guidance for Cox Proportional Hazards Models. Ann Intern Med 2023; 176 (1): 105–14. doi:10.7326/M22-0844

T-02-02 Platelet-derived LTB4 mediates neutrophil recruitment

Authors C. Hackenbroch[1, 2], C. Gross[1, 2], T. Cimen[2], S. Rubenzucker[3], T. Girbl[2], R. Ahrends[3], D. Stegner[1, 2]

Institutes **1** University Hospital Würzburg, Department of Experimental Biomedicine I, Würzburg, Germany; **2** University of Würzburg, Rudolf Virchow Center for Integrative and Translational Bioimaging, Würzburg, Germany; **3** University of Vienna, Department of Analytical Chemistry, Vienna, Austria

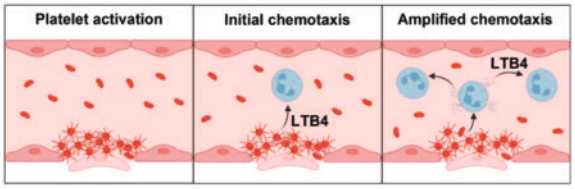
DOI 10.1055/s-0044-1779070

Introduction Leukotriene B4 (LTB4) is a pro-inflammatory lipid mediator derived from arachidonic acid. It is synthesized via the 5-lipoxygenase pathway by the leukotriene A4 hydrolase (Lta4h) [1]. It is primarily secreted by neutrophils and acts as a chemoattractant, drawing more neutrophils to the area by inducing and arresting neutrophil swarming. Additionally, LTB4 triggers accompanying inflammatory responses such as reactive oxygen species production and the formation of neutrophil extracellular traps [1, 2]. Recently, lipidome analyses identified platelets as a potential source of LTB4, suggesting that this might be a mechanism by which activated platelets directly recruit immune cells to sites of injury and drive inflammation [3]

The aim of this study is to investigate the relevance of LTB4 for platelet activation, hemostasis and neutrophil recruitment.

Method Lta4h-deficient mice (*Lta4h*$^{-/-}$), which are unable to form LTB4, were generated and changes in the platelet lipidome were measured by liquid chromatography tandem mass spectrometry (LC-MS/MS). *Lta4h*$^{-/-}$ platelet function was assessed by standard *in-vitro* assays, and an *in vivo* tail bleeding time assay. The *in-vivo* effects of platelet-derived LTB4 on neutrophil recruitment to sites of endothelial damage were studied in different models of sterile inflammation in the liver and the cremaster muscle using intravital microscopy.

Results *Lta4h*$^{-/-}$mice show unaltered blood parameters. LC-MS/MS confirms the lack of LTB4 in the supernatant of activated Lta4h$^{-/-}$ platelets. Injection of the supernatant of stimulated wildtype platelets but not of Lta4h$^{-/-}$ platelets leads to strong recruitment and extravasation of neutrophils in the cremaster muscle. Intravital microscopy shows that neutrophil infiltration in the liver following ischemia reperfusion injury or hot needle injury is significantly reduced, both in the full knockout and after Lta4h$^{-/-}$platelet transfer into previously platelet-depleted animals. Notably, platelet function in classical platelet function tests and the tail bleeding time are not affected by the absence of platelet-derived LTB4 (▶ **Fig. 1**).



▶ **Fig. 1 Platelet-derived LTB4 mediates neutrophil recruitment;** Endothelial damage leads to the adhesion and activation of platelets. Activated platelets secrete LTB4 and attract nearby neutrophils, which in turn release LTB4, leading to the further recruitment of neutrophils. Created with BioRender.com.

Conclusion Our study demonstrates for the first time that platelets secrete LTB4 to directly recruit neutrophils to sites of endothelial damage, making it a potent and previously unknown driver of thrombo-inflammation.

Conflict of Interest I do not have an affiliation (financial or otherwise) with a for-profit/not-for-profit organization.

References

[1] Nakamura M, Shimizu T 'Therapeutic target of leukotriene B4 receptors, BLT1 and BLT2: Insights from basic research'. Biochimie Epub ahead of print 2023

[2] Lämmermann T *et al* 'Neutrophil swarms require LTB4 and integrins at sites of cell death. in vivo' , Nature 2013; 498: 371–375

[3] Peng B *et al* 'Identification of key lipids critical for platelet activation by comprehensive analysis of the platelet lipidome'. Blood 2018; 132 (5): e1–e12

T-02-03 Dietary supplements: Anti-coagulatory and anti-oxidative effects of powdered fruit, vegetable and berry juice powder concentrates.

Authors M. Tsiountsioura[1], T. Ziegler[1, 3], L. Meixner-Götz[3, 4], A. Schlagenhauf[2], F. Schmutz[1], G. Kager[1], G. Ledinski[1], M. Lamprecht[3, 4], G. Cvirn[1]

Institutes **1** Medical University of Graz, Division of Medicinal Chemistry, Graz, Austria; **2** Medical University of Graz, Department of General Pediatrics and Adolescent Medicine, Graz, Austria; **3** Juice Plus + Science Institute, Collierville, USA; **4** Green Beat – Institute of Nutrient Research and Sports Nutrition, Graz, Austria

DOI 10.1055/s-0044-1779071

Introduction Micronutrients are involved in many important functions of the human body and an adequate intake is necessary for optimal health. They could be supplied either by the intake of food or through supplements. The aim of this in-vitro study was to examine the anti-coagulatory as well as the anti-oxidative properties of four supplements, namely a fruit, a vegetable and a berry powder concentrate, and a powder concentrate containing a combination of the three (FVB).

Method Dried extracts were resuspended with NaCl solution, left in an ultrasonic bath, rotated for 30 min in a rotary shaker, and centrifuged. Their anti-oxidative properties were estimated via their capability to attenuate the Cu^{2+}-in-

duced oxidation of low-density lipoprotein (LDL). The anti-coagulatory properties of the concentrates were estimated via thrombelastometry (TEM) and platelet aggregation measurements, performed in whole blood samples. Thrombin generation was assessed by using calibrated automated thrombogram (CAT).

Results The berry concentrate most efficiently impeded the Cu^{2+}-induced LDL oxidation and thus, possessed the highest antioxidant capacity, followed by the FVB concentrate. The vegetable and fruit concentrates had only low antioxidant capacities. Correspondingly, the berry concentrate exerted the most efficient anticoagulant action: The TEM-value "Coagulation time" was significantly prolonged from 218 ± 18 to 249 ± 23 s (P = 0.017). Moreover, the Impedance Aggregation value "Amplitude" was significantly decreased from 13 ± 1.7 to 4.3 ± 1.5 ohm (P = 0.017) by the berry concentrate. CAT measurements revealed that the berry concentrate is able to inhibit the thrombin activity in plasma samples. The fruit, vegetable, as well as the FVB concentrate affected all the coagulation parameters to a much lesser degree.

Conclusion Our in-vitro study indicates that particularly the berry concentrate has potent anti-coagulatory as well as anti-oxidative properties. We have therefore launched a randomized, controlled, open-labelled, parallel-grouped, clinical trial to investigate the anti-oxidative and anti-coagulatory effects of the four concentrates in 112 elderly individuals [1].

Conflict of Interest ML, TZ and LMG are affiliated to the Juice Plus + Science Institute which is funded by NSA LLC/The Juice Plus + ® Company, Memphis, Tennessee, USA, provider of the nutritional supplements utilised in this project. There are no other conflicts to declare.

References

[1] Tsiountsioura M, Cvirn G, Meixner-Goetz L, Ziegler T, Lamprecht M. Effects of long-term consumption of two plant-based dietary supplements on cardiovascular health and low-grade inflammation in middle-aged and elderly people: study protocol for a randomised, controlled trial. J Health Popul Nutr 2023; 42 (1): 100. doi:10.1186/s41043-023-00434-x

T-02-04 Insights into the molecular basis and mechanism of heme-triggered TLR4 signaling

Authors M.-T. Hopp[1, 3], J. Holze[2], D. C. Rathod[1], G. Weindl[2], D. Imhof[1]

Institutes **1** University of Bonn, Pharmaceutical Biochemistry and Bioanalytics, Pharmaceutical Institute, Bonn, Germany; **2** University of Bonn, Pharmacology and Toxicology, Bonn, Germany; **3** University of Koblenz, Department of Chemistry, Institute for Integrated Natural Sciences, Koblenz, Germany

DOI 10.1055/s-0044-1779072

Introduction Large amounts of labile heme are known to be pro-inflammatory, pro-thrombotic, and cytotoxic by e.g. binding to biomolecules such as proteins [1–3]. One such example is TLR4, which has been poorly understood with regards to its activation by heme. TLR4 forms a complex with MD2 and the complex then assembles in a dimeric state. In this study, we report *in vitro*, cellular and *in silico* analysis of the heme binding to TLR4, MD2, and their complex.

Method Heme binding was investigated on the peptide as well as the protein level by employing prediction of heme-binding motifs by HeMoQuest [4], peptide synthesis and characterization, as well as binding studies by UV/vis spectroscopy experiments. Surface plasmon resonance (SPR) and UV/vis spectroscopic studies were used to analyze heme binding to the proteins. These experiments were further complimented by molecular docking and molecular dynamics simulations of the free and heme-bound proteins TLR4, and MD2, as well as the TLR4-MD2 complex.

Results Our study revealed motifs with high to moderate heme-binding affinity in both proteins, with TLR4 possessing 4 HBMs and MD2 2 HBMs. In contrast to an earlier report [5], two other heme-binding sites were identified in MD2. Cellular experiments analyzing heme-mediated cytokine responses are partially via TLR4 and independently of CD14, which activates TLR4 signaling in the absence of MD2. The further characterization of the most promising and experimentally validated HBMs in TLR4 using mutagenesis studies revealed that heme interacts with TLR4 through distinct binding sites, and individual HBMs are dispensable for full receptor activation.

Conclusion To summarize, a stepstone analysis in the direction of TLR4 signaling activation via transient heme binding was performed. HBMs available in the TLR4-MD2 complex can mediate TLR4 signaling upon heme binding via different mechanisms [6]. Additionally, we also found that TLR4 has higher heme binding affinity as compared to MD2. A detailed exploration of the effects of heme on immune receptors with regard to proinflammatory effects may contribute to a better understanding of hemolytic disorders.

Conflict of Interest The authors declare no conflict of interest.

References

[1] Roumenina LT, Rayes J, Lacroix-Desmazes S, Dimitrov JD. Heme: Modulator of Plasma Systems in Hemolytic Diseases. Trends Mol Med 2016; 22 (3): 200–213. doi:10.1016/j.molmed.2016.01.004

[2] Hopp M-T, Imhof D. Linking Labile Heme with Thrombosis. Journal of Clinical Medicine 2021; 10 (3): 427. doi:10.3390/jcm10030427

[3] Mubeen S, Domingo-Fernández D, Díaz del Ser S, Solanki DM, Kodamullil AT, Hofmann-Apitius M, Hopp M-T, Imhof D. Exploring the Complex Network of Heme-Triggered Effects on the Blood Coagulation System. Journal of Clinical Medicine 2022; 11 (19): 5975. doi:10.3390/jcm11195975

[4] Paul George AA, Lacerda M, Syllwasschy BF, Hopp MT, Wißbrock A, Imhof D. HeMoQuest: a webserver for qualitative prediction of transient heme binding to protein motifs. BMC Bioinformatics 2020; 21 (1): 124. Published 2020 Mar 27. doi:10.1186/s12859-020-3420-2

[5] Belcher JD, Zhang P, Nguyen J et al. Identification of a Heme Activation Site on the MD-2/TLR4 Complex. Front Immunol 2020; 11: 1370. Published 2020 Jun 30. doi:10.3389/fimmu.2020.01370

[6] Hopp M-T, Holze J, Lauber F, Holtkamp L, Rathod DC, Miteva MA, Prestes EB, Geyer M, Manoury B, Merle NS, Roumenina LT, Bozza MT, Weindl G, Imhof D. Insights into the molecular basis and mechanism of heme-triggered TLR4 signaling: The role of heme-binding motifs in TLR4 and MD2. Immunology 2023 (accepted)

T-02-05 Diet and gut microbiota orchestrate the hepatic endothelial transcriptome in atherosclerosis

Authors O. Dremova[1], M. P. Khuu[1], H. Rupprecht[1], L. Bury[1], S. Boujataoui[1], F. Sommer[2], F. Marini[3], C. Reinhardt[1, 4]

Institutes **1** University Medical Center Mainz, Center for Thrombosis and Hemostasis, Mainz, Germany; **2** Christian-Albrechts-University, Institute of Clinical Molecular Biology, Kiel, Germany; **3** University Medical Center Mainz, Institute of Medical Biostatistics, Epidemiology and Informatics, Mainz, Germany; **4** Partner Site RhineMain, German Center for Cardiovascular Research (DZHK), Mainz, Germany

DOI 10.1055/s-0044-1779073

Introduction The gut-liver axis is a bidirectional system, which markedly influences remote metabolic functions of the host and is involved in the development of atherosclerosis. By delivering microbial components through the portal vein, the gut microbiota was shown to modulate the cholesterol and lipid metabolism in the hepatic endothelium. Yet, how diet, a significant modifier of gut microbial communities, affects the hepatic endothelium remains limitedly understood. In this study, we shed light on the diet-dependent effects on the transcriptome of liver endothelial cells (LECs) during the progression of both early and late-stage atherosclerosis.

Method Low-density lipoprotein receptor-deficient mice (*Ldlr*$^{-/-}$) were housed in either germ-free (GF) or conventionally raised (CONV-R) conditions and fed with high-fat Western diet (HFD) or standard chow diet (ND) for 16 weeks. Following isolation of LECs using a magnetic cell separation technique, whole-transcriptome sequencing analysis was performed. To dissect the influence of microbiome and diet at early time points, the effect of 8 weeks of feeding with ND or HFD on the LEC transcriptome, mRNA and protein expression was investigated. Furthermore, the aortic root and aortic arch lesion size was histologically quantified.

Results In CONV-R *Ldlr*$^{-/-}$ mice, fed with HFD for 16 weeks, cholesterol homeostasis and inflammatory responses in the hepatic endothelial transcriptome were deregulated. Interestingly, no significant regulation in the sphingolipid metabolism was identified. However, in early atherosclerosis *Ldlr*$^{-/-}$ mice exhibited diet-dependent regulations of the key enzymes sphingomyelin synthase-1 (Sms1) and sphingomyelin phosphodiesterase 3 (Smpd3) in LECs, suggesting dynamic changes of endothelial lipid synthesis pathways in the progression of atherosclerosis. Since sphingolipids were shown to be involved in atherogenesis, atherosclerotic plaques in aortic roots and aortic arch of GF and CONV-R *Ldlr*$^{-/-}$ mice were histologically analyzed. However, atherosclerotic lesion size in early atherosclerosis was unchanged at the studied conditions.

Conclusion Here, we report that the gut microbiota and diet dynamically modify the expression profile of enzymes involved in cholesterol and sphingolipid metabolism in the hepatic endothelium in a mouse model of early atherosclerosis.

Conflict of Interest The authors declare that they have no conflict of interest.

T-03. Pathomechanisms of thrombosis

T-03-01 GSDMD- and PAD4-Independent NET Formation Mediates Vascular Occlusions in Septicemia

Authors H. Englert[1], C. Rangaswamy[1], M. Divivier[1], J. Göbel[1], I. Hermans-Borgmeyer[2], U. Hermans-Borgmeyer[2], K. A. Mowen[3], T. Fuchs[1], T. Renné[1, 4]

Institutes **1** University Medical Center Hamburg-Eppendorf, Institute of Clinical Chemistry and Laboratory Medicine, Hamburg, Germany; **2** University Medical Center Hamburg-Eppendorf, Transgenic Mouse Unit, Center for Molecular Neurobiology Hamburg, Hamburg, Germany; **3** The Scripps Research Institute, Department of Chemical Physiology, La Jolla, USA; **4** Royal College of Surgeons in Ireland, Irish Centre for Vascular Biology, School of Pharmacy and Biomolecular Sciences, Dublin, Ireland

DOI 10.1055/s-0044-1779074

Introduction Generation of Neutrophil Extracellular Traps (NETs), also termed NETosis, contribute to host defense; however, excessive NETosis leads to hyperinflammatory, autoimmune and thrombotic disease states. Histone citrullination by peptidyl arginine deiminase 4 (PAD4), and pore formation in the plasma membrane by gasdermin D (GSDMD) are believed to be involved in the formation and release of NETs [1, 2]. In this study, we aimed to study the role of GSDMD and PAD4 in NET formation in experimental models of septicemia and sterile neutrophilia.

Method We generated *Gsdmd*$^{-/-}$ and *Pad4*$^{-/-}$gene deficient mice on a *Dnase1*$^{-/-}$ *Dnase1l3*$^{-/-}$ deficient background to amplify intravascular NET occlusions in these mice. Animals were challenged in two models of NET formation, sterile neutrophilia, and septicemia, triggered by hepatic transgene expression of granulocyte colony stimulating factor (G-CSF) and injection of LPS and heat-killed *E. coli*, respectively [3]. The survival rate, body weight, and peripheral body temperature of the mice were measured. The murine lungs were stained with H&E and the NET markers chromatin, myeloperoxidase and citrullinated histone 3 to detect intravascular NET occlusions. We also assessed *ex vivo* NET formation from isolated murine neutrophils using phorbol-12-myristate-13-acetate (PMA) and calcium ionophore in the presence or absence of GSDMD and PAD4.

Results Here we tested the importance of PAD4, and GSDMD for NETosis *in vivo*. *Gsdmd*/*Dnase1*/*Dnase1l3*$^{-/-}$ and *Pad4*/*Dnase1*/*Dnase1l3*$^{-/-}$ mice were not protected from death in sterile neutrophilia, and septicemia. Histological analysis of lung sections showed that NET-derived clots occluded blood vessels in both mouse strains. Furthermore, we found that the genetic or pharmacological inhibition of GSDMD blocks *ex vivo* NETosis in PMA, but not calcium ionophore-activated murine neutrophils. The inhibition of PAD4 did not block *ex vivo* NETosis, independent of the respective stimulus.

Conclusion Taken together, we challenge the current role of GSDMD by newly reporting that intravascular NETosis is independent of GSDMD in experimental models of sterile neutrophilia and septicemia.

Conflict of Interest The authors declare no conflict of interest.

References

[1] Li P, Li M, Lindberg MR, Kennett MJ, Xiong N, Wang Y. PAD4 is essential for antibacterial innate immunity mediated by neutrophil extracellular traps. J Exp Med 2010; 207 (9): 1853–62

[2] Sollberger G, Choidas A, Burn GL, Habenberger P, Di Lucrezia R, Kordes S et al. Gasdermin D plays a vital role in the generation of neutrophil extracellular traps. Sci Immunol 2018; 3 (26): eaar6689

[3] Jiménez-Alcázar M, Rangaswamy C, Panda R, Bitterling J, Simsek YJ, Long AT et al. Host DNases prevent vascular occlusion by neutrophil extracellular traps. Science. 2017; 358 (6367): 1202–6

T-03-02 Novel diversity of plasmatic thrombin pools to regulate blood clotting: interference by an anti-fibrin nanobody

Authors S. Sun[1, 2], J. Konings[1], R. T. Urbanus[3], D. Huskens[1], F. Swieringa[1], R.D. L. Kremers[1], J. Zou[1, 2], P. G. de Groot[1], M. Roest[1], J. W. Heemskerk[1, 2], B. de Laat[1]

Institutes **1** Synapse Research Institute, Maastricht, Netherlands; **2** Maastricht University, Department of Biochemistry, Maastricht, Netherlands; **3** University Medical Centre Utrecht, Van Kreveldkliniek, Utrecht, Netherlands

DOI 10.1055/s-0044-1779075

Introduction Thrombin has a multitude of roles in the initiation and propagation of (anti)coagulation, in platelet activation and fibrin clot formation. The assessment of thrombin generation (TG) has provided in-depth understanding of the plasma factors contributing to the (in)activation of thrombin in triggered plasma and whole-blood. By default, the generated thrombin is considered to act as a single pool. Here, we present evidence for the diversity of two major proteolytically active thrombin pools with non-overlapping functions in blood clotting.

Method A novel Syn-Nb-AF106 anti-fibrin nanobody (Nb106) was characterized, as described in Sun et al. (abstract this GTH meeting). TG was assessed by calibrated automated thrombography using various triggers with control (multi-donor) and (reconstituted) coagulation factor-deficient plasmas, platelet-rich plasma (PRP) or whole blood. Fibrin clot formation was evaluated by scanning electron microscopy (SEM). Clot retraction was assessed macroscopically.

Results In tissue factor-triggered TG,we discovered that Nb106 dose-dependently reduced the thrombin peak level and endogenous thrombin potential (ETP) to 54-62 %, without effect on kinetic TG parameters. We observed a similar reduction in platelet-free plasma, PRP and whole blood. We found that Nb106 inhibited tissue factor-induced TG using plasma deficient in factor IX, XI or XII, but not using plasmas deficient in fibrinogen or antithrombin. Reconstitution experiments showed that the inhibition depended on the fibrinogen concentration. Together, the fibrin(ogen)-dependent and thrombin-inhibiting effect indicated that Nb106 operates by releasing proteolytically-active thrombin from fibrin to allow inactivation by antithrombin. Time-dependent, late spiking experiments revealed that Nb106 reduced TG only when added at 0-15 minutes after trigger, but no longer after 30 minutes, suggesting that the inhibitory capacity was due to a shortened thrombin binding to fibrin rather than to a continued protection towards antithrombin. In plasmas from a cohort of 64 healthy subjects, application of Nb106 showed a consistent reducing effect on TG parameters (peak, ETP), with a moderate positive correlation with the fibrinogen level. Strikingly, brightfield microscopy and SEM indicated that Nb106, but not control nanobodies, in triggered plasma, PRP and whole-blood, fully abrogated the formation of elongating fibrin fibers. In agreement with this, it strongly suppressed whole-blood clot retraction.

Conclusion Collectivity, these data point to the existence of two major pools of proteolytically active thrombin, both of which contributing to TG, that are formed upon plasma and blood coagulation. One pool of soluble thrombin steers the proteolytic coagulation cascade, while a second pool is bound to growing fibrin fibers. The latter pool still cleaves TG substrate, is temporarily protected against antithrombin, and is required for fibrin fiber extension and blood clotting and clot retraction.

Thieme

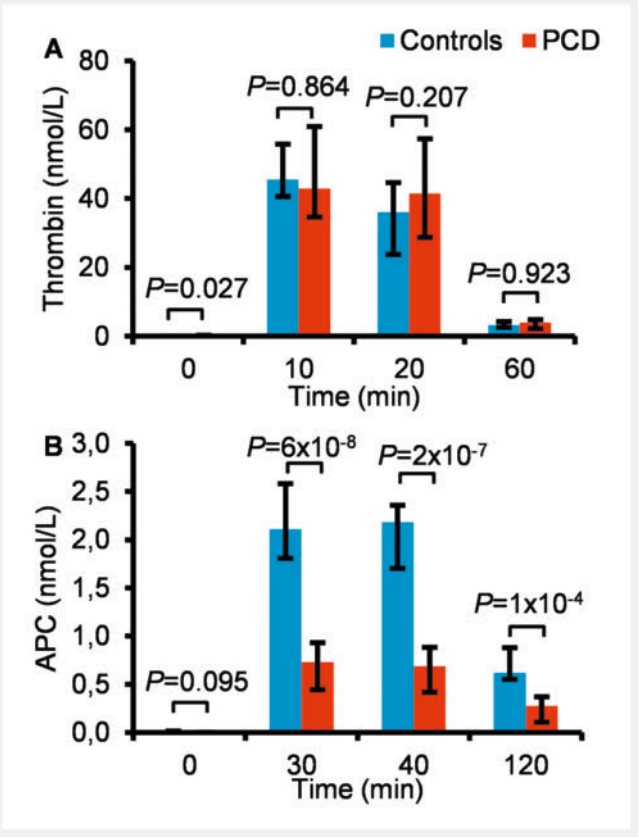
Conflict of Interest BdL JK, DH, FS, RdLK and MR are employees of Synapse Research Institute Maastricht, part of the Diagnostica Stago group, where PGdG and JWMH are advisors. SS and JZ are founded by the China Scholarship Council.

T-03-03 Activated Protein C Formation on Endothelial Cells is Impaired in Hereditary Protein C Deficiency Independent of the Type of Protein C Gene Mutation and Residual Activity

Authors N. Schwarz[1], H. L. McRae[1], J. Müller[1], J. Oldenburg[1], S. Reda[1, 2], B. Pötzsch[1], H. Rühl[1]

Institutes **1** University Hospital Bonn, Institute of Experimental Haematology and Transfusion Medicine, Bonn, Germany; **2** University Hospital Cologne, Department of Transfusion Medicine, Cologne, Germany

DOI 10.1055/s-0044-1779076



▶ **Fig. 1 Thrombin and HUVEC-dependent APC formation in plasma.**; Thrombin and APC levels were measured in supernatant plasma by oligonucleotide-based enzyme capture assays (OECA) over 60 and 120 minutes, respectively. Healthy control group (Controls, n = 11) shown in blue; protein C deficiency group (PCD, n = 22) shown in red. Columns and error bars show median and interquartile ranges, respectively. Obtained data was compared using the two-tailed Student's t-test.

Introduction Protein C deficiency (PCD) is a severe hereditary thrombophilia caused by various variants in the *PROC* gene. The endothelium is involved in the protein C (PC) pathway by promoting activated protein C (APC) formation and thereby regulating thrombin generation. However, it is not clear to what degree different *PROC* gene mutations and plasma levels of mutant PC affect APC formation. Aim of this study was the evaluation of human umbilical vein endothelial cell (HUVEC)-dependent APC formation in plasma of PCD patients with various *PROC* mutations as compared to healthy controls.

Method HUVECs were cultured in 48-well plates and overlaid with citrated, defibrinated plasma from patients with hereditary PCD not on anticoagulation (n = 22) and healthy donors (n = 11). Thrombin formation was initiated by the addition of 1 pM tissue factor. Concentrations of generated thrombin and APC in the supernatant plasma were measured over 60 and 120 minutes, respectively, via oligonucleotide-based enzyme capture assays (OECAs). *PROC* variants were identified by Next Generation Sequencing. Plasma levels of PC, free protein S (PS), antithrombin (AT), and coagulation factors II, V, VII, VIII, IX, X, and XI were measured in all plasma samples applied in experiments. In cases in which no *PROC* mutation was found, diagnosis of hereditary PCD was established by repeated measurements of low PC levels in the patient and in at least one first grade relative.

Results The concentration of free thrombin did not differ significantly between patients and controls 10, 20, or 60 minutes after coagulation activation. The concentration of APC was significantly lower in PCD patients at the 30, 40, and 120 minute timepoints ($P = 6x10^{-8}$, $P = 2x10^{-7}$, and $P = 1x10^{-4}$, respectively) (▶ **Fig. 1**). PS, AT, and coagulation factor levels were within normal ranges in all samples and did not differ significantly between groups. There were 14 previously published *PROC* variants among the 22 patients. Twelve variants were unique, whereas four patients carried 512 C > T and two patients carried 815 G > A. In four PCD patients, no mutation could be identified. Functional PC levels ranged between 35.5 and 74.6 % and the area under the curve (AUC) of APC formation ranged between 19.7 and 113.1 nmol x min/L in PCD patients. Both PC levels and AUC APC were lower in all patients compared to controls (mean 110.7 % and 166.3 nmol x min/L, respectively) (▶ **Fig. 2**).

Group	*PROC* mutation	n	PC level (%)	AUC APC (nmol x min/L)
Controls	none	11	79.2 - 137.8	122.3 - 235.5
Patients with hereditary PC deficiency	520 C>T	4	35.5 - 62.1	24.0 - 71.2
	815 G>A	2	65.4 - 74.6	58.1 - 59.0
	319G>A	1	70.1	113.1
	965A>G	1	58.3	87.0
	479G>C	1	41.9	66.1
	1332G>A	1	62.1	95.3
	925G>A	1	45.1	62.4
	373G>T	1	58.3	79.2
	892A>G	1	51.3	65.1
	400G>T	1	38.2	66.8
	262G>A	1	54.3	81.4
	169C>T	1	74.3	40.2
	412A>T	1	55.7	19.7
	902C>T	1	64.8	26.0
	not detectable	4	47.6 - 73.2	28.0 - 35.3

AUC, area under the curve; APC, activated protein C; PC, protein C

▶ **Fig. 2 PROC variants, PC level, and APC formation in PCD patients and healthy controls.**; Gene variants were measured in all patients and healthy controls by Next Generation Sequencing. PC levels were measured using the Atellica® COAG 360 System. APC formation was measured in supernatant plasma by oligonucleotide-based enzyme capture assays over 120 minutes and the area under the curve was calculated.

Conclusion Regardless of *PROC* mutation or residual PC level, we found decreased APC formation in the endothelial cell model in all 22 patients. This might indicate that PCD patients have a higher risk of thrombosis than residual PC activity levels in plasma suggest. Further studies are warranted to understand the interplay between PCD and APC formation on the endothelial level and its influence on thrombotic risk.

Conflict of Interest None

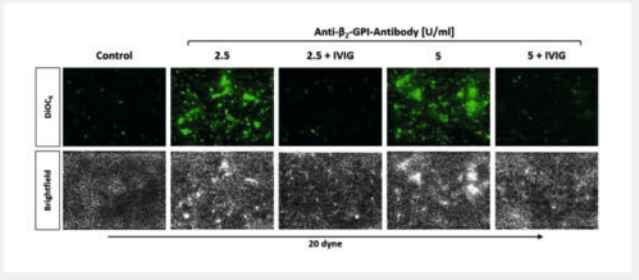
T-03-04 Impact of autoantibody-mediated procoagulant platelets and thrombus formation in antiphospholipid syndrome

Authors C. Schnaidt[1], J. Zlamal[1, 2], T. Bakchoul[1, 2]
Institutes **1** University Hospital Tübingen, Institute for Clinical and Experimental Transfusion Medicine, Tübingen, Germany; **2** University Hospital Tübingen, Center for Clinical Transfusion Medicine, Tübingen, Germany
DOI 10.1055/s-0044-1779077

Introduction Antiphospholipid syndrome (APS) is a prothrombotic autoimmune disease that is associated with recurrent thrombosis and pregnancy loss [1]. The thrombotic risk in patients remains high despite the use of plasmatic anticoagulants [2]. APS is caused by circulating autoantibodies (AAbs) that recognize phospholipids and phospholipid-binding proteins [2]. Anti-β_2-glycoprotein-I APS antibodies (anti-β_2GPI AAbs) are increasingly identified to harbor prothrombotic potential [3–5]. However, the impact of anti-β_2GPI APS antibody interactions with platelets (PLT) and subsequent relevance on thrombus formation are not well explored. This study aims to investigate the relevance of anti-β_2GPI antibody interactions with procoagulant platelets and the impact on thrombus formation *ex vivo*.

Method For the investigation of anti-β_2GPI AAb-mediated PLT alterations, a flow cytometry (FC)-based protocol was employed. To study the impact of anti-β_2GPI AAb-mediated PLT changes on thrombus formation, a microfluidic *ex vivo* thrombosis model was developed with tetra staining to analyze the multicellular interaction in APS.

Results FC analysis revealed that human anti-β_2GPI AAbs have the potential to induce increased formation of procoagulant (CD62p and PS double positive) PLTs compared to control (p-value 0.0026). Anti-β_2GPI-AAb-induced procoagulant PLT formation was PLT Fc-gamma-RIIA (FcyRIIA)-dependent as specific FcyRIIA blockade resulted in a nearly complete inhibition of AAb-induced procoagulant PLT formation (p = 0.0161). Notably, the prothrombotic PLT changes were not only of phenotypical nature, as the reconstitution of anti-β_2GPI AAb-induced procoagulant PLTs into healthy whole blood resulted in formation of multicellular thrombus *ex vivo* (p = 0.0004). Interestingly thrombus formation was prevented when PLTs were pretreated with therapeutic doses of intravenous immunoglobulin G (IVIG) prior to anti-β_2GPI AAb incubation (p = 0.0018) (▶ **Fig. 1**).



▶ **Fig. 1 Ex vivo thrombus formation induced by Anti-β2-GPI antibody and inhibited with IVIG;** Whole blood from healthy individuals was spiked with plasma that was preincubated with IgG isolates from healthy control, Anti-β_2-GPI antibody (2.5 U/ml or 5 U/ml) or a mixture of antibody and IVIG. Samples were stained with $DiOC_6$ and perfused through collagen coated microfluidic channels for 15 minutes at an arterial shear rate of 20 dyne. Images were acquired in the green fluorescent channel (upper panel) as well as in the brightfield channel (lower panel). *IVIG, Intravenous Immunoglobulin; GPI, Glycoprotein I.*

Conclusion Findings from our studies indicate that human anti-β_2GPI AAbs have the potential to induce formation of procoagulant PLT phenotype that harbors dramatic prothrombotic potential. The observation that IVIG treatment could prevent anti-β_2GPI AAb-mediated multicellular thrombus formation directs towards a therapeutic potential of IVIG in the prothrombotic condition often observed in APS patients.

Conflict of Interest Nothing to declare

References

[1] Ruiz-Irastorza G, Crowther M, Branch W, Khamashta MA. 'Antiphospholipid syndrome'. Lancet. 2010; 376 (9751): 1498–509. doi:10.1016/S0140-6736(10)60709-X
[2] Knight JS, Kanthi Y. 'Mechanisms of immunothrombosis and vasculopathy in antiphospholipid syndrome'. Semin Immunopathol 2022; 44 (3): 347–362. doi:10.1007/s00281-022-00916-w
[3] Ho Y., Ahuja K., Körner H., Adams M. 'β2GP1, Anti-β2GP1 Antibodies and Platelets: Key Players in the Antiphospholipid Syndrome'. Antibodies (Basel) 2016; 5 (2): 12. doi:10.3390/antib5020012
[4] McDonnell T., Wincup C., Buchholz I. et al. 'The role of beta-2-glycoprotein I in health and disease associating structure with function: More than just APS'. Blood Rev 2020; 39: 100610. doi:10.1016/j.blre.2019.100610
[5] Baroni G, Banzato A, Bison E, Denas G, Zoppellaro G, Pengo V. 'The role of platelets in antiphospholipid syndrome'. Platelets 2017; 28 (8): 762–766. doi:10.1080/09537104.2017.1280150

T-03-05 Treatment with reversible coagulation inhibitors does not provide full coagulation inhibition.

Authors C. Kluft
Institute Good Biomarker Sciences, Diagnostics, Leiden, Netherlands
DOI 10.1055/s-0044-1779078

Introduction Coagulation is not only characterized by the clotting time. But also, amongst others, by the growth of the clot that is relevant for mural thrombi and obstruction of vessels. The growth is not observed in normal coagulation testing in a tube.

Method The growth, layer by layer, can be studied in the thrombodynamics analyzer with the start of coagulation from immobilized tissue factor and video recording of fibrin formation. Commercially available plasmas were filtered or centrifuged to remove microparticles and inhibitors were from various commercial sources.

Results We tested the effects of the natural mechanism of damping coagulation by activated protein C (inactivating cofactors V and VIII) and observed a delay in growth and eventually stop of growth.

Testing oral anticoagulation with vitamin K antagonists (Warfarin) or with heparinoids (unfractionated heparin, LMW heparin such as Fragmin, Enoxaparin, Bemiparin, and Fondaparinux) showed for heparinoids a similar delay and stop of growth, but surprisingly for vitamin K antagonists a reduced but not a stop of growth. The stop was also induced by the irreversible inhibitor of factor Xa, GGACK. It is concluded that irreversible inhibition of factor Xa is effective in stopping clot growth.

Testing reversible inhibitors of factor Xa (Rivaroxaban, Apixaban, Edoxaban) and of factor IIa (Dabigatran, Argatroban) showed a maximal reduction of about 50 % in growth for quite a prolonged time (> 1 hr), but no stop. On the contrary, irreversible factor IIa inhibition by hirudin showed a stop.

Potential effects of inhibitors of factor XIa were deduced from studies in factor XI deficient plasma, showing no stop of growth.

Reduction of growth by Xa-DOACs was counteracted by factor VIII and required a higher inhibitor at high factor VIII. The growth was found to be mediated by the intrinsic tenase complex (factor IX, factor VIII) which was inhibited in a clotting assay in plasma starting with factor XIa at 4x higher concentrations with Apixaban and Edoxaban than the clotting with the extrinsic tenase complex started with tissue factor. Factor VIII qualifies as antidote for the intrinsic activation route.

Conclusion We concluded that the stop of coagulation, as with the natural protein C pathway, is only realized with treatment with heparinoids and irre-

versible thrombin inhibition, but not with reversible inhibitors such as the DOACs for factor Xa or IIa, and not by Warfarin and factor XIa inhibitors.
As far as these *in vitro*, no flow experiments can be translated to *in vivo* effects, the consequences are that the reversible inhibitors are not complete inhibitors of coagulation events. The profile of the inhibitors may be used to match the clinical need and the pathological process.
The suggestion can be made that new Xa-DOACs may be selected to be more potent, tight inhibitors of the factor Xa formed by the intrinsic tenase (factor IX, factor VIII) which is involved in clot growth.

Conflict of Interest none

T-03-06 Platelet factor 4 mediates procoagulant platelet and increased thrombus formation in vaccine-induced thrombotic thrombocytopenia via SYK signaling pathway

Authors J. Zlamal[1, 2], F. Rigoni[2], G. Uzun[1, 2], K. Althaus[1, 2], T. Bakchoul[1, 2]
Institutes **1** Institute for Clinical and Experimental Transfusion Medicine, University Hospital of Tübingen, Tübingen, Germany; **2** Centre for Clinical Transfusion Medicine ZKT GmbH, Tübingen, Germany
DOI 10.1055/s-0044-1779079

Introduction Vaccine-induced thrombotic thrombocytopenia (VITT) is a rare but life-threatening prothrombotic syndrome observed in individuals following vaccination with vector-based SARS-CoV-2 (ChAdOx1 CoV-19) vaccine. Laboratory findings indicate that VITT is caused by antibodies (Abs) that recognize specific heparin-independent epitopes on the endogenous chemokine platelet factor 4 (PF4) which results in the initiation of a multicellular driven prothrombotic condition and onset of thrombosis at unusual sites. Despite PF4 has been identified as the primary target of anti-PF4 VITT patient antibodies, a deeper understanding regarding the mechanisms of anti-PF4 VITT Ab-mediated prothrombotic conditions is missing. In this study we investigate the regulatory and mechanistic role of PF4 in anti-PF4 VITT Ab-induced procoagulant platelet (PLT) and thrombus formation *ex vivo*.

Method For the investigation of anti-PF4 VITT Ab-induced PLT alterations, a flow cytometry (FC)-based protocol was employed. To study the impact of VITT Ab-mediated PLT alterations on thrombus formation, a microfluidic *ex vivo* thrombosis model that utilizes tetra staining and multiparameter assessment of thrombus formation was developed.

Results FC revealed that anti-PF4 VITT patient IgG induce significant formation of (CD62p/phosphatidylserine [PS] double positive) procoagulant PLTs in a PF4-dependent manner as increased CD62p and PS was only detected in the presence of PF4 and not in vehicle control ($p = 0.0184$). Additionally, PF4-dependent VITT patient IgG-induced procoagulant PLT formation was detected to be strictly dependent on PLT Fc-gamma-RIIA as specific Fc-gamma-RIIA blockade via monoclonal blocking Ab IV.3 resulted in a nearly complete inhibition of anti-PF4 VITT IgG-induced procoagulant PLT formation ($p = 0.0009$). Most importantly, via the utilization of an *ex vivo* model of antibody-mediated thrombosis PF4 was revealed to be a key regulator of VITT patient IgG-induced thrombus formation. Of note, compared to vehicle control, only PLTs that were incubated with VITT patient IgG in the presence of exogenous PF4 and developed a procoagulant PLT phenotype showed the potential to induce significant multicellular thrombus *ex vivo* upon reconstitution into autologous whole blood and perfusion through the collagen coated microfluidic system($p = 0.0022$). Interestingly, and of potential therapeutic interest, VITT Ab-mediated procoagulant PLT as well as increased thrombus formation were nearly completely prevented by the pretreatment of PLTs with the selective spleen tyrosine kinase (SYK) inhibitor Lanraplenib ($p = 0.0199$).

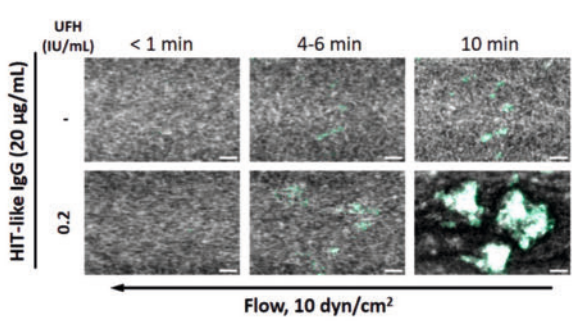
Conclusion Taken together, our data indicate that PF4 is the key mediator of anti-PF4 VITT Ab-induced procoagulant PLT and thrombus formation *ex vivo*. The observation that SYK inhibition in PLTs via Lanraplenib prevents from VITT Ab-mediated prothrombotic conditions directs towards a therapeutic potential of SYK inhibitors in prothrombotic conditions observed in anti-PF4-mediated disorders.

Conflict of Interest There are no potential conflicts of interest to declare.

T-03-07 HIT-IgGs induce a heparin-dependent pro-thrombotic phenotype in a novel endothelialized microfluidic disease model

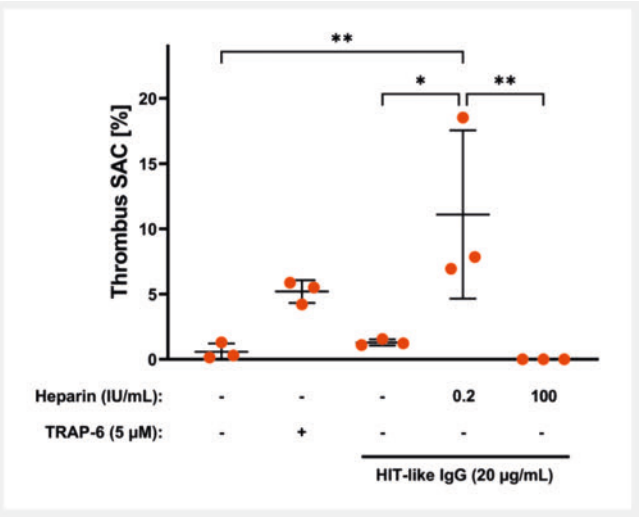
Authors A. Witzemann[1], N. Wolska[1], K. Althaus[1, 4], L. Pelzl[1], J. Amiral[3], J. Zlamal[1, 2], T. Bakchoul[1, 4]
Institutes **1** Institute for Clinical and Experimental Transfusion Medicine, Medical Faculty, Tuebingen, Germany; **2** University Hospital of Tuebingen, Tuebingen, Germany; **3** Hyphen Biomed, Neuville Sur Oise, France; **4** Center for Clinical Transfusion Medicine GmbH, Tuebingen, Germany
DOI 10.1055/s-0044-1779080

Introduction Heparin-induced thrombocytopenia (HIT) is a serious adverse reaction to heparin. Heparin/PF4/IgG complexes have been reported to activate platelets, neutrophils, and possibly endothelial cells, resulting in thrombocytopenia, hypercoagulability and thromboembolic complications. While the impact of anti-PF4/heparin antibodies on blood cell activation has been extensively studied, the role of endothelial cells in HIT-associated thrombosis remains underexplored. In particular, the interactions between HIT-induced procoagulancy and endothelial cells under flow conditions is not completely elucidated. In this report, we investigated how HIT antibodies induce thrombosis in an endothelialized microfluidic system.



▶ **Fig. 1 HIT-IgG together with prophylactic-dose heaprin induce thrombosis on pre-activated endothelial cells;** Representative images of thrombi formed by whole blood incubated with HIT-IgG in absence and under 0.2 IU/mL unfractionated heparin (UFH). Endothelial cells were primed with TNF-α and perfused with whole blood under a shear stress of 10 dyn/cm² for 10 minutes. Overlay of brightfield and fluorescent channel image. Platelets: green, Calcein-AM. Scale bar: 50 µm. [if !supportLineBreakNewLine]

Method Microfluidic channels were coated with monolayers of human umbilical vein endothelial cells (HUVECs). Cells were primed with low-dose TNF-α, before perfusion with whole blood samples. Unstimulated or Thrombin Receptor Activator Peptide 6 (TRAP-6) activated whole blood was perfused at a venous shear rate. The HIT-thrombosis model was established and tested utilizing monoclonal anti-PF4/heparin antibodies. In brief, whole blood was pre-incubated with unfractionated heparin (UFH, 0.2 IU/mL or 100 IU/mL). Anti-PF4/heparin antibodies were introduced to the whole blood mixture and incubated for 30 minutes at 37 °C, under rotation. Whole blood was recalcified and perfused over unstimulated or primed endothelial cells at a venous shear stress. Thrombus formation was recorded over time.



▶ **Fig. 2 HIT-IgG together with prophylactic-dose heaprin induce thrombosis on pre-activated endothelial cells;** Maximal thrombus surface area coverage of TNF-α primed endothelial cells, perfused with unstimulated, TRAP-6 activated or HIT-like IgG/heparin challenged whole blood. HIT-like IgG did not increase thrombus formation in absence of heparin. Addition of low-dose heparin exerted a strong pro-thrombotic effect, that was fully reversible with a super-therapeutic concentration of heparin (HIT-IgG vs. HIT-IgG + 0.2 IU/mL UFH, Thrombus SAC in %, mean ± SD: 0.93 ± 0.82 % vs. 11.10 ± 6.45 %, p = 0.0118, HIT-IgG vs. HIT-IgG + 100 IU/mL UFH: 11.10 ± 6.45 % vs. 0.00 ± 0.00 %, p = 0.0066).

Results The endothelialized microfluidic model successfully captures sub-thrombotic conditions under venous shear, activated platelets induced a procoagulant shift and three-dimensional thrombi. We applied our thrombosis model to investigate thrombus formation induced by HIT mimicking monoclonal antibodies. We observed that the anti-heparin/PF4 antibody-dependent platelet activation predicts the thrombotic response in the microfluidic system: HIT antibodies in absence of heparin did not exert a prothrombotic effect on activated endothelial cells. Co-incubation with 0.2 IU/mL UFH induced thrombosis, embolization of thrombi and channel occlusion only when endothelial cells were primed with TNF-α. The pro-thrombotic effect of HIT antibodies was fully reversed at the super-therapeutic dose of 100 IU/mL UFH.

Conclusion HIT antibodies induce thrombosis and embolization in a system emulating physiological environment. For the first time, we present a comprehensive thrombosis model that incorporates the enhanced thrombogenicity of HIT-mimicking antibodies in presence of heparin. We show that our thrombosis model incorporates both endothelial- and blood-based modulation of thrombosis. Primed endothelial cells and antibody-induced procoagulancy trigger thrombosis in a concerted fashion, allowing the investigation of underlying mechanisms and possible preclinical evaluation of potential inhibitors of HIT-thrombosis (▶ **Fig. 1**, ▶ **Fig. 2**).

Conflict of Interest The authors declare no conflict of interests.

T-03-08 Concurrent interference of thrombin and fibrin fiber generation by a novel nanobody

Authors S. Sun[1,2], J. Konings[1], D. Huskens[1], R.D. L. Kremers[1], F. Swieringa[1], J. Zou[1,2], P. G. de Groot[1], B. de Laat[1], J. W. Heemskerk[1,2], M. Roest[1]

Institutes **1** Synapse Research Institute, MAASTRICHT, Netherlands; **2** Maastricht University, Department of biochemistry, MAASTRICHT, Netherlands

DOI 10.1055/s-0044-1779081

Introduction Thrombin generation (TG) is required for fibrin formation, but the generated thrombin retains activity also after initial clot formation. Here, we present evidence that a substantial proportion of the generated thrombin serves to ensure the continued fibrin growth in plasma systems. This thrombin pool is fibrin-bound and temporarily protected against antithrombin inactivation.

Method A novel nanobody Syn-Nb-AF106 (Nb106) was isolated from llamas injected with human clotting factor concentrate. Selection and characterization proved that Nb106 recognizes thrombin bound to fibrin but not fibrinogen. Calibrated thrombography was used for assessment of thrombin generation (TG) with tissue factor and collagen-related peptide (CRP, platelet glycoprotein VI agonist) using platelet-free plasma, platelet-rich plasma (PRP) and whole blood. Conventional Z-Gly-Gly-Arg aminomethyl coumarin (AMC) was used as thrombin substrate. Fibrin clot formation was evaluated from mechanical clotting kinetics, turbidity assays, and scanning electron microscopy. Interference was tested of the fibrin modulators, GPRP, ancrod and protease III .

Results In tissue factor-triggered TG,we discovered that Nb106 dose-dependently reduced thrombin peak levels and endogenous thrombin potential (ETP) with > 50 %. We observed a similar reduction in human platelet-free plasma, PRP and whole blood, independent of the trigger. Atypical control nanobodies did not alter TG parameters. In mouse PRP, Nb106 was most effective upon co-stimulation with tissue factor and CRP. Mechanistically, Nb106 still suppressed TG in the presence of either GPRP (fibrin cross-linking inhibitor) or ancrod (serine protease, specifically cleaving fibrinopeptide A from fibrinogen). However, it did no longer affect TG parameters in the presence of protease III (serine protease cleaving the Bβ chain of fibrinogen, including fibrinopeptide B). This indicated that Nb106 specifically targets a novel site thrombin-binding site on fibrin, which becomes exposed upon fibrinogen cleavage of fibrinopeptide A.

In fibrin clotting experiments, ancrod shortened the Ca^{2+}-dependent clotting time, whereas protease III prolonged it. Markedly, Nb106 failed to affect plasmatic clotting times, but it significantly and strongly blocked plasma turbidity changes induced by tissue factor. Furthermore, brightfield and scanning electron microscopy revealed that Nb106 dose-dependently suppressed the lateral and longitudinal fibrin fiber extension until complete inhibition, although non-fibrillar fibrin structures were still visible.

Conclusion Collectivity, these data point to the existence of a pool of fibrin-bound thrombin , which is protected against antithrombin inactivation. The Nb106, by binding to the thrombin binding site on fibrin exposed by fibrinopeptide A cleavage, can replace this thrombin, and hence prevents the extension of fibrin fibers, to ultimately abrogate the clotting process.

Conflict of Interest BdL JK, DH, FS, RdLK and MR are employees of Synapse Research Institute Maastricht, part of the Diagnostica Stago group, where PGdG and JWMH are advisors. SS and JZ are founded by the China Scholarship Council.

T-03-09 Thromboembolic (TE) Events in Cold Agglutinin Disease (CAD): Post-hoc Analysis PRE- and ON-sutimlimab Treatment in the Phase 3 CARDINAL and CADENZA Studies

Authors A. Röth[1], Y. Ueda[2], K. McCrae[3], U. Khan[4], K. Kralova[5], M. Wardęcki[6], F. Shafer[7], R. Yoo[8], C. Broome[9]

Institutes **1** University Hospital Essen, Essen, Germany; **2** Osaka University Graduate School of Medicine, Osaka, Japan; **3** Cleveland Clinic Foundation, Cleveland, USA; **4** Sanofi, San Diego, USA; **5** Sanofi, Paris, France; **6** Sanofi, Warsaw, Poland; **7** Sanofi, Bridgewater, USA; **8** Sanofi, Boston, USA; **9** Medstar Georgetown University Hospital, Washington, USA

DOI 10.1055/s-0044-1779082

Introduction Cold agglutinin disease (CAD), a rare chronic autoimmune hemolytic anemia mediated by classical complement pathway activation, is associated with increased risk of thromboembolic (TE) events compared with the general population. Sutimlimab inhibits complement C1s, providing a therapeutic approach for CAD. Here, we assess the TE events PRE- and ON-sutimlimab treatment from the Phase 3 CARDINAL (NCT03347396) and CADENZA (NCT03347422) studies.

Method TE event analysis included participants who had initiated sutimlimab, had a CAD diagnosis date, and had a treatment start and end date. TE events recorded during the study were medically adjudicated before inclusion. ON-sutimlimab events include all events from treatment initiation until 17 days post last dose of sutimlimab. PRE-sutimlimab and ON-sutimlimab follow-up times were matched for each patient.

Results 66 participants (24 from CARDINAL and 42 from CADENZA), were included in this analysis (▶ **Fig. 1**). The median (min, max) follow-up time in each period was 1.8 (0.1, 3.4) years **(▶ Fig. 2)**. In the PRE-sutimlimab period, the TE incidence rate was 7.5 per 100 patient-years compared to 4.4 ON-sutimlimab (p = 0.3056). TE events in the ON-sutimlimab period included cerebral venous sinus thrombosis (CVST) (n = 1), device-related thrombosis (n = 1), peripheral artery thrombosis (n = 1), transient ischemic attack (TIA) (n = 1), and deep vein thrombosis (n = 1). CVST and peripheral artery thrombosis were reported as serious events, and all other events were reported as nonserious. Only the CVST was assessed as related to sutimlimab by the investigator; sutimlimab was temporarily interrupted due to the event. Of the participants that experienced a TE event in the ON-sutimlimab period, 4/5 had a history of TE risk factors and of those, 1 had a confirmed previous TIA.

	ON-sutimlimab - No TE n=61	ON-sutimlimab - Yes TE n=5	Overall N=66
Summary of demographics for targeted prior therapy			
Age (years)			
Mean (SD)	68 (10)	75 (10)	68 (10)
Median	68	71	70
Min, Max	46, 85	62, 88	46, 88
Sex, n (%)			
Male	18 (29.5)	0	18 (27.3)
Female	43 (70.5)	5 (100)	48 (72.7)
Summary of concomitant medications for targeted prior therapy			
Subjects with >1 targeted prior therapy within last 5 years	42 (68.9%)	4 (80.0%)	46 (69.7%)
Corticosteroids	26 (42.6%)	4 (80.0%)	30 (45.5%)
Single Agent Therapy	30 (49.2%)	3 (60.0%)	33 (50.0%)
Ibrutinib	1 (1.6%)	0	1 (1.5%)
Rituximab	30 (49.2%)	3 (60.0%)	33 (50.0%)
Combination Regimens	12 (19.7%)	0	12 (18.2%)
Bendamustine/Rituximab	8 (13.1%)	0	8 (12.1%)
CHOP-R[b]	1 (1.6%)	0	1 (1.5%)
CVP[c]	1 (1.6%)	0	1 (1.5%)
Fludarabine/Rituximab	3 (4.9%)	0	3 (4.6%)
Rituximab/corticosteroid	1 (1.6%)	0	1 (1.5%)
Other combination regimen	4 (6.6%)	0	4 (6.1%)
Summary of lab parameters at baseline			
Hemoglobin (g/dL)			
Mean (SD)	9.1 (1.2)	7.6 (1.6)	9.0 (1.3)
Bilirubin (µmol/L)			
Mean (SD)	42.5 (22.7)	51.4 (11.2)	43.1 (22.1)
Lactate dehydrogenase (U/L)			
Mean (SD)	410.1 (240.4)	477.8 (287.4)	415.2 (242.4)
D-Dimer (µg/L FEU)			
Mean (SD)	647.5 (1058.2)	701.4 (403.6)	551.6 (1021.1)
Thrombin/antithrombin (µg/L)			
Mean (SD)	16.0 (19.7)	8.0 (6.5)	15.4 (19.1)

[a]For the matched analysis, minimum follow-up time between PRE-sutimlimab and ON-sutimlimab is considered.
[b]Combination of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone.
[c]Combination of cyclophosphamide, vincristine, and prednisolone.
n, number; SD, standard deviation.

▶ **Fig. 1** Summary of Baseline Characteristics of Patients That Met the Inclusion Criteria for the Post-hoc Analysis by Matcheda Thromboembolic (TE) Events After Treatment in the CARDINAL and CADENZA Studies

Prevalence Activity	PRE-sutimlimab (N=66)	ON-sutimlimab (N=66)
Number of patients with >1 event, n (%)	8 (12.1)	5 (7.6)
p-value[b]		0.3657
Number of events, n	9	5
p-value[c]		0.2885
Follow-up Time (years)		
Mean (SD)	1.7 (1.0)	1.7 (1.0)
Median	1.8	1.8
Min, Max	0.1, 3.4	0.1, 3.4
Total Patient-Years (years)[d]	113.7	113.7
Incidence Rate (per 100 py) (95 % CI)[e]	7.5 (4.0, 14.2)	4.4 (1.9, 10.1)
Rate Ratio (95 % CI)[e]		0.6 (0.2, 1.6)
p-value[e]		0.3056

[a]For the matched analysis, minimum follow-up time between PRE-sutimlimab and ON-sutimlimab is considered.
[b]p-value calculated based on McNemar's test for comparing proportions with ≥1 event.
[c]p-value calculated based on Paired t-test for difference in number of events (ON-sutimlimab – PRE-sutimlimab).
[d]Total patient-years is the sum of follow-up time (measured in years) for all patients from full analysis set.
[e]Derived from Poisson regression model with exchangeable covariance type.
CI, confidence interval; n, number; py, patient-years; SD, standard deviation.

▶ **Fig. 2** Summary of Matched[a] Adjudicated Thromboembolic (TE) Events for CARDINAL and CADENZA Studies

Conclusion Analysis of matched adjudicated TE events from the CARDINAL and CADENZA studies suggests a trend toward a reduced risk of TE ON-sutimlimab compared to the PRE-sutimlimab period in this medically complex CAD cohort.

Conflict of Interest **AR** has received honoraria from and participated in a data safety monitoring board or advisory board for Alexion, Amgen, Apellis, Novartis, Roche, Sanofi, and Sobi; partook in a data safety monitoring board or advisory board for Bioverativ; and has had support provided for meetings from Sobi. **YU** has received research support from Chugai Pharma, consultancy fees from Alexion, Asahi Kasei, Chugai Pharma, Incyte, Novartis, Sanofi and Sobi, and Janssen Pharmaceutical, honoraria from Alexion, Novartis and Sanofi, participation in an advisory board from Novartis and has a membership on an entity's board of directors or advisory committees from Sanofi and Sobi. **KM** has participated in data and safety monitoring boards for Argenex and Principia, and consulted/participated in advisory boards for Sanofi and Novartis. **CMB** has received grants from Alexion, Argenx, Incyte, and Rigel; honoraria from Alexion, Argenx, Novartis; and Sanofi; and partook in a data safety monitoring board or advisory board for Alexion, Argenx, Incyte, Novartis, and Sanofi. **UK**, **KK**, **MW**, **FS**, and **RY** are employees of Sanofi and may hold stock options in the company. This work and the CADENZA and CARDINAL studies were funded by Sanofi.

T-03-10 Investigating platelet-leukocyte-aggregate formation under shear stress conditions in vitro and in vivo

Authors T. Flieder[1], K. von Bargen[1], M. Zimmermann[1], J. Schmitz[2], A. Hohbein[1], C. Knabbe[1], I. Birschmann[1]
Institutes **1** Herz- und Diabeteszentrum NRW, Institut für Laboratoriums- und transfusionsmedizin, Bad Oeynhausen, Germany; **2** Universität Bielefeld, Multiscale Bioengineering, Technische Fakultät, Bielefeld, Germany
DOI 10.1055/s-0044-1779083

Introduction Platelets are primarily known for their function in hemostasis. However, they also play a role in the field of immunology. When inflammatory or thrombotic processes occur in the body, platelets can form aggregates (PLA) with leukocytes. A distinction can be made between platelet-neutrophil aggregates (PNA) and platelet-monocyte aggregates (PMA). The occurrence of PLAs has been frequently studied in inflammatory disease patterns and has already been considered as potential biomarkers for e.g. chronic obstructive pulmonary disease, consumptive coagulopathy, or sepsis.

Hämostaseologie 2024; 44: S1–S125

Patients with a left ventricular assist device (LVAD) often suffer from infections due to immunosuppression and the exit site of the driveline. In addition, platelets are subjected to higher shear stress by the pump, which may lead to platelet activation and consequently to the formation of PLAs. Therefore, this study investigated the extent to which shear stress alone may contribute to the formation of PLAs.

Method D-Phenylalanyl-L-prolyl-L-arginine chloromethyl ketone (PPACK) anticoagulated blood was collected from healthy donors and patients with an LVAD. For part of the experiments a platelet inhibitor was added to the blood of the donors 15 min before shear stress exposure. Whole blood (from donors) was pumped at a defined shear rate through a PDMS-glass-based flow chamber (height 100 µm, width 1000 µm, length 5 cm, with or without a 75 % stenosis), applying a defined shear stress to the cells. After shear stress exposure, PLA, PNA and PMA were determined by flow cytometry.

The study received a positive vote from the ethics committee (AZ: 2022-1015).

Results The formation of cell aggregates in the flow chamber model without stenosis showed a significant increase exclusively for the formation of PMAs. When 75 % stenosis was present in the model, increased levels of PLA, PNA, and PMA were measured. The values were increased at both the lower and higher shear rates relative to the control. However, no significant difference was observed between the lower and higher shear rates.

Increased levels of PLAs could be measured in the patient samples compared to control samples.

Conclusion It could be shown here that an increased shear rate can contribute to the formation of PLAs in general. Thereby, not the absolute level of the shear rate but an acceleration in the flow system seems to be decisive. Increased levels of PLAs were also measured in the patients with implanted LVAD. Because PLAs also have a prothrombotic effect, the detection of PLAs in this patient population could be a marker for an increased risk of thrombosis. Whether there is an association between elevated PLAs and thromboembolic events in these patients needs to be explored in further studies.

Conflict of Interest Ingvild Birschmann received speaker's honoraria from Bristol-Myers Squibb/Pfizer, CSL Behring, LFB biomedicaments, Octapharma AG and Siemens Healthcare and performed contract research for Siemens Healthcare. Ingvild Birschmann is supported by means of medical writing from CSL Behring and is a member of the advisory board/expert testimony of LFB biomedicaments, Portola Pharmaceuticals, Siemens Healthcare and CSL Behring. All other authors have no competing interests.

T-03-11 Prevalence of GPIIbIIIa-enriched membrane protrusions (Tether) in hospitalized patients

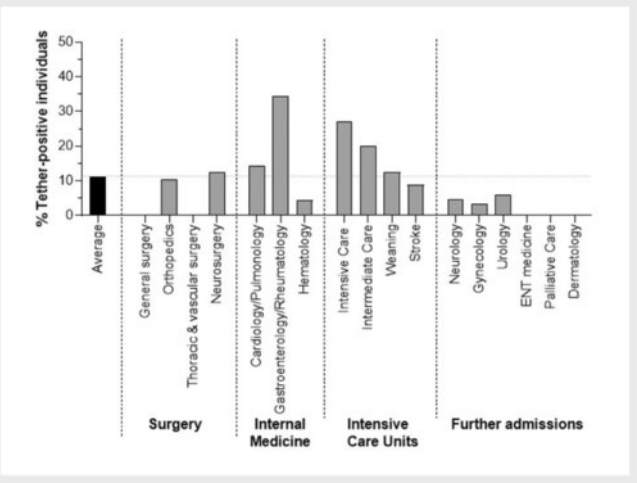
Authors M. Wolff, L. Swensson, L. Wollermann, J. Fuhrmann, J. Klauke, A. Greinacher

Institute Universitätsmedizin Greifswald, Institut für Transfusionsmedizin, Greifswald, Germany

DOI 10.1055/s-0044-1779084

Introduction Platelets mediate adhesion and aggregation via membrane glycoproteins such as Integrin *αIIbβ3*. Formation of GPIIbIIIa-enriched membrane-protrusions (Tether) is a new characteristic of human platelets and has been previously found in patients with an increased risk for thrombosis (e.g. patients with myeloproliferative diseases) [1]. Tethers in humans are comparable to Platelet-derived Integrin & Tetraspanin-rich Tethers (PITTs) described in mouse models in response to anti-platelet antibodies [2]. So far it is still unknown whether this alteration is exclusively observed under special pathological conditions or may be found in a broader spectrum of diseases or even in healthy individuals. We assessed the presence of tethers in different groups of patients and healthy controls.

Method We analyzed blood smears from a representative cohort of 388 in-hospital patients from more than 10 departments. Healthy blood donors (n = 261) served as control group. Blood smears were aceton fixated, labeled with a GPIIbIIIa-antibody (Clone Gi5) and stained with an Alexa Fluor-488 conjugated secondary antibody. Two observers assessed at least 10 fields of view of each slide by immunofluorescence microscopy. Presence of tethers was documented according to different morphological categories (e.g. length and distribution).



▶ **Fig. 1** Percentage of tether-positive individuals and their distribution between different departm

Results Male and female patients were evenly included in the cohort (205 and 179, respectively) and the mean age was 64.6 years. Tethers were found in 11.3 % of all screened patients and more often in females (15.1 % vs 8.3 %). Tethers were more frequently detected in patients from intensive care units (27 %) and patients from internal medicine, especially gastroenterology/rheumatology (34.5 %). In comparison, tethers were observed in less than 15 % of healthy individuals (mean age 41.4 years, 59.8 % male). However, most tethers where short and observed in less than 10 % of platelets (▶ **Fig. 1**).

Conclusion Glycoprotein IIbIIIa-enriched membrane protrusions of platelets are a new phenomenon that can be found in a broader spectrum of hospitalized patients. The prevalence is higher in patients with greater risk for thrombosis and infections especially in intensive care unit patients and patients with gastroenterological disease.

Conflict of Interest No conflict of interest

References

[1] Wolff M, Vater L et al. Platelet GPIIbIIIa enriched membrane protrusions (Tether) in healthy and diseased individuals. GTH abstracts 2023

[2] Johnsson D, Kusch C et al. Platelet-derived Integrin and Tetraspanin-enriched Tethers (PITTs) orchestrate neutrophil recruitment and drive pulmonary thrombo-inflammation. ISTH abstracts 2023

T-03-12 The polyphosphate/factor XII pathway drives the thrombo-inflammatory fat embolism syndrome

Authors S. Konrath[1], S. A. Hemkemeyer[1], B. Ondruschka[2], R. K. Mailer[1], T. Renné[1, 3]

Institutes **1** University Medical Center Hamburg-Eppendorf, Institute of Clinical Chemistry and Laboratory Medicine, Hamburg, Germany; **2** University Medical Center Hamburg-Eppendorf, Institute of Legal Medicine, Hamburg, Germany; **3** Royal College of Surgeons in Ireland, Irish Centre for Vascular Biology, Dublin, Ireland

DOI 10.1055/s-0044-1779085

Introduction Fat embolism syndrome (FES) is a life-threatening complication of traumatic bone fractures or orthopaedic procedures, such as intramedullary nailing, with a mortality rate of close to 10 %. Thrombo-inflammatory reactions are triggered by fat released from the bone marrow, which enters the circulation

and eventually causes fatal obstruction in the pulmonary microvasculature. The disease mechanisms and rational therapy of FES remain elusive.

Method Lipid-mediated cleavage of the factor XII-driven plasma contact system was analysed by Western blotting and chromogenic assays. Intrinsic coagulation activation was assessed with calibrated automated thrombinography and recalcification clotting time analysis in normal and contact system protein-deficient plasma. A mouse model for lethal pulmonary embolism triggered by bone marrow-derived lipids was established and lipid-triggered thrombosis was studied by histochemistry and immunofluorescence analysis.

Results Lipids isolated from human and bovine bone marrow activated the factor XII-driven plasma contact system in human plasma in a concentration- and time-dependent manner. Fluorogenic real-time thrombin generation and recalcification clotting time assays demonstrated lipid-stimulated coagulation activation in plasma and whole blood samples through activation of FXII. Pharmacological inhibition and genetic targeting of the contact system proteases factor XII and kallikrein abolished plasma clotting in vitro. Anionic polyphosphate bound to lipids induces factor XII contact activation. Conversely, digestion of lipid-bound polyphosphate by an exopolyphosphatase abrogated the potential of lipids to activate factor XII and the contact system. Exopolyphosphatase-treatment also blunted lipid-stimulated thrombin generation. Intravenous injection of bone marrow-derived lipids led to lethal pulmonary embolism in wild-type mice. In contrast, mice with genetic deficiency in *F12* or its substrate *F11* were protected from occlusive thrombus formation upon bone marrow-derived lipid infusion. Immunostaining of post mortem lung tissue from FES patients revealed activation of factor XII in close proximity to lipid deposits in the pulmonary vasculature.

Conclusion The data identify the pathomechanism underling FES. Bone marrow-derived lipids activate the factor XII-driven contact system in vitro and in vivo in a polyphosphate-dependent manner. Consequently, blocking the polyphosphate/ factor XII axis might be a promising therapeutic strategy for interference with FES.

Conflict of Interest The authors declare that there is no conflict of interest.

T-04. Coagulation and cardiovascular complications

T-04-01 Outcomes of patients with suspected heparin-induced thrombocytopenia in a contemporary cohort of patients

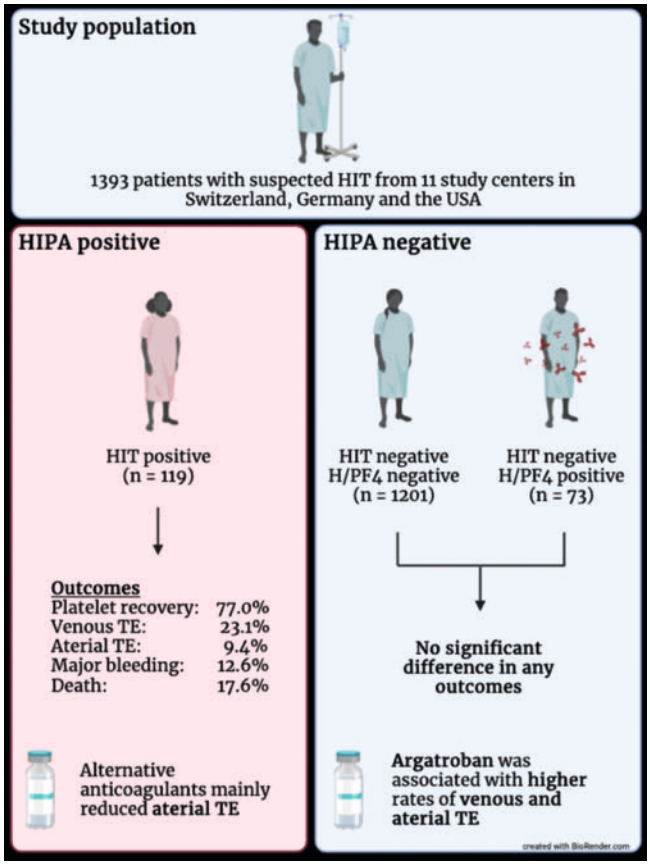
Authors H. Nilius[1, 2], E. Sinitsa[1], J.-D. Studt[3], D. A. Tsakiris[4], A. Greinacher[5], A. Mendez[6], A. Schmidt[7], W. A. Wuillemin[8], B. Gerber[9], J. A. Kremer-Hovinga[10], P. Vishnu[11], L. Graf[12], T. Bakchoul[13], M. Nagler[1, 14]

Institutes **1** Inselspital, Bern University Hospital, Department of Clinical Chemistry, Bern, Switzerland; **2** University of Bern, Graduate School for Health Sciences, Bern, Switzerland; **3** University and University Hospital of Zurich, Division of Medical Oncology and Hematology, Zurich, Switzerland; **4** Basel University Hospital, Diagnostic Haematology, Basel, Switzerland; **5** Universitätsmedizin Greifswald, Institut für Immunologie und Transfusionsmedizin, Greifswald, Germany; **6** Kantonsspital Aarau, Department of Laboratory Medicine, Aarau, Switzerland; **7** Municipal Hospital Zurich Triemli, Clinic of Medical Oncology and Hematology, Zurich, Switzerland; **8** Cantonal Hospital of Lucerne and University of Bern, Division of Hematology and Central Hematology Laboratory, Lucerne, Switzerland; **9** Oncology Institute of Southern Switzerland, Clinic of Hematology, Bellinzona, Switzerland; **10** Inselspital, Bern University Hospital, Department of Hematology and Central Hematology Laboratory, Bern, Switzerland; **11** CHI Franciscan Medical Group, Division of Hematology, Seattle, USA; **12** Cantonal Hospital St. Gallen, Department of Hematology, St. Gallen, Switzerland; **13** University Hospital Tübingen, Centre for Clinical Transfusion Medicine, Tübingen, Germany; **14** University of Bern, Medical Faculty, Bern, Switzerland

DOI 10.1055/s-0044-1779086

Introduction Managing patients with suspected heparin-induced thrombocytopenia (HIT) remains a major clinical challenge. Knowledge of the effects of management decisions on clinical outcomes is sparse and treatment recommendations are often based on low certainty. In a prospective multicenter cohort study, we studied the treatment and outcomes of patients with suspected HIT.

Method We included consecutive patients with suspected HIT and report the outcomes of (a) patients with HIT, (b) patients without HIT but positive heparin/PF4 antibodies, and (c) patients without HIT. Comprehensive clinical and laboratory data were collected in detail and the washed-platelet heparin-induced platelet activation test (HIPA) served as the reference standard test defining HIT.



▶ **Fig. 1** **Graphical abstract**; From 1393 patients with suspected HIT from 11 study centers 119 (8.5 %) were HIT positive. Eighteen percent of HIT patients died during follow up. Alternative anticoagulants mainly reduced the risk of aterial thrombembolism (TE) in HIT patients. Of the 1274 patients without HIT 73 (5.7 %) had a positive heparin/pf4 (H/PF4)-CLIA. However, there was no difference in outcomes between patients with and without H/PF4-CLIA. Argatroban was associated with higher rates of venous and aterial TE in HIT negative patients.

Results Among 1393 patients included in 11 study centers (46 % female, median age of 67), HIT was confirmed in 119 patients (prevalence 8.5 %). The setting was intensive care unit (37 %) and cardiac surgery (32 %) in the majority of patients. The predominant treatment was argatroban (70 %), and complete platelet recovery was observed in 77 % of HIT patients. Of the patients with HIT, 23 % developed subsequent venous thromboembolism (TE), 9 %

arterial TE, and 18 % died. Major bleeding occurred in 13 % of HIT patients and did not differ significantly between drugs. Treatment with argatroban, bivalirudin or DOAC markedly reduced the risk of subsequent arterial TE. HIT-negative patients with and without H/PF4 antibodies did not differ with regard to any outcome (Graphical abstract) (▶ **Fig. 1**).

Conclusion Our results indicate that HIT is still a serious disease with a high risk of major adverse events. In the absence of randomized controlled trials, our results add further evidence on the effectiveness of DOAC, argatroban, and bivalirudin treatment.

Conflict of Interest The institution of B.G. received grant support and CME support from Pfizer, Thermo Fisher Scientific, Axonlab, Sanofi, Alnylam, Bayer, BMS, Daiichi-Sankyo, Octapharma, Takeda, SOBI, Janssen, Novo Nordisk, and Mitsubishi Taneba, outside of the current work. The institution of J.A.K.H. received grant support, consultancy fees, or honoraria from SNSF, Baxter/Takeda, Bayer, CSL-Behring, NovoNordisk, Octapharma, Roche, SOBI, Roche, Sanofi, FOPH, and Swiss Hemophilia Society, outside of the current work. M.N. received research grants from Bayer Healthcare, Roche diagnostics, Siemens Healthineers, Pentapharm, and Bühlmann laboratories as well as lecture fees from Sysmex, Siemens Healthineers, and Euroimmun, outside of the current work. A.G. reports personal fees from Aspen, grants from Ergomed, grants from Boehringer Ingelheim, personal fees from Bayer Vital, grants from Rovi, grants from Sagent, personal fees from Chromatec, personal fees from Instrumentation Laboratory, grants and personal fees from Macopharma, grants from Portola, grants from Biokit, personal fees from Sanofi-Aventis, grants from Blau Farmaceutics, grants from Prosensa/Biomarin, grants and other support from DRK-BSD NSTOB, grants from DRK-BSD Baden-Würtemberg/Hessen, personal fees from Roche, personal fees from GTH e.V., grants from Deutsche Forschungsgemeinschaft, grants from Robert Koch Institute, nonfinancial support from Veralox, personal fees from Dilaflor, nonfinancial support from Vakzine Projekt Management GmbH, grants from GIZ Else-Körner-Stiftung, nonfinancial support from AstraZeneca, nonfinancial support from Janssen Vaccines & Prevention B.V., personal fees from Takeda Pharma, personal fees from Falk Foundation e.V., grants from European Medicines Agency, and personal fees from Mylan Germany, outside the submitted work. In addition, A.G. has a patent Screening Methods for transfusion-related acute lung injury (TRALI) with royalties paid to EP2321644 (May 18, 2011) and a patent Verfahren und Vorrichtung zur Herstellung von Universalplasma, licensed to DE 10 2020 212 609 B3 (April 4, 2022). T.B. reports grant support, consultancy fees, honoraria, or support for attending meetings from DFG, Stiftung Transfusionsmedizin und Immunhämatologie e.V, DRK Blutspendedienst, Deutsche Herzstiftung, Ministerium für Wissenschaft, Forschung und Kunst Baden Würtemberg, Gesellschaft für Thrombose- und Hämostaseforschung, Berufsverband Deutscher Internisten, CoaChrom Diagnostica GmbH, Robert Bosch GmbH, Ergomed, Bayer, Bristol-Myers Squibb, Doctrina Med AG, Leo Pharma GmbH, Schöchl Medical Education GmbH, Mitsubishi Tanabe GmbH, Novo Nordisk GmbH, and Swedish Orphan Biovitrium GmbH. All other authors have no competing interests to disclose.

T-04-02 Haemostatic response to different types of exercise training in patients with atherosclerotic vascular disease

Authors D. Kosuta[1,2], M. Novakovic[1,2], M. Bozic Mijovski[1,3], B. Jug[1,2]

Institutes 1 University Medical Centre Ljubljana, Department of Vascular Diseases, Ljubljana, Slovenia; 2 University of Ljubljana, Faculty of Medicine, Ljubljana, Slovenia; 3 Univeristy of Ljubljana, Faculty of Pharmacy, Ljubljana, Slovenia

DOI 10.1055/s-0044-1779087

Introduction Exercise training in patients with atherosclerotic vascular disease in general and coronary artery disease (CAD) in particular has been shown to reduce recurrent cardiovascular events, improve quality of life and exercise capacity. However, it seems to have also transient unfavourable consequences such as vascular dysfunction, increased inflammation and increased coagulation. Moderate intensity continuous protocols (MICT) are widely used, high intensity interval protocols (HIIT) while more effective, are perceived as having a higher risk of adverse events. Haemostatic derangements in particular may have an important role in recurrent cardiovascular events in these patients. We aimed at investigating the effects of HIIT in comparison to MICT on haemostasis appraised by the overall haemostatic potential in patients with CAD.

Method Patients after acute myocardial infarction were randomized into HIIT or MICT group. The study was conducted in accordance with the Declaration of Helsinki (1964) and approved by the National Medical Ethics Committee (approval number 0120-77/2020/3).

Patients had three blood samples drawn: before the exercise (T0), after the exercise (T2) and one hour after the exercise (T2). Overall haemostatic potential (OHP), overall coagulation potential (OCP) and overall fibrinolytic potential (OFP) were measured.

Between-group differences were assessed by t-test for normally distributed variables and proportions were compared using the χ^2 test. Mixed effects linear models were constructed to assess the effects of exercise on haemostatic measures, with fixed effects [1–5].

Results A total of 117 patients were included. Baseline characteristics are listed in ▶ **Fig. 1**. We found a significant increase in OCP, OHP, and a significant decrease in OFP after exercise. The values of all measured biomarkers returned to baseline after a one-hour rest in both groups. To compare the effect of HIIT and MICT on coagulation parameters a linear mixed model for repeated measurements was constructed (random effects: subjects; fixed parameters: repeated measurements of coagulation parameters, exercise type; covariates: and gender, age). Time (before exercise training, immediately after exercise training and one hour after exercise training) was found to be significant for all measurements while there was no treatment effect for OCP, OHP and OFP (▶ **Fig. 2**).

	All (n=117)	HIIT (n=59)	MICT (n=58)	P value
Women *	23 (19.7)	8 (13.6)	15 (25.9)	0.108
Age, years **	56 (10.3)	55 (11.5)	57 (9.0)	0.293
BMI, kg/m² **	28.8 (4.6)	28.3 (4.4)	29.3 (4.8)	0.244
Arterial hypertension *	86 (73.5)	46 (78.0)	40 (69.0)	0.301
Diabetes mellitus *	10 (8.5)	6 (10.2)	4 (6.9)	0.743
Dyslipidemia *	75 (64.1)	38 (64.4)	37 (63.8)	1.000
Family history *	46 (39.3)	23 (39.0)	23 (39.7)	1.000
Smoking history *	54 (46.2)	27 (45.8)	27 (46.6)	1.000

▶ **Fig. 1** Baseline characteristics; HIIT – high intensity interval training, MICT – moderate intensity interval training. * values represented as number (%); * * values represented as mean (standard deviation).

	Baseline	After exercise	1h after exercise	p value for time	p value for time*group interaction
OCP					
- HIIT	23.5 (22.1-25.0)	24.3 (22.8–25.7)	23.9 (22.4–25.4)	<0.001	0.678
- MICT	23.9 (22.5–25.3)	24.8 (23.4–26.2)	24.3 (22.8–25.7)		
OHP					
- HIIT	7.9 (7.2–8.7)	8.4 (7.6–9.2)	7.9 (7.2–8.7)	<0.001	0.424
- MICT	8.4 (7.6–9.1)	8.8 (8.0–9.5)	8.4 (7.7–9.1)		
OFP					
- HIIT	66.3 (63.6–69.1)	65.3 (62.5–68.1)	67.1 (64.3–69.8)	<0.001	0.370
- MICT	64.5 (61.8–67.2)	64.3 (61.6–67.0)	64.9 (62.3–67.6)		

▶ **Fig. 2** Coagulation parameters; HIIT – high intensity interval training, MICT – moderate intensity interval training, OCP – overall coagulation potential, expressed as abs-sum, OHP – overall hemostatic potential, expressed as abs-sum, OFP – overall fibrinolytic potential, expressed as %.

Conclusion Our study has shown that exercise training is associated with a procoagulant imbalance in haemostasis in patients with CAD. However, no

difference was found between HIIT and MICT, suggesting that both exercise methods are equally safe.

Conflict of Interest The authors have no conflict of interest.

References

[1] Ambrosetti M, Abreu A, Corra U, Davos CH, Hansen D, Frederix I et al. Secondary prevention through comprehensive cardiovascular rehabilitation: From knowledge to implementation. 2020 update. A position paper from the Secondary Prevention and Rehabilitation Section of the European Association of Preventive Cardiology. Eur J Prev Cardiol 2021; 28 (5): 460–495

[2] Visseren SLJ, Mach F, Smulders YM, Carballo D, Koskinas KC, Bäck M et al. ESC Guidelines on cardiovascular disease prevention in clinical practice. Eur Heart J. 2021; 42 (34): 3227–3337

[3] Verschueren S, Eskes AM, Maaskant JM, Roest AM, Latour CHM, Reimer WS. The effect of exercise therapy on depressive and anxious symptoms in patients with ischemic heart disease: A systematic review. J Psychosom Res 2018; 105: 80–91

[4] Spiel AO, Gilbert JC, Jilma B. von Willebrand factor in cardiovascular disease: focus on acute coronary syndromes. Circulation. 2008; 117 (11): 1449–59

[5] Peng Y, Wang H, Li YM, Huang BT, Huang FY, Xia TL et al. Relation between admission plasma fibrinogen levels and mortality in Chinese patients with coronary artery disease. Sci Rep 2016; 6: 30506

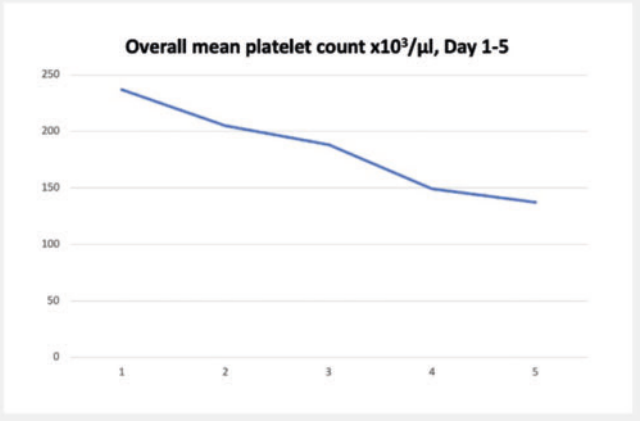
T-04-03 The impact of extracorporal life support and therapeutic anticoagulation on the platelet count in the acute phase of cardiogenic shock

Authors A. Huseynov[1], U. Hoffmann[1], M. Kittel[2], D. Dürschmied[1], I. Akin[1]

Institutes **1** University Medical Centre Mannheim, Medical Clinic I Cardiology, Intensiv Care, Angiology and Hemostasis, Mannheim, Germany; **2** University Medical Centre Mannheim, Institute for Clinical Chemistry, Mannheim, Germany

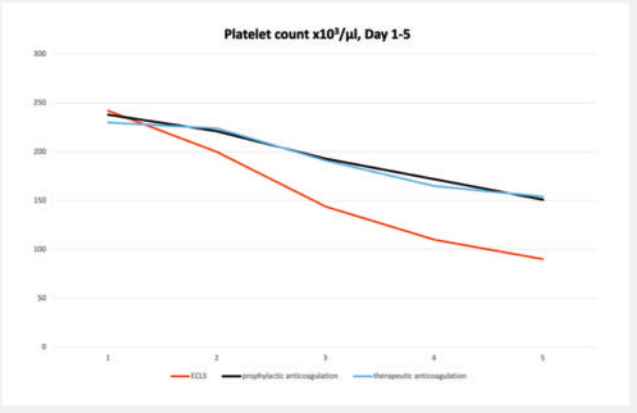
DOI 10.1055/s-0044-1779088

Introduction The treatment of cardiogenic shock is a challenge of modern medicine. Anticoagulation drugs such as unfractionated heparin or low molecular weight heparins are the most common drugs used in cardiogenic shock to prevent thromboembolic complications. Despite years of experience with these substances, there is a lack of data on the effects of anticoagulation strategies with heparins on the occurrence of thrombocytopenia in the acute phase of cardiogenic shock. This study investigated the occurrence of thrombocytopenia in patients with cardiogenic shock depending on therapeutic strategies with plasmatic anticoagulation.



▶ **Fig. 1** Overall mean platelet count Day 1-5

Method We reviewed the retrospectively collected data patients admitted to our hospital (2019-2023) with cardiogenic shock. Some patients were treated using extracorporeal life support systems (ECLS). All patients received anticoagulation therapy with unfractionated or low molecular heparin: the patients with ECLS or other indications for therapeutic anticoagulation with therapeutic dosage, all other patients with prophylactic dosage. Thrombocyte count in day 1 to day 5 was evaluated during hospitalization.



▶ **Fig. 2** Platelet count in subgroups Day 1-5

Results The study population consists of 97 patients with infarct-related cardiogenic shock. Of these, 53 (54.6 %) were admitted with an ST-elevation myocardial infarction, 44 (45.4 %) with a non-ST-elevation myocardial infarction or malignant cardiac arrhythmias leading to cardiogenic shock. All patients had coronary artery disease and were treated emergently using revascularization strategies. Approximately 75.2 % of patients (73 patients) survived out-of-hospital cardiac arrest on admission. The overall in-hospital mortality rate was 55 %. The mean duration of ECLS therapy was 49.8 hours (95 % CI 33.6 to 66.0). The overall mean platelet count at admission was 237 x 103/µl (95 % CI 221 to 253 x 103/µl). A drop of platelet count occurred in all patients with cardiogenic shock, but this was significantly more pronounced in the ECLS group (36 % of the study population) than in patients without ECLS (on day 3: 144 vs. 192 x 103/µl, $p < 0.0001$ and on Day 5 90 vs. 153 x 103/µl, $p = 0.0014$). Comparison of conservative patient groups with prophylactic and therapeutic anticoagulation (37 % and 27 % of the study population) showed no significant differences in platelet counts over 5 days after admission (▶ **Fig. 1**, ▶ **Fig. 2**).

Conclusion Thrombocytopenia is common in cardiogenic shock and occurs more frequently in patients with ECLS treatment than with conservative treatment. Therapeutic anticoagulation alone has no significant effect on platelet counts.

Conflict of Interest The Authors declare no conflict of interests.

T-04-04 Structural analysis of VWF gain-of-function variants by atomic force microscopy

Authors J. Derksen[1], M. Grützmann[1], A. Mojzisch[2], M. Brehm[1]

Institutes **1** University of Siegen, Department of Digital Health Sciences and Biomedicine, Siegen, Germany; **2** Universitätsklinikum Eppendorf, Institute for Dermatology and Venerology, Hamburg-Eppendorf, Germany

DOI 10.1055/s-0044-1779089

Introduction The multimeric glycoprotein von Willebrand factor (VWF) is a highly adhesive protein, found in the bloodstream [1]. At sides of vascular injury, VWF is subjected to elevated hydrodynamic forces, leading to the activation of VWF's A1 domain, initiating primary hemostasis via binding to platelet GPIbα. Later on, the thrombus is further stabilized by crosslinking of platelet receptor GPIIb/IIIa with VWF – via its C4 domain – and fibrin [2].

Recently, VWF gain-of-function (GOF) variants were identified exhibiting an increased prothrombotic potential [3]. For example, Huck et al. characterized variant p.Pro2555Arg, located in the C4 domain, which exhibits increased VWF-platelet-complex size at the same critical shear rate as wildtype (wt)VWF. The GOF effect was hypothesized to be induced by a decreased reformation rate of the closed stem conformation within VWF dimers [4].

Method We performed conformational studies using atomic force microscopy (AFM) imaging of different recombinant VWF dimer and multimer constructs. The proteins were immobilized in a near physiological buffer on a poly-L-lysine functionalized mica surface and dried by a gentle stream of nitrogen. AFM imaging in air was performed to analyze the distribution of VWF stem conformations employing a NanoWizard® ULTRA Speed 2 (JPK, Bruker) and MFP3D (Asylum Research) in tapping mode with silicon nitride probes. The resulting height profiles were evaluated by measuring the stem length and the distance between the CK domain and the abrupt increase in height of the N-terminal domains to normalize the length of the closed stem. Based on the stem conformation distribution and the statistical analysis, three groups of conformations were determined. The initial tertial of the normalized stem length distribution was defined as fully-opened. Closed stem segments with a minimum length of 80 % relative to the normalized full stem length were considered to be fully-closed. The length distribution in between was classified as flexible intermittent.

Results AFM imaging revealed for wtVWF that 14 % of the stems were present in a fully-opened conformation while 52 % exhibited a flexible intermittent conformation and 33 % were fully-closed. In contrast, p.Pro2555Arg dimers showed 36 % fully-opened, 33 % flexible intermittent and 31 % fully-closed stems. The full-length multimeric variants exhibited a similar tendency: within the multimers, wtVWF and p.Pro2555Arg exhibited 20 % and 36 % fully-opened stems, respectively.

Conclusion In this study, we used AFM imaging to visualize and analyze the structural variation of stem conformations in wtVWF and p.Pro2555Arg. The results of this analysis support the hypothesis that p.Pro2555Arg stems are more prone to an open confirmation. This effect was observed in isolated dimers as well as in dimers within multimers. If this effect can also be observed in additional, newly identified, GOF variants is subject of an ongoing study.

Conflict of Interest The authors have no conflicts of interest to declare.

References

[1] Sadler J.E. Biochemistry and genetics of von Willebrand factor. In: Annual review of biochemistry .1998: 67: S 395–424

[2] Wagner D.D. Cell biology of von Willebrand factor. In: Annual review of cell biology.1990: 6: S 217–246

[3] Schneppenheim R. et al. The von Willebrand factor Tyr2561 allele is a gain-of-function variant and a risk factor for early myocardial infarction. In: Blood.2019: 133 (4): S. 356–365

[4] Volker H et al. Gain-of-Function Variant p.Pro2555Arg of von Willebrand Factor Increases Aggregate Size through Altering Stem Dynamics. In: Thrombosis and haemostasis.2022: 122 (2): S. 226–239

T-05. Coagulation management in extracorporal circulation and cardiac assist devices

T-05-01 Telomere length is associated with increased risk of bleeding in patients on hemodialysis

Authors R. Vostatek[1], P. Hohensinner[2, 3], S. Schmaldienst[4], M. Lorenz[5], R. Klauser-Braun[6], I. Pabinger[1], M. Säemann[7], C. Ay[1], O. Königsbrügge[1]

Institutes **1** Medical University of Vienna, Department of Internal Medicine I; Clinical Division of Hematology and Hemostaseology, Vienna, Austria; **2** Medical University of Vienna, Center for Biomedical Research, Vienna, Austria; **3** Medical University of Vienna, Ludwig Boltzmann Institute for Cardiovascular Research, Vienna, Austria; **4** Clinic Favoriten, Department of Medicine I, Vienna, Austria; **5** Vienna Dialysis Center, Vienna, Austria; **6** Clinic Donaustadt, Department of Medicine III, Vienna, Austria; **7** Clinic Ottakring, Department of Medicine VI, Vienna, Austria

DOI 10.1055/s-0044-1779090

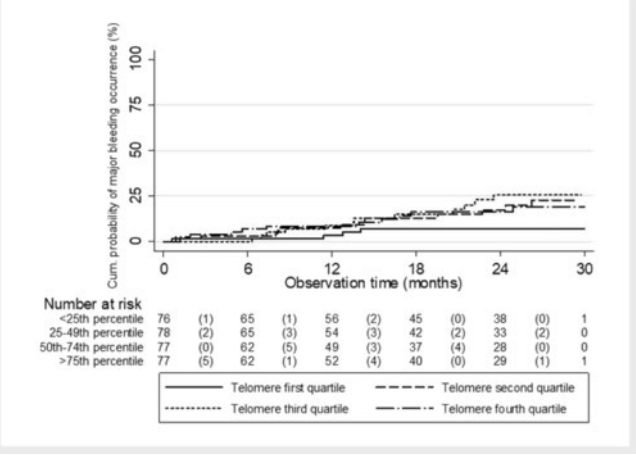
Introduction Patients with end-stage kidney disease (ESKD) on hemodialysis (HD) have a high risk for bleeding complications. The tendency of bleeding events in ESKD patients is multifactorial and influenced by impaired platelet-vessel wall interaction, dysfunctional platelets, and treatment like anticoagulation therapy. Telomere length is a recognized surrogate parameter for biological and early vascular aging. Healthy persons have a chronological age-dependent telomere length approximately between 5 to 15 kb per diploid cell. Our aim is to elucidate the role of bleeding in association with biological aging, expressed by telomere length.

Method The Vienna Investigation of Atrial Fibrillation and Thromboembolism in Hemodialysis (VIVALDI) study is a prospective population-based cohort study of prevalent HD patients. Adult patients were recruited and followed-up for a maximum of 1350 days during which the occurrence of major bleeding events was recorded. The VIVALDI study was approved by the local ethics committees and all patients consented to participate in written form. The DNA from whole blood, sampled at baseline, was isolated and analyzed for average telomere length via qPCR-based method. The risk of major bleeding occurrence was calculated using competing risk regression with consideration of the competing endpoint all-cause death.

Results In 308 patients (193 males (62.7 %) and 115 females (37.3 %)) with ESKD and a median age of 67 years (25th to 75th percentile 56.4-76) years, the median telomere length was 1.51 kb (25th to 75th percentile (0.60 to 3.18kb). Major bleeding events occurred in 39 patients (incidence rate 5.7 per 100 patient-years). There was no relevant correlation between telomere length and chronological age (correlation coefficient of -0.117, p = 0.040). Per 1kb increase in telomere length, the risk of major bleeding occurrence increased by 9 % (SHR 1.094, 95 % confidence interval 1.021-1.172, p = 0.011) in a multivariable competing risk regression model adjusted for age, BMI, and anticoagulation use (▶ **Fig. 1**). Compared to patients in the quartile with the shortest telomere lengths, patients in the second quartile had a 3.3-fold increased risk of major bleeding (95 % CI 1.02-10.56, p = 0.046), patients in the third quartile had a 3.4-fold increased risk (95 %CI 1.09-10.77, p = 0.036), and patients in the fourth quartile had a 3.1-fold increased risk of major bleeding (95 %CI 0.98-10.1, p = 0.053) (▶ **Fig. 2**).

Covariate	Hazard ratio	p-value
Telomere length per kb increase	1.093 (1.019 – 1.173)	0.013
Age per year increase	1.003 (0.982 – 1.025)	0.749
BMI per 1 kg/m^2 increase	0.991 (0.939 – 1.046)	0.740
Anticoagulation use	2.105 (1.050 – 4.222)	0.036

▶ **Fig. 1** Competing risk regression model of major bleeding risk occurrence



▶ **Fig. 2** Cumulative probability of major bleeding occurrence according to quartile of telomere leng

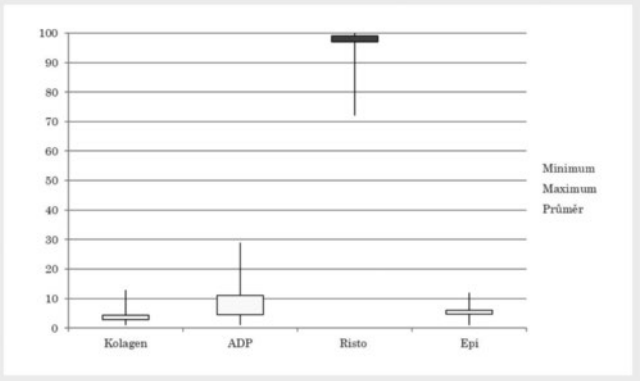
Thieme

Conclusion In a cohort of patients with ESKD on HD and overall short telomere lengths, the risk of major bleeding increased with increasing telomere lengths.
Conflict of Interest The authors declare that they have no competing interests.

T-05-02 Platelet function during platelet-rich plasma squestration in complex cardiac surgery

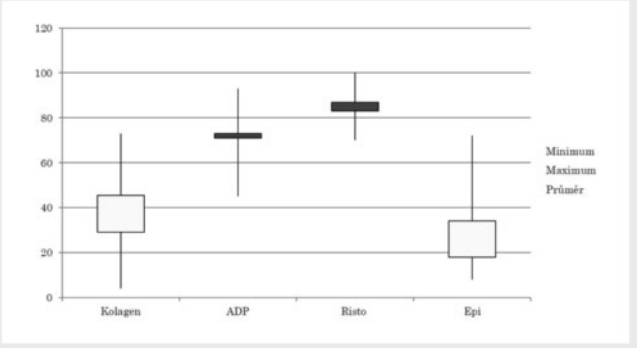
Authors R. Hajek[1], I. Fluger[1], V. Lonsky[1], P. Santavy[1], O. Zuscich[1], P. Caletka[1], L. Slavík[2], J. Úlehlová[2]
Institutes **1** University Hosptal Olomouc, Cardiac Surgery, Olomouc, Czech Republic; **2** University Hospital Olomouc, Hemato-oncology, Olomouc, Czech Republic
DOI 10.1055/s-0044-1779091

Introduction Platelets are the most fragile components of hemostasis in cardiac surgery with cardiopulmonary bypass (CPB).
Contemporary cell-salvage technology can preserve not only red blood cells (RBC) but also platelets in platelet-rich plasma (PRP) sequestration during complex surgical procedures. We tested the presevation of platelet functions during and after PRP sequestration.



▶ **Fig. 1** Aggregation in PRP

Method We obtained informed consent and local ethic commitee approval. In 15 elective adult patients (13 male, 2 female, average age 68 years) scheduled for complex cardiac surgery surgery (aortic, combined and redo, with average CPB time 135 min, cross-clamp time 96 min) with preoperative haematocrit > 0,35 we performed RBC and PRP sequestration (800 ml preoperative autologous blood was processed). Standard mild hypotermic CPB with centrifugal pump, biocompatible surface – X- coating, heparin dose 3mg/kg and protamin reversal 1:1 was used. All the patients were treated with tranexamic acid 30mg/kg i.v. before surgery and 15mg/kg into the CPB priming volume. The Sorin X.tra® cell-saver (175 ml Latham bowl, 2 port system, CPDA bags, prime flow 100 ml/min, PRP spill volume 60 ml/min, spill flow 20 ml/min) was used. PRP was re-transfused immediately after the end of CPB, RBC according to the hemoglobin level at the end of surgery or in ICU. Platelet count and optical aggregometry (aggregation induced by ristocetin, epinephrin, ADP and collagen) before procedure, in processed plasma, at the end of surgery and after PRP retransfusion were assessed. Thromoelastographic parametres (TEG 5000) were assessed before and after CPB. Transfusion therapy was provided by the TEG – guided algorithm.



▶ **Fig. 2** Aggregation after PRP re-transfusion

Results The average amount of autologous blood processed was 874 ± 76ml, the average re-transfused PRP volume 538 ± 57 ml. The average blood loss during the 24 hour period was 676 ± 281 ml. Reexploration due to haemorrhage occurred in 3 patients. Transfusion with FFP was required in 5 cases (range 2 to 6 units), and with RBC in 4 cases (range 2 to 6 units). No platelet transfusions were required. PCC was used in 3 patients, fibrinogen in 1 case. TEG parameters did not differ between the groups. The mean (SD) platelet count before surgery was 187 (35), in PRP 113 (36), after PRP transfusion 126 (31), and after surgery 122 (35) x 109/L. There were statistically significant decreases of collagen-, ADP- and epinephrine-mediated, but not ristocetin-mediated aggregation (97 % of baseline) in PRP compared with the pre-CPB sample. The partial restoration of aggregation after PRP retransfusion was assessed.(median reactivity: collagen 33 to 29 %, ADP 68 to 73 %, ristocetin 97 to 87 %,and epinephrine 19 to 18 % of baseline) [1–2] (▶ **Fig. 1**, ▶ **Fig. 2**).
Conclusion PRP sequetrastion is a safe method. It preserved platelet count and ristocetin-mediated pletelet aggregation, and partially restored other activator- mediated aggregation after CPB.
Conflict of Interest This study was supported by the institutional grant of FN Olomouc 87-55. No conflict of interests is declared by authors.

References
[1] Karkouti K, O´Farrel R, Yau TM, Beattie WS Prediction of massive blood transfusion in cardiac surgery. Can J Anaesth 2006; 53 (8): 781–94
[2] Zhou SF, Estrera AL, Loubser P et al. Autologous pletelet-rich plasma reduces transfusions during ascendent aortic arch repair: a prospective randomized controlled trial. Ann Thorac Surg 2015; 99: 1282–90

T-05-03 Acquired δ-storage pool deficiency and increased GPV shedding impair thrombus formation and predict outcome in ECMO patients

Authors L. J. Weiss[1, 2], J. Herrmann[3], M. Kristina[1], P. Meybohm[3], H. Schulze[1]
Institutes **1** University Hospital Würzburg, Institute of Experimental Biomedicine, Würzburg, Germany; **2** University Hospital Würzburg, Department of Internal Medicine I, Würzburg, Germany; **3** University Hospital Würzburg, Department of Anaesthesiology, Intensive Care, Emergency and Pain Medicine, Würzburg, Germany
DOI 10.1055/s-0044-1779092

Introduction ECMO is a life-saving rescue therapy in patients with ARDS. Bleeding and thrombosis are major complications that are associated with bad outcome. Platelets, the key mediators of hemostasis, can sense matrix proteins like collagen or von Willebrand factor (vWF) by surface receptors like GPIb/V/IX causing activation of integrin GPIIb/IIIa, release of internal stored α-/δ- granules, aggregation and lastly thrombus formation. Recent studies have deciphered a yet unexpected function of GPV, a subunit of the vWF receptor. Thrombin can cleave GPV and release its soluble (s)GPV ectodomain, which finally blocks thrombin activity and fibrin formation. Antibody mediated tar-

Hämostaseologie 2024; 44: S1–S125

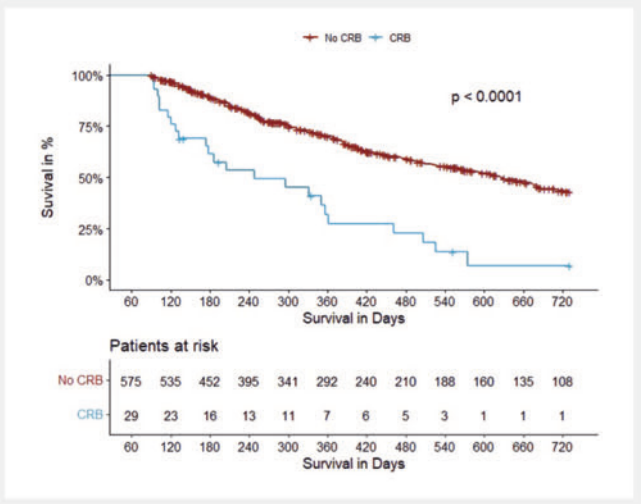
geting of GPV was shown to be a powerful strategy in reverting a bleeding phenotype. In this study we wanted to assess whether ECMO has a specific impact on platelet reactivity including the GPV–sGPV axis.

Method 36 patients with ARDS were recruited (n = 13 ECMO vs. n = 23 non-ECMO patients). Blood was withdrawn at 3 time points (t_1 = day 1-3; t_2 = day 4-6; t_3 = day 7-14 after cannulation/study inclusion) and compared to controls. We assessed platelet phenotype and function using flow cytometry. δ-granule release was assessed by flow cytometry using a kinetically resolved mepacrine assay, the δ-granule count by whole mount-transmission electron microscopy (WM-TEM) and the in vitro bleeding time by PFA-200.

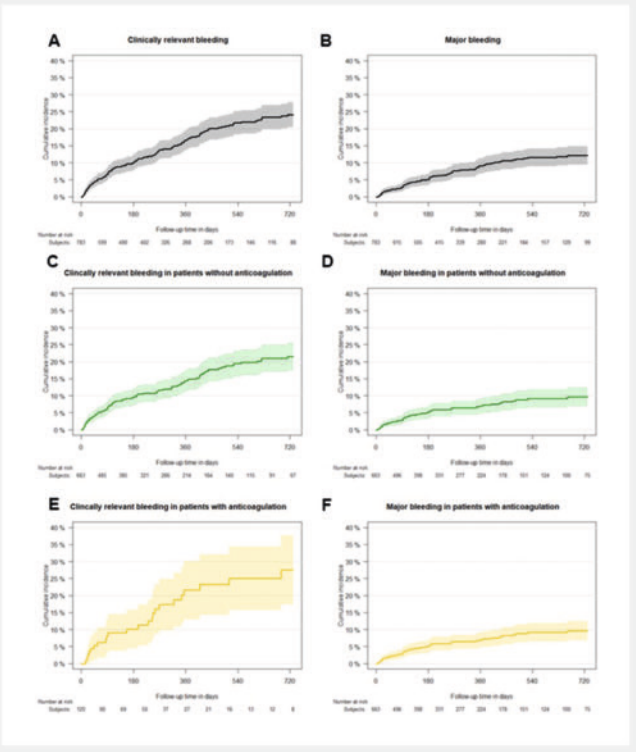
Results GPIIb/IIIa activation and CD62P neo-exposition in response to ADP, TRAP-6 or CRP-X_L were reduced in all patients independent of ECMO. We found no differences in the t-SNE-based platelet supopulation analysis after automated clustering between cohorts. During the course of ECMO treatment, uptake and TRAP-6-induced release of mepacrine continuously decreased (mean GeoMFI ECMO t_1: 37.2 vs t_3: 15; $p < 0.05$), while we observed no differences within follow-up measurements in non-ECMO patients. Concomitantly, δ-granule number per platelet continuously decreased during the course of treatment, which was associated with a prolonged in vitro bleeding time assessed by PFA-200. The expression of the vWF receptor subunits GPIX and GPIb remained overall unaltered, but GPV expression was decreased by 21 % in ECMO compared to non-ECMO patients. Plasma sGPV levels were selectively increased in ECMO patients, suggesting increased shedding in response to the extracorporal device. Decreased sGPV levels were strongly associated with patient survival (ECMO survivor: 0.042; non-survivor: 0.016 $p < 0.05$), implying an adverse effect of sGPV for device-related hemostatic alterations.

Conclusion Agonist induced GPIIb/IIIa activation was markedly impaired in ARDS patients regardless of the use of ECMO. We found a specific paucity of platelet δ-granules in ECMO, implying a device-induced δ-storage pool deficiency. This the first study to show pronounced GPV downregulation and concomitantly increased plasma sGPV in ECMO patients which was associated with survival, suggesting therapeutic targeting of GPV shedding in ECMO patients.

Conflict of Interest The authors declare no conflict of interest.



▶ **Fig. 1** Cumulative risk for major bleeding (MB) and clinically relevant bleeding over 24 months of CRNMB – clinically relevant non-major bleeding



▶ **Fig. 2** Landmark analysis of overall survival according to the occurrence of clinically relevant b Predicted survival times are displayed stratified by the occurrence of CRB within 30 days after study inclusion.

T-06. Cancer and thrombosis

T-06-01 Incidence and outcomes of bleeding events in patients with cancer: results from a prospective cohort study

Authors C. Englisch[1], F. Moik[1, 2], D. Steiner[1], A. Berghoff[3], M. Preusser[3], I. Pabinger[1], C. Ay[1]

Institutes **1** Medical University of Vienna, Department of Medicine I, Clinical Division of Hematology and Hemostaseology, Vienna, Austria; **2** Medical University of Graz, Department of Internal Medicine, Division of Oncology, Graz, Austria; **3** Medical University of Vienna, Department of Medicine I, Clinical Division of Oncology, Vienna, Austria

DOI 10.1055/s-0044-1779093

Introduction Hemostatic imbalances are frequent in patients with cancer. While thrombotic complications have been extensively studied, less is known about baseline bleeding risk and risk factors for bleeding. To allow for a personalized risk-assessment balancing the risk of thrombotic complications and bleeding in patients with cancer, more knowledge on bleeding risk is needed.

Method We aimed to investigate bleeding events and risk factors for bleeding in a prospective cohort study including patients with cancer initiating systemic anti-cancer therapies. Bleeding events were classified according to the ISTH definition as major bleeding (MB) and clinically relevant non-major bleeding (CRNMB) and the composite outcome of both (clinically relevant bleeding, CRB) was used as the outcome of interest as well. All cause-mortality was considered a competing event and therefore competing risk analyses were performed.

Results In total, 791 patients (median age [interquartile range, IQR]: 63 [54-70] years) were included, of which 48 % were female. During a median follow-up time of 19 months (IQR: 8.9-27.9), 366 (46.3 %) patients died. Hundred-twenty patients (15.2 %) were under therapeutic anticoagulation and 124 (15.7 %) patients received anti-platelet therapy at study inclusion. We observed 310 bleeding events of any type in 228 patients. Seventy patients (8.8 %) experienced a MB, 87 (11 %) a CRNMB, and 106 (13.4 %) a minor bleeding. In total, this translated to 140 (17.7 %) first CRB and 70 (8.8 %) first MB events that were

used for further analyses. The 6-month cumulative CRB incidence was 9.9 % (95 % confidence interval [CI]: 7.8-12.2) and the 6-month MB incidence was 5.1 % (95 % CI: 3.4-6.7) (▶ **Fig. 1**). Low hemoglobin (SHR per doubling of hemoglobin: 0.55, 95 % CI: 0.31-0.98), and albumin (SHR per doubling of albumin: 0.28, 95 % CI: 0.11-0.71) at study inclusion as well as head & neck cancer (SHR head & neck cancer versus others: 2.38, 95 % CI: 1.46-3.88) were associated with increased bleeding risk in patients without anticoagulation. We observed 6 (4.3 %) fatal bleedings and furthermore, the occurrence of CRB was associated with an increased risk of all-cause mortality (age, sex, stage, and tumor category adjusted transition hazard ratio [THR]: 5.80; 95 % CI: 4.53-7.43; ▶ **Fig. 2**: landmark analysis of overall survival according to the occurrence of CRB within 30 days of observation).

Conclusion Bleeding events are frequent in unselected patients with cancer. Low hemoglobin and albumin, and head & neck cancer were associated with a higher risk of bleeding in patients receiving no anticoagulation. Furthermore, bleeding events are associated with a poor overall-survival in this patient cohort.

Conflict of Interest IP has received honoraria for lectures and advisory board meetings from Bayer, Sanofi and Pfizer. AB has research support from Daiichi Sankyo, Roche and honoraria for lectures, consultation or advisory board participation from Roche Bristol-Meyers Squibb, Merck, Daiichi Sankyo, AstraZeneca, CeCaVa, Seagen as well as travel support from Roche, Amgen and AbbVie. MP has received honoraria for lectures, consultation or advisory board participation from the following for-profit companies: Bayer, Bristol-Myers Squibb, Novartis, Gerson Lehrman Group (GLG), CMC Contrast, GlaxoSmithKline, Mundipharma, Roche, BMJ Journals, MedMedia, Astra Zeneca, AbbVie, Lilly, Medahead, Daiichi Sankyo, Sanofi, Merck Sharp & Dome, Tocagen, Adastra, Gan & Lee Pharmaceuticals, Janssen, Servier, Miltenyi, Böhringer-Ingelheim

T-06-02 Do arterial and venous thrombotic events have different molecular risk factors in patients with myelofibrosis?

Authors O. Morath[1], C. Crodel[1], K. Schilling[1], M. Meggendorfer[2], C. Baer[2], A. Hochhaus[1], T. Ernst[1]

Institutes **1** Universitätsklinikum Jena, Hämatologie, Internistische Onkologie und Hämostaseologie, Jena, Germany; **2** MLL Münchner Leukämielabor GmbH, Molekulargenetik, München, Germany

DOI 10.1055/s-0044-1779094

Introduction The risk of thrombotic events in myelofibrosis (MF), compared with patients with other myeloproliferative neoplasms (MPN), has been underestimated for a long time. Patients with MF are rarely studied as separate in most cases small cohorts. We sought to independently explore potential molecular risk factors for venous (VTE) and arterial (ATE) events in a substantial cohort of MF patients.

Method Monocentric retrospective data of 122 MF patients were analyzed. 27 (22.1 %) patients had prefibrotic MF, 69 (56.6 %) – overt MF, and 26 (21.3 %) had secondary MF. All patients were tested for driver mutations (*JAK2-V617F*, *CALR*, and *MPL*). *JAK2* allele burden was evaluated in 57/69 mutated patients. Non-driver mutations were analyzed by next-generation sequencing. The primary outcome was the occurrence of a thrombotic event, acute leukemia transformation, or death at any time.

Results The median age at diagnosis was 62 years (range, 26-82), 55.7 % of patients were male. The median follow up was 4.75 years. Nine (7.4 %) patients had prior thrombosis: history of ATE had 3 of them, prior VTE – 4, both – 2. At the time of the diagnosis of MF and during follow-up, 14 (11.5 %) patients had ATE, 22 (18 %) had VTE, and 2 patients had both. Driver mutations: 68 (55.7 %) patients showed a *JAK2V617F*, 39 (32 %) *CALR*, and 4 (3.3 %) *MPL* mutation. Other patients were triple negative or had a combined mutation (n = 3). The median *JAK2* allele burden was 38 % (range, 0.28-96). In total, 92 mutations of these genes were found: *ASXL1* (n = 29), *DNMT3A* (n = 11), *TET2* (n = 25), *IDH2* (n = 2), *SRSF2* (n = 5), *SF3B1* (n = 10), and *U2AF1* (n = 10). A significant association between *JAK2* mutation (OR 2.49, 95 % CI 1.11-5.59) and an increased risk of venous, but not arterial, thrombotic events was found. The *JAK2* allele burden did not impact the risk of VTE. Patients with co-occurrence of *JAK2* and *TET2* or *JAK2* and *ASXL1* mutations did not have a higher risk of VTE (p = 0.22 and p = 0.42, retrospectively). The multivariable analysis showed a significant association between a *DNMTA3* mutation (OR 5.2, 95 % CI 1.3-21.5) and increased risk of ATE [1–4].

Conclusion Arterial and venous thrombotic events had different molecular risk factors in patients with myelofibrosis in this cohort. *JAK2* mutation was associated with an increased risk of venous thrombosis. Patients have also a high risk of VTE even if they carry a low *JAK2* allelic burden. *DNMT3A* mutation showed an independent effect on the risk of arterial thrombosis. Despite the small number of patients with non-driver mutations, these findings warrant consideration of arterial and venous thrombosis separately for future risk stratification and preventive treatment recommendations in patients with MF.

Conflict of Interest None

References

[1] Barbui T, Carobbio A "Thrombosis in myeloproliferative neoplasms during cytoreductive and antithrombotic drug treatment". Research and Practice in Thrombosis and Haemostasis 2022; 6 (1): E12657. Italy

[2] Hernández-Boluda JC, Pastor-Galán I "Predictors of thrombosis and bleeding in 1613 myelofibrosis patients from the Spanish Registry of Myelofibrosis". British Journal of Haematology 2022; 199 (4): 529–538. Spain

[3] Pasquer H, Daltro De Oliveira R "Distinct clinico-molecular arterial and venous thrombotic scoring systems for MPN patients risk stratification. Blood.2022: 140 (1): 830–831. France

[4] Bankar A, Smith E "Clinical and molecular factors associated with thrombosis in myelofibrosis", HemaSphere.2021: 5: e566. Canada

T-06-03 Thrombosis rates in patients with cancer receiving immune checkpoint inhibitors: results from a prospective cohort study

Authors C. Englisch[1], F. Moik[1, 2], D. Steiner[1], A. Starzer[3], A. Berghoff[3], M. Preusser[3], I. Pabinger[1], C. Ay[1]

Institutes **1** Medical University of Vienna, Department of Medicine I, Clinical Division of Hematology and Hemostaseology, Vienna, Austria; **2** Medical University of Graz, Department of Internal Medicine, Division of Oncology, Graz, Austria; **3** Medical University of Vienna, Department of Medicine I, Clinical Division of Oncology, Vienna, Austria

DOI 10.1055/s-0044-1779095

Introduction Cancer is associated with an increased risk of venous thromboembolism (VTE), which is in part attributable to systemic cancer therapies. Immune checkpoint inhibitors (ICIs) have changed the treatment landscape in oncology but their impact on VTE risk is still debated.

Method We assessed VTE risk in a single-center prospective cohort study including patients with cancer initiating systemic anti-cancer therapies and comparing the risk of VTE between patients receiving different types of therapy. Patients recruited between July 2019 and April 2023 were included in this analysis. All-cause mortality was considered a competing event of interest and thus, competing risk analyses were performed.

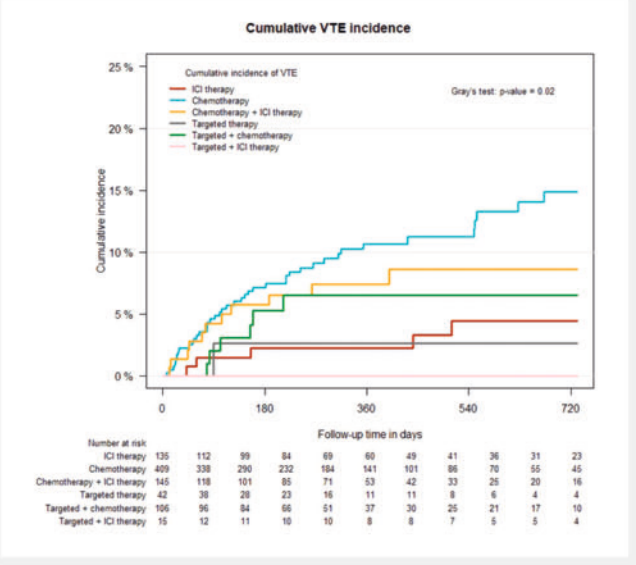
Results In total, 880 patients (median age: 62 years [interquartile range, IQR: 53-70], 47 % women) were included, compromising 459 (52.2 %) patients with newly diagnosed cancer and 421 (47.8 %) patients with recurrent or progressive disease. Of 831 patients with solid tumors, 551 (62.6 %) had metastatic disease at study inclusion. After study inclusion, systemic treatment with chemotherapy alone was initiated in 427 (48.5 %), with combined chemotherapy and ICI therapy in 147 (16.7 %), with ICI therapy alone in 138 (15.7 %), with targeted therapy and chemotherapy in 109 (12.4 %), with targeted therapy alone in 44 (5 %), and with targeted therapy and ICI therapy in 15 patients (1.7 %). During a median follow-up time of 15.8 months (IQR: 9.3-27.7), 68 (7.7 %) were diagnosed with VTE, including 11 catheter-related thrombosis, 20 deep vein thrombosis (DVT), 24 pulmonary embolism (PE), 4 superficial vein thrombosis (SVT),

4 portal vein thrombosis, and 4 patients with PE and DVT as index event. VTE was diagnosed in 43 (10.1 %) patients receiving chemotherapy, 11 (7.5 %) patients receiving chemotherapy and ICI therapy, 6 (4.3 %) patients receiving ICI therapy alone, 7 (6.4 %) patients receiving targeted therapy and chemotherapy, 1 (2.3 %) receiving targeted therapy and in none (0 %) receiving targeted therapy and ICI therapy. This translated into 6-month cumulative VTE incidences of 7.2 % (95 % confidence interval [CI]: 4.6-9.8) in patients with chemotherapy, of 5.8 % (95 % CI: 1.8-9.6) with ICI and chemotherapy, of 2.3 % (95 % CI: 0-4.8) with ICI therapy, of 5.2 % (95 % CI: 0.8-9.6) with targeted therapy and chemotherapy, and of 2.6 % (95 % CI: 0.0-7.7) with targeted therapy (▶ **Fig. 1**, ▶ **Fig. 2**).

Table 1. Cumulative VTE incidence after 6-, 12-, 24-month follow-up stratified according to systemic therapy. ICI – immune checkpoint inhibitors

	Cumulative VTE incidence (95% confidence interval)		
	6-month	12-month	24-month
Chemotherapy (n=427)	7.2 (4.6-9.8)	10.7 (7.4-14)	14.9 (10.3-19.4)
Chemotherapy + ICI therapy (n=147)	5.8 (1.8-9.6)	7.4 (3-11.9)	8.6 (3.7-13.6)
ICI therapy (n=138)	2.3 (0-4.8)	2.3 (0-4.8)	4.4 (0.6-8.3)
Targeted therapy + chemotherapy (n=109)	5.2 (0.8-9.6)	6.5 (1.4-11.4)	6.5 (1.4-11.4)
Targeted therapy (n=44)	2.6 (0.0-7.7)	2.6 (0.0-7.7)	2.6 (0.0-7.7)
Targeted therapy + ICI therapy (n=15)	0	0	0

▶ **Fig. 1** Cumulative VTE incidence after 6-, 12-, 24-month follow-up stratified according to systemic ICI – immune checkpoint inhibitors



▶ **Fig. 2** Cumulative VTE incidence stratified according to systemic therapy that patients started at

Conclusion In our single-center prospective cohort study, patients with cancer receiving systemic anti-cancer therapies had a substantial VTE risk. Patients with cancer receiving chemotherapy had the highest cumulative incidence, while the VTE incidence was lower in those treated with ICI therapy. In the future, further analyses considering other VTE risk factors are needed.

Conflict of Interest This project was supported by a GTH Early Career Research Grant. IP has received honoraria for lectures and advisory board meetings from Bayer, Sanofi and Pfizer. AB has research support from Daiichi Sankyo, Roche and honoraria for lectures, consultation or advisory board participation from Roche Bristol-Meyers Squibb, Merck, Daiichi Sankyo, AstraZeneca, CeCaVa, Seagen as well as travel support from Roche, Amgen and AbbVie. MP has received honoraria for lectures, consultation or advisory board participation from the following for-profit companies: Bayer, Bristol-Myers Squibb, Novartis, Gerson Lehrman Group (GLG), CMC Contrast, GlaxoSmithKline, Mundipharma, Roche, BMJ Journals, MedMedia, Astra Zeneca, AbbVie, Lilly, Medahead, Daiichi Sankyo, Sanofi, Merck Sharp & Dome, Tocagen, Adastra, Gan & Lee Pharmaceuticals, Janssen, Servier, Miltenyi, Böhringer-Ingelheim

T-06-04 Binding of circulating melanoma cell-derived extracellular vesicles to ultra-large von Willebrand factor induces cancer-associated thrombosis

Authors Y. Wang, X. Liu, A. T. Bauer, S. W. Schneider, C. Gorzelanny
Institute University Medical Center Hamburg-Eppendorf, Institute for Dermatology and Venerology, Hamburg, Germany
DOI 10.1055/s-0044-1779096

Introduction Extracellular vesicles (EVs) released by tumor cells are currently investigated as potential biomarkers for cancer detection using liquid biopsy. EVs are cell-derived vesicles with heterogeneous contents, including genetic materials, proteins, lipids, and small metabolites. Tissue factor–-positive (TF)-EVs are considered as biomarkers predicting tumor progression. They were also shown to enhance cancer-associated thrombosis (CAT) and to be linked to elevated plasma levels of von Willebrand factor (vWF). VWF is a large multimeric glycoprotein promoting platelet aggregation. This study aimed to investigate whether circulating melanoma cell-derived TF-EVs can promote CAT by interacting with ultra-large vWF (ULvWF).

Method Binding of melanoma cell-derived EVs or melanoma cells to ULvWF and subsequent thrombosis formation was investigated by microfluidic experiments. To this end, microfluidic channels coated with human umbilical vein endothelial cells or vWF were perfused with whole human blood supplemented with purified and fluorescently labeled EVs. EVs were characterized by nanoparticle tracking analysis, flow cytometry, and electron microscopy. The expression of TF on different melanoma cells and melanoma cell-derived EVs was studied by fluorescence microscopy.

Results We found that melanoma cell-derived EVs, but not intact cells, can bind to ULvWF. The diameter of EVs ranged from 40 nm to 5000 nm, with the majority of EVs being around 165 nm. In microfluidic experiments, mimicking a tumor-activated vascular system, we found that EVs smaller than 1000 nm had the highest probability of interacting with ULvWF. Binding rates of larger EVs were very low. ADAMTS13, the cleavage enzyme of vWF, reduced the binding of EVs significantly. Experiments with different vWF mutants identified the A1 domain as the EV binding site. Accordingly, EV binding was abolished by an inhibitory peptide blocking the A1 domain. Furthermore, the binding of TF-EVs to ULvWF activated platelets and induced the formation of microthrombi. Although intact melanoma cells could not bind to ULvWF directly, melanoma cells were trapped in microthrombi.

Conclusion In conclusion, this study demonstrates that melanoma cell-derived EVs induce CAT by binding to ULvWF. While TF-EVs may serve as a promising biomarker, blocking ULvWF formation or EV binding may prevent metastasis.

Conflict of Interest The authors declare that they have no conflict of interest.

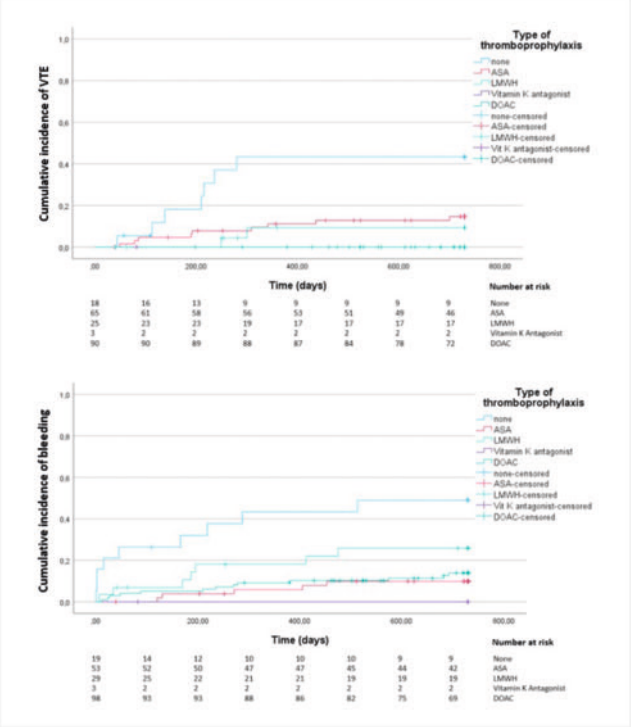
T-06-05 Thromboembolism and bleeding in newly diagnosed Multiple Myeloma – Rates, risk profile and patterns of thromboprophylaxis

Authors L. Vospernik[1], H. Agis[1], C. Ay[1], L. Z. Rüsing[1], H. Gisslinger[1], I. Simonitsch-Klupp[2], M. T. Krauth[1], J. Riedl[1]
Institutes **1** Medical University of Vienna, Department of Medicine 1, Clinical Division of Haematology and Haemostaseology, Vienna, Austria; **2** Medical University of Vienna, Clinical Institute of Pathology, Vienna, Austria
DOI 10.1055/s-0044-1779097

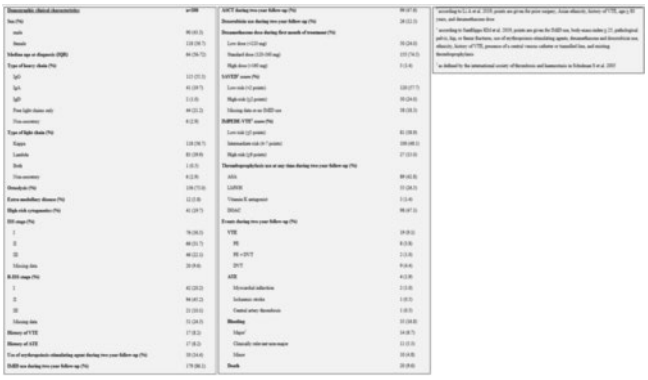
Introduction Patients with multiple myeloma (MM) treated with immunomodulatory drugs (IMiDs) have a high risk of venous thromboembolisms (VTE). Guidelines suggest pharmacological thromboprophylaxis in all patients treated with IMiD-based regimens, using aspirin for patients at low, and low molecular weight heparin (LMWH) or vitamin K antagonists (VKA) for those at high VTE risk. Direct oral anticoagulants (DOACs) have not yet been sufficiently studied in this indication but could be an effective and convenient alternative and have since 2012 been offered as such at our centre.

Method For this retrospective cohort study, we screened all patients that presented at our centre between January 2012 and January 2022 with MM or related plasma cell disorders. Inclusion criteria were newly diagnosed biopsy-proven MM within this time range and induction treatment at our centre. Data were collected through review of medical records. Baseline disease characteristics and risk factors for VTE according to the IMPEDE-VTE and SAVED scores were assessed at time of diagnosis. Anti-myeloma treatment, thromboprophylaxis and thrombotic as well as bleeding events were recorded during a two-year observational period. In those with multiple VTE or bleeding events, only the first event, respectively, was counted. For analysis of outcomes according to type of thromboprophylaxis, the antithrombotic drug prescribed prior to the event of interest was considered in an intention-to-treat manner [1–5].

Results Of 292 patients screened, 208 were included in the analysis based on predefined inclusion and exclusion criteria. Characteristics of patients are shown in ▶ **Fig. 2**. During the 2-year follow up, 19 (9.1 %) patients developed VTE, 4 (1.9 %) had arterial thromboembolism, 35 (16.8 %) had bleeding events and 20 (9.6 %) patients died (▶ **Fig. 1**).



▶ **Fig. 2** 2-Year Cumulative Incidence of VTE and bleeding stratified by type of thromboprophylaxis



▶ **Fig. 1** Demographic and clinical characteristics of 208 patients diagnosed with MM between January

A high IMPEDE-VTE score was associated with risk for VTE (HR for high (≥ 8 points) vs. low (≤ 3 points) IMPEDE-VTE: 3.994 [95 %CI 1.072-14.881], p = 0.039; HR increase per 1 point increase: 1.193 [95 %CI 1.04-1.369], p = 0.012). The SAVED score did not predict VTE in our cohort (HR for high vs. low SAVED: 1.006 [95 %CI 0.354-2.855], p = 0.992).

The type of thromboprophylaxis was significantly associated with both risk of VTE and risk of bleeding (▶ **Fig. 2**). Seven out of 18 patients (38.9 %) had a VTE while not on any type of thromboprophylaxis, 9 out of 65 (13.8 %) while on aspirin, 2 out of 25 (8 %) while on LMWH. None of the 90 patients on DOACs or the 3 patients on VKAs had a VTE. Bleeding occurred in 47.4 % of patients without thromboprophylaxis, in 9.4 % of those using aspirin, in 24.1 % of those using LMWH, in 13.3 % of those using DOACs, and in none of those using VKAs.

Conclusion We observed a high rate of VTE and bleeding events in our cohort. While the IMPEDE-VTE score predicted VTE risk, the SAVED score did not. No patient had a VTE while on oral anticoagulation. Patients who were not prescribed thromboprophylaxis had a high risk of both thrombosis and bleeding.

Conflict of Interest CA has received personal fees for lectures and/or participation in advisory boards from Bayer, BM/Pfizer, Daiichi Sankyo and Sanofi. The other authors declare that they have no conflicting interests with regard to this abstract.

References

[1] Carrier M, Le Gal G, Tay J, Wu C, Lee AY 'Rates of venous thromboembolism in multiple myeloma patients undergoing immunomodulatory therapy with thalidomide or lenalidomide: a systematic review and meta-analysis.'. J Thromb Haemost 2011; 9 (4): 653–663

[2] Farge D, Frere C, Connors JM, Khorana AA, Kakkar A, Ay C, Muñoz A, Brenner B, Prata PH, Brilhante D, Antic D, Casais P, Guillermo Esposito MC, Ikezoe T, Abutalib SA, Meillon-García LA, Bounameaux H, Pabinger I, Douketis J '2022 international clinical practice guidelines for the treatment and prophylaxis of venous thromboembolism in patients with cancer, including patients with COVID-19.'. Lancet Oncol 2022; 23 (7): e334–e347

[3] Li A, Wu Q, Luo S, Warnick GS, Zakai NA, Libby EN et al. 'Derivation and Validation of a Risk Assessment Model for Immunomodulatory Drug-Associated Thrombosis Among Patients With Multiple Myeloma.'. J Natl Compr Canc Netw 2019; 17 (7): 840–7

[4] Sanfilippo KM, Luo S, Wang TF, Fiala M, Schoen M, Wildes TM et al. 'Predicting venous thromboembolism in multiple myeloma: development and validation of the IMPEDE VTE score.'. Am J Hematol 2019; 94 (11): 1176–84

[5] Schulman S, Kearon C 'Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients.'. J Thromb Haemost 2005; 3 (4): 692–4

T-06-06 Immune checkpoints are upregulated on activated platelets and monocytes

Authors C. Rolling, J. Regenhardt, C. Lehr, L. Beckmann, C. Bokemeyer, F. Langer
Institute University Medical Center Hamburg-Eppendorf, Oncology, Hematology and BMT with section of Pneumology, Hamburg, Germany
DOI 10.1055/s-0044-1779098

Introduction While immune checkpoint inhibitors (ICI) have revolutionized the therapeutic landscape of various cancers, most importantly non-small cell lung cancer (NSCLC) and malignant melanoma, recent meta-analyses indicate that patients under ICI are at increased risk of developing venous thromboembolism (VTE) and cardiovascular adverse events. Importantly, platelets and monocytes are crucial cellular mediators in promoting coagulation activation. To delineate their role in ICI-induced thrombosis, we first aimed to investigate if both cell types express immune checkpoints on their surface.

Method We established flow cytometry panels to characterize platelets and monocytes in whole blood. In short, venous blood from healthy donors was drawn without torniquet and after an initial discard into plastic tubes containing 3.2 % (0.109 M) sodium citrate. Blood samples were allowed to rest for 15 min and then incubated with lipopolysaccharide (10 µg/mL) for 4 hours to stimulate monocytes or with ADP (1 µM) for 30 min to activate platelets. To characterize monocyte (activation), whole blood was then stained for CD45, CD14, CD16, PDL1, PD1, tissue factor, CD40, and CD61. Platelets were identified based on side-scatter properties and expression of CD41, P-selectin, PAC-1, CD63, PD1 and PDL1. Platelet releasates (PR) were generated by high-speed centrifugation of platelet rich plasma. PDL1 protein in cell pellets as well as in platelet releasates was measured by commercial PDL1 ELISA.

Results Following stimulation of whole blood with ADP, activated platelets rapidly upregulate PDL1 but not PD1 on their surface. Similarly, isolated platelets increased PDL1 expression when exposed to ADP, pointing towards platelets as the source of PDL1. There was a strong and robust correlation of PDL1 with platelet activation markers P-selectin, PAC-1 and CD63. Importantly, platelet lysates as well as PR from resting platelets contained PDL1 protein. PDL1 levels in PR were further elevated when obtained from activated platelets. Increased expression of PDL1 and PD1 was also confirmed on LPS-stimulated proinflammatory monocytes in whole blood.

Conclusion We show that activated platelets and monocytes upregulate PDL1 on their surface, indicating a potential effector role in inhibiting T cells under inflammatory conditions. Importantly, activated platelets release soluble PDL1, indicating an immunomodulatory effector role which may affect therapeutic outcome in ICI-treated cancer patients. Based on these findings, we are currently assessing monocyte and platelet activation and PDL1 expression in cancer patients before and under ICI. Our ultimate goal is to investigate if monocytes and platelets become activated under ICI, hence resulting in ICI-induced thromboinflammation, and to assess the impact of platelet and monocyte PDL1 expression on treatment outcome

Conflict of Interest The authors declare no conflict of interests.

T-06-07 Cellular Components in Whole Blood Contribute to the Development of Thromboembolic Events in Patients with Pancreatic Cancer

Authors R. A.L. Willems[1, 2], M. Roest[1], J. de Vos-Geelen[4, 6], B. de Laat[1], H. ten Cate[2, 3]
Institutes **1** Synapse Research Institute, Maastricht, Netherlands; **2** Maastricht University Medical Center, Department of Internal Medicine, section Vascular Medicine, Maastricht, Netherlands; **3** Maastricht University Medical Center, Thrombosis Expert Center Maastricht, Maastricht, Netherlands; **4** Maastricht University Medical Center, Department of Internal Medicine, Section of Medical Oncology, Maastricht, Netherlands; **5** Maastricht University Medical Center, Cardiovascular Research Institute Maastricht (CARIM), Maastricht, Netherlands; **6** Maastricht University Medical Center, GROW, Maastricht, Netherlands
DOI 10.1055/s-0044-1779099

Introduction Thromboembolic disease is a major complication in patients with pancreatic ductal adenocarcinoma (PDAC), causing morbidity and mortality. One in five patients with newly diagnosed PDAC develops a venous thromboembolism (VTE), which is associated with a decreased overall survival**.** Patients with PDAC often have altered blood cell counts, which are further affected by chemotherapy. The high thromboembolic risk in patients with PDAC may be caused by procoagulant effects of pancreatic cancer on blood cells. Currently, there are no risk assessment tools or biomarkers available that can identify PDAC patients at highest risk of VTE.

Aims To investigate the impact of PDAC on blood cell dependent coagulation, to characterize the high thrombosis risk in PDAC patients. To predict thromboembolic events in patients with PDAC using blood cell dependent assays, performed in whole blood.

Method Patients with locally advanced or metastatic PDAC (n = 18) were included in a prospective, observational, case-control study (TROCOPAC study). At baseline, before the initiation of chemotherapy, blood was collected to study whole blood (WB) coagulation profiles. Thrombin generation (TG) was measured in WB and platelet poor plasma (PPP) of 18 patients with PDAC and 18 healthy individuals matched for age and sex. The capacity of platelets to release granules was measured in a time-dependent manner upon stimulation with a PAR1 receptor agonist in WB. Patients were followed for 6 months upon inclusion. The primary endpoint was the onset of thromboembolic events, both VTE and arterial thromboembolism (ATE).

Results Patients with PDAC have an increased endogenous thrombin potential (ETP) in whole blood. This difference was not observed in PPP, indicating a procoagulant effect caused by blood cells. Furthermore, patients with PDAC have a prolonged lag time in whole blood, while the lag time in PPP was not different between patients and controls. Platelet and white blood cell counts (WBC) significantly correlated with the WB ETP and peak in patients with PDAC, which was not observed in healthy controls. Of the 18 PDAC patients, four developed a VTE (22 %) and one an ATE (6 %). At baseline, a shorter lag time of WB-TG and an increased maximum capacity of platelets to release granule were associated with incident thromboembolic events.

Conclusion Patients with PDAC have an imbalanced WB-TG profile. The lag time in WB-TG and the granule release capacity of platelets predict an increased incidence of thromboembolic events. Red blood cell and white blood cell abnormalities in PDAC are associated with this disturbance in thrombin generation. Platelets and white blood cells hence appear to be a driving force in the development of thrombosis in patients with PDAC. As such, measuring hemostasis in whole blood would improve the thrombosis risk estimation in PDAC patients.

Conflict of Interest The authors declare no conflict of interest.

T-06-08 Growth differentiation factor-15 is associated with bleeding risk in patients with cancer: results from a prospective cohort

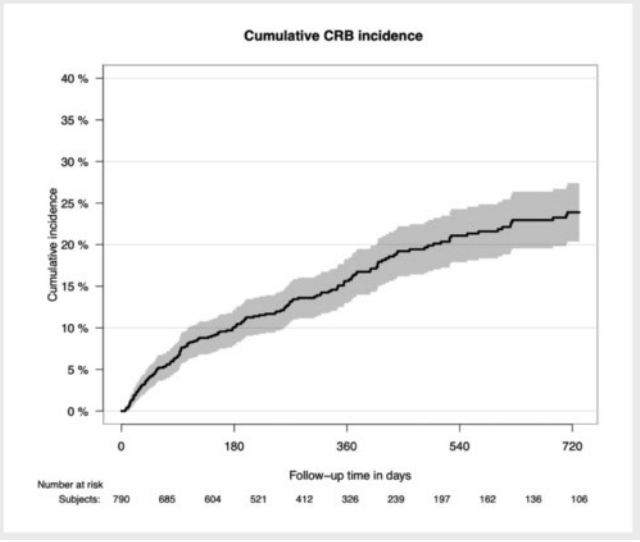
Authors C. Englisch[1], S. Nopp[1], I. Pabinger[1], F. Moik[1, 2], D. Steiner[1], M. Fritzer-Szekeres[4], M. Preusser[3], A. Berghoff[3], C. Ay[1]
Institutes **1** Medical University of Vienna, Department of Medicine I, Clinical Division of Haematology and Hemostaseology, Wien, Austria; **2** Medical University of Graz, Department of Internal Medicine, Division of Oncology, Graz, Austria; **3** Medical University of Vienna, Department of Medicine I, Clinical Division of Oncology, Wien, Austria; **4** Medical University of Vienna, Department of Laboratory Medicine, Wien, Austria
DOI 10.1055/s-0044-1779100

Introduction In patients with cancer, dysregulation of hemostasis is present. While there is extensive knowledge on risk, risk factors, and predictive biomark-

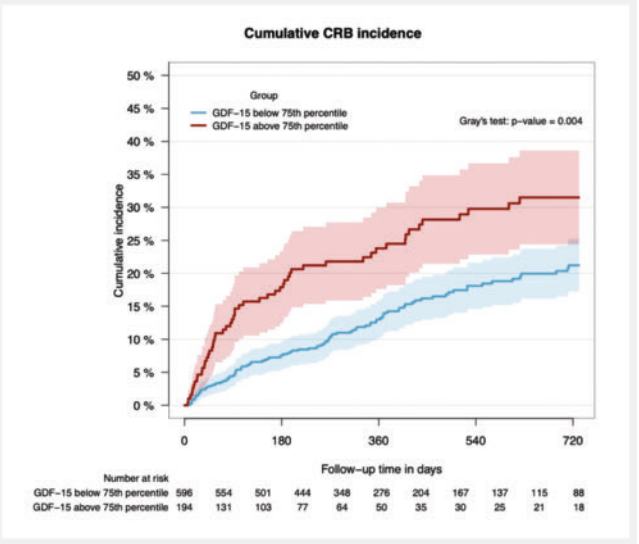
ers for venous thromboembolism (VTE), there is limited understanding of bleeding risk and associated biomarkers. Prior research has indicated that growth differentiation factor-15 (GDF-15) holds promise as a predictive biomarker for bleeding risk in various patient populations, including a preliminary study focused on patients with cancer.

Method We aimed to investigate the association between GDF-15 and bleeding risk in a prospective cohort study including patients with cancer initiating systemic anti-cancer therapies. Bleeding events were classified according to the ISTH definition as major bleeding (MB) and clinically relevant non-major bleeding (CRNMB). The composite outcome of both, i.e., clinically relevant bleeding (CRB), and MB were specified as the outcomes of interest. Serum GDF15 levels were measured in samples drawn at study inclusion (prior to treatment) using the Elecsys® GDF-15 assay (Roche Diagnostics) performed on the Cobas e801 Module. The association between GDF-15 and CRB/MB was analyzed in a Fine and Gray model accounting for all cause-mortality as a competing risk.

Results In total, 792 patients (48 % women) were included in this analysis (median age: 62, interquartile range [IQR]: 53-70). At study inclusion, 121 (15.3 %) patients received therapeutic anticoagulation and 125 (15.8 %) antiplatelet therapy. During a median follow-up of 18 months (IQR: 11-28), 240 patients (30.3 %) experienced any type of bleeding. We observed CRB in 156 (19.7 %) patients and MB in 81 (10.4 %) patients. This translated into a 6-, 12-, 24-month cumulative incidence of CRB of 10.1 % (95 % confidence interval [CI]: 8-12.2), 15.8 % (13.2-18.5), 23.9 % (17.9-27.4) and of MB of 5.4 % (3.8-6.9), 8.5 % (6.5-10.5), 12.2 % (9.5-14.8), respectively (▶ **Fig. 1**). The median GDF-15 level was 1863.5 ng/L (IQR: 1061-3490). Elevated GDF-15 levels were significantly associated with an increased risk of CRB (SHR per doubling: 1.29 [95 % CI: 1.16-1.44]) and MB (SHR per doubling: 1.40 [95 % CI: 1.22-1.62]). In a 12-month comparison, patients with GDF-15 levels above the 75th percentile (> 3490 ng/L) had a higher cumulative bleeding incidence than those with levels at or below the 75th percentile (≤ 3490 ng/L). Specifically, the cumulative incidence for CRB was 23.8 % versus 13.3 %, and the cumulative incidence for MB was 13.6 % versus 6.8 %. The difference in CRB incidence remained consistent throughout the entire observation period (▶ **Fig. 2**).



▶ **Fig. 1** Cumulative clinically relevant bleeding (CRB) incidence.



▶ **Fig. 2 Cumulative CRB incidence of patients (n = 898) with GDF-15 levels ≤ 75th (n = 598) (≤ 3490 ng/L) versus > 75th percentile (n = 194) (> 3490ng/L)** Patients were divided according to their GDF-15 level and the group with levels equal to or below 3490 ng/L (≤ 75th percentile) was compared to the group with levels above 3490 ng/L (> 75th percentile) within a Fine and Gray subdistribution hazard model, p = 0.004

Conclusion Patients with cancer are at high risk for bleeding events and elevated GDF-15 levels were significantly associated with an increased risk of bleeding. Therefore, GDF-15 is a promising candidate biomarker for bleeding risk prediction in patients with cancer.

Conflict of Interest This project was supported by an unrestricted grant from Roche Diagnostics to the Medical University of Vienna. This project was supported by a GTH Early Career Research Grant. AB has research support from Daiichi Sankyo, Roche and honoraria for lectures, consultation or advisory board participation from Roche Bristol-Meyers Squibb, Merck, Daiichi Sankyo, AstraZeneca, CeCaVa, Seagen as well as travel support from Roche, Amgen and AbbVie. MP has received honoraria for lectures, consultation or advisory board participation from the following for-profit companies: Bayer, Bristol-Myers Squibb, Novartis, Gerson Lehrman Group (GLG), CMC Contrast, GlaxoSmith-Kline, Mundipharma, Roche, BMJ Journals, MedMedia, Astra Zeneca, AbbVie, Lilly, Medahead, Daiichi Sankyo, Sanofi, Merck Sharp & Dome, Tocagen, Adastra, Gan & Lee Pharmaceuticals, Janssen, Servier, Miltenyi, Böhringer-Ingelheim

T-06-09 Endogenously produced factor XI (FXI) contributes to hepatic cancer cell-induced coagulation activation

Authors L. Beckmann[1], K. Riecken[2], J. Mäder[1], C. Rolling[1], M. Voigtländer[1], A. Schulenkorf[1], C. Lehr[1], J. Regenhardt[1], B. Fehse[2], C. Bokemeyer[1], F. Langer[1]

Institutes 1 Universitätsklinikum Eppendorf, II. Medizinische Klinik und Poliklinik, Hubertus Wald Tumorzentrum, Universitäres Cancer Center Hamburg (UCCH), Hamburg, Germany; 2 Universitätsklinikum Eppendorf, Forschungsabteilung Zell- und Gentherapie, Klinik für Stammzelltransplantation, Hamburg, Germany

DOI 10.1055/s-0044-1779101

Introduction Elevated FXI levels are a risk factor for venous thromboembolism (VTE) [1]. While FXI/FXIa inhibition is safe and efficacious in preventing post-

operative VTE in non-oncologic patients [2–5], its role in tumor cell-induced coagulation activation is less clear [6]. We hypothesize that some tumor cells, especially those of hepatic cancers, aberrantly produce FXI, which potentially contributes to a hypercoagulable state and reduced efficacy of FXI/FXIa-directed anticoagulation in these patients.

Method Several cancer cell lines were characterized for FXI and tissue factor (TF) expression by quantitative PCR, ELISA and flow cytometry. FXI activity was recorded by a chromogenic assay. Tumor cell-induced coagulation activation was measured by single-stage clotting assay in the presence or absence of the FXIa inhibitor, BMS-262084 (BMS), or peak and trough concentrations of rivaroxaban (270 and 26 ng/mL) and tinzaparin (0.85 and 0.2 IU/mL). We also designed a lentiviral vector to stably express FXI in hepatoma HepG2 and hepatocellular Huh7 cells.

Results Only in hepatic cancer cell lines, HepG2 and Huh7, relevant FXI mRNA and antigen expression were measured. FXI released by these cell lines showed potent amidolytic activity in a chromogenic assay. Both cell lines had also marked TF antigen expression and procoagulant activity (PCA). Compared to FXa inhibition by rivaroxaban and tinzaparin, FXIa inhibition with BMS was significantly less potent in mitigating tumor cell-induced fibrin clot formation, and only in the presence of low HepG2 and Huh7 cell concentrations. Lentiviral expression of FXI in HepG2 and Huh7 cells resulted in significantly increased production of FXI, but not of TF. In these cells, endogenous FXI amplified tumor cell-induced fibrin clot formation.

Conclusion Dysregulated FXI production is presumably a common finding only in hepatic cancers, but may contribute to a hypercoagulable state in these patients with potential therapeutic implications for FXI/FXIa-directed anticoagulation.

Conflict of Interest The submitted work was supported in part by an Early Career Research Grant from the GTH e.V. to L.B. L.B., K.R., J.M., A.S., C.L., J.R., and B.F. declare no conflicts of interest relevant to the content of this article. C.C.R. has received travel support from Pfizer. M.V. declares personal fees for lectures from Bris-tol Myers Squibb and travel support from Bayer, Bristol Myers Squibb and LEO Pharma. C.B. has received personal fees for consultancy, research support, and/or travel support from Bayer, Boehringer Ingelheim, Bristol Myers Squibb, Daiichi Sankyo, Novartis, Pfizer, Roche, and Sanofi. F.L. has received personal fees for lectures or consultancy and/or research support from Bayer, Boehringer-Ingelheim, Bristol Myers Squibb, Daiichi Sankyo, LEO Pharma, Pfizer, Sanofi, and Viatris.

References

[1] Meijers JC, Tekelenburg WL, Bouma BN, Bertina RM, Rosendaal FR. High levels of coagulation factor XI as a risk factor for venous thrombosis. N Engl J Med 2000; 342: 696–70

[2] Büller HR, Bethune C, Bhanot S, Gailani D, Monia BP, Raskob GE, Segers A, Verhamme P, Weitz JI. Factor XI antisense oligonucleotide for prevention of venous thrombosis. N Engl J Med 2015; 372: 232–40

[3] Weitz JI, Bauersachs R, Becker B, Berkowitz SD, Freitas MCS, Lassen MR, Metzig C, Raskob GE. Effect of Osocimab in Preventing Venous Thromboembolism Among Patients Undergoing Knee Arthroplasty: The FOXTROT Randomized Clinical Trial. JAMA 2020; 323: 130–9

[4] Verhamme P, Yi BA, Segers A, Salter J, Bloomfield D, Büller HR, Raskob GE, Weitz JI. Abelacimab for Prevention of Venous Thromboembolism. N Engl J Med 2021; 385: 609–17

[5] Weitz JI, Strony J, Ageno W, Gailani D, Hylek EM, Lassen MR, Mahaffey KW, Notani RS, Roberts R, Segers A, Raskob GE. Milvexian for the Prevention of Venous Thromboembolism. N Engl J Med 2021; 385: 2161–72

[6] Mäder J, Rolling CC, Voigtländer M, Schulenkorf A, Lehr C, Regenhardt J, Bokemeyer C, Beckmann L, Langer F. Effect of factor XI inhibition on tumor cell-induced coagulation activation. J Thromb Haemost 2023; S1538–7836(23): 00717-1. doi:10.1016/j.jtha.2023.09.015

T-07. Haemophilia and von Willebrand disease

T-07-01 Early Changes in Liver Transcriptomic Profiles Following Adeno-Associated Viral Gene Therapy in the Severe Hemophilia A Dog Model

Authors A. M. Ismail[1], P. Batty[2], A. Mo[2], B. Yates[1], L. Harpell[2], A. Menard[3], A. Pender[2], N. Khattak[1], L. Razon[1], A. Winterborn[4], S. Fong[1], D. Lillicrap[2], S. Harris[1]

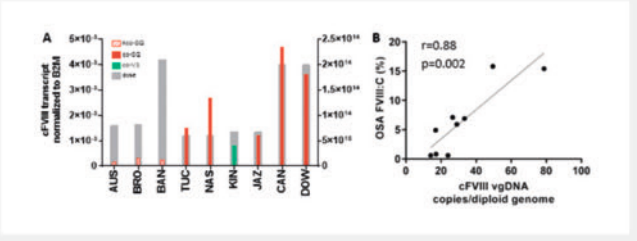
Institutes **1** BioMarin Pharmaceutical Inc., Novato, USA; **2** Queen's University, Department of Pathology and Molecular Medicine, Kingston, Canada; **3** Kingston Health Sciences Center, Kingston, Canada; **4** Queen's University, Animal Care Services, Kingston, Canada

DOI 10.1055/s-0044-1779102

Introduction Recombinant adeno-associated virus vectors (rAAV) are a major gene therapy platform for treatment of monogenic disorders, including hemophilia A. Valoctocogene roxaparvavec (AAV5-HLP-hFVIII-SQ) is an AAV5 vector delivering B-domain-deleted (BDD) human FVIII transgene controlled by a hybrid liver-selective promoter. In a phase 3 trial, a single treatment of AAV5-HLP-hFVIII-SQ (6e13 vg/kg) provided therapeutic expression of FVIII and bleeding control in adult severe hemophilia A patients. However, the mechanistic basis of transaminitis, variability and durability observed in clinical trial participants are not clear. Understanding the molecular changes in the liver are critical to identify appropriate immune modulatory strategies for safety, efficacy, and long-term durability of AAV gene therapy. The aim is to investigate liver gene expression profiles before and after AAV5-HLP-canine-BDD-FVIII (cFVIII) administration in a severe hemophilia A dog model.

Method Nine severe hemophilia A dogs received one of three vectors (non-codon-optimized AAV5-HLP-cFVIII-SQ, codon-optimized AAV5-HLP-cFVIII-SQ and AAV5-HLP-cFVIII-V3 at 6.0e13 – 2.0e14 vg/kg). Liver biopsies were collected at baseline and 3 months after vector administration. FVIII activity (FVIII:C) was measured by one-stage FVIII (OSA) assay using a pooled normal canine plasma standard. Liver cFVIII DNA and RNA levels were quantified using ddPCR. Transcriptomic profiling was performed by RNA-seq followed by pathway enrichment analysis. Cellular immune response in the peripheral blood mononuclear cells (PBMC) was evaluated using an IFN-γ ELISPOT assay.

Results Dose-relatedFVIII expression was observed in dogs treated with the codon-optimized vectors, with significant correlation between liver vector DNA and circulating FVIII:C (▶ **Fig. 1**). Dogs treated with codon-optimized cFVIII vectors demonstrated enhanced transgene expression compared to non-codon-optimized vector. No IFN-γ response was detected in PBMC. Transcriptomic profiling of liver biopsies demonstrated enrichment of integrin pathways, immunological gene signatures for B cells and plasmacytoid dendritic cells (pDCs), and common dendritic cells at 3 months compared to baseline (▶ **Fig. 2**). Expression of inflammatory cytokines involved in NK-cell and T-cell activation were also enriched.



▶ **Fig. 1 AAV5-cFVIII expression and activity in severe hemophilia A dogs at 3 months**; (**a**) AAV5-cFVIII mRNA transcript levels, and (**b**) correlation between liver vector DNA and OSA FVIII:C, one-stage FVIII clotting assay (canine normal pooled standard) with a lower limit of detection of 2 %. co, codon optimized; nco, non-codon optimized; SQ and V3, B-domain-deleted FVIII variants.

Enriched Gene Set	p-value	FDR-adjusted p-value	No. of genes in term	No. of genes that were regulated following AAV5-HLP-cFVIII treatment	No. of up-regulated genes	No. of down-regulated genes	Normalized Gene Set Enrichment Score (NES)
Integrin pathway	0.00058	0.034	22	3	3	0	1.47
B cells and plasmacytoid dendritic cells	3.40E-06	0.014	168	10	9	1	1.80
Common dendritic cells	1.00E-05	0.020	187	10	8	2	1.49
Cytokine production	7.00E-06	0.015	668	19	16	3	1.56

▶ **Fig. 2** Gene set enrichment analysis comparing AAV5-HLP-cFVIII vectors administered to hemophilia A dogs at 3 months and baseline (treatment vs control). A normalized gene set enrichment score (NES) determines whether a gene set is positively regulated or negatively regulated and an NES with Benjamini-Hochberg adjusted P value < 0.05 was considered statistically significant. FDR, False discovery rate.

Conclusion Our data suggests that mild activation of B cells, dendritic cells, NK-cell, and T-cells with an inflammatory cytokine response occurred in the liver of AAV5-HLP-cFVIII treated dogs 3 months post gene transfer; albeit without transaminitis. Transcriptomic profiling of PBMCs is ongoing to compare to liver profiles to better understand kinetics of immune responses to rAAV.

Conflict of Interest **AM Ismail**, **B Yates**, **N Khattak**, and **S Fong** are employees and shareholders of BioMarin Pharmaceutical Inc. **P Batty** has received research support from, and acted in an advisory role for, BioMarin Pharmaceutical Inc. **D Lillicrap** has received research support from CSL Behring and BioMarin Pharmaceutical Inc. **A Mo**, **L Harpell**, **A Menard**, **A Pender**, and **A Winterborn** have no conflicts to declare.

T-07-02 Beyond bleeding: Exploring coagulation factors and cytokines in bone health *in vitro*

Authors A. Bernar[1], J. Gebetsberger[1], M. Bauer[2], M. Schirmer[2], W. Streif[1]
Institutes **1** Medical University of Innsbruck, Department of Pediatrics I, Innsbruck, Austria; **2** Medical University of Innsbruck, Department of Internal Medicine, Clinic II, Innsbruck, Austria
DOI 10.1055/s-0044-1779103

Introduction Hemophilia A (HA) patients are prone to low bone mineral density (BMD), independent of commonly known risk factors [1]. Recent research suggests that coagulation factor VIII (FVIII) could potentially act through direct interactions with key bone-regulating pathways (e.g. RANK/RANKL/OPG axis, Wnt/ß-catenin pathway, cytokines) or indirectly through downstream processes (e.g. thrombin production) [2]. Despite these promising leads, the connection between FVIII and bone health remains elusive. Therefore, cell culture experiments involving primary bone cells are pivotal to understand the impact of coagulation and inflammation on bone health.

Method Human (SaOs-2) and murine (MC3T3-E1) osteoblast cell lines, along with peripheral blood–derived human osteoclasts, were cultured together with human coagulation factors (FVIII, FIX, FX, vWF, vWF-FVIII complex, and thrombin at 1 U/ml each) and cytokines (IL-6 and TNF-α at 50 ng/ml each). We assessed alterations in cell viability using the CCK8 assay, mineralization of osteoblasts using the alizarin red S assay [3], and osteoclast formation using the tartrate-resistant acid phosphatase (TRAP) staining.

Results Coagulation factors had distinct effects on human and murine osteoblasts: In comparison to untreated controls, thrombin reduced cell viability of murine osteoblasts by 18 %, whereas vWF, vWF-FVIII, FIX, FVIII-thrombin, and FX increased viability by 33 %, 24 %, 26 %, 23 % and 31 % respectively. None of the factors changed mineralization of murine osteoblasts. However, mineralization of human osteoblasts decreased in the presence of vWF, vWF-FVIII, thrombin and FX by 44 %, 37 %, 35 % and 56 % respectively. For human osteoclasts, the addition of FX led to increased TRAP activity by 16 %.

In the presence of TNF-α cell viability in both murine and human osteoblasts was reduced by 29 % and 53 %, and mineralization in murine osteoblasts increased by 119 %. In contrast, TRAP activity of osteoclasts was reduced by both TNF- α and IL-6 by 50 % and 60 %.

Conclusion This study shows different responses of human and murine osteoblasts to human coagulation factors. The particular influence of FX on both human osteoblasts and osteoclasts will be further investigated. Also, the impact of inflammatory cytokines on osteogenic cells warrants further studies. Understanding the interplay between coagulation, inflammation and bone metabolism is pivotal for developing future therapeutic strategies to enhance patient health.

Conflict of Interest None.

References

[1] Iorio A, Fabbriciani G, Marcucci M, Brozzetti M, Filipponi P Bone mineral density in haemophilia patients. A meta-analysis. Thrombosis and haemostasis. 2010: 103 (3): 596–603Germany: Thieme;
[2] Gebetsberger J, Schirmer M, Wurzer WJ, Streif W Low Bone Mineral Density in Hemophiliacs. Frontiers in medicine. 2022: 9: 794456Switzerland: Frontiers Media;
[3] Bernar A, Gebetsberger JV, Bauer M, Streif W, Schirmer M Optimization of the Alizarin Red S Assay by Enhancing Mineralization of Osteoblasts. International journal of molecular sciences. 2022 24 (1):Switzerland: MDPI;

T-07-03 Fall risk in patients with haemophilia: A case-control study on clinical motor performance tests

Authors M. Brühl[1, 2], F. Tomschi[2], A. Schmidt[2], P. Ransmann[2], A. C. Strauss[1], T. Hilberg[2]
Institutes **1** University Hospital Bonn, Department of Orthopaedics and Trauma Surgery, Bonn, Germany; **2** University of Wuppertal, Department of Sports Medicine, Wuppertal, Germany
DOI 10.1055/s-0044-1779104

Introduction Due to hemophilic arthropathy, joint health as well as associated strength, mobility, and postural stability are reduced in patients with hemophilia (PwH). These impairments go along with an increased fear and risk of falling. To test these functional abilities effectively, the present study aims (1) to evaluate the feasibility and safety of different clinical motor performance tests in PwH, (2) to evaluate, whether a higher joint impairment results in a lower self-efficacy regarding falling and lower test performance.

Method 29 PwH (55.3 years ± 10.7) and 29 healthy controls (CG; 50.2 years ± 13.9) were examined by different clinical tests: short physical performance battery (SPPB), timed up and go test (TUG), functional reach test (FRT), push and release (PAR), single leg stand (SLS) with open and closed eyes, strength of knee extension (SKE), and hand grip strength (HGS). The subjects were also examined using the Hemophilia Joint Health Score (HJHS) and the Fall Efficacy Scale International questionnaire (FES-I). PwH were divided in two groups based on the HJHS score, resulting in three groups: (1) healthy controls, (2) minorly affected PwH (lower half based on HJHS Score), and (3) majorly affected PwH (upper half based on HJHS Score). Group comparisons were performed using the Kruskal-Wallis test followed by pairwise post-hoc tests.

Results No adverse events occurred during the tests. One PwH did not perform the SLS with closed eyes due to the patient's own uncertainty. Majorly and minorly affected PwH revealed higher FES-I scores than CG. All clinical tests showed significant differences between CG and majorly affected PwH. The comparison between CG and the minorly affected PwH showed a significant

Hämostaseologie 2024; 44: S1–S125

difference only in TUG. Furthermore, significant differences between the minorly and majorly affected PwH were observed in SKE right and SLS with open eyes (▶ **Fig. 1**).

Parameter	Majorly affected PwH (n=14)	Minorly affected PwH (n=15)	CG (n=29)	Kruskal-Wallis-Test p-value
HJHS score (0-124 points)	50.0 [41.5-55.0] (36.0-64.0) *** #	24.0 [19.0-28.0] (14.0-35.0) ***	5.0 [3.0-10.5] (0.0-19.0)	<.001
SPPB total (0-12 points)	9.5 [8.0-11.3] (4.0-12.0) ***	11.0 [10.0-12.0] (9.0-12.0)	12.0 [11.0-12.0] (9.0-12.0)	<.001
Timed up and Go test (seconds)	7.8 [7.0-11.0] (5.8-16.8) ***	7.2 [6.2-8.7] (4.4-9.4) *	5.7 [4.7-6.8] (3.8-8.8)	<.001
Push and release test (0-4 points)	2.0 [1.0-2.0] (0.0-3.0) **	1.0 [1.0-1.0] (0.0-2.0)	1.0 [0.0-1.0] (0.0-3.0)	.011
Functional reach test (cm)	30.0 [26.1-33.8] (19.0-42.3) ***	35.0 [30.0-38.3] (26.0-42.3)	38.0 [34.5-42.8] (29.3-49.7)	<.001
Single-leg stance opened eyes (seconds)	8.5 [2.7-17.7] (2.1-45.0) *** ##	45.0 [31.1-45.0] (3.5-45.0)	45.0 [45.0-45.0] (9.8-45.0]	<.001
Single-leg stance closed eyes (seconds)	2.0 [1.6-3.0] (1.4-17.1) ***	3.7 [2.7-7.7] (1.8-22.6)	8.2 [3.5-14.7] (1.9-43.3)	<.001
Knee extension right (N)	188.5 [120.0-280.0] (48.0-371.7] *** #	357.2 [219.9-500.4] (143.7-611.0)	395.6 [343.3-499.3] (183.7-719.9)	<.001
Knee extension left (N)	179.0 [138.6-325.2] (60.3-416.2) ***	339.5 [300.7-429.8] (172.0-674.0)	429.4 [338.0-493.3] (201.3-667.4)	<.001
Hand strength right (kg)	33.3 [25.9-38.5] (10.7-42.0) ***	38.7 [32.2-49.7] (25.0-54.3)	43.3 [38.3-50.7] (28.0-58.0)	<.001
Hand grip strength left (kg)	34.0 [26.7-39.7] (11.3-44.7) **	36.3 [30.7-47.3] (16.7-51.0)	40.7 [38.0-48.5] (29.7-55.3)	.003
FES-I (16-64)	20.0 [18.0-31.1] (16.4-37.5) **	20.0 [17.0-22.0] (16.0-29.0) *	17.0 [16.0-18.0] (16.0-26.0)	<.001

▶ **Fig. 1 Comparison between majorly affected PwH (upper half based on HJHS Score), minorly affected**; Data presented as median [25 %-quartile; 75 %-quartile] (min–max). PwH = Patients with haemophilia; CG = control group; SPPB = short physical performance battery; FES-I = Falls Efficacy Scale-International; HJHS = haemophilia joint health score; * = p < 0.05 vs. CG; ** = p < 0.01 vs. CG; *** = p < 0.001 vs. CG; # = p < 0.05 vs. Minorly affected, ## = p < 0.01 vs. Minorly affected, ### = p < 0.001 vs. Minorly affected.

Conclusion The performed clinical tests are feasible in PwH as no adverse events occurred. Results reveal that majorly and minorly affected PwH reported a higher subjective concern and a lower self-efficacy of falling compared to the CG. Yet, this observation is accompanied by the fact that only majorly affected PwH exerted impaired performance in all clinical tests compared to the CG, while minorly affected PwH only presented higher TUG values compared to the CG. Largest discrepancies were observed in the single-leg stance with eyes open and knee extensor strength, where majorly affected PwH showed worse performance.

These clinical tests offer safe and feasible time-saving tools to evaluate the functional motor performance in the context of the risk of falling in PwH without much equipment, especially in clinical settings, and can be used as a basis for further individual tailored physical and sport therapies.

Conflict of Interest All authors stated that they had no interests, which might be perceived as posing a conflict or bias.

T-07-04 Prevalence of synovitis and osteochondral damage in relation to age and BMI in patients with haemophilia

Authors A. Schmidt[1], M. Sigl-Kraetzig[2], H. Richter[3], T. Vogler[4], F. Tomschi[1], T. Hilberg[1]

Institutes **1** University of Wuppertal, Department of Sports Medicine, Wuppertal, Germany; **2** Institut für pädiatrische Forschung und Weiterbildung, Blaubeuren, Germany; **3** Hämophiliezentrum, Münster, Germany; **4** Zentrum für Sportmedizin, Münster, Germany

DOI 10.1055/s-0044-1779105

Introduction Haemarthroses lead to changes of the synovium, cartilage, and bone in patients with haemophilia (PwH). Although this is widely recognized, little data exist on the prevalence of the distinct joint pathologies. Hence, the joint status was evaluated by sonography and prevalence rates were clustered based on of the risk factors age and BMI.

Method 80 patients with moderate or severe haemophilia A or B (PwH) (severe A = 55, B = 11; moderate A = 13, B = 1) were included. Thus, a total of 480 joints (ankle, knee, and elbow) were sonographically examined. The Haemophilia Early Arthropathy Detection with Ultrasound (HEAD-US) protocol was used to systematically assess structural alterations. This protocol discriminates between *synovitis* (Syn) and changes of *cartilage* (Cart) and *bone*. The degree of Syn (0 = none/minimal, 1 = moderate, 2 = severe) and the extent of Cart (0 = none, 1 = less than 50 % of cartilage loss, 2 = 50 % or more of cartilage loss) and bone damage (0 = none, 1 = mild irregularities, 2 = deranged subchondral bone) were scored on a 3-point scale. The scoring for cartilage was slightly modified to be consistent with the other parameters. Additionally, parameters were dichotomized (0 = no pathology, 1 = pathology) and prevalence rates were determined based on the score (> 0) of joint pathology. Age groups were clustered as follows: Age1: 13-17 years (n = 60), Age2: 18-29 years (n = 186), Age3: 30-39 years (n = 120), Age4: 40-49 years (n = 48), Age5: 50-70 years (n = 66). For BMI, the conventional classification system was used (underweight: n = 36, normal: n = 264, overweight: n = 132, obese: n = 48).

Results The prevalence of Syn, Cart, and bone alterations across all joints amount to 76 %, 35 % and 35 %, respectively. For all arthropathy-related parameters, the highest prevalence was observed for the ankle joint (Syn: 81 %, Cart: 63 %, bone: 61 %), while the knee joint demonstrated the lowest prevalence rates (Syn: 69 %, Cart: 17 %, bone: 20 %). Breakdown by age groups revealed a significant increase in the prevalence of Syn (from age1: 62 % to Age5: 99 %), Cart (from Age1: 15 % to Age5: 73 %) and bone (from Age1: 12 % to Age5: 75 %) across all joints examined (all p < .001). When comparing the prevalence of Syn in PwH with normal weight (75 %) to overweight (75 %, p = .997) and obese patients (85 %, p = .129), no significant differences could be observed. In terms of joint degeneration, PwH with normal weight (Cart: 31 %, bone: 39 %) demonstrated significantly lower prevalence rates when compared to overweight (bone: 43 %, p = .017) and to obese patients (Cart: 58 %, p < .001; bone: 50 %, p = .008).

Conclusion The prevalence of Syn as well as degeneration of cartilaginous and osseous tissues are alarmingly high even in adolescent patients. Prevalence of synovial inflammation increases steadily across age groups, however, appears to not be affected by higher BMI. On the other hand, both Cart and bone degeneration increase with age and in relation to BMI, particularly in obese PwH.

Conflict of Interest None of the authors have potential conflicts of interest to declare.

Thieme

T-07-05 Frequency of Pathogenic Variants for Hereditary Bleeding Disorders in General Czech Population

Authors K. Stano Kozubik[1, 2], J. Hynst[1], H. Svozilova[1], J. Stika[1], Z. Vrzalova[1, 2], P. Likavcova[1], I. Blahakova[1], S. Pospisilova[1, 2], M. Doubek[2, 1]

Institutes **1** Masaryk University, Central European Institute of Technology, Brno, Czech Republic; **2** University Hospital, Department of Inetrnal Medicine – Hematology and Oncology, Brno, Czech Republic

DOI 10.1055/s-0044-1779106

Introduction Genome and exome sequencing is becoming a standard method for finding the genetic cause of phenotypic problems in genes, where pathogenic variants may be located in multiple sites. The variant interpretation process suffers from a lack of information on benign population variants and an unknown incidence of bleeding disorders with mild symptoms. The Analysis of Czech Genomes for Theranostics (A-C-G-T) project was designed to offer the possibility of accurate classification of gene variants in a local context. The database contains genomic data from 1016 Czech individuals selectedon the basis of their parents' place of birth with an equal representation of people within individual regions.

Method Genomic data from the A-C-G-T (https://database.acgt.cz/) project were screened for variants in the haemophilia genes F8 and F9, and VWF. The pathogenicity of variants was determined based on the HGMD database v.2023.2 search.

Results Most variants were detected in the VWF gene (▶ **Tab. 1**). Only a few variants classified as pathogenic were present in the F8 and F9 genes (▶ **Tab. 2**).

Conclusion The number of VWF gene variants was as expected. In the F8 and F9 genes, some pathogenic variants found appear to be non-pathogenic based on population frequency, sex ratio, and ClinVar reports. However, some of them may cause mild and clinically insignificant bleeding symptoms and therefore are still undiagnosed. The frequency of gene variants in the general local population could help to assess the impact of the variant.

Conflict of Interest No conflict of interest regarding this topic.

▶ **Table 1** Variant incidence for hereditary bleeding disorders. VUS – variant of uncertain significance.

Gene	Variant count ACGT	Pathogenic variants	Pathogenic variants %	VUS variants	VUS variants %	Polymorphism variants	Polymorphism variants %
VWF	3358	78	4.14	43	1.28	16	0.61
F8	1511	5	0.33	11	0.73	2	0.13
F9	217	3	1.38	0	0	1	0.46

▶ **Table 2** Pathogenic variants from HGMD for F8 and F9 genes. Conflicting interpretation of pathogenicity in the ClinVar database means benign/likely benign interpretation.

Gene	hg38 coordinate	HGMD variant	HGVS	GnomAD NFE AF	A-C-G-T AF	Variant occurence in males	ClinVar variant classification
F8	chrX:154905041	ins 1 bp non-coding DNA	NM_000132.4: c.5374-19dupT	6.32 %	3.10 %	34 %	not reported
F8	chrX:154928925	del 1 bp codon 1622	NM_000132.4: c.4865delC	unknown	0.09 %	100 %	not reported
F8	chrX:154929926	ins 1 bp codon 1288	NM_000132.4: c.3863dupC	8.83 %	8.86 %	35.10 %	not reported
F8	chrX:154930010	del 1 bp codon 1260	NM_000132.4: c.3780delC	16.69 %	19.20 %	36.70 %	1098530 Likely_pathogenic
F8	chrX:154966633	Arg355Gln	NM_000132.4: c.1064G>A	0.03 %	0.05 %	0 %	82145 Likely_benign
F9	chrX:139530772	Arg3His	NM_000133.4: c.8G>A	0.01 %	0.05 %	0 %	695909 Conflicting_interpretat ions_of_pathogenicity
F9	chrX:139530783	Ile7Phe	NM_000133.4: c.19A>T	0.17 %	0.25 %	66.70 %	367997 Conflicting_interpretat ions_of_pathogenicity
F9	chrX:139541193	IVS4 ds A-G+4	NM_000133.4: c.391+4A>G	0.00 %	0.09 %	100 %	695795 Conflicting_interpretat ions_of_pathogenicity

T-07-06 Pregnancy Outcome in Women with von Willebrand Disease Following Fertility Treatment: A Retrospective Cohort Analysis

Authors L. Haack[1], B. Dasenbrook[2], B. Krammer-Steiner[3], J. Stubert[1]
Institutes **1** University of Rostock, Department of Obstetrics and Gynecology, Rostock, Germany; **2** University of Rostock, Institute of Computer Science, Faculty of Mathematics and Computer Science, Rostock, Germany; **3** Klinikum Südstadt Rostock, Department of Internal Medicine, Rostock, Germany
DOI 10.1055/s-0044-1779107

Introduction Von Willebrand disease (vWD) is the most prevalent inherited bleeding disorder, with conflicting results regarding the bleeding risk during pregnancy. The aim of the present study was to investigate the bleeding risk during and after pregnancy after fertility treatment in women with vWD.

Method Between January 2010 and December 2019, a cohort of women presenting with an unfulfilled wish for children at a fertility center underwent vWF antigen (vWF:Ag) and vWF ristocetin cofactor activity (vWF:RCo) assessment. The vWD group included 41 pregnancies of 30 patients with vWF:Ag and/or vWF:RCo levels at or below 50 IU/dL at initial presentation, irrespective of bleeding symptoms, as well as three patients with a prior vWD diagnosis. The control group consisted of 143 women with vWF:Ag and vWF:RCo levels above 50 IU/dl, resulting in a total of 171 pregnancies in the control group. Only pregnancies with live births were included. Postpartum hemorrhage (PPH) was defined as an estimated blood loss (EBL) of ≥ 500 mL in vaginal births and ≥ 1000 mL in caesarean sections.

Results In the vWD group, 43.9 % (18/41) of pregnancies experienced antepartum bleeding events, compared to 19.9 % (34/171) in the control group ($p < 0.01$). Median peripartal EBL was 350 mL in the vWD group and 450 mL in the control group ($p < 0.05$). The prevalence of PPH was significantly higher in the control group (22.9 % vs. 7.3 %, $p < 0.05$). Of the vWD group pregnancies, 68.3 % resulted from fertility treatment, including assisted reproductive technology (ART) treatment (11/41) and non-ART treatment (17/41). In the control group, 64.9 % of pregnancies were a result of fertility treatment, including ART treatment (66/171) and non-ART treatment (45/171). No differences were observed in neonatal outcomes as assessed by APGAR-5, APGAR-10, birth weight, birth length and small for gestational age status.

Conclusion Screening for vWD in women undergoing fertility therapy without clinical bleeding symptoms is not recommended. Despite a higher incidence of antepartum hemorrhage, women with low vWF values were less likely to experience PPH. We conclude that increased intra-operative awareness for bleeding complications in women with low vWF values may have contributed to the significantly lower prevalence of PPH and lower EBL.

Conflict of Interest This project was supported by a doctoral scholarship from CSL Behring.

T-07-07 Etranacogene Dezaparvovec Shows Sustained Efficacy and Safety in Adult Patients With Severe or Moderately Severe Haemophilia B 3 Years After Administration in the HOPE-B Trial

Authors S. Pipe[1], P. van der Valk[2], P. Verhamme[3], P. Kampmann[4], F. Leebeek[5], M. Coppens[6], K. Meijer[7], P. Raheja[8], N. Key[9], N. Visweshwar[10], G. Young[11], R. Lemons[12], R. Klamroth[13], W. Miesbach[14], J. Astermark[15], N. O'Connell[16], R. Kazmi[17], N. Galante[18], S. le Quellec[18], P. Monahan[18], C. Hermans[19]
Institutes **1** University of Michigan, Departments of Pediatrics and Pathology, Ann Arbor, USA; **2** University Medical Center Utrecht, Van Creveldkliniek, Utrecht, Netherlands; **3** University of Leuven, Center for Molecular and Vascular Biology, KU Leuven Department of Cardiovascular Sciences, Leuven, Belgium; **4** Rigshospitalet, Department of Haematology, Copenhagen, Denmark; **5** Erasmus University, Erasmus University Medical Center Rotterdam, Rotterdam, Netherlands; **6** University of Amsterdam, Amsterdam University Medical Centers, Amsterdam, Netherlands; **7** University of Groningen, Department of Haematology, University Medical Centre, Groningen, Netherlands; **8** Department of Haematology, The Royal London Hospital, London, UK; **9** University of North Carolina, Chapel Hill, USA; **10** University of South Florida, Tampa, USA; **11** University of Southern California Keck School of Medicine, Children's Hospital Los Angeles, Los Angeles, USA; **12** University of Utah, Salt Lake City, USA; **13** Vivantes Klinikum im Friedrichshain, Berlin, Germany; **14** University Hospital Frankfurt, Frankfurt, Germany; **15** Lund University Skåne University Hospital, Institution of Translational Medicine and Department of Hematology, Oncology and Radiation Physics, Malmö, Sweden; **16** St. James's Hospital, National Coagulation Centre, Dublin, Ireland; **17** University Hospital Southampton, NHS Foundation Trust, Southampton, UK; **18** CSL Behring, King of Prussia, USA; **19** Université Catholique de Louvain (UCLouvain) Cliniques Universitaires Saint-Luc, Division of Haematology, Brussels, Belgium
DOI 10.1055/s-0044-1779108

Introduction Etranacogene dezaparvovec (formerly AMT-061) is the first approved gene therapy for haemophilia B in the EU and US. The HOPE-B pivotal phase 3 clinical trial (NCT03569891) demonstrated superior bleed protection compared to FIX prophylaxis up to 24 months post treatment with ongoing follow-up from Year 2 onward. Here, we report efficacy and safety during Years 1-3.

Method In this pivotal phase 3 open-label, single-arm trial, adult male patients (pts) with severe or moderately severe haemophilia B, with or without preexisting adeno-associated virus serotype 5 (AAV5) neutralising antibodies, received a single dose of etranacogene dezaparvovec (2×10^{13} gc/kg, an AAV5 vector containing factor IX [FIX] Padua R338L transgene under the control of the liver-specific LP-1 promoter) following ≥ 6-month lead-in period of FIX prophylaxis.

Of 54 pts receiving etranacogene dezaparvovec, 52 completed 36 months' follow-up. Mean annualized bleeding rate (ABR) for all bleeds during Months 7-36 was reduced by 64 % versus lead-in (1.52 and 4.17, respectively; $P = 0.0004$). Mean ± SD endogenous FIX activity was sustained at 41.5 IU/dL ± 21.7 (n = 50), 36.7 IU/dL ± 19.0 (n = 50), and 38.6 IU/dL ± 17.8 (n = 48) at Years 1, 2, and 3 posttreatment, respectively. At 3 years posttreatment, 51 pts (94 %) remained free of continuous FIX prophylaxis; mean annualized FIX consumption decreased by 96 % versus lead-in ($P < 0.0001$). One pt's FIX levels eventually declined to 2-5 %; his bleeding phenotype returned, and he resumed prophylaxis per protocol at Month 30 post-treatment.

Results All pts experienced at least 1 adverse event (treatment-emergent AE), with no serious AEs related to treatment (1 case of hepatocellular carcinoma [HCC] and 1 death were reported before Year 2 and unrelated to treatment). A total of 38/54 (70 %) pts experienced 96 treatment-related AEs. The most common AE was increased alanine transaminase (ALT). Nine pts (16.7 %) received reactive corticosteroids for mean ± SD 81.4 ± 28.6 days. No new deaths, HCC, or late treatment-related ALT elevations were reported during Year 3.

Conclusion A single dose of etranacogene dezaparvovec provides long-term FIX Padua expression and superior bleed protection compared to prophylaxis, with a favourable safety profile over 3 years post administration.

Conflict of Interest **Disclosure of interest**: S Pipe has received consultancy fees from Apcintex, ASC Therapeutics, Bayer, BioMarin, CSL Behring, Equilibra Bioscience, GeneVentiv, HEMA Biologics, Freeline, LFB, Novo Nordisk, Pfizer, Regeneron/Intellia, Roche/Genentech, Sanofi, Takeda, Spark Therapeutics and uniQure; research funding from Siemens; and holds a membership on a Scientific advisory committee for GeneVentiv and Equilibra Bioscience; P van der Valk has received consultation fees from Bayer; P Verhamme has received consultant fees from CSL Behring, Roche, CAP-DCF, Bayer HealthCare; LeoPharma; Boehringer Ingelheim; Daiichi Sankyo; Pfizer; Sanofi-Aventis; ThromboGenics; P Kampmann has received consultant fees from BioMarin Pharmaceuticals, CSL

Behring, NovoNordisk AS, and speaker fees from CSL Behring; F Leebeek has received research support from CSL Behring, Takeda, Sobi and uniQure; and is a consultant for uniQure, Sobi, Biomarin and Takeda, of which the fees go to the institution. He was a DSMB member for a study by Roche; M Coppens has received financial support for research from Anthos, Bayer, CSL Behring/uniQure, Novo Nordisk and Roche; and honoraria for lecturing or consultancy from Alexion/AstraZeneca, Bayer, CSL Behring, Daiichi Sankyo, Sobi and Viatris. All funds were received by his institution; K Meijer has received speaker fees from Alexion pharmaceuticals Bayer, CSL Behring, and consultant fees from UniQure, Octapharma USA Inc., Bayer; P Raheja has no conflict of interest to declare; N Key has received consultant fees from BioMarin Pharmaceutical Inc., CSL Behring, Genetech USA Inc., NovoNordisk AS; N Visweshwar has received consultant fees from Biogen Idec; G Young has received consultant fees from BioMarin, Genentech, NovoNordisk, Pfizer Pharmaceuticals LLC Sanofi US Service Inc., Spark Therapeutics, Takeda California, Inc.; R Lemons has received consultant fees from CSL Behring, NovoNordisk; R Klamroth has received consultant fees from Bayer Healthcare, BioMarin Pharmaceuticals, Biotest Pharmaceutical Corporation, CSL Behring, .Hoffman-La Roche, Grifols Biologicals, Inc., NovoNordisk, Octapharma USA, Inc., Sanofi Pasteur Inc., SOBI Inc., Takeda Development Center America Inc.; W Miesbach has received honoraria from Bayer, Biomarin, Biotest, CSL Behring, Chugai, Freeline, LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Takeda/Shire, and uniQure; J Astermark has received consultation fees and speaker fees from BioMarin, Pfizer, Sparks, uniQure, CSL Behring, SOBI, Sanofi, Novo Nordisk, Bayer, Roche, Takeda/Shire and Octapharm; and research grants from SOBI, Bayer, Takeda/Shire and CSL Behring; N O'Connell has received consultant fees from CSL Behring, F.Hoffman-La Roche, Novo Nordisk, Sanofi, and speaker fees from Takeda. All funds were received by a charitable organisation; R Kazmi has received consultant fees from BioMarin Pharmaceuticals, CSL Behring; N Galante is a full-time employee of CSL Behring; S le Quellec is a full-time employee of CSL Behring; P Monahan is a full-time employee of CSL Behring; C Hermans has received consultancy and/or lecture fees from Bayer, Takeda, Roche, CSL Behring, Novo Nordisk, Pfizer, Sobi, LFB, OctaPharma, Uniqure and Biomarin.

T-07-08 A simulation study to provide guidance for individuals transitioning from emicizumab to valoctocogene roxaparvovec

Authors S. Agarwal[1], C. Hermans[2], W. Miesbach[3], S. Pipe[4], R. Sidonio[5], D. Osmond[1], V. Newman[1], J. Henshaw[1], F. Peyvandi[6, 7]

Institutes **1** BioMarin Pharmaceutical Inc., Novato, USA; **2** Division of Adult Haematology, St-Luc University Hospital, Brussels, Belgium; **3** Medical Clinic 2, University Hospital, Frankfurt, Germany; **4** University of Michigan, Departments of Pediatrics and Pathology, Ann Arbor, USA; **5** Hemostasis and Thrombosis at Children's Healthcare of Atlanta at Emory University, Atlanta, USA; **6** Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center and Fondazione Luigi Villa, Milan, Italy; **7** Università degli Studi di Milano, Department of Pathophysiology and Transplantation, Milan, Italy

DOI 10.1055/s-0044-1779109

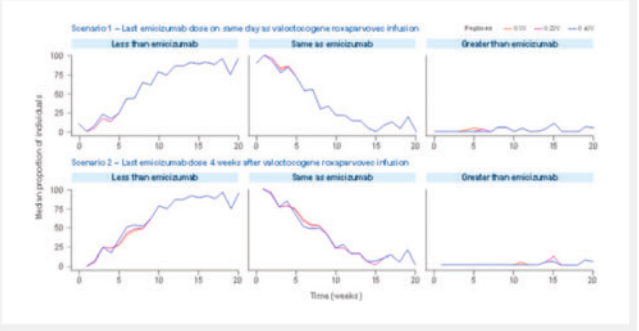
Introduction Valoctocogene roxaparvovec (AAV5-hFVIII-SQ) is a gene therapy evaluated in the phase 3 GENEr8-1 trial that provides endogenous factor VIII (FVIII) production to prevent bleeding in people with severe hemophilia A and represents an alternative to emicizumab, a recombinant antibody mimicking the function of FVIII. Individuals receiving emicizumab were excluded from GENEr8-1 enrollment since emicizumab was then an investigational therapy. The aim is to utilize pharmacokinetic simulations to provide guidance on maintaining hemostatic control while transitioning patients from emicizumab to valoctocogene roxaparvovec gene therapy.

Method A published emicizumab pharmacokinetic model, based on data from the HAVEN clinical trials, was used to simulate in vivo emicizumab concentrations and merged with FVIII activity time-course data for 134 GENEr8-1 participants to estimate bleeding risk at weekly intervals post-infusion with valoctocogene roxaparvovec. The analysis examined 3 approved emicizumab dosing regimens for 2 transition scenarios; first, the last emicizumab dose given the same day as infusion, and second, given 4 weeks after valoctocogene roxaparvovec. Bleeding risk was based on the assumptions in ▶ **Fig. 1**.

FVIII activity levels (IU/dL)	Average emicizumab levels at each week (µg/mL)	Bleeding risk category
<5	<15	Greater than emicizumab
	15 to <100	Same as emicizumab
	≥100	Less than emicizumab
5 to <15	<65	Same as emicizumab
	≥65	Less than emicizumab
≥15	Any	Less than emicizumab

▶ **Fig. 1 Assumptions for the estimation of bleeding risk categories**; If emicizumab concentrations fell below 15 µg/mL and FVIII activity levels had not reached 5 IU/dL, then individuals were assumed to have a greater bleeding risk. FVIII activity levels > 15 IU/dL were assumed to provide hemostatic efficacy.; FVIII, factor VIII.

Results Discontinuation of emicizumab the day of valoctocogene roxaparvovec infusion compared with continued dosing for 4 weeks offered similar levels of hemostatic control (▶ **Fig. 2**). The time course for bleeding risk was comparable across the emicizumab dosing regimens for both scenarios. An algorithm to provide guidance for discontinuing emicizumab was developed based on these results. To guide how treatment decisions for emicizumab discontinuation may vary among individuals, theoretical case examples were developed based on participants of GENEr8-1.



▶ **Fig. 2 Proportion of individuals with bleeding risk over time**; The bleeding risk categories of less than emicizumab, same as emicizumab, and greater than emicizumab are based on the assumptions regarding FVIII activity level and emicizumab blood concentration outlined in ▶ **Fig. 1**; FVIII, factor VIII; QW, once weekly; Q2W, once every 2 weeks; Q4W, once every 4 weeks

Conclusion Regardless of the emicizumab dose or dosing regimen, pharmacokinetic simulations showed no meaningful difference in the risk of bleeding related to FVIII and FVIII equivalent activity determined by the dynamic balance of decaying emicizumab levels and increasing gene therapy–derived endogenous FVIII. These original data suggest individuals on emicizumab prophylaxis can safely transition to valoctocogene roxaparvovec.

Conflict of Interest Suresh Agarwal, Dane Osmond, Vanessa Newman, Josh Henshaw are employees and shareholders of BioMarin Pharmaceutical Inc. Cédric Hermans reports consulting fees and honoraria as a speaker for educa-

tional symposia for Shire, Pfizer, Bayer, Octapharma, LFB, CAF-DCF, Roche, Novo Nordisk, CSL Behring, Sobi, Bioverativ, and Kedrion Wolfgang Miesbach reports grant and research support from Bayer, Biotest, CSL, LFB, Novo Nordisk, Octapharma, Pfizer, Sobi, Takeda, and uniQure; reports consulting fees from BioMarin Pharmaceutical Inc., Freeline, LFB, Octapharma, Sobi, Novartis, Novo Nordisk, Pfizer, and Roche; and reports honoraria as a speaker for educational symposia for Shire, Pfizer, Bayer, Sobi, Amgen, Novartis, Alexion Pharmaceuticals, LEO Pharma, Grifols, Roche, and Novo Nordisk. Steven Pipe reports consulting fees from ApcinteX, ASC Therapeutics, Bayer, BioMarin Pharmaceutical Inc., CSL Behring, Equilibra Bioscience, HEMA Biologics, Freeline, LFB, Novo Nordisk, Pfizer, Roche/Genentech, Sanofi, Spark Therapeutics, Takeda, and uniQure; and service as a clinical trial investigator for BioMarin Pharmaceutical Inc., Freeline, Genentech/Roche, Sanofi, and uniQure. Robert Sidonio, Jr. reports grants and consulting fees from Bayer, Genentech, HEMA Biologics, Novo Nordisk, BioMarin Pharmaceutical Inc., Octapharma, Pfizer, Roche, Sanofi, Sigilon, Sobi, Guardian Therapeutics, and Takeda.Flora Peyvandi reports honoraria as a speaker for educational symposia for Grifols, Sanofi, and Takeda; and reports honoraria as an advisory member for Sanofi and Roche.

T-07-09 Bleeding Outcomes in Participants with Factor VIII Activity < 5 IU/dL post–gene transfer in GENEr8-1

Authors J. Mahlangu[1], M. Ozelo[2], C. W. Tan[3, 4], A. Giermasz[5], J.-D. Wang[6], S. Harris[7], D. Osmond[7], H. Yu[7], T. Robinson[7]

Institutes **1** Hemophilia Comprehensive Care Center, Charlotte Maxeke Johannesburg Academic Hospital, University of the Witwatersrand and NHLS, Johannesburg, South Africa; **2** School of Medical Sciences, University of Campinas, Hemocentro UNICAMP, Department of Internal Medicine, Campinas, Brazil; **3** Royal Adelaide Hospital, Department of Haematology, Adelaide, Australia; **4** University of Adelaide, Adelaide, Australia; **5** Hemophilia Treatment Center, University of California Davis, Sacramento, USA; **6** Center for Rare Disease and Hemophilia, Taichung Veterans General Hospital, Taichung, Taiwan; **7** BioMarin Pharmaceutical Inc., Novato, USA

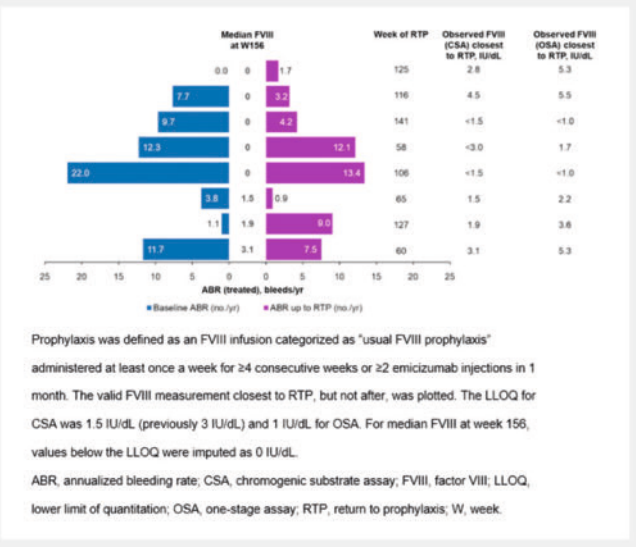
DOI 10.1055/s-0044-1779110

Introduction Valoctocogene roxaparvovec is expected to transfer a functional FVIII coding sequence that would eliminate the need for regular prophylaxis for people with severe hemophilia A (HA). At the same valoctocogene roxaparvovec dose, FVIII levels varied among participants in the phase 3 GENEr8-1 trial. The protective effect of low transgene-derived FVIII is unknown. The aim is to determine clinical outcomes for participants with low FVIII activity 3 years post–gene transfer in GENEr8-1.

Method In the open-label, single-arm, phase 3 GENEr8-1 trial (NCT03370913), 134 adult males (intention-to-treat [ITT] population) with severe HA (FVIII activity < 1 IU/dL) previously receiving regular FVIII prophylaxis and with no history of FVIII inhibitors received a single dose of $6x10^{13}$ vg/kg valoctocogene roxaparvovec. Plasma FVIII activity was measured via chromogenic substrate assay (reported in text; lower limit of quantitation [LLOQ], 1.5 [previously 3.0] IU/dL) or one-stage assay (LLOQ, 1.0 IU/dL). RTP was defined per-protocol as usual FVIII prophylaxis administered ≥ 1 time/week for ≥ 4 consecutive weeks or ≥ 2 emicizumab injections/month. Outcomes are reported for up to 156 weeks.

Results Of 134 ITT participants, 131 completed the week 156 visit. Mean (standard deviation [SD]) FVIII activity at week 156 was 18.2 (30.6) IU/dL in the ITT population. At week 156, 46 of 134 (34.3 %) ITT participants had median FVIII activity < 5 IU/dL (range, 0 to 4.9 IU/dL). These 46 participants had annualized bleeding rate (ABR) for treated spontaneous and traumatic bleeds at baseline between 0 and 27.5 (mean, 4.8) bleeds/year and from post-prophylaxis to week 156 between 0 and 12.0 (mean, 1.7) bleeds/year.

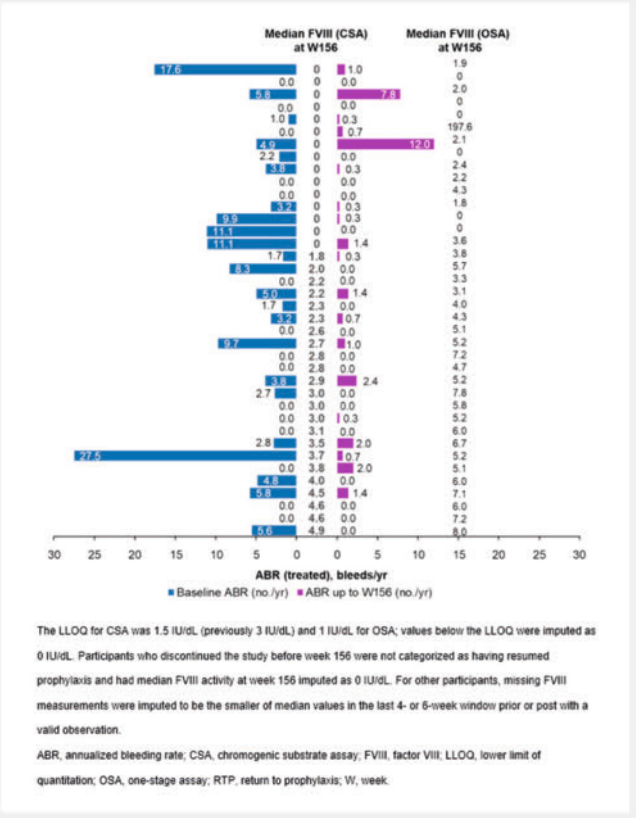
Of these 46 participants, 8 resumed prophylaxis before week 156 (range, 58–141 weeks; ▶ **Fig. 1**) and 38 did not RTP before week 156 (▶ **Fig. 2**).



Prophylaxis was defined as an FVIII infusion categorized as "usual FVIII prophylaxis" administered at least once a week for ≥4 consecutive weeks or ≥2 emicizumab injections in 1 month. The valid FVIII measurement closest to RTP, but not after, was plotted. The LLOQ for CSA was 1.5 IU/dL (previously 3 IU/dL) and 1 IU/dL for OSA. For median FVIII at week 156, values below the LLOQ were imputed as 0 IU/dL.

ABR, annualized bleeding rate; CSA, chromogenic substrate assay; FVIII, factor VIII; LLOQ, lower limit of quantitation; OSA, one-stage assay; RTP, return to prophylaxis; W, week.

▶ **Fig. 1** ABR for treated bleeds at baseline and post-prophylaxis up to RTP for participants with week 156 FVIII activity < 5 IU/dL who resumed prophylaxis before week 156



The LLOQ for CSA was 1.5 IU/dL (previously 3 IU/dL) and 1 IU/dL for OSA; values below the LLOQ were imputed as 0 IU/dL. Participants who discontinued the study before week 156 were not categorized as having resumed prophylaxis and had median FVIII activity at week 156 imputed as 0 IU/dL. For other participants, missing FVIII measurements were imputed to be the smaller of median values in the last 4- or 6-week window prior or post with a valid observation.

ABR, annualized bleeding rate; CSA, chromogenic substrate assay; FVIII, factor VIII; LLOQ, lower limit of quantitation; OSA, one-stage assay; RTP, return to prophylaxis; W, week.

▶ **Fig. 2** ABR for treated bleeds at baseline and during post-prophylaxis up to week 156 for participants with week 156 FVIII activity < 5 IU/dL who did not RTP.

For 2 of 8 participants who resumed prophylaxis before week 156, treated ABR was higher during the post-prophylaxis period up to RTP than at baseline; treated ABR was higher post-prophylaxis to week 156 compared with baseline for 5 of 38 participants who did not RTP before week 156. Most of the 38 participants

who did not RTP before week 156 had lower ABR for treated bleeds compared with baseline, low post-prophylaxis ABRs for treated bleeds, or no substantial treated spontaneous bleeds.

Conclusion Most participants with low FVIII activity had low bleeding rates, suggesting that low endogenous FVIII expression may provide protective hemostatic benefits. Many participants who resumed prophylaxis had clinical presentation consistent with moderate hemophilia; the individual decision to RTP was multifactorial and influenced by FVIII activity, bleeding rates, desired physical activity levels, and personal preferences.

Conflict of Interest **JM** has received research funding from BioMarin Pharmaceutical Inc., Catalyst, Novo Nordisk, Pfizer, Roche, Sandoz, Sanofi, and Spark Therapeutics. **MO** has participated in advisory boards for Bayer, BioMarin Pharmaceutical Inc., Grifols, Pfizer, Sanofi, and Takeda and received honoraria from BioMarin Pharmaceutical Inc., Biotest, Novo Nordisk, Pfizer, and Roche. **AG** reports honoraria for service on advisory boards from American Thrombosis and Hemostasis Network, Bayer, BioMarin Pharmaceutical Inc., Genentech, Novo Nordisk, Pfizer, Sanofi Genzyme, and uniQure and travel grants from BioMarin Pharmaceutical Inc. **JDW** reports consulting fees from Bayer, Chugai, Novo Nordisk, Pfizer, Sanofi, Sobi, and Takeda; speaker fees from Bayer, Behring, Chugai, CSL, Novo Nordisk, Pfizer, Sanofi, and Takeda; and service as a clinical trial investigator for Bayer, BioMarin Pharmaceutical Inc., Novo Nordisk, Pfizer, Roche/Chugai, and Sanofi. **DO, HY**, and **TMR** are employees and shareholders of BioMarin Pharmaceutical Inc. **CWT** has no conflicts to declare.

T-07-10 New inSight Joint Health tool: Focusing on long-term joint health

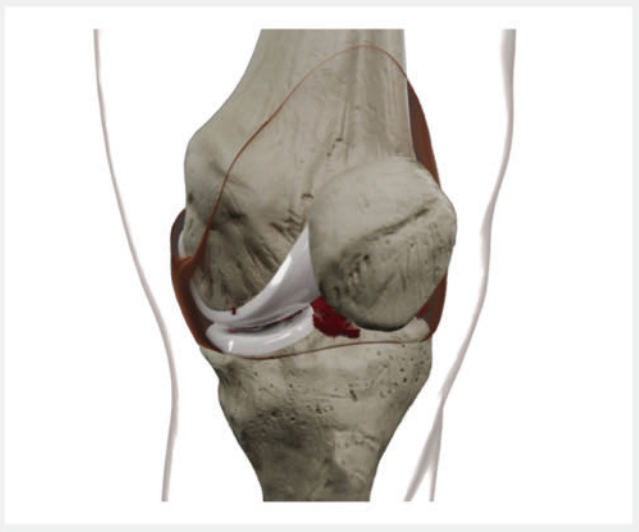
Authors G. Goldmann, N. Marquardt, J. Oldenburg
Institute Universitätsklinikum Bonn, Institut f. Exper. Hämatologie und Transfusionsmedizin, Bonn, Germany
DOI 10.1055/s-0044-1779111

Introduction Recurrent joint bleeding is the most frequent clinical manifestation of severe **haemophilia**. Unless appropriately managed, even subclinical hemarthrosis can lead to the development of **hemophilic arthropathy** and chronic pain [1]. Even today, arthropathy is a major comorbidity for people with haemophilia. Despite prophylaxis with factor and non-factor replacement therapies, many of them still develop joint damage that causes pain, limited mobility and poor quality of life.Non-adherence often occurs. Problems with acceptance or self-management on the patient side appear to be the main reasons for this challenge [2]. The WFH recommends the use of personalised prophylaxis to optimise protection for each individual patient and to provide adequate factor levels that allow the patient's desired level of physical activity [3].

Method To raise awareness for joint health and support communication between physicians and their patients, an innovative tool has been developed to help HCPs and patients explore how to optimise long-term joint health. Using innovative technology, the **inSight Joint Health** tool lets users explore immersive 3D models, detailed animated videos, medical scan images and informative messaging about joint health in haemophilia. The tool explains the pathophysiology behind subclinical and clinical bleeds and provides guidance on how long-term joint health can be preserved. It enables to communicate how both, clinical and subclinical bleeds, can contribute to joint damage and highlight that an optimized and individualized prophylaxis therapy supports joint health.

Results Even a single joint bleed can trigger a vicious cycle of recurrent bleedings, which can lead to synovitis and arthropathy. Clinical bleeds can cause synovial hypertrophy which changes the structure of the synovial tissue, making it more likely to experience future bleeds. To address chronic synovitis, the German S2k guidelines on synovitis recommend maintaining factor trough levels of ≥ 30 % for 6 months [4–5].

Rapid resolution of bleeds with **factor replacement** is key to minimizing long term damage in the event of bleeds occurring. A non-promotional version of the tool can be used to highlight the unmet need in joint health management and how steps can be taken to preserve joint health. Expert advice and information to patients with haemophilia should also be based on individual functional prevention diagnoses, advice on available therapies and sports practice, as well as general health recommendations [6] (▶ **Fig. 1**).



▶ **Fig. 1** inSight Joint Health tool visualizing a subclinical bleed

Conclusion The approach to haemophilic patients should be interdisciplinary. Assessment of the processes, that affect pain in these patients and the development of pain education models should be implemented [6]. The new inSight Joint Health tool provides a beneficial tool for the visualization of joint health in haemophilic patients, contributing to disease awareness and **adherence**.

Conflict of Interest GG: Payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from SOBI, Takeda, Bayer, Octapharma, Novo Nordisk, Biotest and Roche; support for attending meetings and/or travel from Novo Nordisk, Biotest and Swedish Orphan Biovitrum SOBI. NM: Speaker/consultant and grant/research support from Bayer, Chugai, CSL Behring, Novo Nordisk, Octapharma, Pfizer, Roche, Shire/Takeda, SOBI. JO: Research Support/P.I. from Bayer, Biotest, Chugai, CSL-Behring, Novo Nordisk, Octapharma, Pfizer, Roche, SOBI, Takeda; Consultant for Bayer, Biogen Idec, Biomarin, Biotest, Chugai, CSL-Behring, Freeline, Grifols, LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Sparks, SOBI, Takeda; Speakers Bureau for Bayer, Biogen Idec, Biomarin, Biotest, Chugai, CSL-Behring, Freeline, Grifols, LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Sparks, SOBI, Takeda; Scientific Advisory BoardBayer, Biogen Idec, Biomarin, Biotest, Chugai, CSL-Behring, Freeline, Grifols, LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Sparks, Takeda, SOBI.

References

[1] Gualtierotti R, Solimeno LP, Peyvandi F Hemophilic arthropathy: Current knowledge and future perspectives. J Thromb Haemost 2021; 19 (9): 2112–21
[2] Hoefnagles JW, Fischer K et al. et al. A feasibility study on two tailored interventions to improve adherence in adults with haemophilia. Pilot Feasibility Stud 2020; 6 (1): 189
[3] Srivastava A, Santagostini E, Dougall A et al. WFH Guidelines for the Management of Hemophilia, 3rd edition. Haemophilia: 2020: 26 (6): 1–158
[4] Iorio A, Iserman E, Blanchette V et al. Target plasma factor levels for personalized treatment in haemophilia: a Delphi consensus statement. Haemophilia 2017; 23 (3): e170–e179
[5] AWMF-Register-Nr 086/005 S2k-Leitlinie Synovitis bei Haemophile 2022
[6] Cuesta-Barriuso R, Donoso Úbeda E, Merono-Gallut J et al. Hemophilic Arthropathy: Barriers to Early Diagnosis and Management. J Blood Med 2022; 13: 589–601

T-07-11 Molecular, structural, and functional insights into the interaction of coagulation factor VIII with hemorrhage-derived heme

Authors M.-T. Hopp[1], D. Ugurlar[2], B. Pezeshkpoor[3], J. Oldenburg[3], D. Imhof[4]

Institutes **1** University of Koblenz, Department of Chemistry, Institute for Integrated Natural Sciences, Koblenz, Germany; **2** Thermo Fisher Scientific, Center for Electron Microscopy, Eindhoven, Netherlands; **3** University Hospital Bonn, Institute of Experimental Haematology and Transfusion Medicine, Bonn, Germany; **4** University of Bonn, Pharmaceutical Biochemistry and Bioanalytics, Pharmaceutical Institute, Bonn, Germany

DOI 10.1055/s-0044-1779112

Introduction Hemarthrosis episodes in hemophilia A patients are associated with hemorrhagic events that lead to the breakdown of erythrocytes and, thus, the release and massive accumulation of labile heme. To avoid consequences, such as hemophilic arthropathy, exogenous substitution therapy with factor VIII (FVIII) is an option for affected patients. In this regard, it has been reported that direct FVIII injection into the joint bleeding, consequently into the heme-rich environment, shows significantly fewer side reactions. As such, FVIII and heme get in direct contact, however, basic information about this interaction is missing.

Method UV/Vis spectroscopic studies were used to characterize the heme-binding capacity of FVIII. To identify the respective heme-binding sites, FVIII was screened for potential heme-binding motifs by using the webserver HeMoQuest. Subsequently, these motifs were synthesized as FVIII-derived peptides and analyzed for heme binding using UV/vis spectroscopy. Promising sites were further evaluated by molecular docking simulations of the respective heme-protein complexes. Cryo-EM studies and clinical assay systems revealed functional aspects of heme binding to FVIII.

Results It is demonstrated that FVIII has with a binding capacity of seven heme molecules the so far highest known heme-binding capacity among all heme-binding proteins. The heme-binding regions are found in interaction sites. Furthermore, Cryo-EM studies as well as in silico studies suggest that heme binding induces higher flexibility in defined regions of FVIII. Finally, heme is thus able to diminish the procoagulant cofactor activity of FVIII.

Conclusion These results characterize FVIII as an extraordinary heme-binding protein and, thus, highlight the importance of the consideration of the FVIII-heme complex formation in the context of FVIII substitution therapy in hemophilia A patients with joint bleeding.

This research is funded by the Society for Thrombosis and Haemostasis Research e.V. (GTH) and the German Research Foundation (DFG).

Conflict of Interest The authors declare no conflict of interest.

T-07-12 Detection and domain characterization of anti-FVIII antibodies: Comparative evaluation of NBA and immunoassays

Authors B. Pezeshkpoor, A.-C. Berkemeier, K. Herbst, T. Albert, J. Müller, J. Oldenburg

Institute University Hospital Bonn, Institute of Experimental Hematology and Transfusion Medicine, Bonn, Germany

DOI 10.1055/s-0044-1779113

Introduction In severe cases of Hemophilia A (HA), the presence of antibodies against factor VIII (FVIII) poses significant treatment challenges. While the Nijmegen Bethesda assay (NBA) is the established standard for detecting inhibitors, conventional immunoassays like ELISA and Luminex™-based assays can inadvertently detect non-neutralizing antibodies (NNAs) along with inhibitors.

Method A cohort of 573 citrate plasma samples from 205 HA patients (cohort 1) were analyzed. For domain specificity and genetic correlation, 448 samples from cohort 1 and an additional 525 samples formed cohort 2 (973 samples, 233 patients). Samples were concurrently assessed using NBA, a commercial ELISA method, and our in-house Luminex-based domain epitope assay: LumiTope. The LumiTope method involved using full-length and B-domain deleted FVIII, as well as nine purified FVIII single or multi-domains. These proteins were attached to magnetic beads to identify domain-specific Ig (G, $G_{1\text{-}4}$) anti-FVIII antibodies in a cohort of HA patients, both with and without inhibitors. Genetic analysis was performed to identify the underlying genetic defect.

Results he LumiTope assay demonstrated high sensitivity (94.9 %) and specificity (91.2 %), particularly in patients with low-titer inhibitors, as compared to ELISA (72.2 % vs. 27.7 %). Pearson's correlation coefficient confirmed linearity. IgG_4 (76.9 %) and IgG_1 (57.7 %) were predominant IgG subclasses. All patients with a BU/ml > 0.6 had IgG_4 anti-FVIII antibodies. Patients positive and negative for NBA exhibited distinct domain profiles. Moreover, patients with heavy-chain variant showed antibodies specific to this chain. Light-chain variant patients had heterogeneous domain specificity. Strong correlations to NBA was observed for patients with anti-FVIII antibodies towards the A2, C1, and C2 domains. Conversely, no notable correlation was found for the A1, B, and a3A3 domains.

Conclusion In summary, the LumiTope assay is a sensitive and rapid method for characterization of anti-FVIII antibodies in HA patients. It provides higher sensitivity and specificity than a commercial ELISA, especially in detecting anti-FVIII antibodies in patients with low inhibitor titers (< 1 BU/ml). In addition, the LumiTope assay offers the possibility of simultaneous, domain-specific detection of anti-FVIII antibodies, allowing for more streamlined and comprehensive testing and better interpretation of antibody profiles in both, NBA-positive and NBA-negative patients. Better characterization of anti-FVIII antibodies may also allow identification of biomarkers that can guide individualized antibody eradication protocols.

Conflict of Interest None

T-07-13 Lonoctocog alfa vs. octocog alfa: incremental recovery and extended coagulation analysis

Authors J. Müller, T. Albert, C. Klein, S. Horneff, H. Rühl, B. Pötzsch, G. Goldmann, N. Marquardt, J. Oldenburg

Institute University Hospital Bonn, Institute of Exp. Haematology and Transfusion Medicine, Bonn, Germany

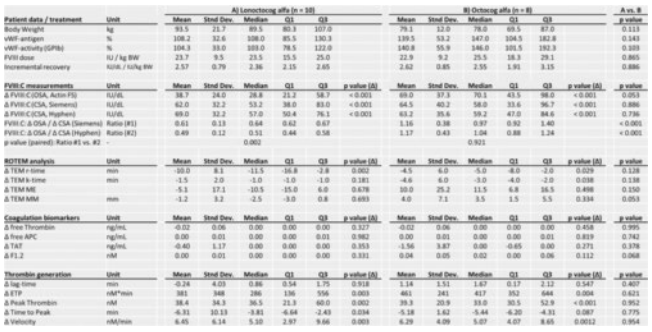
DOI 10.1055/s-0044-1779114

Introduction Lonoctocog alfa (Afstyla) is a recombinant single-chain FVIII molecule with improved binding to vWF, resulting in a higher stability and slightly prolonged half-life [1, 2]. The aim of the present study was to assess the performance of lonoctocog alfa regarding incremental recovery and impact on coagulation assays in comparison to conventional recombinant full-length FVIII (Octocog alfa: Advate, Kovaltry).

Method HA patients treated with lonoctocog alfa (group A, n = 10) or octocog alfa (group B, N = 8 [Advate: 5, Kovaltry: 3]) scheduled for routine assessment of incremental recovery were included in the study. Blood samples were taken at trough levels (before) and 30 min after factor substitution. Besides basic coagulation analysis, an extended test panel was applied. FVIII activity (FVIII:C) was measured by an one-stage clotting assay (OSA, Actin FS, Siemens) and two chromogenic FVIII assays (CSA, Siemens, Hyphen). Whole blood thromboelastometry was assessed after re-calcification of samples using a ROTEM delta system. Thrombin generation (TG) was measured by CAT using the low tissue factor reagent. Thrombin-antithrombin-complexes (TAT) and Prothrombin fragments 1 + 2 (F1.2) were determined by respective ELISA (Siemens). Circulating thrombin and activated protein C (APC) were measured using oligonucleotide-based enzyme capture assays (OECA) as described elsewhere [3, 4]. The Kolmogorov-Smirnov- and the Levene's Test were used for analysis of data normality and homogeneity of variance, respectively. For assessment of statistical significances ($p < 0.05$), the Student's t-Test, the Mann-Whitney U Test, or the Wilcoxon Signed-Ranks Test were applied accordingly.

Results The patient characteristics and results are summarized in ▶ **Fig. 1**. FVIII dosing and observed incremental recoveries (based on Siemens CSA) as well as plasma vWF-antigen and -activity were comparable between groups. Besides FVIII:C levels, FVIII substitution led to significant and comparable changes (Δ) of the ROTEM r-time and TG parameters ETP, peak thrombin, and velocity in both groups. Interestingly, there was a trend to differences of the ROTEM

r- and k-times between groups. Levels of circulating coagulation biomarkers were generally low to non-detectable and did not significantly change after substitution in both groups. As expected, FVIII:C levels measured by OSA were underestimated for lonoctocog alfa [5].



▶ **Fig. 1** Patient characteristics and results

Conclusion Lonoctocog alfa appears to be comparable to octocog alfa with respect to achievable incremental recovery and corresponding effects on coagulation assays. The observed trend to differences in ROTEM analysis warrants further investigations. The found difference between OSA/CSA ratios highlights the known imponderables associated with use of FVIII OSAs for post-infusion measurements of lonoctocog alfa.

Conflict of Interest JM has received honoraria from Octapharma and Siemens Healthineers. JO has received research funding from Bayer, Biotest, CSL Behring, Octapharma, Pfizer, Swedish Orphan Biovitrum, and Takeda; consultancy, speakers bureau, honoraria, scientific advisory board, and travel expenses from Bayer, Biogen Idec, BioMarin, Biotest, Chugai Pharmaceutical Co., Ltd., CSL Behring, Freeline, Grifols, LFB, Novo Nordisk, Octapharma, Pfizer, F. Hoffmann-La Roche Ltd., Sanofi, Spark Therapeutics, Swedish Orphan Biovitrum, and Takeda.

References

[1] Schmidbauer S, Witzel R, Robbel L. et al. Physicochemical characterisation of rVIII-SingleChain, a novel recombinant single-chain factor VIII. Thromb Res 2015; 136: 388–395

[2] Klamroth R, Simpson M, von Depka-Prondzinski M. et al. Comparative pharmacokinetics of rVIII-SingleChain and octocog alfa (Advate®) in patients with severe haemophilia A. Haemophilia 2016; 22: 730–73

[3] Müller J, Becher T, Braunstein J, Berdel P, Gravius S, Rohrbach F, Oldenburg J, Mayer G, Pötzsch B. Profiling of active thrombin in human blood by supramolecular complexes. Angew Chem Int Ed Engl 2011; 50: 6075–8

[4] Müller J, Friedrich M, Becher T, Braunstein J, Kupper T, Berdel P, Gravius S, Rohrbach F, Oldenburg J, Mayer G, Pötzsch B. Monitoring of plasma levels of activated protein C using a clinically applicable oligonucleotide-based enzyme capture assay. J Thromb Haemost 2012; 10: 390–8

[5] St Ledger K, Feussner A, Kalina U. et al. International comparative field study evaluating the assay performance of AFSTYLA in plasma samples at clinical hemostasis laboratories. J Thromb Haemost 2018; 16: 555–564

T-07-14 An update on real-world use of rVIII-SingleChain in patients with haemophilia A in Germany: Interim results from a prospective, non-interventional study

Authors M. Olivieri[1], S. Juranek[1], F. Langer[2], C. Escuriola-Ettingshausen[3], R. Klamroth[4], U. J. Sachs[5], C. Niekrens[6], M. von Depka Prondzinski[7], U. Scholz[8], M. Kalus[9], T. Linhoff[10], J. Oldenburg[11]

Institutes **1** Dr. von Hauner Children´s Hospital LMU Munich, Paediatric Thrombosis and Haemostasis Unit, Munich, Germany; **2** Universitäres Cancer Center Hamburg (UCCH), Universitätsklinikum Eppendorf, II. Medizinische Klinik und Poliklinik, Hubertus Wald Tumorzentrum, Hamburg, Germany; **3** Hämophilie Zentrum Rhein Main (HZRM), Mörfelden-Walldorf, Germany; **4** Vivantes Klinikum im Friedrichshain, Department for Internal Medicine, Haemophilia Treatment Centre, Berlin, Germany; **5** Giessen University Hospital, Department of Thrombosis and Haemostasis, European Haemophilia Comprehensive Care Center (EHCCC), Giessen, Germany; **6** Stadtkrankenhaus Delmenhorst GmbH, Klinik für Kinder- und Jugendmedizin, Delmenhorst, Germany; **7** Werlhof-Institut für Hämostaseologie GmbH, Hannover, Germany; **8** MVZ Labor Dr. Reising-Ackermann und Kollegen, Leipzig, Germany; **9** Paediatric Practice, Berlin-Schöneberg, Germany; **10** CSL Behring, Hattersheim, Germany; **11** University Clinic Bonn, Institute of Experimental Haematology and Transfusion Medicine, Bonn, Germany

DOI 10.1055/s-0044-1779115

Introduction rVIII-SingleChain is a B-domain truncated recombinant factor VIII (FVIII), indicated for the treatment and prevention of bleeding in patients with haemophilia A. Previous studies have shown that rVIII-SingleChain is efficacious and well tolerated in patients with haemophilia A; however, long-term studies are needed to confirm real-world effectiveness and tolerability in routine clinical practice.

Method Data are reported from a sub-analysis of patients from Germany included in a non-interventional study of patients with haemophilia A from six European countries receiving rVIII-SingleChain prophylaxis or on-demand treatment. All patients with haemophilia A were eligible for enrolment. The study was initiated in August 2019, enrolment was completed in December 2021, and patients were followed-up for up to 3 years. Annualised bleeding rates (ABR), annualised spontaneous bleeding rates (AsBR), infusion frequency, dosage and haemostatic effectiveness were recorded. Patients with < 12 weeks on a specific regimen were excluded from efficacy analyses. Patients could switch treatment regimen so may appear in multiple categories.

Results At the second interim data cut-off on 1 February 2023, 43 previously treated patients with a mean (SD) age of 32.7 (16.9) years were enrolled in the study over a mean (SD) observation period of 25.5 (5.9) months. Patient characteristics are provided in ▶ **Fig. 1**. The median (IQR) ABR and AsBR for patients on any prophylaxis regimen (n = 38) were 0.0 (0.0–2.1) and 0.0 (0.0–1.1), respectively. For patients on 2x weekly prophylaxis (n = 8) median (IQR) ABR was 0.0 (0.0–0.2) and AsBR was 0.0 (0.0–0.2). The majority of the reported bleeding episodes were mild in the prophylaxis and on-demand groups (50.0 % and 44.9 %, respectively). Overall haemostatic effectiveness was considered excellent (45.5 %) or good (34.5 %) in patients with available data from at least one assessment. In total, 22 adverse events were reported in 12 patients; of these, 6 events were serious, but none were considered related to rVIII-SingleChain. No patients developed inhibitors to FVIII during treatment with rVIII-SingleChain.

	Children <12 years (N=4)	Adolescents 12 to <18 years (N=7)	Adults ≥18 years (N=32)	Total (N=43)
Sex, N				
Male	4	7	32	43
Mean Age, Years (SD)	9.3 (1.0)	14.1 (1.7)	39.7 (13.7)	32.7 (16.9)
Disease Severity: FVIII levels, N				
Mild: >5%	0	0	5	5
Moderate: 1–5%	0	2	6	8
Severe: <1%	3	5	20	28
Not Reported	1	0	1	2

N, number; SD, standard deviation

▶ **Fig. 1** Patient characteristics at baseline

Hämostaseologie 2024; 44: S1–S125

Conclusion This second interim analysis verifies the efficacy and safety of rVIII-SingleChain for use in routine prophylaxis and on-demand therapy in patients with haemophilia A. Data collection is ongoing to evaluate long-term effectiveness and tolerability.

Conflict of Interest **MO**: has received grants/research support from Bayer, Biomarin, Biotest, Takeda, CSL Behring Octapharma, Pfizer, Shire, Roche, Stago and Swedish Orphan Biovitrium, consultancy and speaker fees from Bayer, BioMarin, Biotest, Novo Nordisk, Takeda, CSL Behring, Pfizer, Roche and Swedish Orphan Biovitrium. **SJ**: has received grants/research support from Bayer, Biotest, Takeda, CSL Behring Octapharma, Pfizer, Shire, Roche and Swedish Orphan Biovitrium, consultancy and speaker fees from Bayer, Biotest, Novo Nordisk, Takeda, CSL Behring, Pfizer, Roche and Swedish Orphan Biovitrium. **FL**: has received personal fees for lectures or consultancy from Bayer, BioMarin, Chugai, CSL Behring, Grifols, Pfizer, Roche, SOBI and Takeda, and research support from Bayer, CSL Behring, Intersero, Novo Nordisk and SOBI. **CEE**: has acted as a consultant, received speaker's fees and/or research funding from Bayer Healthcare, Biomarin, Biotest, CSL Behring, Grifols, LFB, Octapharma, Novo Nordisk, Shire/Takeda, SOBI, Roche/Chugai and Kedrion. **RK**: has received grant/research support, consultancy fees and/or served on speakers' bureaus for Bayer, BioMarin, CSL Behring, Novo Nordisk, Octapharma, Pfizer, Shire, Biotest, Grifols, Roche and Sobi. **UJS**: has no competing interests to declare. CN: has no competing interests to declare. **MvDP**: has received grants/research support from Baxter, Bayer, Biotest, CSL Behring, Octapharma and Pfizer, consultancy fees from Baxter, Bayer, Biotest, CSL Behring, Novo Nordisk, Octapharma, Pfizer, Roche and Swedish Orphan Biovitrum, speaker bureau from Baxter, Bayer, Biotest, CSL Behring, Grifols, Novo Nordisk, Octapharma, Pfizer and Swedish Orphan Biovitrum. **US**: has no competing interests to declare. **MK**: has no competing interests to declare.**TL**: is an employee of CSL Behring. **JO**: has received grants/research support from Baxter, Bayer, Biotest, CSL Behring, Grifols, Novo Nordisk, Octapharma and Pfizer, consultancy fees from Baxter, Bayer, Biogen Idec, Biotest, Chugai, CSL Behring, Grifols, Novo Nordisk, Octapharma, Pfizer, Roche and Swedish Orphan Biovitrium and speaker fees from Baxter, Bayer, Biogen Idec, Biotest, CSL Behring, Grifols, Novo Nordisk, Octapharma, Pfizer and Swedish Orphan Biovitrium.

T-07-15 *Staphylococcus aureus* infection restimulates inhibitor production in tolerized hemophilia A mice

Authors A. Abdelmageed[1], B. Jurado-Mestre[1], V. Kotov[1], S. L. Schötz[1], H. Gerritzen[3], T. Albert[2], J. Oldenburg[2], A. Heine[3], C. Kurts[1], J. Becker-Gotot[1]

Institutes **1** University Clinic Bonn, Institute of Molecular Medicine and Experimental Immunology, Bonn, Germany; **2** University Clinic Bonn, Institute of Exp. Haematology and Transfusion Medicine, Bonn, Germany; **3** University Clinic Bonn, Institute of Haematology and Oncology, Bonn, Germany

DOI 10.1055/s-0044-1779116

Introduction Hemophilia A (HA) is the most common inherited bleeding disorder characterized by a deficiency in coagulation factor VIII (FVIII). Upon replacement therapy, over 30 % of patients develop inhibitory antibodies (inhibitors), rendering the treatment ineffective [1, 2]. An immune tolerance induction (ITI) therapy has been developed, wherein patients receive frequent, high-doses of FVIII, to build up tolerance and prevent inhibitor formation. Recently, antigen-specific induced regulatory T cells (Tregs) expressing PD-L1 were identified as the cell population responsible for mediating suppression [3].

Several case studies have associated ITI failure or prolonged tolerization periods with immunologic challenge i.e., *Staphylococcus aureus (S. aureus)* infection. Identifying risk factors and mechanisms of tolerance breakdown could help discover potential novel biomarkers to predict ITI success [4, 5].

Method To understand the impact of infectionon the FVIII-specific immune response the following setting was applied: HemA mice intravenously received 2IU recombinant human (rh)FVIII twice a week for 3 weeks to develop tolerance. On day 21, one group was additionally infected with *S. aureus (USA 300 lac)* through an intravenous injection. At alternate time points, splenocytes were aseptically harvested for further analysis by flow cytometry, ELISPOT and RT-qPCR. Blood was also collected before and after infection to analyze inhibitor abundance and FVIII activity.

Results Importantly, upon infection a significant increase in inhibitor titres was observed in comparison to the non-infected controls. Enhanced antibody production was accompanied by an increase in FVIII-specific B cells with an abrogated PD-1 expression and increased survival. Furthermore antigen-specific Treg function seemed to be dampened by the infection, but interestingly Treg numbers remained stable. On the contrary the T helper cell number and function was amplified. Overall, this led to a significant reduction in active FVIII in the serum and indicated that infection disrupted induced tolerance.

Conclusion These initial results suggest that *S. aureus* infection could destroy induced tolerance by promoting a pro-inflammatory environment and preventing suppression of the FVIII-specific immune response. By identifying specific infection-mediated risk factors, involved in ITI breakdown, like cytokines, the treatment can be adapted and resulting complications might be minimised.

Conflict of Interest None.

References

[1] Schep SJ, Schutgens REG, Fischer K, Boes ML. 'Review of immune tolerance induction in hemophilia A'. Blood Rev. 2018; ; 32 (4): : 326–338. doi:10.1016/j.blre.2018.02.003. Epub 2018 Feb 15PMID: 29482894

[2] Qian J, Borovok M, Bi L, Kazazian HH Jr, Hoyer LW 'Inhibitor antibody development and T cell response to human factor VIII in murine hemophilia A'. Thromb Haemost 1999; 81 (2): 240–4

[3] Becker-Gotot J, Meissner M, Kotov V, Jurado-Mestre B, Maione A, Pannek A, Albert T, Flores C, Schildberg FA, Gleeson PA, Reipert BM, Oldenburg J, Kurts C. 'Immune tolerance against infused FVIII in hemophilia A is mediated by PD-L1 + regulatory T cells'. J Clin Invest 2022 15. doi:10.1172/JCI159925

[4] Crary SE, Buchanan GR, Journeycake JM 'Fatal central venous catheter-related infection in haemophilia'. Haemophilia 2006; 12 (2): 183–186. doi:10.1111/J.1365-2516.2006.01176.X

[5] DiMichele DM, Hoots WK, Pipe SW, Rivard GE, Santagostino E 'International workshop on immune tolerance induction: consensus recommendations'. Haemophilia 2007; 13: (Suppl 1): 1–22

T-07-16 Change in Hemophilia Joint Health Score (HJHS) During the Phase 3 XTEND-1 Study of Efanesoctocog Alfa in Patients With Severe Hemophilia A

Authors A. von Drygalski[1], C. Königs[2], C. Nagae[3], J. Dumont[4], L. Bystricka[5], A. Willemze[6], E. Santagostino[7], J. Oldenburg[8]

Institutes **1** University of California, Division of Hematology/Oncology, Department of Medicine, San Diego, USA; **2** University Hospital Frankfurt, Goethe University, Frankfurt, Germany; **3** St Marianna University School of Medicine, Kawasaki, Japan; **4** Sanofi, Cambridge, USA; **5** Sobi, Stockholm, Sweden; **6** Sanofi, Amsterdam, Netherlands; **7** Sobi, Basel, Switzerland; **8** Universitätsklinikum Bonn, Institute of Experimental Hematology and Transfusion Medicine, Bonn, Germany

DOI 10.1055/s-0044-1779117

Introduction Development of hemophilic arthropathy and chronic joint pain occurs even with the current standard of care (SoC) for hemophilia A. The completed Phase 3 study XTEND-1 (NCT04161495) showed once-weekly efanesoctocog alfa was well tolerated in adults and adolescents with severe hemophilia A and provided superior bleed prevention to prior FVIII prophylaxis. Here, we report changes in joint health from baseline to Week 52 using the Hemophilia Joint Health Score (HJHS) v2.1 in patients from the XTEND-1 study.

Method Patients on prior FVIII prophylaxis were enrolled into Arm A (52 weeks of once-weekly efanesoctocog alfa prophylaxis [50 IU/kg]). Patients receiving prior on-demand therapy entered Arm B (26 weeks of on-demand efanesoctocog alfa [50 IU/kg], then 26 weeks of once-weekly prophylaxis). Six joints (left and right ankle, elbow, and knee) were scored according to 9 HJHS domains.

Gait was scored based on walking and climbing stairs. Total score was the sum from all 6 joints plus gait score. Total HJHS change from baseline to Week 52 was estimated in both study arms by mixed-effects model with repeated measures. Changes from baseline to Week 52 in HJHS domains are presented descriptively.

Results Baseline mean (standard deviation [SD]) HJHS was 18.1 (18.4) in Arm A (n = 116) and 26.3 (13.2) in Arm B (n = 25). Significant improvements in total HJHS from baseline to Week 52 were observed; least squares (LS) mean (95 % confidence interval [CI]) change was –1.54 (–2.70, –0.37; P = 0.0101; n = 107) in Arm A and –4.1 (–7.94, –0.25; P = 0.0382; n = 22) in Arm B. In Arm A, the HJHS domains with greatest mean (SD) change from baseline to Week 52 were swelling –0.3 (1.2), muscle atrophy –0.3 (1.2), crepitus on motion –0.3 (1.2), and flexion loss –0.3 (1.6). In Arm B, HJHS domains with greatest mean (SD) change from baseline to Week 52 were swelling –0.6 (1.1), duration of swelling –0.4 (0.7), crepitus on motion –0.6 (1.8), flexion loss –0.7 (3.1), joint pain –0.4 (1.5), and strength –1.2 (2.4).

Conclusion Significant improvements in joint health were observed within 1 year of starting efanesoctocog alfa treatment in both study arms. These data suggest that once-weekly efanesoctocog alfa prophylaxis may improve joint health in adults and adolescents with severe hemophilia A, and offer benefits above current SoC FVIII prophylaxis.

- von Drygalski: Grant/Research support from: Bioverativ/Sanofi-Genzyme and Pfizer. Consultant for: Biomarin, Bioverativ/Sanofi-Genzyme, Novo Nordisk, Takeda, and Uniqure, Employee of: Hematherix Inc.
- Königs: Personal fees from Bayer, CSL Behring, Novo Nordisk, Roche/Chugai, Sanofi/Sobi, and Takeda. Dr Königs's institution has also received grants for research and clinical trials from Bayer, Biotest, CSL Behring, Intersero, Novo Nordisk, Pfizer, Roche/Chugai, Sanofi/Sobi, and Takeda.
- Nagae: Grant/Research support from: Chugai and Sanofi. Speaker Bureau of: Chugai and Sanofi.
- J. Dumont: Shareholder of: Sanofi. Employee of: Sanofi.
- L. Bystrická: Shareholder of: Sobi. Employee of: Sobi.
- Willemze: Shareholder of: Sanofi. Employee of: Sanofi.
- Santagostino: Shareholder of: Sobi. Employee of: Sobi.
- J. Oldenburg: has received research funding from Bayer, Biotest, CSL Behring, Octapharma, Pfizer, Sobi and Takeda; consultancy, speakers bureau, honoraria, scientific advisory board and travel expenses from Bayer, Biogen Idec, BioMarin, Biotest, Chugai, CSL Behring, Freeline, Grifols, LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Spark Therapeutics, Sobi and Takeda.

T-07-17 Development of a digital patient decision aid for bleeding prophylaxis in severe hemophilia A

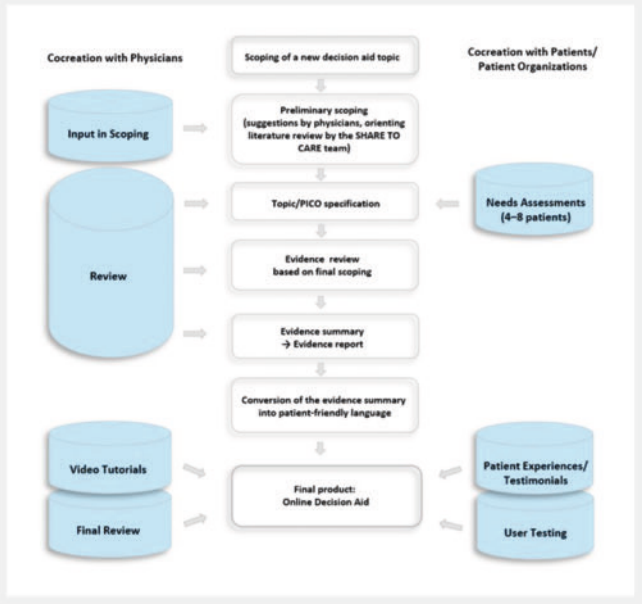
Authors F. Langer[1], C. Escuriola-Ettingshausen[2], C. Pfrepper[3], W. Miesbach[4], R. Stähler[5], C. Schepperle[6], K. Weik[7], F. Scheibler[8, 9]

Institutes **1** Universitätsklinikum Eppendorf, II. Medizinische Klinik und Poliklinik, Bereich Hämostaseologie (Gerinnungsambulanz und Hämophiliezentrum), Hamburg, Germany; **2** HZRM Hämophilie Zentrum Rhein Main GmbH, Walldorf-Mörfelden, Germany; **3** Universitätsklinikum Leipzig, Klinik für Hämatologie, Zelltherapie und Hämostaseologie, Bereich Hämostaseologie, Leipzig, Germany; **4** Universitätsklinikum Frankfurt, Medizinische Klinik 2, Hämostaseologie/Hämophiliezentrum, Frankfurt, Germany; **5** Deutsche Hämophiliegesellschaft (DHG) zur Bekämpfung von Blutungskrankheiten e.V., Hamburg, Germany; **6** Interessensgemeinschaft Hämophiler (IGH) e.V., Rottenburg am Neckar, Germany; **7** TAKEPART Media + Science GmbH, Köln, Germany; **8** SHARE TO CARE, Patientenzentrierte Versorgung GmbH, Köln, Germany; **9** Universitätsklinikum Schleswig-Holstein, National Competency Center for Shared Decision Making, Kiel, Germany

DOI 10.1055/s-0044-1779118

Introduction In patients with severe hemophilia A (HA), bleeding prophylaxis is standard of care to prevent life-threatening hemorrhages and disabling arthropathy and to improve health-related quality of life. Intravenous replacement therapy with standard (SHL) or extended half-life (EHL) factor VIII (FVIII) concentrates, subcutaneous administration of a FVIIIa-mimetic bispecific monoclonal antibody, and AAV5-based *F8* gene transfer to hepatocytes are prophylactic treatment options for adult patients with severe HA who fulfill the respective eligibility criteria. Active participation of patients and their relatives in a structured shared decision-making (SDM) process has been shown to improve treatment satisfaction and outcomes in various clinical indications other than severe HA.

Method Physicians specialized in the treatment of inherited bleeding disorders, representatives of two hemophilia patient organizations, and members of a professional SDM program used International Patient Decision Aids Standards (IPDAS) criteria to provide evidence-based, plain language answers to the following questions related to the three existing treatment options replacement therapy with SHL/EHL FVIII concentrates, FVIIIa-mimetic therapy with a bispecific antibody, and AAV5-based *F8* gene therapy (▶ **Fig. 1**): What does the treatment involve? Will it help my bleeding tendency? How long will the treatment effect last? Can the treatment prevent joint damage? How will treatment impact my quality of life? Will the treatment prolong my life? What are the risks or side effects? Are there long-term negative effects of treatment to be expected? The project was supported by Roche/Chugai Pharma.



▶ **Fig. 1** Flow chart for the creation of a decision aid

Results A web-based patient decision aid for bleeding prophylaxis in severe HA was developed to identify individual patient needs, to facilitate communication with healthcare professionals, and to support efficient decision-making for improved treatment satisfaction and outcomes. Written information on the natural course of the bleeding disorder and available treatment options is supplemented with short videos of patients and physicians explaining their personal expectations and treatment-specific aspects, respectively. By answering and hierarchically ordering questions on their values and preferences, patients are assisted in identifying the prophylaxis regimen that best fits their individual needs. The patient decision aid will be regularly updated when novel evidence on existing or new treatment options become available. The structure and content of the decision aid and initial data on its acceptance by patients, relatives, and healthcare professionals will be presented. The decision aid is available free of charge on the internet.

Conclusion Using validated IPDAS criteria, an evidence-based, easy understandable digital patient decision aid was developed with the potential to improve patient care in severe HA.

Conflict of Interest **FL** has received honoraria for lectures or consultancy from AstraZeneca, Bayer, BioMarin, Bristol Myers Squibb, Chugai, CSL Behring, Daiichi Sankyo, Grifols, LEO Pharma, Novo Nordisk, Octapharma, Pfizer, Roche, SOBI, Takeda, and Viatris. **CEE** has acted as a consultant, received speaker's fees and/or research funding from Bayer Healthcare, BioMarin, Biotest, CSL Behring, Grifols, Kedrion, LFB, Novo Nordisk, Octapharma, Pfizer, Roche/Chugai, Sanofi, SOBI, and Takeda. **CP** reports institutional grants for research and studies from Chugai/Roche, Takeda, Zacros, and LEO Pharma, and honoraria for lectures or consultancy from Bayer, BioMarin, Chugai/Roche, CSL Behring, Novo Nordisk, Pfizer, BMS, SOBI, and Takeda. **WM** reports honoraria for lectures or consultancy from Bayer, BioMarin, Biotest, CSL Behring, Chugai, Freeline, LFB, Novo Nordisk, Octapharma, Pfizer, Regeneron, Roche, Sanofi, Sigilon, SOBI, Takeda/Shire, and uniQure. **RS** reports no COIs. **CS** reports no COIs. **KW** and **FS** received funding from the German Innovations Fund; **FS** is co-founder of the SHARE TO CARE Patientenzentrierte Versorgung GmbH.

T-07-18 Treatment of Bleeding Episodes With Efanesoctocog Alfa in Patients With Severe Haemophilia A in the Phase 3 XTEND-1 Study

Authors A. Weyand[1], C. Königs[2], S. Meunier[3], K. Amano[4], L. Bystricka[5], G. Neill[6], L. Abad-Franch[7], A. Willemze[8], A. Tosetto[9]

Institutes **1** University of Michigan, Division of Hematology/Oncology, Department of Pediatrics, Ann Arbor, USA; **2** University Hospital Frankfurt, Goethe University, Frankfurt, Germany; **3** Hospices Civils de Lyon, Groupement Hospitalo Universitaire Est, Unité Hémostase Clinique, Bron, France; **4** Tokyo Medical University, Department of Laboratory Medicine, Tokyo, Japan; **5** Sobi, Stockholm, Sweden; **6** Sanofi, Reading, UK; **7** Sobi, Basel, Switzerland; **8** Sanofi, Amsterdam, Netherlands; **9** San Bortolo Hospital, Hemophilia and Thrombosis Center, Hematology Department, Vicenza, Italy

DOI 10.1055/s-0044-1779119

Introduction Efanesoctocog alfa is a new class of factor VIII replacement designed to overcome the von Willebrand factor-imposed half-life ceiling. The completed Phase 3 study XTEND-1 (NCT04161495) showed once-weekly efanesoctocog alfa was well tolerated in adults and adolescents with severe hemophilia A and provided superior bleed prevention to prior FVIII prophylaxis. Here, we report on the use of efanesoctocog alfa for the treatment of bleeding episodes (BEs) during XTEND-1.

Method Patients on prior prophylaxis before XTEND-1 were enrolled into Arm A (52 weeks of once-weekly efanesoctocog alfa prophylaxis [50 IU/kg]). Patients receiving prior on-demand therapy entered Arm B (26 weeks of on-demand efanesoctocog alfa [50 IU/kg], then 26 weeks of once-weekly prophylaxis). BEs were treated with single-dose efanesoctocog alfa (50 IU/kg) and additional doses (30 or 50 IU/kg) as needed every 2–3 days. The number and location of treated BEs, and dose and number of efanesoctocog alfa injections to resolve BEs, were evaluated. Patient assessment of the response to bleed treatment was evaluated using the 4-point ISTH scale (excellent, good, moderate, and none).

Results Overall, 133 patients enrolled in Arm A and 26 in Arm B. Median (range) annualized bleed rate was 0.0 (0.0–11.0) in Arm A, and 21.1 (8.3–33.4) and 0.0 (0.0–4.1) for the 6-month on-demand and prophylaxis periods of Arm B, respectively. There were 362 BEs treated with efanesoctocog alfa, of which 86 occurred in Arm A, 268 occurred during Arm B on-demand treatment, and 8 occurred during the subsequent Arm B prophylaxis period. Of the BEs in Arm A, 33 (38 %) were spontaneous, 45 (52 %) traumatic, and 8 (9 %) of unknown aetiology. Corresponding values in Arm B were 197 (74 %), 62 (23 %), and 9 (3 %) for the on-demand period, and 5 (63 %), 2 (25 %), and 1 (13 %) for the prophylaxis period. Most BEs occurred in joints (79 %) and muscles (14 %). A single injection was sufficient to resolve 96.7 % of BEs. All but 1 BE were controlled by ≤ 2 injections. Median (interquartile range) total efanesoctocog alfa dose for BE resolution was 50.9 (50.0–51.8) IU/kg. Most (94.9 %) patient assessments of the BE treatment response were excellent or good.

Conclusion A single 50 IU/kg dose of efanesoctocog alfa effectively treated 96.7 % of BEs regardless of bleed type and location in patients receiving prophylaxis or on-demand treatment.

Conflict of Interest A. Weyand: Grant/Research support from: Pfizer, Novo Nordisk, Takeda, and Sanofi. Consultant for: Takeda, Sanofi, Genentech, Spark, Pfizer, and Bayer. C. Königs: Personal fees from Bayer, CSL Behring, Novo Nordisk, Roche/Chugai, Sanofi/Sobi, and Takeda. Dr Königs's institution has also received grants for research and clinical trials from Bayer, Biotest, CSL Behring, Intersero, Novo Nordisk, Pfizer, Roche/Chugai, Sanofi/Sobi, and Takeda. S. Meunier: Clinical Trials for CSL Behring, Novo Nordisk, Octapharma, Roche, Sanofi, Takeda; The institution of S Meunier received honoraria for consulting/advisory board participation for LFB, Sanofi, Novo Nordisk and personal consultancy fees for consulting/advisory board participation for Sanofi and Takeda. K. Amano: Grant/Research support from: KM Biologics. Consultant for: Chugai Pharmaceutical Co., Ltd. Speaker Bureau of: Bayer, Chugai Pharmaceutical Co., Ltd., CSL Behring, Japan Blood Products Organization, KM Biologics, Novo Nordisk, Pfizer, Sanofi, and Takeda. L. Bystrická: Shareholder of: Sobi. Employee of: Sobi. G. Neill: Shareholder of: Sanofi. Employee of: Sanofi. L. Abad-Franch: Shareholder of: Sobi. Employee of: Sobi. A. Willemze: Shareholder of: Sanofi. Employee of: Sanofi. A. Tosetto: Speaker Bureau of: Bayer, CLS Behring, GSK, and Novo Nordisk.

T-07-19 Real-world use of rIX-FP in patients with haemophilia B: Interim results from a prospective, non-interventional study in Germany

Authors J. Oldenburg[1], M. Olivieri[2], S. Wenning[3], S. Holzhauer[4], B. Krammer-Steiner[5], C. Escuriola-Ettingshausen[6], R. Klamroth[7], W. Miesbach[8], T. Linhoff[9], C. Pfrepper[10]

Institutes **1** University of Bonn, Institute of Experimental Hematology and Transfusion Medicine, University Hospital Bonn, Medical Faculty, Bonn, Germany; **2** LMU Munich, Paediatric Thrombosis and Haemostasis Unit, Paediatric Haemophilia Centre, Munich, Germany; **3** SRH Kurpfalzkrankenhaus, Heidelberg, Germany; **4** Charité University Medicine, Department of Pediatric Hematology and Oncology, Berlin, Germany; **5** Klinikum Südstadt Rostock, Department of Internal Medicine III, Rostock, Germany; **6** Haemophilia Centre Rhein Main (HZRM), Mörfelden-Walldorf, Germany; **7** Vivantes Hospital Friedrichshain, Department of Haemophilia and Haemostasis, Berlin, Germany; **8** University Hospital Frankfurt, Frankfurt, Germany; **9** CSL Behring, Hattersheim, Germany; **10** University Hospital Leipzig, Division of Hemostaseology, Leipzig, Germany

DOI 10.1055/s-0044-1779120

Introduction The efficacy and safety of a long-acting recombinant factor IX albumin fusion protein (rIX-FP) in patients with haemophilia B has been previously demonstrated in clinical trials. rIX-FP provides dosing flexibility allowing selected patients to receive prophylaxis at intervals up to 21 days. Data from routine clinical practice on the use of rIX-FP are required. Therefore, prospective, multicentre studies are ongoing to obtain data on the effectiveness and tolerability of rIX-FP when used to treat patients with haemophilia B during routine clinical practice.

Method A non-interventional study was initiated in Germany in March 2018. All patients with haemophilia B receiving rIX-FP for treatment and prophylaxis of bleedings were eligible for enrolment. The treatment fell within current clinical practice of the investigator. Patients were excluded in the presence of inhibitors and multi-factor deficiency. Annualised bleeding rates (ABR), annualised spontaneous bleeding rates (AsBR), infusion frequency, dosage and haemostatic effectiveness were recorded. Patients are routinely monitored every 3–12 months and followed-up for 3 years.

Results At the interim data cut-off on 18 September 2023, 73 patients were enrolled in the study across 22 sites, which includes 9 previously untreated patients (PUPs). The recruitment was completed in March 2021, and follow-up is ongoing. At baseline, patients ranged in age from 1–80 years with a mean (SD) of 27.4 (19.6) years. Patients with available data had an estimated mean (SD) of 105.5 (75.8) exposure days to rIX-FP (n = 65), and the majority had moderate or severe haemophilia B (n = 59). To date, 54 patients completed the study, 10 are ongoing and 9 discontinued. 82 adverse events (AEs) in 34 patients have been reported including 19 events assessed as serious, none of which were considered related to rIX-FP. No patients have developed inhibitors. Analyses of bleeding rates, haemostatic effectiveness and rIX-FP consumption are currently being performed.

Conclusion To date, interim results from this study indicate that rIX-FP prophylaxis is well tolerated in both adults and paediatrics in routine clinical practice. Further data collection is ongoing to assess the long-term effectiveness and tolerability of rIX-FP in the real-world clinical setting.

Conflict of Interest JO has received research funding from Bayer, Biotest, CSL Behring, Octapharma, Pfizer, Swedish Orphan Biovitrum and Takeda; consultancy, speakers bureau, honoraria, scientific advisory board and travel expenses from Bayer, Biogen Idec, BioMarin, Biotest, Chugai, CSL Behring, Freeline, Grifols, LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Spark Therapeutics, Swedish Orphan Biovitrum and Takeda. MO received grants/research support from Bayer, Biotest, Takeda, CSL Behring, Octapharma, Pfizer, Shire, Roche and Swedish Orphan Biovitrum, consultancy and speaker fees from Bayer, Biotest, Novo Nordisk, Takeda, CSL Behring, Pfizer, Roche and Swedish Orphan Biovitrum. SW received study support and consultancy and speaker fees from Bayer, Biotest, Takeda, CSL Behring, Novo Nordisk, Octapharma, Roche and Swedish Orphan Biovitrum, consultancy and speaker fees from Bayer, Novo Nordisk, Roche, Swedish Orphan Biovitrum and Takeda. SH have no conflicts of interest to declare. BKS received study support by CSL Behring. CEE has acted as a consultant and received speaker's fees and/or research funding from Bayer, Biomarin, Biotest, CSL Behring, Grifols, Kedrion, LFB, Octapharma, Novo Nordisk, Pfizer, Roche/Chugai, SOBI, Takeda. RK received grants and research support from Bayer, Novo Nordisk and Sobi; honoraria and consultation fees from Bayer, BioMarin, CSL Behring, Novo Nordisk, Octapharma, Pfizer, Roche, Sobi and Takeda, participates in a company sponsored speakers bureau for Bayer, BioMarin, CSL Behring, Novo Nordisk, Octapharma, Pfizer, Roche, Sobi and Takeda. WM has received grant and research support from Bayer, Biotest, CSL, LFB, Novo Nordisk, Octapharma, Pfizer, Sobi, Takeda and UniQure; received consultation fees from BioMarin, Freeline, LFB, Octapharma, Sobi, Novartis, Novo Nordisk, Octapharma, Pfizer and Roche; and participated in speaker bureau for Shire, Pfizer, Bayer, Sobi, Amgen, Novartis, Alexion, Leo Pharma, Grifols, Roche and Novo Nordisk. TL is CSL Behring employee. CP received speaker honoraria from BMS, CSL Behring, Pfizer, Roche, Shire Takeda, acted as a medical advisor for Bayer HealthCare, Chugai, CSL Behring, Novo Nordisk, Pfizer, Roche, Shire Takeda, received research grants from Fujimori, LeoPharma, Takeda, Roche.

T-07-20 In-detail AFM analysis of the different conformational states of full-length coagulation Factor VIII and FVIII-vWF complex.

Authors S. U. Ramaraje Urs[1, 2], J. L. Pellequer[2], J. M. Teulon[2], S. Singh[1], J. Oldenburg[1], A. Biswas[1]

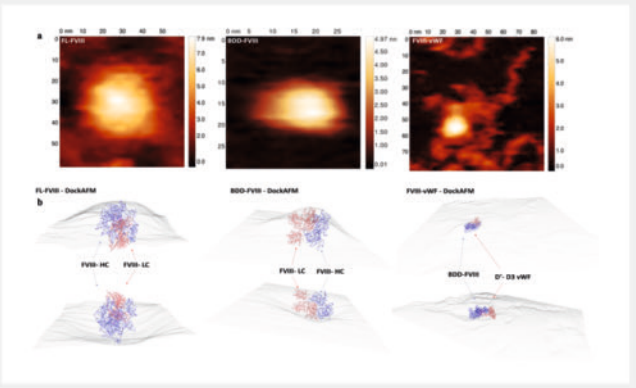
Institutes **1** Universitätsklinikum Bonn, Institut für Experimentelle Hämatologie und Transfusionsmedizin, Bonn, Germany; **2** Univ. Grenoble Alpes, CEA, CNRS, IBS (Institut de Biologie Structurale), Grenoble, France

DOI 10.1055/s-0044-1779121

Introduction Full-length factor VIII protein (FL-FVIII) is biochemically composed of A, B, and C domains [1]. Current structural information is skewed towards the B domain deleted FVIII (BDD-FVIII), leaving most of the heavily glycosylated B domain to be structurally unresolved [2]. In this study, we combined atomic force microscopic (AFM) techniques with molecular modeling and a unique AFM assembly pipeline to unravel the spatial orientation of the FVIII B-domain with respect to the rest of the FVIII protein in the FL-FVIII. Our goal is to understand the effect of the B domain in the binding of interacting partners vWF, Thrombin, and inhibitory antibodies were performed using refined FL-FVIII and BDD-FVIII.

Method AFM imaging of highly pure recombinant and plasma-derived FL-FVIII and FVIII-vWF complex was performed. Samples were imaged with the off-resonance oscillation mode in liquid (imaging buffer) using a Multimode-8 microscope (Bruker). Refinement of the previously simulation equilibrated glycosylated and non-glycosylated FL-FVIII computational models was performed using x-plor and Coot. The refined models of FL-FVIII and BDD-FVIII were then fit onto the topographic images from AFM using DockAFM [3]. Comparative surface area accessibility studies also were performed with YASARA.

Results In the AFM topographs, FL-FVIII and BDD-FVIII appeared globular/spherical whereas the FVIII-vWF complex appeared to have longer vWF tails connected to globular FVIII (▶ **Fig. 1a**). These topographs of the FL-FVIII and FVIII-vWF complex showed an average height of 6-7 nm in liquid, and the BDD-FVIII showed an average height of 4 nm. Appropriate parameters were defined to dock the FVIII structure under the AFM topography (128 pixels [3], grid space of 4 Å, threshold of 30 Å, favorable region of 40 Å). Spatial positioning and various orientations of the FL-FVIII, BDD-FVIII, and FVIII-vWF complex were identified by the fits obtained from DockAFM (▶ **Fig. 1b**). Accessible surface area analysis suggests that FL-FVIII has a decreased interaction with known reported inhibitors compared to BDD-FVIII. Additionally, a FL-FVIII – D'-D3-von Willebrand factor complex has been built.



▶ **Fig. 1 AFM analysis of the FL-FVIII, BDD-FVIII and FVIII-vWF complex**; **a**. AFM topographs of FL-FVIII and BDD-FVIII showing globular conformations and FVIII-vWF complex showing globular FVIII connected to vWF tails (Left to Right, respectively). **b**. Spatial positioning of the FL-FVIII, BDD-FVIII, and FVIII-vWF complexes identified by the fits obtained from DockAFM (Left to Right, respectively).

Conclusion The refined FL-FVIII models show the significance of glycosylation in stabilizing the orientation of the B domain and the overall structure of FL-FVIII. Our current three-dimensional atomic model of FL-FVIII is compatible with experimental topographic surfaces of FVIII obtained with AFM. The difference in height is likely due to the B domain of FL-FVIII. It also suggests that the conformation of the B domain is distributed over all the FVIII structure and does not form a separate domain. Differences in surface area accessibility between FL-FVIII and BDD-FVIII indicate that the binding of FVIII interacting partners or neutralizing antibodies might structurally and functionally differ. The D'-D3 domains of vWF in FL-FVIII – D'-D3-von Willebrand complex fit easily into our FL-FVIII structural model.

Conflict of Interest This project was awarded with the Rudolf Marx Scholarship at GTH 2023, in Frankfurt supporting the research stay of Ms.Samhitha Urs Ramaraje Urs. This work has been funded by grants from Takeda-Shire under the IIR Program awarded to PD Dr. Arijit Biswas and Prof. Dr. med. Johannes Oldenburg

References

[1] Pipe SW. Functional roles of the factor VIII B domain. Haemophilia. 2009; 15 (6): 1187–96

[2] Bonazza K, Rottensteiner H, Schrenk G, Fiedler C, Scheiflinger F, Allmaier G, Turecek PL, Friedbacher G. Ca(2+) concentration-dependent conformational change of FVIII B-domain observed by atomic force microscopy. Anal Bioanal Chem 2015; 407 (20): 6051–6

[3] Rui C. Chaves, Jean-Luc Pellequer, DockAFM: benchmarking protein structures by docking under AFM topographs, Bioinformatics, Volume 29, Issue 24, December 2013, Pages 3230–3231.

T-07-21 Evaluation of primary haemostasis in children and young adults with haemophilia

Authors M. Kürschner[1], J. Lohse[1], F. Boiti[1], O. Tiebel[2], R. Knoefler[1]

Institutes **1** Medical Faculty Carl Gustav Carus of TU Dresden, Dept. of Pediatric Haemostaseology, Dresden, Germany; **2** Medical Faculty Carl Gustav Carus of TU Dresden, Dept. of Clinical Chemistry and Laboratory Medicine, Dresden, Germany

DOI 10.1055/s-0044-1779122

Introduction Haemophilia patients show interindividual differences in bleeding tendencies despite comparable values of coagulation factor activities VIII and IX. This led to the assumption that other factors may influence the bleeding phenotype, among them the function of primary haemostasis. Contradictory data can be found in the literature with increased platelet activities as a compensatory mechanism in haemophilia and a lack of differences compared to healthy controls. We comprehensively investigated the primary haemostasis in children and young adults with haemophilia using different methods.

Method Platelet aggregation and release of adenosine triphosphate were investigated in citrated whole blood using the Chronolog lumi-aggregometer with the agonists adenosine diphosphate (ADP, final concentration: 20 µM), arachidonic acid (AA, 0.5 mM), collagen (1 µg/ml), ristocetin (1 mg/ml) and thrombin (0.5 U/ml). Samples from 38 patients (33 with haemophilia A: severe n = 24, moderate n = 1, mild n = 8 and 5 with hemophilia B: severe n = 3, moderate n = 1, mild n = 1) with an average age of 11 years (range: 1 to 32 years) and 30 healthy controls (mean age 5.5 years, range: 1 to 19 years) were included. Platelet function was also analyzed in platelet-rich plasma using light transmission aggregometry (ADP 2.5 and 5 µM, AA 1.0 mM, collagen 2.0 and 10.0 µg/ml) in 45 patients and controls, and flow cytometry (CD42, PAC-1 CD62P, CD63, mepacrine-uptake and -release) in 45 patients and 39 controls. In addition, PFA-100® closure times, von Willebrand factor parameters, blood cell counts and blood group were determined.

Results We found a significantly higher maximum platelet aggregation in whole blood induced by arachidonic acid and collagen in the haemophilia group compared to the controls. However, the median values of maximum aggregation were within the reference range in both groups. A significant difference was also observed in the analysis of all patients with haemophilia A, as well as in the subgroup consisting of patients with severe and moderate disease. Platelet flow cytometry showed a significantly decreased ADP-induced CD63 expression and mepacrine release in the haemophilia group compared to controls. Concerning the blood cell count, haemophilia patients showed a significantly lower median platelet count, but in both groups platelet counts were in the normal range. For the other parameter no differences between both groups were detected.

Conclusion Our data show clinically non-relevant differences in primary haemostasis function between patients with haemophilia and healthy controls. However, an influence of the function of primary hemostasis, including platelet function, on the clinical phenotype cannot be ruled out, at least in some patients. Therefore, it makes sense to carry out comprehensive coagulation diagnostics in haemophilia patients in order to identify the individual factors influencing the bleeding phenotype and, if necessary, to take them into account in bleeding risk situations.

Conflict of Interest None

T-07-22 Interleukin-6 as a Plasma Marker of Bleeding in Patients with Hemophilia

Authors L. M. Knowles[1], C. Wolter[1], M. D. Menger[2], M. W. Laschke[2], F. Haegele[1], H. Eichler[1], J. Pilch[1]

Institutes **1** Saarland University Hospital, Institute of Clinical Hemostaseology and Transfusion Medicine, Homburg/Saar, Germany; **2** Saarland University Hospital, Institute for Clinical and Experimental Surgery, Homburg/Saar, Germany

DOI 10.1055/s-0044-1779123

Introduction The development of arthrofibrosis following one or more joint bleeds remains an unsolved problem for hemophilia patients and their health care providers. We hypothesize that the risk of bleeding in hemophilia results in aberrant wound healing and increased inflammation.

Method Recognizing the role of macrophages in wound healing, we systematically assessed macrophage function in the context of hemophilia.

Results The ensuing studies revealed a deficit in regenerative macrophage markers in connection with reduced wound healing in monocytes isolated from the blood of a randomly selected cohort of hemophilia patients. In a mouse model of hemophilia, we observed a delayed influx and reduced phagocytic activity of hemophilia macrophages after joint bleeding, which resulted in delayed resorption of blood and increased inflammation due to neutrophil persistence. Inflammation coincided with increased interleukin-6 levels in blood samples from hemophilia patients with acute bleeding.

Conclusion IL-6 could be a useful biomarker for joint bleeds in hemophilia patients.

Conflict of Interest None

T-07-23 Development of a low-titer inhibitor in a boy with congenital mild hemophilia A: clinical and immunological follow up of 1 year

Authors I. Wieland[1], K. Becker[1], D. Stichel[2], K. Christoph[2]

Institutes **1** Hannover Medical School, Department of Pediatric Hematology and Oncology, Hannover, Germany; **2** Goethe-Universität Frankfurt am Main, Department of Pediatric Hematology and Oncology, Frankfurt am Main, Germany

DOI 10.1055/s-0044-1779124

Introduction The inhibitor development in mild hemophilia A [HA] is rare. Due to cross-reactivity of the inhibitor to the endogenous FVIII, plasma FVIII activity may decrease and bleeding complication can increase. The INSIGHT Study reported a cumulative incidence of 5.3 % after a median of 28 exposure days. The inhibitors disappeared in around 70 % of patients after a median of 45 weeks and in 70 % of them without a specific eradication treatment [1, 2].

Method We report the clinical and immunological course of a 4 year old boy with mild HA (FVIII 20 %) caused by a missense mutation (M2164V), not described as high-risk mutation for inhibitor development in mild HA [1, 2] .

Results He developed low titer inhibitor (4.6BU) following tonsillectomy with rFVIII substitution (50IU/kgBW) for 10 days. Routine inhibitor monitoring revealed an FVIII level of 1.1 % and an inhibitor of 4.6 BU with clinically increased bleeding. After diagnosis of inhibitor and change of endogenous FVIII activity to levels comparable to moderate/severe hemophilia, we started a treatment with emicizumab as bleeding prophylaxis. Bleeding symptoms disappeared. The immunological characterization of the inhibitory antibody at first detection showed IgG1, IgG3 and IgG4 specific for FVIII, but with IgG3 highest, and a binding to FVIII domains HC (A2) and LC (C1 and C2).

During the 9 month follow up (August 2023) without FVIII substitution, the inhibitor disappeared (< 0.6BU) in May 2023 and FVIII levels slowly increased to 11.5 %. While IgG1 and IgG3 decreased, IgG4 increased over time, reached a plateau and has started to decrease since May. The binding to FVIII domains decreased for all domains but the A1 domain. After 9 month, IgG4 and binding to A1 decreased further, but remained borderline positive.

Conclusion Although no high-risk mutation was present, the mild HA patient developed low-titer inhibitor after FVIII substitution for surgery, changing mild HA to a moderate to severe phenotype. This underlines the importance of regular inhibitor analysis following FVIII substitution also in patients with mild HA.

The immunological profile at beginning seemed comparable with that of severe HA with inhibitor. Despite negative inhibitor testing and increasing FVIII activity but not to the initial levels, the FVIII antibody screen remained positive with highly positive IgG4 subclass and increasing binding to domain A1. This demonstrates a specific antibody response against FVIII despite negative inhibitor testing. It is unknown if this is a predictor for inhibitor reoccurrence after another FVIII exposure, but should be carefully considered.

Therefore, in addition to the inhibitor analysis a more detailed analysis of immune response to FVIII, for instance the testing for FVIII-specific IgG and IgG subclasses may give further insight into the actual immune response to FVIII in patients receiving intermittent FVIII, including moderate and mild patients or severe patients on emicizumab prophylaxis.

Disclosures IW: Clinical studies: Boehringer-Ingelsheim, Pfizer, Roche/Chugai, Shire, Sobi,; Consultings: Bayer, Biotest, CSL Behring, Novo Nordisk, Octapharma, Pfizer, Roche/Chugai, Shire/Takeda, Sobi,

References

[1] Eckhardt C.L. et al. Factor VIII gene (F8) mutation and risk of inhibitor development in nonsevere hemophilia A. Blood. 2013; 122: 1954–1962

[2] Castaman G., Fijnvandraat K. Molecular and clinical predictors of inhibitor risk and its prevention and treatment in mild hemophilia A. Blood. 2014; 124: 2333–2336

T-07-24 A Pooled Analysis of Treatment with a Plasma-Derived von Willebrand Factor (VWF) Almost Devoid of Factor VIII in Paediatric Patients with Von Willebrand Disease (VWD)

Authors A. Hüsing[1], J. Goudemand[2], A. Borel Derlon[3], C. Henriet[4], B. Boiffin[5], Y. Repesse[6], S. Susen[7]

Institutes **1** LFB GmbH, Medical Affairs, Münster, Germany; **2** Lille University Hospital, Department of Haematology, Lille, France; **3** Caen University Hospital, Department of Haematology, Caen, France; **4** LFB, Department of medical and clinical development, Les Ulis, France; **5** LFB GmbH, Medical Affairs, Münster, Germany; **6** Caen University Hospital, Department of Haematology, Caen, France; **7** Lille University Hospital, Department of Haematology, Lille, France

DOI 10.1055/s-0044-1779125

Introduction Von Willebrand factor (VWF) replacement therapy is commonly used in the treatment of patients with von Willebrand Disease (VWD) who do not respond to or cannot use desmopressin. While its effectiveness in adults is well-known, it is important to investigate its usage in the paediatric population, as it is a key element in the clinical trial programme of a VWF product because children may respond differently to treatment.

Method This pooled analysis gives new data from five clinical studies conducted by LFB between 1999 and 2014, using a plasma-derived VWF concentrate almost devoid of factor VIII (FVIII) in patients under 18 years old. A total of 56 paediatric patients were included in the analysis, categorised into three age groups (< 6 years, 6-11 years, and 12- < 18 years). These patients received at least one injection of VWF for the treatment of bleeding episodes (BE), prevention during surgery, as well as short or long-term prophylaxis to prevent BE.

Results Among the patient population, 54 % were female. Most patients had type 3 (25) or type 2 (27) VWD. Basal FVIII:C (factor VIII coagulant activity) levels were < 20 IU/dL in 59 % of patients. The treatment efficacy and safety were assessed based on 122 BEs (including gastrointestinal, musculoskeletal, epistaxis, intrabuccal, and other types) and 29 surgical/invasive procedures (including orthopaedic, urological, dental, and general procedures). Investigator assessment of treatment was rated as excellent/good in 89.3 % of BEs and 100 % of surgical/invasive procedures. The dosages of VWF varied between age groups and specific clinical situations. FVIII was co-administered in 23 % of treatment events. Long-term prophylaxis was given for 22 occasions in 17 patients, approximately twice a week with a median dose of 40 IU/kg (patients aged ≥ 12 years) and 50 IU/kg (other age groups). Adverse drug reactions were reported in only one 17-year-old patient (1.8 %), consisting of two non-serious episodes of dizziness that resolved within 15 minutes without any corrective treatment. No inhibitors were reported in any of the trials.

Conclusion This data suggests that a plasma-derived VWF concentrate with low FVIII content, is effective and has a favourable safety profile in paediatric patients with VWD. Similar to adults, the dosage and duration of treatment should be adjusted based on the patient's clinical condition and VWF:RCo (VWF Ristocetin cofactor) and FVIII:C plasma levels during treatment.

Conflict of Interest Prof. Goudemand: LFB, Roche-Chugai; Dr. Borel-Derlon: LFB, Takeda, Roche-Chugai; Prof. Yohann Repesse: CSL Behring, LFB, Octapharma, Shire, Takeda; Prof. Sophie Susen: Biomarin, CSL Behring, Roche, LFB, NovoNordisk, Sanofi, siemens Healthiners, Sobi, Stago, Takeda.

T-07-25 Association of telomere length, a marker of biological aging, with hemophilia severity

Authors M. Trappl[1], R. Vostatek[1], D. Kraemmer[1], P. Hohensinner[2, 3], I. Pabinger[1], C. Ay[1]

Institutes **1** Medical University of Vienna, Department of Internal Medicine I, Clinical Division of Haematology and Haemostaseology, Vienna, Austria; **2** Medical University of Vienna, Department of Internal Medicine II, Division of Cardiology, Vienna, Austria; **3** Medical University of Vienna, Center for Biomedical Research, Vienna, Austria

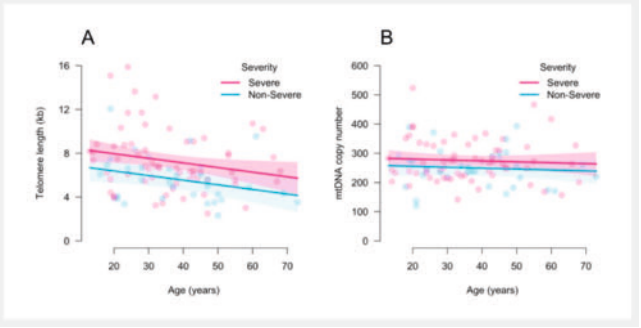
DOI 10.1055/s-0044-1779126

Introduction Hemophilia is a rare X-linked bleeding disorder caused by mutations in the factor VIII or IX gene (hemophilia A or B). The hallmark of hemophilia is bleeding into joints, which leads to pathological changes and painful joint damage, which progresses with increased age, adding to morbidity and impacting functionality and quality of life. We hypothesized that hemophilia as a chronic disease is associated with accelerated biological aging. Therefore, we aimed to investigate biomarkers of biological aging (human mitochondrial DNA (mtDNA) copy number and absolute human telomere length) in patients with hemophilia of different severity.

Method We performed a cross-sectional study at our hemophilia treatment center for adults utilizing blood that was collected within the Austrian Haemophilia Registry and stored at the biobank of our hospital. DNA was isolated with the Promega Maxwell RSC Blood DNA Kit AS1400 for the Maxwell automated system. The average human mtDNA copy number and telomere length were measured with qPCR-based assay kits. We investigated the association of biological markers of aging with hemophilia severity by fitting ordinary least squares regression models, adjusting for chronological age.

Results We included 97 hemophilia patients (median age: 35 [IQR 24.0-46.5] years; 86 hemophilia A and 11 hemophilia B) in this study, of whom 64 (66 %) had severe, 11 (11 %) moderate, and 22 (23 %) mild hemophilia. Telomere length (median: 6.31 [IQR: 4.82–8.16] kb) and chronological age showed a weak negative correlation (Spearman's ρ = -0.27 [95 % CI -0.45, -0.06]; ▶ **Fig. 1a**). The median telomere length was 5.28 (IQR 4.02–6.81) kb in mild, 5.35 (IQR 3.88–6.69) kb in moderate, and 6.65 (IQR 5.57–8.82) kb in severe hemophilia patients. When adjusting for chronological age, severe hemophilia was associated with a 1.58 (95 % CI 0.49, 2.66) kb higher mean telomere length

compared to non-severe hemophilia (▶ **Fig. 1a**). Linear regression of mtDNA copy number (median: 255.7 [IQR: 219.0 – 308.1]; ▶ **Fig. 1b**) on age and severity remained inconclusive.



▶ **Fig. 1** **a**) *telomere length of patients with non-severe and severe hemophilia in relation to age*: A weak inverse correlation between telomere length and age can be seen. **b**) *mtDNA copy number of patients with non-severe and severe hemophilia in relation to age*: No correlation between mtDNA copy number and age was found. The lines and shaded region depict age-adjusted fitted values and accompanying 95 % confidence bands for severe (pink) and non-severe hemophilia (blue), respectively.

Conclusion We have observed a weak correlation of telomere length with chronological age in patients with hemophilia. Interestingly, age-adjusted mean telomere length was significantly shorter in patients with mild and moderate hemophilia compared to those with severe hemophilia. This result questions our initial hypothesis of increased biological aging in severe compared to non-severe hemophilia patients. Further investigations are needed to better characterize biological aging in hemophilia.

Conflict of Interest The authors declare that they have no competing interests.

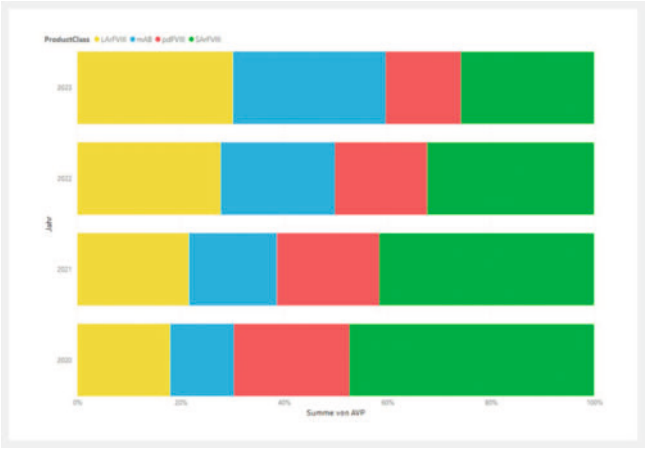
T-07-26 The change in factor VIII therapy since the introduction of mandatory pharmacy reporting in the GSAV in Germany – a retrospective data analysis from smart medication ScanDoc over 3 years

Authors A. Rösch, D. Schmoldt
Institute smart medication eHealth Solutions GmbH, Frankfurt Am Main, Germany
DOI 10.1055/s-0044-1779127

Introduction Since the introduction of the long acting recombinant factor VIII preparations (LArFVIII) and the monoclonal antibodies (mAB), these product classes are increasingly replacing the previously used plasmatic (pdFVIII) and short acting recombinant factor preparations (SArFVIII). Starting in 9/2020 factor dispensing is documented in about 100 specialized hemophilia pharmacies with the smart medication ScanDoc software tool. The retrospective analysis of documentation shows the change over time in product classes in Germany.

Method: With the introduction of the GSAV in 2019 and the elimination of the special distribution channel directly through hemophilia centers, factor preparations are provided to patients exclusively through pharmacies. The reporting obligation of the pharmacy to the prescribing physician (regulated in ApBertrO) starting in 9/2020 has since been supported by the smart medication ScanDoc software tool and is used in about 100 pharmacies in Germany. The present analysis covers a period of 37 months (9/2020 – 9/2023) with a total of 22794 prescription records. The pharmacy sales price (AVP) of the dispensed factor preparations amounts to almost EUR 630 million.

Results Since 9/2020 the following changes could be observed (details see ▶ **Fig. 1**):



▶ **Fig. 1** Change in product classes in the period under analysis

LArFVIII increase of total AVP from 18 % to 30 % (+ 12 %)
mAB increase of total AVP 12 % to 30 % (+ 17 %)
pdFVIII decrease of total AVP from 22 % to 15 % (- 8 %)
SArVFIII decrease of total AVP from 47 % to 26 % (- 22 %)(AVP = Apothekenverkaufspreis, pharmacy sales price)

Conclusion The retrospective data analysis shows that the modern therapy options (LArFVIII, mAB) are gradually replacing the previously established preparations (pdFVIII, SArFVIII). SArVFIII preparations declined with -22 % and pdVFIII with -8 % in total value over the observation period of 37 months. Interestingly, the decline of pdFVIII preparations is significantly slower than that of SArVFIII preparations. In contrast modern therapy options each increased by + 12 % (LArVFIII) and + 17 % (mAB) in total value. Back in 2020 classical therapy options (pdFVII, SArFVIII) still accounted for 70 % of the total value, but in 2023 modern therapy options (LArFVIII, mAB) already have a share of 60 % with a still increasing relevance.

Conflict of Interest none.

T-07-27 Platelet-dependent thrombin generation in hemophilia A patients may compensate low FVIII levels

Authors S. Kieninger[2], N. Wolska[2], J. Zlamal[1, 2], T. Bakchoul[1, 2], K. Althaus[1, 2]
Institutes **1** Medical Faculty and University Hospital of Tuebingen, Center for Clinical Transfusion Medicine, Tuebingen, Germany; **2** Medical Faculty and University Hospital of Tuebingen, Institute for Clinical and Experimental Transfusion Medicine, Tuebingen, Germany
DOI 10.1055/s-0044-1779128

Introduction In hemophilia patients the bleeding phenotype generally correlates with the residual FVIII levels, which depend on the genetic mutation underlying the condition. However, different bleeding tendencies can be observed in patients with equal FVIII levels, raising the question which other parameters contribute to the variable bleeding phenotype in these patients. We investigate the role of platelets (PLTs) in patients with hemophilia. Our focus lies on the procoagulant subpopulation of PLTs, which are characterized by P-selectin expression and exposition of phosphatidylserine (PS).

Method We measured thrombin generation (TG) in a calibrated automated thrombinoscope for TG in platelet rich plasma (PRP) and platelet poor plasma (PPP) of hemophilia patients. We inhibited PLT surface PS (with lactadherin) in PRP with different FVIII levels. Further, we investigated TG in PRP of healthy controls upon PLT activation (TRAP-6, Convulxin (CVX), and a combination of both). Lastly, we measured hemophilia PRP spike-in samples with hemophilia drugs (plasma derived human FVIII, Efmoroctocog alfa and Octocog alfa).

Results An endogenous thrombin potential (ETP) is detectable with all FVIII levels in PPP. Whereas in PRP an ETP was not measurable in low FVIII concentrations. PS inhibition showed that in a low FVIII environment (FVIII 10 %) the lagtime (p = 0.0037) and time to peak (ttpeak) (p = 0.0148) are significantly prolonged and the thrombin peak is significantly lower (p = 0.0095), indicating that TG is PS dependent in a low FVIII environment.

Contrasting to low FVIII environments, lagtime and ttpeak are not affected by PS inhibition under high FVIII activity (FVIII 70 %) (p = 0.3, and p = 0.07, respectively) indicating that the PS dependency is stronger in low FVIII environments. Only the thrombin peak was significantly lower with PS inhibition and high FVIII activity (p = 0.001). Activation of PRP samples with TRAP-6 + CVX heightened the thrombotic potential of healthy controls, as well as in hemophilia patients.

In the FVIII spike-in samples the thrombin peak (p = 0.001), lagtime (p = 0.0028) and ttpeak (p < 0.0001) steadily improved with higher FVIII levels, as well as ETP (p = 0.0158).

Conclusion Our findings show steadily improving parameters in TG in FVIII spike in studies, indicating that TG on PLTs depends on FVIII concentration and that there is a minimum FVIII level necessary for sufficient TG on PLTs, which we saw in hemophilia patients. TG on PLTs depends on PS exposure. The PS dependency is stronger in low FVIII environments (FVIII 10 %) in comparison to high FVIII environments (FVIII 70 %), however high FVIII concentrations cannot fully compensate for PS inhibition. Induction of procoagulant platelet formation improves TG, indicating the crucial role of procoagulant PLTs in allowing hemophilia patients to compensate their FVIII deficiency.

Conflict of Interest Nothing to declare.

T-07-28 Artrial fibrillation – a challenge in patients with hemophilia A

Authors K. Althaus[1, 2], J. Schreieck[3], S. Hammer[1, 2], T. Bakchoul[1, 2]

Institutes **1** University Hospital of Tuebingen, Center for Clinical Transfuion Medicine, Tuebingen, Germany; **2** University Hospital of Tuebingen, Institute for Clinical and Experimental Transfusion Medicine, Tuebingen, Germany; **3** University Hospital of Tuebingen, Department of Cardiology and Angiology, Tuebingen, Germany

DOI 10.1055/s-0044-1779129

Introduction With the aging population of hemophilic patients, cardiovascular diseases are also increasing among these patients. Atrial fibrillation is one of these increasing diseases. Despite the reduction in a clotting factor, these patients are at risk of a stroke. Permanent anticoagulation may be necessary despite bleeding tendency. In this case, we report a patient with mild hemophilia A (residual activity 10 %) and atrial fibrillation.

Method The therapeutic options in patients with atrial fibrillation were anticoagulation with, for example, apixaban 2x5mg or 2x2.5mg in a reduced dose. The second option would be pulmonary vein isolation with subsequent medium-term anticoagulation until the atrium has completely healed or cardioversion, as a third option, with short-term anticoagulation. If no thrombus can be detected in TEE after 4 weeks of anticoagulation, anticoagulation is stopped again. The final option would be an atrial occluder, which prevents a thrombus from forming. It is particularly suitable for patients where long-term anticoagulation is contraindicated. However, long-term secondary prophylaxis with aspirin is usually indicated.

Results After unsuccessful rhythm control with medication, cardiologist decided to undergo cardioversion with amiodarone within 1 year of the onset of persistent atrial fibrillation. This results in an improvement in morbidity and a reduction in cardiovascular events. Mortality remains unaffected in this procedure.

For this purpose, the patient received recombinant factor VIII, which he had already received regularly in the past as on-demand therapy. After one week, anticoagulation with apixaban was reduced to 2x2.5mg and continued for another 3 weeks. Factor therapy was also continued during this time (trough level 30 %). A TEE check was carried out after 4 weeks. If there was no evidence of cardiac thrombi and sinus rhythm was present, anticoagulation therapy was stopped. So far the patient remains in sinus rhythm. Neither thromboembolic complications nor bleeding complications occurred during therapy or afterwards.

Conclusion Although patients with hemophilia have an increased risk of bleeding, diseases such as atrial fibrillation also have an increased risk of stroke. A lack of guidelines in this area leads to difficult therapy decisions, which are sometimes certainly wrong for the individual patient. Therefore, the consistent collection of individual cases like this is important in order to increase the evidence piece by piece.

Conflict of Interest none

T-07-29 Downregulation of von Willebrand factor induces endothelial barrier dysfunction

Authors M. Arshad, E. Dreisbach, M. A. Brehm

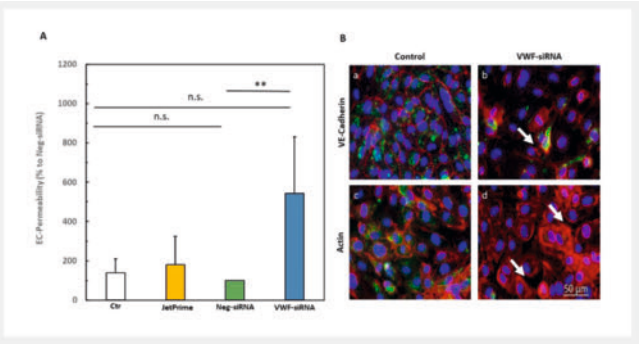
Institute University of Siegen, Department of Digital Health Sciences and Biomedicine, Siegen, Germany

DOI 10.1055/s-0044-1779130

Introduction Von Willebrand factor (VWF) is a plasma glycoprotein mainly known for its function in hemostasis but also involved in (patho)-physiological processes, such as angiogenesis and inflammation. In association with inflammation, VWF deficiency or inhibition was suggested to decrease vascular permeability. Deficiency of VWF can lead to the bleeding disorder von Willebrand disease (VWD). VWD arises from mutations in the von Willebrand factor (VWF) gene, leading to qualitative or quantitative defects in the VWF protein. Angiodysplasia is the most common vascular abnormality in the gastrointestinal tract, characterized by fragile and leaky blood vessels with increased permeability, and associated with von Willebrand disease (VWD). The mechanisms by which VWF modulates endothelial cell permeability are poorly studied.

Method The study was conducted in cultured human umbilical vein endothelial cells (HUVECs), in which a VWD phenotype was mimicked by siRNA knockdown of VWF. HUVEC-permeability experiments were carried out after si-RNA-VWF transfection by seeding knock-down cells on filter membranes (pore size 0.4 µm) in a two-compartment system. HRP-conjugated streptavidin-containing medium was collected from the lower compartment after passing through the EC monolayer. For measuring absorption of HRP-conjugated streptavidin, a colorimetric tetramethylbenzidine (TMB) substrate assay was performed using an ELISA plate reader. Effect of downregulation of VWF on actin cytoskeleton and changes in VE-Cadherin (VE-Cad) localization at the cell-cell junctions was analyzed by fluorescence microscopy. Expression levels were determined by Western blotting.

Results si-RNA transfection significantly reduced VWF expression (86 ± 8,08 %) in HUVECs compared to si-Neg controls. Downregulation of VWF induced an increase in permeability (544,15 ± 287,21 %) of endothelial monolayers compared to controls (▶ **Fig. 1a**) which was accompanied by derangements of actin cytoskeleton and loss of VE-Cad from cellular junctions. Downregulation of VWF enhanced stress fiber formation and loss of peripheral actin from cell borders and increased intercellular gap formation in HUVEC monolayers compared to controls. A reduced expression of VE-Cad was observed in VWF downregulated cells (▶ **Fig. 1b**).



▶ **Fig.1 Endothelial cell permeability and intercellular gap formation after downregulation of VWF expression;** (A) Endothelial cell permeability after 48 h of siRNA-transfection (si-VWF, blue) or control siRNA (si-Neg, green) using the JetPRIME transfection reagent (orange) or non-transfected (Ctr, White) in HUVECs. 100 % confluent monolayers were incubated with HRP-conjugated streptavidin and absorption was measured using an ELISA plate reader and considered as relative permeability. (B) Representative fluorescence images of F-Actin (red), VWF (green) and VE-cadherin (red) were taken using fluorescence microscope (BZ-X810, Keyence corporation Osaka, Japan). Scale bar: 50 µm.

Conclusion These data indicate that VWF plays an important role in the integrity of endothelial monolayers, as its downregulation induces loss of endothelial barrier function. Preservation of endothelial monolayer integrity may offer a new therapeutic maneuver for the treatment of angiodysplasia.

Conflict of Interest None

T-07-30 Von Willebrand disease in Hungary – results of the national von Willebrand registry

Authors A. Selmeczi[1], L. Nemes[1], Z. Boda[2], Á. Schlammadinger[2], I. Szegedi[3], J. Göncziné Kiss[3], M. Zombori[5], K. Vezendi[4], I. Marton[4], H. Alizadeh[7], Z. Bereczky[6], C. Kiss[3]

Institutes **1** Central Hospital of Northern Pest, National Haemophilia Center, Budapest, Hungary; **2** University of Debrecen, Faculty of Medicine, Department of Internal Medicine, Division of Haematology, Debrecen, Hungary; **3** University of Debrecen, Faculty of Medicine, Department of Pediatrics, Division of Pediatric Hematology-Oncology, Debrecen, Hungary; **4** University of Szeged, Albert Szent-Györgyi Medical School, Department of Transfusion Medicine, Szeged, Hungary; **5** Heim Pal National Institute of Pediatrics, Hemato-oncology Unit, Budapest, Hungary; **6** University of Debrecen, Faculty of Medicine, Department of Laboratory Medicine, Division of Clinical Laboratory Science, Debrecen, Hungary; **7** University of Pécs Medical School, Clinical Center, 1st Department of Medicine, Division of Haematology, Pécs, Hungary

DOI 10.1055/s-0044-1779131

Introduction The Hungarian von Willebrand patient registry contains data on patient demographic characteristics and treatment modalities.

Method The registry collected data on 1201 patients from 9 treatment centers, representing about 85 % of all patients diagnosed with von Willebrand disease (vWD) in Hungary. With the participation of 4 treatment centers, all events requiring treatment were recorded in the registry over a 24-month period from 1 June 2019 to 31 May 2021. The 4 centers included the largest adult and pediatric care centers, and two additional comprehensive centers, which together provide care for 1047 patients, covering around 87 % of patients in the registry.

Results The median age was 45 years and 13 % of patients (155) were under 18 years of age. The majority of registered patients, 66 % (798), were women. The distribution of the ABO blood group system among the patients was as follows: 34 % O, 32 % A, 10 % B, and 4 % AB. Typing and subtyping of the disease were carried out in 61 % (732) of the patients. 41 % of patients (494) were registered with type 1, 17 % (204) type 2, of which 2A 12 %, 2M 2 %, 2N 2 %, 2B 1 %, and 3 % (36) with type 3 vWD. In 14 % of type 1 patients, von Willebrand factor activity – in terms of ristocetin cofactor activity (vWF:RCo) was below 15 %. 85 patients were infected with HCV during their lifetime, 657 patients were found to be negative, while in 461 cases HVC status was not reported in the registry. 32 patients have been receiving continuous prophylaxis with a von Willebrand factor concentrate.

Over the 24-month period, a total of 268 bleeding events in 75 patients required treatment in the participating 4 centers. The most common bleeding manifestation was nosebleeds, which accounted for 33 % of all bleeding events. This was followed by gingival bleeding and gynecological bleeding. In terms of the quantity of coagulation factor concentrate used for the bleeding events, the most significant condition was angiodysplasia, which was reported in 3 patients, but consumed 67 % of the total amount of factor used for bleeding control. A total of 242 interventions were performed in 24 months in the 4 large treatment centers, of which 179 were minor interventions and 42 were major surgeries. In addition, 15 deliveries were considered to require hematological support. In 30 cases DDAVP, while in the other cases factor concentrate was used to support the intervention. Bleeding complication requiring further treatment occurred in 4 cases.

Conclusion With 1021 patients registered, vWD is the most frequent bleeding disorder in Hungary. The Hungarian von Willebrand patient registry, covering 85 % of patients, provides representative data on demographics and treatment needs of patients with vWD. Based on these data, treatment centers together with the National Health Insurance Fund Administration may optimize patient care.

Conflict of Interest Data collection was sponsored by CSL Behring.

T-07-31 Physical activity, bone mineral density, and lean mass in patients with haemophilia

Authors P. Ransmann[1], M. Brühl[1, 2], J. Hmida[1, 2], F. Tomschi[1], G. Goldmann[3], J. Oldenburg[3], D. Wirtz[2], T. Hilberg[1], A. C. Strauss[2]

Institutes **1** University of Wuppertal, Department of Sports Medicine, Wuppertal, Germany; **2** University Hospital Bonn, Department of Orthopedics and Trauma Surgery, Bonn, Germany; **3** University Hospital Bonn, Haemophilia Care Centre, Bonn, Germany

DOI 10.1055/s-0044-1779132

Introduction Prior investigations point towards the fact that patients with haemophilia are affected by reduced bone mineral density (BMD), which is directly linked to physical activity (PA). Based on prior literature, it is assumed that patients with severe haemophilia are physically less active compared to patients with moderate or mild haemophilia. This study aims to objectively evaluate the PA with regard to BMD and lean mass in dependance to severity phenotype.

Method This investigation was part of a large prospective cohort study examining the relation between osteoporosis and haemophilia (registered at clinicaltrials.gov (ID: NCT04524481)). 223 Patients underwent a dual X-ray screening to examine both bone mineral density (BMD) and lean mass of the four extremities. This assessment was conducted and analyzed using Horizon™ (Hologic, USA; Apex Software, V. 13.6.0.7). Supporting to the lean mass, circumferences of the extremities were examined via the haemophilia joint health score (HJHS). Further, step tracked electronically for 7 days consecutive after examination, augmented by a 7-day subjective activity diary.

Results Data of 197 patients with either mild (N = 45), moderate (N = 46), and severe (N = 132) haemophilia A or B were analyzed. PwH had a mean age of 43.6 ± 15.6 years. Step activity was similar between severity phenotypes ($p = 0.162$). Subjective activity data revealed that the most frequent type of physical activity in all severities was walking (n = 72), cycling (n = 60), and strength training (n = 60). Lean mass of the upper and lower (mean value of left and right) extremities differed significantly between severity phenotypes (legs: $p = 0.028$; arms: $p = 0.017$; Bonferroni corrected post-hoc: legs: $p > 0.005$, arms

p = 0.022 between severe and moderate phenotype). The circumference of thighs and arms did not differ between severities, though the shanks are significantly smaller in patients with severe haemophilia compared to patients with moderate haemophlia (p = 0.005; Bonferroni corrected post-hoc: left shank p = 0.010, right shank: p = 0.020). Both, lean mass and circumference of the extremities correlated positively with BMD (see ▶ **Fig. 1**), though not with step activity nor subjective activity data (p > 0.05).

Variable	BMD (neck left)
Activity (min)	r=0.001 p=0.987
Lean mass arms (g)	r=0.336 p<0.001**
Lean mass legs (g)	r=0.390 p<0.001**
Circumference arms (cm)	r =0.213 p=0.003*
Circumference shanks (cm)	r =0.341 p<0.001**
Circumference thighs (cm)	r =0.339 p<0.001**

▶ **Fig. 1 Spearmans's correlation coefficient of activity, lean mass, and circumference of extremity**; Explanation: BMD = bone mineral density *indicates significant difference of p < 0.05, **indicates significant difference of p < 0.001

Conclusion The present study shows that step activity does not differ between the severity phenotypes, though lean mass of the extremities does. Higher lean mass seems to positively influence BMD. However, neither step activity nor the duration of subjective reported activity seems to affect BMD. It needs to be highlighted that only step activity is tracked electronically and most of the patients performed walking as PA. Though, it is assumed that the type of activity plays an important role on bone metabolism, as strength training is most efficient in increasing extremity circumference as well as lean mass and has been shown to oppose reduction of BMD. Hence, it is highly recommended to promote whole body strength training in PwH.

Conflict of Interest This study was supported by Bayer Vital AG

T-07-32 Emicizumab Prophylaxis for the Treatment of Infants with Severe Haemophilia A without Factor VIII Inhibitors: Primary Analysis of the HAVEN 7 Study

Authors J. Oldenburg[1], S. W. Pipe[2], P. Collins[3], C. Dhalluin[4], G. Kenet[5, 6], C. Schmitt[4], M. Buri[4], V. Jiménez-Yuste[7], F. Peyvandi[8, 9], G. Young[10], M. E. Mancuso[11, 12], K. Kavakli[13], A. Kiialainen[3], M. Niggli[3], T. Chang[14], M. Lehle[3], K. Fijnvandraat[15]

Institutes **1** University Hospital Bonn, Institute of Experimental Hematology and Transfusion Medicine, Bonn, Germany; **2** University of Michigan, Ann Arbor, USA; **3** Cardiff University, School of Medicine, Cardiff, UK; **4** F. Hoffmann-La Roche Ltd, Basel, Switzerland; **5** Sheba Medical Center, Ramat Gan, Israel; **6** Tel Aviv University, Tel Aviv, Israel; **7** Universidad Autónoma, Hospital Universitario La Paz-IdiPaz, Madrid, Spain; **8** Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Milan, Italy; **9** Università degli Studi di Milano, Milan, Italy; **10** Children's Hospital Los Angeles, Los Angeles, USA; **11** IRCCS Humanitas Research Hospital, Rozzano, Italy; **12** Humanitas University, Milan, Italy; **13** Ege University Faculty of Medicine Children's Hospital, Izmir, Turkey; **14** Spark Therapeutics, Inc, San Francisco, USA; **15** University of Amsterdam, Amsterdam, Netherlands

DOI 10.1055/s-0044-1779133

Introduction Venous access presents a significant challenge in infants with haemophilia A (IwHA) requiring prophylaxis.Subcutaneous emicizumab enables prophylaxis from birth, reducing bleed risk. The HAVEN 7 (NCT04431726) primary analysis evaluates emicizumab prophylaxis over ≥ 52 weeks (wks) in IwHA.

Method IwHA in the Phase 3b, open-label study were aged ≤ 12 months (m) without factor (F)VIII inhibitors. They received emicizumab 3mg/kg maintenance dose every 2wks (Q2W) for 52wks, continuing emicizumab for 7 years' planned follow-up. Endpoints include efficacy (negative binomial regression model-based annualised bleed rates [ABR] for treated, all, treated spontaneous, and treated joint bleeds), safety, pharmacokinetics, anti-emicizumab antibodies (ADAs), FVIII inhibitors, and biomarkers (biomarker results are not detailed here, but will be included in the event of an associated presentation).

Results At data cut-off (22 May, 2023),55 male IwHA had received emicizumab for ≥ 52wks (54.5 % previously minimally treated [≤ 5 exposure days, EDs], and 45.5 % previously untreated [PUP]). Median (range) age: 4m (9 days–11m 30 days) at enrolment; 29 (12–39) m at cut-off. Median (range) treatment duration: 100.3 (52–118) wks.

Mean ABRs (95 % confidence interval) for treated, all, and treated joint bleeds were 0.4 (0.30–0.63), 2.0 (1.49–2.66), and 0.0 (0.01–0.09), respectively. Overall, 207 bleeds occurred in 46 IwHA (83.6 %), 87.9 % of which were traumatic. Of the 207 total bleeds, 42 bleeds in 25 IwHA were treated, all traumatic. Thirty (54.5 %) IwHA had zero treated bleeds, and no IwHA had > 3 treated bleeds. No intracranial haemorrhage occurred. One IwHA was up-titrated (Day 374) to 3mg/kg weekly per investigator request based on locally assessed decreasing emicizumab levels. Nine IwHA (16.4 %) had ≥ 1 treatment-related adverse event (AE), all Grade 1 injection-site reaction. No AE led to emicizumab change/withdrawal. No deaths/thrombotic events/thrombotic microangiopathies occurred. Mean steady-state emicizumab concentrations were 57–66µg/mL, above those with the same regimen in HAVEN 2/3 (46–48µg/mL). No IwHA developed ADAs. Two PUPs developed confirmed inhibitors after three and ten FVIII EDs, respectively.

Conclusion This analysis suggests that emicizumab is efficacious and well tolerated in IwHA without FVIII inhibitors.

Conflict of Interest **JO:** Consultancy: Working Group Blood of the Ministry of Health, Bayer, Biogen Idec, Biomarin, Biotest, Chugai, CSL-Behring, Freeline, Grifols, LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Sparks, Swedish Orphan Biovitrum, Takeda; Employment: University Clinic Bonn; Research Funding: Bayer, Biotest, Chugai, CSL-Behring, Novo Nordisk, Octapharma, Pfizer, Roche, Swedish Orphan Biovitrum, Takeda; Honoraria: Bayer, Biogen Idec, Biomarin, Biotest, Chugai, CSL-Behring, Freeline, Grifols, LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Sparks, Swedish Orphan Biovitrum, Takeda; Speakers Bureau: Bayer, Biogen Idec, Biomarin, Biotest, Chugai, CSL-Behring, Freeline, Grifols, LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Sparks, Swedish Orphan Biovitrum, Takeda; Membership on an entity's Board of Directors or advisory committees: Stiftung Hamotherapie-Forschung, Working Group Richtlinien zur Gewinnung von Blut und Blutbestandteilen und zur Anwendung von Blutprodukten (Hamotherapie) of the Scientific Advisory Board of the German Medical Association; Other: Reimbursement of travel expenses: Bayer, Biogen Idec, Biomarin, Biotest, Chugai, CSL-Behring, Freeline, Grifols, LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Spark, Swedish Orphan Biovitrum, Takeda; **SWP:** Consultancy: Apcintex, ASC Therapeutics, Bayer, BioMarin, CSL Behring, NEMA Biologics, Freeline, LFB, Novo Nordisk, Pfizer, Regeneron/Intellia, Roche/Genentech, Sanofi, Takeda, Spark Therapeutics,

Uniqure; Research Funding: Siemens, Yewsavin; Membership on an entity's Board of Directors or advisory committees: GeneVentiv, Equilibrium Biosciences; **PC:** Membership on an entity's Board of Directors or advisory committees: HAVEN 7 trial steering committee; **CD:** Employment: F. Hoffmann-La Roche Ltd; **GK:** Employment: Sheba Medical Center and Sackler Faculty of Medicine, Tel Aviv University; Consultancy: ASC Therapeutics, Bayer, BioMarin, Novo Nordisk, Pfizer, Roche, Sanofi- Genzyme, Sobi, Takeda; Research Funding Research Funding: BSF, Opko Biologics, Pfizer, Roche, Shire; Membership on an entity's Board of Directors or advisory committees: PedNet foundation; **CS:** Employment: F. Hoffmann-La Roche Ltd; Patents & Royalties: Co-inventor of a patent related to an anti- FIXa/FX bispecific antibody: Anti- FIXa/FX bispecific antibody; **MB:** Employment: F. Hoffmann-La Roche Ltd; **VJY:** Consultancy/ Research Funding/ Honoraria: Grifols, Novo Nordisk, F. Hoffmann-La Roche Ltd, Takeda, Bayer, CSL Behring, Pfizer, BioMarin, Sanofi, Sobi, Spark, Octapharma; FP: Membership on an entity's Board of Directors or advisory committees: Biomarin, Sanofi, Sobi, CSL Behring; F. Hoffmann-La Roche Ltd; Speakers Bureau: Spark, Takeda; **GY:** Patents & Royalties: Viatris; Research Funding: Genentech, Inc.; Consultancy and Research Funding: Takeda, Consultancy and Speakers Bureau: CSL Behring, Sanofi Genzyme, Spark, Consultancy: Hema Biologics/LFB, Genentech/ Roche, Novo Nordisk; Speakers Bureau: Hema Biologics; **MEM:** Consultancy/ Honoraria: Bayer, CSL Behring, Novo Nordisk, Pfizer, Sobi, Sanofi, Biomarin, Octapharma, Roche, LFB, Takeda; Employment: IRCCS Humanitas Research Hospital; Research Funding: CSL Behring, Bayer, Novo Nordisk, Takeda; Speakers Bureau: Bayer, CSL Behring, Novo Nordisk, Pfizer, Sobi, Sanofi, Biomarin, Octapharma, Roche; **KK:** Consultancy, Honoraria and Research Funding: F. Hoffmann-La Roche Ltd, Novo Nordisk, Takeda, Pfizer; Membership on an entity's Board of Directors or advisory committees: BioMarin, CSL Behring, Novo Nordisk, Pfizer; **AK:** Employment: F. Hoffmann-La Roche Ltd; TC: Employment: Spark Therapeutics; Current equity holder: F. Hoffmann-La Roche Ltd.; **ML, MN:** Employment: F. Hoffmann-La Roche Ltd; **KF:** Consultancy and Research Funding: Sobi, Novo Nordisk; Consultancy: Sanofi, F. Hoffmann-La Roche Ltd; Research Funding: CSL Behring

T-07-33 Unraveling the Molecular Pathogenesis and Mutation Spectrum of Quantitative VWD: Type 1, Low VWF, and Type 3

Authors A. Krahforst[1], H. Yadegari[1], B. Pezeshkpoor[1], A. Pavlova[1], S. Halimeh[2]

Institutes **1** University Hospital Bonn, Institute of Experimental Hematology and Transfusion Medicine, Bonn, Germany; **2** Gerinnungszentrum Rhein-Ruhr (GZRR), Duisburg, Germany

DOI 10.1055/s-0044-1779134

Introduction Von Willebrand disease (VWD) is the most prevalent hereditary bleeding disorder, stemming from either a quantitative deficiency or functional defect in von Willebrand factor (VWF), a vital plasma protein crucial for hemostasis. This study presents a comprehensive analysis of laboratory phenotypes and mutation spectra in a substantial cohort of patients with quantitative VWF deficiencies.

Method Our analysis involved 221 individuals, all of Caucasian origin, with diagnoses of VWD. We conducted a comprehensive assessment, including multiple VWF assays (VWF:Ag, VWF:GPIbR/or VWF:RCo, VWF:CB, and multimer analysis), as well as FVIII coagulant activities. Extensive genetic analyses were undertaken, encompassing Sanger sequencing/ or targeted panel next-generation sequencing, copy number variation analysis, and predictive bioinformatic evaluations.

Results Upon examination of laboratory tests, the index patients (IPs) were classified based on the updated ISTH-SSC VWF guidelines. Within this cohort, 77 IPs were diagnosed with type 1 VWD, with VWF:Ag below 30 IU/dL (%). An additional 111 patients displayed VWF levels ranging from 30 % to 50 % and were categorized as type 1/Low VWF. An additional 33 IPs were classified as having type 3 VWD. The mutation detection rates for these subcohorts were 88 %, 66 %, and 92 %, respectively. A total of 228 VWF variants were identified in the cohort with quantitative VWF deficiencies, including 60 novel variants (26 %). In the type 1 VWD cohort with VWF levels below 30 %, we discovered 81 gene variations, comprising 57 % missense variants, 41 % null alleles, and rare gene conversion and promoter variants. Large deletions and splice site (ss) variants predominated among null alleles. In the Low VWF/type 1 subgroup, featuring VWF levels between 30 % and 50 %, we identified 84 variants. These included 62 % missense variants, 34 % null alleles, and 4 % promoter variants. In this group, small deletions and ss variants constituted the majority of null alleles. Remarkably, overexpression of blood group O prevalence (67 % compared to 45 % in the Caucasian population) was observed within the Low VWF/type 1 subgroup, especially among mutation-negative individuals (83 %). This suggests a potential association between the O blood group and reduced VWF:Ag levels in this subgroup. In the type 3 VWD cohort, a total of 63 variants were detected, including 25 % missense variants and 75 % null alleles. Small deletions and nonsense mutations were the most prevalent null allele variants.

Conclusion Data from this extensive patient cohort enhance our understanding of the molecular underpinnings and pathophysiological mechanisms underlying of quantitative VWD. Significant distinctions in mutation patterns were observed among subgroups of quantitative VWD. Notably, the contrast between Type 1 VWD with VWF levels under 30 % and Low VWF/Type 1 VWD with VWF levels between 30-50 % is increasingly prominent in contemporary research.

Conflict of Interest The authors declare no conflicts of interest.

T-07-34 A Unique Clinical Case of Using Emicizumab as an Effective Therapeutic Approach for Refractory Acquired Hemophilia

Authors L. Hambardzumyan[1], I. Pabinger[2], G. Tamamyan[3, 4], S. Danelyan[6], S. Araqelyan[1], L. Petrosyan[1], E. Vardumyan[1], N. Sargsyan[1, 3], H. Khachatryan[1, 3]

Institutes **1** Yeolyan Hematology and Oncology Center, Hemophilia and Thrombosis Center, Yerevan, Armenia; **2** Medical University of Vienna, Clinical Division of Hematology and Hemostaseology, Vienna, Austria; **3** Yerevan State Medical University, Department of Pediatric Oncology and Hematology, Yerevan, Armenia; **4** Yeolyan Hematology and Oncology Center, Pediatric Cancer and Blood Disorders Center of Armenia, Yerevan, Armenia; **5** Immune Oncology Research Institute, Yerevan, Armenia; **6** Yeolyan Hematology and Oncology Center, Yerevan, Armenia

DOI 10.1055/s-0044-1779135

Introduction Acquired Hemophilia A (AHA) is a rare hemorrhagic disorder characterized by spontaneous bleeding resulting from autoantibodies targeting clotting factor VIII. While the standard treatment for AHA involves immunosuppressive therapy, a subset of patients remains unresponsive to these conventional approaches, necessitating alternative treatment strategies. Emicizumab, a bispecific antibody mimicking activated factor VIII, has demonstrated promise in managing congenital Hemophilia A. Nevertheless, limited evidence exists regarding its efficacy in treating AHA.

This case report seeks to provide compelling evidence supporting emicizumab as an effective alternative treatment for refractory AHA patients.

Method We present a case of a 30-year-old female patient with the anamnesis of undifferentiated connective tissue disease and psoriasis diagnosed with AHA at 6 months postpartum. At the time of diagnosis, the patient presented with extensive ecchymoses and heavy uterine bleeding. Factor VIII activity was 2,8 % and the inhibitor level was 4,3 Bethesda unit. The patient also reported a family history of systemic lupus erythematosus diagnosed in her mother. Treatment with corticosteroid, hydroxychloroquine therapy, and plasmapheresis were ineffective, in addition to above mentioned unresolved bleeding manifestations she periodically developed hematuria. Emicizumab was administered subcutaneously at a weekly dosage of 3 mg/kg during the first 4 weeks, followed by 2 additional injections every 2 weeks in conjunction with methylprednisolone 8 mg for 3 weeks. Continuous monitoring was conducted through physical examinations and laboratory assessments over a six-month period.

Results Following the administration of emicizumab and during the subsequent six-month follow-up period after emicizumab discontinuation, the patient's activated partial thromboplastin time (APTT) normalized from 96.1 to 27.8 seconds, factor VIII activity improved to 108-112 % and inhibitor level decreased to 0,73-0,66 Bethesda unit. Over the course of the six-month follow-up period after stopping emicizumab, the patient remained free from any bleeding episodes and reported significant enhancement in her quality of life with no emicizumab-related adverse effects observed.

Conclusion Emicizumab emerges as a secure and efficacious therapeutic option for patients grappling with AHA who manifest resistance to conventional immunosuppressive interventions. Further investigations are warranted to deepen our comprehension of the intricate relationship between autoimmune disorders and acquired hemophilia. Nevertheless, this distinctive clinical case from Armenia contributes to the accumulating body of evidence affirming the therapeutic potential of emicizumab in addressing AHA. Notably, the patient's sustained remission without detectable antibodies and restored factor VIII levels post-treatment further distinguishes this clinical case.

Conflict of Interest The authors declare no conflict of financial or personal interests.

T-07-35 Phenotype-genotype correlation in patients with non-severe haemophilia A and intronic variants of *F8*

Authors D. Provaznikova[1], A. Radovska[1], M. Simonikova[1], P. Salaj[2], E. Drbohlavova[3], P. Smejkal[4], E. Zapotocka[5], I. Hrachovinova[1]

Institutes **1** Institute of Hematology and Blood Transfusion, NRL for Disorders in Hemostasis, Prague, Czech Republic; **2** Institute of Hematology and Blood Transfusion, Center for Thrombosis and Hemostasis (CTH), Prague, Czech Republic; **3** Hospital Liberec, Department of clinical hematology, Liberec, Czech Republic; **4** University Hospital Brno, Department of Clinical Hematology, Brno, Czech Republic; **5** University Hospital Motol, Department of Pediatric Hematology/Oncology, Prague, Czech Republic

DOI 10.1055/s-0044-1779136

Introduction Despite the high causal variants detection rate in haemophilia A (HA) overall, there was still about 26 % of Czech non-severe haemophiliacs, where no causal variant was detected in exons. We were looking for pathological variants in these cases in the non-coding part of the gene *F8*.

Method We examined 278 patients from 182 families with non-severe HA in total. VWF activity, antigen and binding to FVIII were established for excluding VWD type Normandy. FVIII coagulation activity was measured with chromogenic and coagulation method (FVIII:C). DNA was isolated; where available cDNA was also obtained. Sanger sequencing was used for the causal variant detection. Where no variant was found, we used NGS for screening the whole *F8* gene focusing on introns. The method MLPA was performed to exclude whole exons deletion or duplication. The pathogenic impact of identified candidate variants was assessed using *in silico* analysis and functional analysis cDNA. Segregation analyses was performed where the relatives were available.

Results The causal variants of 205 non-severe haemophiliacs were found in exons and very close intronic region (within 3 bp from exons). The analysis of the whole *F8* intronic region was performed in remaining 73 (26 %) patients. We found nine different intronic variants. These variants included six single nucleotide substitutions in introns 6, 10, 16, 22 and 25, two deletions in intron 13, one insertion in intron 4 and deletion of exon 19. Six of them were described as associated with non-severe HA before [1–4]. Our most common causal variant detected was c.778-14T > G in intron 6 first reported by Reitter in association with severe form of HA and causing skipping the whole exon 7. However, our results differ from his. We detected this variant only in non-severe haemophiliacs, their FVIII:C was in range 6-30 IU/dL. The analysis of cDNA revealed that this variant probably creates de novo splicing site within the exon, which leads to partial deletion of the exon 7 from the 5'end and is predicted as in frame deletion. We found three novel variant: 1. A single nucleotide insertion in intron 4, other causal variants were already detected in this region. 2. Two short deletions close together in intron 13. 3. Third novel variant was skipping exon 19 detected on cDNA level, which is predicted as delins variant. The cause was not yet identified. Phenotype of all patients inside of our four most common intronic variants is highly various, FVIII:C range from 6 to 44 IU/dL, even in one family is wide range of FVIII:C results. Cause of this might be a different amount of co-expression of normal cDNA lengths.

Conclusion The intronic variants are associated with 26 % of non-severe Czech haemophiliacs. The phenotype of these patients is very variable depending on expression of nonhaemophiliac allele. Probably this finding might be used for a novel concept of personal treatment of this non-severe HA.

Conflict of Interest None.

References

[1] Klopp N, Oldenburg J et al. '11 hemophilia A patients without mutations in the factor VIII encoding gene', Thromb Haemost. 2002: 88 (2): 357–60 Thieme;

[2] Reitter S, Sturn R et al. 'Spectrum of causative mutations in patients with haemophilia A in Austria', Thromb Haemost. 2010: 104 (1): 78–85 Thieme;

[3] Bach JE, Wolf B et al. 'Identification of deep intronic variants in 15 haemophilia A patients by next generation sequencing of the whole factor VIII gene', Thromb Haemost. 2015: 114: 757–767 Thieme;

[4] Jourdy Y, Janin A et al. 'Reccurrent F8 Intronic Deletion Found in Mild Hemophilia A Causes Alu Exonization'. Am J Hum Gen 2018; 102 (2): 199–206. PMC

T-07-36 Assessing Adherence to Prophylactic Treatment in Patients with Haemophilia from Five European Countries

Authors P. Milanov[1], S. Roth[1], A. Griesheimer[2], J. Weiss[3], K. B. Holm[4], A. J. Sumera[5], B. M. Ziemele[6], W. Miesbach[1]

Institutes **1** University Hospital Frankfurt, Goethe University, Haemophilia Care Centre, Frankfurt am Main, Germany; **2** Deutsche Hämophiliegesellschaft (DHG), Hamburg, Germany; **3** Österreichische Hämophilie Gesellschaft (ÖHG), Wien, Austria; **4** Danish Haemophilia Society, Taastrup, Denmark; **5** Polish Haemophilia Society, Warsaw, Poland; **6** Latvia Hemophilia Society, Riga, Latvia

DOI 10.1055/s-0044-1779137

Introduction Adherence to prophylactic treatment is a critical factor in the management of haemophilia, impacting patient outcomes. Prophylactic treatment not only reduces bleeding episodes but also prevents debilitating haemarthropathy. Reported adherence rates vary widely across countries, ranging from 4.1 % to 94.2 % [1–5], highlighting the critical importance of adherence in haemophilia care. Higher adherence has consistently been linked to improved clinical outcomes, especially in severe haemophilia. We employed the VERITAS-Pro method [6] to quantitatively assess adherence in patients of all ages from five different countries.

Method We employed the validated Haemophilia Regimen Treatment Adherence Scale-Prophylaxis (VERITAS-Pro) questionnaire, which assesses various adherence subscales, including time, dose, plan, remember, skip, and communicate. Participants from Germany and Austria completed paper-based questionnaires, while those from Denmark, Poland, and Latvia responded to an online survey. Data collection occurred between 2017 and 2020, and data analysis was performed using the R programming language for statistical computing.

Results Our analysis included a total of 363 patients from Germany, 59 from Austria, 32 from Denmark, 122 from Poland, and 18 from Latvia, as presented in ▶ **Fig. 1**. The percentage of young participants (0-14 years) ranged from 16.3 % to 50 %, with the highest number of participants from Latvia and the lowest from Germany and Austria. Reported regular prophylactic treatment varied from 79.7 % to 88.9 %. The rate of treatment in Haemophilia Centres ranged from 16.7 % to 100 %, with the lowest rate in Latvia and the highest in Denmark. Adherence rates calculated in our study ranged from 77.8 % to 90.6 %, with the lowest adherence observed in Latvia and the highest in Germany. As

Hämostaseologie 2024; 44: S1–S125

previously reported [5], the median total score for younger patients (0-14 years) was lower than the total score for other age groups (▶ **Fig. 2**) among all countries, indicating a higher adherence tendency in younger patients compared to adolescents. Additional results, including an analysis of scores across the six distinct subscales and the influence of specific factors on treatment adherence, will be presented at the congress meeting.

Table 1. Patient characteristics

Variable	% (No. of patients)				
Age of patients	**Germany (n = 363)**	**Austria (n = 59)**	**Denmark (n = 32)**	**Poland (n = 122)**	**Latvia (n = 18)**
0-14	16.3 (59)	15.3 (9)	21.9 (7)	17.2 (21)	50.0 (9)
15-19	6.1 (22)	6.8 (4)	0	1.6 (2)	5.6 (1)
20-59	55.1 (200)	67.8 (40)	56.2 (18)	66.4 (81)	33.3 (6)
Within: 20-29	10.2 (37)	20.3 (12)	9.4 (3)	12.3 (15)	11.1 (2)
30-39	10.2 (37)	16.9 (10)	15.6 (5)	17.2 (21)	0
>=60	21.8 (79)	6.8 (4)	15.6 (5)	14.8 (18)	11.1 (2)
No data	0.8 (3)	3.4 (2)	6.2 (2)	0	0
Type of haemophilia					
haemophilia A	82.6 (300)	88.1 (52)	81.2 (26)	82.0 (100)	77.8 (14)
haemophilia B	14.9 (54)	11.9 (7)	18.8 (6)	17.2 (21)	22.2 (4)
no comment	2.5 (9)	0	0	0.8 (1)	0
Degree of severity					
severe	87.1 (316)	89.8 (53)	87.5 (28)	94.3 (115)	66.7 (12)
moderate	7.2 (26)	8.5 (5)	3.1 (1)	4.1 (5)	27.8 (5)
mild	4.4 (16)	0	6.2 (2)	1.6 (2)	5.6 (1)
no comment	1.1 (4)	0	0	0	0
No data	0.3 (1)	1.7 (1)	3.1 (1)	0	0
Treatment modalities					
Haemophilia Centre	78.0 (283)	66.1 (39)	100.0 (32)	41.8 (51)	16.7 (3)
prophyl. subst. regulary	81.8 (297)	79.7 (47)	78.1 (25)	81.1 (99)	88.9 (16)
prophyl. subst. occasionally	8.0 (29)	11.9 (7)	12.5 (4)	13.1 (16)	11.1 (2)
Pain level					
independent without pain	40.8 (148)	49.2 (29)	37.5 (12)	29.5 (36)	44.4 (8)
independent with mild pain	42.1 (153)	40.7 (24)	40.6 (13)	52.5 (64)	44.4 (8)
independent with strong pain	14.6 (53)	6.8 (4)	18.8 (6)	14.8 (18)	0
in need of wheelchair	9.9 (36)	3.4 (2)	3.1 (1)	2.5 (3)	11.1 (2)
Pain level (pain face 0 - 10)					
0	32.2 (117)	45.8 (27)	46.9 (15)	27.0 (33)	50.0 (9)
2	17.9 (65)	25.4 (15)	21.9 (7)	27.0 (33)	33.3 (6)
4	19.3 (70)	10.2 (6)	21.9 (7)	26.2 (32)	5.6 (1)
6	11.6 (42)	6.8 (4)	6.2 (2)	12.3 (15)	11.1 (2)
8	2.8 (10)	1.7 (1)	3.1 (1)	5.7 (7)	0
10	0.6 (2)	0	0	0.8 (1)	0
No data	15.7 (57)	10.2 (6)	0	0.8 (1)	0
Co-morbidities					
Hepatitis C	40.5 (147)	37.3 (22)	34.4 (11)	40.2 (49)	22.2 (4)
HIV	19.3 (70)	20.3 (12)	12.5 (4)	0	0
other	44.6 (162)	22.0 (13)	15.6 (5)	78.7 (96)	16.7 (3)

▶ **Fig. 1** Patient characteristics

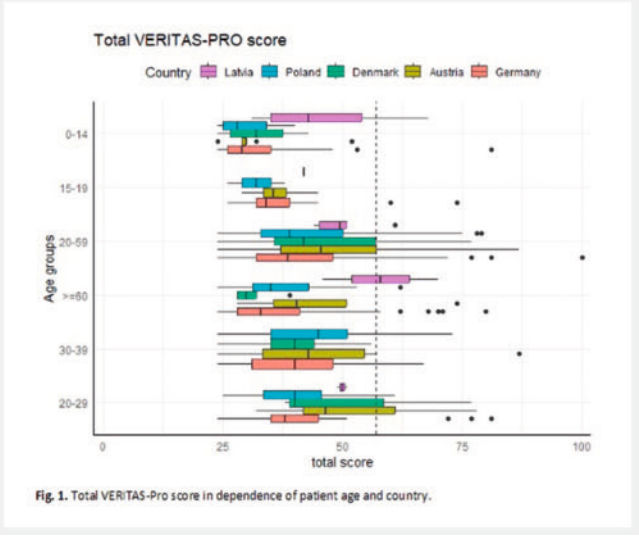


Fig. 1. Total VERITAS-Pro score in dependence of patient age and country.

▶ **Fig. 2** Total VERITAS-PRO score

Conclusion Our findings reveal substantial variability in adherence to prophylactic treatment among haemophilia patients across five different countries and different ages. These differences underscore the need for tailored interventions to improve adherence in specific patient populations. Understanding the factors influencing adherence can help healthcare providers develop strategies to enhance patient outcomes and reduce the burden of haemophilia-related complications. Further research is necessary to explore the determinants of adherence and to design effective interventions for enhancing adherence to prophylactic therapy in patients with haemophilia.

Conflict of Interest Funding by the HERO Research Grant from Novo Nordisk. There is no conflict of interest.

References

[1] Mokhtar FM, Sathar J, Huri HZ. Medication Adherence for Haemophilia Patients: Outcome of Prophylaxis Treatment Intervention. Healthcare (Basel) 2021; 9 (12): 1702. doi:10.3390/healthcare9121702.PMID: 34946428; PMCID: PMC8701723

[2] Mokhtar FM, Sathar J, Huri HZ. Medication Adherence for Haemophilia Patients: Outcome of Prophylaxis Treatment Intervention. Healthcare (Basel) 2021; 9 (12): 1702. doi:10.3390/healthcare9121702.PMID: 34946428; PMCID: PMC8701723

[3] Zdziarska J, Górska-Kosicka M, Jamrozik M, Kozińska J, Podolak-Dawidziak M, Stefańska-Windyga E, Zawilska K, Chojnowski K. Adherence to prophylactic treatment in adult patients with severe hemophilia in Poland. Pol Arch Intern Med. 2022; 132 (7/8): 16280. doi:10.20452/pamw.16280. Epub 2022 Jun 28PMID: 35925049

[4] van Os SB, Troop NA, Sullivan KR, Hart DP. Adherence to Prophylaxis in Adolescents and Young Adults with Severe Haemophilia: A Quantitative Study with Patients. PLoS One 2017; 12 (1): e0169880. doi:10.1371/journal.pone.0169880.PMID: 28103266; PMCID: PMC5245860

[5] Miesbach W, Kalnins W. Adherence to prophylactic treatment in patients with haemophilia in Germany. Haemophilia 2016; 22 (5): e367–74. doi:10.1111/hae.13003. Epub 2016 Jun 28PMID: 27353213

[6] Duncan N, Kronenberger W, Roberson C, Shapiro A. VERITAS-Pro: a new measure of adherence to prophylactic regimens in haemophilia. Haemophilia 2010; 16 (2): 247–55. doi:10.1111/j.1365-2516.2009.02129.x. Epub 2009 Nov 18PMID: 19925631

T-07-37 The miRNAs present in LSECs play pivotal role in defining their cellular identity and have an impact on the regulation of F8 gene expression

Authors R. Al Rifai[1], M. A. Jamil[1], N. Nüsgen[1], J. Altmüller[2, 3], J. Oldenburg[1], O. El-Maarri[1]

Institutes **1** University Hospital Bonn, Institute of Experimental Haematology and Transfusion Medicine, Bonn, Germany; **2** University Medicine Berlin, Berlin Institute of Health at Charité, Berlin, Germany; **3** Faculty of Medicine and University Hospital Cologne, Cologne Center for Genomics (CCG), Cologne, Germany

DOI 10.1055/s-0044-1779138

Introduction Liver sinusoidal endothelial cells (LSECs) play a crucial role in producing coagulation Factor VIII (FVIII), a vital component for blood clotting. Hemophilia A, characterized by a deficiency in FVIII. Current treatments include FVIII replacement therapy, gene therapy employing AAV vectors, and the promising avenue of cellular therapy. However, the success of these treatments requires a deep understanding of the physiological and genetic regulation of FVIII-producing cells. MicroRNAs (miRNAs) are small RNA molecules that regulate gene expression. While specific miRNAs related to LSECs have been identified in rat studies, human-specific information is still lacking. Additionally, some studies have suggested miRNAs' involvement in hemophilia A. Previous miRNA profiling has identified unique markers distinguishing LSECs from other liver cell types, yet comprehensive exploration of miRNA's influence in the context of LSECs remains limited. This study bridges this gap by conducting a comparative analysis of miRNA expression in LSECs and other endothelial cells, offering valuable insights into unique miRNA profiles and their role in the regulation of the FVIII gene expression.

Method This study involved the preparation, sequencing, and analysis of microRNAs, with an in-depth bioinformatics analysis to identify specific microRNAs. Primary LSECs were obtained from Biozol Diagnostica Vertrieb GmbH, cultured in a specialized medium, and transfected with the selected miRNAs. After transfection, quantitative real-time PCR was conducted to measure FVIII expression. Total RNA was extracted, specific primers were used, and the data underwent statistical analysis.

Results The outcome of this exploration highlights discernible differences in microRNA expression that distinguish LSECs from both hepatocytes (193 microRNAs at $p < 0.05$) and other endothelial cells (72 microRNAs at $p < 0.05$). Notably, 134 and 35 microRNAs were found to be overexpressed in LSECs compared to hepatocytes and other endothelial cells, respectively, shedding light on the unique functions of LSECs in the liver. Our investigation identified a panel of 10 microRNAs (miR-429, miR-200b-3p, miR-200a-3p, miR-216b-5p, miR-1185-5p, miR-19b-3p, miR-192-5p, miR-122-5p, miR-30c-2-3p, and miR-30a-5p) that distinctly define LSEC identity. Furthermore, our scrutiny extended to microRNAs implicated in F8 regulation, revealing a subset – miR-122-5p, miR-214-3p, miR-204-3p, and miR-2682-5p – whose expression intricately correlates with F8 expression within LSECs.

Conclusion In summary, we have conducted the first-ever characterization of the miRNA expression profile of isolated human primary LSECs. We have identified LSEC-specific microRNAs that are either shared with the host liver organ or unique to LSECs. These LSEC-specific miRNAs play a role in regulating F8 gene expression. The identified LSEC-specific miRNAs could be potential targets for F8 gene therapy or small molecules for reprogramming into FVIII-producing LSECs.

Conflict of Interest None

T-07-38 Vitamin D and Parathyroid Hormone in Hemophilia

Authors D. Kraemmer, O. Königsbrügge, I. Pabinger, C. Ay

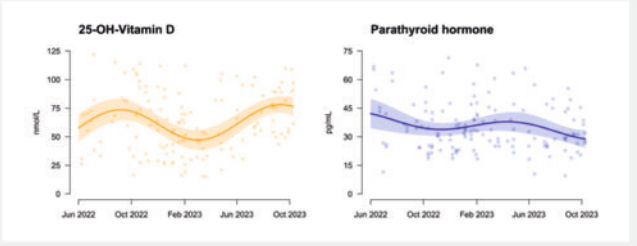
Institute Medical University of Vienna, Department of Medicine I, Clinical Division of Haematology and Haemostaseology, Vienna, Austria

DOI 10.1055/s-0044-1779139

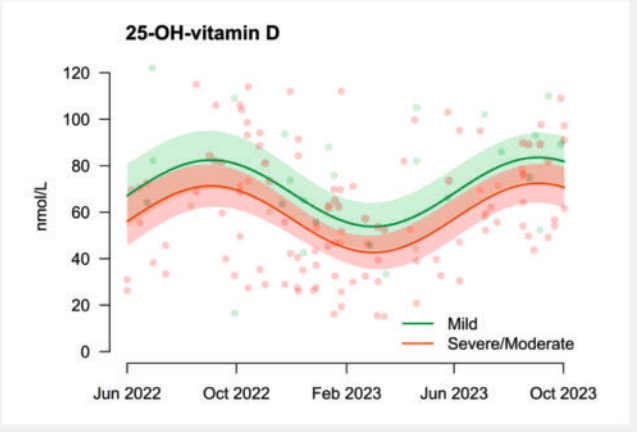
Introduction Hemophilia is a rare congenital bleeding disorder caused by deficiencies of coagulation factor VIII (A) or IX (B). The hallmark of hemophilia is recurrent hemarthroses, leading to progressive joint damage and disabling hemophilic arthropathy. Hemophilia has been frequently associated with reduction in bone mineral density, osteopenia, and osteoporosis. Here, we aimed to describe 25-OH-vitamin D (25(OH)D) and parathyroid hormone (PTH) concentrations measured in persons with hemophilia attending our clinic over more than one year.

Method We prospectively measured 25(OH)D and PTH in all persons with hemophilia of any type and severity attending the outpatient clinic of our hemophilia treatment center. To model and describe the expected cyclic trends over time, we used ordinary least squares regression fitting time as a periodic function by including a sine and a cosine term assuming a cycle length of 365.24 days. To account for the inclusion of correlated responses from cluster sampling due to multiple measurements from the same patients, we estimated a robust variance-covariance matrix using the Huber-White method.

Results Starting on 2022-06-08 up to 2023-10-09, we measured 25(OH)D and PTH (142 measurements) in 110 patients with hemophilia A (n = 98; 89 %) and B (n = 12; 11 %). The mean (SD) age of the study cohort was 41.2 years (16.3) years and comprised 70 (64 %), 13 (12 %), and 27 (25 %) subjects with severe, moderate, and mild hemophilia, respectively. Ten (9 %) subjects (eight with severe hemophilia) were on vitamin D substitution therapy at time of measurement. Over all visits, 25(OH)D and PTH were measured at a median (IQR) concentration of 62.2 nmol/L (42.8-82.2) and 32 pg/mL (25.3-43.2), respectively, showing a weak inverse correlation with a Spearman's ρ of -0.33 (95 % CI -0.47, -0.17). Mean 25(OH)D followed a circannual cycle whereas periodic changes in PTH concentrations were less apparent (▶ **Fig. 1**). Fifteen (14 %) subjects, all with hemophilia A, had 25(OH)D measurements < 30 nmol/L, the cut-off defined as indicating vitamin D deficiency [1]: 13 with severe (19 %), and one each with moderate (8 %) and mild (4 %) hemophilia. After adjustment for age, vitamin D substitution, and the cyclic time trend, severe/moderate hemophilia was associated with decreased mean 25(OH)D concentrations of -11.1 nmol/L (95 % CI -21.6, -0.6; ▶ **Fig. 2**) compared to mild hemophilia. A multiple regression of PTH concentration on the same covariates remained inconclusive.



▶ **Fig. 1** The cyclic trend of 25-OH-vitamin D and parathyroid hormone over time



▶ **Fig. 2** Mean 25-OH-vitamin D concentrations stratified by mild and severe/moderate hemophilia

Conclusion We observed a circannual change in particular of 25(OH)D and lower levels in patients with severe or moderate hemophilia. Similarly, vitamin D deficiency was more frequent in patients with severe hemophilia. This could at least partially explain an often-described higher bone loss in patients with severe hemophilia and, consequently, might suggest the need to measure vitamin D and initiate vitamin D substitution in patients with a deficiency.

Conflict of Interest The authors have no conflicts of interest to declare with regard to the present study.

References

[1] Holick MF, Binkley NC 'Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society Clinical Practice Guideline'. Clin Endocrinol Metab. 2011; 96: 1911–30. doi:10.1210/jc.2011-0385

T-07-39 Evaluation of Quantitative Osteodensitometry in children and young adults with hemophilia – Results of the EQUOHÄM Study of the KHDO

Authors L. Kanthack[1], G. Brodt[1], H.-J. Mentzel[3], R. Knoefler[4], S. Kim[5], C. Kühnöl[6], N. Hagedorn[7], K. Kentouche[1]

Institutes 1 Universitätsklinikum Jena, Klinik für Kinder- und Jugendmedizin, Jena, Germany; 2 Universitätsklinikum Jena, Klinik für Kinder- und

Hämostaseologie 2024; 44: S1–S125

Jugendmedizin, Jena, Germany; **3** Universitätsklinikum Jena, Institut für Diagnostische und Interventionelle Radiologie Sektion pädiatrische Radiologie, Jena, Germany; **4** Universitätsklinikum Dresden, Klinik und Poliklinik für Kinder- und Jugendmedizin, Dresden, Germany; **5** Kinderzentrum am Johannisplatz, Praxis, Leipzig, Germany; **6** Universitätsklinikum Halle (Saale), Klinik und Poliklinik für Pädiatrie, Halle, Germany; **7** Charité University Medicine, Department of Pediatric and Adolescent Medicine, Berlin, Germany; **8** Universitätsklinikum Jena, Klinik für Kinder- und Jugendmedizin, Jena, Germany
DOI 10.1055/s-0044-1779140

Introduction Patients with hemophilia (PWH) have an increased risk for bone fractures and decreased bone density. However, it is unclear, whether this is a result of repetitive bleeding, the development of hemophilia arthropathy or immobilisation. Recently the lack of coagulation factor itself was named to be involved in the modelling of bone architecture. Therefore, it is recommended to measure bone density in PWH with repetitive fractures. The standard for measuring bone density are DXA or pQCT, both of them radiographic methods, that utilize the absorption of x-rays by the content of calcium apatite in the bone. Quantitative Ultrasound (QUS) is another method for measuring bone density, where broadband ultrasound attenuation (BUA in dB/MHz) and Speed of Sound (SOS in m/s) and calculated stiffness index (SI) give information about density, structure and elasticity of the bone. This method is easily applied at the calcaneus and can be used repetitively and in children where x-ray exposure is of concern. Aim of the study was to measure bone density by QUS in children and adolescents with hemophilia and to compare the results to age adjusted mates without hemophilia.

Method PWH treated at 4 pediatric KHDO centers had bone density measurement by QUS at their right and left calcaneus. Their treater reported epidemiologic and anamnestic data (age, type and severity of hemophilia, treatment schedule, annual bleeding rate, history of joint bleeds). Data was compared to the results of 376 healthy males to serve as a control. The study was approved by the local ethic board.

Results 82 PWH (mean age 10.5 ± 4.1 years, HA: n = 71, HB = 11, severe = 63, moderate = 8, mild = 7, unknown = 4) were divided in 4 age groups and compared to corresponding groups without hemophilia (mean age 10.1 ± 4.5 years). All probands show a gradual increase over time in BUA from 70 (3 years) to 125 (18 years) dB/MHz and 1555 (3 years) to 1587 (18 years) m/s for SOS respectively. Whereas the youngest patients show equal BUA and SOS to their non-bleeding age mates, there is a significant difference in BUA (120 dB/MHz in controls, 105 dB/MHz in PWH) and close to significance for SOS 1595 vs 1577 m/s in the age group 15 -18 years.

Conclusion Quantitative Ultrasound Osteodensitometry is a readily available method for measuring bone density even in childhood. We have established reference values in a 3 – 18 year population. Differences between non-bleeding probands and PWH are seen from young adulthood on. This method might be useful to define patients at risk for fractures or in interventional studies on bone density when repetitive measurement is warranted.

Conflict of Interest No conflicts to declare The study was sponsored by the "joint health program" of BAYER GmbH

T-07-40 Angiodysplasia and other Vascular Structural Changes in Von Willebrand Disease

Authors R. Möller[1, 2], U. Baulain[3], B. Petersen[3], C. Detering[1], M. Ekhlasi-Hundrieser[1], K. Kaiser[1, 2], R. Zimmermann[4], C. Mühlfeld[4], M. von Depka Prondzinski[1], K. Gutensohn[1], S. Lehner[1], C. Pfarrer[2]
Institutes **1** Werlhof-Institut MVZ GmbH, Hannover, Germany; **2** University of Veterinary Medicine Hannover, Institute for Anatomy, Hannover, Germany; **3** FLI, Institute of Farm Animal Genetics, Mariensee, Germany; **4** Hannover Medical School, Institute of Functional and Applied Anatomy, Hannover, Germany
DOI 10.1055/s-0044-1779141

Introduction Von Willebrand factor (VWF) is crucial for blood coagulation, and patients with von Willebrand disease (VWD) type 3 (T3) suffer mucosal bleeding due to a complete VWF deficiency. VWF also significantly influences angiogenesis, leading to angiodysplasia in VWD patients. Our porcine model for VWD T3 clinically matches the human phenotype. Our study explores the impact of VWD on blood vessel structure. We analysed the vascular elastic fibers (EF) and vascular smooth muscle cell (VSMC) architecture in different tissues of porcine VWD T3 subjects, comparing them with wildtype pigs. Our findings illuminate intricate vascular alterations due to VWF deficiency.

Method Three pigs with a homozygous VWF genotype for VWD T3 and three wildtype pigs were chosen. Samples from uterus, stomach, and Kiesselbach plexus were processed for histology and stained with Resorcin Fuchsin and Nuclear Fast Red. The staining intensity and distribution of EF and organization of VSMC in phenotypically unremarkable blood vessels was evaluated semi-quantitatively. The EF in arterial blood vessels were investigated separately for the internal (IEL) and external elastic lamina (EEL), while in venous vessels EF were evaluated as whole. Additionally, we observed typical angiodysplasia on macroscopic and microscopic level in several VWD T3 animals.

Results In every investigated organ, EF of unremarkable blood vessels were significantly more organized in T3 animals, and the staining intensity was significantly higher (e.g. stomach veins: $p < 0.0001$, IEL stomach: $p = 0.0002$, EEL stomach: $p = 0.002$). In contrast, VSMC were significantly more organized in WT animals (e.g. stomach veins: $p < 0.0001$, stomach arteries $p = 0.0005$). Next to these findings, typical angiodysplastic vessels were seen. Macroscopic angiodysplasia, observed as pinhead to pea-sized tortuous patches infiltrated with blood beneath and within the mucosa, was identified in almost the complete gastrointestinal tract. Microscopic angiodysplasia, characterized by dilated, tortuous vessels with irregular VSMC coverage, was observed in the esophagus, stomach, jejunum, caecum, and Kiesselbach plexus. The structure was notably non-uniform, with disrupted VSMC layers and elastic fibers displaying varying staining intensity.

Conclusion Angiogenesis in VWD-affected animals seems to be altered and angiodysplasia is present in many tissues. The enhanced staining intensity and organization of EF in unremarkable blood vessels of T3 animals may indicate a compensation mechanism for the generally compromised organization of VSMC. Thus, not only obviously angiodysplastic vessels, but also the at first glance unremarkable vessels are affected by structural changes. The observed alterations could lead to destabilization, which may facilitate bleeding events in VWD affected individuals.

Conflict of Interest This study was supported financially by Octapharma, Langenfeld, Germany and Biotest AG, Dreieich, Germany.

T-07-41 Changes in hemophilia treatment in the eastern part of Germany between 2015 and 2021 – data from the Kompetenznetz Hämorrhagische Diathese Ost (KHDO)

Authors C. Pfrepper[1], A. Siecke[1], R. Klamroth[2], C. Kühnöl[3], K. Kentouche[4], S. Holzhauer[5], L. Fischer[6], V. Aumann[7], K. Trautmann-Grill[8], U. Scholz[9], I. Halm-Heinrich[10], B. Krammer-Steiner[11], K. Schilling[12], N. Hagedorn[5], R. Knoefler[13]
Institutes **1** University Hospital Leipzig, Division of Hematology and Cellular Therapy, Department of Hematology, Cellular Therapy, Hemostaseology and Infectiology, Leipzig, Germany; **2** Vivantes Clinic Friedrichshain, Internal Medicine, Vascular Medicine and Coagulation Disorders, Berlin, Germany; **3** University Hospital Halle, Department of Pediatrics, Halle (Saale), Germany; **4** University Hospital Jena, Department of Pediatrics, Jena, Germany; **5** Charité University Medicine, Department of Pediatric Hematology and Oncology, Berlin, Germany; **6** University Hospital Leipzig, Department of Pediatric Oncology, Hematology and Hemostaseology, Leipzig, Germany; **7** University Hospital Magdeburg, Department of

Pediatrics, Magdeburg, Germany; 8 University Hospital Carl Gustav Carus, Technische Universität Dresden, Medical Clinic I, Dresden, Germany; 9 Centre of Coagulation Disorders, Leipzig, Germany; 10 University Hospital Magdeburg, Department of Transfusion Medicine, Magdeburg, Germany; 11 Rostock South City Medical Centre, Department of Hematology and Oncology, Rostock, Germany; 12 University Hospital Jena, Department of Hematology and Oncology, Jena, Germany; 13 University Hospital Dresden, Technische Universität Dresden, Department of Pediatric Hematology and Oncology, Dresden, Germany
DOI 10.1055/s-0044-1779142

Introduction Treatment options for patients with hemophilia (PWH) have changed substantially in the last years. Since 2015, extended half-life factor concentrates (EHL) and the bispecific antibody emicizumab have been approved. The aim of this epidemiologic study was to compare hemophilia treatment in the eastern part of Germany in 2021 with data from 2015 prior to the approval of EHL and emicizumab.

Method Data from patients with hemophilia A (HA) and hemophilia B (HB) of the year 2021 has been retrospectively collected from 13 hemophilia care centers from the „Kompetenznetz Hämorrhagische Diathese Ost“ (KHDO) and compared with data from the KHDO from 2015 [1]. Substitution diaries, e-diaries, patient records and patient specific reports to the German hemophilia registry were analyzed.

Results A total of 487 patients (411 HA and 76 HB) were included in 2021, 359 of them were already analyzed in 2015. Among the 130 children and the 357 adults analyzed in 2021, 93 (71.5 %) children and 237 (66.4 %) adults had severe, 16 (12.3 %) children and 50 (14.0 %) adults had moderate, and 21 (16.2 %) children and 70 (19.6 %) adults had mild hemophilia.

At the end of 2021, 97.8 % of children and 95.4 % of adults with severe hemophilia were on prophylactic therapy compared to 98.8 % and 80.2 % in 2015.

Among the 86 children and the 207 adults with severe HA, 25.6 % and 21.7 % were on plasma derived, 30.2 % and 32.9 % on recombinant factor concentrates, 24.4 % and 30.9 % on EHL, and 19.8 % and 14.0 % on emicizumab, respectively. One adult with HA and inhibitor had prophylaxis with a bypassing agent. All children with severe HB and 93.3 % of the adults with severe HB were on EHL. In contrast, 55.4 % of the children and 47.5 % of the adults with severe hemophilia were using plasma derived concentrates in 2015.

Conclusion The use of new treatment options has changed hemophilia treatment. About 50 % of the patients with severe HA were switched to EHL or emicizumab and almost all severe HB patients are on EHL. The proportion of adult patients with severe HA receiving prophylaxis has increased. Data on bleeding rates and factor consumption will be presented.

Conflict of Interest CP reports institutional grants for research and studies from Chugai/Roche, Takeda, Zacros, and LeoPharma, and honoraria for lectures or consultancy from Bayer, Biomarin, Chugai/Roche, CSL Behring, Novo Nordisk, Pfizer, BMS, SOBI, and Takeda. RK reports institutional grants for research and studies from Bayer, CSL Behring, Novo Nordisk, Octapharma, and SOBI, and honoraria for lectures or consultancy from Bayer, Biotest, Biomarin, CSL Behring, Grifols, LFB, Novo Nordisk, Octapharma, Pfizer, Roche/Chugai, Sanofi, SOBI, and Takeda. CK reports research grants from Bayer and honoraria for lectures or consultancy from Bayer, Biotest, CSL Behring, Novo Nordisk, Sobi. KTG received consulting fees from Pfizer, Takeda, Roche, Grifols, Sobi and Honoraria for lectures from Takeda, Roche, Grifols, Sobi.

References

[1] Mahn R, Schilling K, Klamroth R, Kentouche K, Aumann V, Fischer L et al. Development of Haemophilia Treatment in the Eastern Part of Germany over the Last Decade in the Kompetenznetz Hämorrhagische Diathese Ost (KHDO). Hamostaseologie 2020; 40 (1): 119–27

T-07-42 Enhancing Joint Health in Hemophilic Patients: A Retrospective Cohort Study on Early Detection and Intervention for Synovitis

Authors S. Halimeh[1, 2], J. Zappek[1], M. Siebert[1], A. Daoud[1], B. Habermann[3, 4]
Institutes 1 Gerinnungszentrum Rhein-Ruhr (GZRR), Duisburg, Germany; 2 Universitätsuniklinik Essen, Essen, Germany; 3 Orthopädie am Fürstenhof, Frankfurt, Germany; 4 Universitätsklikum Mainz, Mainz, Germany
DOI 10.1055/s-0044-1779143

Introduction Hemophilic patients often face critical health challenges stemming from joint damage caused by untreated joint bleeding. The ankle joint is frequently the initial site affected in these patients. To address this issue, it is imperative to improve strategies for the early detection and treatment of bleeding in hemophilic patients, a matter that requires wider recognition among healthcare practitioners.

Method

Objective This retrospective, single-center cohort study aimed to compare the outcomes of a proactive versus reactive approach to joint ultrasound-guided adjustments of treatment regimens in severe hemophilia patients treated at our Hemophilia Comprehensive Care Center (HCCC) in Duisburg, Germany.

Methods Before 2020, we followed the 2018 German hemophilia-synovitis guidelines, performing joint ultrasounds on patients when they reported joint discomfort or bleeding. Prophylactic hemophilia treatment was promptly adjusted if needed, with radiosynoviorthesis (RSO) used if necessary.

In 2020, our diagnostic strategy shifted towards prevention. We conducted annual ultrasound examinations on all ankle and knee joints in severely hemophilic patients. Joints displaying "silent symptoms" or signs of degradation underwent quarterly ultrasounds, with prophylactic factor treatment adjusted accordingly.

Results Between 2020 and 2022, we conducted ultrasound assessments on 1193 joints in 688 patients with various coagulation disorders. Notably, for 103 patients with severe hemophilia A or B, we performed 656 joint ultrasounds, including 281 ankle joints and 175 knees. Therapy regimens were adjusted as needed.

Before 2020, seven RSOs of ankle joints and three RSOs of knees were performed. After 2020, only two RSOs of ankle joints were required.

Conclusion Our findings, focused on our HCCC in Duisburg, Germany, during 2020-2022, demonstrate that proactive ultrasound monitoring of ankle and knee joints in patients with severe hemophilia A or B, coupled with appropriate adjustments in prophylactic factor therapy, resulted in reduced joint degradation in terms of severity and frequency.

Conflict of Interest No conflict of interst

T-07-43 Understanding the Psychosocial Impact on Hemophilia Carriers

Authors D. Heinrichs, M. Siebert, S. Halimeh
Institute Gerinnungszentrum Rhein-Ruhr, Duisburg, Germany
DOI 10.1055/s-0044-1779144

Introduction Genetic counseling, testing, and prenatal diagnosis play a crucial role in comprehensive hemophilia care. Hemophilia carriers often suffer from reduced quality of life (QoL), especially in terms of mental health [1–3]. However, the comprehensive evaluation of QoL, encompassing physiological, psychological, and social aspects, remains limited in both literature and clinical practice. Addressing the unique needs of carriers is essential for improving their QoL throughout their lives.

Method In a groundbreaking single-center study, we enrolled 20 confirmed hemophilic carriers in a coaching program. This program involved comprehensive physician examinations, including the documentation of patient characteristics and medical histories. It also assessed the physiological and psychological burdens they faced. Following WHO guidelines [4], we collected

Hämostaseologie 2024; 44: S1–S125

qualitative data on QoL across all life stages, incorporating both objective and subjective perspectives. The patients' qualitative statements about their QoL were clustered, with higher priority given to frequently mentioned topics.

Results The participants, predominantly mothers with an average age of 28.7, each had an average of 2.2 children. Notably, there were 12 participants with hemophilia A and 8 with hemophilia B, with no discernible differences in their QoL. Participants' experiences and concerns were categorized into nine thematic clusters:

1. Heavy menstrual bleeding (HMB)
2. Empathy for their own children
3. Negative self-perception as mothers/wives
4. Challenges in communication with:
   a) Partners
   b) Doctors
   c) Social circles
   d) Authorities
5. Shortages in supplies
6. Apprehensions regarding:
   a) Their own children's health
   b) The well-being of their own children
   c) The stability of their partnerships or the search for a partner
7. Uncertainty about family planning
8. Insufficient social support
9. Stigmatization related to supposed "child maltreatment due to hematomas."

Conclusion In conclusion, being a hemophilia carrier significantly impacts women's QoL, necessitating innovative management strategies. Currently, minimal support and resources are available to women facing this burden. We recommend enhancing support, allocating more resources, and conducting further research to gain a deeper understanding of the challenges faced by hemophilia carriers.

Conflict of Interest No conflict of interest

References

[1] Torres-Ortuno A et al. Hemophilia carriers: quality of life and management at different life stages. Blood Coagul Fibrinolysis 2020; 31 (1S): S12–S14

[2] Wiedebusch S et al. Quality of life, psychosocial strains, and coping in parents of children with hemophilia. Haemophilia 2008; 14: 1014–1022

[3] Limperg PF et al. Psychosocial functioning of mothers of boys with hemophilia. Haemophilia 2016; 22: e57–e60

[4] [No authors listed] The World Health Organization Quality of Life assessment (WHOQOL): position paper from the World Health Organization. Soc Sci Med 1995; 41: 1403–1409. Lastname, FN, Lastname, FN YYYY, 'Article', *Journal*, Edition, Page, Place of publication: publishers

T-07-44 Investigating the Role of GABARAP in FVIII Trafficking: A Comparative Study of Cellular Responses to Pathway Disturbances

Authors S. El Hazzouri, N. Nüsgen, J. Oldenburg, O. El-Maarri

Institute Universitätsklinikum Bonn, Institut für Exp. Hämatologie und Transfusionsmedizin (IHT), Bonn, Germany

DOI 10.1055/s-0044-1779145

Introduction We have previously shown that the absence of the GABARAP protein decreased FVIII secretion and rearranged its cellular distribution in the ER-to-Golgi compartment and in endosomal organelles. In fact, the knockout of GABARAP decreased the secretion of FVIII, similarly to LMAN1-, MCFD2- and Calreticulin- KOs, in contrast to Calnexin-, GABARAPL1- and GABARAPL2-KOs that led to an increase in FVIII secretion. Furthermore, GABARAP which co-localizes with FVIII intracellularly, was in parallel found to associate with proteins in the ERGIC compartment (COPI, COPII, LMAN1 and GM130), suggesting that GABARAP might have a coordinated role with the protein secretory machinery, in facilitating and regulating FVIII secretion.

Aim: To better interpret the role of GABARAP in FVIII trafficking we employed a comparative approach using the established CRISPR/Cas9-KOs in HEK293 cell lines expressing FVIII. We exposed our cell lines to four chemical treatments to disturb specific cellular pathways. Using this methodology, our first objective was to monitor how each cell line responded to the treatments. We wanted secondarily to compare the behavior of the GABARAP-KO cells with other cell lines to better situate the role of the GABARAP protein in FVIII trafficking.

Method We have **1)** first performed a Brefeldin A treatment to hinder the vesicular flow in the ERGIC compartment, **2)** then chloroquine to inhibit autophagy, **3)** glucose starvation to disturb cellular metabolism and **4)** finally Rab7 inhibition to further disturb endosomal and lysosomal compartments. We focused our downstream analysis on tracking changes in FVIII secretion levels (chromogenic assay).

Results All four treatments affected FVIII secretion in both wild type and KO-cell lines. Notable effects include: **1)** Inhibition of autophagy by chloroquine treatment decreased FVIII secretion in all cell lines and completely abolished it in the LMAN1-KO cells. Inducing autophagy by glucose starvation in the same LMAN1-KO-cells led to a 36 % increase in FVIII secretion, suggesting an alternative route for FVIII. **2)** CRT- and GABARAP-KO clones exhibited similar trends in FVIII activities in response to the treatments, including a 50 % reduction under glucose deprivation and a 30 % decrease with chloroquine exposure. Conversely, both clones were the least affected by Brefeldin A treatment, showing a 30 % decrease in CRT-KO and almost no change in GABA-KO (2 %). Finally, both clones were most affected when Rab7, a marker of late endosomes, was inhibited (40 % decrease).

Conclusion The data analysis of the four chemical treatments affecting specific intercellular functions and machineries place GABARAP closer to Calreticulin in its effect on FVIII biogenesis.

Conflict of Interest None

T-07-45 Blood viscoelastic testing could support the management of patients with hemophilia A

Authors S. Hammer[1, 2], M. Wekerle[2], M. Strauss[2], S. Kieninger[2], L. Heuer[2], K. Althaus[1, 2], T. Bakchoul[1, 2]

Institutes **1** Medical Faculty and University Hospital of Tübingen, Blood Transfusion Centre, Tübingen, Germany; **2** Center for Clinical Transfusion Medicine GmbH, Blood Transfusion Centre, Tübingen, Germany

DOI 10.1055/s-0044-1779146

Introduction Measurement of Factor VIII activity is decisive for the bleeding management of patients with hemophilia A in emergency situations. However, in most laboratories the determination of factor VIII activity takes several hours and is not available as an emergency parameter especially during holidays and weekends. Thrombelastography is a point-of-care method that provides data on different coagulation parameters in whole blood samples. It is well established so that the coagulation system can be monitored in emergency situations using this easy to use method. It gives a rapid overview of all phases of coagulation and fibrinolysis. Most results are available within a few minutes. In this paper, we report on the use of thrombelastography in the management of patients with hemophilia.

Method Consecutive 9 patients with hemophilia A were included in this retrospective study. Whole blood samples of patients with and without substitution of different factor VIII preparations were investigated by using a viscoelastic test system to assess coagulation and fibrinolysis systems. Additionally, standard routine coagulation testing and factor VIII activity were performed.

Results 27 samples from 9 patients were tested in this study. Thrombelastographic measurements revealed longer clotting time in hemophilia patients compared to healthy donors (clotting time in IN-test: 198.7 ± 38.9 seconds vs. 146.7 ± 12.0 seconds, respectively, $p < 0.0001$). Interestingly, viscoelastic pa-

rameters were corrected after factor substitution (clotting time in IN-test: 157.8 ± 23.7 seconds vs. 146.7 ± 12.0 seconds, respectively, p = 0.1016). Significant correlations between factor VIII activity as measured by the routine aPTT-based clotting test and the clotting time of the intrinsic (r = -0.7188, p = < 0.0001) and also the extrinsic test (r = -0.3887, p = 0.0451) were observed. Maximum of clot firmness and maximum lysis in both assays didn't correlate with factor VIII activity. Interestingly, also lysis time of the formed clot and factor VIII activity correlated significantly (r = -0.4759, p = 0.0252).

Conclusion Our data support that viscoelastic testing could be useful to diagnose factor VIII deficiency and to support the management of coagulation treatment of patients with hemophilia A in emergency situations.

Conflict of Interest nothing

T-07-46 HEM-POWR study: Fourth interim analysis evaluating real-world effectiveness and safety of damoctocog alfa pegol in previously treated patients with haemophilia A in Germany

Authors J. Oldenburg[1], S. Wenning[2], K. Holstein[3], J. Filip[4], W. Miesbach[5], K. Severin[6], H. Eichler[7], S. Halimeh[8, 9]

Institutes **1** University Clinic Bonn, Bonn, Germany; **2** SRH-Kurpfalzkrankenhaus Heidelberg, Heidelberg, Germany; **3** Universitätsklinikum Hamburg-Eppendorf (UKE), Hamburg, Germany; **4** Bayer, Leverkusen, Germany; **5** Universitätsklinikum Frankfurt a.M., Frankfurt am Main, Germany; **6** MV-Zentrum für Onkologie und Hämatologie, Cologne, Germany; **7** Universität und Universitätsklinikum des Saarlandes, Homburg/Saar, Germany; **8** Universitätsklinikum Essen, Essen, Germany; **9** Gerinnungszentrum Rhine Ruhr, Duisburg, Germany

DOI 10.1055/s-0044-1779147

Introduction Damoctocog alfa pegol (BAY 94-9027) is an extended half-life PEGylated recombinant factor VIII product approved for treatment of previously treated patients (PTPs) aged ≥ 12 years with haemophilia A. Real-world effectiveness and safety of damoctocog alfa pegol are being assessed in the HEM-POWR study (NCT03932201). Here we present the fourth interim analysis of the HEM-POWR study for a subgroup of PTPs from German study sites.

Method HEM-POWR, a Phase IV, open-label, prospective cohort study, included PTPs with mild, moderate or severe haemophilia A receiving damoctocog alfa pegol prophylactically or on demand. Primary endpoint was annualised bleeding rate (ABR); secondary endpoints included joint health and safety. The safety analysis set (SAF) included PTPs with ≥ 1 study dose in the observation period. PTPs fulfilling all inclusion criteria with a documented dose of damoctocog alfa pegol in the study and ≥ 1 documented infusion during the observation period were included in the full analysis set (FAS). Data were captured from patient diaries and physician records. Statistical analyses were explorative and descriptive. Patients provided informed consent and ethical approval was obtained for all study sites.

Results At data cut-off (1 August 2023), 73 and 48 PTPs were included in the SAF and FAS of this sub-population, respectively. The median (Q1, Q3) observation periods in the SAF and FAS were 814.0 (482.0, 903.0) and 874.5 (675.5, 933.5) days, respectively. In the FAS, most patients were aged ≥ 18 to < 65 years (40/48, 83.3 %) and had severe disease (39/48, 81.3 %; moderate 9/48, 18.8 %; mild 0.0 %, ▶ **Fig. 1**). Total median (Q1, Q3), mean (SD) ABR during the observation period was 0.5 (0.0, 1.6), 1.4 (2.4), with a change in ABR of 0.0 (-1.5, 0.5), -0.9 (3.3) compared with prior to damoctocog alfa pegol initiation. Data for bleed subtypes are summarised in ▶ **Fig. 2**. During the observation period, 21/48 patients (43.8 %) had no bleeds, 37/48 (77.1 %) had no spontaneous bleeds and 29/48 (60.4 %) had no joint bleeds. In the SAF, 27/73 patients (37.0 %) reported treatment-emergent adverse events (TEAEs). Overall, 1 TEAE, a transient inhibitor that resolved, and 1 TEAE-related death (septic shock in aspiration pneumonia and not related to study drug administration) were reported.

Total number of patients in the HEM-POWR study, n	SAF, n (n=332)	FAS, n (n=227)
Characteristics for subgroup of patients from German study sites	**SAF, n (n=73)**	**FAS, n (n=48)**
Observation period, days, median (Q1, Q3)	814.0 (482.0, 903.0)	874.5 (675.5, 933.5)
Sex, male, n (%)	73 (100.0)	48 (100.0)
Age at enrolment, years, median (Q1, Q3)	37.0 (25.0, 51.0)	38.50 (26.5, 52.0)
Age at enrolment, years, n (%)		
<12	0	0
≥12 to <18	4 (5.5)	4 (8.3)
≥18 to <65	63 (86.3)	40 (83.3)
≥65	6 (8.2)	4 (8.3)
Weight at baseline, kg, median (min, max)	80.0 (44.0, 185.0)*	80.0 (44.0, 185.0)[†]
Severity of haemophilia at initial diagnosis, n (%)		
Mild	2 (2.7)	0
Moderate	13 (17.8)	9 (18.8)
Severe	58 (79.5)	39 (81.3)
Patient history of inhibitors, yes, n (%)	16 (21.9)	12 (25.0)
Prescribed prophylaxis regimen of damoctocog alfa pegol at baseline, n (%)		
Every day	4 (5.5)	4 (8.3)
Every 2 days	18 (24.7)	15 (31.3)
Every 3–4 days	25 (34.2)	14 (29.2)
Every 5 days	12 (16.4)	10 (20.8)
Every 7 days	10 (13.7)	5 (10.4)
Prophylactic treatment prior to enrolment, yes, n (%)	68 (93.2)	46 (95.8)
Dose per kg per infusion for most recent FVIII treatment prior to baseline, IU/kg, median (Q1, Q3)	25.0 (21.1, 31.3)[‡]	25.0 (19.1, 31.1)[§]

*Data missing for 24 patients; [†]data missing for 14 patients; [‡]data missing for 22 patients; [§]data missing for 16 patients. FAS, full analysis set; FVIII, factor VIII; IU, international units; Q1, 1st quartile; Q3, 3rd quartile; SAF, safety analysis set.

▶ **Fig. 1 Patient demographics, baseline characteristics and treatment exposure in the FAS and SAF**; Patient demographics and characteristics during baseline and observation period in a subgroup analysis of patients from German clinical study sites. Baseline data include sex, age, weight and disease severity. Exposure in the SAF and FAS includes patients pretreated with damoctocog alfa pegol prior to initial visit, as well as the most recent dosing modality and dose per infusion.

Characteristic	FAS (n=48)
ABR during the observation period, median (Q1, Q3), mean (SD)	
Total bleeds	0.5 (0.0, 1.6), 1.4 (2.4)
Spontaneous bleeds	0.0 (0.0, 0.0), 0.5 (1.4)
Trauma bleeds	0.0 (0.0, 0.8), 0.8 (2.0)
Joint bleeds	0.0 (0.0, 1.4), 0.8 (1.3)
Spontaneous joint bleeds	0.0 (0.0, 0.0), 0.3 (0.8)
Difference in ABR during the observation period compared with prior to damoctocog alfa pegol initiation, median (Q1, Q3), mean (SD)	
Total bleeds	0.0 (-1.5, 0.5), -0.9 (3.3)
Spontaneous bleeds	0.0 (-0.1, 0.0), -0.6 (2.5)
Trauma bleeds	0.0 (-1.0, 0.6), -0.4 (2.1)
Joint bleeds	0.0 (-1.0, 0.0), -0.8 (2.7)
Spontaneous joint bleeds	0.0 (0.0, 0.0), -0.5 (1.9)

ABR, annualised bleeding rate; FAS, full analysis set; Q1, 1st quartile; Q3, 3rd quartile; SD, standard deviation.

▶ **Fig. 2 ABR and difference in ABR throughout the study in the FAS**; A summary of ABR during the observation period and ABR compared with prior to damoctocog alfa pegol initiation for total, spontaneous, trauma, joint and spontaneous joint bleeds in a German subgroup analysis in the FAS.

Conclusion Results from the fourth interim analysis of HEM-POWR continue to provide valuable insights into real-world clinical practice in Germany, inform German stakeholders, and provide evidence of effectiveness and safety of damoctocog alfa pegol in PTPs with mild, moderate or severe haemophilia A.

Conflict of Interest **JO:** reimbursed for attending symposia/congresses and/or received honoraria and/or funds for research from Bayer, Biogen Idec, Biotest, Chugai, CSL Behring, Novo Nordisk, Octapharma, Pfizer, Roche, Swedish Orphan Biovitrum and Takeda. **SW:** reimbursed for attending symposia/congresses and/or received honoraria and/or funds for research from Bayer, Biotest, Chugai, CSL Behring, Novo Nordisk, Octapharma, Pfizer, Roche, Swedish Orphan Biovitrum and Takeda. **KH:** received grants for studies and research from Bayer, Chugai/Roche, CSL Behring, Pfizer and Sobi, and personal fees for lectures or consultancy from Bayer, Biotest, Biomarin, Chugai/Roche, CSL Behring, LFB, Novo Nordisk, Pfizer, Sobi, and Takeda. **JF:** employee of Bayer. **WM:** acted as a paid consultant to Bayer, BioMarin, Biotest, CSL Behring, Chugai Pharma, Freeline, LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Takeda Pharmaceutical/Shire and UniQure, and received funding for research from Bayer, Biotest, CSL Behring, LFB, Novo Nordisk, Octapharma, Pfizer and Takeda/Shire. **KS:** none to disclose. **HE:** reimbursed for attending symposia/congresses and/or received honoraria and/or funds for research from Bayer Vital, BioMarin,

Biotest, CSL Behring, Novo Nordisk, Pfizer, Roche and Sobi. **SH:** received grants for studies and research from Bayer, Biotest, CSL Behring, Novo Nordisk, Octapharma, Pfizer Pharma, Swedish Orphan Biovitrum and Takeda, and personal fees for lectures or consultancy from Bayer, Biotest, CSL Behring, Chugai, Novo Nordisk, Octapharma, Pfizer, Roche, and Swedish Orphan Biovitrum.

Acknowledgements Editorial support (in the form of writing assistance, including development of the initial draft based on author direction, collating authors' comments and grammatical editing) was provided by Linda Wang and Laura Buck of Fishawack Communications Limited, part of Avalere Health, and was funded by Bayer. This study was funded by Bayer.

T-07-47 HIV Comorbid Infection and Etranacogene Dezaparvovec Therapy: Efficacy and Safety Results From Phase 2b and Pivotal Phase 3 HOPE-B Trials 3 Years after Administration

Authors S. Pipe[1], E. Gomez[2], C. Hermans[3], A. Giermasz[4], P. Kampmann[5], R. Lemons[6], N. Galante[7], S. le Quellec[7], P. Monahan[7]

Institutes **1** University of Michigan, Departments of Pediatrics and Pathology, Ann Arbor, USA; **2** The Center for Inherited Blood Disorders, Orange, USA; **3** Division of Haematology, Université Catholique de Louvain (UCLouvain) Cliniques Universitaires Saint-Luc, Brussels, Belgium; **4** University of California Davis Department of Medicine, Hemophilia Treatment Center Division of Hematology/Oncology, Sacramento, USA; **5** Rigshospitalet, Copenhagen, Denmark; **6** University of Utah, Salt Lake City, USA; **7** CSL Behring, King of Prussia, USA

DOI 10.1055/s-0044-1779148

Introduction Potential hepatotoxicity of liver-directed adeno-associated viral (AAV) vectors in patients (pts) receiving HIV medications have excluded those with HIV from participating in gene therapy haemophilia trials. Pts with controlled HIV comorbid infection were enrolled in phase 2b (NCT03489291) and phase 3 HOPE-B (NCT03569891) trials of etranacogene dezaparvovec (formerly AMT-061); here we evaluate the individual efficacy and safety outcomes in the subset of pts with comorbid HIV infection.

Method A single injection of etranacogene dezaparvovec (2×10^{13}gc/kg, an AAV5 vector containing factor IX [FIX] Padua R338L transgene under the control of the liver-specific promoter LP-1) was administered to adult pts with severe or moderately severe haemophilia B. Pts with uncontrolled HIV (CD4 + counts ≤ 200/µL) were excluded.

Results Of 57 pts in phase 2b and phase 3 HOPE-B trials, 5 had comorbid HIV infection (median [range] age 49 years [38-54]), 4 of whom had a history of hepatitis C virus with a negative viral load. Three of 5 pts with comorbid HIV infection had preexisting AAV5 neutralising antibodies with median (range) titer of 20 (0-99). Annualised bleeding rate (ABR) decreased compared to previous extended half-life FIX prophylaxis; median (range) ABR during FIX prophylaxis was 5 (1-10.4). Two pts recorded no bleeds in the 36 months after receiving etranacogene dezaparvovec; the overall median (range) ABR was 0.64 (0-5.0). Median (range) uncontaminated endogenous FIX at 36 months post treatment was 32.3 % (31.5 %-58 %). Three pts received no FIX infusions through 36 months.

Seven treatment-related adverse events (AEs) were reported in 3 pts, and no treatment-related serious AEs were reported. Treatment-related alanine aminotransferase (ALT) elevation of moderate severity occurred in 1 pt (20 %, similar to pts without comorbid HIV) 35 days post treatment and resolved within 15 days with use of corticosteroids. His FIX levels later declined to 2-5 %, and he resumed prophylaxis per protocol at Month 30 post treatment.

Conclusion Etranacogene dezaparvovec was found safe and effective in a subset of pts with controlled comorbid HIV infection. Owing to the small number of pts with HIV enrolled in trials, long-term collection of data and special attention in the real-world setting is recommended.

Conflict of Interest S Pipe has received consultancy fees from Apcintex, ASC Therapeutics, Bayer, BioMarin, CSL Behring, Equilibra Bioscience, GeneVentiv, HEMA Biologics, Freeline, LFB, Novo Nordisk, Pfizer, Regeneron/Intellia, Roche/Genentech, Sanofi, Takeda, Spark Therapeutics and uniQure; research funding from Siemens; and holds a membership on a Scientific advisory committee for GeneVentiv and Equilibra Bioscience.; E Gomez has received consultant fees from Chieisi USA, Inc., and Global Blood Therapeutics Inc.; C Hermans has received consultancy and/or lecture fees from Bayer, Takeda, Roche, CSL Behring, Novo Nordisk, Pfizer, Sobi, LFB, OctaPharma, Uniqure and Biomarin., A Giermaz has received Consultant fees from Bioverativ, Genentech/Roche, Biomarin, uniQure, and has been a member of speaker Bureau of Bioverativ, Genentech/Roche; P Kampmann has received consultant fees from CSL Behring, Novo Nordisk, and BioMarin pharmaceuticals; R Lemons has received consultant fees from CSL Behring and NovoNordisk; N Galante is a CSL Behring full time employee; S Le Quellec is a CSL Behring full-time employee; P Monahan is a CSL Behring full time employee

T-07-48 German Paediatric Haemophilia Research Database – Update 2023

Institute University Hospital Frankfurt, Pediatrics and Adolescent Medicine, Frankfurt am Main, Germany

DOI 10.1055/s-0044-1779149

Introduction Haemophilia A (HA) and B (HB) are rare, X-linked bleeding disorders. The normal standard therapy is a primary prophylaxis to prevent bleeding events and subsequent sequelae. The major complication in the initial treatment period in haemophilia is the development of inhibitors during replacement therapy. This happens in up to one third of the patients with HA and in about 10 % with HB. Treatment and outcome data for previously untreated patients (PUPs) with HA or HB in Germany to date are very limited. Questions remain about the optimal timing and treatment regimens for PUPs to prevent bleeds and adverse events in a constantly evolving treatment landscape. The German Paediatric Haemophilia Research Data Base – GEPHARD – is a multicenter observational cohort study to collect and analyze data on PUPs in Germany.

Method GEPHARD prospectively includes all children and adolescents (< 18 years) diagnosed with HA or HB (FVIII or FIX levels < 25 %) since 1st January 2017. The registry concentrates on outcomes including development of inhibitors, provides quality assurance as well as serving as a basis for future studies.

Results From January 1st, 2017, 472 children and adolescents from 41 participating centers have been reported (on September 20th, the 500th patient was recorded). An average of 55 patients with HA and 11,3 patients with HB were reported annually. Of these, 391 were diagnosed with HA, 81 were diagnosed with HB. The allocation in severity levels shows 230 severe (59 %), 45 moderate (12 %) and 112 mild (29 %) patients with an HA and 39 severe (48 %), 15 moderate (19 %) and 26 mild (32 %) patients with an HB. Age of diagnosis inversely correlates with severity. A significant drop of new diagnosis was noted early 2020.

Conclusion The GEPHARD registry successfully enrols patients from an increasing number of participating centers. The GEPHARD community is actively collecting data on the German treatment situation. The longitudinal documentation is quickly increasing and allows further analyses.

Conflict of Interest GEPHARD is being funded by Bayer, Biotest, CSL Behring, Intersero, Novo Nordisk, Pfizer, Sobi, Takeda

T-08. Other congenital bleeding disorders

T-08-01 Impact of Next Generation Sequencing on molecular genetic analysis in patients with multiple coagulation factor deficiencies

Authors B. Preisler[1], B. Pezeshkpoor[1], R. Fischer[2], A. Pavlova[1], J. Oldenburg[1]
Institutes **1** University Clinic Bonn, Institute of Experimental Hematology and Transfusion Medicine, Bonn, Germany; **2** SRH Kurpfalzkrankenhaus Heidelberg, Hemophilia Care Center, Heidelberg, Germany
DOI 10.1055/s-0044-1779150

Introduction Familial multiple coagulation factor deficiencies (FMCFDs) are a group of rare inherited hemostatic disorders characterized by a simultaneous reduction of plasma activity of at least two coagulation factors. The detection of multiple deficiencies was based on systematic screening of coagulation factors in patients exhibiting atypical bleeding patterns, in pre-surgery management, or familial investigations using Sanger Sequencing. The introduction of Next Generation Sequencing (NGS) in routine molecular genetic testing has significantly increased the identification of multiple genetic alterations, albeit often as incidental findings, posing challenges.

Method Approximately, 7500 index patients (IPs) with known deficiencies in one or more than one coagulation factors have been screened by Sanger Sequencing and Multiplex Ligand Probe Analysis, with around 3000 IPs analyzed by NGS and Copy Number Variant Analysis.

Results The use of NGS has led to a twofold rise in the detection rate of multiple coagulation factor deficiencies (82 FMCFD IPs in 13 years – 1,1 % against 54 FMCFD IPs in 4,5 years – 1,9 %), particularly in FMCFD IPS with variants present in at least two different genes. Within the NGS-based cohort, 106 genetic variants were identified, with 23 % classified as variant of uncertain significance (VUS) and 33 % of them were incidental findings. Notably, male severe hemophilic IPs show the incidental finding in the additional affected gene – due to the dominant clinical phenotype of F8 or F9 defect. In contrast, incidentals findings in hemophilia carriers were predominantly found in the F8- and F9 gene – explainable with the X-linked inheritance. The highest rate of incidental findings was observed in IPs with genetic variants in one pro- and one anticoagulant factor, where 50 % of the variants would have remained undetected without NGS. Remarkably, 17 % of all genetic variants in the entire NGS cohort affected the VWF gene, and 63 % of IPs with defects in one pro- and one anticoagulant factor showed involvement of the VWF gene. Interestingly, in one family with VWD type 3, a variant in the second gene (SERPINC1) completely mitigated the phenotype of VWS [1–2].

Conclusion The implication of NGS in molecular genetic diagnostics has nearly doubled the detection rate of FMCFDs with a predominance of VWD. NGS not only supports the detection of carriers for hemophilia, but also enables the identification of VWD in IPs with no bleeding symptoms due to a secondary defect in an anti-coagulant factor. Additionally, family segregation analysis and expression studies will help to improve the assessment of VUS pathogenicity. Overall, NGS supports tailored treatment approaches of FMCFD IPs and facilitates effective family counselling.

Conflict of Interest This research project was funded by CSL Behring.

References

[1] Ver Donck F, Downes K, Freson K. Strengths and limitations of high-throughput sequencing for the diagnosis of inherited bleeding and platelet disorders. J Thromb Haemost 2020; 18: 1839–1845
[2] Preisler B., Pezeshkpoor B., Banchev A., Fischer R., Zieger B., Scholz U., Rühl H., Kemkes-Matthes B., Schmitt U., Redlich A. et al. Familial Multiple Coagulation Factor Deficiencies (FMCFDs) in a Large Cohort of Patients—A Single-Center Experience in Genetic Diagnosis. J. Clin. Med. 2021; 10: 347

T-08-02

Authors K. Kentouche[1], G. Brodt[1], C. Wollenhaupt[1], N. Gassler[4], H. Proquitte[1], E. Schleußner[6]
Institutes **1** Universitätsklinikum Jena, Klinik für Kinder- und Jugendmedizin, Jena, Germany; **2** Universitätsklinikum Jena, Klinik für Kinder- und Jugendmedizin, Jena, Germany; **3** Universitätsklinikum Jena, Klinik für Kinder- und Jugendmedizin, Jena, Germany; **4** Universitätsklinikum Jena, Institut für Pathologie, Jena, Germany; **5** Universitätsklinikum Jena, Klinik für Kinder- und Jugendmedizin, Jena, Germany; **6** Universitätsklinikum Jena, Klinik für Geburtsmedizin, Jena, Germany
DOI 10.1055/s-0044-1779151

T-08-03 Genetic Background of Plasminogen Deficiency in 65 patients

Authors S. Körber[1], C. Sucker[2], E. Fleck[1], M. Krause[3], B. Bomke[4], C. Heller[5], C. Geisen[1]
Institutes **1** DRK Blood Donor Service, Molecular Haemostaseology, Frankfurt am Main, Germany; **2** COAGUMED Gerinnungszentrum, Haemostaseology, Berlin, Germany; **3** DKD Helios Klinik Wiesbaden, Department for Internal Medicine I, Wiesbaden, Germany; **4** University Hospital Düsseldorf, Department of Haemostaseology and Transfusion Medicine, Düsseldorf, Germany; **5** University Hospital Frankfurt, Department of Pediatrics and Haemostaseology, Frankfurt am Main, Germany
DOI 10.1055/s-0044-1779152

Introduction Plasminogen is a plasma protein that exists in various inactive zymogenic forms, that are converted in the active form plasmin by urokinase and tissue plasminogen activator. Plasmin belongs to the class of serine proteases and its main function is the degradation of fibrin clots, but also plays a role in wound healing, cell migration, angiogenesis, and embryogenesis. Plasminogen deficiency is a genetic autosomal disorder and severe forms can lead to ligneous conjunctivitis and hydrocephalus.

Method DNA of 65 patients was analysed by Sanger Sequencing of the coding regions of plasminogen gene (*PLG*)including the exon intron boundaries. The characterisation of the variants was performed using *in silico* evaluation tools, including Polyhen-2, SIFT and molecular graphic imaging by mapping mutations to the x-ray structure of plasminogen.

Results We detected mutations in 40 out of 65 patients in the *PLG*, corresponding to a mutation detection rate of 61 %. We identified a total of 23 different mutations. Thereof 10 were previously described in the Human Genome Mutation database, 7 in the dbSNP (Polymorphism-Database) and five were novel mutations. Two nonsense mutations and one frameshift mutation (small deletion) were detected. All other mutations were missense mutations. From the unknown variants, 4 were potentially missense changes and one was a nonsense change. All *in silico* prediction tools classified the unknown variants as mutations with pathogenic effect, according to the plasminogen levels of the patients. Two of the mutations mentioned in the dbSNP database, were described as pathogenic by all prediction tools. The remaining five were predicted with conflicting interpretations. One of the mutations published already in the Human Genome mutation database, was interpreted as pathogenic by all prediction tools. Seven of them were mentioned with conflicting interpretations. To get more information about the functional impact, variants were mapped to the x-ray structure of plasminogen [1–2].

Conclusion*In silico* methods and molecular graphic imaging are useful tools to predict the effect of genetic variants on the protein function and/or structure and by this to predict the pathogenic effect of certain variants. Nevertheless, these methods cannot replace *in vitro* analysis by laboratory experiments, phenotypic studies of patients and cosegregation analysis within families.

Conflict of Interest I have no conflict of interest.

References

[1] Capriotti E, Altman RB, Bromberg Y Collective judgment predicts disease-associated single nucleotide variants mutations in proteins. BMC Genomics 2013; (Suppl 3): S2
[2] Law RHP et al. The X-ray Crystal Structure of Full-Length Human Plasminogen. Cell Reports 2012; 1: 185–90

T-08-04 Novel non-synonymous coding MPL-gene variants in two unrelated infants with congenital amegakaryocytic thrombocytopenia

Authors D. Boeckelmann, A. Yoshimi-Nöllke, B. Strahm, H. Georgiev, K. Reinsberger, M. Erlacher, B. Zieger
Institute Medical Center-University of Freiburg, Faculty of Medicine, Department of Pediatrics and Adolescent Medicine, Division of Pediatric Hematology and Oncology, Freiburg, Germany
DOI 10.1055/s-0044-1779153

Introduction Congenital amegakaryocytic thrombocytopenia (CAMT) is a rare inherited bone marrow failure syndrome presenting as an isolated thrombocytopenia at birth with development to pancytopenia due to exhaustion of hematopoietic progenitors. The disease is primarily caused by biallelic pathogenic variants in the thrombopoietin (TPO) receptor c-Mpl, encoded by the MPL-gene. TPO signaling is essential for adequate thrombopoiesis as well as hematopoietic stem cell homeostasis. Next generation sequencing (NGS) is an important tool to analyze genetic alterations in multiple genes associated with inherited thrombocytopenia.

Method We investigated two unrelated infants with congenital severe thrombocytopenia using platelet flow cytometry (for Pt.1), NGS (95-gene panel, Illumina) and bone marrow analysis.

Results Pt.1 presented as a newborn girl with multiple hematomas and pre- and perinatal intracranial bleeding (Plt. 9 G/Lafter birth). The girl suffered from left-sided tetraplegic spastic cerebral palsy, post-hemorrhagic hydrocephalus and epilepsy and needed regular platelet transfusions. Flow cytometry revealed reduced fibrinogen binding and surface exposure of CD62 and CD63. NGS identified two variants in the MPL-gene (NM_005373): a pathogenic canonical splice-site variant in intron 11 (c.1653 + 1delG) [1, 2] and a missense variant in exon 2 (c.118T > A, p.Cys40Ser). Family genotyping confirmed compound heterozygosity. Bone marrow analysis showed reduced megakaryopoiesis and unremarkable myelo- and erythropoiesis. During the following months Pt.1 developed an increasing bone marrow failure with severe neutropenia. Therefore, she received allogeneic stem cell transplantation at the age of 13 month. Unfortunately she passed away at day 39 after HSCT due to multi-organ failure. Pt.2 (1 month-old boy) presented with isolated thrombocytopenia and needed regular platelet transfusions. Bone marrow analysis showed hypoplasia of the megakaryopoiesis. NGS identified the same pathogenic canonical splice-site variant in intron 11 (paternal inherited) combined with a missense variant in exon 3 (c.269G > C; p.Arg90Pro, maternal inherited).
Both missense variants are classified as VUS (ACMG citeria). The first variant c.118T > A (p.Cys40Ser) is absent in dbSNP and gnomAD. The second variant c.269G > C (rs766638870, Arg90Pro) is listed in ClinVar as likely pathogenic with one entry (Accession: VCV000812958.1). In gnomAD it has been found in 5 alleles (only heterozygous) out of 282862. CADD scores of 25.4 and 25.3, respectively indicate that the variants are among the top 1 % of the deleterious variants in the human genome.

Conclusion Congenital severe thrombocytopenia is heterogeneous and therefore, early-performed molecular genetic analysis using NGS is indispensable. Together with bone marrow and platelet analysis, NGS may lead to an early diagnosis and helps to choose the adequate treatment option. In the case of CAMT-MPL early hematopoietic stem cell transplantation is still the only treatment option.

Conflict of Interest The authors declare no conflict of interest.

References

[1] Germeshausen M., Ballmaier M. CAMT-MPL: congenital amegakaryocytic thrombocytopenia caused by MPL mutations – heterogeneity of a monogenic disorder – a comprehensive analysis of 56 patients. Haematologica 2021; 106 (9): p 2439–2448
[2] Stoddart M.T. et al. Congenital amegakaryocytic thrombocytopenia (CAMT) presenting as severe pancytopenia in the first month of life. Pediatr Blood Cancer 2013; 60 (9): p E94–6

T-08-05 Heterozygous RUNX1-gene deletion in a patient with mild thrombocytopenia and a platelet granule secretion defect

Authors H. Glonnegger[1], D. Boeckelmann[1], U. Koehler[2], M. Wolter[1], M. Erlacher[1], M. Sparber-Sauer[3, 4], B. Zieger[1]
Institutes **1** Medical Center University of Freiburg, Department of Pediatric and Adolescent Medicine, Freiburg, Germany; **2** MGZ, Medical Genetics Center, Munich, Germany; **3** Klinikum der Landeshauptstadt Stuttgart, Zentrum für Kinder-,Jugend- und Frauenmedizin, Stuttgart, Germany; **4** University of Medicine Tübingen, Medical Faculty, Tübingen, Germany
DOI 10.1055/s-0044-1779154

Introduction Inherited platelet disorders (IPD) are a heterogynous group of disorders affecting platelet number and function. Next generation sequencing (NGS) has been an important tool to analyze genetic alterations in multiple genes. However, platelet function analysis performed with light transmission aggregometry (LTA), flow cytometry, and microscopy are important to identify patients with an IDP and to characterize the platelet phenotype. We investigated a young girl, who presented with multiple hematoma and mild thrombocytopenia (Plt. 109 G/L). Case history revealed that a few of her mother´s relatives had suffered from leukemia as adults.

Method LTA and flow cytometry for platelet function analysis, multi gene panel (custom, Illumina) for genetic analysis, microarray analysis (Infinium® CytoSNP-850K, Illumina)

Results LTA showed reduced aggregation after stimulation with low dose collagen (2.0 µg/mL), ADP and epinephrine. Flow cytometry revealed slightly reduced VWF- and fibrinogen binding of the platelets. Surface exposure of CD62 and CD63 after stimulation with thrombin was significantly reduced, indicating a α- and δ-granule secretion disorder.
The NGS-Panel analysis performed, identified a heterozygous RUNX1-gene deletion in the copy number variation (CNV) analysis (SeqPilot, JSI). To validate the finding, microarray analysis was performed and identified a 1.56 Mb deletion (21q22.11q22.12) including RUNX1. The deletion could not be identified in the parents of the young girl. However, a germline cell mosaic could not safely be ruled out. Platelet count of the mother was within normal range (288 G/L), LTA showed only a slightly reduced platelet aggregation after stimulation with low dose ADP (4 µmol/L), flow cytometry analysis remained without pathological findings. Repeated measurements of the platelet count of the index patient showed mild thrombocytopenia (109, 133, 136, 132G/L) over 2 years.

Conclusion Our findings led to the diagnosis of RUNX1 haploinsufficiency for the young girl. In summary, in addition to platelet dysfunction and thrombocytopenia, there is an increased risk of developing hematologic malignancies (RUNX1-FPDMM, RUNX1 familial platelet disorder with associated myeloid malignancies). It is estimated that ≈30-40 % of individuals with RUNX1-FPDMM will develop acute myeloid leukemia (AML) or myelodysplastic syndromes, with a median age of onset of 33 years [1, 2]. Therefore, we recommend regular hematological check-up in an experienced center. Possible bleeding occurs more often than expected due to qualitative platelet defect and should be considered in case of surgery, especially in mucous area.

Conflict of Interest The authors declare no conflict of interest. This research project was partially funded by CSL Behring (ZVT Nr.: ZVS-2019092402).

References
[1] Latger-Cannard V. et al. Haematological spectrum and genotype-phenotype correlations in nine unrelated families with RUNX1 mutations from the French network on inherited platelet disorders. Orphanet J Rare Dis 2016; 11: p 49
[2] Luo X. et al. ClinGen Myeloid Malignancy Variant Curation Expert Panel recommendations for germline RUNX1 variants. Blood Adv 2019; 3 (20): p 2962–2979

T-08-06 Endemic Struma Nodosa in European Patients with Factor XI Deficiency

Authors S. Halimeh[1, 2], M. Siebert[1, 2], S. Alecsi[3]
Institutes **1** Gerinnungszentrum Rhein-Ruhr, Duisburg, Germany; **2** Universitätsklinikum Essen, Essen, Germany; **3** Gerinnungszentrum Hochtaunus, Bad Homburg, Germany
DOI 10.1055/s-0044-1779155

Introduction Factor XI deficiency (FXI def) is an exceedingly rare coagulation disorder, prevalent at 1:10^6 in the general population. However, among communities with a history of segregated religious practices and intermarriage like Ashkenazi Jews and Israeli Arabs, the prevalence escalates to 1:450 [1]. Previous research predominantly focused on these communities, leaving a gap in understanding the disorder's broader implications.
Factor deficiencies are linked to thyroid functions; hypothyroidism often correlates with bleeding tendencies, while hyperthyroidism associates with thromboembolic events [2]. An older study involving 43 FXI def patients, primarily Ashkenazim, found a 9 % prevalence of Struma nodosa [3].
Method Our study comprehensively assessed 212 FXI def patients, conducting thorough anamnesis, genetic and blood testing, FXI activity evaluation, and ISTH-Bleeding Score assessment. Statistical correlations were explored.
Results In our cohort, 51 patients exhibited Struma nodosa, indicating a prevalence of 24 %. Notably, both hemi- and homozygote patients displayed significantly lower FXI activity and higher ISTH Bleeding Scores compared to heterozygote patients.
Conclusion Rosen et al. previously suspected a shared genetic origin between FXI def and Struma nodosa in their limited cohort of 4 patients but lacked resources for testing[3]. Our ongoing research aims to bridge this gap, conducting extensive genetic and laboratory analyses on our cohort of 51 patients with Struma nodosa and FXI deficiency.
Conflict of Interest No conflict of interest

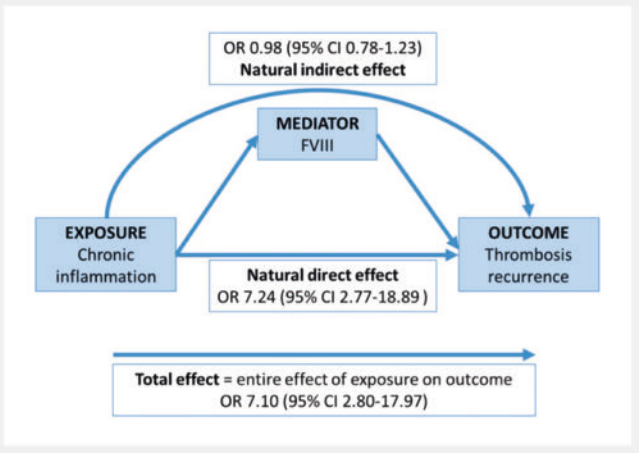
References
[1] Lastname,1 Preis M, Hirsch J, Kotler A et al. Factor XI deficiency is associated with lower risk for cardiovascular and venous thromboembolism events. Blood 2017; 129 (9): 1210–1215. doi:10.1182/blood-2016-09-742262
[2] Franchini M. Hemostatic changes in thyroid diseases: haemostasis and thrombosis. Hematol Amst Neth 2006; 11 (3): 203–208. doi:10.1080/10245330600667591
[3] Rosen IB, Anderson I, Musclow CE. The factor of factor XI deficiency in thyroid neoplasia. *Surgery*. 1986;100(6):1062-1067. FN, Lastname, FN YYYY, 'Article', *Journal*, Edition, Page, Place of publication: publishers

T-09. Paediatric haemostasis and thrombosis

T-09-01 The mediating role of coagulation factor VIII in the effect of chronic inflammation on recurrent thrombotic events in children with non-central line deep vein thrombosis

Authors A. Bosch[1, 2], K. Jarvis Brunsvig[1, 3], L. Brandao[1, 4], Y. Zou[4], J. Vincelli[1], N. Amiri[1], L. Avila[1, 4]
Institutes **1** The Hospital for Sick Children, Division of Haematology / Oncology, Toronto, Canada; **2** Universitäts Kinderspital Zürich, Department of Haematology, Zürich, Switzerland; **3** Oslo University Hospital, Department of Pediatric Hematology and Oncology, Oslo, Norway; **4** University of Toronto Dalla Lana School of Public Health, Toronto, Canada; **5** The Hospital for Sick Children, Child Health Evaluative Sciences, Research Institute, Toronto, Canada
DOI 10.1055/s-0044-1779156

Introduction Pediatric patients who had a first venous thromboembolic event are at risk of experiencing a thrombosis recurrence, which in turn is associated with high morbidity and mortality. Thus, predicting recurrence is relevant for clinical management. In a previous study, we have shown that chronic inflammation independently predicted recurrent thrombotic events in children with an index non-central venous catheter (non-CVC) related deep vein thrombosis (DVT). In that study, coagulation factor VIII (FVIII) did not predict thrombosis recurrence. However, FVIII is an acute phase protein that can be increased in the context of inflammatory conditions. Therefore we investigated the extent to which the effect of chronic inflammation on the outcome of thrombosis recurrence is mediated by FVIII.
Method Children aged 0-18 years diagnosed with an index non-CVC related DVT between 1993-2020 were included in this single-center retrospective cohort study. Ethics approval was obtained.
The primary outcome was thrombosis recurrence as per ISTH definitions. Two chart reviewers independently assessed the underlying conditions of each child and adjudicated whether there was an underlying chronic inflammatory condition present. FVIII was measured ≥ 30 days after acute DVT diagnosis.
Regression-based causal mediation analysis was conducted to assess the extent to which the effect of chronic inflammation on the outcome of thrombosis recurrence was mediated by FVIII (▶ **Fig. 1**), adjusting for sex and age at the time of the index DVT. Blood group was included in an exploratory mediation analysis. The natural indirect effect, natural direct effect, total effect, and proportion mediated were calculated and measured on the odds ratio scale.



▶ **Fig. 1** **Mediation Analysis**; Direct acyclic graph of the assumed causal effects between underlying diseases with chronic inflammation (exposure) on recurrent thrombosis (outcome) through factor VIII (mediator). The natural indirect effect, natural direct effect, and total effect are depicted by arrows. Their effect size is shown on the Odds ratio scale with 95 % confidence intervals. The proportion mediated by FVIII reduced the total effect by 2 %. Covariates: sex, age; Legend: Factor VIII (FVIII), Odds Ratio (OR), Confidence Interval (CI)

Results A total of 139 children with an index non-CVC related DVT were included. Of these, 39 (28 %) children had a recurrent thrombosis at a median of 206 days (P25-75 56-642 days) after the index DVT. Demographics and clinical characteristics are shown in ▶ **Fig. 2**.

Hämostaseologie 2024; 44: S1–S125

	Thrombosis recurrence N = 39[1]	No thrombosis recurrence N = 100[1]	p-value[2]
Age at time of index DVT (years)	14 (12 – 16)	15 (13 – 17)	0.19
Male sex	24 (62%)	48 (48%)	0.15
FVIII (U/mL)	1.66 (1.24- 2.04)	1.61 (1.13-2.24)	0.90
Underlying condition with chronic inflammation	17 (44%)	11 (11%)	<0.001
Blood group O[3]	9 (26%)	17 (25%)	0.91

▶ **Fig. 2 Demographics and clinical characteristics of study population by thrombosis recurrence**; [1] Median (P25 – P75); n (%); [2] Wilcoxon rank sum test; Pearson's Chi-squared test;[3] n = 101; Legend: deep vein thrombosis (DVT), Factor VIII (FVIII)

The mediation effect of FVIII on the association of chronic inflammation and recurrent thrombosis is shown in ▶ **Fig. 1**. Chronic inflammation had a significant total effect and a significant natural direct effect on recurrent thrombosis, with no evidence of mediation by FVIII (natural indirect effect). The proportion mediated indicated that FVIII reduced the total effect by 2 %. When adding blood group as a covariate in exploratory analysis, the results of the mediation analysis did not change (data not shown) [1–9].

Conclusion Mediation analysis showed no evidence that factor VIII acts as a mediator in the effect of chronic inflammation on recurrent thrombosis. Hence, assessing the presence or absence of underlying conditions with chronic inflammation was more informative than measuring factor VIII to predict the outcome of recurrent thrombosis in children with non-central venous catheter-related thrombosis. These findings have implications for clinical practice.

Acknowledgements: A.B. was supported by the Rudolf-Marx-Research-Grant of the German Society for Thrombosis and Haemostasis Research e.V. (GTH) and by the Clinical Medicine Plus Scholarship of the Prof. Dr. Max Cloetta Foundation. K.J.B. was supported by the Norwegian Childhood Cancer association.

Conflict of Interest K.J.B. reports honoraria from Bayer, outside the topic of the current study. The authors A.B., L.B., Y.Z., J.V., N.A., and L.A. declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this study.

References

[1] Bosch A, Uleryk E, Avila L. Role of factor VIII, IX, and XI in venous thrombosis recurrence risk in adults and children: A systematic review.. Res Pract Thromb Haemost 2023; 7 (2): 100064

[2] Mitchell LG, Goldenberg NA, Male C, Kenet G, Monagle P, Nowak-Göttl U et al. Definition of clinical efficacy and safety outcomes for clinical trials in deep venous thrombosis and pulmonary embolism in children. J Thromb Haemost 2011; 9 (9): 1856–8

[3] Mountain States Regional Thrombophilia GroupGoldenberg NA, Knapp-Clevenger R, Manco-Johnson MJ Elevated plasma factor VIII and D-dimer levels as predictors of poor outcomes of thrombosis in children. N Engl J Med 2004; 351 (11): 1081–8

[4] Branchford BR, Carpenter SL. The Role of Inflammation in Venous Thromboembolism. Front Pediatr 2018; 6: 142

[5] Lee H, Cashin AG, Lamb SE, Hopewell S, Vansteelandt S, VanderWeele TJ et al. A Guideline for Reporting Mediation Analyses of Randomized Trials and Observational Studies: The AGReMA Statement. JAMA 2021; 326 (11): 1045

[6] VanderWeele TJ. Mediation Analysis: A Practitioner's Guide. Annu Rev Public Health 2016; 37: 17–32

[7] Cashin AG, McAuley JH, VanderWeele TJ, Lee H. Understanding how health interventions or exposures produce their effects using mediation analysis. BMJ. 2023; 382: e071757

[8] Shrier I, Suzuki E. The primary importance of the research question: implications for understanding natural versus controlled direct effects. Int J Epidemiol 2022; 51 (4): 1041–6

[9] Avila ML, Amiri N, Zavareh ZT, Al-Saoud SA, Sabapathy C, Vincelli J et al. Characterization of recurrent thrombosis in pediatric antiphospholipid syndrome. Am J Hematol 2022; 97 (7): E268–70

T-09-02 A deep look at the fragile coagulation balance in children with acute lymphoblastic leukemia at high risk of thrombosis enabled by a spatio-temporal analysis of coagulation

Authors C. Betticher[1], D. Bertaggia Calderara[2], E. Matthey Guirao[2], F. J. Gomez[2], A. Aliotta[2], E. Lemmel[1], F. Ceppi[1], L. Alberio[2], M. Rizzi[1]

Institutes **1** Lausanne University Hospital (CHUV) and University of Lausanne (UNIL), Pediatric Hematology-Oncology Unit, Department Woman-Mother-Child, Lausanne University Hospital (CHUV), Lausanne, Switzerland; **2** Lausanne University Hospital (CHUV) and University of Lausanne (UNIL), Division of Hematology and Central Hematology Laboratory, Lausanne, Switzerland

DOI 10.1055/s-0044-1779157

Introduction Children with acute lymphoblastic leukaemia (ALL) are at highest risk for venous thromboembolism (VTE) during induction therapy (IT).[1] Thromboprophylaxis in this population is currently not standard of care, and its unselective use is not justified and remains challenging considering the fragile thrombo-hemorrhagic balance (e.g. hypofibrinogenemia, frequent thrombocytopenia and/or intrathecal procedures).[2–4] Conventional coagulation assays are unable to identify patients at VTE-risk.[5] We aim to assess the utility of the Thrombodynamics-Analyzer (TD) [6, 7] an innovative thrombin generation (TG) and fibrin clot formation (FCF) assay enabling to monitor the dynamics of tissue factor-dependent and independent coagulation, for assessing the haemostatic state of children with ALL during IT at high risk of VTE.

Method TG and FCF were measured with TD (Hemacore) as Endogenous Thrombin Potential (ETP, AU/ * min/L) and rate of clot-growth (V, mm/min) respectively, in 15 children with ALL treated according to the AIEOP-BFM protocol at baseline (day d0), d12 (prior to PEG-Asparaginase), d22 (d10 after PEG-Asparaginase treatment) and at d33 of IT, as well in 15 healthy controls. In addition, prothrombin fragments 1 + 2 (F1 + 2), thrombin-antithrombin complexes (TAT) and D-dimers (DD) were assessed as in vivo conventional TG biomarkers. Kruskall-Wallis or Mann-Whitney test were used for statistical analysis to compare patient baseline to different endpoints and control group.

Results In vivo conventional TG markers were significantly higher in patients at baseline prior to treatment (d0) compared to the control group (F1 + 2 $p < 0.0001$; TAT $p = 0.0001$; DD $p < 0.0001$). However, TG in vivo markers were not able to consistently reflect the changes towards a procoagulant state during IT (d0 vs d33, F1 + 2 $p = 0.4$; TAT $p = 0.9$; DD decrease, $p = 0.047$).

Differently, TD parameters for TG and FCF were significantly increased in children with ALL during IT. In particular, for TG, ETP [median (IQR)] changed from 1612 (1376-2013) at d0 to 2386 (1928-2990; $p = 0.0037$) at d12, to 3308 (2456-3460; $p < 0.0001$) at d22, and to 3017 (2650-3975; $p < 0.0001$) at d33. For FCF, V [median (IQR)] significantly increased from 51 (45-55) at d0 to 67 (62-82; $p = 0.021$) at d12, to 82 (75-91; $p < 0.0001$) at d22, and to 80 (66-90, $p = 0.0005$) at d33. In addition, ETP (TD) tend to be higher in patients with VTE (n = 5) at baseline and early during IT, well before the development of thrombotic events.

Conclusion Our data show that TG as well as FCF parameters assessed by TD in pediatric patients with ALL significantly increase during induction treatment and are very sensitive to changes of coagulation state. This global hemostasis assay may be able, alone or in combination with other clinical and/or laboratory parameters, to identify children at high risk for thrombosis, who could profit from a primary pharmacological thromboprophylaxis.

Conflict of Interest None

References

[1] Athale Uma H, Chan Anthony KC. Thrombosis in children with acute lymphoblastic leukemia: part I. Epidemiology of thrombosis in children with acute lymphoblastic leukemia. Thromb Res 2003; 111 (3): 125–31. doi:10.1016/j.thromres.2003.10.013

[2] Greiner J, Schrappe M et al. THROMBOTECT – a randomized study comparing low molecular weight heparin, antithrombin and unfractionated hep-

arin for thromboprophylaxis during induction therapy of acute lymphoblastic leukemia in children and adolescents. Haematologica 2019; 104 (4): 756–765. doi:10.3324/haematol.2018.194175
[3] Pelland Marcotte et al. Effectiveness and Safety of Primary Thromboprophylaxis in Children with Cancer: A Systematic Review of the Literature and Network Meta-Analysis. Thromb Haemost 2019; 119 (12): 2034–2042. doi:10.1055/s-0039-1697027. Epub 2019 Oct 10.
[4] O'Brien S, Rodriguez V, et al. PREVAPIX-ALL: Phase 3 Study of the Safety and Efficacy of Apixaban for Thromboprophylaxis versus Standard of Care in Newly Diagnosed Pediatric Acute Lymphoblastic Leukemia or Lymphoma (ALL/LL) [abstract]. Abstract Number: OC 15.1, Meeting: ISTH 2022 Congress
[5] Burley K, Salem J et al. Evaluation of coagulopathy before and during induction chemotherapy for acute lymphoblastic leukaemia, including assessment of global clotting tests. Blood Cancer J 2017; 7 (6): e574. doi:10.1038/bcj.2017.54
[6] Sinauridze Elena I, Vuimo Tatiana A et al. Thrombodynamics, a new global coagulation test: Measurement of heparin efficiency. Talanta 2018; 180: 282–291. doi:10.1016/j.talanta.2017.12.055. Epub 2017 Dec 19PMID: 29332812
[7] Bertaggia Calderara D, Aliotta A et al. Hyper-coagulability in obese patients accurately identified by combinations of global coagulation assay parameters. Thromb Res 2020; 187: 91–102. doi:10.1016/j.thromres.2020.01.012

T-09-03 Successful thrombolysis in a preterm newborn with acute Leriche syndrome caused by spontaneous thrombotic aortoiliac occlusion: a case report and review of the literature

Authors D. Gorodezki[1], S. Schober[1], I. Tsiflikas[2], U. Holzer[1], C. F. Poets[3], J. H. Schulte[1], J. Nordmeyer[4], V. Icheva[4]
Institutes **1** University Children's Hospital Tuebingen, Department of Hematology and Oncology, Tuebingen, Germany; **2** University Hospital Tuebingen, Department of Diagnostic and Interventional Radiology, Tuebingen, Germany; **3** University Children's Hospital Tübingen, Department of Neonatology, Tuebingen, Germany; **4** University Children's Hospital Tübingen, Department of Pediatric Cardiology and Intensive Care Medicine, Tuebingen, Germany
DOI 10.1055/s-0044-1779158

Introduction Neonatal aortic thromboses represent rare, yet serious conditions commonly associated with severe morbidity and a high mortality rate [1, 2]. Their management, however, issues major challenges due to limited experience and lacking treatment recommendations [2, 3]. Although systemic thrombolysis is considered first-line treatment in case of organ-threatening ischemia, a limited number of attempted thrombolytic treatment approaches, particularly in preterm neonates, have been reported to this day [2, 5, 6, 8].

Method We report a case of successful thrombolysis in a preterm newborn presenting with acute Leriche syndrome caused by spontaneous aortic thrombosis.

Results A preterm newborn of 35 weeks gestational age presented with signs of severe symmetric lower limb ischemia with cyanosis, reduced oxygen saturation and undetectable blood pressure in both lower limbs at first examination. An immediately performed echocardiogram showed no evidence of intracardiac thrombosis, congenital heart defects or aortic anomalies, while a thrombotic occlusion with absence of arterial blood flow in the aortic bifurcation and both iliac arteries was detected via Doppler ultrasound. After interdisciplinary discussion, systemic thrombolysis with tissue plasminogen activator (rtPA) at a dose of 0.05 mg/kg/h was started after application of a bolus of 0.1 mg/kg body weight, and was later on increased up to 0.075 mg/kg/h. An incremental dissolution of thrombosis and restoration of blood flow in sequentially performed ultrasound examinations could be observed. Parallel to thrombolysis, fresh frozen plasma was administered to raise plasminogen levels. Systemic thrombolysis with rtPA was ended after 48 hours with overlapping continuation of anticoagulation therapy with subcutaneously applied low molecular weight heparin (anti-Xa activity 0.6 – 1 IU/ml). No hemorrhagic complications have been observed. Laboratory screening showed no indication for acquired or hereditary thrombophilia. The patient was discharged at the twelfth day of life in good clinical condition without residual symptoms, while a follow-up examination two months after discharge showed a sufficient lower limb perfusion despite the presence of a persistent residual thrombus.

Conclusion Spontaneous neonatal aortic thromboses in absence of umbilical artery catheterization, aortic anomalies or prothrombotic disorders represent a rare and potentially life-threatening condition [2, 6, 7]. Particularly in preterm newborns, thrombolytic therapy may be complicated due to limited experience and both lacking dose recommendations and reliable laboratory monitoring options of thrombolytic activity [2, 4, 7]. Our case underlines the successful use of thrombolysis in preterm infants under careful risk-benefit consideration. Although several case reports outline the feasibility of thrombolysis in these patients, further studies are required to confirm the efficacy and safety of systemic thrombolysis in neonatal aortic thrombosis [5–8].

Conflict of Interest No potential conflict of interest was reported by the authors.

References

[1] Nowak-Göttl U, von Kries R, Göbel U. Neonatal symptomatic thromboembolism in Germany: two year survey. Arch Dis Child Fetal Neonatal Ed. 1997; 76 (3): F163–7. doi:10.1136/fn.76.3.f163
[2] Nagel K, Tuckuviene R, Paes B, Chan AK. Neonatal aortic thrombosis: a comprehensive review. Klin Padiatr 2010; 222 (3): 134–9. doi:10.1055/s-0030-1249662
[3] Yee DL, Chan AK, Williams S, Goldenberg NA, Massicotte MP, Raffini LJ. Varied opinions on thrombolysis for venous thromboembolism in infants and children: findings from a survey of pediatric hematology-oncology specialists. Pediatr Blood Cancer 2009; 53 (6): 960–6. doi:10.1002/pbc.22146
[4] Streif W, Mitchell LG, Andrew M. Antithrombotic therapy in children. Curr Opin Pediatr 1999; 11: 56–64. doi:10.1097/00008480-199902000-00011
[5] Grizante-Lopes P, Garanito MP, Celeste DM, Krebs VLJ, Carneiro JDA. Thrombolytic therapy in preterm infants: Fifteen-year experience. Pediatr Blood Cancer 2020; 67 (10): e28544. doi:10.1002/pbc.28544
[6] Mulcaire-Jones JP, Bailly DK, Frank DU, Verma AR, Barney BJ, Siefkes HM. Spontaneous aortic thrombosis in neonates: a case report and review of literature. Cardiol Young 2020; 30: 95–99. doi:10.1017/S1047951119003093
[7] Wieland I, Jack T, Seidemann K, Boehne M, Schmidt F, Happel CM, Koeditz H, Bertram H, Sasse M. Neonatal aortic arch thrombosis: analysis of thrombophilic risk factors and prognosis. Cardiol Young 2014; 24: 33–9. doi:10.1017/S1047951112002077
[8] Samji N, Twiss J, Chan AKC, Bhatt MD. Successful Use of Thrombolysis in an Extremely Low Birth Weight, Premature Infant With Aortic Thrombosis. J Pediatr Hematol Oncol 2022; 44 (5): e888–e891. doi:10.1097/MPH.0000000000002455

T-09-04 Anti-HPA-1a antibody RLYB212 depletes transfused HPA-1a positive platelets in HPA-1a negative volunteers: Results of a phase 1b PK/PD study towards development of a fetal and neonatal alloimmune thrombocytopenia (FNAIT) prophylaxis

Authors C. Geisen[1], E. Fleck[1], S.M. G. Schäfer[2], C. Walter[2], J. Sondergaard Jensen[3], D. Sheridan[4], K. Patki[4], R. Armstrong[4], F. Behrens[2], E. Seifried[1], M. Kjaer[7, 8], M. Köhm[2], J. Kjeldsen-Kragh[5, 6]
Institutes **1** Goethe University Frankfurt, Institute of Transfusion Medicine and Immunohaematology, Molecular Haemostasis, Frankfurt am Main, Germany; **2** Goethe University Frankfurt, Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Frankfurt am Main, Germany; **3** Larix A/S, Herlev, Denmark; **4** Rallybio, New Haven, USA; **5** Lund University and Regional Laboratories, Lund, Sweden; **6** University Hospital of North Norway, Tromso, Norway; **7** UiT – the Arctic University of Norway, Hammerfest, Norway; **8** Finnmark Hospital Trust, Hammerfest, Norway
DOI 10.1055/s-0044-1779159

Introduction Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a rare bleeding disorder driven by development of maternal alloantibodies against fetal human platelet antigens (HPAs), predominantly HPA-1a, potentially resulting in uncontrolled bleeding in the fetus/newborn. Currently there are no treatments available to prevent maternal alloimmunization and FNAIT. This proof-of-concept study was designed to assess the concentration-effect relationship of subcutaneous (SC) RLYB212, a monoclonal anti-HPA-1a antibody, to eliminate HPA-1a positive platelets transfused to HPA-1a negative male subjects.

Method Subjects (HPA-1a and HLA-A2–negative) were randomized to receive a single SC dose of RLYB212 (0.09 or 0.29 mg) or placebo on day 1 in a single-blinded manner, followed by transfusion of 10×10^9 HPA-1a and HLA-A2 positive platelets on day 8. Of 11 subjects, 4 were assigned to 0.09 mg SC RLYB212, 5 to 0.29 mg SC RLYB212, and 2 to SC placebo. The proportion of HPA-1a and HLA-A2 positive platelets in circulation over 7 days was determined by flow cytometry using HLA-A2 antibodies.

Results RLYB212 drove rapid and complete elimination of HPA-1a positive platelets in a concentration-dependent manner, with RLYB212 meeting the prespecified proof-of-concept criterion of ≥ 90 % mean reduction in platelet elimination half-life vs placebo. Platelet elimination kinetics were dose-dependent and biphasic, with a mean terminal half-life of 5.8 h in the 0.09 mg dose group and 1.5 h in the 0.29 mg dose group vs 71.7 h for placebo. The mean duration of the lag phase was 4.8 h (0.09 mg) and 2.0 h (0.29 mg), respectively.

Conclusion Subcutaneous RLYB212 treatment resulted in dose-dependent and rapid elimination of HPA-1a positive platelets in HPA-1a negative subjects. Platelet elimination kinetics were consistent with those of RhD positive erythrocytes transfused to RhD negative individuals after intramuscular administration of anti-RhD, which is known to prevent RhD alloimmunization when administered within 72 h of a suspected fetal-maternal hemorrhage. The data from this study support the potential use of SC RLYB212 as a prophylactic treatment in pregnant women at risk of HPA-1a alloimmunization and FNAIT.

Conflict of Interest M.Kj. and J.K.-K. are stockholders of Prophylix AS, a Norwegian biotech company, that obtained the licence of the monoclonal antibody 26.4 from UiT – the Arctic University of Norway, Tromsø, Norway, which was later purchased by Rallybio. M.Kj. and J.K.-K. are currently consultants for Rallybio, which is the Sponsor of the study. R.A., D.S., and K.P. are employees of Rallybio. J.K.-K. also provides consultancy service for Johnson & Johnson. German Red Cross Blood Donor Service Baden-Württemberg-Hessen gGmbH has a contract with Rallybio for the presented study. The remaining authors declare no competing financial interests.

T-09-05 First patient with hereditary thrombomodulin-deficiency and transaldolase-deficiency

Authors M. Raum, T. Prell, A. Lotz-Havla, E. Lurz, J. Hölz, S. Juranek, M. Olivieri

Institute University Hospital, LMU Munich, Department of Pediatrics, Dr. von Hauner Children's Hospital, Munich, Germany

DOI 10.1055/s-0044-1779160

Introduction We herein report the first case of a patient with hereditary thrombmodulin deficiency and transaldolase 1 deficiency presenting with penile hematoma after primary c-sectio, coagulopathy, pancytopenia, cutis laxa and hypersplenism. Global coagulation parameters QUICK and aPTT were not measurable with low levels of all coagulation factors. Thrombelastometry-analysis showed abnormalities of intrinsic and extrinisc pathway. Peripher blood smear showed tripenia, anisozytose and poikilozytose. Bone marrow puncture revealed suppressed bone marrow but excluded leukemia. Due to gastrointestinal bleeding symptoms transfusion of fresh frozen plasma, fibrinogen, vitamin k and transfusion of platelet concentrate was administrated. Due to anemia transfusion of erythrocyte concentrate was necessary.

Method Fast exom sequencing revealed a new heterozygote frame shift mutation of one nucleotide (c.1670del) in the thrombmodulin gene. This variant has not been described yet. Thrombmodulin plays an important role as an anticoagulant through protein c activation and thrombin binding. Phenotype of described patients with thrombmodulin-mutation were presenting with a history of myocardial infarction or pulmonary embolism, cerebral venoussinus thrombosis, and stroke. Analysis of the TALDO1 gene revealed a new homozygote frame shift mutation of two nucleotides (c.643_644del). Transaldolase 1 is an enzyme in the pentose phosphate pathway that plays a role in supplying ribose-5-phosphate for nucleic acid biosynthesis. Furthermore it is involved in production of reducing equivalents and therewith in regulation of the cellular redox potential via maintenance of the reduced glutathione pool and neutralization of reactive oxygen intermediates. Clinical manifestation of patients with TALDO1 deficiency present with thrombocytopenia, hemolytic anemia, hepatopathia, hepatosplenomegaly, and dysmorphic features (cutis laxa, low-set ears). Urine analysis confirmed diagnosis wiht increased levels of polyol derivates [1–7].

Results After genetic results and intedisciplinary consultation including experts in pediatric coagulation, pediatric hepatology and pediatric metabolism management of the patient included administration of N-acetylcystein to prevent hepatopathy, avoiding hepatotoxic medication including paracetamol. Furthermore administration of cotrimoxazol preventing bacterial infection due to neutrocytopenia was indicated. Coagulation parameters were improving in the course. There were no more bleeding symptoms. Currently there is no therapy necessary. Perioperative management because of recurrent prolapse of inguinal hernia included administration of fresh frozen plasma without any complications.

Conclusion We report the first patient with hereditary thrombmodulin deficiency and transaldolase 1 deficiency with hope of increasing awareness of rare disorders that should be considered in differential diagnosis of uncelar coagulopahty, liver disease or tripenia.

Conflict of Interest The authors have no conflicts of interest to declare. All co-authors have seen and agree with the contents of the manuscript and there is no financial interest to report.

References

[1] Ohlin A.K. et al. The first mutation identified in the thrombomodulin gene in a 45year-old man presenting with thromboembolic disease. Blood 1995

[2] Norlund L. et al. A novel thrombomodulin gene mutation in a patient suffering from sagittal sinus thrombosis. Thromb Haemostasis 1997

[3] Weiler H., Isermann B.H. Thrombomodulin. Journal of Thrombosis and Haemostasis 2003

[4] Okada M. et al. A case of thrombomodulin mutation causing defective thrombin binding with absence of protein C and TAFI activation. Blood advances 2020

[5] Balasubramaniam S. et al. Novel Heterozygous Mutations in TALDO1 Gene Causing Transaldolase Deficiency and Early Infantile Liver Failure. JPGN 2011

[6] Grammatikopoulos T. et al. Liver Disease and Risk of Hepatocellular Carcinoma in Children With Mutations in TALDO1. Hepatology Communications 2022

[7] Lance H. et al. NAcetylcysteine Therapy in an Infant with Transaldolase Deficiency Is Well Tolerated and Associated with Normalization of Alpha Fetoprotein Levels. JIMD Reports 2020

T-09-06 Complex malformation of the inferior vena cava vein can be caused by thrombosis in utero

Authors G. Brodt[1], M. Waginger[2], P.-C. Krüger[2], H.-J. Mentzel[2], H. Proquitte[1], K. Kentouche[1]

Institutes **1** Universitätsklinikum Jena, Klinik für Kinder- und Jugendmedizin, Jena, Germany; **2** Universitätsklinikum Jena, Sektion Kinderradiologie, Jena, Germany

DOI 10.1055/s-0044-1779161

Introduction Malformations of the vena cava inferior (VCI) are rare findings, exceptional in children, and are usually asymptomatic. They may caused by disturbances in the embryological formation of the venous system or can de-

velop as result of perinatal venous thrombosis with secondary impairment of the venous development.

Method We report the case of a 15 years old girl admitted for haemostaseological assessment before starting hormonal contraception. A prenatal ultrasound raised the suspicion of atresia of VCI already. Postnatally an echocardiography showed absence of VCI and drainage by the vena azygous system.

Results The diagnosis of a malformation of the abdominal deep venous system (subhepatic vena cava atresia, vena azygos drainage) was established by MRI angiography. The agenesis shows an astonishing finding, rather in the sense of a standard variant with prolonged elevated iliac veins and drainage via the azygos vein. A collateralization via e.g. lumbar veins, as it is seen in acute thrombotic events, was not found. Haemostaseological diagnostics revealed no pathological findings.

Conclusion Most malformations of the VCI are usually asymptomatic and sometimes are diagnosed prenatally. More often the diagnosis of a VCI atresia is made in the context of an acute thrombosis of the lower extremities, then showing typical signs of flow obstruction like collateralization or the development of post thrombotic syndrome. In these cases the clinically overt thrombosis might be secondary to an occult VCI thrombosis in the past. These patients usually require anticoagulant treatment. The patient presented here, shows no signs of obstruction and may rather present with a variant of the norm. We recommended the use of oral contraceptives without restrictions.

Conflict of Interest no conflict of interest

T-09-07 Cerebral sinus venous thrombosis in a teenage girl with heterozygeous Protein C deficiency – Experience with rivaroxaban for secondary thromboprophylaxis

Authors J. Lohse[1], J. Beyer-Westendorf[2], M. Smitka[3], G. Hahn[4], R. Knoefler[1]
Institutes **1** Medical Faculty Carl Gustav Carus of Technical University Dresden, Dept. of Pediatric Haemostaseology, Dresden, Germany; **2** Medical Faculty Carl Gustav Carus of Technical University Dresden, Dept. of Internal Medicine, Haemostaseology, Dresden, Germany; **3** Medical Faculty Carl Gustav Carus of Technical University Dresden, Dept. of Pediatric Neuropediatrics, Dresden, Germany; **4** Medical Faculty Carl Gustav Carus of Technical University Dresden, Pediatric Radiology, Dresden, Germany; **5** Medical Faculty Carl Gustav Carus of Technical University Dresden, Dept. of Pediatric Haemostaseology, Dresden, Germany
DOI 10.1055/s-0044-1779162

Introduction Cerebral vein and sinus thrombosis (CVST) is a rare and challenging disease in children and adolescents. The anticoagulation treatment regimen includes low molecular heparin (LMWH) at a therapeutic dose in the acute phase followed by a secondary prophylaxis with heparin or a vitamin K antagonist for 3 to 12 months to avoid a recurrence of CVST [1]. Recently, with the license of rivaroxaban for the treatment of venous thromboembolism in children [2], another treatment alternative became available for pediatric patients. However, rivaroxaban is not specifically licensed for secondary prophylaxis in CVST patients of all age groups and data for rivaroxaban use in clinically relevant thrombophilias are scarce.

Method A case of a 16-years-old girl who suffered from an extensive CVST, treated with and switched from low molecular weight heparin (LMWH) to rivaroxaban for secondary prophylaxis is presented.

Results Three months after starting oral contraception the patient was admitted to our hospital with severe headaches and was diagnosed with an extensive CVST involving the sinus sagittalis superior and parietal bridging veins on both sides with an accompanying brain congestion edema.

Anticoagulant therapy was started with enoxaparin at therapeutic dosage and estrogen contraception was stopped. Because of persistent severe headaches a lumbar puncture was performed revealing a secondary pseudotumor cerebri which was successfully treated by a single liquor draining. The girl was discharged from the hospital after 10 days. Four weeks later, scheduled MRI showed beginning vein recanalization, leading to enoxaparin dose reduction to a prophylactic dose. Thrombophilia diagnostics revealed a genetically confirmed heterozygeous protein C deficiency with a protein C activity of 40 %. Because of the burden of daily s.c. injections and pronounced hair loss, which the patient attributed to enoxaparin, anticoagulation was switched to rivaroxaban (due to the established protein C deficiency at therapeutic dosage of 20 mg OD) approximately 12 weeks after index diagnosis. Six months later the affected cerebral veins were nearly completely recanalized and the rivaroxaban dose was stepwise reduced to 15 mg OD and, after another 3 months, to 10 mg OD. The patient had major concerns regarding the option to stop anticoagulation and was actively involved in the decision for long-term secondary prevention. 18 months after the index event, the patient is still taking rivaroxaban without VTE recurrence or relevant bleeding complications.

Conclusion Our complex case demonstrates that rivaroxaban can be safely and effectively used for treatment and secondary prophylaxis of CVST in adolescents, even in the presence of protein C deficiency. However, given the complexity of CVST in under-aged populations, use of rivaroxaban should remain case-by-case and expert decision.

Conflict of Interest JBW: Steering Committee member of EINSTEIN junior studies, honoraria from Bayer AG for lectures and advisory boards.

References

[1] Weimar C. Kurth T., et al., Zerebrale Venen- und Sinusthrombose, S2k-Leitlinie, 2018; in: *Deutsche Gesellschaft für Neurologie (Hrsg.)*, Leitlinien für Diagnostik und Therapie in der Neurologie.

[2] Male C et al. Rivaroxaban compared with standard anticoagulants for the treatment of acute venous thromboembolism in children: a randomised, controlled, phase 3 trial. Lancet Haematol 2020; 7 (1): e18–e27. doi:10.1016/S2352-3026(19)30219-4. Epub 2019 Nov 5.

T-09-08 Joint Bleed as an uncommon bleeding event in a 7-years old Patient with Bernard-Soulier-Syndrom

Authors J. Holzapfel[1], N. Stadler[1], M. Frühwald[1], S. Halimeh[3], M. Olivieri[2]
Institutes **1** Universitätsklinikum Augsburg, Schwäbisches Kinderkrebszentrum, Augsburg, Germany; **2** Dr. von Hauner Children´s Hospital LMU Munich, Hemophilia Care Center, Munich, Germany; **3** Gerinnungszentrum Rhein-Ruhr (GZRR), Duisburg, Germany
DOI 10.1055/s-0044-1779163

Introduction Bernard-Soulier-Syndrom (BSS) is a rare inherited bleeding disorder characterised by thrombocytopenia, giant platelets and prolonged bleeding time. It is caused by genetic mutations that affect the function of the GPIb-IX-V platelet GP receptor complex. Clinical manifestations are commonly reported as frequent episodes of epistaxis, gingival and cutaneous bleeding and menorrhagia.

Method Case Report

Results Here we report a seven year old boy with genetically confirmed diagnosis of BSS, who presented with painful swelling of the right knee after playing soccer without noticed trauma. Ultrasound examination and MRI-Scan revealed haemarthrosis of the knee joint. Treatment consisted in i.v. substitution of activated factor VIIa and tranexamic acid in short intervals over a long period of time. Despite consistent substitution of FVIIa and regularly physiotherapy the patient developed chronic synovitis assosciated with recurrent pain, restriction of range of motion and swollen knee. In a multidisciplinary team different treatment options were discussed. Similar to chronic synovitis in hemophilia patients a radiosynoviorthesis was perfomed and the patient recovered.

Conclusion Haemarthrosis is rarely described as a bleeding manifestation in patients with BSS. Because of the rareness of disease, the lack of data regarding the management of joint bleeds in this patients, the role of haemarthrosis and

Hämostaseologie 2024; 44: S1–S125

chronic synovitis may be underestimated. A therapeutic approach similar to arthopathy in hemophilia might be assessed.

Conflict of Interest None

T-09-09 A congenital disorder of fibrinogen consumption associated with neonatal bleeding and thrombosis

Authors C. Male, K. Thom

Institute Medical University of Vienna, Dept. of Pediatrics, Vienna, Austria

DOI 10.1055/s-0044-1779164

Introduction Consumptive coagulopathy in neonates is usually due to sepsis but, rarely, may result from a congenital disorder. Parallel bleeding and thrombotic manifestations present a therapeutic dilemma.

Method Case report

Results The consanguineous healthy parents had their ninth pregnancy. There had been one early abortion and two intrauterine deaths at 24 and 38 weeks gestation. Two older siblings had died at age day 10 (intracerebral hemorrhage) and 5 months (cerebral infarction and bleeding, disseminated intravascular coagulation, hypofibrinogenemia). There are three healthy siblings.

Our patient was born at term per cesarian section (polyhydramnion), showed respiratory distress and needed ventilatory support for 7 days, as well as medical closure of a patent arterial duct. Cranial ultrasound revealed bleeding of the coronoid plexus, and cranial magnetic resonance imaging showed sino-venous thrombosis of the transverse sinus. Laboratory results showed low fibrinogen levels around 50 mg/dL, and high levels of D-Dimer. Treatment was by plasma initially, followed by fibrinogen concentrate which was required frequently because levels dropped rapidly. Assuming consumptive coagulopathy, low-dose heparin and tranexamic acid were started. Fibrinogen plasma levels stabilized somewhat but DD remained high. Subsequently, the patient developed convulsions, requiring anticonvulsive medication and intensive care. Cranial MR showed, apart from the intraventricular hemorrhage, signs of venous congestion and hemorrhagic infarction of the brain parenchyma. Over the next days, the patient deteriorated with hemodynamic instability and progressive neurological impairment and finally died on day 35.

Conclusion We report on a case of neonatal intracerebral bleeding and thrombosis. The family history suggested a congenital disorder. Coagulation parameters indicated consumptive coagulopathy but gave no clue on the underlying defect. Replaced fibrinogen was rapidly consumed, and neither anticoagulation nor inhibiting fibrinolysis could control the coagulopathy which subsequently lead to fatal brain damage. The highly unusual diagnosis could only be made post-mortem based on whole genome sequencing and will be revealed at the abstract presentation.

Conflict of Interest none

T-10. Bleeding of unknown origin

T-10-01 Activated protein C and free protein S in patients with mild to moderate bleeding disorders

Authors D. Mehic[1], T. Schramm[1], B. Forstner-Bergauer[1], H. Haslacher[2], C. Ay[1], I. Pabinger[1], J. Gebhart[1]

Institutes **1** Medical University of Vienna, Department of Medicine I, Clinical Division of Hematology and Hemostaseology, Vienna, Austria; **2** Medical University of Vienna, Department of Laboratory Medicine, Vienna, Austria

DOI 10.1055/s-0044-1779165

Introduction Bleeding disorder of unknown cause (BDUC) is a diagnosis of exclusion, after extensive investigation of plasmatic coagulation and platelet function [1]. The bleeding phenotype as well as severity is similar as in other patients with a mild-to-moderate bleeding disorders (MBD) such as von Willebrand disease (VWD), platelet function defects (PFD) and coagulation factor deficiencies (CFD). The underlying mechanisms for bleeding and impaired thrombin generation (TG) and plasma clot formation (PCF) in BDUC is unknown [2]. Natural anticoagulants might play an important role in BDUC patients [3]. The contribution of the pivotal anticoagulants activated protein C (APC) and protein S (PS) to the hemostatic profile of patients with BDUC has yet not been explored [4].

Method APC levels and free PS activity were measured by ELISA , and TG and PCF were determined in 262 MBD patients from the Vienna bleeding biobank (VIBB), a single-center cohort study, and compared to 61 healthy controls (HC). Bleeding severity was determined by the ISTH-BAT, the Vicenza bleeding score and the number of bleeding manifestations.

Results In total, 262 MBD patients, of whom 69 % had BDUC were compared to 61 HC (▶ **Fig. 1**). Levels of APC were higher in MBD patients than in HC when adjusted for age, sex and BMI (median [IQR] 33.1 [20.6-52.6] and 28.6 [16.4-47.2] ng/mL, ▶ **Fig. 2**). This was most pronounced in patients with BDUC (35.3 [21.7-54.3] ng/mL), while in PFD there was only a trend towards increased APC levels and no difference was seen when comparing VWD and CFD to HC. The percentage of all MBD patients and BDUC specifically above the 95th percentile of APC levels in healthy controls (≥ 70.5 ng/mL) was twice as high but lacking statistical significance (▶ **Fig. 2**).

Characteristic	All MBD* n = 262	BDUC n=180	PFD n=38	VWD n=32	CFD n=11	HC n=61
	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
Female sex	217.0 (82.8%)	157.0 (87.2%)	27.0 (71.1%)	27.0 (84.4%)	5.0 (45.5%)	48.0 (78.7%)
Positive family history	101.0 (38.5%)	71.0 (39.4%)	11.0 (28.9%)	15.0 (46.9%)	4.0 (36.4%)	-
Blood group O†	140.0 (53.4%)	87.0 (48.3%)	22.0 (57.9%)	25.0 (78.1%)	5.0 (45.5%)	16.0 (32.0%)
Above the Vicenza score cutoff ∑	242.0 (92.4%)	170.0 (94.4%)	32.0 (84.2%)	30.0 (93.8%)	9.0 (81.8%)	-
	median (IQR)	median (IQR)	median (IQR)	median (IQR)	median (IQR)	median (IQR)
Age, years	38.5 (29.0, 52.0)	40.5 (30.8, 53.2)	43.0 (28.0, 53.8)	31.5 (22.8, 41.8)	29.0 (26.5, 38.0)	47.3 (28.2, 54.6)
BMI, kg/m^2	23.4 (21.4, 26.3)	23.5 (21.4, 26.3)	23.7 (21.5, 25.4)	23.5 (20.9, 26.1)	23.1 (21.4, 23.8)	23.4 (20.8, 25.5)
Vicenza score	5.0 (4.0, 7.0)	5.0 (4.0, 8.0)	6.0 (3.2, 7.0)	6.0 (4.8, 7.2)	5.0 (3.0, 6.5)	-
ISTH-BAT¶	6.0 (4.0, 8.0)	6.0 (4.0, 8.0)	6.5 (3.8, 8.0)	5.0 (4.0, 8.0)	5.0 (2.5, 7.5)	-
Number of bleeding manifestations	3.0 (2.0, 4.0)	3.0 (2.0, 4.0)	3.0 (2.0, 4.0)	3.5 (3.0, 5.0)	2.0 (1.5, 4.0)	-
Platelets, G/L	238.0 (208.2, 276.0)	239.5 (217.0, 276.0)	216.5 (189.5, 261.5)	246.0 (204.2, 288.0)	244.0 (181.5, 265.0)	253.0 (224.0, 281.8)
Hemoglobin, g/dL	13.6 (12.8, 14.3)	13.5 (12.8, 14.1)	14.1 (12.9, 15.1)	13.4 (12.4, 14.4)	14.0 (12.9, 14.7)	13.8 (13.1, 14.2)
APTT, sec	35.9 (34.0, 38.7)	35.5 (33.7, 37.4)	36.6 (34.4, 38.1)	38.4 (35.8, 42.0)	44.0 (42.5, 49.8)	34.4 (33.2, 35.8)
PT, %	92.0 (86.0, 101.0)	94.0 (86.0, 102.0)	94.0 (86.0, 99.0)	91.0 (84.8, 97.0)	89.0 (85.5, 93.5)	101.5 (95.0, 108.5)
Fibrinogen, mg/dL	298.5 (263.2, 352.0)	307.0 (273.5, 365.0)	290.0 (257.2, 318.5)	271.0 (244.8, 321.8)	273.0 (245.5, 308.0)	287.5 (258.0, 331.5)
sTM, ng/mL	5.1 (3.7, 6.3)	5.2 (3.9, 6.3)	5.4 (3.7, 6.6)	4.1 (3.0, 5.9)	4.5 (4.4, 5.2)	-

** 1 patient with dysfibrinogenemia was not analyzed in subgroups*
† Blood group information missing in 11 HC
¶ missing in 136 patients (94 BDUC, 18PFD, 18 VWD, 5 CFD)
∑ ≥3 for male patients, ≥ 5 for female patients

MBD, mild to moderate bleeding disorders; BDUC, bleeding disorder of unknown cause; PFD, platelet function defect; VWD, von Willebrand disease; CFD, clotting factor deficiency; HC, healthy controls APTT, activated partial thromboplastin time; PT, prothrombin time; sTM, soluble thrombomodulin; IQR, interquartile range

▶ **Fig. 1** Patients characteristics in all MBD patients, and according to diagnoses, and HC

		APC, ng/mL			APC ≥ 95th percentile of HC (≥ 70.5 g/mL)	
	n (%)	median (IQR)	p	p*	n (%)	OR*(95% CI)
Healthy controls	61 (100)	28.6 (16.4, 47.2)	na	na		
All patients with MBD	262 (100)	33.1 (20.6, 52.6)	0.059	**0.046**	26 (10.0)	1.5 (0.6-5.4)
BDUC	180 (69)	35.3 (21.7, 54.3)	**0.026**	**0.033**	19 (10.6)	1.6 (0.6-6.0)
PFD	38 (15)	35.8 (21.0, 53.4)	0.064	0.064	5 (13.2)	2.1 (0.5-9.5)
VWD	32 (12)	21.5 (14.2, 35.7)	0.062	0.942	2 (6.3)	1.0 (0.1-5.7)
CFD	11 (4)	26.7 (18.1, 44.4)	0.994	0.925	0 (0)	na
		Free protein S, %			Free protein S ≥ 95th percentile (≥74.6 %)	
	n (%)	median (IQR)	p	p*	n (%)	OR* (95% CI)
Healthy controls	60 (100)	48.4 (44.3, 58.3)	na	na		
All patients with MBD	254 (100)	49.3 (40.9, 59.6)	0.485	0.604	15 (5.9)	1.1 (0.4-5.3)
BUC	174 (68.5)	48.4 (40.8, 61.5)	0.387	0.759	9 (5.2)	1.0 (0.3-4.9)
PFD	37 (14.6)	51.9 (45.9, 58.5)	0.702	0.664	1 (2.7)	0.6 (0.0-5.2)
VWD	32 (12.6)	49.3 (39.9, 56.4)	0.515	0.578	4 (12.5)	2.7 (0.5-15.3)
CFD	11 (4.3)	47.1 (41.6, 60.8)	0.721	0.831	1 (9.1)	2.0 (0.1-19.5)

*adjusted for age, sex and BMI

IQR, interquartile range; OR, odd ratio; na, not applicable; CI, confidence interval; MBD, mild to moderate bleeding disorders; BDUC, bleeding disorder of unknown cause; PFD, platelet function defect; VWD, von Willebrand disease; CFD, clotting factor deficiency; HC, healthy controls

▶ **Fig. 2** Activated protein C levels and free protein S activity in patients and healthy controls

No differences in free PS activity between patients and HC were seen overall, or according to specific diagnoses (▶ **Fig. 2**).

Further, no association between APC or PS and bleeding severity or TG were identified, while paradoxically APC weakly correlated with shorter lag time ($r = -0.29$, $p < 0.001$) and time to peak (TTP; $r = -0.24$, $p < 0.001$) of PCF in all MBD patients and also in BDUC. Free protein S activity only showed a weak positive correlation with TTP in BDUC patients only ($r = 0.20$, $p < 0.01$).

There was no significant correlation between APC levels with its co-factor free protein S ($r = -0.02$, $p = 0.76$) or with soluble thrombomodulin ($r = 0.08$, $p = 0.21$), which together with thrombin activates PC to APC, in all MBD patients and according to diagnoses.

Conclusion Our data demonstrate increased levels of APC in BDUC compared to HC, while no differences were found in free PS activity. APC may act as a modifier of bleeding risk and reduced hemostatic capacity within a holistic understanding of a multifactorial aggregation of distinct risk factors in BDUC patients. Targeting APC could be a potential therapeutic strategy to mitigate bleeding complications in BDUC, as already investigated in hemophilia patients.

Conflict of Interest The authors have no conflict of interest with regards to this study. D.M. received honoraria for advisory board meetings from CSL Behring. C.A. received honoraria from Bayer, CSL Behring, Novo Nordisk, Pfizer, Roche, Sobi, and Takeda for lectures and/or participation in advisory board meetings. I.P. has occasionally received honoraria from Bayer, CSL Behring, Novo-Nordisk, Pfizer, Roche, Sobi, and Takeda for lectures and advisory board meetings. J.G. received honoraria for lectures, advisory board meetings, and research funding for the Medical University of Vienna from CSL Behring, Novartis, Amgen, and Sobi. A.T., J.B., P.Q., and H.H. have no conflicts of interest to declare.

References

[1] Baker RI, O'Donnell J. How I treat. Bleeding Disorder of Unknown Cause (BDUC). Blood. 2021 blood.2020010038.

[2] Hofer S, Ay C, Rejtö J et al. Thrombin-generating potential, plasma clot formation, and clot lysis are impaired in patients with bleeding of unknown cause. Journal of Thrombosis and Haemostasis 2019; 17 (9): 1478–1488

[3] Mehic D, Colling M, Pabinger I, Gebhart J. Natural anticoagulants: A missing link in mild to moderate bleeding tendencies. Haemophilia : the official journal of the World Federation of Hemophilia 2021

[4] Mehic D, Tolios A, Hofer S et al. Elevated levels of tissue factor pathway inhibitor in patients with mild to moderate bleeding tendency. Blood advances 2021; 5 (2): 391–398

T-10-02 The prevalence and impact of iron deficiency and iron deficiency anemia in patients with mild-to-moderate bleeding disorders and bleeding disorder of unknown cause

Authors T. Dreier[1], D. Mehic[1], J. Rast[1], H. Haslacher[2], C. Ay[1], I. Pabinger[1], J. Gebhart[1]

Institutes 1 Medical University of Vienna, Department of Medicine 1, Clinical Division of Hematology and Hemostaseology, Vienna, Austria; 2 Department of Laboratory Medicine, Section for Preanalytics and Biobank, Vienna, Austria

DOI 10.1055/s-0044-1779166

Introduction Iron deficiency (ID) and iron-deficiency anemia (IDA) are common complications in patients with bleeding disorders. However, their prevalence and impact on the bleeding phenotype in patients with mild to moderate bleeding disorders (MBD) and especially bleeding disorder of unknown cause (BDUC) has not yet been conclusively investigated.

Method Patients from the Vienna Bleeding Biobank, a single-center prospective cohort study on patients with MBD were investigated and compared with healthy controls (HC). ID was defined as ferritin < 30µg/L and/or transferrin-saturation ≤ 15 % without the presence of anemia [1]. ID combined with anemia, defined as hemoglobin < 12g/dL for women and < 13g/dL for men, was classified as IDA.

Results Characteristics of 646 MBD patients, including 432 patients with BDUC (66.9 %), and 202 HC are reported in ▶ **Fig. 1**. Women made up the majority of both patients (83.5 %) and HC (70 %). MBD patients had significantly lower hemoglobin levels and prothrombin time than HC, as well as a longer aPTT, while ferritin and transferrin-saturation did not differ significantly.

Groups	Cohorts		Diagnoses			
	Controls	Patients	BDUC	PFD	vWD	CFD
n	202	646	432	133	63	18
Sex female	142 (70%)	540 (84%)	371 (86%)	109 (82%)	54 (86%)	6 (33%)
Age [years] (Median ± IQR)	48 ± 22	40 ± 24	41 ± 24	37 ± 26	33 ± 19	45 ± 34
BMI [kg/m²] (Median ± IQR)	23.4 ± 4.2	23 ± 5.8	23.1 ± 5.9	22.9 ± 6	22.7 ± 5.7	24.6 ± 7.6
ISTH-BAT-Score (n=475) (Median ± IQR)	n.a.	6 ± 5	6 ± 4	6 ± 5	5 ± 4	8 ± 7
Vicenza-Score (Median ± IQR)	0 ± 0	5 ± 4	5 ± 4	5 ± 5	6 ± 4	5 ± 5
Hemoglobin [g/dL] (mean ± SEM)	13.9 ± 0.09	13.6 ± 0.05	13.6 ± 0.06	13.7 ± 0.1	13.5 ± 0.18	13.7 ± 0.46
MCV [fL] (mean ± SEM)	87.3 ± 0.29	87.4 ± 0.18	87.6 ± 0.22	87.5 ± 0.4	86.1 ± 0.6	87.3 ± 0.75
MCH [pg/cell] (mean ± SEM)	29.7 ± 0.12	29.5 ± 0.07	29.5 ± 0.08	29.4 ± 0.17	29 ± 0.25	29.9 ± 0.28
Platelets [G/L] (mean ± SEM)	252.8 ± 3.8	251 ± 2.6	251 ± 2.9	243 ± 5.4	266 ± 13	245 ± 20
aPTT [seconds] (mean ± SEM)	35.3 ± 0.2	36.6 ± 0.2	36.1 ± 0.25	35.8 ± 0.3	39.4 ± 0.6	45.9 ± 1.6
Prothrombin time [%] (mean ± SEM)	108.3 ± 1.3	95.1 ± 0.5	92.5 ± 1.4	92.5 ± 1.1	92.5 ± 1.4	95.7 ± 2.6
Ferritin [ng/mL] (mean ± SEM)	83 ± 5.3	82.6 ± 3.8	84 ± 4.9	89.8 ± 7.6	47.5 ± 5.4	116.7 ± 33.1
Transferrin saturation [%] (mean ± SEM)	25.1 ± 0.8	24.5 ± 0.45	24.1 ± 0.5	24 ± 0.92	27.6 ± 2.1	24.2 ± 2
	Controls	patients	BDUC	PFD	VWD	CFD
Cohort	202	646	432	133	63	18
Ferritin ≤ 30 [n (%)]	48 (23.8%)	188 (29.1%)	125 (28.9%)	31 (23.3%)	30 (47.6%)	2 (11.1%)
Transferrin saturation ≤ 15 [n (%)]	30 (14.9%)	108 (16.7%)	79 (18.2%)	21 (15.8%)	7 (11.1%)	1 (5.6%)
Iron deficiency [n (%)] p-value (compared w. controls)	57 (28.2%)	224 (34.7%) 0.089	157 (36.3%) **0.044**	34 (25.6%) 0.593	30 (47.6%) **0.004**	3 (16.7%) 0.292
IDA [n (%)] p-value (compared w. controls)	12 (5.9%)	39 (6%) 0.960	26 (6%) 0.969	7 (5.2%) 0.793	6 (9.5%) 0.324	0 0.288

▶ **Fig. 1 cohort characteristics and prevalence of ID and IDA.** Bold p-values indicated significant differences; Abbreviations: BDUC – bleeding disorder of unknown cause. PFD – platelet function disorder. VWD – von Willebrand-disease. CFD – coagulation factor deficiency. BMI – body mass index. MCV – mean corpuscular hemoglobin. MCH – mean corpuscular volume. aPTT – activated partial thromboplastin time. IQR – interquartile range. SEM = standard error of mean. IDA – iron deficiency anemia.

iron deficiency		
Factor	univariate	multivariable
	OR, [significance, 95% CI of OR]	OR [significance, 95% CI of OR]
Female sex	**OR=5.063** [p<0.001*, 2.71;9.46]	**OR=5.945** [p<0.001*, 3.132;11.286]
Age	**OR=0.967** [p<0.001*, 0.956;0.979]	**OR=0.962** [p<0.001*, 0.950;0.974]
BMI	OR=1.007 [p=0.695, 0.974; 1.04]	**OR=1.037** [p=0.047*, 1.0005;1.076]
Diagnosis	OR=0.939 [p=0.559, 0.762;1.159]	n.a.
BDUC	OR=1.253 [p=0.206, 0.883;1.776]	OR=2.855 [p=0.1, 0.814;10.01]
PFD	**OR=0.584** [p=0.014*, 0.38;0.896]	OR=1.717 [p=0.415, 0.468;6.297]
VWF	**OR=1.823** [p=0.025*, 1.08;3.077]	**OR=4.545** [p=0.026*, 1.197;17.265]
CFD	OR=0.368 [p=0.117, 0.105;1.286]	n.a.
ISTH-BAT-Score	OR=1.018 [p=0.483, 0.968;1.071]	OR=1.01 [p=0.827, 0.923;1.105]
Vicenza-Score	OR=0.995 [p=0.859, 0.946;1.048]	OR=1.013 [p=0.808, 0.912;1.126]

▶ **Fig. 2 Uni- and multivariable logistic regression models of risk factors for iron deficiency**; Bold ORs are statistically significant. Abbreviations: OR – odds ratio. CI – confidence interval. BDUC – bleeding disorder of unknown cause. PFD – platelet function disorder. VWD –von Willebrand-disease. CFD – coagulation factor deficiency. BMI – body mass index.

Overall, ID was more common in MBD patients than HC with 224 cases in patients (34.7 %) and 57 cases in HC (28.2 %), nevertheless not reaching statistical significance (▶ **Fig. 1**). According to MBD diagnosis, ID was more frequent in patients with VWD (30/63, 47.6 %, p = 0.004) and BDUC (157/432, 36.3 %, p = 0.044) compared to HC, whereas ID occurred in a similar or even lower frequency in PFD (24.3 %) and CFD (16.7 %). There was no difference in the occurrence of IDA in all MBD patients or according to diagnoses compared to HC (▶ **Fig. 1**).

In MBD patients, female sex and diagnosis of VWD correlated positively, while age and diagnosis of PFD correlated negatively with ID in univariate binary logistic regression analysis (▶ **Fig. 2**). In multivariable analysis female sex, age, and VWD diagnosis prevailed as significant risk factors for ID, and BMI was also significantly associated with ID in MBD. Bleeding scores were not associated with ID in MBD patients in either uni- or multivariable binary logistic regression.

Conclusion Patients with MBD, especially with VWD and BDUC, show an increased risk for iron-deficiency but not IDA. Younger age, female sex and VWD diagnosis were identified as risk factors for ID. While a low iron storage does not seem to lead to more severe bleeding phenotypes, ID itself can present with significant comorbidities, both physical and mental, which the established questionnaires do not screen for [2].

Conflict of Interest The Vienna Bleeding Biobank is financed by CSL Behring with an unrestricted grant.

References

[1] Ning S, Zeller M.P. Management of iron deficiency. Hematology Am Soc Hematol Educ Program 2019; 2019 (1): 315–322. doi:10.1182/hematology.2019000034

[2] Percy L, Mansour D, Fraser I. Iron deficiency and iron deficiency anaemia in women. Best Pract Res Clin Obstet Gynaecol 2017; 40: 55–67. doi:10.1016/j.bpobgyn.2016.09.007

T-10-03 Platelet function analyzer (PFA-100) in patients with bleeding disorder of unknown cause

Authors D. Mehic[1], B. Eichinger[1], T. Dreier[1], J. Rast[1], A. Tolios[2], B. Eichelberger[2], A. Kaider[3], C. Ay[1], I. Pabinger[1], J. Gebhart[1]

Institutes 1 Medical University of Vienna, Department of Medicine I, Clinical Division of Hematology and Hemostaseology, Vienna, Austria; 2 Medical University of Vienna, Department of Transfusion Medicine and Cell Therapy, Vienna, Austria; 3 Medical University of Vienna, Center for Medical Data Science, Vienna, Austria

DOI 10.1055/s-0044-1779167

Introduction Bleeding disorder of unknown cause (BDUC) is a diagnosis of exclusion. BDUC patients exhibit a clinical phenotype similar to that of established mild-to-moderate bleeding disorders (MBDs) such as platelet function defects (PFD), von Willebrand Disease (VWD), or coagulation factor deficiencies (CFD). While the PFA-100 has demonstrated sensitivity in the detection of VWD, data regarding its clinical utility in PFD are conflicting and data on PFA-100 in BDUC patients are scarce. The aim of this study was to evaluate the diagnostic utility of PFA-100 in BDUC compared to other MBDs, and to investigate the influence of various parameters on PFA-100 closure time (CT).

Method We analyzed PFA-100 measurements of 821 MBD patients from in the Vienna Bleeding Biobank, who were included until December 2022. Patients receiving anticoagulation or antiplatelet treatments were excluded from the study. PFA-100® (Dade Behring, Newark, Delaware, USA) analyses with collagen-epinephrine (EPI) and collagen-adenosine diphosphate (ADP) cartridges were conducted on citric acid anticoagulated whole blood samples at study inclusion.

Results In total, 535 BDUC patients (65.1 %) were compared to 79 patients (9.6 %) with VWD, 180 (21.9 %) with PFD, and 27 (3.3 %) with CFD (▶ **Fig. 1**).

	BDUC§	VWD¶	PFD†	CFD¢
N (%)	535 (65.2)	79 (9.6)	180 (21.9)	27 (3.3)
	n (%)	n (%)	n (%)	n (%)
Blood group O*	238 (45.0)	56 (70.9)	89 (50.0)	13 (48.2)
Positive family history for bleeding*	193 (36.9)	44 (57.1)	60 (34.3)	9 (34.6)
	median (IQR)	median (IQR)	median (IQR)	median (IQR)
Age, years	40 (29-52)	33 (25-45)	38 (27-53)	37 (26-57)
Hemoglobin, g/dL	13.5 (12.8-14.2)	13.4 (12.5-14.4)	13.7 (12.8-14.6)	13.9 (13.1-14.9)
Hematocrit, %	40.1 (38.3-42.0)	40.0 (37.7-42.6)	40.5 (38.2-43-4)	41.2 (38.3-43.3)
Platelet count, x 10^9	246 (213-286)	248 (205-319)	231 (194-272)	222 (170-255)
Fibrinogen, mg/dL	312 (270-361)	276 (247-320)	297 (250-342)	278 (246-330)
APTT, sec	35.5 (33.3-37.7)	38.3 (35.5-42.0)	36.4 (33.7-39.2)	44.0 (41.9-48.0)
PT, %	96 (88-105)	92 (86-97)	92 (85-98)	95 (88-105)
VWF:Ag, IU/dL	100 (81-126)	50 (40-58)	97 (76-118)	80 (66-109)
VWF:RCo, IU/dL	90 (69-129)	42 (30-49)	84 (66-115)	74 (61-124)
PFA-Epinephrine, sec Reference range: 82-170 sec	102 (88-117)	149 (131-216)	111 (94-130)	108 (91-131)
PFA-ADP, sec Reference range: 74-130 sec	135 (112-163)	212 (163-289)	153 (119-196)	127 (103-169)
Number of patients with pathological CT above 170 sec for PFA-Epinephrine or 130 sec for PFA-ADP				
0 pathological	180 (33.8)	7 (8.9)	44 (24.4)	9 (33.3)
1 pathological	341 (64.1)	46 (58.2)	124 (68.9)	7 (63.0)
2 pathological	11 (2.1)	26 (32.9)	12 (6.7)	1 (3.7)

BDUC, bleeding disorder of unknown cause; VWD; von Willebrand disease; PFD, platelet function defect; CFD, clotting factor deficiency
APTT, activated partial thromboplastin time; PT, prothrombin time; VWF:Ag, von Willebrand factor antigen; VWF:RCo, von Willebrand factor ristocetin cofactor activity; PFA, platelet function defects, CT, closure time

*blood group missing in 8 patient, family history missing in 20 patients
§ diagnosis of exclusion after extensive investigation of plasmatic coagulation and platelet function
† including patients with possible (n=125; at least one pathological LTA with one agonists at one time point), definite PFD (n=55; two pathological LTA with two agonists at two time points)
¶ including all patients with VWF antigen and/or activity ≤50 IU/dL at study inclusion
¢ including patients with FVIII (n=15), FIX (n=8), FXI (n=3) and FXIII (n=1) deficiency

▶ **Fig. 1** Clinical characteristics and PFA-100 in MBD patients

In BDUC patients, median (interquartile range [IQR]) CTs were 102 sec (88-117) in PFA-EPI and 135 sec (112-163) in PFA-ADP (▶ **Fig. 1**). Both CTs in BDUC patients were shorter than those of VWD (p < .001) and PFD patients (p < .001),

though no significant difference was observed in comparison to CFD (PFA-EPI: p = 0.2; PFA-ADP: p = 0.7).

Applying internal reference standards (PFA-EPI: 82-170 sec; PFA-ADP: 74-130 sec), 341 BDUC patients (64 %) exhibited an abnormal CT in PFA-ADP or PFA-EPI, and 11 patients (2 %) displayed abnormalities in both, similar to CFD patients (▶ **Fig. 1**). The rate of pathological CTs was much higher in VWD (no abnormal CT: 9 %, 1 abnormal CT: 58 %, 2 abnormal CTs: 33 %; p < .001), as well PFD (no abnormal CT: 24 %, 1 abnormal CT: 69 %, 2 abnormal CTs:7 %; p = 0.003) compared to BDUC.

In BDUC patients there was no correlation between PFA-EPI and PFA-ADP CTs and the Vicenza bleeding score (PFA-EPI: Spearman rho (r) = 0.03, p = 0.5; PFA-ADP: r = 0.04, p = 0.4), ISTH-BAT (PFA-EPI: r = 0.06, p = 0.3; PFA-ADP: r = 0.10, p = 0.06), or the number of bleeding manifestations (PFA-EPI: r = 0.01, p = 0.8; PFA-ADP: r = -0.01, p = 0.8).

Employing a Tobit regression model, we identified increasing age, reduced hematocrit, lower VWF:Ag/VWF:RCo levels, lower platelet count, and elevated fibrinogen levels to be associated with extended CTs in PFA-EPI and PFA-ADP (▶ **Fig. 2**).

	Epinephrine						ADP					
	Univariable			Multivariable			Univariable			Multivariable		
	Estimate	95% CI	p	Estimate	95% CI	p	Estimate	95% CI	p	Estimate	95% CI	p
Age*	-0.009	-0.04, 0.03	0.6	**0.05**	**0.02, 0.08**	**0.003**	0.01	-0.04, 0.06	0.6	**0.09**	**0.04-0.14**	**<.001**
Sex *M vs. F*	-0.003	-0.06, 0.05	0.9	0.05	-0.002, 0.11	0.06	0.005	-0.08, 0.09	0.9	0.07	-0.02, 0.16	0.1
Blood group *non-O vs. O*	**-0.10**	**-0.13, -0.06**	**<.001**	0.01	-0.02, 0.05	0.5	**-0.08**	**-0.13, -0.02**	**0.005**	0.05	-0.0004, 0.11	0.05
Hematocrit	**-0.01**	**-0.02, -0.004**	**0.002**	**-0.01**	**-0.02, -0.01**	**<.001**	**-0.01**	**-0.02, -0.003**	**0.007**	**-0.02**	**-0.03, -0.01**	**<.001**
Platelet count*	-0.02	-0.08, 0.04	0.5	**-0.07**	**-0.13, -0.02**	**0.003**	-0.05	-0.14, 0.03	0.2	**-0.12**	**-0.19, -0.04**	**0.002**
VWF:Ag*	**-0.22**	**-0.25, -0.18**	**<.001**	**-0.08**	**-0.14, -0.01**	**0.031**	**-0.22**	**-0.27, -0.16**	**<.001**	0.03	-0.07,0.14	0.5
VWF:RCo*	**-0.21**	**-0.24, -0.18**	**<.001**	**-0.19**	**-0.24, -0.13**	**<.001**	**-0.25**	**-0.30, -0.20**	**<.001**	**-0.33**	**-0.41, -0.25**	**<.001**
Fibrinogen*	0.01	-0.06, 0.07	0.9	**0.12**	**0.06, 0.18**	**<.001**	0.002	-0.09,0.09	0.9	**0.12**	**0.03,0.21**	**0.01**

VWF:Ag, von Willebrand antigen; VWF:RCo, von Willebrand ristocetin co-factor activity; M, male; F, female; ADP, adenosine diphosphate

** log2-transformed*

▶ **Fig. 2** Univariable and multivariable Tobit regression models for the association of clinical and laboratory parameters with PFA-Epinephrine and PFA-ADP in all BDUC patients

Conclusion Two thirds of BDUC patients had prolongations in any PFA-100 CT. Nevertheless, pathologic PFA-100 measurements were less common than in patients with known defects of primary hemostasis. Factors known to influence PFA-100 CTs were also identified as relevant in BDUC patients.

Conflict of Interest The authors have no conflict of interest with regards to this study. D.M. received honoraria for advisory board meetings from CSL Behring. C.A. received honoraria from Bayer, CSL Behring, Novo Nordisk, Pfizer, Roche, Sobi, and Takeda for lectures and/or participation in advisory board meetings. I.P. has occasionally received honoraria from Bayer, CSL Behring, Novo-Nordisk, Pfizer, Roche, Sobi, and Takeda for lectures and advisory board meetings. J.G. received honoraria for lectures, advisory board meetings, and research funding for the Medical University of Vienna from CSL Behring, Novartis, Amgen, and Sobi. A.T., J.B., P.Q., and H.H. have no conflicts of interest to declare.

T-11. Platelet dysfunction and associated bleeding disorders

T-11-01 Pregnancy Outcomes in Hereditary Thrombotic Thrombocytopenic Purpura – Room for (Further) Improvement

Authors M. Schraner[1, 2], K. D. Friedman[3], J. N. George[4], I. Hrachovinova[5], P. N. Knöbl[6], M. Matsumoto[7], R. Schneppenheim[8], A. S. von Krogh[9, 10], B. Lämmle[1, 11], J. A. Kremer-Hovinga[1, 2]

Institutes **1** Inselspital, Bern University Hospital, Department of Hematology and Central Hematology Laboratory, Bern, Switzerland; **2** University of Bern, Department for BioMedical Research (DBMR), Bern, Switzerland; **3** Medical College of Wisconsin, Division of Hematology and Oncology, Milwaukee, USA; **4** University of Oklahoma Health Sciences Center, College of Public Health, Department of Biostatistics & Epidemiology, Oklahoma City, USA; **5** NRL for Hemostasis, Institute of Hematology and Blood Transfusion, Prague, Czech Republic; **6** Medical University of Vienna, Department of Medicine 1, Division of Hematology and Hemostasis, Vienna, Austria; **7** Nara Medical University, Department of Blood Transfusion Medicine, Kashihara, Japan; **8** University Medical Center Hamburg-Eppendorf, Department of Pediatric Hematology & Oncology, Hamburg, Germany; **9** St. Olavs Hospital, Trondheim University Hospital, Department of Hematology, Trondheim, Norway; **10** Norwegian University of Science and Technology, Department of Clinical and Molecular Medicine, Trondheim, Norway; **11** University Medical Center Mainz, Center for Thrombosis and Hemostasis, Mainz, Germany

DOI 10.1055/s-0044-1779168

Introduction Hereditary thrombotic thrombocytopenic purpura (hTTP) is a rare thrombotic microangiopathy that results from bi-allelic *ADAMTS13* mutations and severe congenital ADAMTS13 deficiency. Affected patients are particularly vulnerable during infancy and pregnancy, where acute episodes are often severe, presenting with thrombocytopenia, haemolytic anemia and symptoms of organ ischemia. Standard of care consists of replenishing ADAMTS13 using infusions of plasma/plasma-derived blood products to treat acute episodes as well as prophylactically to prevent morbidity (i.e. strokes) and mortality. We aimed to characterize the impact of hTTP diagnosis and the effect of plasma prophylaxis on pregnancy outcomes.

Method Observational study on female patients with confirmed hTTP diagnosis (ADAMTS13 activity < 10 %, bi-allelic *ADAMTS13* mutations) enrolled in the International hTTP Registry (clinicaltrials.gov #NCT01257269) before June 30, 2023. Documented pregnancies were divided into before (retrospective, incl. index pregnancy) and after (prospective) hTTP diagnosis.

Results By the end of June 2023, the Registry had enrolled 131 female hTTP patients. Of them, 87 (66.4 %) had been pregnant one or several times, resulting in 214 documented pregnancies. Half of these patients (n = 44, 50.6 %) received their hTTP diagnosis because of obstetrical complications (index pregnancy) at a median maternal age of 26.7 (IQR, 23.5-31.2). The live-birth rate for the 125 retro- and 89 prospectively followed pregnancies was 52.8 % (n = 66) and 79.8 % (n = 71), respectively. Miscarriage was the main adverse outcome in retro- and prospectively followed pregnancies (n = 30, 50.8 %; vs. n = 13, 72.2 %), followed by late abortion (n = 14, 23.7 %; n = 4, 22.2 %). Stillbirth (n = 7, 11.9 %) and neonatal death (n = 8, 13.6 %) were observed only before hTTP diagnosis. Continued plasma prophylaxis, or prophylactic plasma infusions started in on-demand treated patients when pregnancy was recognized, increased live-birth rates to 85.7 % and 76.2 %, respectively, compared to 37.5 % in patients without treatment. In 10/88 (11.4 %) pregnancies after hTTP diagnosis, aspirin was given in addition (live-birth rate 80 %). Acute TTP episodes and occurrence of preeclampsia were lowest in patients on plasma prophylaxis before becoming pregnant.

Conclusion The International hTTP Registry cohort substantiates that a diagnosis of hTTP and associated plasma prophylaxis during pregnancy reduces maternal morbidity and increases the live-birth rate considerably. The earlier plasma prophylaxis is started, the larger the positive effect. As hTTP confers an increased risk for preeclampsia, the low prescription rate of aspirin leaves room for improvement.

Conflict of Interest NA

T-11-02 Identification of key regulators of procoagulant COAT platelet generation by quantitative temporal phosphoproteomic analysis

Authors L. Veuthey, A. Aliotta, D. Bertaggia Calderara, C. Pereira Portela, L. Alberio
Institute Lausanne University Hospital (CHUV) and University of Lausanne (UNIL), Division of Hematology and Central Hematology Laboratory, Lausanne, Switzerland
DOI 10.1055/s-0044-1779169

Introduction Decreased or enhanced procoagulant platelet generation may lead to bleeding or thrombotic events, respectively. The intracellular program underlying the dichotomous generation of aggregating (AGG) and procoagulant (COAT) platelets upon combined activation by Collagen-And-Thrombin is only partially described. In this study, we investigated the utility of timelapse phosphoproteomics to identify potential early regulators of the procoagulant response and further describe the procoagulant phenotype.

Method Human platelets from 3 to 5 healthy controls were activated at RT simultaneously with convulxin (agonist of the collagen receptor GPVI) plus thrombin in presence or absence of calcium, which generated procoagulant or aggregating phenotypes, respectively. Platelets were sampled at baseline and at different timepoints up to 8 min after activation. The phosphoproteomes of resting, AGG and COAT platelets were analysed by isobaric Tandem-Mass-Tag based Mass Spectrometry strategy. Phosphosites significantly changing compared to baseline and differentially regulated among AGG versus COAT platelets were identified by non-parametric ANOVA test.

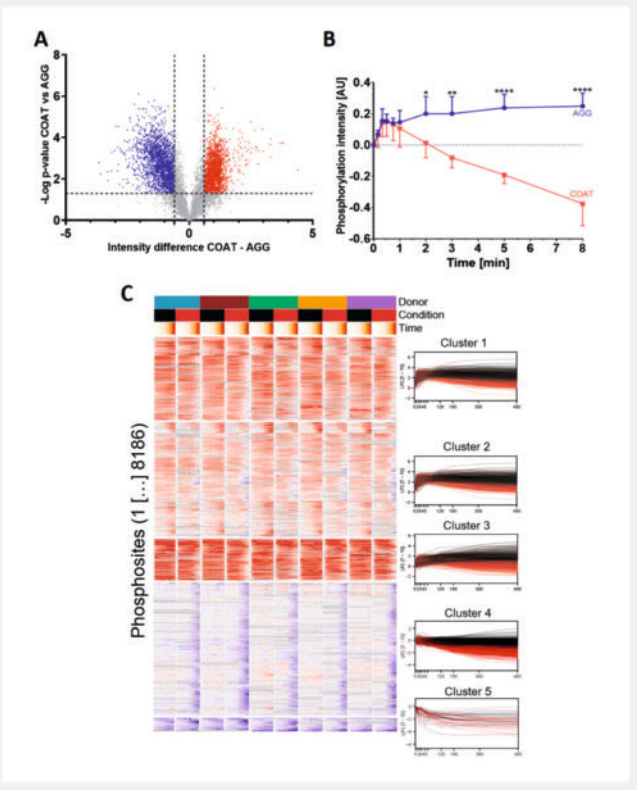
Results We identified 4223 differently regulated phosphosites corresponding to 1643 unique proteins showing significant changes at 8 min after stimulation (▶ **Fig. 1a**). Starting at 1 min after activation, proteins gradually dephosphorylated in COAT platelets and hyper-phosphorylated in AGG platelets (▶ **Fig. 1b**). Phosphosites were clustered in 5 groups based on their common patterns of phosphorylation changes over 8 min (▶ **Fig. 1c**). All clusters showed decreased phosphorylation in COAT platelets at late timepoints.Potential regulators of the dichotomous program may be proteins showing early (during the first 45 sec after activation) differential phosphorylation, such as actors of cytoskeleton remodelling (DOCK families and small GTPase regulators), lipid transferases (DAPP1 and C2CD2L), ion exchangers (SLC9A1, TRPC6) and mitochondrial fission (DNM1L, MFF).

Conclusion The present study highlighted the utility of phosphoproteome analysis to detect time-dependent changes of key molecular regulators of the dichotomous response leading to the generation of COAT besides AGG platelets. We showed a global dephosphorylation in COAT platelets, possibly initiated by profound membrane remodelling and translocation of phosphatidylserine. We hypothesize that this decrease is due to the disassembly in the inner leaflet of platelet membrane of several protein kinase (PK) complexes requiring phosphatidylserine for binding, such as Fyn, PKC, and phospholipase A2 [1–3]. Moreover, the global dephosphorylation may also be explained by the differential phosphorylation of several actin phosphatase regulators and by the activation of a proteasome pathway. Finally, we identified several potential early regulators of the dichotomous platelet response upon combined convulxin and thrombin activation.

Conflict of Interest No conflict of interest to disclose.

References

[1] Stahelin RV, Cho W. Roles of calcium ions in the membrane binding of C2 domains. Biochem J 2001; 359 (Pt 3): 679–685
[2] Verdaguer N, Corbalan-Garcia S, Ochoa WF, Fita I, Gómez-Fernández JC. Ca2 + bridges the C2 membrane-binding domain of protein kinase Cα directly to phosphatidylserine. The EMBO Journal 1999; 18 (22): 6329–6338
[3] Wu N, Song H, Veillette A. Plasma membrane lipid scrambling causing phosphatidylserine exposure negatively regulates NK cell activation. Cellular & Molecular Immunology 2021; 18 (3): 686–697

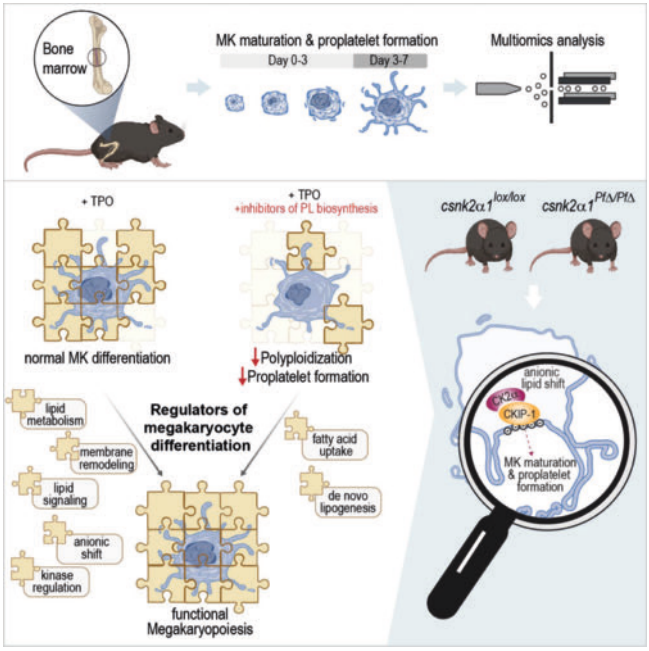


▶ **Fig. 1 Snapshot and timelapse phosphoproteome analysis of aggregant and procoagulant platelets**; (**a**) Volcano plot of all 7219 phosphosites mean intensity values. Procoagulant (COAT) platelet proteins demonstrated downregulated phosphorylation status compared to aggregant (AGG) ones at 8 min (n = 3). (**b**) Time-dependent changes of the overall phosphorylation status during convulxin-plus-thrombin stimulation in AGG (blue circle) compared to COAT (red square) platelets. (n = 5, 1865 phosphosites). (**c**) Heatmap for each phosphosite up to 8 min of activation, in which we could identify 5 clustering groups (n = 3-5, all 8186 phosphosites). P-values, *<0.05, **<0.01, ****<0.0001

T-11-03 Decoding Blood Platelet Production: The Intricate Role of Lipids

Authors B. de Jonckheere[1, 2], F. Kollotzek[3, 4], O. Borst[3, 4], R. Ahrends[1]
Institutes **1** University of Vienna, Institute of Analytical Chemistry, 1090, Austria; **2** University of Vienna, Vienna Doctoral School in Chemistry, 1090, Austria; **3** Eberhard-Karls University of Tuebingen, Department of Cardiology and Angiology, 72074, Germany; **4** Eberhard-Karls University of Tuebingen, DFG Heisenberg Group Thrombocardiology, 72074, Germany
DOI 10.1055/s-0044-1779170

Introduction During megakaryopoiesis and consecutive platelet production, megakaryocytes undergo cellular morphological changes which are associated with the reprogramming of signaling pathways. Moreover, membrane composition and lipid signaling are expected to be strongly modified. However, the knowledge of how lipids are modulated and which pathways are involved is still lacking.



▶ **Fig. 1 Lipid-driven functional regulation and underlying mechanisms of MK maturation and thrombopoiesis;** The study focuses on the functional analysis and regulation of MK maturation, using a multiomics approach and incorporating both in vitro and in vivo methodologies. Isolated hematopoietic stem cells from murine bone marrow were subjected to a 7-day differentiation protocol with TPO. The SIMPLEX workflow was used to comprehensively determine the general molecular composition of MKs. The results revealed significant anionic lipid membrane remodeling and relocalization of the CKIP-1/CK2α complex to the plasma membrane, which appear to be essential for adequate platelet biogenesis.

Method Here, we adopt a lipid-centric multiomics approach applying the SIMPLEX protocol [1], which allows for simultaneous lipid and protein sample preparation, to create a quantitative map of the murine megakaryocyte lipidome during maturation and proplatelet formation. Mass spectrometry-based findings were combined with both in vitro and in vivo methodologies to functionally analyse and elucidate the underlying mechanisms of megakaryocyte maturation and proplatelet formation.

Results Our data reveal that megakaryocyte differentiation is associated with enhanced expression of lipid-related enzymes and driven by an increased fatty acyl import and *de novo* lipid synthesis, resulting in the modulation towards an anionic membrane phenotype. Pharmacological perturbation of fatty acid import and phospholipid synthesis proved to block membrane remodeling and directly reduced megakaryocyte polyploidization and proplatelet formation, leading to thrombocytopenia.

Furthermore, the anionic lipid shift during megakaryopoiesis is accompanied by the relocalization of the scaffold protein CKIP-1 and recruitment of the kinase CK2α to the plasma membrane, which is essential for platelet biogenesis (▶ **Fig. 1**).

Conclusion Overall, this study provides a framework to understand how the megakaryocyte lipidome is altered during maturation and proplatelet formation and the effect of membrane lipid remodeling on megakaryocyte kinase signaling involved in thrombopoiesis.

Conflict of Interest We declare no conflict of interest.

References

[1] Coman C. et al. Simultaneous Metabolite, Protein, Lipid Extraction (SIMPLEX): A Combinatorial Multimolecular Omics Approach for Systems Biology. Mol Cell Proteomics 2016; 15 (4): p 1453–66

T-11-04 Identification of an Unmet Medical Need – Height of Depression, Hypersomnia and Sleep Apnea Positively Correlate with the Level of Fatigue in Patients with Immune Thrombocytopenia

Authors R. S. Alesci[1], C. Hecking[1], M. V. Weißmann[2], C. E. Dempfle[2]
Institutes 1 IMD Blood coagulation centre, Hochtaunus, Bad Homburg, Germany; 2 IMD Blood coagulation centre, Mannhei, Mannheim, Germany
DOI 10.1055/s-0044-1779171

Introduction Immune thrombocytopenia (ITP) is a rare chronic disease, frequently accompanied by fatigue, which is an important comorbidity associated with this disease [1]. Patients experience difficulties to manage their daily activities and a reduction in their overall quality of life (QoL) [2]. The causes of fatigue in ITP are not clarified yet and underlying causes seem to be multifactorial. The development of fatigue may not solely be influenced by a decrease in platelet count, but also by unknown factors as well as psychological reasons [3, 4].

Method This prospective, multicenter, exploratory, pilot study aimed to investigate which parameters contribute to the occurrence of fatigue in patients with ITP. Adult patients with ITP and with or without fatigue, who visited the study center for their regular appointments were asked to complete questionnaires pertaining to patient-reported outcome measures regarding depression, sleep apnea and hypersomnia and the ISTH-BAT. Blood tests included platelet count as well as different parameters like vitamin D (▶ **Fig. 1**).

Results A total of 36 patients (27 females, nine males) with primary ITP, with a median age of 46.5 years (min-max 19-83) were analyzed. Median duration of ITP was 4.5 years (min-max 0-21). Approximately one-third of patients (29.4 %) had no comorbidities.The two most frequently used current treatment options were 'watch-and-wait' (38.9 %) and thrombopoietin-receptor-agonists (58.3 %); eight patients needed rescue therapy. There was a statistically negative correlation between fatigue and year of diagnosis ($r = -0.41$, $p = 0.014$). Results indicated no statistically significant relationship in fatigue and age or gender. Ferritin predicted fatigue with statistical significance while platelet count was not correlated. A significant correlation was obvious between fatigue, depression and obstructive sleep apnea syndrome as well as sleep-related problems ($p < 0.01$).

Scale	Items (n)	Range of scores	Mean (SD)	Alpha
FACIT-F	13	4-47	1.67 (0.99)	0.95
BDI	21	0-26	0.54 (0.51)	0.93
Hypersomnia	8	3-19	0.85 (0.45)	0.83
OSAS	8	2-22	1.09 (0.62)	0.83
STOP-BANG	5	1-6	0.26 (0.20)	0.55
ISTH/SSC-Bleeding Assessment Score	10	0-56	0.17 (0.10)†	0.063†

†Reliability of the ISTH/SSC-Bleeding Assessment Score could not actually be calculated as the items had no variance.
BDI: Beck Depression Inventory; FACIT-F: Functional Assessment of Chronic Illness Therapy–Fatigue; FAS: full-analysis set; ISTH/SSC: International Society on Thrombosis and Haemostasis/Scientific and Standardization Committee; n: number; OSAS: obstructive sleeping apnea syndrome; SD: standard deviation; STOP-BANG: snore, tired, observed apneas, pressure, body mass index, age, neck circumference, gender

▶ **Fig. 1 Internal consistency of total score, trial outcome index, and subscales (FAS);** BDI: Beck Depression Inventory; FACIT-F: Functional Assessment of Chronic Illness Therapy–Fatigue; FAS: full-analysis set; ISTH/SSC: International Society on Thrombosis and Haemostasis/Scientific and Standardization Committee; n: number; OSAS: obstructive sleeping apnea syndrome; SD: standard deviation; STOP-BANG: snore, tired, observed apneas, pressure, body mass index, age, neck circumference, gender

Conclusion Patient characteristics were comparable to that of other studies [1–4]. The level of fatigue negatively impacts the lives of patients with ITP. Age and gender were not correlated with fatigue in ITP, which is in line with other reports. Interestingly, the fatigue level was higher in patients presenting with

an additional depression and poor sleeping quality due to, e.g., hypersomnia, which seems not uncommon [5]. Fatigue levels seem independent from thrombocyte levels, which was reported elsewhere [3].

Patients diagnosed with ITP several years ago cope with their condition better than patients with a more recent diagnosis, who have higher levels of fatigue. Concurrent depression, hypersomnia and sleep apnea are important underestimated factors, which do have a negative effect on the QoL of patients with ITP. We were able to show that patients with ITP might face an unmet medical need in terms of delayed diagnosis and supportive therapy. To our knowledge this is the first report on combined findings of depression, hypersomnia and sleep apnea in patients with ITP.

Conflict of Interest This study was supported by Novartis Pharma GmbH, Nuremberg, Germany. RSA acted also as a speaker and advisory board member within the last two years. CHE and CED were member of advisory board organised by Novartis Pharma GmbH.

References

[1] Matzdorff A, Alesci SR, Gebhart J et al. Expert Report on Immune Thrombocytopenia: Current Diagnostics and Treatment – Recommendations from an Expert Group from Austria, Germany, and Switzerland. Oncol Res Treat 2023; 46: (Suppl 2): 5–44. doi:10.1159/000529662.

[2] Hill QA, Newland AC Fatigue in immune thrombocytopenia. Br J Haematol 2015; 170: 141–149. doi:10.1111/bjh.13385.

[3] Cooper N, Kruse A, Kruse C et al. Immune thrombocytopenia (ITP) world impact survey (iWISh): patient and physician perceptions of diagnosis, signs and symptoms, and treatment. Am J Hematol 2021; 96: 188–198. doi:10.1002/ajh.26045.

[4] Newton JL, Reese JA, Watson SI et al. Fatigue in adult patients with primary immune thrombocytopenia. Eur J Haematol 2011; 86: 420–429. doi:10.1111/j.1600-0609.2011.01587.x.

[5] Farney RJ, Walker BS, Farney RM, Snow GL, Walker JM The STOP-Bang equivalent model and prediction of severity of obstructive sleep apnea: relation to polysomnographic measurements of the apnea/hypopnea index. J Clin Sleep Med 2011; 7: 459–465b. doi:10.5664/jcsm.1306.

T-11-05 USP25 Regulates Platelet Function During Aging by Stabilizing Talin-1

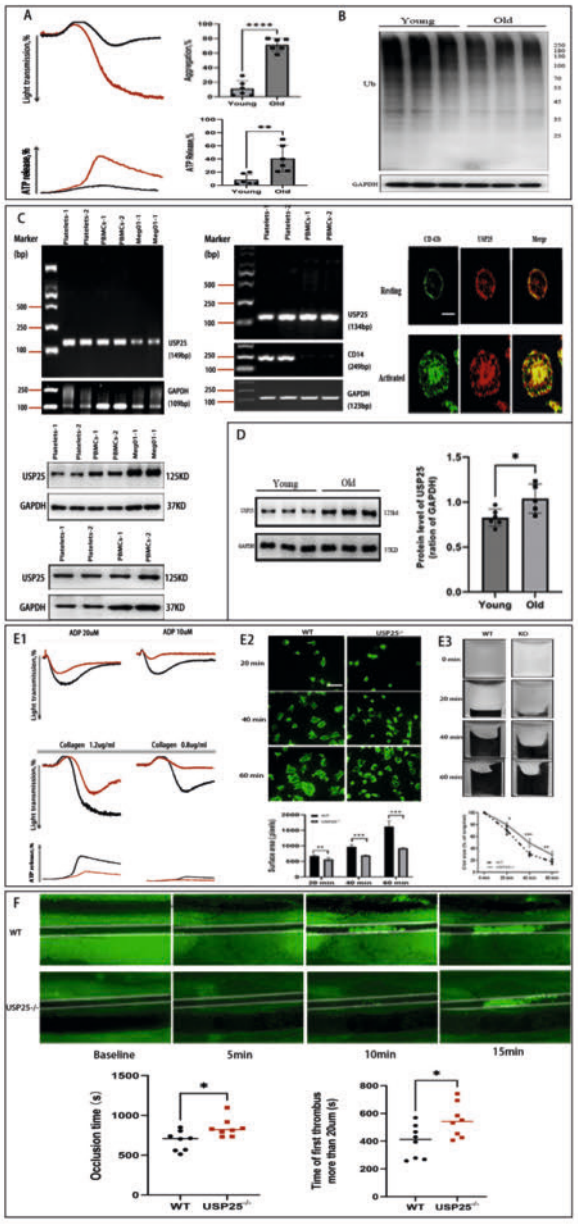
Authors X. Jia[1], S. Jiang[2], Z. Chen[3], R. Xia[1]

Institutes 1 Fudan University, Huashan hospital, Shanghai, China; 2 Fudan University, Department of Biochemistry and Molecular Biology, School of Basic Medical Sciences, Shanghai, China; 3 Fudan University, School of Basic Medical Sciences, Shanghai, China

DOI 10.1055/s-0044-1779172

Introduction Aging is an independent risk factor for the incidence of arterial thrombotic events. Loss of protein homeostasis is one of hallmarks for many aged-related diseases. Sing cell RNA sequencing of megakaryocyte in previous study have identified different patterns of protein ubiquitination between old and young murine. However, regulation of protein ubiquitination in platelet during aging remain poorly understood.

Method Platelet functions including aggregation, ATP release were compared in old (>18 months) and young (2-3 months) mice. The level of total ubiquitination was evaluated by western blot. Available datasets were dig out to identify the most abundant deubiquitinating enzymes (DUB) and finally USP25 was chosen. Then, western blot, RT-PCR and immunofluorescence were used to confirm the expression of USP25. The platelet function was conducted in WT and USP25 knock-out mice. To characterize the mechanism of USP25 in the regulation of platelet function, ubiquitinated proteomics were conducted to identify the substrate of USP25 in platelet. Later co-immunoprecipitation and western blot were used to confirm the deubiquitinase activity of USP25 in platelet. Eventually, small molecular compound AZ-1, an USP25 inhibitor, was tested in terms of platelet function [1–10].



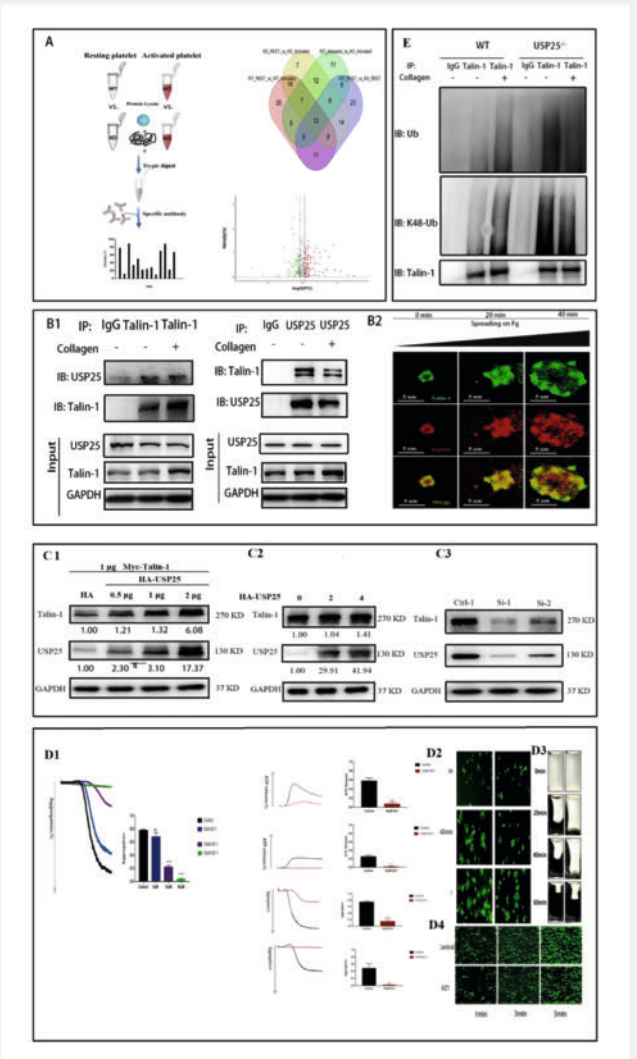
▶ **Fig. 1** Enhanced platelet function and decreased level of ubiquitination were observed in old mice. Expression of USP25 were confirmed in platelet. Upregulation of USP25 was found in old mice platelet. In vitro, aggregation, ATP release, spreading and clot retraction were inhibited in USP25-knockout mice, compared with age- and sex- matched wild type mice.In vivo, decreased final occlusion time of FeCl3-induced mesenteric arteriole thrombosis were observed in USP25 knock-out mice

Results Enhanced platelet function and decreased level of ubiquitination were observed in old mice. The loss of ubiquitination can be caused by upregulation of DUBs. Then we screened DUBs in three datasets and determined that USP25 is one of the most abundant DUBs in both human and murine platelet. Expression of USP25 were confirmed in platelet . Upregulation of USP25 was found in old mice platelet. To determine the effect of USP25 in the regulation of platelet function, we constructed USP25-knockout mice. In vitro, aggregation, ATP release, spreading and clot retraction were inhibited in USP25-knockout mice, compared with age- and sex- matched wild type mice. In vivo, decreased final

occlusion time of $FeCl_3$-induced mesenteric arteriole thrombosis were observed in USP25 knock-out mice. Mechanistically, by using ubiquitinated proteomics, we found that talin-1 served as one target of USP25. The association of USP25 and talin-1 were confirmed by co-immunoprecipitation and western blot. We then observed that USP25 can maintain the expression of talin-1. The expression of talin-1 was significantly down-regulated when silencing USP25 in CHO-K1 cells, as well as in USP25 knock-out platelet. Further, the results showed that USP25 can directly deubiquitinate talin-1. Finally, we found that small molecular compound AZ-1, one USP25 inhibitor, can significantly decrease platelet function including aggregation, ATP release, spreading and chamber in human platelet (▶ **Fig. 1**, ▶ **Fig. 2**).

Conclusion Overall, our study found that upregulation of USP25 may serve as a mechanism of platelet hyperactivity during aging. Furthermore, AZ1, an USP25 inhibitor, could be a potential target for thromboembolism.

Conflict of Interest All authors disclosed no relevant relationships



▶ **Fig. 2** By using ubiquitinated proteomics, we found that talin-1 served as one target of USP25. The association of USP25 and talin-1 were confirmed by co-immunoprecipitation and western blot. We then observed that USP25 can maintain the expression of talin-1. The expression of talin-1 was significantly down-regulated when silencing USP25 in CHO-K1 cells, as well as in USP25 knock-out platelet.Further, the results showed that USP25 can directly deubiquitinate talin-1.Finally, AZ-1, an USP25 inhibitor, can decrease the platelet function

References

[1] Heron M. Deaths: Leading Causes for 2019. Natl Vital Stat Rep 2021; 70 (9): p. 1–114

[2] Di Micco R. et al. Cellular senescence in ageing: from mechanisms to therapeutic opportunities. Nat Rev Mol Cell Biol 2021; 22 (2): p 75–95

[3] López-Otín C. et al. Hallmarks of aging: An expanding universe. Cell 2023; 186 (2): p 243–278

[4] Hipp M.S., Kasturi P., Hartl F.U. The proteostasis network and its decline in ageing. Nat Rev Mol Cell Biol 2019; 20 (7): p 421–435

[5] Galves M. et al. Ubiquitin Signaling and Degradation of Aggregate-Prone Proteins. Trends Biochem Sci 2019; 44 (10): p 872–884

[6] Davizon-Castillo P. et al. TNF-α-driven inflammation and mitochondrial dysfunction define the platelet hyperreactivity of aging. Blood 2019; 134 (9): p 727–740

[7] Zhong B. et al. Negative regulation of IL-17-mediated signaling and inflammation by the ubiquitin-specific protease USP25. Nat Immunol 2012; 13 (11): p 1110–7

[8] Zhong B. et al. Ubiquitin-specific protease 25 regulates TLR4-dependent innate immune responses through deubiquitination of the adaptor protein TRAF3. Sci Signal 2013; 6 (275): p ra35

[9] Teo Q.W. et al. Usp25-Erlin1/2 activity limits cholesterol flux to restrict virus infection. Dev Cell 2023

[10] Lin D. et al. Induction of USP25 by viral infection promotes innate antiviral responses by mediating the stabilization of TRAF3 and TRAF6. Proc Natl Acad Sci U S A 2015; 112 (36): p 11324–9

T-11-06 GPVI deficiency in a 17-year-old girl, congenital or acquired?

Authors P. Bradáčová[1], L. Slavík[2], J. Ullrychová[1], J. Úlehlová[3], D. Procházková[4]

Institutes **1** Masaryk Hospital, Department of clinical hematology, Ústi nad Labem, Czech Republic; **2** Faculty of Medicine and Dentistry, Palacky University Olomouc, Olomouc, Czech Republic; **3** University Hospital Olomouc, Department of Hemato-oncology, Olomouc, Czech Republic; **4** Masaryk Hospital, Department of pediatric hematology, Ústí nad Labem, Czech Republic; **5** Institute of Hematology and Blood Transfusion, Praha, Czech Republic

DOI 10.1055/s-0044-1779173

Introduction The diagnosis of the bleeding complications causes remains a very complicated part of the diagnostic process. In particular, distinguishing whether the disorder affects primary haemostasis or the plasma factor system can be very complicated, as can the question of whether it is a congenital or acquired condition. In our case report, we demonstrate inhibition of glycoprotein VI.

Glycoprotein VI (GPVI) is specifically expressed on megakaryocytes and platelets. It is a receptor for collagen-mediated platelet aggregation. GPVI deficiency is associated with bleeding manifestations. In congenital GPVI deficiency in Gray platelet syndrome (GPS), alpha-granules do not mature in platelets, the cause being a mutation in the NBEAL2 gene. GPS is manifested by mild macro-thrombocytopenia, platelet hypogranularity and reduced aggregation after collagen. The cause of acquired GPVI deficiency may be due to anti-GPVI autoantibodies that occur in association with autoimmune diseases such as autoimmune thyroiditis, autoimmune thrombocytopenia and systemic lupus erythematosus.

Method We report an unusual case of a young girl with sudden onset of moderate bleeding. The following tests were repeatedly performed: blood count, platelet count (PLT) and morphology, PFA-200, optical platelet aggregation, anti-platelet antibody ELISA, coagulation tests, platelet glycoprotein determination by flowcytometry. Genetic testing for congenital mutations was performed.

Results A 17-year-old girl presents to the pediatric hematology department for sudden onset of excessive bruising. The patient has autoimmune thyroiditis, otherwise healthy. 14 days ago she was vaccinated with Comirnaty Pfizer against Covid 19.

Hämostaseologie 2024; 44: S1–S125

Her platelet count is decreased 113; 96; 108 x10^9/L. On microscopy, we observe hypogranular macro-thrombocytes, PFA-200 COL-EPI > 300s, COL-ADP 120s pathology is present, there is very reduced aggregation after collagen 2 %, positive (+) anti-PLT, positive antinuclear antibody titer 1: 80, positive anti-TPO 62.88 kU/L, positive anti-TG 281 kU/L There is significantly decreased expression of GPVI 854 sABC. Coagulation tests are without pathology. Genetic testing did not show NBEAL2 mutation.

Over 1.5 years, PLT counts have gradually normalized, but some PLTs are still hypogranular, PFA200 pathology persists, and post-collagen aggregation is no longer normal. Clinical manifestations have diminished, however, extensive bruising still forms after insult.

Conclusion On the basis of the investigations performed, we excluded congenital Gray platelet syndrome as no NBEAL2 mutation was found. We are inclined towards acquired GPVI deficiency, which is described among others in autoimmune thyroiditis. We explain the reduced PLT count by vaccination with Comirnata Pfizer, which may cause autoimmune thrombocytopenia. In combination with the reduced PLT count and reduced GPVI expression, the patient may have developed significant bleeding manifestations. After normalization of PLT, the bleeding manifestations subsided.

Conflict of Interest No conflict of interest.

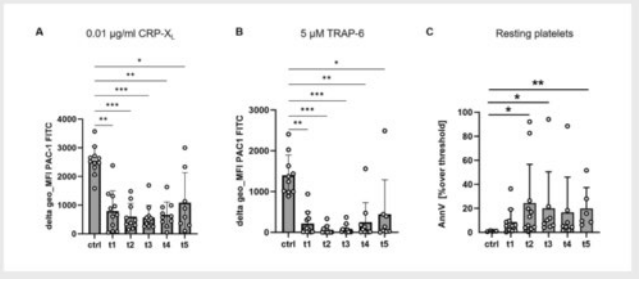
T-11-07 Myeloablation and allogeneic hematopoietic stem cell transplantation causes a marked GPIIb/IIIa activation defect and procoagulant platelet phenotype

Authors K. Mott[1], L. J. Weiss[1, 2], M. Drayss[1, 2], B. Just[1], S. Kraus[3], T. Steinbrunn[3, 4], H. Schulze[1]

Institutes **1** University Hospital Würzburg, Institute of Experimental Biomedicine, Würzburg, Germany; **2** University Hospital Würzburg, Department of Internal Medicine I, Würzburg, Germany; **3** University Hospital Würzburg, Department of Internal Medicine II, Würzburg, Germany; **4** Harvard Medical School, Dana-Farber Cancer Institute, Department of Medical Oncology, Boston, USA

DOI 10.1055/s-0044-1779174

Introduction In response to allogeneic hematopoietic stem cell transplantation (HSCT), all blood cell lineages become replaced by donor-derived cells. During this process delayed engraftment results in a period of low platelet counts. Paradoxically, despite thrombocytopenia and associated bleedings, there is also an increase of thrombotic events including veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS), of which the underlying mechanisms are yet unknown. While platelet counts are routinely monitored to evaluate their engraftment, platelet function after HSCT has not been studied so far. Here, we aimed to comprehensively assess platelet phenotype and function during the course of transplantation and engraftment (▶ **Fig. 1**).



▶ **Fig. 1 GPIIb/IIIa activation defect and procoagulant platelets in HSCT patients;** (A) Stimulation of platelets from HSCT patients with high doses of CRP-XL or (B) thrombin receptor activator peptide 6 (TRAP-6) revealed markedly impaired GPIIb/IIIa activation. (C) Platelets in HSCT patients exhibit a procoagulant phenotype as indicated by increased annexin V (AnnV) binding.

Method 13 adult allogeneic HSCT patients treated for acute leukemia were recruited at the University Hospital Würzburg (EV 301/21). Patients were conditioned by total body irradiation (TBI) with chemotherapy or chemotherapy alone before receiving 3.8 – 9.6 x 10^6/kg mobilized CD34$^+$cells. Platelet function was studied by flow cytometry before conditioning (t_1), after platelet engraftment (3 consecutive days over 20/nL, t_2) and after 30 (t_3), 60 (t_4), and 100 days (t).

Results Surface expression of GPIb/V/IX and GPIIb/IIIa was overall unaltered after HSCT compared to healthy controls (HCs). CD9 exhibited a transient decrease at t_2 while GPVI was downregulated at all time-points. We detected no differences in the number of PAC-1$^+$or CD62P$^+$resting platelets, indicating that platelets were not pre-activated in response to myeloablation and HSCT. Upon stimulation with high doses of ADP, TRAP-6, CRP-X_L or convulxin, we detected massively impaired integrin activation, still present at t_5, whereas α-granule release was only mildly compromised compared to HCs. Subpopulation analysis of activated platelets revealed a decreased percentage of CD62P$^-$PAC-1$^+$and CD62P$^+$PAC-1$^+$but not CD62P$^+$PAC-1$^-$ events in HSCT patients, suggesting that integrin activation and P-selectin exposure become decoupled. Platelet δ-granule loading and release was monitored indirectly by flow cytometry using mepacrine. Mepacrine uptake was markedly impaired in platelets of HSCT patients at t_2, t_3 and t_4. In line with this, the relative mepacrine release in response to activation was significantly impaired until t_5, implying that there is a sustained paucity of δ-granules in these platelets. HSCT promotes activation of plasmatic coagulation. Intriguingly, we found massively upregulated annexin V binding and reduced mitochondrial membrane potential, indicative of procoagulant platelets.

Conclusion We demonstrate for the first time that platelets in HSCT patients exhibit a severe integrin activation defect and a paucity of δ-granules, which is uncoupled from α-granule release. Further, we provide evidence that this is associated with a massive increase of procoagulant platelets, which are prone to promote thrombosis. Our data suggest a mechanism why both bleeding and thrombotic events can occur after HSCT.

Conflict of Interest No conflict of interest to declare.

T-11-08 Differential binding of recombinant factor VIII concentrates to platelets may impact platelet functionality

Authors A. Strebel[1], F. A. Pennacchio[1], S. Lickert[1], K. Selçuk[1], R. Klamroth[2], V. Vogel[1]

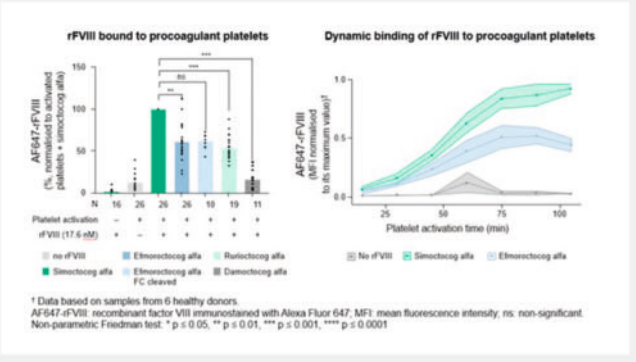
Institutes **1** ETH Zurich, Laboratory of Applied Mechanobiology, Department of Health Sciences and Technology, Zurich, Switzerland; **2** Vivantes Klinikum im Friedrichshain, Zentrum für Hämophilie und Hämostaseologie, Berlin, Germany

DOI 10.1055/s-0044-1779175

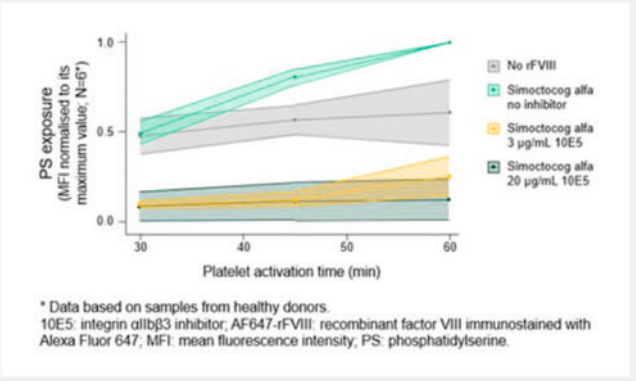
Introduction After vascular injury, platelets become activated to a pro-aggregatory state, with a subpopulation undergoing a phenotype shift to a pro-coagulant state. Pro-coagulant platelets bind and localize factor VIII (FVIII) at injury sites. It is unclear whether modifications in recombinant (r)FVIII products impact FVIII-platelet binding and platelet signalling. Here we examined the binding of different rFVIII products to platelets *in vitro*, and the impact of rFVIII binding on the platelet phenotype and intracellular signalling.

Method Platelets isolated from healthy donors and people with severe haemophilia A were activated with thrombin and cross-linked collagen-related peptide.Activated platelets were incubated with simoctocog alfa, efmoroctocog alfa, rurioctocog alfa pegol or damoctocog alfa pegol. Binding of rFVIII to platelets was quantified by flow cytometry using immunostaining or by direct measurement of labelled rFVIII concentrates. The extent of phosphatidylserine (PS) exposure on the platelet surface was used to measure the shift to a pro-coagulant state. Annexin V-BV421 was used as a molecular probe to assess PS exposure levels. To assess the role of integrin αIIbβ3 signalling in the response of platelets to FVIII binding, platelets were concurrently activated and incubated with the integrin αIIbβ3 inhibitory antibody 10E5.

Results Binding to platelets of healthy donors was significantly higher with simoctocog alfa than with efmoroctocog alfa, rurioctocog alfa pegol or damoctocog alfa pegol irrespective of the detection method (▶ **Fig. 1**). The rFVIII-platelet interactions observed in the static assay were validated for simoctocog alfa and efmoroctocog alfa using a dynamic platelet-binding assay (▶ **Fig. 1**). Incubation of activated platelets with rFVIII resulted in increased PS exposure, with a greater effect observed with simoctocog alfa than with efmoroctocog alfa. Immunostaining revealed co-localization of simoctocog alfa and integrin αIIbβ3 in pro-aggregatory platelets. Inhibition of integrin αIIbβ3 decreased the binding of simoctocog alfa to platelets in a dose-dependent manner (▶ **Fig. 2**). Similar trends were observed for the analysis of both platelet binding and phenotype shift in platelets from people with severe haemophilia A.



▶ **Fig. 1** Simoctocog alfa shows increased binding to activated platelets vs other rFVIII products



▶ **Fig. 2** Simoctocog alfa increases PS exposure, which is reduced by integrin αIIbβ3 inhibition

Conclusion Simoctocog alfa demonstrated higher binding to activated platelets from healthy donors and people with haemophilia A *in vitro* compared with other rFVIII products. The increased binding was associated with a phenotypic shift in platelets and was disrupted with inhibition of integrin αIIbβ3, suggesting a role of integrin αIIbβ3 signalling following binding of FVIII to platelets. Variations in platelet binding and signalling between different rFVIIIs might impact their therapeutic efficacy for the prevention of bleeds in people with haemophilia A.

Conflict of Interest KS and KW declare no conflict of interest. AS and SL have received research funding from Octapharma AG. FAP has received research funding and travel grants from Octapharma AG. RK has been on an advisory board and has received honoraria from Octapharma AG. VV has received research funding for this work from ETH Zurich and Octapharma AG, and has received travel grants from Octapharma AG.

T-11-09 Mitochondrial calcium uniporter as a Key Regulator in the Generation of Procoagulant COAT Platelets

Authors D. Shehwar[1, 2], A. Aliotta[1], S. Barki[2], D. Bertaggia Calderara[1], L. Veuthey[1], C. Pereira Portela[1], M. Rizwam Alam[2], L. Alberio[1]
Institutes **1** Lausanne University Hospital (CHUV) and University of Lausanne (UNIL), Division of Hematology and Central Hematology Laboratory, Lausanne, Switzerland; **2** Quaid-i-Azam University, Department of Biochemistry, Islamabad, Pakistan
DOI 10.1055/s-0044-1779176

Introduction Procoagulant platelets represent a subpopulation of platelets that are phenotypically and functionally different from aggregating platelets. The combined activation of platelets with collagen-plus-thrombin induces the formation of procoagulant COAT platelets, which expose phosphatidylserine (PS) and down-regulate integrin alphaIIb/beta3. This dual agonist stimulation mobilizes high level of cytosolic calcium, which is taken up by mitochondria through mitochondrial calcium uniporter (MCU). A significant rise in mitochondrial calcium results in the depolarization of mitochondrial membrane potential, which triggers the opening of mitochondrial permeability transition pore (mPTP) to achieve the supramaximal cytosolic calcium level required for PS exposure. Thus, in this study, we aimed to explore the function of MCU in the generation and modulation of procoagulant COAT platelets.

Method Platelets were activated with thrombin (THR) or convulxin (CVX, collagen GPVI agonist) or a combination of these. Flow cytometry was employed to monitor procoagulant and aggregatory properties of platelets using Annexin-V and PAC-1 binding, respectively. Function of MCU was modulated with specific inhibitors, namely mitoxantrone (MTX) or Ru265.

Results Our results demonstrated that MTX had non-significant effect on single THR stimulated platelets as this agonist triggered poor procoagulant platelet formation. In contrast, platelets stimulated with single CVX generated moderate procoagulant platelets and MTX showed a significant inhibition in the generation of Annexin V positive platelets. Furthermore, when we co-stimulated the platelets with THR-plus-CVX, our results demonstrated that MTX exhibited significant decrease (-23 % ± 1.26) in Annexin V positive platelets and inversely increase (+ 36 % ± 4.6) in PAC-1 positive platelets. Data were replicated and validated by blocking MCU with Ru265.

Conclusion Altogether, the present study revealed that CVX pathway alone and synergistically with THR enhances the formation of procoagulant platelets through MCU-driven mitochondrial calcium uptake. MCU is as a key regulator in the generation of procoagulant platelets.

Conflict of Interest No conflict of interest to disclose.

T-11-10 High risk of thromboembolic events in adult with primary immune thrombocytopenia: results from a prospective cohort study

Authors J. Rast[1], T. Schramm[1], D. Mehic[1], M. Fillitz[2], B. Dixer[2], C. Ay[1], I. Pabinger[1], J. Gebhart[1]
Institutes **1** Medical University of Vienna, Department of Internal Medicine I; Clinical Division of Hematology and Hemostaseology, 1090, Austria;
2 Hanusch Hospital, Third Department of Internal Medicine, 1140, Austria
DOI 10.1055/s-0044-1779177

Introduction Patients with primary immune thrombocytopenia (ITP) have an increased risk of bleeding but paradoxically also an increased thromboembolic risk

Method Between March 2017 and July 2023, 160 patients with primary ITP were enrolled in the Vienna ITP Biobank, a prospective dual-center cohort study (Medical University of Vienna; Hanusch Hospital, EC 1843/2016). Descriptive analysis was performed and the associations between clinical risk factors and prospective thrombotic events (TE) were identified using univariate and multivariate logistic regression analyses.

Results 160 ITP patients were followed for median duration of 35 months (inter-quartile range (IQR): 26-52). Of them 17 (10.6 %) patients experienced a TE after a median of 32 months (IQR: 12-53). These events consisted of 6 ATE (35 %), including 3 acute myocardial infarctions, 1 cerebral vascular events (stroke or transient ischemic attack) and 2 peripheral artery thrombosis and 11 (64.7 %) VTE, inlcuding 4 superficial vein thromboses, 4 deep vein thromboses and 3 cases of isolated pulmonary embolism. The cumulative 1- and 5-year incidence of TE accounting for competing mortality were 4.4 % and 9.4 %. Four (23.5 %) of the patients with thrombosis died during the follow-up period. The cause of death was unclear for three patients, while one patient died from pulmonary embolism. Cardiovascular risk factors were more prevalent in ATE patients (▶ **Fig. 1**). At time of thrombosis the median (IQR) platelet count in patients was 197 (66-218) and 11 (64.7 %) patients received ITP-speciffic treatment, of whom 9 (81.8 %) received thrombopoeitin-receptor agonists. In univariate regression analysis, age over 60 years (OR 6.8 95 % CI 1.4-11.1, p = 0.009), the presence of antiphospholipid antibodies (APLA) (OR 3.5 95 %CI 1.0-11.4, p = 0.035), particularly lupus anticoagulant positivity (OR 2.80; 95 % CI 1.8-9.8, p = 0.022) and anti-cardiolipin antibody positivity (OR 4.7 95 % CI 1.2-25.1, p = 0.030), and previous thrombosis (OR 10.2; 95 % CI 3.4-30.5, p = 0.001), were significantly associated with a higher risk of arterial and venous TE among ITP patients (▶ **Fig. 2**). After conducting multivariate logistic regression analysis, age ≥ 60 and previous thrombosis were found to be the only independent significant risk factors for developing TE (OR 3.8 95 % CI 1.34-43.28, *p* = 0.044; OR 13.2 95 % CI 3.1-45.2 *p* = 0.001).

	Univariate analysis		Multivariate analysis	
Risk factors	**OR (95% CI)**	**P value**	**OR (95% CI)**	**P value**
Age ≥60 years	6.8 (1.4-11.1)	0.009*	3.8 (1.0-13.6)	0.044*
Sex, M vs. F	0.0 (0.3-2.4)	0.801	1.3 (0.4-4.6)	0.07
Smoking	1.5 (0.7-4.2)	0.433	1.6 (0.4-6.1)	0.464
Hypertension	2.2 (0.8-6.7)	0.185	3.3 (0.8-14.7)	0.106
Diabetes mellitus	1.6 (0.1-4.5)	0.062	1.8 (0.1-3.5)	0.052
History of thrombosis	10.2 (3.4-30.5)	0.001*	13.2 (3.1-45.2)	0.001*
Antiphospholipid antibodies	3.5 (1.0-11.4)	0.035*	2.5 (0.6-10.2)	0.453
LA-positive	2.8 (1.8-9.8)	0.022*	2.5 (0.6-11.0)	0.201
aCL Ab	4.7 (1.2-25.1)	0.030*	5.2 (0.8-30.2)	0.081
aβ2GPI Ab	1.1 (0.5-3.5)	0.301	1.5 (0.3-4.2)	0.562
Splenectomy	1.4 (0.6-9.3)	0.232	0.8 (0.1-6.4)	0.821

▶ **Fig. 1 Univariate and multivariate Logistic regression Analysis.** IQR, interquartile range; LA, lupus anticoagulant , aCL Ab, anti-cardiolipin antibodies; ab2GPI Ab, anti-b2 glycoprotein I antibodies; TPO-RAs, thrombopoietin-receptor agonist statistically significant . p-value < 0.05

		All ITP patients (n=160)	All TE patients (n=17)	ATE patients (n=6)	VTE patients (n=11)
Female, n (%)		100 (62.5)	13 (76.5)	4 (66.7)	9 (81.8)
Age, years, mean ± SD		47 ± 18	62 ± 17	63 ± 11	62 ± 21
BMI, kg/m2, median (IQR)		26 (23.0-29.9)	26 (23.3-30.4)	26 (25.2-32.0)	26 (23.0-31.0)
Blood group O, n (%)		52 (32.5)	5 (29.4)	2 (33.3)	3 (27.3)
Smoking, n (%)		20 (12.5)	6 (35.3)	3 (50.0)	3 (27.3)
Hypertension, n (%)		14 (8.75)	8 (47.1)	4 (66.7)	4 (36.4)
Diabetes mellitus, n (%)		35 (21.8)	3 (17.6)	1 (16.7)	2 (18.2)
History of thrombosis n (%)		26 (16.3)	9 (52.9)	4 (66.7)	5 (45.5)
Disease duration, median (IQR)		44 (27.5)	82 (18-186)	55 (2-550)	83 (51-170)
acute, n (%)		16 (10.0)	4 (23.5)	2 (33.3)	2 (20.0)
chronic, n (%)		100 (62.5)	15 (88.2)	4 (66.7)	9 (81.8)
Platelet count, x109/L, median (IQR)		57 (26-101)	40 (18-102)	35 (16-89)	44 (22-110)
LA-positive, n (%)		18 (11.3)	3 (17.6)	2 (33.3)	1 (9.1)
aCL Ab, n (%)		8 (5.0)	4 (23.5)	2 (33.3)	2 (18.2)
aβ2GPI Ab, n (%)		9 (5.6)	2 (12.5)	2 (33.3)	0 (0.0)
Single positive, n (%)		14 (8.9)	3 (17.6)	0 (0.0)	3 (27.3)
Double positive, n (%)	ACA+Aβ2GPI	3 (1.8)	0 (0.0)	0 (0.0)	0 (0.0)
	LA+ACA	2 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)
Triple positive (%)		4 (2.5)	2 (12.5)	2 (33.3)	0 (0.0)
ITP treatment n (%)		75 (48.9)	12 (70.6)	3 (50.0)	8 (72.7)
Corticosteroids, n (%)		38 (23.8)	3 (17.6)	0.0 (0.0)	3 (27.3)
TPO RA, n (%)		22 (14.3)	1 (5.8)	0 (0.0)	1 (9.0)
Splenectomy, n (%)		14 (8.8)	3 (17.6)	2 (33.3)	1 (9.1)

▶ **Fig. 2 Baseline characteristics at time of study inclusion of patients with ITP;** interquartile range; BMI, body mass index; LA, lupus anticoagulant, aCL Ab, anti-cardiolipin antibodies; ab2GPI Ab, anti-b2 glycoprotein I antibodies; TPO-RAs, thrombopoietin-receptor agonist * statistically significant . p-value < 0.05

Conclusion We observed high rates of arterial and venous TE in our patients. Previous thrombosis, APLA positivity, age, confirmed as thrombosis risk factors in our study. Although, ITP patients commonly present with cardiovascular risk factors, our study did not find that these factors affected thrombotic ris. Close monitoring and tailored treatment strategies should be applied to minimize the risk of thrombotic events in ITP patients at risk.

Conflict of Interest The biobank from which this data derives is supported by Novartis, Swedish Orphan Biovitrum (SOBI), MedMedia Verlag und Mediaservice GmbH and AMGEN GmbH

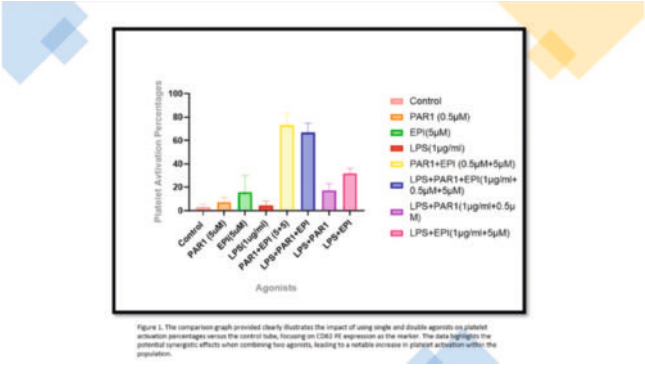
T-11-11 The role of Inflammation in platelet activation (and in anti-neutrophil cytoplasmic antibody (ANCA) vasculitis)

Authors S. Rashvand, A. Dishkelov, S. Jurcevic
Institute University of Westminster, Department of life Science, London, UK
DOI 10.1055/s-0044-1779178

Introduction Although the platelets' role in haemostasis is well established, their contribution to the inflammatory response remains poorly understood. This is particularly true for the complex interactions between inflammatory factors and platelet agonists.

ANCA vasculitis is a group of rare autoimmune diseases that cause inflammation in small blood vessels throughout the body, leading to damage in various organs. It is characterized by the presence of anti-neutrophil cytoplasmic antibodies (ANCA) that target white blood cells, contributing to excessive inflammation and tissue injury.

Therefore, this study aims to determine the effects of inflammatory factors such as different cytokines and hormones on their own and in combination with platelet agonists.



▶ **Fig. 1** The comparison graph provided clearly illustrates the impact of using single and double agonists on platelet activation percentages versus the control tube, focusing on CD62 PE expression as the marker. The data highlights the potential synergistic effects when combining two agonists, leading to a notable increase in platelet activation within the population.; The comparison graph of different agonists on platelet activation

Method The platelets from healthy donors are exposed to well-defined agonists, including ADP, PAR1, TRAP, Epinephrine, IL-4, IL-12 and serotonin alone or in combination to achieve a high level of platelet activation in an *in vitro* environment. The resulting changes in platelet activation will be analysed using flow cytometry to measure various markers.

Results Low concentrations of different individual agonists did not lead to significant platelet activation according to CD62P expression by activated platelets. PAR1 agonist with 0/5μM concentration caused 7.3 % ± 4.2 % (P = 0.3365) CD62P expression in comparison with Control Tube (3.06 % ± 2.3 %). 5μM Epinephrine agonist led to 15.8 % ± 14.26 % CD62P expression by activated platelet in contrast to no-agonist condition (P = 0.4594). The presence of 1μg/ml

LPS agonist, displayed a very mild activation of 4.5 % ± 3.5 % in CD62P expression (P = 0.9999) in parallel with the absence of agonists test.
However, the combination of two and three agonists illustrates a significant platelet activation level increase and a remarkable synergy. The mixture of 1µg/ml LPS and 0/5µM PAR1 led to 17.37 % ± 5.6 % (P = 0.3305), 1µg/ml LPS and 5µM Epinephrine caused 31.83 % ± 4.4 % (P = 0.0045) and also, the combination of 5µM Epi and 5µM led to 73 % ± 9.8 % (P = < 0.0001) CD62P expression by activated platelets.
The combination of all named agonists with the same concentrations could reach 66.8 % ± 7.8 % (P = < 0.0001) CD62P expression which is a noticeable platelet activation level (▶ **Fig. 1**).
Conclusion In conclusion, the early results from the first phase of the study using *in vitro* condition display that different agonists in combination together lead to a significant activation percentage in the platelet population, however, a similar process should be examined during *in vivo* models that would be evaluated in further study.
Conflict of Interest N/A

T-11-12 Platelets with antibacterial properties – a case for smaller platelets?

Authors S. L. Schwarz[1], S. Handtke[2, 1], L. Steil[3], L. Lux[1], M. Gesell-Salazar[4], M. Wolff[1], A. Greinacher[1], T. Thiele[2, 1]
Institutes **1** Universitätsmedizin Greifswald, Institut für Transfusionsmedizin, Greifswald, Germany; **2** Universitätsmedizin Rostock, Institut für Transfusionsmedizin, Rostock, Germany; **3** Universität Greifswald Zentrum, Interfakultäres Institut für Genetik und Funktionelle Genomforschung, Greifswald, Germany; **4** Universität Greifswald Zentrum, Department of Biology, Greifswald, Germany
DOI 10.1055/s-0044-1779179
Introduction Platelets differ in size and function. We have previously characterized large and small platelet subpopulations and found that smaller platelets carry increased amounts of immunoglobulins, inflammatory and immunomodulatory proteins compared to large platelets. This raises the question, whether platelets with smaller size could play a special role in host-bacteria immune responses and inflammation. In this study, we aimed to investigate the influence of large and small platelet fractions and their releasates on the growth of *Staphylococcus aureus*.
Method Large and small platelets of healthy donors (n = 8) were isolated from whole blood by differential centrifugation. Releasates were obtained from of thrombin-receptor-activating-peptide-6 and collagen activated large and small platelets. The supernant was fractionated into 3 molecular weight (MW) fractions (low: < 10kDa, middle:10-100kDa, and high: > 100kDa) using Amicon Ultra centrifugal filter units. Intact platelets, complete and MW-fractionated platelet releasates were incubated with *S. aureus* (*SA 113Δspa*) and was cultured on blood agar plates (12h at 37 °C). Bacterial growth was assessed by counting colony forming units (CFU). Releasates from large and small platelets were further analysed by quantitative LC-MS/MS (*liquid chromatography–mass spectrometry*).
Results Large platelets and their releasates did not alter the growth of *S. aureus*. In contrast,intact small platelets and their releasates reduced bacterial growth by median 12 % and 38 %, respectively, when CFUs were compared to buffer control (p < 0.01). While MW-fractionated releasates of small platelets alone could not reduce the growth of *S. aureus*, the combination all 3 MW-fractions restored the antibacterial effect. Removal of the high MW fraction completely abolished the antimicrobial effect whereas a combination of low/high or middle/high MW fractions could both inhibit bacterial growth. Proteome analyses identified 151 proteins significantly more abundant in releasates of small compared to large platelets (2-fold difference, p-value < 0.05, ≥ 2peptides identified), with a high proportion of plasma proteins including immunoglobulins (appearing in the > 100kDa fraction) and complement factors (appearing in the > 100kDa and 10-100kDa fractions).
Conclusion Small platelets inhibit the growth of *S. aureus* by releasing antibacterial proteins. The antibacterial effect requires proteins of low, middle, and high MW, suggesting a role of immunoglobulins and complement factors. Platelets with smaller size could serve as reservoir for antimicrobial plasma proteins and as vehicle for these proteins to sites of infection. Our data support a special role of platelets with smaller size in the host defence against bacteria.
Conflict of Interest No conflict of interest to disclose.

T-11-13 Evaluation of the MCMDM-1 VWD Bleeding Questionnaire score for the detection of coagulation abnormalities in an outpatient setting

Authors S. Metternich[1], K. Holstein[1], A. Beitzen-Heineke[1], M. Voigtländer[1], L. Beckmann[1], C. Bokemeyer[1], T. Renné[2], P. Kuta[2], F. Langer[1], C. Rolling[1]
Institutes **1** University Medical Center Hamburg-Eppendorf, Oncology, Hematology and BMT with section of Pneumology, Hamburg, Germany; **2** University Medical Center Hamburg-Eppendorf, Institute of Clinical Chemistry and Laboratory Medicine, Hamburg, Germany
DOI 10.1055/s-0044-1779180
Introduction The condensed MCMDM-1 Bleeding Questionnaire score has originally been designed to investigate for type 1 von Willebrand Disease (VWD). However, its diagnostic utility in detecting other hemorrhagic disorders is unknown. Since patients with a suspected bleeding diathesis usually undergo an extensive laboratory assessment including platelet function tests and measurement of coagulation factor activities, we wanted to assess the association between MCMDM-1 VWD bleeding score and other laboratory parameters.
Method We conducted a retrospective study at the University Medical Center Hamburg-Eppendorf, Germany. Patients presented to our department between January 2012 and December 2022 with a suspected bleeding diathesis. Patients receiving anticoagulant or antiplatelet therapy were excluded. Clinical and laboratory datawere retrieved fromelectronic patient charts. MCMDM-1 VWD bleeding score higher than three was defined as pathologic.
Results In total, 1806 patients (median age: 39 years) were analyzed. Seventy-nine percent (n = 1431) were female. A bleeding score > 3 was detected in 51 % (n = 929) of patients. An abnormal bleeding score was associated with lower levels of factor VIII (FVIII) activity (127.2 % vs 137.7 %, p < 0.001), von Willebrand factor (VWF) activity (98.98 % vs 105 %, p < 0.005) and VWF antigen (103.33 % vs 111.8 %, p < 0.001). However, frequency of manifest VWD (VWF:Ag or VWF:Ac < 50 %), VWF activity to antigen ratio < 0.7, or FVIII deficiency did not differ between both groups. In contrast, patients with a bleeding score > 3 had longer median closure times (CTs) in the platelet function analyzer (PFA-100®) using the collagen/ADP or the collagen/epinephrine cartridges (101 sec vs 97 sec, p < 0.001, and 146 sec vs 139 sec, p < 0.001, respectively) and were more frequently found to have abnormal CT prolongation above the upper limit of each test system (23 % vs 17 %, p < 0.005, and 40 % vs 31 %, p < 0.001, respectively). There were no differences in platelet aggregation by different platelet agonists as assessed by light transmittance aggregometry (LTA) but lower levels of factor VII activity (94.5 % vs 100.25, p < 0.01) in patients with a bleeding score < 3.
Conclusion An MCMDM-1 VWD bleeding score > 3 is associated with lower VWF and FVIII levels but not with a higher detection rate of VWD or FVIII deficiency in outpatients presenting to a tertiary center for the diagnostic work-up of a suspected bleeding disorder. In contrast, CTs in the PFA-100® were longer and findings of abnormal CT prolongation indicating impaired primary hemostasis were more frequent in patients with a bleeding score > 3.
Conflict of Interest The authors declare no conflict of interests.

Hämostaseologie 2024; 44: S1–S125

T-12. Gene and cell therapy

T-12-01 Exploring actionable strategies to improve AAV5-hFVIII-SQ durability and optimize gene expression

Authors B. Yates, D. Groeneveld, S. Scheeler, B. Handyside, I. Villalpando, S. Agarwal, S. Bunting, S. Fong, S. Harris
Institute BioMarin Pharmaceutical Inc., Novato, USA
DOI 10.1055/s-0044-1779181

Introduction Roctavian (AAV5-HLP-hFVIII-SQ) is an approved gene therapy in the EU for the treatment of severe hemophilia A. A single infusion provides therapeutic levels of FVIII expression in adult men. The precise mechanisms contributing to variability and durability in transgene expression are unknown. Multiple lines of evidence suggest low RNA production contributes to the decline of FVIII expression and low-response to AAV gene therapy. Previously, we have shown that decreased interaction of active histones with episomal genomes may mediate the decline in transgene expression. Additional studies suggested hepatocyte capacity to fold and secrete FVIII may contribute to variability. We hypothesize (1) modifying the chromatin interaction with AAV-episomes using epigenic regulators may increase accessibility of vector genomes and (2) the use of molecular chaperones may improve FVIII folding and secretion.

Method Using in vitro hepatocyte models, epigenetic modifiers and molecular chaperones were screened to evaluate effects on FVIII RNA and secreted protein levels. Subsequently, we tested a histone deacetylase inhibitor in vivo.

Results We identified a molecular chaperone capable of significantly increasing FVIII protein secretion. Furthermore, three epigenetic modifiers significantly potentiated (>10-fold) and re-activated FVIII RNA expression (>40-fold) following drug-induced transgene silencing in vitro. Significantly increased AAV-mediated transgene expression was observed in mice treated with the histone deacetylase inhibitor, at much lower doses than indicated for currently approved use.

Conclusion These results suggest the potential use of epigenetic modifiers to enhance AAV-mediated expression and reactivation, if silenced; and the use of chemical molecular chaperones to improve FVIII secretion; thereby optimizing the hemostatic benefit for hemophilia A patients.

Conflict of Interest All are employees and shareholders of BioMarin Pharmaceutical Inc.

T-12-02 Immunoadsorption plasmapheresis for the removal of plasma immunoglobulins to enable repeat dose administration with an AAV5 gene therapy vector

Authors B. Long[1], B. Hock[1], C. O'Neill[1], J. Arens[1], T. Seitel[1], L. Crockett[1], F. Relouzat[2], C.-M. Fovet[2], N. Dereuddre-Bosquet[2], P. Maisonnasse[2], H. Letscher[2], R. Le Grand[2], C. Vettermann[1], S. Gupta[1]
Institutes **1** BioMarin Pharmaceutical Inc., Novato, USA; **2** Commissariat à l'Energie Atomique et aux Energies Alternatives, IMVA-IDMIT Center DRF/Institut de Biologie François Jacob, Fontenay-aux-Roses, France
DOI 10.1055/s-0044-1779182

Introduction The investigation of adeno associated virus (AAV) vectored gene therapies has increased exponentially over the past decade for the treatment of monogenic disorders. However, the presence of pre-existing anti-AAV neutralizing antibodies (AAV NAb) may limit the efficacy of gene therapy. Moreover, a first administration of an AAV vector induces high titers of treatment emergent AAV NAb, which may compromise repeat dose administration with the same vector. Depletion of AAV NAb by immunoadsorption plasmapheresis (IAP) is a strategy that could allow successful vector administration in recipients with either pre-existing or treatment-emergent antibodies. The objective of this study was to evaluate the effectiveness of IAP to remove AAV serotype 5 (AAV5) NAb from animals sensitized by an initial gene therapy dose.

Method Five cynomolgus macaques (*Macaca fascicularis*) were included in this study; four were sensitized by administration of an AAV5 capsid encoding for the beta subunit of cyno chorionic gonadotropin (AAV5-βCG) at a dose of 6E13vg/kg, and one control animal was naïve. All were subjected to IAP for a minimum of 1 day of 4 runs (plasma volume exchanges) to a maximum of 3 days of 3 runs. All 5 animals were challenged with the exact same AAV5 capsid encoding a different protein, human factor IX (AAV5-hFIX) at a dose of 6E13 vg/kg, administered within 10 minutes of the last run IAP. Efficacy of the IAP procedure was functionally evaluated by laboratory measures of plasma IgG and AAV5 total binding antibody (AAV5 TAb) titer, hFIX plasma protein concentration, and quantitation of vector genomes and transcripts in liver tissue.

Results Maximal depletion of AAV5 TAb titer (>99%) was achieved in two animals resulting in a nadir titer of 61 and 59. These two animals achieved approximately 25% and 50% of hFIX plasma protein levels, respectively, compared to the naïve animal (0.8 IU/mL), and a proportional percentage of vector genome copies measured in liver tissue compared to the naïve animal (1.6 x 10^7 cp/mg tissue). Following a variable number of IAP sessions (plasma volume exchanges over consecutive days) and administration of the AAV5-hFIX challenge dose, there was significant perturbation of hematological and biochemical blood parameters; however, all parameters returned to baseline levels within hours or days of the procedure.

Conclusion These results demonstrate the viability of IAP as an immune modulation procedure to deplete AAV5 capsid-specific antibody titers sufficient to allow repeat dose administration. As such, IAP may enable AAV-based vector gene therapy in patients currently excluded from gene therapy clinical trials or commercial product use due to pre-existing antibodies. Furthermore, additional evaluation of the efficacy of this procedure may be worthwhile in subjects with pre-existing antibody titers resulting from natural exposure to AAV infections, which result in lower antibody titers than the treatment-emergent titers observed here.

Conflict of Interest Brian R. Long, Benjamin M. Hock, Charles A. O'Neill, Jeremy Arens, Theresa Seitel, Lucy Crockett, Christian Vettermann and Soumi Gupta are employees and shareholders of BioMarin Pharmaceutical Inc. Francis Relouzat, Claire-Maëlle Fovet, Nathalie Dereuddre-Bosquet, Pauline Maisonnasse, Helene Letscher, Roger Le Grand are employees of Commissariat à l'Energie Atomique et aux Energies Alternatives, IMVA-IDMIT Center DRF/Institut de Biologie François Jacob.

T-12-03 Long-Term FVIII Expression with Reduced Bleeding Following Gene Transfer for Hemophilia A: Follow-up on the Dirloctocogene Samoparvovec Phase I/II Trial

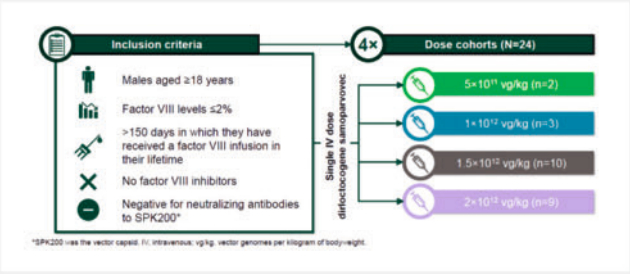
Authors W. Miesbach[1], S. E. Croteau[2], E. Eyster[3], H. Tran[4], M. V. Ragni[5], B. J. Samelson-Jones[6], L. George[6], S. Sullivan[7], J. E. Rasko[8], J. Moormeier[9], P. Angchaisuksiri[10, 11], J. Teitel[12], G. Kenet[13], T. Wynn[14], K. Jaworski[15], A. MacDougall[15], S. Jaeger[15], T. Trivedi[15], F. Mingozzi[15], T. Chang[15], G. Levy[15]
Institutes **1** Goethe University Frankfurt, Goethe University Frankfurt Hospital, Frankfurt, Germany; **2** Boston Children's Hospital, Department of Pediatrics, Harvard Medical School, and the Division of Hematology and Oncology, Boston, USA; **3** Penn State Milton S. Hershey Medical Center, Department of Medicine, Division of Blood and Vascular Disorders, Hershey, USA; **4** Monash University, Melbourne, Australian Centre for Blood Diseases, Victoria, Australia; **5** University of Pittsburgh, Department of Medicine, Pittsburgh, USA; **6** Children's Hospital of Philadelphia, Division of Hematology and the Raymond G. Perelman Center for Cellular and Molecular Therapeutics, Philadelphia, USA; **7** Mississippi Center for Advanced Medicine, Madison, USA; **8** University of Sydney, Department of Cell and Molecular Therapies, Royal Prince Alfred Hospital, Camperdown,

Australia; **9** UMKC School of Medicine, Department of Medicine, Kansas City, USA; **10** Ramathibodi Hospital, Department of Medicine, Bangkok, Thailand; **11** University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, USA; **12** University of Toronto, St Michael's Hospital Haemophilia Treatment Centre, Toronto, Canada; **13** Sheba Medical Center, Israel National Hemophilia Center and Thrombosis Institute, Tel Hashomer, Israel; **14** University of Florida, Division of Hematology & Oncology, Department of Pediatrics, Gainesville, USA; **15** Spark Therapeutics, Inc., Philadelphia, USA

DOI 10.1055/s-0044-1779183

Introduction Hemophilia A (HA) is managed with factor (F)VIII replacement, or non-factor replacement therapy such as emicizumab. More recently, gene therapies have been under investigation for HA. These use a modified viral vector to deliver a functional FVIII gene to liver cells, aiming to safely provide long-term, stable FVIII expression to prevent bleeding. Dirloctocogene samoparvovec (SPK-8011), an investigational gene therapy, uses a modified adeno-associated viral vector designed to achieve clinically meaningful and sustained FVIII expression at the lowest possible dose. The ongoing Phase I/II trial (NCT03003533/NCT03432520) is evaluating the safety and efficacy of dirloctocogene samoparvovec, assessed by FVIII activity levels and patient-reported bleeding events.

Method This open-label, multicenter, non-randomized trial enrolled participants into four dose cohorts, receiving a single intravenous dose (▶ **Fig. 1**).



▶ **Fig. 1** Study design: a Phase I/II, open-label, multicenter, non-randomized trial

Results At data cut-off (Oct 4, 2022), 24 participants were included across dosing cohorts. Median observation time was 191 (range: 2–285) weeks. Thirteen participants (54.2 %) experienced 28 adverse events (AEs) related to dirloctocogene samoparvovec, including one serious AE, a Grade 2 alanine transaminase (ALT, a liver enzyme) elevation resulting in elective hospitalization for intravenous corticosteroid administration. Eight participants (33.3 %) experienced 26 immunomodulatory therapy-related AEs. No FVIII inhibitors or thrombotic events were reported. Fourteen participants had transient ALT elevations per CTCAE Grade 1–3, with these being Grade 1 in 12/14 participants. Among 18 participants with ≥ 1 year of follow-up, 16 showed sustained FVIII activity, with most in the mild HA range, and two lost FVIII expression within the first year following a presumed capsid immune response. These two participants were not included in efficacy analyses. In participants previously using prophylaxis (n = 17) who received dirloctocogene samoparvovec, mean all-bleeds annualized bleeding rate (ABR) was reduced by 82 % (95 % confidence interval [CI]: 55–93 %): ABR of 6.88 (95 % CI: 4.17–11.37) pre-vector infusion versus 1.21 (0.55–2.66) post infusion. In participants previously using on-demand treatment (n = 5), there was a 99 % reduction (95 % CI: 98–100 %) in all-bleeds ABR: 27.17 (95 % CI: 18.46–39.98) pre-vector infusion versus 0.23 (0.13–0.42) post infusion. Median annualized FVIII infusion rates (AIRs) starting 28 days post-vector infusion were 0.3 (interquartile range: 0.0–1.4) versus 85.5 (40.0–104.0) pre infusion.

Conclusion With up to 5 years of follow-up (range: 2–285 weeks), a dirloctocogene samoparvovec infusion in participants with HA resulted in sustained year-to-year FVIII expression and clinically meaningful ABR and AIR reductions. No major safety signals were reported, indicating dirloctocogene samoparvovec was well tolerated.

Conflict of Interest **WM**:Bayer, Biomarin, Biotest, CSL Behring, Chugai, Freeline, LFB, Novo Nordisk, Octapharma, Pfizer, Roche, Sanofi, Sigilon, sobi, Takeda/Shire, uniQure; **SEC**: consultancy: Bayer, Pfizer, Sanofi, BioMarin, Hema Biologics; research funding: Spark Therapeutics, Inc., Genentech, Inc., Sanofi; honoraria: Bayer, Pfizer; membership on an entity's Board of Directors or advisory committees: ATHN, HemophiliaAlliance, THSNA; **MEE**: employment: Penn State Hershey Medical Center; research funding: Spark Therapeutics, Inc., F. Hoffmann-La Roche Ltd.; Novo Nordisk Inc.; Baxalta Inc.; **HT**: research funding: Sanofi, AstraZeneca; honoraria: AstraZeneca, CSL Behring; speaker's bureau: Pfizer, Takeda; **MVR**: grant/research support: Biomarin, Sanofi, Spark Therapeutics, Inc., Takeda; consultancy: honoraria: BeBio, Takeda, Biomarin, TPG (HemabTherapeutics); membership on an entity's board of directors or advisory committees: FWGBD, Blood Advances Editorial Board; **BJS**: grant/research support: Clinical trial support to institution by Spark Therapeutics, Inc., Pfizer; sponsored research support:Accugen; consultancy: Genentech, Inc., Biomarin; honoraria: Pfizer; membership on an entity's board of directors or advisory committees: GeneVentiv, Amarna; patents and royalties: Accugen, Cabaletta; employment: The University of Pennsylvania; **LG**: scientific advisory board: STRM Bio; consultancy: Avrobio, Intellia, BioMarin, Spark Therapeutics, Inc., Bayer; licensing fees: Ask Bio Therapeutics; **SS**: consultancy: CSL, Genentech, Inc., Octapharma, Pfizer; membership on an entity's board of directors or advisory committees: Make-A-Wish Mississippi, employment: Mississippi Center for Advanced Medicine; **JEJR**: shareholder: shareholdings with Rarecyte, Woke; DSMB for Fanconi anaemia trial; grant/research support: NHMRC, NSWCC, CINSW, MRFF, Therapeutic Innovation Australia, philanthropic foundations; supply of material (MTA) or consultancy or honoraria: Rarecyte, Novartis, Bluebird Bio, Spark Therapeutics, Inc., Cynata, Pfizer, CRISPRTx; membership on an entity's board of directors or advisory committees: co-Founder AAVecBio, non-executive director Woke Pharmaceuticals, non-executive director KennertonCapital; employment: Sydney Local Health District at Royal Prince Alfred Hospital; **JM**: employment: University Health Physicians, University of Missouri-Kansas City; **PA**: employment: RamathibodiHospital; **JT**: consultancy: Regeneron, Bayer, Takeda, Novo Nordisk, Pfizer, F. Hoffmann-La Roche Ltd., Sanofi, BioMarin, Vega Therapeutics; research funding: Pfizer, Spark Therapeutics, Inc., Bayer; honoraria: Regeneron, Bayer, Takeda, Novo Nordisk, Pfizer, F. Hoffmann-La Roche Ltd., Sanofi; membership on an entity's board of directors or advisory committees: Pfizer; **GK**: employment: Sheba Medical Center and Sackler Faculty of Medicine, Tel Aviv University, Israel; consultancy: ASC therapeutics, Bayer, Biomarine, Novo Nordisk, Pfizer, F. Hoffmann-La Roche Ltd., Sanofi-Genzyme, Sobi, Takeda, uniQure; research funding: **BSF**: Opko Biologics, Pfizer, F. Hoffmann-La Roche Ltd., Shire; honoraria: Bayer, BioMarin, BPL, CSL, Pfizer, Novo Nordisk, Roche, Sanofi-Genzyme, Sobi, Spark Therapeutics, Inc., Takeda, Uniquore; scientific advisory board: PedNetfoundation; **TW**: Spouse was employed by Novartis full time until October 2022 and is now employed full time by Takeda; research/clinical trial support: Takeda, Sanofi, AMAG, Sobi, and Spark Therapeutics, Inc.; **TT**: employment: Spark Therapeutics, Inc., equity: F. Hoffmann-La Roche Ltd.; **FM**: employment: Spark Therapeutics, Inc., equity: F. Hoffmann-La Roche Ltd.; **TC**: shareholder: F. Hoffmann-La Roche Ltd.; employment Spark Therapeutics, Inc; **GL**: shareholder: F. Hoffmann-La Roche Ltd.; employment: Spark Therapeutics, Inc. Board of Directors (non-voting).

Hämostaseologie 2024; 44: S1–S125

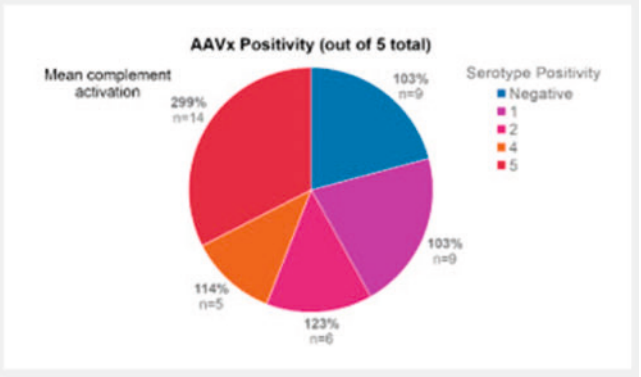
T-12-04 Characterization of Pre-existing Immunity to AAV5

Authors K. Lau[1], C. Vettermann[1], G. de Hart[1], B. Hock[1], A. Frazer-Abel[2], B. Long[1], S. Gupta[1]
Institutes **1** BioMarin Pharmaceutical Inc., Novato, USA; **2** Exsera BioLabs, University of Colorado School of Medicine, Aurora, USA
DOI 10.1055/s-0044-1779184

Introduction Seroprevalence studies indicate many prospective gene therapy (GT) patients have been previously exposed to adeno-associated viruses (AAV) and harbor pre-existing AAV antibodies which may impact the administration and efficacy of GT. The objective of this study was to further characterize pre-existing antibody responses to AAV serotype 5 (AAV5) in severe hemophilia A patients (n = 540) who are GT naïve.

Method We evaluated AAV5 neutralizing factors (transduction inhibition, TI) using a cell-based assay, AAV5 total binding antibodies (TAb) using a bridging immunoassay, AAV serotype specificity using a bridging immunoassay, AAV5 complement activation, and AAV5 antibody isotypes [1].

Results Most subjects with pre-existing immunity to AAV5 were positive for TAb and TI, with a small subset showing discordant results. Pre-existing AAV5 TAb titers were much lower than AAV5 post-treatment titers. We previously[1] assessed the seroprevalence of pre-existing immunity to AAV5 and four other AAV serotypes (AAV2/6/8/rh10). Using those data, we assigned subjects to AAVx positivity groups (Negative, 1, 2, 3, 4, 5) based on the number of positive serotypes. There was no association between higher AAV5 TAb titers and the presence of antibodies reactive with other serotypes, suggesting that AAV5-specific TAb titers were not broadly a result of previous AAV exposures and subsequent cross-reactivity. In a subset of subjects (n = 43), AAV5-induced complement activation was assessed in vitro by measuring complement split products (C3a, C4a, C5a, Bb). In some samples with AAV5 TAb, a > 200 % increase in activated complement split products was observed; however, this was not correlated with AAV5 TAb titer magnitude. Complement activation was not observed in TAb-negative subjects, and not all TAb-positive subjects exhibited complement activation. Based on AAVx positivity, mean complement activation to AAV5 > 200 % in vitro was measured only in subjects that were positive for all five serotypes (AAV5/2/6/8/rh10). Activation of complement may enhance complement receptor-mediated clearance, alter the biodistribution away from the target tissue, and therefore reduce transduction (▶ **Fig. 1**).



▶ **Fig. 1** AAVx Positivity (out of 5 total)

In another subset of subjects (n = 78) with pre-existing AAV5 TAb positivity, we characterized the isotype and subtype of the anti-AAV5 response (IgM, IgG1, IgG2, IgG3, IgG4, IgA). Responses were predominantly the IgG isotype. AAV5 TAb titers > 100 and TI titers > 60 were associated with detection of IgG1, but AAV5 TAb titers > 100 that were TI negative/titer < 60 did not reveal a similar trend. Consequently, highly neutralizing antibody responses were associated with detection of AAV5-specific IgG1.

Conclusion Our in-depth characterization of pre-existing immunity to AAV5 can help elucidate the most important immunological determinants that limit AAV vectored transduction of target tissues. Understanding these determinants of pre-existing immunity could enable more patients to gain access to GT.

Conflict of Interest Kelly Lau, Christian Vettermann, Greg de Hart, M. Benjamin Hock, Brian Long, Soumi Gupta are employees and shareholders of BioMarin Pharmaceutical Inc. Ashely Frazer-Abel is an employee of the University of Colorado School of Medicine. She consult for the following companies; Ionis, CSL Behring, but all funds have gone to the laboratory therefore the University.

References

[1] Klamroth R, Hayes G, Andreeva T, Gregg K et al. Global Seroprevalence of Pre-existing Immunity Against AAV5 and Other AAV Serotypes in People with Hemophilia A. Hum Gene Ther 2022; 33 (7/8): 432–44

T-12-05 Efficacy and Safety of Fidanacogene Elaparvovec in Adults with Moderately Severe or Severe Hemophilia B: Results from the Phase 3 BENEGENE-2 Gene Therapy Trial

Authors R. Klamroth[1], A. Cuker[2], H. Alzahrani[3], J. Astermark[4, 5], L. Frenzel[6], O. Katsarou[7], K. Kavakli[8], D. Matino[9, 10], A. Shapiro[11], J.-D. Wang[12], J. Fuiman[13], J. McKay[14], P. Sun[15], J. Rupon[13]
Institutes **1** Vivantes Hospital Friedrichshain, Berlin, Germany; **2** Perelman School of Medicine, University of Pennsylvania, Department of Medicine and Department of Pathology and Laboratory Medicine, Philadelphia, USA; **3** King Faisal Specialist Hospital and Research Centre, Adult Haematology, Riyadh, Saudi Arabia; **4** Skåne University Hospital, Department of Hematology, Oncology and Radiation Physics, Malmö, Sweden; **5** Lund University, Department of Translational Medicine, Malmö, Sweden; **6** Institut Necker, Department of Haematology, Paris, France; **7** National Reference Centre for Congenital Bleeding Disorders, Laiko General Hospital, Blood Transfusion Centre, Athens, Greece; **8** Ege University Faculty of Medicine, Division of Hematology, Department of Pediatrics, Bornova-İzmir, Turkey; **9** Thrombosis and Atherosclerosis Research Institute, McMaster University, Hamilton, Canada; **10** McMaster University, Department of Medicine, Hamilton, Canada; **11** Indiana Hemophilia & Thrombosis Center, Inc., Indianapolis, USA; **12** Taichung Veterans General Hospital, Center for Rare Disease and Hemophilia, Taichung, Taiwan; **13** Pfizer Inc, Collegeville, USA; **14** Pfizer Inc, Groton, USA; **15** Pfizer Inc, Cambridge, USA
DOI 10.1055/s-0044-1779185

Introduction Fidanacogene elaparvovec (PF-06838435, formerly SPK-9001) is an adeno-associated virus-based gene therapy vector transferring the high-activity variant of human factor IX (FIX) FIX-R338L for the treatment of hemophilia B. The aim of this study was to evaluate the efficacy and safety of fidanacogene elaparvovec in participants with moderately severe or severe hemophilia B.

Method BENEGENE-2 (NCT03861273) is a phase 3, open-label, single-arm trial that enrolled adult males with hemophilia B (FIX:C ≤ 2 IU/dL) who had completed ≥ 6 months of FIX prophylaxis prior to administration of 5e11 vg/kg fidanacogene elaparvovec. The primary endpoint was non-inferiority on annualized bleeding rate (ABR) for total (treated and untreated) bleeds from Week 12 to Month 15 post-infusion vs the pre-infusion prophylaxis period. Key secondary endpoints included FIX activity, ABR for treated bleeds, and annualized infusion rate. Other secondary endpoints included annualized FIX consumption, ABR of specific bleed types (eg, spontaneous), and frequency of target joint bleeds. Adverse events (AEs) were monitored. Participants provided written informed consent and the study was approved by the relevant regulatory/ethics committees.

Results Forty-five participants (median [range] age, 29 [18, 62] years) were dosed with fidanacogene elaparvovec. Total ABR was reduced by 71 % post-fi-

danacogene elaparvovec treatment vs prophylaxis (mean ABR, 1.3 vs 4.4; $P<0.0001$; ▶ **Fig. 1**) and was stable over time (0.4 at Year 3; n = 21). Mean FIX activity, measured by one-stage SynthASil, one-stage Actin-FSL, and chromogenic assays, is shown in ▶ **Fig. 2**. FIX activity remained relatively stable at Month 24 (▶ **Fig. 2**). Data for other secondary efficacy endpoints are shown in ▶ **Fig. 1**. Twenty-eight participants (62 %) received corticosteroids for presumed immune response. No deaths, infusion-related serious AEs, thrombotic events, or FIX inhibitors were reported.

	Pre-infusion FIX prophylaxis period	Post-infusion period
Total bleeds		
Mean ABR[a] (95% CI)	4.4 (1.81–7.05)	1.3 (0.59–2.02)
Median ABR (min, max)	1.3 (0.0, 53.9)	0.0 (0.0, 10.4)
Participants without bleeds, % (n)	28.9 (13)	64.4 (29)
Treated bleeds		
Mean ABR[a] (95% CI)	3.4 (1.71–4.98)	0.7 (0.25–1.21)
Median ABR (min, max)	1.0 (0.0, 20.5)	0.0 (0.0, 8.5)
Participants without bleeds, % (n)	35.6 (16)	73.3 (33)
Annualized infusion rate (SD)	58.8 (29.06)	4.5 (10.03)
Participants who resumed FIX prophylaxis, n (%)	n/a	6 (13.3)

[a] Model-derived ABR.

Min, max-minimum, maximum

▶ **Fig. 1** Total bleeds, treated bleeds, annualized infusion rate and resumption of prophylaxis in the pre-infusion prophylaxis and post-infusion periods

	FIX activity level[a], %		
	One-Stage SynthaSil	One-Stage Actin-FSL	Chromogenic
Week 12	n=44	n=43	n=44
Mean (SD)	27.8 (15.23)	13.5 (8.13)	13.9 (9.30)
Median (min, max)	26.5 (3.2, 68.6)	13.5 (1.7, 35.1)	12.1 (1.4, 36.3)
Month 6	n=39	n=41	n=40
Mean (SD)	27.7 (21.34)	13.1 (11.14)	14.8 (12.96)
Median (min, max)	23.2 (1.9, 99.7)	10.1 (0.6, 55.0)	10.3 (1.0, 57.7)
Month 15	n=35	n=34	n=35
Mean (SD)	27.5 (25.74)	13.1 (12.79)	15.8 (17.00)
Median (min, max)	23.3 (1.9, 119.0)	10.3 (1.8, 62.0)	10.2 (1.9, 74.2)
Month 24	n=22	n=22	n=22
Mean (SD)	25.0 (22.63)	12.7 (11.88)	15.4 (18.83)
Median (min, max)	22.8 (1.9, 95.3)	9.2 (0.9, 46.6)	9.8 (1.2, 80.3)

[a] FIX was imputed to 1.9% for the period post-resumption if a participant resumed prophylaxis.

Min, max-minimum, maximum

▶ **Fig. 2** Mean FIX activity levels (%) by one-stage SynthASil, one-stage Actin-FSL and chromogenic assays

Conclusion Fidanacogene elaparvovec yielded endogenous FIX expression in participants with moderately severe to severe hemophilia B, resulting in significant decreases in bleeding and was generally well tolerated.

Conflict of Interest Robert Klamroth has received honoraria and/or been a member of Advisory Committees for Bayer, BioMarin, Biotest, CSL Behring, Grifols, Novo Nordisk, Octapharma, Pfizer, Roche/Chugai, Sanofi, Sobi, and Takeda. Adam Cuker has received consulting fees from MingSight, the New York Blood Center, and Synergy and has received authorship royalties from UpToDate. Hazzaa Alzahrani declares no conflicts of interest. Jan Astermark has received consulting fees and honoraria from Bayer, BioMarin, CSL Behring, Novo Nordisk, Octapharma, Pfizer, Roche, SOBI, Sanofi, Uniqure and Shire/Takeda. Laurent Frenzel has received grants from CSL Behring and Pfizer; and consulting fees BioMarin, CSL Behring, and Pfizer. Olga Katsarou-Fasouli has received honoraria from Bayer and Sobi; and has participated on a Data Safety Monitoring Board/Advisory Board for Novo Nordisk and Sobi. Kaan Kavakli has participated in Scientific Advisory Board meetings for BioMarin, CSL Behring, Novo Nordisk, Pfizer, Roche, and Takeda. Davide Matino has received research grants to the institution from Bayer, Octapharma, Pfizer, Novo Nordisk, Roche, Sanofi, Spark; advisory boards, lectures and preparation of educational materials with Bayer, Novo Nordisk, Octapharma, Pfizer, Sanofi, Sobi. Amy D. Shapiro has participated as a clinical trial investigator for Freeline, Genzyme/Bioverativ, Novo Nordisk, and Sanofi; has received consulting fees from Novo Nordisk; has participated in speaker's bureaus for Sanofi-Genzyme/Bioverativ; has participated in Advisory Boards for CSL Behring, Novo Nordisk, Pfizer, and Sanofi-Genzyme/Bioverativ; and is on the Board of Directors for the Novo Nordisk Haemophilia Foundation. Jiaan-Der Wang has received honoraria from and conducted clinical trials on behalf of Bayer, Chugai, CSL Behring, Pfizer, Novo Nordisk, and Sanofi. Joanne Fuiman, John McKay, Pengling Sun, Jeremy Rupon, and Francesca Biondo are employees and shareholders of Pfizer.

Funding statement This study was sponsored by Pfizer.

T-12-06 smart medication Gene – a collaboration and documentation tool for the patient journey according to hub & spoke modell in gene therapy of hemophilia A/B

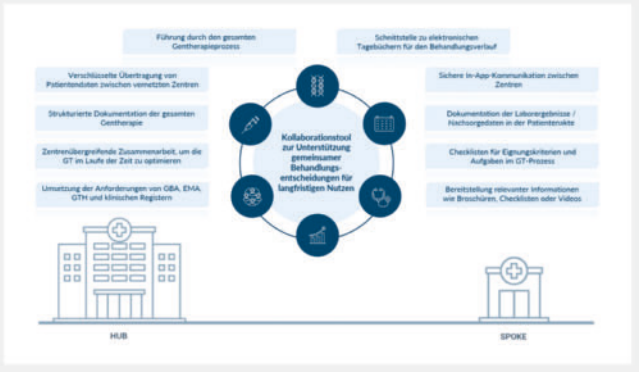
Authors A. Rösch, D. Schmoldt

Institute smart medication eHealth Solutions GmbH, Frankfurt Am Main, Germany

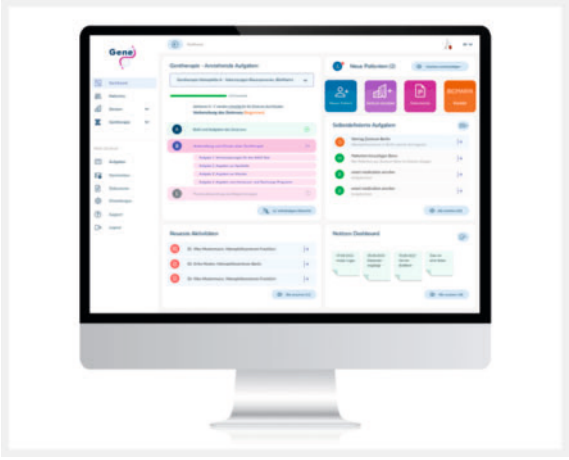
DOI 10.1055/s-0044-1779186

Introduction Since the EMA approval of gene therapy for hemophilia A and B, two new gene therapies are immediately available as treatment options. The gene therapies are characterized by a complex patient initiation and follow-up process. The smart medication GENE software platform is a collaboration and documentation tool for the entire patient journey from the initial consultations between physician and patient at the home center to the application of the gene therapy at the dosing center as well as follow-up care of the patient after therapy has been applied.

Method smart medication Gene covers all documentation and collaboration requirements between home center and dispensing center according to the Hub & Spoke modell (see ▶ **Fig. 1**). The complex treatment process can be individually configured and adapted. For example, individual tasks can be assigned to the home center or the dosing center respectively. Tasks and results can be tracked, edited and marked as completed by the centers (see ▶ **Fig. 2**). The entire patient journey is mapped: from initial discussions, patient education and proper documentation of a informed consent, outcome of the AAV test, preparation and application of gene therapy and follow-up after application of gene therapy.



▶ **Fig. 1** **Hub & Spoke Model;** Requirements of the Hub & Spoke Modell



▶ **Fig. 2 Dashboard Patient Journey;** Dashboard of smart medication Gene software tool to visualize the patients journey

Results smart medication Gene was developed in close collaboration with pharmaceutical manufacturers to take into account and map all requirements for the application of gene therapy. In a further step, the software tool was extensively validated in an advisory board consisting of physicians from future home centers and dosing centers. HCPs are currently being trained in the use of smart medication Gene, so that patients can then be documented and gene therapy applied in collaboration between the centers.

Conclusion smart medication Gene covers the entire patient journey of gene therapy in hemophilia A and B. The tool was developed in collaboration with physicians and the pharmaceutical industry for physicians. It is expected that gene therapies will be performed in Germany in a sisgnificant number after the conclusion of negotiations with the health insurance companies and that the tool smart medication Gene will be used as standard tool in Gene therapy.

Conflict of Interest Biomarin, CSL Behring

T-12-07 Adults With Haemophilia B and History of Chronic HCV/HBV Infection Receiving Etranacogene Dezaparvovec Gene Therapy in the HOPE-B Clinical Trial Demonstrate Long-Term Bleeding Protection and Sustained FIX Activity 3 Years After Administration

Authors A. von Drygalski[1], N. O'Connell[2], P. Verhamme[3], K. Meijer[4], P. van der Valk[5], R. Kazmi[6], P. Raheja[7], N. Galante[8], S. le Quellec[8], R. Church[8], S. Lucas[8], G. Castaman[9], P. Monahan[8]

Institutes **1** University of California San Diego, Division of Hematology/Oncology, Department of Medicine, San Diego, USA; **2** St. James's Hospital, National Coagulation Centre, Dublin, Ireland; **3** University of Leuven, Center for Molecular and Vascular Biology, KU Leuven Department of Cardiovascular Sciences, Leuven, Belgium; **4** University of Groningen, Department of Haematology, University Medical Centre, Groningen, Netherlands; **5** University Medical Center Utrecht, Van Creveldkliniek, Utrecht, Netherlands; **6** University Hospital Southampton, NHS Foundation Trust Southampton, Southampton, UK; **7** The Royal London Hospital, Department of Haematology, London, UK; **8** CSL Behring, King of Prussia, USA; **9** Careggi University Hospital, Center for Bleeding Disorders and Coagulation, Department of Oncology, Florence, Italy

DOI 10.1055/s-0044-1779187

Introduction Etranacogene dezaparvovec (formerly AMT-061) has recently become a therapeutic option for patients (pts) with haemophilia B (HB) and comorbid chronic hepatitis C virus (HCV) and hepatitis B virus (HBV). The pivotal phase 3 HOPE-B trial (NCT03489291) evaluated etranacogene dezaparvovec in pts with severe or moderately severe HB; here we evaluate the efficacy and safety in the subset of pts with a history of chronic HCV and/or HBV over 3 years post treatment.

Method Adult males with haemophilia B (factor IX [FIX] ≤ 2 %), were infused with a single dose of etranacogene dezaparvovec (2×10^{13} gc/kg; an adeno-associated virus serotype 5 (AAV5) vector containing FIX Padua R338L transgene under the control of the liver-specific promoter LP-1), following a ≥ 6-month lead-in period of FIX prophylaxis. Exclusion criteria included baseline liver chemistries > 2 × upper limit of normal; active HCV/HBV or uncontrolled HIV infection; or advanced liver fibrosis (FibroScan score ≥ 9 kPa).

Results Of 54 pts in the HOPE-B trial, 31 (57.4 %) had comorbid chronic HCV without active disease. Of these, 7 had a history of chronic HBV infection without active disease. Two were HCV/HIV co-infected. Two pts were HBV + /HCV–/HIV–. The mean age in HCV/HBV pts (n = 33) was 50.0 years (range, 31-75). Mean ± SD FIX activity was 46.5 ± 23.0, 40.2 ± 20.8, and 44.5 ± 19.0 at Years 1, 2, and 3 in HCV/HBV pts and 34.0 ± 17.8, 31.3 ± 14.6, and 31.0 ± 13.1 at Years 1, 2, and 3 in HCV–/HBV– pts (n = 21). Mean FibroScan score for HCV/HBV pts was 5.2 kPa (range 2.8-8.0). Excluding 1 pt with AAV5 neutralising antibody (NAb) titre of 1:3212, HCV/HBV pts (n = 32) demonstrated ABR ratio of 0.31 (95 % CI, 0.13, 0.72), indicating 69 % reduction in all bleeding, sustained from months 7-36 post treatment.

In the HCV/HBV subgroup, 5/33 (15.2 %) had ALT elevations, of which 4/33 (12.1 %) were treated with corticosteroids versus 11/54 (20.4 %) and 9/54 (16.7 %) in the whole HOPE-B population, respectively. As reported previously HCV/HBV pt developed a hepatocellular carcinoma (HCC) deemed unrelated to treatment.

Conclusion Patients in the HOPE-B trial with HCV and/or HBV infection show comparable efficacy and safety to the rest of the study population.

Conflict of Interest A von Drygalski has received consultant fees from Biomarin, Sanofi Genzyme, Novo Nordisk, Pfizer, uniQure, Hematherix; N O'Connell has received consultant fees from CSL Behring, F.Hoffman- La Roche, Novo Nordisk, Sanofi, and speaker fees from Takeda – All funds were received by a charitable organisation; P Verhamme has received consultant fees from CSL Behring, Roche, CAP-DCF, Bayer HealthCare; LeoPharma; Boehringer Ingelheim; Daiichi Sankyo; Pfizer; Sanofi-Aventis; ThromboGenics; K Meijer has received speaker fees from Alexion pharmaceuticals Bayer, CSL Behring, and consultant fees from UniQure, Octapharma USA Inc., Bayer ; P van der Valk has received consultation fees from Bayer; R Kazmi has received consultant fees from BioMarin Pharmaceuticals, CSL Behring; P Raheja has no conflict of interest to declare; N Galante is a full-time CSL Behring employee; S Le Quellec is a full-time CSL Behring employee; R Church is a full-time CSL Behring employee; S Lucas is a full-time CSL Behring employee, G Castaman has received consultant fees from G. Castaman has received Grant/Research support from: CSL Behring, Pfizer, Sobi, Speaker Bureau of: Bayer, Biomarin, Roche, Sobi, Grifols, LFB, Novo Nordisk, Werfen, Kedrion, uniQure; P Monahan is a full-time CSL Behring employee.

T-13. Acquired coagulation disorders

T-13-01 Management of Immune Thrombotic Thrombocytopenic Purpura without Therapeutic Plasma Exchange: the Austrian-German Experience

Authors L. Kühne[1], P. Knöbl[2], K. Eller[3], L. A. Völker[1], P. T. Brinkkötter[1]

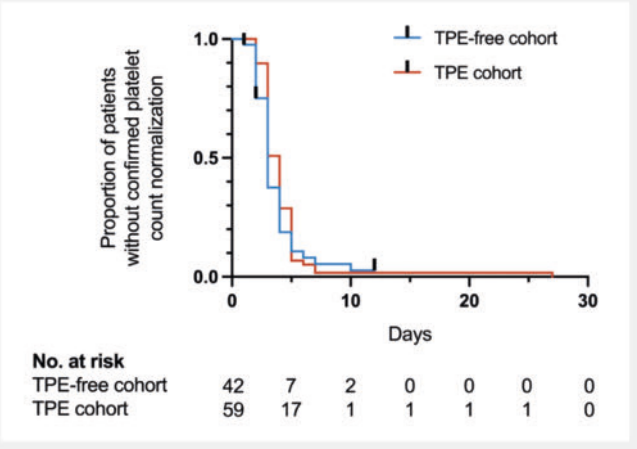
Institutes **1** University Hospital Cologne, epartment II of Internal Medicine and Center for Molecular Medicine Cologne (CMMC), Faculty of Medicine, Cologne, Germany; **2** Medical University of Vienna, Division of Hematology and Hemostasis, Department of Medicine 1, Vienna, Austria; **3** Medical University of Graz, Division of Nephrology, Department of Internal Medicine, Graz, Austria

DOI 10.1055/s-0044-1779188

Introduction Immune thrombotic thrombocytopenic purpura (iTTP) is a rare, life-threatening autoimmune disorder caused by ADAMTS13 deficiency. Caplacizumab, an anti-VWF nanobody, is approved for iTTP treatment, reducing the need for therapeutic plasma exchange (TPE) and improving platelet recovery and survival.

Method All patients in this study were identified from the Austrian Thrombotic Microangiopathy Registry (ATMAR) and the German REACT-2020 TTP registry (ClinicalTrials.gov identifier: NCT04985318). A total of 42 acute iTTP episodes in 41 patients were managed with a treatment regimen aimed at avoiding first-line TPE if the platelet count increased after the first dose of 10 mg caplacizumab, and the patient's condition and organ function remained stable (TPE-free cohort). This treatment modification was implemented based on shared decision making. An extensive efficacy and safety analysis of this approach was performed, in comparison with a control group of 59 patients with iTTP, receiving frontline caplacizumab treatment with TPE and immunosuppression (TPE cohort). The main outcome was time to platelet count normalization. Secondary outcomes included clinical response, exacerbation, refractory iTTP, and iTTP-related deaths. Retrospective safety assessments with evaluation of complications were performed in both cohorts.

Results The median time to platelet count normalization was similar between the two cohorts (3 and 4 days; P = 0.31). There were no significant differences in clinical response, exacerbations, refractoriness, or iTTP-related deaths. Four patients did not respond to the first dose of caplacizumab, and TPE was initiated. CMV infection, HIV/hepatitis B co-infection, and ovarian teratoma may have hindered immediate treatment response (▶ **Fig. 1**).



▶ **Fig. 1 Time to platelet count normalization after the first caplacizumab administration**; Symbols indicate censored data. P value for time to platelet count normalization is 0.31 according to log-rank test.

During overall follow-up at least one complication was observed in 11 patients (26.2 %) in the TPE-free group and 16 patients (27.1 %) in the control group. Serious complications associated with therapeutic plasma exchange were observed in 3 patients (5.1 %) exclusively in the SOC cohort, comprising severe anaphylactic reactions. Bleeding complications were reported without a significant difference and mainly comprised gingival bleeding and epistaxis. Two patients in the TPE-free cohort (4.8 %) experienced major bleeding events, namely subdural hematoma requiring surgical intervention and gastrointestinal bleeding with mass transfusion (▶ **Fig. 2**).

Conclusion Our treatment approach was successfully implemented in 38 of 42 acute iTTP episodes (90.5 %). We observed no significant difference in the time to platelet count normalization between patients with iTTP treated with and without TPE. The analysis of key secondary outcomes did not reveal significant differences in the proportion of patients who achieved a clinical response or experienced exacerbations, refractoriness, or TTP-related deaths. In conclusion, Caplacizumab and immunosuppression, without TPE, rapidly controlled microvascular thrombosis and achieved a sustained clinical response in iTTP.

Conflict of Interest L. K. received consulting fees from Alexion. P. K. received consultancy and advisory board fees, speaker honoraria, and travel grants from Ablynx/Sanofi, Alexion, CLS Behring, Novo-Nordisk, Roche and Shire/Takeda. L. A. V. received research funding and consulting fees from Alexion, AstraZeneca, Bayer, and Sanofi-Genzyme. P. T. B. received speaker honoraria and consultant fees from AstraZeneca, Alexion, Bayer, Novartis, Roche, Sanofi-Genzyme, Travere, Vifor CSL and participated in advisory boards for Alexion, Sanofi-Genzyme, Novartis, Travere, Takeda, Vifor CSL and Bayer. He declares research funding from the German Research Foundation BR-2955/8 and Sanofi-Genzyme. All other authors report no conflict of interest.

Table 1. Outcome parameters and safety analysis of the TPE-free and TPE cohorts treated with frontline caplacizumab.*

Parameter	TPE-free cohort (n=42)	TPE cohort (n=59)	P Value
Primary outcome			
Median time to platelet count normalization – days (range; IQR)	3 (1-12; 2-4)	4 (2-27; 3-5)	0.31
Key secondary outcomes			
Patients achieving a clinical response without requiring TPE – no. (%)	38 (90.5)		
Patients achieving a clinical response – no. (%)	41 (97.6)	57 (96.6)	
Patients with a clinical exacerbation – no. (%)	2 (4.8)	9 (15.3)	
Patients refractory to therapy – no. (%)	0 (0)	1 (1.7)	
TTP-related death – no. (%)	0 (0)	1 (1.7)	
Other secondary outcomes			
Median time to recovery of ADAMTS13 activity to ≥20% after treatment initiation – days (IQR)	25 (13-33)	37 (19-51)	0.01
Patients without confirmed recovery of ADAMTS13 activity to ≥20% at end of follow-up – no. (%)	4 (9.5)	10 (16.9)	
Days in hospital Median (IQR)	11 (5-18)	14 (9-21)	0.03
Patients admitted to ICU – no. (%) Data missing – no. (%)	6 (14.3) 7 (16.7)	42 (71.2) 8 (13.6)	<0.001
Days on ICU, if admitted Median (IQR)	4 (2-4)	4 (2-6)	
Safety			
Patients with at least one reported complication during overall follow-up period – no. (%)	11 (26.2)	16 (27.1)	
Reported complications on TPE – no. (%)	0 (0)	4 (6.7)	
Any bleeding – no. (%)	5 (11.9)	2 (3.4)	
Major bleeding according to ISTH – no. (%)	2 (4.8)	0 (0)	

* There were no significant differences between the two cohorts in the parameters listed in this table except as noted.

▶ **Fig. 2** Outcome parameters and safety analysis of the TPE-free and TPE cohorts

T-13-02 Severity of prothrombotic state modulates the effect of direct oral anticoagulants in liver cirrhosis

Authors C. Pereira Portela[1], M. G. Zermatten[1], É. Mdawar-Bailly[2], D. Bertaggia Calderara[1], A. Aliotta[1], L. Veuthey[1], D. Moradpour[2], M. Fraga[2], L. Alberio[2]

Institutes 1 Lausanne University Hospital and University of Lausanne, Division and Central Laboratory of Hematology, 1011, Switzerland;
2 Lausanne University Hospital and University of Lausanne, Division of Gastroenterology, 1011, Switzerland

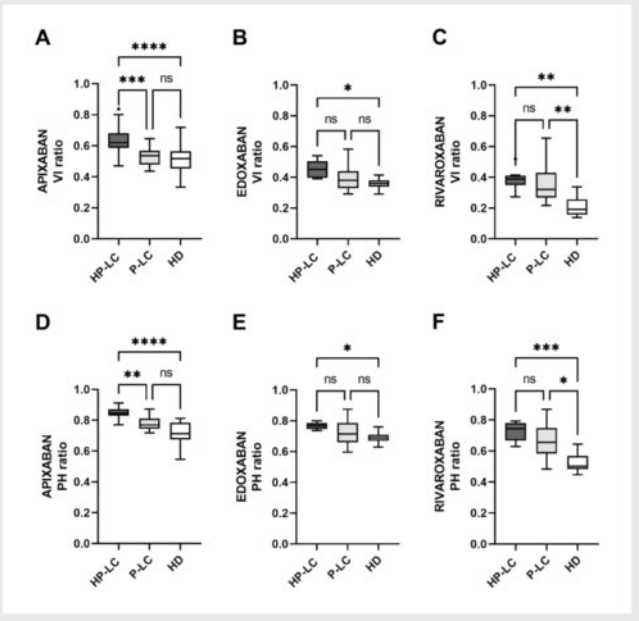
DOI 10.1055/s-0044-1779189

Introduction Liver cirrhosis (LC) induces alterations in haemostatic systems usually in favour of a prothrombotic state. The effect of direct oral anticoagu-

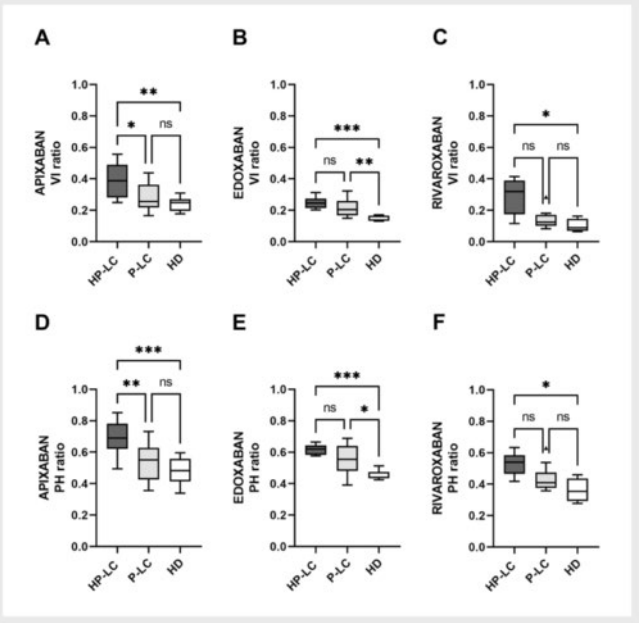
lants (DOAC) in this population remains to be investigated. The "one dose fits all" approach used for DOAC might not always be adequate for patients with complex prothrombotic conditions, as highlighted in triple positive antiphospholipid syndrome [1, 2]. With this rationale, we aimed to compare the anticoagulant effect of DOAC (anti-Xa inhibitors) on ex vivo thrombin generation in plasma from patients with LC and a prothrombotic (P-LC) or highly-prothrombotic (HP-LC) profile compared to healthy donors (HD).

Method We included 72 patients with LC and 10 HD. Plasma samples were divided into P-LC (n = 43) or HP-LC (n = 29) according to ex vivo thrombin generation (TG) as assessed by ST Genesia (ThromboScreen with and without thrombomodulin; Stago, France). HP-LC plasma was defined as an endogenous thrombin potential inhibition mediated by thrombomodulin < 20 %. Plasmas were spiked with either vehicle, apixaban, edoxaban or rivaroxaban (final concentrations: 50 and 150 ng/ml). After spiking, DOAC concentrations were verified and ex vivo thrombin generation was analysed using ST Genesia with DrugScreen. Ratios for velocity index (VI) and peak height (PH) were defined as the TG parameter of interest with DOAC divided by the same parameter without DOAC. Comparisons between P-LC, HP-LC and HD plasma were performed for each concentration using Kruskal-Wallis test.

Results At a concentration of 50 ng/ml (▶ **Fig. 1**), no statistical difference between P-LC and HD was observed except for rivaroxaban. However, both VI and PH ratios for HP-LC plasma were significantly higher in samples with apixaban, edoxaban and rivaroxaban compared to HD plasma. At a concentration of 150 ng/ml (▶ **Fig. 2**), no statistical difference between P-LC and HD was observed (excepted for edoxaban). However, similar to 50 ng/ml, both VI and PH ratios for HP-LC plasma were significantly higher in samples with apixaban, edoxaban and rivaroxaban compared to HD plasma.



▶ **Fig. 1** Effect of DOAC at 50 ng/ml. Ratios for velocity index (VI) and peak height (PH) after spiking with apixaban (panel A, D), edoxaban (panel B, E) and rivaroxaban (panel C, F) in prothrombotic (P-LC) or highly-prothrombotic (HP-LC) patients with liver cirrhosis (LC) compared to healthy donors (HD). Ns, non significant (p-value > 0.05); *, p-value ≤ 0.05; **, p-value < 0.01; ***, p-value < 0.001; ****, p-value < 0.0001.



▶ **Fig. 2** Effect of DOAC at 50 ng/ml. Ratios for velocity index (VI) and peak height (PH) after spiking with apixaban (panel A, D), edoxaban (panel B, E) and rivaroxaban (panel C, F) in prothrombotic (P-LC) or highly-prothrombotic (HP-LC) patients with liver cirrhosis (LC) compared to healthy donors (HD). Ns, non significant (p-value > 0.05); *, p-value ≤ 0.05; **, p-value < 0.01; ***, p-value < 0.001; ****, p-value < 0.0001

Conclusion We demonstrated that DOAC with anti-Xa activity exhibit a variable anticoagulant effect in LC. At similar spiked concentrations, anti-Xa inhibitors (apixaban, edoxaban and rivaroxaban) showed a significantly lower anticoagulant effect in HP-LC compared to HD plasma. These findings raise the question whether dose-adjustments and monitoring may be clinically useful in selected patients with LC demonstrating a highly prothrombotic potential.

Conflict of Interest Stago (Asnière-sur-Seine, France) supported the study with discounts for ST Genesia reagents. No other relevant conflict of interest to disclose.

References

[1] Peleg H, Naparstek Y, Regola F, Tincani A, Pengo V, Denas G. Can we use NOACS in APS? Autoimmun Rev 2019; 18: 102408

[2] Pengo V, Denas G, Zoppellaro G, Jose SP, Hoxha A, Ruffatti A, Andreoli L et al. Rivaroxaban vs warfarin in high-risk patients with antiphospholipid syndrome. Blood 2018; 132: 1365–1371

T-13-03 Prevalence of Anti-PF4/Heparin Antibodies After Second COVID-19 Vaccination in Healthcare Workers

Authors G. Uzun[1, 2], F. Lyshy[1], K. Althaus[1, 2], T. Bakchoul[1, 2]

Institutes 1 Centre for Clinical Transfusion Medicine, Tübingen, Germany; 2 Medical Faculty and University Hospital of Tuebingen, Institute for Clinical and Experimental Transfusion Medicine, Tübingen, Germany

DOI 10.1055/s-0044-1779190

Introduction Anti-platelet factor 4 (PF4) antibodies have been identified as the cause of vaccine-induced immune thrombotic thrombocytopenia following adenoviral vector-based COVID-19 vaccines. Previous studies have primarily focused on determining the frequency of anti-PF4 antibodies shortly after the first COVID-19 vaccination, leaving the rates of anti-PF4 antibody positivity after second vaccinations largely unknown.

Method In this study, we analyzed serum samples from healthcare workers who had received at least two vaccines, with the first vaccine being the AstraZeneca (AZ) vaccine. Anti-PF4/heparin IgG antibody levels were determined using a commercially available ELISA kit. An optical density (OD) greater than 0.5 was considered a positive result. Heparin and PF4 dependent procoagulant platelet formation was analyzed with flow cytometry.

Results A total of 444 study participants (356 (80 %) females and 88 (20 %) males) with a median age of 48 years (ranging from 21 to 67 years) were included in this study. Serum samples were collected a median of 158 days (range: 76 to 209 days) after the second vaccination. Of the samples analyzed, 27 (6 %) were positive by ELISA with a median OD of 0.728 ranging from 0.5 to 2.8. Specifically, there were 5 of 93 cases (5.3 %) in individuals who received two AZ vaccinations, 15 of 226 cases (6.6 %) in individuals who received AZ-Biontech/Pfizer vaccinations, and 7 of 125 cases (5.6 %) in individuals who received AZ-Moderna vaccinations. None of the positive serum samples (OD > 0.5) induced procoagulant platelet formation in flow cytometry.

Conclusion The observed frequency of positive cases is consistent with findings from previous studies conducted after the first vaccination. However, the presence of cases with high OD values and the time elapsed since the second vaccination suggest the need for further studies to fully evaluate the clinical significance of vaccine-induced anti-PF4 antibodies.

Conflict of Interest No conflict interest

T-13-04 Emicizumab for the treatment of acquired hemophilia A – consensus recommendations from the GTH-AHA working group

Authors C. Pfrepper[1], R. Klamroth[2], J. Oldenburg[3], K. Holstein[4], H. Eichler[5], C. Hart[6], P. Moehnle[7], K. Schilling[8], K. Trautmann-Grill[9], M. Alrifai[10], C. Ay[11], W. Miesbach[12], P. Knoebl[11], A. Tiede[13]

Institutes **1** University Hospital Leipzig, Division of Hemostaseology, Leipzig, Germany; **2** Vivantes Clinic Friedrichshain, Department of Angiology and Clotting Disorders, Hemophilia Treatment Center, Berlin, Germany; **3** University Clinic Bonn, Institute of Experimental Hematology and Transfusion Medicine, Bonn, Germany; **4** University Medical Center Hamburg-Eppendorf, Hematology and Oncology, Hamburg, Germany; **5** Saarland University Hospital, Clinical Hemostaseology and Transfusion Medicine, Homburg/Saar, Germany; **6** University Hospital Regensburg, Department of Hematology and Oncology, Regensburg, Germany; **7** University Hospital, Ludwig Maximilian University, Division of Transfusion Medicine, Cell Therapeutics and Hemostaseology, München, Germany; **8** University Hospital Jena, Department of Hematology and Oncology, Jena, Germany; **9** University Hospital Carl Gustav Carus, Medical Clinic I, Dresden, Germany; **10** University Hospital Giessen and Marburg GmbH, Department of Thrombosis and Hemostasis, Giessen, Germany; **11** Medical University of Vienna, Clinical Division of Hematology and Hemostaseology, Wien, Germany; **12** Goethe University Frankfurt, Medical Clinic II, Frankfurt, Germany; **13** Hannover Medical School, Hematology, Hemostasis, Oncology, and Stem Cell Transplantation, Hannover, Germany

DOI 10.1055/s-0044-1779191

Introduction Acquired hemophilia A (AHA) is a severe bleeding disorder caused by autoantibodies against coagulation factor VIII (FVIII). Standard treatment consists of bleeding control with bypassing agents and immunosuppressive therapy. Emicizumab is a bispecific antibody that mimics the function of FVIII irrespective of the presence of neutralizing antibodies. Recently, the GTH-AHA-EMI study demonstrated that emicizumab prevents bleeds and allows to postpone immunosuppression, which may influence future treatment strategies.

Method A Delphi procedure was conducted among 33 experts from 16 German and Austrian hemophilia care centers to provide clinical practice recommendations on the use of emicizumab in AHA. Statements were scored on a scale of 1 to 9, and agreement was defined as a score of ≥ 7. Consensus was defined as ≥ 75 % agreement among participants, and strong consensus as ≥ 95 % agreement.

Results Strong consensus was reached that emicizumab is effective for bleed prophylaxis and should be considered from the time of diagnosis (100 % consensus). A fast-loading regimen of 6mg/kg on day 1 and 3mg/kg on day 2 should be used if rapid bleeding prophylaxis is required (94 %). Maintenance doses of 1.5mg/kg once weekly should be given (91 %). Immunosuppression should be offered to patients on emicizumab if they are eligible based on physical status (97 %). Emicizumab should be discontinued when remission of AHA is achieved (97 %).

Conclusion These GTH consensus recommendations provide guidance to physicians on the use of emicizumab in AHA and follow the results of clinical trials that have shown emicizumab is effective in preventing bleeding in AHA.

Conflict of Interest CP reports institutional grants for research and studies from Chugai/Roche, Takeda, Zacros, and LeoPharma, and honoraria for lectures or consultancy from Bayer, Biomarin, Chugai/Roche, CSL Behring, Novo Nordisk, Pfizer, BMS, SOBI, and Takeda. RK reports institutional grants for research and studies from Bayer, CSL Behring, Novo Nordisk, Octapharma, and SOBI, and honoraria for lectures or consultancy from Bayer, Biotest, Biomarin, CSL Behring, Grifols, LFB, Novo Nordisk, Octapharma, Pfizer, Roche/Chugai, Sanofi, SOBI, and Takeda. JO reports institutional grants for research and studies from Bayer, Biotest, CSL Behring, Octapharma, Pfizer, SOBI, and Takeda, and honoraria for lectures or consultancy from Bayer, Biogen Idec, Biomarin, Biotest, CSL Behring, Chugai, Freeline, Grifols, Novo Nordisk, Octapharma, Pfizer, Roche, Sparks, SOBI, and Takeda. KH reports institutional grants for research and studies from Bayer, CSL Behring, Novo Nordisk, Pfizer, and SOBI, and honoraria for lectures or consultancy from Bayer, Biotest, Chugai, CSL Behring, LFB, Novo Nordisk, Pfizer, Roche, SOBI, and Takeda. HE reports grants or contracts from Bayer, BioMarin, Biotest, CSL Behring, NovoNordisk, Pfizer, Sobi, consulting fees from Bayer, BioMarin, CSL Behring, Novo Nordisk, Pfizer, Sobi, payment of honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Novo Nordisk, Pfizer, support for attending meetings and/or travel from Bayer, BioMarin, Biotest, Novo Nordisk, participation on a Data Safety Monitoring Board or Advisory Board from Bayer, BioMarin, CSL Behring, Novo Nordisk, Pfizer, Sobi. CH reports honoraria for lectures or consultancy from Bayer, SOBI, Roche, Pfizer, and Takeda. PM reports institutional grants for research and studies from Baxter Innovations, Bayer, LFB, SOBI, Octapharma, Pfizer, and Roche, and honoraria for lectures or consultancy from Alexion, AstraZeneca, Biotest, CSL Behring, Shire, Octapharma, Pfizer, Roche, and Takeda. KS has nothing to disclose. KTG reports honoraria for lectures or consultancy from Grifols, SOBI, Takeda and Roche. MA has nothing to disclose. CA reports consulting fees from Bayer, Novo Nordisk, CSL Behring, Pfizer, Octapharma, LFB, and Swedish Orphan Biovitrium,Payment of honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from Bayer, Novo Nordisk, Takeda, CSL Behring, Pfizer, Octapharma, Roche, LFB, and Swedish Orphan Biovitrium. WM reports institutional grants for research and studies from Bayer, Biotest, CSL Behring, LFB, Novo Nordisk, Octapharma, Pfizer, and Takeda/Shire, and honoraria for lectures or consultancy from Bayer, Biomarin, Biotest, CSL Behring, Chugai, Freeline, LFB, Novo Nordisk, Octapharma, Pfizer, Regeneron, Roche, Sanofi, Takeda/Shire, and uniQure. PK reports institutional grants for research and studies from Ablynx/Sanofi, Novo Nordisk, Roche, and Takeda, and honoraria for lectures or consultancy from Ablynx/Sanofi, Alexion, Biotest, CSL Behring, Novo Nordisk, Roche, Takeda, and Technoclone. AT reports institutional grants for research and studies from Bayer, Biotest, Chugai, Novo Nordisk, Octapharma, Pfizer, Roche, SOBI, and Takeda, and honoraria for lectures or consultancy from Bayer, Biomarin, Biotest, Chugai, CSL Behring, Novo Nordisk, Octapharma, Pfizer, Roche, SOBI, and Takeda.

T-13-05 Long term observation of therapy and outcome in acquired hemophilia A

Authors L. Dreier, F. Gatzke, S. Roth, S. Rauchfuß, W. Miesbach
Institute Goethe-Universität Frankfurt am Main, Medizinische Klinik 2/Hämostaseologie/Universitätsklinikum Frankfurt am Main, Frankfurt am Main, Germany
DOI 10.1055/s-0044-1779192

Introduction Acquired hemophilia A (AHA) is a rare autoimmune disorder caused by autoantibodies against factor VIII (FVIII) and can lead to potential life-threatening bleeding events. Primary therapy goals are prevention and control of bleeding, inhibitor eradication by immunosuppression and therapy of a potential underlying disease. While predictors for remission have been investigated, there is still very few data available for recurrences and predictors which may help to monitor patients.

Method The study was a retrospective and in parts prospective study of 79 AHA patients treated between 10/1995 and 01/2021 at the Hemophilia Comprehensive Care Center of University Hospital Frankfurt am Main. Research protocol was approved by the ethics committee. Definition of remission was used according to GTH criteria [1]. Recurrence was defined as renewed FVIII acitivity decrease to < 50 IU/dl and renewed neutralizing FVIII inhibitor ≥ 0.4 Bethesda units (BU)/mL after initial complete (CR) or partial remission (PR). Data collection was based on existing patient files and on structured interviews.

Results The baseline characteristics of the 79 patients are summarized in ▶ **Fig. 1**.

Table 1. *Baseline characteristics of the cohort.*

	All patients n = 79 (%)
Gender	
Female	40 (50.6)
Male	39 (49.4)
Age in y, median (range)	74 (28-91)
Underlying main disorder	
None/Idiopathic	52 (65.8)
Malignancy	16 (20.3)
Autoimmunity	6 (7.6)
Postpartum	5 (6.3)
Comorbidities	
Arterial hypertension	56 (70.9)
Cardiovascular (Heart failure, CAD, PAOD)	27 (34.2)
Renal failure	19 (24.1)
Neurological disorder (Alzheimers disease, Parkinsons disease, ...)	19 (24.1)
Diabetes mellitus type 2	17 (21.5)
Liver failure	11 (13.9)
Initial FVIII activity in IU/dl, median (range)	2.0 (0.0-35.8)
Initial Inhibitor concentration in BU/ml, median (range)	18.16 (0.44-9000.0)

Abbreviations: CAD, coronary artery disease; PAOD, peripheral arterial occlusive disease; IU, international unit; BU, bethesda units.

▶ **Fig. 1** Baseline characteristics of the cohort.

Time in remission

All relapsing patients with initial CR had their first relapse < 1 year after achieving CR. Median time in CR was 44.5 days and 75 days for PR.

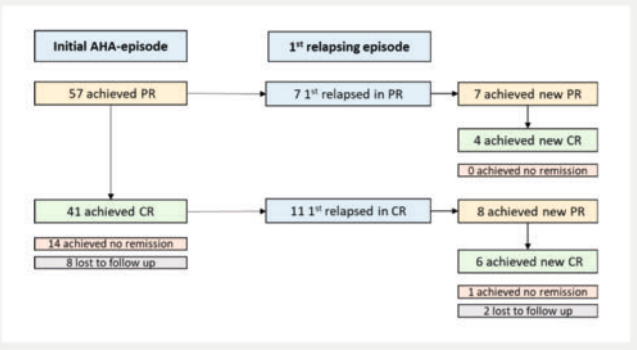
Recurrence rate

18 patients recurred after initial CR or PR. In total 22 patients did not achieve remission or state of remission was unknown, 11 patients died in close period around the initial AHA diagnosis and 5 patients could not be followed up ≥ 1 year. Therefore the remission rate of the remaining 41 patients (100.0) was 43.9 %.

Remission process from initial episode to 1st relapsing episode

After initial AHA episode 41 patients achieved CR, while 16 patients stayed in initial PR. 14 patients achieved no remission and 8 were lost to follow up. 11 of 18 1st time relapsing patients relapsed in CR (61.1 %) and 7 in PR (38.9 %). Out of the initial CR relapsing patients 75.0 % achieved a new CR out of initial CR, whereas the initial PR relapsing patients achieved new CR in 57.1 % (▶ **Fig. 2**), $p = 0.659$.

Conclusion Here we report the first results of the only study so far, that reports recurrences of AHA in detail and will evaluate possible predictors in further analysis.



▶ **Fig. 2** Remission process from initial episode to 1st relapsing episode.

The following findings have been obtained so far:

- Our study showed a AHA-relapsing rate of 43.9 %
- Due to the fact, that all relapsing patients relapsed < 1 year after achieving initial CR, we highly recommend a follow up period of at least 1 year after CR.
- Achievement of initial CR was no protective factor against recurrences.
- Patients who went from PR to relapse were able to regain PR and also often CR afterwards.

Conflict of Interest Institutional budget, no external funding (budget of sponsor/PI).

References

[1] Tiede A, Klamroth R, Scharf RE, Trappe RU, Holstein K, Huth-Kühne A, Gottstein S, Geisen U, Schenk J, Scholz U, Schilling K, Neumeister P, Miesbach W, Manner D, Greil R, von Auer C, Krause M, Leimkühler K, Kalus U, Blumtritt JM, Werwitzke S, Budde E, Koch A, Knöbl P. 'Prognostic factors for remission of and survival in acquired hemophilia A (AHA): results from the GTH-AH 01/2010 study.'. Blood 2015; 125 (7): 1091–1097. doi:10.1182/blood-2014-07-587089

T-13-06 Cerebral venous sinus thrombosis and thrombocytopenia after adenovirus infection due to heparin-independent anti-PF4 antibodies

Authors G. Uzun[1,2], J. Zlamal[1,2], K. Althaus[1,2], A. Bevot[3], F. Hennersdorf[4], N. Wolska[1], A. Jock[5], J. Kern[5], V. Icheva[5], S. Poli[6,7], U. Ernemann[4], A. Neu[8], T. Bakchoul[1,2]
Institutes **1** University Hospital of Tuebingen, Institute for Clinical and Experimental Transfusion Medicine, Tübingen, Germany; **2** Center for Clinical Transfusion Medicine, Tübingen, Germany; **3** University Children's Hospital, University of Tuebingen, Department of Neuropediatrics and Developmental Medicine, Tübingen, Germany; **4** University Hospital Tuebingen, Department of Diagnostic and Interventional Neuroradiology, Tübingen, Germany; **5** University Children's Hospital, University of Tuebingen, Department of Pediatric Cardiology, Pulmonology and Intensive Care Medicine, Tübingen, Germany; **6** University Hospital of Tuebingen, Department of Neurology & Stroke, Tübingen, Germany; **7** Eberhard-Karls University of Tuebingen, Hertie Institute for Clinical Brain Research, Tübingen, Germany; **8** University Children's Hospital, University of Tuebingen, Department of Pediatric Endocrinology and Diabetes, Tübingen, Germany
DOI 10.1055/s-0044-1779193

Introduction Cerebral venous sinus thrombosis (CVST) is a rare cerebrovascular disease that primarily affects adults. However, its occurrence in paediatric patients presents unique challenges. This paper discusses a paediatric case of severe CVST and thrombocytopenia following adenovirus infection, resembling vaccine-induced immune thrombotic thrombocytopenia (VITT). The aim of this study is to serologically characterize antibodies against platelet factor 4 (PF4)/heparin detected in the serum of patients and to investigate their ability to induce thrombosis ex vivo.

Method Serum sample was investigated for anti-PF4/heparin antibodies using a commercially available enzyme immunoassay (EIA). The functionality of anti-PF4 antibodies were investigated using modified heparin induced platelet agregation (HIPA) assay as well as procoagulant platelet assay in flow cytometry. The ability of patient's serum to induce thrombosis was investigated using an ex vivo model for antibody-mediated thrombosis.

Results A 7-year-old girl presented with sudden onset of severe headache and vomiting after adenovirus infection. Extensive thrombosis in the superior sagittal sinus and thrombocytopenia were noted. Thrombectomy was performed, but recurrent thrombosis occurred. Therapeutic anticoagulation with unfractionated heparin was started and a second mechanical thrombectomy was performed. Due to thrombocytopenia and bleeding complications, the patient received high-dose intravenous immunoglobulin (IVIG), which rapidly normalised the platelet count. Examination of the sample taken before heparin therapy showed a strong IgG PF4/heparin EIA reaction (OD 2.8; normal range: 0-0.5). However, the HIPA assay was negative with a low concentration of heparin, making heparin-induced thrombocytopenia (HIT) very unlikely. In contrast, modified HIPA was positive (platelet aggregation within 5 minutes in the presence of PF4 and within 15-35 minutes without exogenous PF4), a serological pattern that mimics VITT. Patient's serum induced procoagulant platelet formation, which was inhibited by IV.3 (Fcγ receptor IIA [FcγRIIA] blocking monoclonal antibody) and IVIG, indicating FcγRIIA dependence. In a novel ex vivo model of antibody-mediated thrombosis, patient serum induced significant thrombus formation with increased fibrin deposition compared to healthy control, which was prevented by IV.3 and IVIG.

Conclusion This case suggests that anti-PF4 antibodies can develop after adenovirus infection without prior heparin or COVID-19 vaccine exposure, leading to severe CVST and thrombocytopenia. The mechanisms behind this phenomenon warrant further investigation, potentially impacting the management of unexplained thrombosis and thrombocytopenia.

Conflict of Interest no relevant conflict of interest

T-13-07 Thrombin generation parameters accurately predict liver cirrhosis decompensation

Authors M. G. Zermatten[1], M. Fraga[2], D. Bertaggia Calderara[1], A. Aliotta[1], C. Pereira Portela[1], É. Mdawar-Bailly[2], F. Artru[3, 2], D. Moradpour[2], L. Alberio[1]

Institutes **1** Lausanne University Hospital and University of Lausanne, Division and Central Laboratory of Hematology, 1011, Switzerland; **2** Lausanne University Hospital and University of Lausanne, Division of Gastroenterology, 1011, Switzerland; **3** King's College Hospital, Institute of Liver Studies, SE5 9RS, UK

DOI 10.1055/s-0044-1779194

Introduction Liver cirrhosis (LC) is a complex condition that is associated, among others, with a prothrombotic state and episodes of decompensation. While hypercoagulability is secondary to the altered equilibrium in coagulation factors, the causes of liver decompensation are multiple. The aim of this study is to investigate the relationship between hemostatic parameters anDariusd the occurrence of LC decompensation.

Method We performed a prospective single-centre study at Lausanne University Hospital (CHUV) including 302 non-anticoagulated adult patients with LC of all aetiologies and stages. The primary outcome was LC decompensation, defined by the development of ascites, encephalopathy, hepato-renal syndrome, spontaneous bacterial peritonitis, or variceal bleeding. We studied clinical and laboratory parameters including *in vivo* and *ex vivo* thrombin generation. Statistical analyses were performed using univariate and multivariate logistic regression.

Results Among the 302 patients, 67 (22.2 %) developed LC decompensation. Results of logistic regression are presented in ▶ **Fig. 1**. Six parameters were found to be significantly and independently associated with LC decompensation: Child-Turcotte-Pugh score, alkaline phosphatase, gamma-GT, hemoglobin, prothrombin fragments 1 and 2 (F1 + 2) and thrombomodulin-mediated (TM) endogenous thrombin potential (ETP) inhibition. The combination of these six variables predicted liver decompensation with an area under the ROC curve of 0.93.

Table 1 : Patient characteristics and results of univariate and multivariate logistic regressions. *Numbers are mean (standard deviation), resp. numbers of patients (percentage). NASH, nonalcoholic steatohepatitis; ETP, endogenous thrombin potential; TM, thrombomodulin.*

		All	Liver cirrhosis decompensation			
			0	1	p-value (univariate)	p-value (multivariate)
	Events	302	235 (77.8%)	67 (22.2%)		
Clinical parameters	Age	58.1 (11.1)	58.2 (10.6)	57.8 (13.0)	0.810	
	Female	66 (21.8)	51 (77.3%)	15 (22.4%)	0.905	
	Aetiology					
	Alcoholic	153 (50.5%)	109 (71.7%)	43 (28.3%)	0.011	
	NASH	25 (8.2%)	18 (72.0%)	7 (28.0%)	0.467	
	HBV	24 (7.9%)	18 (75.0%)	6 (25.0%)	0.730	
	HCV	73 (24.0%)	68 (93.2%)	5 (6.9%)	0.001	
	HBV + HCV	2 (0.6%)	2 (100.0%)	0 (0.0%)	0.000	
	Autoimmune	18 (5.9%)	14 (77.8%)	4 (22.2%)	0.997	
	Others	8 (2.6%)	6 (75.0%)	2 (25.0%)	0.846	
	Porta vein flow >10 (cm/s)	122 (63.2%)	96 (78.7%)	26 (21.3%)	0.519	
	Thrombocytopenia	169 (56.9%)	120 (71.4%)	48 (28.6%)	0.004	
	Portal hypertension	238 (78.6%)	176 (74.3%)	61 (25.7%)	0.007	
	Child-Turcott-Turcott score	5.9 (1.5)	5.49 (1.1)	7.3 (2.0)	0.000	0.028
	MELD score	9.7 (4.1)	8.8 (3.4)	12.7 (4.9)	0.000	
	Ascites					
	Absence	253 (83.8%)	216 (85.4%)	37 (14.6%)		
	Moderate	42 (13.9%)	17 (41.5%)	24 (58.5%)	0.000	
	Refractory	7 (2.3%)	2 (28.6%)	5 (71.4%)	0.002	
	Presence of esophageal varices	175 (59.5%)	124 (71.3%)	50 (28.7%)	0.000	
Laboratory parameters	AST [U/l]	48.7 (35.9)	44.7 (32.5)	62.3 (43.5)	0.001	
	ALT [U/l]	40.5 (32.1)	40.7 (33.8)	39.7 (25.8)	0.821	
	Alkaline phosphatase [U/l]	115.8 (77.2)	103.5 (47.4)	159.2 (129.4	0.000	0.007
	Gamma-GT [U/l]	162.1 (191.2)	143.5 (174.4)	228.8 (231.2)	0.003	0.009
	Total bilirubin [umol/l]	22.6 (29.9)	17.4 (20.0)	40.7 (47.3)	0.000	
	Hemoglobin [g/l]	131.1 (22.4)	136.6 (18.7)	112.7 (23.9)	0.000	0.000
	Thrombocyte count [G/l]	145.5 (74.0)	155.9 (73.8)	109.2 (63.4)	0.000	
	Prothrombin time [%]	77.1 (18.6)	80.9 (16.7)	64.0 (18.7)	0.000	
	aPTT [s]	34.9 (7.0)	33.5 (5.6)	39.2 (9.1)	0.000	
	Fibrinogen [g/l]	2.7 (1.0)	2.9 (1.0)	2.3 (0.9)	0.000	
	Factor V activity [%]	81.9 (30.3)	87.4 (28.9)	63.9 (27.8)	0.000	
	Prothrombin fragment 1 and 2 > normal	83 (12.0%)	57 (68.7%)	26 (31.3%)	0.010	0.043
	Thrombin-antithrombin complexes [pmol/l]	3.6 (3.2)	3.4 (3.3)	4.0 (2.9)	0.208	
	D-dimers [ng/ml]	1415 (2872)	947 (2368)	3003 (3789)	0.000	
	Albumin [g/l]	40.3 (5.6)	41.7 (4.8)	35.5 (6.6)	0.000	
	Creatinin [umol/l]	83.4 (31.9)	80.3 (28.2)	93.4 (41.0)	0.007	
Ex vivo thrombin generation	Lag time normalized	1.2 (0.32)	1.3 (0.3)	1.1 (0.2)	0.002	
	Peak height normalized [%]	77.6 (22.0)	77.9 (22.5)	77.0 (20.0)	0.787	
	Time to peak normalized	1.1 (0.3)	1.2 (0.3)	1.0 (0.2)	0.000	
	ETP normalized [%]	77.4 (16.4)	78.6 (16.2)	73.7 (16.7)	0.035	
	Velocity index normalized [%]	90.4 (41.8)	86.6 (42.7)	103.4 (36.0)	0.005	
	Lag time with TM [min]	2.7 (0.8)	2.7 (0.9)	2.4 (0.4)	0.007	
	Peak height with TM [nM]	164.1 (63.1)	157.8 (64.1)	186.1 (55.0)	0.002	
	Time to peak with TM [min]	4.4 (1.1)	4.5 (1.1)	4.1 (0.6)	0.001	
	ETP with TM [nM*min]	738.9 (288.7)	701.1 (290.1)	871.3 (244.7)	0.000	
	Velocity index with TM [nM/min]	136.2 (70.6)	127.6 (69.4)	165.6 (67.3)	0.000	
	TM-mediated ETP inhibition [%]	37.1 (21.5)	41.7 (20.9)	21.4 (15.2)	0.000	0.002

▶ **Fig. 1 Patient characteristics and results of univariate and multivariate logistic regression.** Numbers are mean (standard deviation), resp. numbers of patients (percentage). NASH, nonalcoholic steatohepatitis; ETP, endogenous thrombin potential; TM, thrombomodulin.

Conclusion We developed a score that accurately predicts LC decompensation. Interestingly, several hemostasis parameters, including *in vivo* and *ex vivo* thrombin generation, were found to be significantly associated with the occurrence of LC decompensation in univariate analyses. More specifically, F1 + 2 and TM inhibition were found to be significantly and independently associated with LC decompensation. This observation supports a major role of a prothrombotic state in the pathophysiology of LC decompensation, suggesting a rationale for the use of anticoagulation as a preventive measure. After external validation, the proposed score shall be used in a large prospective study assessing the utility of targeted prophylactic anticoagulation to prevent decompensation in patients with LC.

Conflict of Interest No conflict of interest to declare

T-13-08 Dynamic changes in Factor XIII activity as predictors of mortality in critically ill COVID-19 patients

Authors C. L. Cibis, W. Miesbach, E. Adam, F. Gatzke, S. Roth, S. Rauchfuß
Institute Goethe University Frankfurt, Clinical Hemostaseology and Transfusion Medicine, Frankfurt am Main, Germany
DOI 10.1055/s-0044-1779195

Introduction Severe COVID-19 disease is characterized by a proinflammatory state with multiple organ dysfunction and a significant derangement of haemostasis. Critically ill patients are at increased risk of developing venous and arterial thrombosis, as well as extensive fibrin deposition and microthrombi formation in the lungs, suggesting a disbalance in fibrin generation and degradation often resulting in death. Given the role of FXIII in fibrin cross-linking and clot stabilization, this study investigates the impact of FXIII activity on overall in-hospital mortality as well as dynamic changes in activity during the course of disease.

Method This analysis includes 68 patients who were treated at the ICU of Frankfurt University Hospital between March 01, 2020 and December 31, 2021. Patients had to be over 18 years of age with laboratory confirmed SARS-CoV-2 infection requiring mechanical ventilation or high-flow oxygen therapy without having undergone ECMO-therapy.

We prospectively collected blood coagulation data from each patient at day one and day five as well as relevant clinical data from electronic medical records. This includes clinical characteristics, age, gender, relevant comorbidities, and therapeutic interventions [1–2].

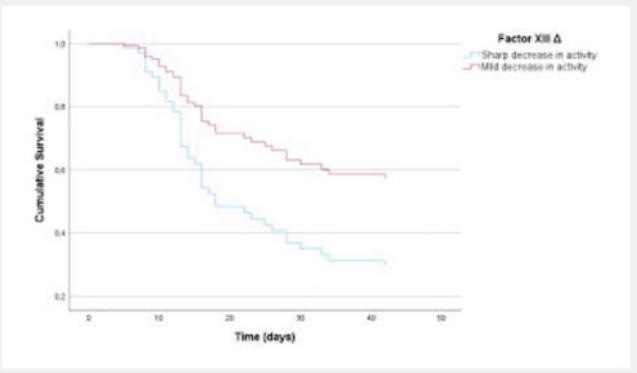
Results Levels of Factor XIII activity decreased significantly over the course of hospitalisation ($p < .001$). Activity was decreased below the normal range of 70-130 % in 14 out of 68 Patients (20.6 %) upon admission to the ICU. On day five, this reduction was even more pronounced with 35 out of 68 patients (51.5 %) below normal range. On average, mean factor XIII activity decreased by 22.4 % during this period.

We divided the patient cohort into "Median low" and "Median high" groups using a median split at each respective time point (▶ **Fig. 1**). While FXIII levels on day one and day five did not show an association with in-hospital mortality in univariate logistic regression analysis, the dynamic change in FXIII activity (FXIII Δ) emerged as a predictor of survival, with a sharp decrease in activity indicating a higher risk of death. In the multivariate survival analysis, FXIII Δ was identified as an independent predictor of mortality (HR = 2.174; 95 % CI [1.075, 4.386]; p = .031). ▶ **Fig. 2** illustrates the survival curve adjusted for group differences.

FXIII-Activity (%)	Total N = 68	Median low	Median high
FXIII Day One	89.4 (71.4-119.5)	71.8 (53.5-83.4)	118.5 (103.7-143.7)
FXIII Day five	66.2 (49.9-89.1)	50.0 (37.8-57.0)	89.1 (77.2-116.2)
FXIII Δ	-22.4 ((-35.1)-(-6.5))	-34.7 ((-45.6)-(-24.1))	-6.1 ((-12.0)-(9.6))

▶ **Fig. 1** Median FXIII activity

Conclusion Low FXIII-activity was found in a relevant portion of patients and further decreased with prolongation of hospitalization. A sharp decline in activity was associated with higher risk of in-hospital mortality. This decrease may be attributed to factor consumption resulting from increased activation of procoagulant pathways. However, alternative mechanisms, such as reduced synthesis due to liver dysfunction or the development of autoantibodies against FXIII, cannot be ruled out. To establish a causal relationship between our observations and the underlying mechanisms, further research is required.



▶ **Fig. 2** Survival curve adjusted for age and lung function (paO2/FiO2-ratio)

Conflict of Interest All authors declare no conflict of interest.

References

[1] Fan BE, Ng J, Chan SSW et al. COVID-19 associated coagulopathy in critically ill patients: A hypercoagulable state demonstrated by parameters of haemostasis and clot waveform analysis. Journal of Thrombosis and Thrombolysis 2021; 51 (3): 663–674. doi:10.1007/s11239-020-02318-x

[2] Ackermann M, Verleden SE, Kuehnel M et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. The New England journal of medicine 2020; 383 (2): 120–128. doi:10.1056/NEJMoa2015432

T-13-09 Daratumumab as a Well-Tolerated Treatment Option for Acquired Hemophilia A in a Frail Elderly Patient

Authors L. E. Kausche[1, 2], G. Schuster[3], J. Tomasits[4], C. A. Schmitt[1, 2]
Institutes **1** Kepler Universitätsklinikum, Universitätsklinik für Hämatologie und Internistische Onkologie, Linz, Austria; **2** Johannes Kepler Universität Linz, Universitätsklinik für Hämatologie und Internistische Onkologie, Linz, Austria; **3** Rotes Kreuz Oberösterreich, Blutzentrale, Linz, Austria; **4** Kepler Universitätsklinikum, Institut für Medizinische und Chemische Labordiagnostik, Linz, Austria
DOI 10.1055/s-0044-1779196

Introduction Acquired hemophilia A (AHA) is a rare serious bleeding disorder caused by autoantibodies targeting coagulation factor VIII (FVIII). The general one-year survival rate is about 68 %, with considerably worse survival if the inhibitor cannot be eliminated. Alongside the risk of severe bleeding, complications arising from the immunosuppressive treatment of AHA are of particular concern. AHA primarily affects elderly patients, and comorbidities and frailty can limit treatment options and increase susceptibility to complications. Currently, there is a need to develop treatment regimens for treatment of AHA in frail patients [1]. We present here a case of relapsed AHA with high levels of FVIII inhibitor in a 83-year-old female patient with multiple health conditions. To treat the AHA the patient received subcutaneously administered daratumumab, a monoclonal antibody targeting CD38.

Method Recombinant coagulation factor VIIa (rFVIIa) was administered as initial hemostatic therapy. The hemostatic treatment was then continued with emicizumab to further bridge the hemophilia. However, initial immunosuppressive therapy was not sufficient to treat the inhibitor in a patient who was too frail for more intense immunosuppression. We therefore decided to treat the AHA off-label with daratumumab on an outpatient basis.

Results The patient presented with spontaneous subcutaneous bleeds and was at risk of developing a compartment syndrome of the left arm. Bleeding was controlled using rFVIIa, and hemostasis was then maintained with emici-

zumab. An accidental minor skin lesion on the lower left leg did not exhibit increased bleeding during hemostatic therapy with emicizumab. However, the inhibitor titer rose from 1733 Bethesda Units (BU) per ml to 2653 BU/ml after starting the immunosuppressive treatment with prednisolone and one dose of rituximab. Due to the patient's multiple health conditions, she was not eligible for intensive outpatient immunosuppressive therapy, and the initiated therapy with cyclophosphamide was discontinued after 10 days. Instead, the patient received subcutaneous daratumumab on a weekly basis for 12 weeks, followed by every 2nd week thereafter. Prednisolone was tapered off after 3 weeks and completely discontinued after another 6 weeks. Apart from supportive dexamethasone administered shortly before each dose of daratumumab, no further immunosuppressant therapy was continued. After 16 weeks of daratumumab treatment the peak inhibitor titer of 2653 BU/ml decreased to 50 BU/ml. No infusion-related reaction and no infection was observed.

Conclusion The subcutaneous administration of daratumumab, in combination with hemostatic treatment using emicizumab, was a well-tolerated and efficacious option for the reported elderly patient with AHA. However, clinical studies are necessary to develop and assess a safe and effective treatment regimen using daratumumab as a treatment option for vulnerable patients with AHA.

Conflict of Interest The authors have no conflicts of interest to declare.

References

[1] Tiede A et al. 'International recommendations on the diagnosis and treatment of aquired hemophilia A', Haematologica. 2020: 105 (7): 1791–1801Italy: Ferrata Storti Foundation;

T-13-10 Thromboprophylaxis in 474 pregnancies in women with a positive family history or symptomatic VTE: a prospective two center follow-up study

Authors R. Beck[1], K. Lattmann[1], M. Ritgen[3], T. P. Bajorat[1], S. Kügler[2, 1], D. Juhl[2], U. Nowak-Göttl[1]

Institutes **1** Univ.-Hospital Schleswig-Holstein, Institute of Clinical Chemistry, 24105, Germany; **2** Univ.-Hospital Schleswig-Holstein, Institute of Blood Transfusion, 24105, Germany; **3** Univ.-Hospital Schleswig-Holstein, Department of Internal Medicine II, 24105, Germany

DOI 10.1055/s-0044-1779197

Introduction According to national and international guidelines in women with 1) a previous history of venous thromboembolism (VTE) or 2) a family history of VTE a risk adapted VTE prophylaxis is recommended.

Method The course of 474 pregnancies in 413 women with a history of VTE (n = 357 pregnancies) or a positive family history of VTE (n = 111 pregnancies) referred to our outpatient departments were prospectively followed with respect to the study endpoint "recurrent VTE". Starting in the first trimester women with a previous VTE received risk-adapted LMWH alone (n = 298) or LMWH in combination with ASS (n = 59). Women without a previous VTE and a positive family history received LMWH prophylaxis postpartum only (6-8 weeks of duration).

Results Overall, recurrent VTE occurred in 15 women (3.16 %): two events were observed in women without LMWH (2/111: 1.8 %) and 12 cases occurred in the cohort of 298 women with LMWH and a prior thrombotic event (4.02 %). No recurrence was observed in women with LMWH in combination with ASS. Logistic regression adjusted for the number of individual pregnancies, age at present pregnancy, presence of known thrombophilia and performed thromboprophylaxis (yes or no) revealed that the number of pregnancies (OR/95 % CI: 1.4/1.04-1.94) and younger age at pregnancy (OR/95 % CI: 0.85/0.75-0.97) predict recurrent VTE during follow-up. Off note, abortions in the first and second trimester were reported in 7 women with no influence on the study endpoint. No adverse bleedings were observed during LMWH or LMWH plus ASS therapy.

Conclusion*In conclusion*, in the present prospective cohort study in women suffering from 1) a previous VTE prior to the present pregnancy or 2) a positive familiy history of VTE the guided VTE prophylaxis according to present national and international recommendations is safe with a low rethrombosis rate during administration.

Conflict of Interest No

T-13-11 Long-term outcome in patients with acquired von-Willebrand-disease receiving ECMO or LVAD implants: a comparative cohort study

Authors T. P. Bajorat[1], R. Beck[1], B. Panholzer[2], D. Juhl[4], A. Kowalski[2], B. Zieger[3], U. Nowak-Göttl[1]

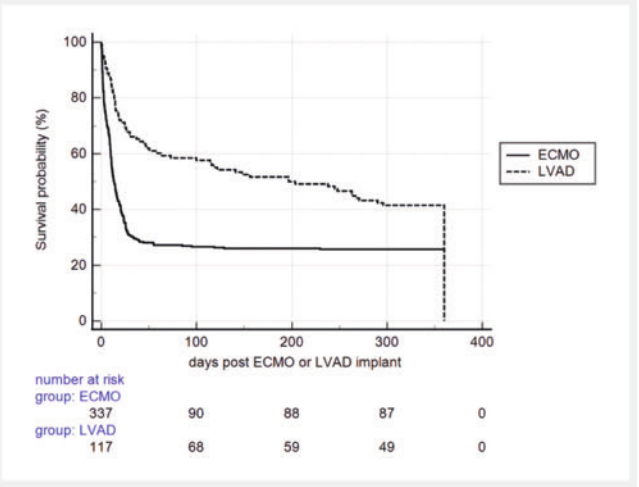
Institutes **1** Univ.-Hospital Schleswig-Holstein, Institute of Clinical Chemistry, 24105, Germany; **2** Univ.-Hospital Schleswig-Holstein, Dept. of Cardiovascular Surgery, 24105, Germany; **3** Medical Center-University of Freiburg, Faculty of Medicine, Division of Pediatric Hematology/Oncology, Department of Pediatrics, 79110, Germany; **4** Univ.-Hospital Schleswig-Holstein, Institute of Blood Transfusion, 24105, Germany

DOI 10.1055/s-0044-1779198

Introduction Approximately 100 % of patients necessitating ECMO or LVAD conduits develop acquired von-Willebrand-disease (aVWD) during the follow-up, with only a small proportion presented with clinically relevant bleeding. The aim of the present cohort study of consecutively enrolled patients admitted to the cardiac surgery department was to collect demographic, medical and laboratory data possibly associated with *i)* development of clinically relevant bleeding and/or *ii)* death during a 12-months follow-up.

Method We performed a comparative cohort study.

Results 507 consecutive Caucasian patients following ECMO (n = 338) or LVAD (n = 169) implantation aged 18-89 years (male 80 %) were enrolled and followed over a period of 12 months. When compared ECMO versus LVAD patients *1)* at the time of device implantation ECMO patients showed significantly elevated liver enzymes (p < 0.001), lower number of platelets (p = 0.03), prolonged aPTT-(p < 0.001) and PT (p = 0.01) values. In contrast, hemoglobin concentration, creatinine and fibrinogen values were similar in both groups. Lethal outcome *2)*within 30 days following device implantation was associated with symptomatic aVWD (OR/95 %CI: 2.63/1.4-5.0) with LVAD patients less commonly affected (OR/95 %CI: 0.55/0.33-0.98). The death rate within 30 days post intervention adjusted for gender and liver function revealed that *ii)* ECMO versus LVAD implantation (OR/95 %CI: 1.9/1.57-2.38), symptomatic aVWD (OR/95 %CI: 2.8/1.5-5.4), the presence of blood group non-0 versus 0 (OR/95 %CI:1.7/1.06-2.7), and increasing age per year (OR/95 %CI: 1.01/1.002-1.03) was independenly associated with lethal outcome. The *3)* follow-up of the surving patients (ECMO n = 86; LVAD n = 51) showed a survival benefit in LVAD patients, which however, did not reach statistical significance mainly due to limited patient numbers (OR/95 %CI: 0.88/0.70-1.1) (▶ **Fig. 1**).



▶ **Fig. 1** Survival 360 days ECMO versus LVAD

Hämostaseologie 2024; 44: S1–S125

Conclusion*In conclusion,* in the present comparative cohort study we found more often a clinical relevant bleeding rate in patients receiving ECMO compared with LVAD devices, which was associtated with lethal 30-day outcome. Following suviving ECMO and LVAD patients over a 12-months period the survival benefit of LVAD versus ECMO patients showed a trent but did not reach statistical significance. Thus, a multicenter prospective follow-up study should be conducted to clarify this issue.

Conflict of Interest No

T-13-12 Prevention of haemostatic complications Myeloproliferative Neoplasms: retrospective mutational and haemostatic profile study in Western Estonia

Authors I. Vaide[1, 2], V. Aabrams[2], C. E. Ursu[4], E. Laane[3, 1]

Institutes **1** University of Tartu, Department of Hemato-Oncology, Institute of Clinical Medicine, Tartu, Estonia; **2** Pärnu Hospital, Department of Internal Medicine, Pärnu, Estonia; **3** Kuressaare Hospital, Department of Internal Medicine, Kuressaare, Estonia; **4** Romanian Academy of Medical Sciences, Onco-Hematology Research Unit, Children Emergency Hospital "Louis Turcanu" Timisoara, European Hemophilia Treatment Centre, Timisoara, Romania

DOI 10.1055/s-0044-1779199

Introduction Patients with myeloproliferative neoplasms (MN) have the complication in both side of hemostasis – related to bleeding and thrombosis. The major goal of therapy is to reduce thrombosis risk, which impacts MN morbidity and mortality. However, in lifelong evaluating quality of life during the disease course, the bleeding has also important role. Acquired von Willebrand disease (AvWD) may result from increased proteolysis together with platelet activation, which leads to reduced von Willebrand factor activity (vWFact). Holding hemostatic balance in prophylactical management to prevent complications is challenging, and understanding predicting factors valuable for physician. Approximately 60 % of patients with MN carry a genetic mutation: it's suggested that in MPN thrombocytosis has a higher risk of bleeding than thrombosis, aspirin may exacerbate this risk of bleeding in CALR-mutated essential thrombocythemia (ET) and JAK2 V617F mutation increases risk of thrombotic complications. We analyzed our MN patient cohort to lab analysis related to hemostasis and genetic testing in order to have better understanding in the patient's profile [1–3].

Method Chart review of case records with diagnosis of D47.3, D45 and D47.1 between years 2016-2022**.**

Results During 2016-2022 in total of 145 patients with MN were in regular follow-up in two centers in Western Estonia. Patients with essential thrombocythemia (ET) consisted of 35.17 %, with polycythemia vera (PV) of 33,79 % and with primary myelofibrosis (PMF) of 31,03 % There where Janus kinase 2 (JAK2), calreticulin (CALR), and ASXL1 mutations with frequencies of these mutations 78,12 %, 0 %, and 0 % in PV; 58 %, 9,67 % and 0 % in ET, and 51,28 %, 7,69 %, and 5,13 % in PMF detected. In 53 patients the analysis of von Willebrand factor antigen (vWFAg), von Willebrand factor activity (vWFact), Factor VIII were measured in case of bleeding tendency or before operation and invasive procedures. In addition, semiautomated von Willebrand factor multimer assay was performed and according the test results (loss of high weight multimers). In 21 from 53 patients (39.6 %) AvWD was diagnosed: 13 from 23 patients with ET, in 5 from 14 patients with PMF and in 3 from 12 patients with PV. In all patients except two the vWFact/vWFAg ratio was below 0.7 and the multimeric assay showed decreased high molecular weight of multimers. In patients with AvWD diagnosis 4 were CALR pos and 15 patients had JAK2 positivity. Seven patients died during the observation period, 3 of them from thrombosis, 1 from bleeding and 1 from thrombosis together with bleeding related courses

Conclusion AvWD can be a serious complication in patients with ET, PV and PMF. Based on our analysis, in MN patients VWF Ag and vWFact testing during the disease cause and also before planned surgical intervention or invasive procedures can help to evaluate the balanced hemostasis and to choose the proper preventive measures

Conflict of Interest No

References

[1] McMahon B, Stein BL. Thrombotic and bleeding complications in classical myeloproliferative neoplasms. Semin Thromb Hemost 2013; 39 (1): 101–11. doi:10.1055/s-0032-1331153. Epub 2012 Dec 23PMID: 23264112

[2] Stein BL, Martin K. From Budd-Chiari syndrome to acquired von Willebrand syndrome: thrombosis and bleeding complications in the myeloproliferative neoplasms. Hematology Am Soc Hematol Educ Program 2019; 2019 (1): 397–406. doi:10.1182/hematology.2019001318.PMID: 31808903; PMCID: PMC6913438.

[3] Pikta M, Banys V, Szanto T, Joutsi-Korhonen L, Vaide I, Varik M, Lehtinen AE, Giangrande P, Laane E. Von Willebrand Factor Multimeric Assay in Acquired von Willebrand Disease Diagnosis: A Report of Experience from North Estonia Medical Centre. J Lab Physicians 2021; 13 (3): 195–201. doi:10.1055/s-0041-1730818. Epub 2021 Jun 28PMID: 34602781; PMCID: PMC8478502.

T-14. Fibrinolysis

T-14-01 Plasmin generation and fibrinolysis in adults with primary immune thrombocytopenia

Authors T. Schramm[1], D. Mehic[1], J. Rast[1], S. E. Reitsma[2], M. Fillitz[3], P. Quehenberger[4], B. de Laat[5, 6], A. S. Wolberg[2], C. Ay[1], I. Pabinger[1], J. Gebhart[1]

Institutes **1** Medical University of Vienna, Department of Medicine I, Clinical Division of Hematology and Hemostaseology, Vienna, Austria; **2** University of North Carolina at Chapel Hill, Department of Pathology and Laboratory Medicine, UNC Blood Research Center, Chapel Hill, USA; **3** Hanusch Krankenhaus, Department of Internal Medicine, Vienna, Austria; **4** Medical University of Vienna, Department of Laboratory Medicine, Vienna, Austria; **5** Maastricht University, Department of Biochemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht, Netherlands; **6** Synapse Research Institute, Maastricht, Netherlands

DOI 10.1055/s-0044-1779200

Introduction Immune thrombocytopenia (ITP) is associated with bleeding but also increased thrombotic risk. Data on the role of imbalanced fibrinolysis in ITP patients are limited. We observed prolonged plasma clot lysis (CLT) in our primary ITP patient cohort, suggesting impaired fibrinolysis as a potential contributor to thrombotic risk (Schramm et al. 2021). This study investigates plasmin generation (PG) as the central protease of fibrinolysis, and fibrinolysis parameters in a large and well characterized cohort of adult primary ITP patients and healthy controls (HC).

Method Patients from the Vienna ITP biobank (EC 1843/2016) and age- and sex-matched HC (EC 039/2006) were studied. Plasma clot formation and lysis and PG were measured using ex vivo kinetic turbidimetric and fluorogenic methods, respectively (Miszta et al. 2021). Fibrinolysis factors including plasminogen activator inhibitor 1 (PAI1) antigen, alpha2-antiplasmin activity, tissue-plasminogen activator (tPA) antigen and activity, tPA-PAI1 complex levels and thrombin activatable fibrinolysis inhibitor (TAFI) antigen were measured by ELISA. D-dimer, antithrombin, and plasminogen antigen levels were assessed via bead-based flow cytometry immunoassays.

Results Eighty-six primary ITP patients and 78 HC were compared (▶ **Fig. 1**). After adjustment for sex, age, BMI, and fibrinogen, ITP patients had a prolonged PG lag time compared to HC (▶ **Fig. 2**). No difference in total PG or other assay parameters between the two groups was observed. Compared to HC, ITP patients had higher PAI-1, lower tPA antigen and activity, but no difference in the other fibrinolysis parameters (▶ **Fig. 2**).

	Primary ITP	HC
	n (%)	n (%)
Female	55 (64.0)	59 (75.6)
Blood type 0*	29 (34.1)	24 (35.8)
	median (IQR)	median (IQR)
Age, years	40.0 (30.0-54.0)	43.0 (28.0-52.0)
BMI, kg/m² **	26.0 (22.9-29.7)	23.4 (21.0-25.5)
Platelet count, x10⁹/L	61.5 (31.0-120.0)	258.0 (230.0-299.0)
Fibrinogen, mg/dL	317.0 (279.0-363.5)	289.0 (258.0-330.0)
aPTT, s ***	33.2 (30.8-37.7)	34.6 (32.7-36.3)
Prothrombin time , % ***	98.0 (91.0-107.0)	103.0 (95.0-112.0)
ITP specific data		
Disease duration, months	61.0 (9.0-130.0)	Na
ITP-SMOG Bleeding score	1.0 (0.0-3.0)	0
	n (%)	n (%)
Current ITP treatment	37 (43.0)	Na
Corticosteroids	23 (62.2)	
TPO-RA	14 (37.8)	
Previous thrombosis ****	9 (10.6)	0 (0)
Thrombosis during follow-up	6 (7.1)	Na

*Abbreviations: ITP, immune thrombocytopenia; HC, healthy controls; IQR, interquartile range [25th-75th percentile]; aPTT, activated partial thromboplastin time; TPO-RA, Thrombopoietin Receptor Agonist; Na, not applicable. *available of 85 ITP patients (99%) and of 67 healthy controls (86%); **available of 83 (97%) ITP patients and all healthy controls; ***available of 78 ITP patients (91%) and all healthy controls; ****available 85 ITP patients (99%) and all healthy controls*

▶ **Fig. 1** Baseline demographic data and laboratory data of primary ITP patients (n = 86) and healthy co

		Primary ITP		HC	
Plasma clot lysis	n	median (IQR)	n	median (IQR)	p*-value
CLT, min	86	28.0 (13.7-34.7)	78	16.7 (12.3-24.0)	**0.003**
Plasmin Generation					
Lag time, min	86	2.9 (2.6-3.3)	78	2.7 (2.6-2.9)	**0.018**
Velocity Index, nmol/min	86	17.1 (14.1-18.6)	78	17.1 (15.3-19.1)	0.414
Peak Plasmin, nmol/L	86	53.9 (42.9-63.4)	78	52.7 (45.0-58.2)	0.630
Time to Peak Plasmin, min	86	6.2 (5.3-6.7)	78	5.8 (5.3-6.1)	0.106
EPP, nmol/L x min	86	555.9 (408.8-709.3)	78	518.8 (421.7-668.5)	0.505
Fibrinolysis Parameters					**p-value**
PAI-1, U/mL	86	1.2 (0.8-2.6)	77	1.1 (0.6-2.1)	**0.049**
alpha2-antiplasmin, %	86	103.0 (91.0-111.0)	77	103.0 (94.0-110.0)	0.905
tPA antigen, ng/mL	86	2.6 (1.1-4.4)	77	3.9 (3.9-4.8)	**<0.001**
tPA activity, U/mL					**0.001**
≤0 U/mL	86	22 (25.6)	77	5 (6.5)	
>0 U/mL	86	64 (74.4)	77	72 (93.5)	
tPA-PAI1 complex, ng/mL	86	5.6 (2.9-8.9)	78	6.4 (3.6-8.8)	0.556
TAFI, µg/mL	86	9.4 (7.7-11.2)	68	8.6 (7.6-10.4)	0.242
D-dimer, ng/mL	86	15.9 (8.8-35.8)	77	18.8 (11.1-28.4)	0.524
Antithrombin, mg/mL	86	0.19 (0.15-0.31)	75	0.19 (0.14-0.27)	0.299
Plasminogen, mg/mL	86	0.18 (0.12-0.31)	75	0.17 (0.13-0.25)	0.502

*Abbreviations: ITP, immune thrombocytopenia; HC, healthy controls; IQR, interquartile range [25th-75th percentile]; CLT, clot lysis time; EPP, endogenous plasmin potential; PAI-1, Plasminogen activator inhibitor 1; tPA, Tissue-plasminogen activator; TAFI, Thrombin activatable fibrinolysis inhibitor. *p-value: adjusted for sex, age, BMI and fibrinogen levels by multiple linear regression analysis*

▶ **Fig. 2** Plasmin generation and fibrinolysis parameters in primary ITP patients (n = 86) compared to h

In ITP, no PG parameters were associated with CLT, but levels of tPA-PAI1 complex (r = 0.275, p = 0.010), antithrombin (r = 0.244, p = 0.024), and plasminogen (r = 0.247, p = 0.022) correlated weakly with CLT. Multiple linear regression showed a positive association of tPA-PAI1 complex with CLT (β = 0.241, 95 % CI 0.147-1.627, p = 0.019). Bleeding severity weakly correlated with antithrombin (r = 0.233, p = 0.031) and TAFI (r = 0.253, p = 0.019). Neither PG parameters, fibrinolysis parameters, nor CLT correlated with thrombosis history. During a median (IQR) observation period of 68 (52-74) months, 6 patients (7.1 %) developed thrombotic events (3 cases of venous and 3 cases of arterial thromboembolism). Thrombosis development positively correlated with tPA-PAI1 complex levels ($\eta^2 = 0.306$, p = 0.004) only. Additionally reflected through higher median (IQR) tPA-PAI1 complex levels in thrombosis patients (11.5 (8.9-17.1) ng/mL) compared to those without thrombosis development (5.6 (2.8-8.6) ng/mL, p = 0.033) [1–2].

Conclusion In addition to our previous findings of impaired clot lysis, adult primary ITP patients also have a prolonged PG lag time. The level of tPA-PAI1 complex, reflecting a prothrombotic state, was associated with delayed clot lysis and future thrombosis in ITP patients, whereas PG was not.

Conflict of Interest No conflict of interest.

References

[1] Schramm T. et al. Delayed Plasma Clot Lysis in Adult Patients with Primary Immune Thrombocytopenia (ITP). Blood 2021; 138: p 3162

[2] Miszta A. et al. Application of a plasmin generation assay to define pharmacodynamic effects of tranexamic acid in women undergoing cesarean delivery. J Thromb Haemost 2021; 19 (1): p. 221–232. Lastname, FN, Lastname, FN YYYY, 'Article', *Journal*, Edition, Page, Place of publication: publishers

T-14-02 The Factor VII activating protease stimulates fibrinolysis by increasing plasmin generation on the fibrin surface

Authors M. Etscheid[1], N. Beer[1], S. Kanse[2]

Institutes **1** Paul Ehrlich Institute, Haematology/Cell and Gene Therapy, Langen, Germany; **2** University of Oslo, Oslo University Hospital, Oslo, Norway

DOI 10.1055/s-0044-1779201

Introduction Cleavage of fibrinogen in the Bβ chain (at Lys53-Lys54) and at several sites in the AαC region by FSAP altered fibrin clot structure and functions in plasma, leading to enhanced tPA-triggered lysis [1]. Clots revealed a less coarse fibrin network with thinner fibers and a smaller pore size, considered to improve resistance to lysis. Since a direct activation of fibrinolytic enzymes by FSAP could be excluded, the true mechanism behind the profibrinolytic activity of FSAP remains unsolved. We extended our studies to pre-formed fibrin and plasma clots, excluding FSAP-related structural alterations during clot formation, to investigate if FSAP also modulates preformed fibrin clots to accelerate tPA-driven lysis. Whether FSAP might be of interest as a thrombolysis-promoting agent was of particular interest.

Method In an ELISA- or ELISA/activity-based assay setups on immobilized fibrin in a MTP, plasminogen binding and plasmin generation by tPA were studied as function of FSAP and TAFI. In laser scanning microscopy of a plasma clot generated in Ibidi µ-slides the velocity of tPA-driven clot lysis was investigated as function of exogenous and endogenous FSAP. The influence of FSAP and/or TAFI on fibrinolysis was also studied in clot lysis time (CLT) assays.

Results Clotting plasma in histone-coated µ-slide channel resulted in activation of endogenous proFSAP and accelerated tPA-triggered lysis, not seen in the presence of an FSAP-inhibitory antibody. Likewise, also exogenous FSAP promoted lysis, if added simultaneously with tPA. Apparently, FSAP interaction with the fibrin mesh facilitated clot lysis. Treatment of immobilized fibrin with increasing concentration of FSAP lead to enhanced plasminogen binding and shortened tPA-driven plasmin generation time (PGT). Activated TAFI (TAFIa), which removes C-terminal Lys residues (plasminogen binding sites) from fibrin, prolonged PGT and caused a 50 % delayed CLT in the absence of FSAP. FSAP accelerated PGT and enhanced tPA-driven lysis of a fibrin clot. This stimulating effect on lysis was completely reversed by TAFIa, but not TAFI precursor or inactivated TAFIa.

Conclusion Cleavage of fibrin(ogen) by FSAP generates new C-terminal Lys-residues, facilitating plasminogen binding and tPA-driven lysis. New C-terminal Lys residues are present on fragment Bβ1-53, released from the Bβ chain by FSAP [1, 2]. Presumably, also in the AαC region, sensitive to proteolytic cleavage, FSAP generates new C-terminal Lys-residues, although such sites need to be confirmed experimentally. Whether removal of an α2-antiplasmin covalent binding site in fibrin (cross-linked by FXIIIa to Lys303) or other non-covalent binding sites in the αC region contribute to enhanced fibrinolysis by FSAP is under investigation. Overall, FSAP not only modulates fibrinogen, but also fibrin to facilitate clot lysis. This opens the potential as a novel pharmacological agent to support established thrombolytic agents like tPA, as recently seen in a mouse stroke model [3].

Conflict of Interest None.

References

[1] Etscheid M, Subramaniam S, Lochnit G, Zabczyk M, Undas A, Lang IM, Hanschmann K-M, Kanse SM Biochimica Biophysica Acta-Molecular Basis of Disease 2018; 1864: 3397–3406

[2] Choi-Miura NH, Yoda M, Saito K, Takahashi K, Tomita M Biological & Pharmaceutical Bulletin 2001; 24: 140–143
[3] Kim JY, Manna D, Etscheid M, Leergaard TB, Kanse SM FASEB Journal 2022; 36: e22564

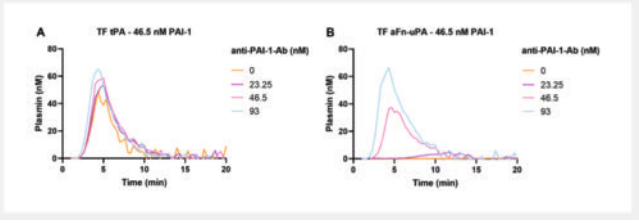
T-14-03 A Novel Plasminogen Activator Anti-fibrin-uPA Increases the Sensitivity of the Plasmin Generation Assay to Therapeutic Target PAI-1

Authors I. De Simone[1], M. Roest[1], A. S. Wolberg[2], B. de Laat[1], D. Huskens[1]
Institutes 1 Synapse Research Institute, Maastricht, Netherlands; 2 University of North Carolina at Chapel Hill, Department of pathology and laboratory medicine, Chapel Hill, USA
DOI 10.1055/s-0044-1779202

Introduction The fibrinolytic system is crucial for clot lysis during wound healing. Clot lysis is initiated by tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA), which convert plasminogen into plasmin. TPA-mediated plasminogen activation requires binding to fibrin, while uPA activates plasminogen independently of fibrin. Both plasminogen activators are inhibited by plasminogen activator inhibitor-1 (PAI-1), although, fibrin-bound tPA is protected from PAI-1. As inadequate regulation of fibrinolysis might lead to thrombotic and bleeding events, there is a clinical need for tools that estimate a patient's fibrinolytic state. We developed anti-fibrin-uPA (aFn-uPA), a construct containing uPA, linked to a nanobody directed against fibrin, to support binding of uPA to fibrin, and to increase effectiveness of plasminogen activation. The aim of this study is to increase the sensitivity of the calibrated, automated PG assay for detection of (anti-)PAI-1 effects in human plasma.

Method PG in normal pooled plasma (NPP) or factor deficient plasma was measured by cleavage of a plasmin-specific fluorogenic substrate, using a calibrated, automated method. In brief, fibrinolysis was started by combining plasma and trigger solution in a 96-well plate. The trigger solution consists of tissue factor (TF) or Russell's viper venom-factor X activator (RVV-X), phospholipids, and tPA or aFn-uPA. PG was measured with a fluorometer (Fluoroscan Ascent, Thrombinoscope, Maastricht, The Netherlands). PG parameters extracted from the data were lag time, time to peak (ttPeak), endogenous plasmin potential (EPP) and peak.

Results High TF, RVV-X, tPA and aFn-uPA were associated with a shorter PG lag time and a shorter PG ttPeak, compared to lower trigger concentrations. For subsequent experiments, 5 pM TF or 10^{-5} RVV-X, and 1.25 µg/mL (17.8 nM) tPA or 1 µg/mL (21 nM) aFn-uPA, were selected as optimal. The assay was specific for plasmin as no PG was observed in plasminogen- nor fibrinogen-deficient plasma. The sensitivity of the PG assay to PAI-1 was higher in PG triggered with the novel plasminogen activator aFn-uPA, compared to tPA, regardless of the affinity of both activators for fibrin. PAI-1 dose-dependently increased the lag time and ttPeak, and reduced the EPP and peak in PG stimulated with aFn-uPA in both NPP and PAI-1-deficient plasma. Using an anti-PAI-1-Ab, the inhibition obtained upon 2 µg/mL (46.5 nM) PAI-1, was reduced or completely abolished, depending on the concentration of anti-PAI-1-Ab (▶ **Fig. 1**).



▶ **Fig. 1 PAI-1-induced Inhibition of Plasmin Generation (PG) Is Abolished by Anti-PAI-1-Ab.** Normal pooled plasma, treated with 46.5 nM (2 µg/mL) PAI-1 was incubated (5 min, room temperature) with a range of anti-PAI-1-Ab concentrations, as indicated. PG induced by tissue factor (TF) and tPA (A) or aFn-uPA (B), was measured using the calibrated, automated PG assay.

Conclusion Overall, the calibrated, automated PG assay, in particular when aFn-uPa is used as trigger for fibrinolysis, is a specific and efficient tool to measure PG in human plasma, and is sensitive to the therapeutic target PAI-1. This tool may help identify abnormal fibrinolytic states in bleeding and thrombotic disorders.

Conflict of Interest Synapse Research Institute is part of the Diagnostica Stago Group.

T-14-04 Dysregulations of fibrinolysis system can be detected in frozen plasma samples using thrombelastography

Authors L. Heuer[1], S. Hammer[1, 2], K. Althaus[1, 2], T. Bakchoul[1, 2]
Institutes 1 University Hospital of Tuebingen, Institute for Clinical and Experimental Transfusion Medicine, Tübingen, Germany; 2 University Hospital of Tuebingen, Center for Clinical Transfuion Medicine, Tübingen, Germany
DOI 10.1055/s-0044-1779203

Introduction Thrombelastography (TE) is a standard lab test which measures coagulation parameters in whole blood samples in real time [1, 2]. The method serves as a useful point-of-care test to diagnose and manage patients with coagulation disorders [3]. The advantage of TE is the rapid overview of all phases of coagulation and fibrinolysis [4]. However, this method is limited by the need for immediate testing and difficulty in dissecting the exact mechanism of the dysregulation.

The aim of the study is to modify the method to enable assessment of specific aspects of coagulation, even retrospectively. In particular, it was investigated whether thrombelastographic studies can be performed using spike-in experiments from frozen plasma samples to measure fibrinolysis parameters (maximum of lysis, lysis time).

Method Frozen platelet poor plasma (PPP) samples were spiked in plasma-depleted blood samples. Both whole blood and spike-in samples were examined using TE. After establishing this method, plasma samples from 20 healthy donors and 35 patients with hypo- (COVID-19, sepsis, occlusion disease) or hyperfibrinolysis (bleeding) were analysed. Additionally, lysis time of plasma from pathological samples was determined in a photometric assay (lysis timer). Furthermore, supplementary investigations to determine plasminogen activator inhibitor-1 (PAI-1), tissue plasminogen activator and plasmin-alpha-2-antiplasmin complex in plasma were conducted.

Results Spike-in with healthy PPP samples and untreated whole blood samples correlate in maximum lysis in Ex-test ($p < 0.0001$; $r = 0.8240$) and in lysis time in TPA-test ($p = 0.004$; $r = 0.6035$), which confirms the feasibility of testing the fibrinolysis system using the spike-in approach in TE.

Especially, there was a statistically significant increase in lysis time of whole blood clots, as measured by the TPA-test, in spike-in samples with plasma from patients with hypofibrinolysis compared to healthy donors (lysis time in TPA-test: 222.2 ± 45.2 seconds vs. 166.7 ± 8.4 seconds, respectively $p < 0.0001$).

Interestingly, the lysis timer confirmed the hypofibrinolytic activity in patients plasma (lysis time in lysis timer: 67.9 ± 18.6 minutes vs. 40.9 ± 6.2 minutes, $p < 0.0001$). Most importantly, we observed that certain hypofibrinolytic samples have increased PAI-1 activity.

Conclusion The modified method is promising for diagnosing hypofibrinolysis retrospectively, especially in laboratories with no immediate access to thrombelastography. Our data suggest that a plasmatic factor might be responsible for the hypofibrinolytic activity, most likely PAI-1.

Conflict of Interest Nothing to declare.

References

[1] Collins S., MacIntyre C., Hewer I. `Thromboelastography: Clinical Application, Interpretation, and Transfusion Management´. AANA Journal Apr 2016; 84: 129–134PMID: 27311154
[2] Da Luz L.T., Bartolomeu N., Rizoli S. `Thrombelastography (TEGW): practical considerations on its clinical use in trauma resuscitation´. Scandinavian Journal of Trauma, Resuscitation and Emergency Medicine 2013; 21: 29PMID: 23587157

[3] Hammer S, Häberle H, Bakchoul T. et al. `Severe SARS-CoV-2 Infection Inhibits Fibrinolysis Leading to Changes in Viscoelastic Properties of Blood Clot: A Descriptive Study of Fibrinolysis in COVID-19´. Thromb Haemost 2021; 121 (11): 1417–1426PMID: 33634444
[4] Lang T., von Depka M. `Diagnostic potential and limitations of thrombelastography/-metry´. LaboratoriumsMedizin 2007; 31 (6): 254–261

T-15. Perioperative haemostasis

T-15-01 Fibrinolysis resistance in a patient with Streptococcus pyogenes associated septic shock and necrotizing fasciitis.

Authors K. M. Hofmann, M. Brauer, A. Kortgen, M. Bauer
Institute Jena University hospital, Department of Anaesthesiology and Intensive Care Medicine, Jena, Germany
DOI 10.1055/s-0044-1779204

Introduction Sepsis- induced coagulopathy (SIC) is a serious complication in patients with sepsis and septic shock. In an observational survay conducted in japan 29 percent of patients treated for sepsis developed SIC.
Systemic inflammation due to host invasion by pathogens is responsible for systemic coagulation activation.SIC is part of a host defence mechanism , leading to a inbalance in clot generation and fibrinolysis. Diffuse activation of endothelium by proinflammatory cytokines, leucocytes and other proteins create a prothrombotic state with excessive thrombin generation, resulting in an insoluble fibrin network, microvascular thrombosis and multiple organ dysfunction. Concomitantly, fibrinolysis can be impaired by increased production and activation of inhibitors such as PAI-1 and TAFI, as well as reduced t- PA.

Method A 66-year-old female patient was admitted to the emergency room with fever, chills and pain in her right arm after an insect bite. After hospitalization, the patient developed fulminant septic shock with severe ARDS, high-dose vasopressor therapy and multiorgan dysfunction. CT scan showed soft tissue infection of the right axilla and upper arm. To monitor SIC, we performed daily standard coagulation tests. In addition, we performed viscoelastic tests including TPA test to monitor fibrinolysis.

Results We treated with local debridement, fasciectomy, penicillin G and clindamycin for confirmed Streptococcus pyogenes infection, as well as guideline-based supportive sepsis therapy. Additionally we used antithrombin concentrate and low-dose heparin to treat the accompanying SIC and lysis resistance with a target range for antithrombin of 70 to 80 percent. Platelet count, antithrombin and Quick were significantly decreased, while D-dimer and aPTT were increased. Platelet count fell to a minimum of 19 gpt/l. If the patient was in an overall prothrombotic state, we refrained from platelet transfusion if there was no clinical bleeding and resistance to lysis. Lysis resistance persisted for a total of 5 days. On day 5, the platelet counts increased spontaneously and antithrombin no longer needed to be substituted. Quick value normalized, the D-dimers decreased. The patient stabilized clinically [1–5].

Conclusion SIC represents a serious complication of sepsis and septic shock. Some of these patients develop resistance to fibrinolysis. Limited data suggest resistance of fibrinolysis correlates with higher markers of cellular damage, higher severity score and worse outcome. The treatment of fibrinolysis resistance is still unclear. In addition to causal and supportive sepsis therapy, pathophysiological considerations discuss a continuous low-dose administration of rt-PA. The ClotPro TPA test represents a way to monitor fibrinolysis resistance in critically ill patients bedside. More studies are needed to investigate monitoring and treatment of fibrinolysis resistance in critically ill patients.

Conflict of Interest Hofmann, KM: Speaker fee, travel expenses (CSL Behring, Bayer HealthCare, LFB, Daiichi- Sankyo)

References

[1] Iba T, Levy JH 'Sepsis-induced Coagulopathy and Disseminated Intravascular Coagulation'. Anesthesiology 2020; 132 (5): 1238–1245. Lastname, FN, Lastname, FN YYYY, 'Article', *Journal*, Edition, Page, Place of publication: publishers
[2] Helms J, Iba T, Connors JM, Gando S, Levi M, Meziani F, Levy JH 'How to manage coagulopathies in critically ill patients'. Intensive Care Med 2023; 49: 273–290
[3] Coupland LA, Rabbolini DJ, Schoenecker JG, Crispin PJ, Miller JJ, Ghent T, Medcalf RL, Aneman AE ' Point-of-care diagnosis and monitoring of fbrinolysis resistance in the critically ill: results from a feasibility study'. Critical Care 2023; 27–55
[4] Panigada M, Zacchetti L, L'Acqua C, Cressoni M, Anzoletti MB, Bader R, Protti A, Consonni D, D'Angelo A, Gattinoni L 'Assessment of Fibrinolysis in Sepsis Patients with Urokinase Modified Thromboelastography'. PLoS One 2015; 26 10 (8): 1–17
[5] Delabranche X, Helms J, Meziani F ' Immunohaemostasis: a new view on haemostasis during sepsis '. Annals of Intensive Care 2017; 7 (1): 117

T-15-02 Incidence and risk factors of portal vein thrombosis in patients who underwent hepatectomy

Authors C. Chai-Adisaksopha, W. Lapisatepun, T. Pipanmekaporn, P. Niprapan, W. Lapisatepun
Institute chaing mai University, chiang mai, Thailand
DOI 10.1055/s-0044-1779205

Introduction Portal vein thrombosis (PVT) is the uncommon complication following hepatectomy, and there is ongoing debate regarding its incidence and associated risk factors. This study aimed to investigate the incidence and identify potential risk factor of PVT in patients undergoing hepatectomy.

Method This was a single center, retrospective cohort study, conducted at a university-based hospital in Thailand. The study included consecutive patients aged 18 years or older scheduled to undergo hepatectomy. Exclusion criteria encompassed patients with pre-existing PVT, those on anticoagulants for any reason, and individuals lacking postoperative CT abdomen scans. Data collected included demographic information, ASA classification, surgical diagnosis, presence of cirrhosis, type of surgery, vascular resection, baseline laboratory results, and transfusion history. PVT diagnosis was confirmed via CT abdomen scans.

Results A total of 874 patients were included with the mean (SD) age of 56.6 (11.4) years, and 62.6 % were male. Hepatocellular carcinoma and cholangiocarcinoma accounted for 74.8 % of cases. The incidence of PVT was 4.8 % (95 %CI 3.58-6.44). Multivariable analysis revealed that patients with presurgical serum albumin level < 4 mg/dL (OR 2.8, 95 %CI 1.1-4.4, P = 0.001) and those who had vascular resection (OR 5.12, 95 %CI 2.00-13.06) were at increased risk of developing postoperative PVT.

Conclusion In the context of hepatectomy, PVT remains relatively uncommon. Low serum albumin levels and the performance of vascular resection emerged as significant risk factors associated with the development of postoperative PVT.

Conflict of Interest I have no conflict of interest.

T-15-03 FXIII – slow recovery after liver transplant surgery?

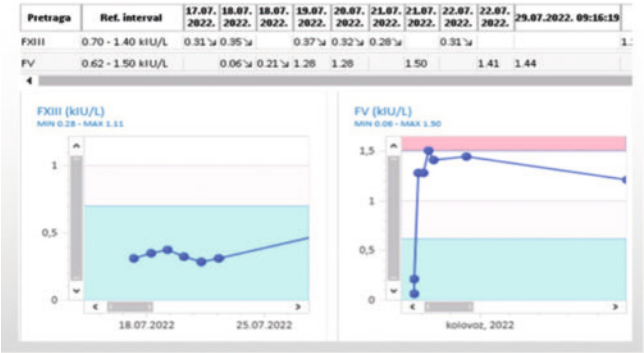
Authors T. Tomić Mahečić[1], I. Hanžek[1], M. Tomičić Nikić[1], L. P. Divjak[1], D. Coen Herak[2], M. Miloš[2]
Institutes **1** UHC Zagreb, Department for anesthesiology and Intensive care medicine, Zagreb, Croatia; **2** UHC Zagreb, Department of Laboratory Diagnostics, Zagreb, Croatia
DOI 10.1055/s-0044-1779206

Introduction The impaired production and disbalance of clotting factors are present in patients with end-stage liver disease (ESLD), candidates for liver transplant (LT) surgery, which lead to perioperative complications, either bleeding or hypercoagulability with a risk of thrombosis. Close monitoring with the standard coagulation tests (SCT) (PT, aPTT, Fibrinogen, INR) is not enough for the majority of patients. These SCTs may not detect factors affecting firm clot formation after initial thrombin generation, or factors contributing to clot strength. The viscoelastic coagulation point of care (POC) devices such as TEM

or TEG, despite allowing us individual approaches for treating our patients still have some „blind spots“ like the functional values of FXIII. In selective cases with continuous bleeding, despite normal SLT measured, we found, especially postoperatively low values of FXIII persisting for more days than anticipated. This can also influence prolonged recovery, longer intensive care unit stays, additional transfusion therapy, and possible infections.

Method In the postoperative period after LT surgery, we measure not only SLT, but we perform viscoelastic POC coagulation tests. We also measure the specific factors' activity, especially for patients with coagulopathy or unexplainable bleeding. This allows us a clearer picture of any coagulation abnormality and treatment needed.

Results We compare the recovery of FV (surrogate of liver function) with FXIII after liver transplant surgery measuring both factors on a daily basis. We realize that there is a relevant difference as FV produced from the new liver reaches normal values 36 to 48 hours after surgery on average. For FXIII we found that this period is prolonged to 5 days in selective patients (▶ **Fig. 1**).



▶ **Fig. 1** Dynamics of factor V and XIII in postoperative period after LT

Conclusion According to available data in the literature, acceptable levels of FXIII for adequate clotting is either 30 or ≥ 60 % (still debatable) and the same values were found as the cut-off level in different guidelines for the correction of bleeding patients. We recommend measuring FXIII activity at different time points after LT surgery. Our goal level in this sensitive period is to reach more than 60 % of activity. More patients are required to strengthen the quality and clinical importance of our results.

Conflict of Interest None

T-15-04 Accuracy of DOAC Dipstick urine test from patients treated peri-operatively with DOACs compared to UHPLC-MS/MS

Authors J. Vassart[1], A.-Y. Boucher[1], L. Morimont[1, 2], A. Tafur[3], J. Walenga[4], J. Harenberg[5, 6], J. Douxfils[1, 2]

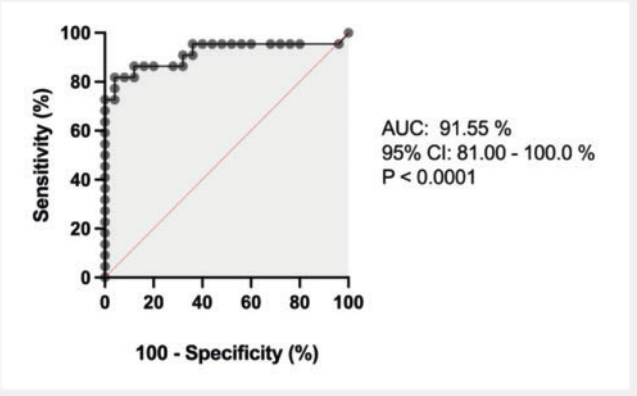
Institutes **1** University of Namur, Clinical Pharmacology Research Group, Department of Pharmacy, Faculty of Medicine, Namur Research Institute for Life Sciences (NARILIS), Namur, Belgium; **2** QUALIblood s.a., Research and Development Department, Namur, Belgium; **3** NorthShore University Health System, Department of Medicine, Evanston, USA; **4** Loyola University Chicago, Cardiovascular Research Institute, Stritch School of Medicine, Health Sciences Division, Maywood, USA; **5** Heidelberg University, Medical Faculty Mannheim, Heidelberg, Germany; **6** DOASENSE GmbH, Heidelberg, Germany; **7** CHU Clermont-Ferrand, Hôpital Estaing, Department of Biological Hematology, Clermont-Ferrand, France

DOI 10.1055/s-0044-1779207

Introduction In the perioperative context, there is an unmet need for a rapid and accurate method to exclude clinically significant levels of DOACs in plasma. The DOAC Dipstick is a test strip designed to detect qualitatively direct oral factor Xa (FXA) or direct oral factor IIa (FIIa) inhibitors in urine.

Method Citrated plasma and urine were collected from 47 patients treated with apixaban (n = 33) and rivaroxaban (n = 14) who interrupted their treatment 1 day before a low-bleeding-risk and 3 days before a high-bleeding-risk scheduled surgical procedure. Plasma and urine concentrations of DOACs were quantified by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS). These results were compared to the DOAC Dipstick results with plasma cut-offs set at ≥ 30 ng/mL and ≥ 50 ng/mL. FXA pads of DOAC Dipstick were evaluated visually as positive or negative (DOACs present or absent) [1].

Results The relation between plasma and urine concentrations of DOACs measured by UHPLC-MS/MS was high and depicted by the following equation: $y = 16.80x - 21.38$ ($R^2 = 0.83$, $r = 0.91$). Mean drug concentrations of paired plasma samples of positive pads were 48.2 ng/mL [27.6–68.7] (95 % confidence interval), and 6.1 ng/mL [3.8–8.4] for negative pads. At the cut-off plasma value of ≥ 30 ng/mL, the DOAC Dipstick exhibited a sensitivity of 100.0 % [73.5–100.0], a specificity of 71.4 % [53.7–85.4], a positive predictive value (PPV) of 38.2 % [26.8–51.1], a negative predictive value (NPV) of 100.0 % [86.3–100.0] and an accuracy of 75.7 % [61.0–87.0]. At ≥ 50 ng/mL threshold, the values were: sensitivity 100.0 % [63.1–100.0], specificity 64.1 % [47.2–78.8], PPV 7.9 % [5.4–11.6], NPV 100.0 % [86.3–100.0] and accuracy 65.2 % [49.9–78.5]. The area under the receiver operating characteristic curve was 0.92 [0.81–1.0] (▶ **Fig. 1**).



▶ **Fig. 1** **Receiver operating characteristic (ROC) curve**; depicting the comparison between DOAC Dipstick results in urine and DOACs determination by UHPLC-MS/MS in plasma. AUC: area under the curve 95 % CI: 95 % confidence interval P: P value

Conclusion The DOAC Dipstick is a simple, rapid method of delivering DOACs results using patient urine. Advantages of the method are that it does not require knowledge of the specific DOAC taken by the patient, is a point-of-care format, and has a rapid turnaround time, thereby enabling use even in small facilities such as community hospitals. This test excludes clinically relevant blood concentrations of DOACs.

Conflict of Interest AT conducted the clinical trial at NorthShore University Medical Center; JHis the general manager and founder of the company producing the DOAC Dipstick (Doasense GmbH, Heidelberg, Germany); JD is CEO and founder of QUALIblood s.a. and reports personal fees and honorarium from Daiichi-Sankyo, Diagnostica Stago, DOASense, Gedeon Richter, Mithra Pharmaceuticals, Norgine, Portola, Roche and Roche Diagnostics. The other authors do not have to report conflict of interest.

References
[1] Merrelaar A.E., Bogl M.S., Buchtele N., Merrelaar M., Herkner H., Schoergenhofer C., Harenberg J., Douxfils J., Siriez R., Jilma B., Spiel A.O. ,Schwameis-Lastname, M. ,2022, 'Performance of a Qualitative Point-of-Care Strip Test to Detect DOAC Exposure at the Emergency Department: A Cohort-Type Cross-Sectional Diagnostic Accuracy Study'. Thrombosis and haemostasis 2022; 122 (10): 1723–1731

T-16. Haemostasis in women

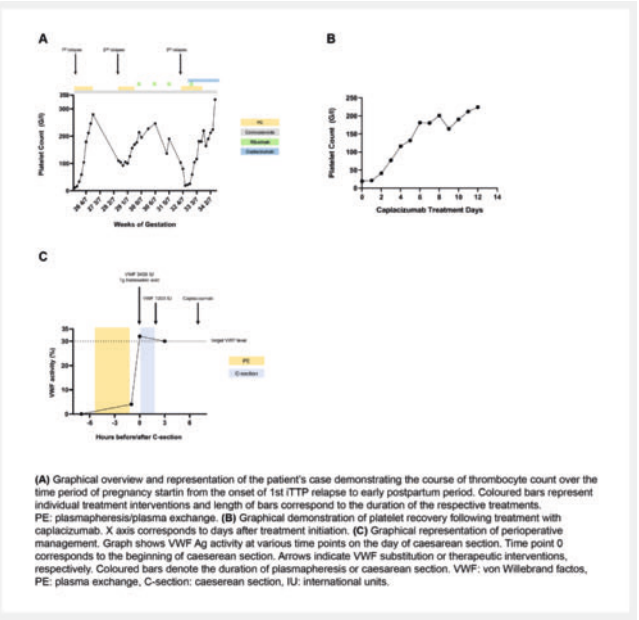
T-16-01 Successful management of refractory immune-mediated thrombotic thrombocytopenic purpura during pregnancy and delivery using the anti-VWF nanobody caplacizumab

Authors R. Schimmer[1], T. Sutter[1], A. Bachofner[1], E. Ranieri[2], A.-K. Rodewald[3], N. Kimmich[2], A. Trinchero[1], J. A. Kremer-Hovinga[4], J.-D. Studt[1]
Institutes **1** University of Zurich and University Hospital Zurich, Department of Medical Oncology and Haematology, Zurich, Switzerland; **2** University of Zurich and University Hospital Zurich, Department of Obstetrics, Zurich, Switzerland; **3** University of Zurich and University Hospital Zurich, Department of Pathology and Molecular Pathology, Zurich, Switzerland; **4** Inselspital, Bern University Hospital, Department of Haematology and Laboratory Haematology, Bern, Switzerland
DOI 10.1055/s-0044-1779208

Introduction Pregnancy is a well-known trigger of acute thrombotic thrombocytopenic purpura (TTP) [1]. Management of pregnancy-associated immune TTP (iTTP) is challenging, especially when it is refractory to standard treatment [2]. Caplacizumab, an anti-VWF nanobody, is a valuable new therapeutic option but is not approved in pregnancy and breastfeeding. We report the successful off-label administration of caplacizumab during pregnancy and delivery in a patient with refractory iTTP.

Method We report a 27-year old woman with iTTP with severe ADAMTS13 deficiency and a high-titre ADAMTS13-inhibitory autoantibody. iTTP relapsed during her advanced pregnancy and eventually was refractory to standard treatment including plasma exchange (PE), glucocorticoids and rituximab. Additional caplacizumab was therefore administered and was maintained throughout the cesarean section. In order to prevent hemorrhage, VWF was substituted before and during delivery tailored to maintain a VWF activity of 30 %.

Results The patient presented with acute recurrent iTTP in her 25th week of gestation. Standard treatment including daily PE, high-dose glucocorticoids, and anti-CD20 therapy with rituximab achieved only transient clinical remission while severe ADAMTS13 deficiency and ADAMTS13-inhibitor persisted (▶ **Fig. 1a**). In her 32nd week of gestation she experienced her third iTTP relapse with persistent thrombocytopenia < 20 G/l. Additional onset of uterine contractions, signs of pre-eclampsia and deterioration of foetal growth required urgent delivery. After the patient's informed consent, additional daily off label-therapy with caplacizumab was initiated and resulted in a rapid increase of the platelet count within 2 days (▶ **Fig. 1b**). To allow for a safe caesarean section despite a severe caplacizumab-induced functional defect of VWF, a tailored approach was planned: on the day of section PE was performed immediately before delivery and caplacizumab was withheld. VWF substitution pre- and post-section was tailored to restore a VWF activity of 30 % (▶ **Fig 1c**). Thereby, blood loss was limited to 700 ml, and no thrombotic or bleeding complications occurred in both mother and child. Daily caplacizumab was continued postpartum, PE was discontinued when the platelet count normalized and glucocorticoids were tapered. However, severe ADAMTS13 deficiency and ADAMTS13 inhibitor persisted.



(A) Graphical overview and representation of the patient's case demonstrating the course of thrombocyte count over the time period of pregnancy startin from the onset of 1st iTTP relapse to early postpartum period. Coloured bars represent individual treatment interventions and length of bars correspond to the duration of the respective treatments. PE: plasmapheresis/plasma exchange. **(B)** Graphical demonstration of platelet recovery following treatment with caplacizumab. X axis corresponds to days after treatment initiation. **(C)** Graphical representation of perioperative management. Graph shows VWF Ag activity at various time points on the day of caesarean section. Time point 0 corresponds to the beginning of caeserean section. Arrows indicate VWF substitution or therapeutic interventions, respectively. Coloured bars denote the duration of plasmapheresis or caesarean section. VWF: von Willebrand factos, PE: plasma exchange, C-section: caeserean section, IU: international units.

▶ **Fig. 1** Safe and effective use of caplacizumab of refractory iTTP during pregnancy and delivery

Conclusion The observed favourable outcome without significant thrombotic or hemorrhagic complications indicates that caplacizumab could be an effective and safe treatment option in pregnant patients with refractory iTTP. Additionally, our observation suggests that caplacizumab may be a treatment option in pregnant patients with refractory iTTP requiring urgent delivery when combined with a VWF substitution which is precisely tailored and closely monitored.

Conflict of Interest The authors have no competing interests.

References
[1] Tzur-Tseva A., Czuzoj-Shulman N., Abenhaim H.A. Thrombotic Thrombocytopenic Purpura and Pregnancy Outcomes: A Cohort Study [A258]. Obstetrics Gynecol 2022; 139: 74S–75S
[2] Scully M. et al. Thrombotic thrombocytopenic purpura and pregnancy: presentation, management, and subsequent pregnancy outcomes. Blood 2014; 124: 211–219

T-16-02 First diagnosed inherited TTP combined with inherited thrombophilia in a pregnant woman

Authors O. Kostenchak-Svystak[1, 2], I. Pabinger[3], O. Puzii[4]
Institutes **1** Uzhhorod national university, physiology and pathophysiology, Uzhhorod, Ukraine; **2** Saint Martin Hospital, Department of Hematology and Oncology, Mukachevo, Ukraine; **3** Medical University of Vienna, Department of Medicine I, Clinical Division of Haematology and Haemostaseology, Vienna, Austria; **4** Dnipropetrovsk Regional Perinatal center, Dnipropetrovsk, Ukraine
DOI 10.1055/s-0044-1779209

Introduction Thrombotic thrombocytopenic purpura (TTP) is a rare, life-threatening thrombotic disorder marked by microvascular platelet clumping, leading to low platelet count, hemolytic anemia, and various systemic complications. TTP can be either acquired (immune) or congenital (hereditary, also known as Upshaw-Schulman syndrome), the latter arising from mutations in the ADAMTS13 gene. Deficient ADAMTS13 activity results in abnormal von Willebrand factor (vWF) multimers, leading to platelet thrombus formation and TTP symptoms [1, 2].

Hämostaseologie 2024; 44: S1–S125

	HELLP syndrome	AFLP	TTP	HUS	Exacerbation of SLE
HTN	+	+-	+-	+	+
Proteinuria	+	+-	-	+	+
Fever	+ (absent)	+-	+-	NR	-
Jaundice	+ (absent)	-	+- (rare)	+ -(rare)	+ (absent)
Nausea and vomiting	+-	-	-	-	-
Abdominal pain	-	+-	-	-	-
Central nervous system	+-	+-	-	NR	-
Thrombocytopenia (less than 100,000/mm^3)	More than 20,000 +	More than 50,000 -	20,000 or less ++	More than 20,000 +	More than 50,000 -
Hemolysis	NR	NR	NR	NR	NR
Anemia	+- (less than ½ of patients)	-	+	+	+-- (1/3 of patients)
DIC	NR	NR	NR	NR	NR
Hypoglycemia	NR	NR	NR	NR	NR
VW factor multimers	NR	NR	NR	NR	NR
ADAMTS13 less than 5%	-	-	+	+ -(rare)	+ -(rare)
Impaired renal function	-	-	-	-	-
LDH	- 600 or more	+- Variable	- More than 1000	- More than 1000	+- May be elevated
Elevated ammonia	NR	NR	NR	NR	NR
Elevated bilirubin	+- ⅓ of patients	+	+	NA	+--- Less than 10%
Elevated transaminases	+	+	+- Usually mild	+- Usually mild	+

▶ **Fig. 1 Differential diagnosis;** We marked "+"- if the symptom or laboratory marker is common for diagnosis, and "-" if it is uncommon. We marked both symbols if the presentation of pathology could have or could not have these parameters. NR – not reported

Method

Case Report

A 26-year-old woman, previously diagnosed with a heterozygous factor II mutation (Prothrombin G20210A) and negative for antiphospholipid syndrome (APS), experienced severe preeclampsia during her first pregnancy in 2021 at seven months gestation. Prophylactic anticoagulation with low molecular weight heparin (LMWH) was initiated upon confirmation of her pregnancy, concerning the risk of thrombosis. We reviewed her anamnesis during her pregnancy, she exhibited no risk factors for thrombosis, maintained a normal BMI, and had no personal or family history of thrombosis or hematological disorders. At 32 weeks, she developed severe anemia, thrombocytopenia (28,000/µL), proteinuria, elevated D-dimers, hyperhomocysteinemia, and hypertension. Among other differential diagnoses (HELLP, HUS, AFLP, SLE (▶ **Fig. 1**)), TTP was suspected and ADAMTS13 activity was measured, revealing levels below 5 %. Urgent delivery was conducted at 32 weeks of gestation, followed by plasma infusion and corticosteroids. Her baby didn't have any complications and was healthy. Four months postpartum, her ADAMTS13 level was 4.7 %, and dropped to 2.8 % in September 2023 year, despite the inhibitor level being within the reference range. Presently, she feels well, apart from anxiety regarding her results and occasional fatigue. Physical examination, laboratory parameters, and vital signs show no abnormalities, and she is not on any medication.

Results

Discussion

This case underscores the challenge of diagnosis and managing congenital TTP, especially during pregnancy. Elevated homocysteine levels and prothrombotic polymorphisms have been associated with TTP, indicating a complex interplay of factors [3]. Pregnancy can lead to exacerbation of TTP manifestations, with recurring complications observed, LMWH is not effective in preventing TTP exacerbation [4]. Limited data on pregnancy outcomes in congenital TTP patients necessitate further research [5–7].

Conclusion This case highlights the complexities of diagnosing and managing congenital TTP during pregnancy. Ongoing research, precise diagnostics, and tailored interventions are crucial. Continued monitoring and genetic analysis are essential, with samples sent to The Hereditary Thrombotic Thrombocytopenic Purpura Registry for further analysis. Documenting cases and advancing research remain pivotal for enhancing TTP management and patient outcomes.

Conflict of Interest There is no conflict of interests

References

[1] Kremer Hovinga JA, George JN. Hereditary Thrombotic Thrombocytopenic Purpura. N Engl J Med 2019; 381 (17): 1653–1662
[2] Joly BS, Coppo P, Veyradier A. Thrombotic thrombocytopenic purpura. Blood. 2017; 129 (21): 2836–2846
[3] Trisolini SM, Capria S, Cafolla A et al. Inherited thrombophilia in patients with thrombotic thrombocytopenic purpura. Thromb Res 2009; 124 (4): 512–513
[4] Kasht R, Borogovac A, George JN. Frequency and severity of pregnancy complications in women with hereditary thrombotic thrombocytopenic purpura. Am J Hematol 2020; 95 (11): E316–E318
[5] Pérez-Rodríguez A, Lourés E, Rodríguez-Trillo Á et al. Inherited ADAMTS13 deficiency (Upshaw-Schulman syndrome): a short review. Thromb Res 2014; 134 (6): 1171–1175
[6] Battinelli EM. TTP and pregnancy. Blood. 2014; 123 (11): 1624–1625
[7] Knöbl P. Inherited and acquired thrombotic thrombocytopenic purpura (TTP) in adults. Semin Thromb Hemost 2014; 40 (4): 493–502

T-16-03 Markers of Coagulation Activation in Pregnant Women from a Thrombosis & Hemostasis Clinic – a First Step Towards "Target Levels"?

Authors S. Marten, L. Tittl, C. Naue, J. Beyer-Westendorf
Institute University Hospital "Carl Gustav Carus" Dresden, Thrombosis Research Unit, Department of Medicine I, Division Hematology, Dresden, Germany

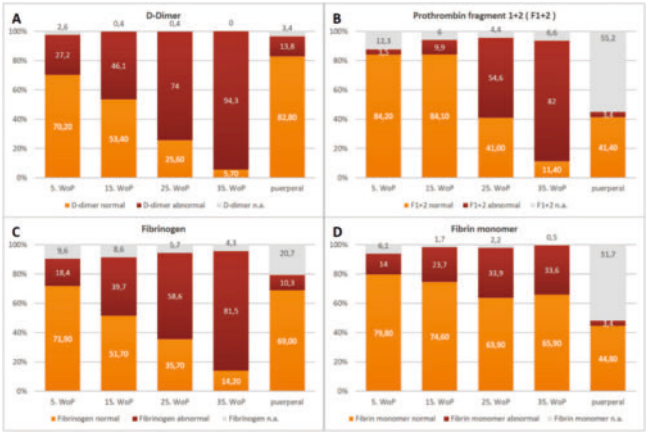
DOI 10.1055/s-0044-1779210

Introduction During pregnancy, a physiological shift of prothrombotic and antithrombotic factors is preparing the mother-to-be for delivery with the intention to limit blood loss. Among many other well-documented changes, d-dimer and fibrinogen rise. However, pathologic pattern changes can lead to hypercoagulable states, followed by clinical complications that can include venous thromboembolism, placentar thrombosis, gestosis or miscarriage. We prospectively analyzed coagulation parameters in a cohort of women with known coagulation abnormalities who were counselled during pregnancy in a Thrombosis & Hemostasis Clinic. In this ongoing project, we aim to establish correlations of these biomarkers of coagulation activation to clinical outcomes and to evaluate the impact from heparin interventions. As a first step, we try to establish overall patterns of changes, which we report here.

Method Pregnant women with either venous or arterial thromboembolism, thrombophilia, recurrent miscarriage or a strong family history for thrombo-

embolism are routinely seen in our Thrombosis & Hemostasis Clinic and levels of d-dimer (DD), fibrinogen (FIB), prothrombin fragments (F1 + 2) and fibrin monomers (FM) are collected as part of our routine at consultations in pregnancy weeks 5-10; 15-18; 25 and 35, with some patients also undergoing a clinical visit at 6-8 weeks post partum.

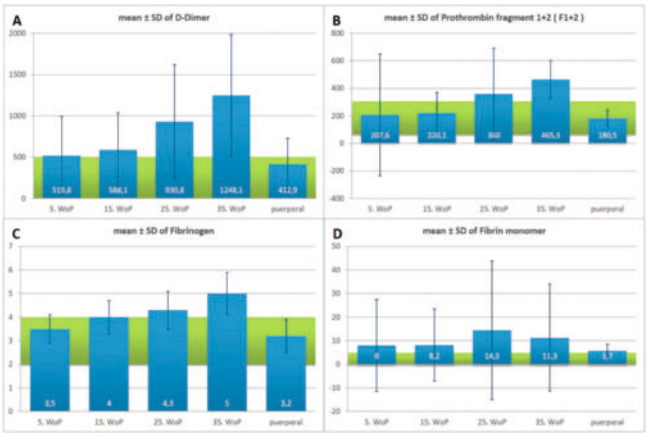
Results Between September 2018–September 2023, 300 pregnancies with at least 1 blood sample and a full set of all 4 parameters were identified. Clinical abnormalities at referral/baseline included a history of VTE in 51.3 %, stroke/TIA in 2.0 % and recurrent miscarriage in 13.0 %. The most common thrombophilias at baseline were factor V Leiden mutation (24.1 %; 67 heterozygous, 1 homozygous), anti-phospholipid antibody syndrome (11.3 %), heterozygous prothrombin mutation (6.7 %), protein C deficiency (2.1 %) and protein S deficiency (1.4 %).As indicated in ▶ **Fig. 1**, all 4 parameters of interest demonstrated significant changes throughout pregnancy. In early pregnancy, at least 80 % of samples showed normal values for f 1 + 2 and FM, whereas only 70 % of samples showed normal values of DD and FIB, both of which also showed the fastest and most constant increase during progression of pregnancy (▶ **Fig. 2**). In contrast, F1 + 2 and FM did not increase in the first trimester, but F1 + 2 considerably increased from the second to third trimester, whereas FM demonstrated peak values in the second trimester and declined again towards the end of pregnancy. All 4 parameters rapidly normalized post partum, with DD remaining elevated in approximately 13 %.



▶ **Fig. 1** Proportions of normal and abnormal D-dimer (A), Prothrombin fragment 1 + 2 (B), Fibrinogen (C) and Fibrin monomer (D) in pregnant patients according to weeks of pregnancy. Ranges of normal are DD < 501 ng/mL; F1 + 2: 63-307 pmol/l; FIB 2-4 g/L; FM: < 5 µg/mL

Conclusion Coagulation parameters, including DD, FIB, F1 + 2 and FM, show differential patters of changes throughout pregnancy of women with increased thrombotic risk. If these differential patterns can be used to predict complications and unfavourable pregnancy outcomes or to guide pharmacoprophylaxis remains to be elucidated in our next analyses of a larger cohort.

Conflict of Interest J.B.-W.: honoraria and research support from Bayer, Boehringer Ingelheim, Daiichi Sankyo, Pfizer, Alexion, Norgine, DOASENSE and Sanofi. L.T.: honoraria and travel support from Daiichi Sankyo and Bayer. S.M.: honoraria from Daiichi Sankyo and Bayer. C.N.: no conflict of interest.



▶ **Fig. 2** Distribution (mean ± SD) of D-dimer (A), Prothrombin fragment 1 + 2 (B), Fibrinogen (C) and Fibrin monomer (D) in pregnant patients according to weeks of pregnancy. Green areas present upper and lower limits of normal

T-16-04 Priming of maternal platelets during pregnancy – the role of pregnancy specific beta-1-glycoprotein 11 in platelet activation

Authors D. Forstner[1], L. Nefischer[1], F. Lyssy[1], J. Guettler[1], C. Daxboeck[1], S. Wernitznig[1], N. Kupper[1], K. Ujčič[1], B. A. Brugger[1], L. Neuper[1], J. Krappinger[1], J. Feichtinger[1], G. Cvirn[2], Z. Mihalic[3], J. Kargl[3], M. Gauster[1]

Institutes **1** Medical University of Graz, Division of Cell Biology, Histology and Embryology, Gottfried Schatz Research Center, Graz, Austria; **2** Medical University of Graz, Division of Medicinal Chemistry, Otto Loewi Research Center, Graz, Austria; **3** Medical University of Graz, Division of Pharmacology, Otto Loewi Research Center, Graz, Austria

DOI 10.1055/s-0044-1779211

Introduction Pro- and anticoagulant mechanisms play an important role during gestation to guarantee a successful implantation of the blastocyst, but also when it comes to term to avoid haemorrhage [1, 2]. Pregnancy is a state of hypercoagulation and platelets underlie certain dynamic changes during pregnancy, like a decrease of the platelet count towards the end of pregnancy [3]. Previous studies revealed that adherence of maternal platelets to the placental villous surface is a common process even in very early stages of gestation, and therefore a tightly regulated cross talk between platelets and the placenta seems to be of high importance [4].

Pregnancy-specific glycoproteins (PSGs) are the most abundant trophoblast-derived proteins in the maternal blood during human pregnancy and several studies indicate that PSGs play a critical role in the regulation of the immune response and platelet activation [5]. Here, we test the hypothesis whether the pregnancy-specific glycoprotein 11 (PSG11) is selectively taken up by platelets and whether platelet priming is a crucial process during pregnancy.

Method Platelets were isolated from whole blood samples of either healthy pregnant women in the first and third trimester, pregnant women suffering from preeclampsia or healthy non-pregnant women. Afterwards, magnetic bead-based purified platelet fractions were subjected to proteomics analysis as well as to RNA Sequencing in order to elucidate dynamic changes of the platelet proteome and transcriptome over the course of gestation and in case

of preeclampsia. Furthermore, isolated platelets from non-pregnant women were incubated with plasma from healthy pregnant women or with recombinant PSG11 and subsequently analysed on protein level, via electron microscopy or via impedance aggregometry.

Results Our proteomics data showed an abundance of PSG11 in platelets from pregnant women and an accumulation over the course of pregnancy. Furthermore, we could detect PSG11 in platelets from healthy non-pregnant women after incubation with plasma from pregnant women. Interestingly, pre-incubation of isolated platelets with PSG11 hampered the Collagen Type I induced platelet aggregation nearly by 50 %compared to controls, whereas the Thrombin Receptor Activator Peptide-6 (TRAP6) induced platelet aggregation was slightly increased.

Conclusion Our data suggests that platelets sequester increased concentrations of PSG11 over gestation, which tempts us to speculate that platelet priming by placenta-derived factors is a common process to adapt maternal platelets to the haemostatic challenges in pregnancy.

Conflict of Interest the authors declare no conflict of interest

References

[1] Martin JF, Wagner GP. The origin of platelets enabled the evolution of eutherian placentation. Biol Lett 2019; 15 (7): 20190374

[2] Moser G, Guettler J, Forstner D, Gauster M. Maternal Platelets—Friend or Foe of the Human Placenta? Int J Mol Sci 2019; 20 (22):

[3] Reese JA, Peck JD, Deschamps DR, McIntosh JJ, Knudtson EJ, Terrell DR et al. Platelet Counts during Pregnancy. N Engl J Med 2018; 379 (1): 32–43

[4] Forstner D, Maninger S, Nonn O, Guettler J, Moser G, Leitinger G et al. Platelet-derived factors impair placental chorionic gonadotropin beta-subunit synthesis. J Mol Med Berl Ger 2020; 98 (2): 193–207

[5] Moore T, Dveksler GS. Pregnancy-specific glycoproteins: complex gene families regulating maternal-fetal interactions. Int J Dev Biol 2014; 58 (2/4): 273–80

T-16-05 Exploratory analysis of thrombophilic risk markers in histology-defined placental thromboembolism.

Authors E. Hertrampf, F. Gatzke, S. Roth, S. Rauchfuß, W. Miesbach

Institute University Hospital Frankfurt, Department of Medicine II, Division of Hemostaseology, Frankfurt am Main, Germany

DOI 10.1055/s-0044-1779212

Introduction Recurrent pregnancy loss (RPL) is defined as 3 miscarriages before 20 weeks of gestation and affects about 3-5 % of all women at reproductive age. Etiology is multifaceted including age, anatomical aberrations and infectious disease.

Thromboembolism of the placental vasculature is also considered as an etiological factor for RPL. Whereas RPL was indeed found to be associated with antiphospholipid syndrome, no other thrombophilic risk markers were reliably associated with abortions. Moreover, the use of heparin or acetylsalicylic acid wasn't found to increase the rate of live births questioning the association of RPL with thrombophilic risk. Prevalence data of placental thromboembolisms in the setting of RPL are scarce. Histological examination of placenta tissue following curettage after an abortion might represent an attractive strategy to confirm placental thromboembolism as the etiology of the observed RPL and inform treatment.

Method 1400 female patients aged 18 to 45 years that presented to the department of hemostaseology at Goethe University Frankfurt between 2012 and 2022 for assessment of thrombophilic risk markers were screened by retrospective chart review. All patients gave informed consent. The screening process allowed for the identification of 13 patients with histologically confirmed placental thromboembolism (PTE) and 29 patients with unrevealing placental histology (UPH).

Results 13 out of 42 (30 %) patients with recurring pregnancy loss had histology findings consistent with placental thromboembolism.

When comparing PTE patients with UPH patients, there were no obvious differences regarding established risk factors for recurrent pregnancy loss such as age or abnormal anatomical findings. Restrictively, it must be mentioned that a complete risk profile could only be retrieved for a minor fraction of all RPL patients from retrospective analysis.

No significant differences were observed for thrombophilic risk markers as well as s/p DVT between PTE patients and UPH patients.

Conclusion To our knowledge, this is the first study to estimate the prevalence of placental thromboembolism as a cause for RPL based on histology and provide an association with risk markers for thrombophilia. PTE was found in 30 % of histological specimens arguing for an important role in the etiology of RPL. However, no profound association of established thrombophilic risk factors with histology-defined placental thromboembolism was observed which might help to explain discrepant results reported so far [1–5].

The low amount of histopathological examinations of abort material is the major limitation of the study. A prospective study design to ensure adequate accrual will determine the generalizability and scientific value of the results presented here.

Further analyses will be necessary to determine the predictive value of histologically-defined placental thromboembolism for a successful preganacy and as a stratification criterion to select patients that could benefit from anticoagulation.

Conflict of Interest The authors declare no conflict of interest.

References

[1] Katzmann PJ, Genest DR Maternal floor infarction and massive perivillous fibrin deposition: histological definitions, association with intrauterine fetal growth restriction, and risk of recurrence. Pediatr Dev Pathol 2002; 5: 159–164

[2] Odendaal HJ. Strong Association Between Placental Pathology and Second-trimester Miscarriage. Arch Obstet Gynaecol 2021; 2 (3): 51–56

[3] Rodger MA, Langlois NJ. Is thrombophilia associated with placenta-mediated pregnancy complications? A prospective cohort study: reply. J Thromb Haemost 2014; 12 (8): 1378–9

[4] Bradley LA, Palomaki GE, Bienstock J, Varga E, Scott JA. Can Factor V Leiden and prothrombin G20210A testing in women with recurrent pregnancy loss result in improved pregnancy outcomes?: Results from a targeted evidence-based review. Genet Med 2012; 14 (1): 39–50

[5] de Jong PG, Kaandorp S, Di Nisio M, Goddijn M, Middeldorp S. Aspirin and/or heparin for women with unexplained recurrent miscarriage with or without inherited thrombophilia. Cochrane Database Syst Rev 2014; 2014 (7): CD004734. doi:10.1002/14651858.CD004734.pub4.PMID: 24995856; PMCID: PMC6769058.

T-16-06 Maternal platelet activation at the maternal-fetal interface affects placental mitochondrial and endocrine activity

Authors N. Kupper[1], J. Guettler[1], D. Forstner[1], B. A. Brugger[1], F. Lyssy[1], K. Ujčič[1], S. Wernitznig[1], L. Neuper[1], C. Daxboeck[1], G. Cvirn[2], B. Obermayer-Pietsch[3], O. Nonn[4], M. Gauster[1]

Institutes **1** Medical University of Graz, Division of Cell Biology, Histology and Embryology, Gottfried Schatz Research Center, Graz, Austria; **2** Medical University of Graz, Division of Physiological Chemistry, Otto Loewi Research Center, Graz, Austria; **3** Medical University of GrazEndoc, Division of Endocrinology and Diabetology, Department of Internal Medicine, Graz, Austria; **4** Charité University Medicine, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt, Berlin, Germany

DOI 10.1055/s-0044-1779213

Introduction As a highly functional organ, the placenta shapes the environment in which the fetus develops, in part through the secretion of autocrine and endocrine factors at several stages of gestation [1, 2]. Its key role in the synthesis of steroid hormones during pregnancy is essential for a healthy out-

come [3]. In early pregnancy, the placenta is not yet perfused by maternal blood because the spiral arteries, veins, and glands are plugged by trophoblast cells. However, these plugs are loosely cohesive, allowing plasma and small particles to pass through, resulting in histotrophic feeding of the fetus [4–6]. Our previous results suggest that maternal platelets are among the small-sized particles that cross these plugs and can adhere to the syncytiotrophoblast surface of the placenta from early gestational stages onwards [2, 7]. In this study, we investigate the effects of maternal platelets on trophoblast physiology with emphasis on modulation of placental steroid hormone synthesis.

Method Differentiated BEWO cells were co-incubated with platelets and or platelet-released factors from healthy donors. The trophoblast transcriptome was analyzed with RNA-sequencing. Protein and mRNA analysis were performed with Western Blot and qPCR respectively. Endocrine analysis of the supernatant was conducted by clinical routine assays. Seahorse analysis was performed to measure mitochondrial respiration and glycolysis of live cells. Steroid sulfatase (STS) was overexpressed in BEWO cells to decipher its effect on the steroid hormone synthesis in trophoblasts upon platelet treatment.

Results RNA sequencing analysis revealed 1462 significantly deregulated genes in BeWo cells upon co-incubation with platelets. Genes involved in the steroid hormone synthesis were significantly modified compared to control conditions. Also, in the supernatant of the cells a significant lower progesterone level was detected after co-incubation. Treatment of trophoblasts with platelet-released factors altered the mitochondrial membrane potential and oxidative phosphorylation activity. Additionally, STS overexpression affected steroid hormone synthesis of trophoblasts.

Conclusion Activation of maternal platelets at the feto-maternal interface alters the trophoblast transcriptome, driving various immunomodulatory responses. In addition, our data suggest deregulatory effects of activated platelets on the placental mitochondrial activity and steroid hormone synthesis, as is known to be affected in pregnancy-related diseases [8]. Thus, maternal platelets appear to have a major impact on the placental steroid hormone synthesis already from early stages of pregnancy. However, further studies are needed to determine whether maternal platelets and their cargo change in pathological pregnancy and whether they contribute to the development of a high-risk pregnancy.

Conflict of Interest The authors declare no conflict of interest.

References

[1] Gude N.M., Roberts C.T., Kalionis B., King R.G. Growth and function of the normal human placenta. Thrombosis Research 2004; 114 (5/6): 397–407. doi:10.1016/j.thromres.2004.06.038

[2] Forstner D. et al. Platelet-derived factors impair placental chorionic gonadotropin beta-subunit synthesis. Journal of Molecular Medicine 2020; 98 (2): 193–207. doi:10.1007/s00109-019-01866-x

[3] Szilagyi A. et al. Placenta-specific genes, their regulation during villous trophoblast differentiation and dysregulation in preterm preeclampsia. International Journal of Molecular Sciences 2020. doi:10.3390/ijms21020628

[4] Weiss G., Sundl M., Glasner A., Huppertz B., Moser G. The trophoblast plug during early pregnancy: a deeper insight. Histochemistry and Cell Biology 2016; 146 (6): 749–756. doi:10.1007/s00418-016-1474-z

[5] Moser G., Gauster M., Orendi K., Glasner A., Theuerkauf R., Huppertz B. Endoglandular trophoblast, an alternative route of trophoblast invasion? Analysis with novel confrontation co-culture models. Human Reproduction 2010; 25 (5): 1127–1136. doi:10.1093/humrep/deq035

[6] Moser G., Windsperger K., Pollheimer J., de Sousa Lopes S.C., Huppertz B. Human trophoblast invasion: new and unexpected routes and functions. Histochemistry and Cell Biology 2018; 150 (4): 361–370. doi:10.1007/s00418-018-1699-0

[7] Guettler J. et al. Maternal platelets pass interstices of trophoblast columns and are not activated by HLA-G in early human pregnancy. Journal of Reproductive Immunology 2021; 144: October 2020), 103280. doi:10.1016/j.jri.2021.103280.

[8] Chatuphonprasert W., Jarukamjorn K., Ellinger I. Physiology and pathophysiology of steroid biosynthesis, transport and metabolism in the human placenta. Frontiers in Pharmacology 2018. doi:10.3389/fphar.2018.01027

T-16-07 Comparison of two automated thrombin generation methods, ST Genesia® and Ceveron® alpha, during pregnancy with inherited thrombophilia.

Authors J. Luterán[1], J. Feriel[2], U. Scholz[1]

Institutes **1** MVZ Labor Dr. Reising-Ackermann und Kollegen, Specialist Haemostasis Unit, Leipzig, Germany; **2** Diagnostica Stago S.A.S., Asnières-sur-Seine, France

DOI 10.1055/s-0044-1779214

Introduction During normal pregnancy, the hemostatic balance shifts toward hypercoagulability to minimize risk of bleeding during birth. The thrombin generation assay (TG assay) captures the entire functional coagulation status of plasma and the dynamic processes of thrombin formation. This allows the assessment of the balance between procoagulant and anticoagulant factors. Therefore, TG assays are considered as global coagulation tests and enable more sensitive and comprehensive detection of coagulation deficiencies in vivo [1].

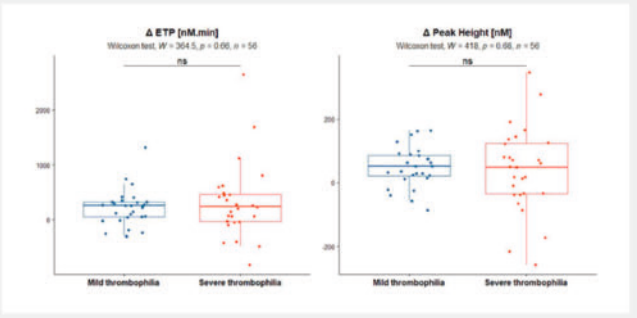
For the first time, both automated TG methods ST Genesia® and Ceveron® were used in a study and the insights gained regarding the TG parameters in pregnant women with inherited thrombophilia were compared.

Method Platelet-poor citrated plasma samples of 56 pregnant women with thrombophilia were assessed with Ceveron® TGA RC Low and with ST Genesia® ThromboScreen assay.

To analyze thrombin generation depending on the coagulation defect present, the patient population was divided into 2 groups: mild and severe thrombophilia. The causes of mild thrombophilia include heterozygous prothrombin G20210A mutation (n = 6) and heterozygous FV Leiden mutation (n = 22). The group of severe thrombophilia includes various hemostaseological disorders, such as protein C or S deficiency (n = 10), antithrombin deficiency (n = 5) and antiphospholipid syndrome (n = 2). Almost half of the patients in this group suffer from combined hereditary thrombophilia (n = 11). Due to the severity of thrombophilia the majority of patients (n = 19) were treated with heparin during pregnancy in accordance with guidelines [2].

Thrombin generation was assessed in two different time-points during early (5-15 weeks) and late (30 – 36 weeks) pregnancy. The wilcoxon rank-sum test was used to compare the TG parameters in the different groups.

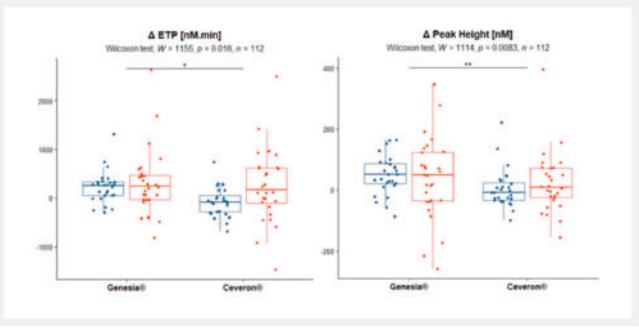
Results During the course of pregnancy there was a significant increase in the TG parameters ETP [nM.min] and Peak Height [nM] in both groups (data not shown). However, it was shown that the changes in ETP and Peak Height were similar in pregnant women with mild thrombophilia compared to those with severe thrombophilia (▶ **Fig. 1**).



▶ **Fig. 1 Comparison of TG parameters evolution in the different groups;** Shown are changes in the TG parameters ETP [nM.min] and Peak Height [nM] during pregnancy for mild and severe thrombophilia. To examine the significance, a Wilcoxon rank-sum test was carried out.

Furthermore, it was investigated whether the device used had an influence on the changes in the TG parameters. It could be determined that Δ ETP and Δ Peak

Height were higher with Genesia® than with Ceveron®, but the changes in ETP between the two groups was more pronounced with Ceveron® (▶ **Fig. 2**).



▶ **Fig. 2 Impact of the analyzer on the evolution of TG parameters;** Shown are changes in the TG parameters ETP [nM.min] and Peak Height [nM] during pregnancy for mild (blue) and severe (red) thrombophilia depending on the used TG analyzer. To examine the significance, a Wilcoxon rank-sum test was carried out.

Conclusion As expected, there was an increase in TG parameters ETP and Peak Height over the course of pregnancy, but the severity of thrombophilia did not impact the evolution of these parameters. Since the majority of patients in the severe thrombophilia group were on heparin therapy, a heparin effect cannot be ruled out with certainty.

Furthermore, the TG analyzer used should be considered when interpreting ETP and Peak Height results, as higher variations for both parameters are observed with the ST Genesia® compared to the Ceveron®.

Conflict of Interest Julia Luterán and Dr. Ute Scholz have no conflicts of interest. Joeffrey Feriel is employed with a company developing in vitro diagnostics including thrombin generation assays.

References

[1] Maughan B.C., Marin M., Han J., Gibbins K.J., Brixey A.G., Caughey A.B., Kline J.A., Jarman A.F. "Venous Thromboembolism During Pregnancy and the Postpartum Period: Risk Factors, Diagnostic Testing, and Treatment.". Obstet Gynecol Surv 2022; 77 (7): 433–444

[2] Hart C., Bauersachs R., Scholz U., Zotz R., Bergmann F., Rott H., Linnemann B. "Prevention of Venous Thromboembolism during Pregnancy and the Puerperium with a Special Focus on Women with Hereditary Thrombophilia or Prior VTE-Position Paper of the Working Group in Women's Health of the Society of Thrombosis and Haemostasis (GTH).". Hamostaseologie 2020; 40 (5): 572–590

T-18. Laboratory issues

T-18-01 Effects of heat treatment on emicizumab and an alternative approach to eliminate interfering FVIII activity prior to functional analysis

Authors N. S. Hamedani[1], A.A.M. T. Donners[2], M. van Luin[2], S. Gasper[1], H. Rühl[1], C. Klein[1], T. Albert[1], M. El Amrani[2], B. Pötzsch[1], J. Oldenburg[1], J. Müller[1]

Institutes 1 University Hospital Bonn, Institute of Experimental Hematology and Transfusion Medicine, Bonn, Germany; 2 University Medical Center Utrecht, Department of Clinical Pharmacy, Utrecht, Netherlands

DOI 10.1055/s-0044-1779215

Introduction Measurement of emicizumab in the presence of FVIII may be required in severe Haemophilia A (HA) patients additionally treated with FVIII or in emicizumab prophylaxis in patients with acquired or moderate to mild HA [1, 2]. However, the presence of FVIII activity (FVIII:C) may lead to falsely elevated functional emicizumab test results [3]. While pre-analytical heat treatment of samples appears to be an obvious measure to annihilate interfering FVIII:C, also partial inactivation of emicizumab has been described under such conditions [3]. Our aim was to address this issue systematically and to evaluate an alternative, FVIII-inhibitor-based approach.

Method Fourteen plasma samples obtained from 12 patients treated with emicizumab and having different intrinsic or substituted levels of FVIII:C (< 1 to 160 IU/dL) were available for analysis [4]. Emicizumab plasma levels were directly assayed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) and by a functional modified FVIII one-stage clotting assay (mOSA) as described elsewhere [4, 5]. Plasma levels of FVIII:C were measured using a chromogenic FVIII assay based on bovine factors (Siemens). For inactivation of FVIII:C prior to functional emicizumab analysis, samples were (i) heat-treated at 56 °C for 40 minutes or (ii) mixed 1 + 1 with Factor VIII Inhibitor Plasma (Technoclone) and incubated for 30 min at room temperature. In order to address the resulting 1:2 dilution of samples, a correspondingly adapted mOSA was applied [4]. All analysis was done using an Atellica Coag 360 analyzer (Siemens).

Results Considering direct quantification of emicizumab by LC-MS/MS as reference, the presence of FVIII:C led to falsely increased emicizumab plasma levels as determined by the functional mOSA (▶ **Fig. 1**). Pre-analytical heat treatment clearly diminished mOSA-based emicizumab plasma levels by -40.7 % ± 6.8 % (mean ± SD, ▶ **Fig. 1**, ▶ **Fig. 2a**). In contrast, the FVIII-neutralization assay not only proved to be efficient to extinguish FVIII:C but also yielded emicizumab plasma levels comparable with that determined by LC-MS/MS (▶ **Fig. 1** and ▶ **Fig. 2b**). Interestingly, further analysis revealed significantly more pronounced heat instability of intrinsic emicizumab (40 % to 50 % reduction [mOSA]) when compared to emicizumab spiked into different plasma matrices (-6 % to -30 %).

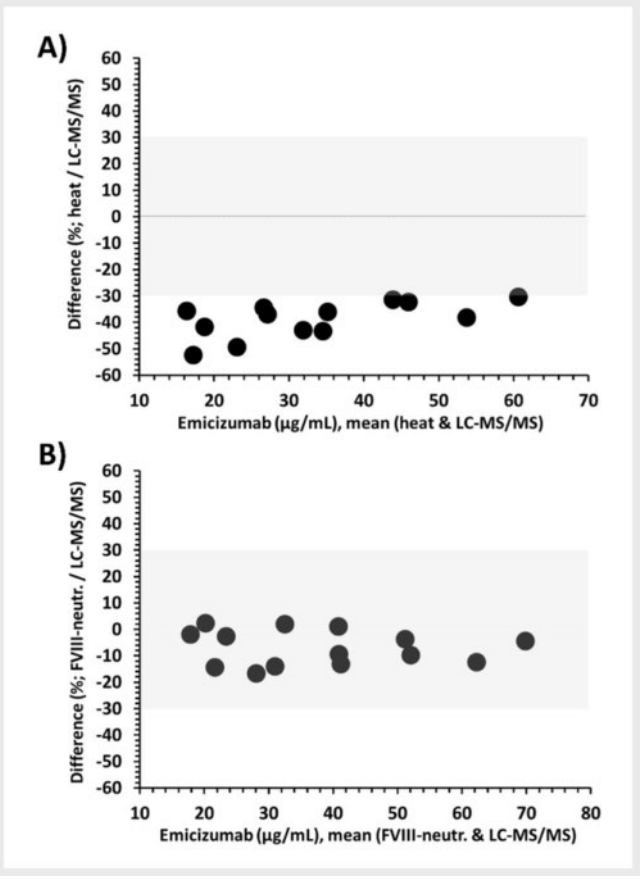
Patient / Sample	Clinic	Drug / intrinsic FVIII	FVIII-inhibitor (BU/mL)	FVIII:C (IU/dL)	Emicizumab (µg/ml)			
			NBA[a]	FVIII CSA[b]	LC-MS/MS	mOSA	HI[c] > mOSA	FVIII-neutr. > adapted mOSA[d]
#1	acquired HA	NovoSeven	366	< [e]	32.2	32.4	21.1	33.5
#2	acquired HA	intrinsic FVIII	3.4	16.6	52.1	56.7	35.7	49.5
#2a		intrinsic FVIII	< [e]	161.7	42.9	67.4	27.4	39.7
#3	acquired HA	intrinsic FVIII	< [e]	5.1	54.8	50.5	37.1	50.8
#4	acquired HA	Haemoctin / intrinsic FVIII	2.0	141.7	18.0	44.0	<9.1	17.9
#5	severe HA	Elocta	N/A[f]	33.4	33.3	40.3	21.0	29.4
#6	acquired HA	intrinsic FVIII	1.5	46.6	23.3	28.6	11.1	19.0
#7	acquired HA	intrinsic FVIII	1.3	57.1	44.1	56.6	25.0	38.5
#8	acquired HA	intrinsic FVIII	3.9	15.3	23.7	25.3	13.8	22.2
#9	acquired HA	intrinsic FVIII	< [e]	93.1	19.9	37.9	12.8	19.5
#10	mild HA	Elocta / intrinsic FVIII	N/A[f]	25.3	71.4	71.3	49.8	66.5
#10a		Elocta / intrinsic FVIII	N/A[f]	30.7	66.4	67.6	41.0	59.5
#11	acquired HA	intrinsic FVIII	1.6	10.8	40.6	43.2	23.2	40.6
#12	acquired HA	intrinsic FVIII	5.8	10.5	30.6	25.8	15.5	23.7

[a] Nijmegen-Bethesda-Assay; [b] chromogenic substrate assay (bovine); [c] heat inactivation (treatment); [d] 1:2 pre-dilution addressed by 1:40 instead of 1:80 dilution of sample during automated analysis; [e] < limit of detection (FVIII CSA: 1 IU/dL; NBA: 0,42 BU/mL); [f] no clinical evidence for inhibitor formation (no analysis).

▶ **Fig. 1** Characteristics of patient samples and results. Modified from [4].

Conclusion The increasing use of emicizumab in patients with acquired or mild to moderate HA, as well as additional substitution of FVIII require a reliable strategy for accurate measurement of emicizumab in presence of FVIII:C. While heat treatment leads to falsified emicizumab results, the proposed preanalytical neutralization of FVIII:C by FVIII inhibitors appears to be a feasible strategy to address this demand.

Conflict of Interest JM has received honoraria from Octapharma and Siemens Healthineers. JO has received research funding from Bayer, Biotest, CSL Behring, Octapharma, Pfizer, Swedish Orphan Biovitrum, and Takeda; consultancy, speakers bureau, honoraria, scientific advisory board, and travel expenses from Bayer, Biogen Idec, BioMarin, Biotest, Chugai Pharmaceutical Co., Ltd., CSL Behring, Freeline, Grifols, LFB, Novo Nordisk, Octapharma, Pfizer, F. Hoffmann-La Roche Ltd., Sanofi, Spark Therapeutics, Swedish Orphan Biovitrum, and Takeda.



▶ **Fig. 2** Relative difference plots of emicizumab results obtained by functional analysis vs. LC-MS/MS. (A) Heat inactivation. (B) Factor VIII neutralization assay. The gray areas represent a relative error of ± 30 %. Modified from [4].

References

[1] Shima M, Amano K, Ogawa Y, Yoneyama K, Ozaki R, Kobayashi R, Sakaida E, Saito M, Okamura T, Ito T, Hattori N, Higasa S, Suzuki N, Seki Y, Nogami K. A prospective, multicenter, open-label phase III study of emicizumab prophylaxis in patients with acquired hemophilia A. J Thromb Haemost 2023; 21: 534–545

[2] Négrier C, Mahlangu J, Lehle M, Chowdary P, Catalani O, Bernardi RJ, Jiménez-Yuste V, Beckermann BM, Schmitt C, Ventriglia G, Windyga J, d'Oiron R, Moorehead P, Koparkar S, Teodoro V, Shapiro AD, Oldenburg J, Hermans C. Emicizumab in people with moderate or mild haemophilia A (HAVEN 6): a multicentre, open-label, single-arm, phase 3 study. Lancet Haematol 2023; 10: e168–e177

[3] Habay C, Auditeau C, Blandinières A, Bentounes NK, Lavenu-Bombled C, Harroche A, Bally C, Frenzel L, Borgel D, Lasne D. Interferences by factor VIII and lupus anticoagulant in the modified one-stage assay for emicizumab. Haemophilia 2023. doi:10.1111/hae.14873

[4] Hamedani NS, Donners AAMT, van Luin M, Gasper S, Rühl H, Klein C, Albert T, El Amrani M, Pötzsch B, Oldenburg J, Müller J. Functional determination of emicizumab in presence of factor VIII activity. J Thromb Haemost 2023; S1538-7836 (23): 00713–4. doi:10.1016/j.jtha.2023.09.011

[5] Donners AAMT, Gerencsér L, van der Elst KCM, Egberts TCG, de Maat MPM, Huisman A, Urbanus RT, El Amrani M. Quantification of emicizumab by mass spectrometry in plasma of people with hemophilia A: A method validation study. Res Pract Thromb Haemost 2022; 6: e12725

T-18-02 Semiautomatic assessment of immunofluorescence microscopy on blood smears in inherited platelet disorders using artificial intelligence: a proof of concept.

Authors R. Scholz[1, 2], C. E. Sigel[1, 2], J. Roggenbuck[1], C. Zaninetti[1], J. Wesche[1], J. Fuhrmann[1], L. Kaderali[3], R. Hiemann[4], D. Roggenbuck[5], A. Greinacher[1]

Institutes **1** Universitätsmedizin Greifswald, Institut für Transfusionsmedizin, Greifswald, Germany; **2** Medical Faculty Carl Gustav Carus of Technical University Dresden, Dresden, Germany; **3** Universität Greifswald Zentrum, Institut für Bioinformatik, Greifswald, Germany; **4** Brandenburg University of Technology Cottbus-Senftenberg, Institute of Biotechnology, Senftenberg, Germany; **5** Brandenburg University of Technology Cottbus-Senftenberg, Institute of Health Sciences Brandenburg, Senftenberg, Germany

DOI 10.1055/s-0044-1779216

Introduction Inherited platelet disorders (IPD) are a group of rare diseases caused by mutations in more than 70 genes. Despite advances in molecular diagnosis, platelet phenotyping still represents a cornerstone in the diagnostic workup of IPD. Immunofluorescence microscopy on the blood smear, combined with light microscopy, has been established as a screening tool for IPD and validated as sensitive and specific for 9 of the most frequent IPD [1]. A major drawback of the method lies in the subjective nature of morphological evaluation. Recent advances in computer vision and artificial intelligence tools offer improved performance and increasing accessibility of these technologies even for diagnostic laboratories.

Method Peripheral blood smears from IPD patients and healthy controls were stained with 13 primary antibodies against platelet structures (granules, cytoskeleton, surface receptors) and 2 fluorescence-labelled secondary antibodies. Immunofluorescence images were taken and processed using a fluorescence microscope (Aklides, Medipan) with bioimage analysis and machine-learning tools such as CLIJ [2], BaSiC [3], ilastik [4] and KNIME [5]. The expression pattern of platelet markers was assessed in IPD patients with respect to controls and eventually compared with the reference reports, which had been previously obtained by traditional manual immunofluorescence analysis.

Results We established a standardized process for the semiautomatic acquisition of immunofluorescence images. The process was able to capture an image stack of 11 images for 6 fields in less than 40 seconds. The image analysis was capable of segmenting platelets and extracting a spectrum of parameters (size, circularity, granularity, fluorescence intensity index). An exposure algorithm for the optimal use of image depth was also applied. Furthermore, artefacts such as bright accumulations of fluorophores were automatically rejected by dividing the image into 16 areas and disregarding the brightest two of them for auto-exposure.

To validate the approach, we blindly assessed 26 healthy controls and 26 patients affected with two IPD characterized by already described immunofluorescence pattern based on the absence or reduced expression of a platelet surface glycoprotein (GP): GP IbIX in Bernard-Soulier syndrome and GP IIbIIIa in Glanzmann thrombasthenia, respectively [1]. The semiautomatic method was able to distinguish all patients (26/26) from controls and to identify the typical diagnostic fluorescence patterns of the disorders.

Conclusion Semiautomatic image analysis allows an unbiased and standardized immunofluorescence based diagnostic process for patients with IPD. Implementation of the method for diagnostic screening routine is becoming a realistic option.

Conflict of Interest The authors state they have no conflicts of interest to declare.

References

[1] Zaninetti C, Leinøe E, Lozano ML, Rossing M, Bastida JM, Zetterberg E, Rivera J, Greinacher A. Validation of immunofluorescence analysis of blood smears in patients with inherited platelet disorders. J Thromb Haemost 2023; 21 (4): 1010–1019

[2] Haase R, Royer LA, Steinbach P, Schmidt D, Dibrov A, Schmidt U, Weigert M, Maghelli N, Tomancak P, Jug F, Myers EW. CLIJ: GPU-accelerated image processing for everyone. Nat Methods 2020; 17: 5–6
[3] Peng T, Thorn K, Schroeder T, Wang L, Theis FJ, Marr C, Navab N. A BaSiC tool for background and shading correction of optical microscopy images. Nat Commun 2017; 8: 14836
[4] Berg S, Kutra D, Kroeger T, Straehle CN, Kausler BX, Haubold C, Schiegg M, Ales J, Beier T, Rudy M, Eren K, Cervantes JI, Xu B, Beuttenmueller F, Wolny A, Zhang C, Koethe U, Hamprecht FA, Kreshuk A. ilastik: interactive machine learning for (bio)image analysis. Nat Methods 2019; 16 (12): 1226–1232
[5] Berthold MR, Cebron N, Dill F, Gabriel TR, Kötter T, Meinl T, Ohl P, Sieb C, Thiel K, Wiswedel B. KNIME: The Konstanz Information Miner. In: Preisach C, Burkhardt H, Schmidt-Thieme L, Decker R, eds. Data Analysis, Machine Learning and Applications. Studies in Classification, Data Analysis, and Knowledge Organization. Springer; 2008: 319–326

T-18-03 Precision study of a fast and fully automated FVIII functional inhibitor test

Authors B. Verbruggen[1], G. W. Moore[3, 4], N. B. Binder[2]
Institutes **1** Hemostad, Nijmegen, Netherlands; **2** Technoclone Herstellung von Diagnostika und Arzneimitteln GmbH, Vienna, Austria; **3** Addenbrooke's Hospital, Specialist Haemostasis Unit, Cambridge, UK; **4** Middlesex University, Department of Natural Sciences, London, UK
DOI 10.1055/s-0044-1779217

Introduction Laboratory detection of Factor (F)VIII inhibitors is preferentially performed with the Nijmegen assay. It requires a 2-hours incubation of test sample with the FVIII-source mixture because of slow FVIII inactivation, due to its reversible binding to von Willebrand Factor (VWF) delaying inhibitor action. Extended incubation time results in non-specific FVIII inactivation, that, together with complicated liquid handling, may contribute to the considerable variability (CV: 30–40 %) seen in inter-laboratory surveys. We hypothesize that testing in a VWF-free assay matrix using recombinant (r)FVIII can dramatically lower incubation time that, together with full automation, will substantially improve standardisation.

We aimed to test the precision of a fast, fully automated FVIII inhibitor test using VWF-free rFVIII and a dedicated analyser.

Method As in the original Nijmegen assay, test samples are heated for at least 30 minutes at 58 °C and centrifuged for 10 minutes to destroy residual FVIII.

The coagulation analyser employed must provide on board ability of three subsequent sample dilution steps and three reagent additions. An application was defined on a Ceveron s100 (Technoclone), as below.

After loading the heat inactivated samples, sequential automated analytical steps occur as follows:

1. Predilution with heat inactivated FVIII/VWF deficient plasma (if needed)
2. Mixing with rFVIII (1.0 IU/mL)
3. Incubation for 20 minutes at 37 °C
4. Dilution of incubated samples 1:10 with Imidazole buffer, pH 7.3. and analysis for residual rFVIII activity

Results Five samples and two lyophilized controls, whose inhibitor activities with the original Nijmegen assay were between 0 and 40 BU, were analysed. In a reproducibility study with three laboratories, the samples and controls were assayed twice a day on five independent days. For all samples and controls, precision was analysed exhibiting a coefficient of variation of less than 15 % for all samples.

Conclusion Rapid, fully automated FVIII-inhibitor testing can be performed with a dedicated coagulation analyser using rFVIII in a VWF-free matrix. Automation and reduced assay time improve viability and potentially the availability of a normally protracted assay, permitting a more rapid and informed clinical response.

Conflict of Interest BV and NBB are emloyees of the respective companies affiliated with and are listed as inventors of the international patent application for this assay. GWM recieves consulting honoraria from Technoclone.

T-18-04 A new method to determine the cleavage of von Willebrand Factor by recombinant ADAMTS13 under physiological shear-stress conditions

Authors P. Liedl, S. Peyrer-Heimstaett, G. Schrenk
Institute Baxalta Innovations GmbH, Pharmaceutical Science, Vienna, Austria
DOI 10.1055/s-0044-1779218

Introduction Regulation of von Willebrand Factor (VWF) activity by a disintegrin and metalloproteinase with a thrombospondin type 1 motif (ADAMTS13) is critical for hemostasis by controlling VWF multimer size. Demonstrating the ADAMTS13-mediated cleavage of VWF under physiological shear has proven difficult. Traditional analytical methods utilize denaturing environments, thereby poorly replicate the natural vascular environment. This research aimed to develop a sensitive methodology to visualize and quantitate VWF proteolysis by a recombinant ADAMTS13 (rADAMTS13) drug candidate under arterial shear flow in human blood.

Method A microfluidics-based approach utilizing the BioFlux 1000Z system (Fluxion Biosciences) was optimized by: coating channels with 143 µg/ml collagen type I, blood sample preheating, applying 20 dyne/cm^2 pulsatile shear, relying on inherent VWF levels in donor blood, inputting hematocrit based viscosity parameters, and implementing timed protocols. rADAMTS13 was added to healthy donor blood at different concentrations and the time course of platelet adhesion to immobilized collagen was determined by microscopy using fluorescent labeled platelets.

Results Optimized analytical techniques enabled visualization and quantification of VWF proteolysis based on platelet binding under shear flow. Addition of 1.875-7.5 U/ml rADAMTS13 to blood reduced the VWF-mediated platelet adhesion to collagen in a concentration dependent manner. Repeated testing validated the sensitivity. Statistical analysis quantified inter-sample variability.

Conclusion This research successfully established a powerful methodology harnessing microfluidics to gain fundamental insights into rADAMTS13 function under physiologically relevant shear flow conditions. Further enhancement of the techniques, increased biological sampling, and exploration of collagen types could build on these findings.

Conflict of Interest Autor and co-autors are full-time employees of Baxalta Innovations GmbH, Vienna, Austria

T-18-05 Detection and quantification of heme and hemoglobin for diagnosis of intravascular hemolysis

Authors M.-T. Hopp[2], S. M. Vaidya[1], K. M. Grimmig[1], J. Müller[3], J. Oldenburg[3], D. Imhof[1]
Institutes **1** University of Bonn, Pharmaceutical Biochemistry and Bioanalytics, Pharmaceutical Institute, Bonn, Germany; **2** University of Koblenz, Department of Chemistry, Institute for Integrated Natural Sciences, Koblenz, Germany; **3** University Hospital Bonn, Institut für Experimentelle Hämatologie und Transfusionsmedizin, Bonn, Germany
DOI 10.1055/s-0044-1779219

Introduction Hemolysis, caused by e.g. disorders like sickle cell disease, paroxysmal nocturnal hemoglobinuria and vascular trauma or by infections and injuries, is often characterized by serious conditions and consequences for the patients, such as kidney injury or vasoocclusion [1–3]. These clinical concerns are mainly attributed to elevated levels of hemoglobin and labile heme from premature rupture of red blood cells during intravascular hemolysis. Diagnosis of hemolysis is usually done based on elevated reticulocyte, lactate dehydrogenase (LDH), and bilirubin levels, as well as reduced haptoglobin plasma concentrations [4, 6]. Additionally, urine hemosiderin and urine/blood extracellular hemoglobin indicate intravascular hemolysis [1]. Although several methods are available for the diagnosis and monitoring of hemolytic disorders, only hemoglobin is currently considered as a diagnostic marker [5, 7]. However, due to the proven inflammation- and thrombosis-triggering effects of heme, quantification of this biomarker should also be considered in clinical settings to

achieve a complete picture of the hemolytic state and the treatment strategies [7, 8].

Method The aim of our study was to evaluate the validity and applicability of the quantification methods for hemoglobin and heme levels as well as to differentiate between hemoglobin-bound heme and labile heme. Indirect and direct approaches, including e.g., chromatography, spectroscopy, and mass spectrometry as well as enzymatic test systems, were used to determine the concentration of heme and hemoglobin as well as mixtures thereof.

Results A clear distinction between hemoglobin-bound heme and labile heme with one method was, however, not possible, suggesting the use of a combined approach. With a specific spectroscopic approach and a newly established equation we were able to determine both analytes in human plasma samples of different hemolytic states.

Conclusion Our study gives a broader perspective by adding to the knowledge about hemoglobin and heme quantification in research and/or clinical diagnosis. The implementation of an amalgamated method is an easy-to use technique requiring low sample volumes and can thus enable fast detection via spectrophotometric tools. This would enable monitoring of heme as a biomarker to understand the molecular basis of hemolytic disorders in clinical diagnosis.

Conflict of Interest The authors declare no competing interests.

References

[1] Rother RP, Bell L, Hillmen P, Gladwin MT. The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: A novel mechanism of human disease. JAMA. 2005; 293: 1653–1662

[2] Oh JY, Williams A, Patel RP. The role of redox-dependent mechanisms in heme release from hemoglobin and erythrocyte hemolysates. Arch Biochem Biophys 2019; 662: 111–120

[3] Cardoso EC, Silva-Neto PV, Hounkpe BW, Chenou F, Albuquerque CCMX, Garcia NP, de Lima F, De Paula EV, Fraiji NA. Changes in heme levels during acute vasoocclusive crisis in sickle cell anemia. Hematol Oncol Stem Cell Ther 2021; 16 (2): 5

[4] Smith A, McCulloh RJ. Hemopexin and haptoglobin: Allies against heme toxicity from hemoglobin not contenders. Front Physiol 2015; 6: 187

[5] Oh JY, Hamm J, Xu X, Genschmer K, Zhong M, Lebensburger J, Marques MB, Kerby JD, Pittet JF, Gaggar A, Patel RP. Absorbance and redox based approaches for measuring free heme and free hemoglobin in biological matrices. Redox Biol 2016; 9: 167–177

[6] Müller-Eberhard U, Javid J, Liem HH, Hanstein A, Hanna M. Plasma concentrations of hemopexin, haptoglobin and heme in patients with various hemolytic diseases. Blood. 1968; 32: 811–815

[7] Hopp MT, Schmalohr BF, Kühl T, Detzel MS, Wißbrock A, Imhof D. Heme determination and quantification methods and their suitability for practical applications and everyday use. Anal Chem 2020; 92: 9429–9440

[8] Hopp MT, Imhof D. Linking labile heme with thrombosis. J Clin Med 2021; 10: 427

T-18-06 Pilot study of ADAMTS13 activity and anti-ADAMTS13 antibodies stability

Authors A. Bogić[1], D. Coen Herak[1, 2], M. Miloš[1, 3], D. Rogić[1, 2]

Institutes **1** University Hospital Centre Zagreb, Deapartment of Laboratory Diagnostics, Zagreb, Croatia; **2** Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia; **3** Faculty of Pharmacy, University of Mostar, Mostar, Bosnia

DOI 10.1055/s-0044-1779220

Introduction Thrombotic thrombocytopenic purpura (TTP) is a rare disease characterized by thrombocytopenia, microangiopathic hemolytic anemia, neurological disorders, renal dysfunction and febrility. TTP occurs due to ADAMTS13 (a disintegrin-like and metalloproteinase with thrombospondin type 1 motif 13) deficiency, which is most often a consequence of anti-ADAMTS13 autoantibodies development, that cause acquired TTP. The less common congenital TTP is caused by mutations in the gene encoding ADAMTS13. The determination of ADAMTS13 activity and anti-ADAMTS13 antibodies is essential (required) to differentiate between congenital and acquired TTP or to distinguish TTP from other thrombotic microangiopathies. Proper sampling, careful handling of samples and sample storage is crucial to avoid loss of *in vitro* ADAMTS13 activity. Therefore, aim of this study was stability evaluation of ADAMTS13 activity and anti-ADAMTS13 antibodies after 24-hour sample storage at + 4 °C [1–2].

Method Pilot study included 16 patients with high clinical suspicion or longitudinally monitored TTP, admitted to University Hospital Centre Zagreb in February 2023. Blood samples were collected into 3.2 % sodium citrate tubes (BD Vacutainer, Becton Dickinson, USA). Upon admission samples were centrifuged 30 minutes at 4000 rpm and plasma was aliquoted. Rest of the plasma was left on the cells in vacutainer and stored at + 4 °C. After 24-hour storage, remaining plasma was aliquoted. All sample aliquots were kept at -80 °C until analysis. ADAMTS13 activity was determined using chromogenic ELISA (Technozym ADAMTS13 activity, Technoclone) in all samples, while anti-ADAMTS13 antibodies were determined in 11 samples using Technozym ADAMTS13 inh ELISA (Technoclone). Wilcoxon test was performed using MedCalc® Statistical Software version 22.013 (Ostend, Belgium) and P-value < 0.05 was considered statistically significant.

Results For ADAMTS13 activity no statistically significant difference (P = 0.330) between plasma samples aliquoted upon admission (median 0.5 kIU/L; range 0.01 – 1.0 kIU/L) and after 24-hour storage at + 4 °C (median 0.5 kIU/L; range 0.01 – 1.0 kIU/L) was revealed. In addition, no significant difference (P = 0.575) between plasma samples aliquoted upon admission (median 4.7 kU/L; range 1.7 – 52.7 kU/L) and after 24-hour storage at + 4 °C (median 4.4 kU/L; range 1.9 – 49.4 kU/L) regarding anti-ADAMTS13 antibodies was observed.

Conclusion Study indicated that ADAMTS13 activity and anti-ADAMTS13 antibodies can be analysed within 24 hours from venepuncture if samples are appropriately handled and stored under the tested conditions. This could be important for patients with complex clinical picture and high clinical suspicion of TTP, that have previously collected sodium citrate tubes for routine coagulation tests, enabling rapid TTP confirmation and patient treatment initiation, while also reducing the need for additional sampling.

Conflict of Interest No conflicts of interest to declare.

References

[1] Sukumar S, Lämmle B, Cataland SR. Thrombotic thrombocytopenic purpura: Pathophysiology, diagnosis, and management. J Clin Med 2021; 10 (3): 536. doi:10.3390/jcm10030536

[2] Rock G, Yousef H, Neurath D, Lu M. ADAMTS-13 levels in fresh, stored, and solvent detergent treated plasma. Transfus Apher Sci 2006; 3: 235–238. doi:10.1016/j.transci.2006.08.006

T-18-07 Assessment of Emicizumab and Factor VIII using Clot Waveform Analysis

Authors T. Siegemund[1], A. Siegemund[1, 2], H. Bönigk[1], C. Pfrepper[2]

Institutes **1** MVZ Limbach Magdeburg, Magdeburg, Germany; **2** University Hospital Leipzig, Medical Department I, Division of Hemostaseology, Leipzig, Germany

DOI 10.1055/s-0044-1779221

Introduction The bispecific antibody emicizumab is widely used as a factor VIIIa mimetic in the prevention of bleeding in hemophilia A. In certain clinical cases, like surgery, trauma or immune tolerance therapy, factor VIII is given on top of emicizumab. In such scenario, the assessment of emicizumab and factor VIII levels is technically challenging. Latter can be assessed in a two-stage chromogenic assay using bovine coagulation factors, which abolishes emicizumab activity. Emicizumab can be assessed after heat inactivation of factor VIII by either one-stage or human-protein based two-stage assay.

In clot waveform analysis we utilize the different behavior that emicizumab and factor VIII show in an aPTT-based one-stage assay. While emicizumab impacts the lag-time of the assay more than the reaction velocity, it is the other way around for factor VIII. With this opposing behavior it should be possible to set up a system of equations using lag-time and reaction velocity and calculate emicizumab and factor VIII levels simultaneously.

Method Factor VIII deficient plasma was spiked with various levels of emicizumab (0-70 µg/mL) and factor VIII (0.0-1.0 IU/mL) yielding concentrations similar for aPTT-based factor VIII assays. aPTT was determine in all samples using Synthasil reagent on an ACLTOP analyzer. Clot waveform analysis was performed using dedicated software. Results of lag-time and peak of 1st derivative were used in a system of linear equations to calculate emicizumab and factor VIII levels.

Results Recovery of emicizumab levels showed low coefficients of variation between 6 % and 8 % over a wide range of concentrations, but increased significantly below 10 µg/mL. Variability was higher for factor VIII recovery between 9 % and 16 %, with higher variability below 0.2 IU/L (< 40 %). Data transformation could be used to improve variability of factor VIII levels but at the price of higher variation for emicizumab.

Conclusion Clot waveform analysis can be used to assess emicizumab and factor VIII levels simultaneously in a standard aPTT-based one-stage clotting assay. The method shows good recovery for clinical relevant levels of both entities.

No sample manipulation or chromogenic two-stage assay is required. The results on require optical measurement data from the aPTT assay and dedicated software for data analysis. Using an adjusted system of equations for the calculation is likely to reduce variability of the obtained emicizumab and factor VIII levels, especially at low concentrations.

Conflict of Interest The study was sponsored by an unrestricted grant from Chugai/Roche. TS and AS report honoraria for lectures from SOBI. CP reports institutional grants for research and studies from Chugai/Roche, Takeda, Zacros, and LeoPharma, and honoraria for lectures or consultancy from Bayer, Biomarin, Chugai/Roche, CSL Behring, Novo Nordisk, Pfizer, BMS, SOBI, and Takeda.

T-18-08 A normal range study for the T-TAS(R) with three different chips

Authors S. Schneppenheim[1], U. Klemm[1], C. Pfrepper[2], C. Franke[2], K. Freitag[2], R. Henschler[3], H. Ohsawa[4], H.-J. Kolde[5]

Institutes **1** MVZ MEDILYS Laborgesellschaft mbH, Paul-Ehrlich-Straße 1 – 22763 Hamburg, Germany, Hamburg, Germany; **2** Division of Hemostaseology, Medical Department I, University Hospital Leipzig, Leipzig, Germany; **3** Universitätsklinik Leipzig, Instituts für Transfusionsmedizin, Leipzig, Germany; **4** FUJIMORI KOGYO CO. LTD, Zacros, Tokyo, Japan; **5** Consulting Diagnostics, Seefeld, Germany

DOI 10.1055/s-0044-1779222

Introduction The T-TAS® (Total Thrombus formation Analysis System) is a device for assessing hemostasis in whole blood under physiological flow conditions. Resembling a modified flow chamber principle, it measures the change of flow during thrombus formation within micro capillaries in disposable microchips. The global assays measure different aspects in hemostasis. We determined the normal ranges of the three available chip types for a typical European healthy population and compared the variability between labs.

Method The T-TAS®01 (CE marked, FUJIMORI KOGYO Co.) contains an integrated pump and pressure sensor. A blood sample, applied to the respective chip, is pumped through the flow path under optimized conditions. Thrombus formation begins, when blood reaches the area, coated with either collagen only (PL-chip) or collagen plus tissue thromboplastin (AR-/ HD-chip). The pressure change inside the flow path is examined, providing the reaction kinetics of the entire thrombus formation process (pressure/time diagram). Derived results such as area under the curve (AUC) are computed. 50 samples from healthy subjects of both sexes (age range 20 to 80 y) with normal CBC, normal VWF and without any medication were enrolled per center (named as L and HH). Blood for the PL-chip (platelet function) was collected into BAPA (Benzylsulfonyl-D-Arg-Pro-4-amidinobenzylamide)- vacuum tubes. For the AR- and HD-chip (AR: platelet and coagulation interaction, HD: thrombogenicity at low platelet counts), one center (HH) collected blood in vacuum tubes (Vacutainer® , Becton Dickinson and Co), the other (L) employed the aspiration technique (Monovette®, Sarstedt AG & Co. KG). Samples were tested within < 2 hours with the three different chip types. Statistically, Wilcoxon-Mann-Whitney test was used, considering $p < 0.05$ as a statistically significant difference. Analyze-it® (Analyse-it Software, Ltd., UK) was used as a statistical analysis software.

Results The data show a very similar ranges for the PL-chip in both centers, though with a wider distribution in one center. The PL-AUC mean values (L; 352 and HH; 364) were not significantly different. The 5-95 % percentiles for 99 samples for PL-AUC were 260- 424. Similarly, for the HD chip the values of the two centers were not statistically different (mean HD-AUC: 1535(L) and 1565(HH), 5-95 % perc.: 1420- 1622). The AR chip values were statistically different between the two centers. AR-AUC mean values were 1262(L) and 1344(HH), 5-95 % perc.: 908 – 1484 [1–2].

Conclusion The similar ranges of results in the two centers show that the method generates reproducible data for the assessment of haemostasis. Minor differences for the AR assay are probably associated to the blood collection methods employed. These data may help other centers in interpreting own T-TAS® data.

Conflict of Interest S. Schneppenheim, U. Klemm and C. Pfrepper, R.Henschler, C.Franke, K. Freitag: no conflict of interest. Ohsawa H is an employee of FUJIMORI KOGYO Co., Ltd., which is the manufacturer of the T-TAS 01. Kolde HJ is a consultant to FUJIMORI KOGYO Co. Ltd.

References

[1] Thromb Hemostas J 2011; 9 (19): 2029–2037
[2] Thromb Hemostas J 2007; 5 (10): 2119–2126

T-18-09 False normalisation of Antithrombin in Type II-Heparin Binding Site defects in DOAC treated patients

Authors K. Hickey, S. Kitchen

Institute Sheffield Teaching Hospitals, Sheffield Haemophilia and Thrombosis Centre, Sheffield, UK

DOI 10.1055/s-0044-1779223

Introduction The increasing use of DOACs particularly in the USA (Navar, 2022) and Europe (Kirchhof P, 2021) increases the complexity for laboratories as more samples arriving containing DOAC. This is compounded by often incomplete clinical information, including the omission of anticoagulation status or incorrect details of anticoagulation type (e.g., LMWH stated when on DOAC).

We had previously investigated DOAC removal agents for use in a modified DRVVT testing algorithm (data not shown) and in our experience, the combination of cost, ease of use (flexible sample volume) and effectiveness at removal of direct IIa and Xa inhibitors made DOAC Stop (Exner et al, 2018) our agent of choice.

A series of patients with incomplete clinical information on anticoagulation status under investigation for thrombophilia warranted an evaluation of this approach when using Xa-based Antithrombin (AT) assays (Van Cott et al, 2020).

Method Our first line AT assay is IIa based – Berichrom AT (AT-IIa) (Siemens UK) and results < 0.91 IU/mL have AT antigen (ATag) (Liatest AT, Stago, UK) to subtype type 1 and type 2 AT deficiency. Both assays performed on ACL TOP 700 (Werfen, UK). Our secondary AT assay is Xa based – Innovance AT (AT-Xa), Siemens, UK) on Sysmex CS5100 (Sysmex, UK) is used to elucidate query type 2 results. Anti Xa assays used Hyphen LRT (Hyphen, UK) on Sysmex CN6000 (Sysmex, UK) using either LMWH assay as a screen or using drug specific calibration curves.

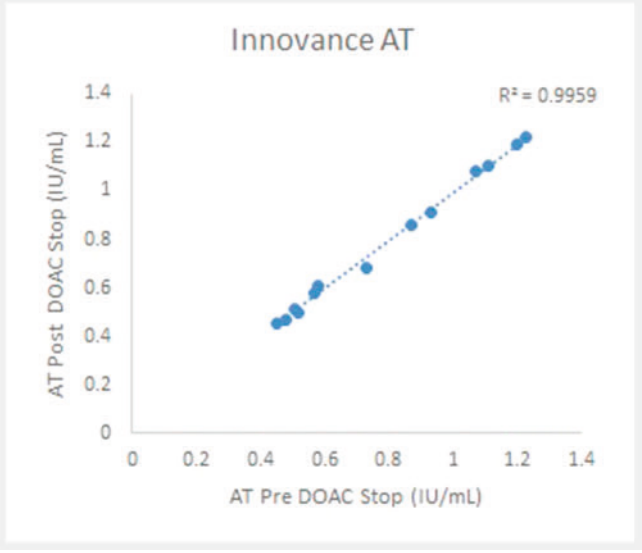
Results Patient under investigation (patient 1a) had low AT-IIa 0.83 IU/mL, ATag 1.04 IU/mL, with an abnormal AT-IIa/ATag ratio 0.80, suggestive of Type II AT deficiency. The AT-Xa was 1.07 IU/mL. This is a pattern of results we have typically seen in AT RS defect – Cambridge II. Confirmatory testing (patient 1b) showed similar pattern for AT-IIa and ATag (see ▶ **Fig. 1**) with a now low AT-IIa 0.68 IU/mL. A pattern of results in keeping with HBS defect. On investigation (patient 1a) was on Rivaroxaban, post DOAC stop AT-Xa was 0.61 IU/mL.

Patient	Pre DOAC STOP					Post DOAC STOP		
	XA screen	AT-IIa (IU/mL) RR 0.85-1.31 IU/mL	AT-Xa (IU/mL) RR 0.85-1.31 IU/mL	ATag (IU/mL) RR 0.83-1.24 IU/mL	ratio RR 0.89 -1.12 IU/mL	XA screen	AT-IIa (IU/mL) RR 0.85-1.31 IU/mL	AT-Xa (IU/mL) RR 0.85-1.31 IU/mL
1a	LMWH curve 2.55iu/ml	0.83	1.08	1.04	0.80	LMWH curve 0.02 iu/ml	not tested	0.61
1b	no DOAC	0.9	0.68	1.11	0.81			
3	Apixaban 141 ng/mL	0.81	0.84	1.09	0.74	Apixaban 11 ng/mL	0.84	0.58
4	Rivaroxaban 179 ng/mL	0.84	0.93	1.05	0.80	Rivaroxaban 9 ng/mL	0.89	0.65

▶ **Fig. 1 Antithrombin Results pre/post DOAC Stop**; Sample investigated for antithrombin deficiency. Results from sample tested on presentation and post DOAC Stop once the presence of a DOAC was established.

A series of AT samples (n = 13) tested with AT-Xa pre (mean 0.79 IU/mL, median 0.73 IU/mL) and post DOAC Stop (mean 0.78 IU/mL, median 0.68 IU/mL) confirm DOAC Stop does affect AT determination, p = 0.952 (graph 1.)
Sourcing other samples (patient 3 & 4) we have had on DOAC and suspected of having AT HBS defect showed similar pattern of DOAC interference (▶ **Fig. 1**).

Conclusion The incomplete information on DOAC treatment in patients results in significant false normalisation in patients with HBS defects leading to under diagnosis of AT deficiency. We adopted an algorithm: any request for AT-Xa is screened for Xa inhibitors (against LMWH curve) and samples with detectable concentration, > 0.05 IU/mL are pretreated with DOAC stop to ensure accuracy of result [1–4] (▶ **Fig. 2**).



▶ **Fig. 2 Antithrombin samples tested pre and post DOAC STOP**; Samples for AT patients pre and post DOAC Stop. Use of DOAC stop does not affect AT results.

More broadly, the increasing use of DOAC in our view necessitates when using any Xa based antithrombin assays, to screen for DOAC presence. When detected, neutralise of the DOAC using appropriate methods prior to testing to ensure accuracy of results.

Conflict of Interest none

References
[1] Navar AM, Kolkailah AA, Overton R, Shah NP, Rousseau JF, Flaker GC, Pignone MP, Peterson ED. Trends in Oral Anticoagulant Use Among 436 864 Patients With Atrial Fibrillation in Community Practice, 2011 to 2020. J Am Heart Assoc 2022; 11 (22):
[2] Kirchhof P, Ammentorp B, Darius H, De Caterina R, Le Heuzey JY, Schilling RJ, Schmitt J, Zamorano JL. Management of atrial fibrillation in seven European countries after the publication of the 2010 ESC Guidelines on atrial fibrillation: primary results of the PREvention oF thromboemolic events--European Registry in Atrial Fibrillation (PREFER in AF). Europace 2014; 16: 1: 6–14
[3] Exner T, Michalopoulos N, Pearce J, Xavier R, Ahuja M. Simple method for removing DOACs from plasma samples. Thromb Res 2018; 163: 117–122
[4] Van Cott EM, Orlando C, Moore GW, Cooper PC, Meijer P, Marlar R Subcommittee on Plasma Coagulation Inhibitors. Recommendations for clinical laboratory testing for antithrombin deficiency; Communication from the SSC of the ISTH. J Thromb Haemost 2020; 18 (1): 17–22

T-18-10 Expert consensus on an algorithm for rapid exclusion of clinically relevant plasma levels of direct oral anticoagulants in patients using the DOAC Dipstick

Authors J. Harenberg[1, 2], R. Gosselin[3], I. Pabinger[4], C. Becattini[5], A. Cuker[6], S. Hetjens[9], C. Weiss[7], R. Klamroth[9], H. Herkner[10], F. Langer[9]
Institutes **1** DOASENSE GmbH, Heidelberg, Germany; **2** University of Heidelberg, Heidelberg, Germany; **3** University of California, Thrombosis and Hemostasis Center, Sacramento, USA; **4** Medical University of Vienna, Department for Internal Medicine, Vienna, Austria; **5** University of Perugia, Internal and Emergency Medicine, Perugia, Italy; **6** University of Pennsylvania, Department of Medicine and Department of Pathology & Laboratory Medicine, Pennsylvenia, USA; **7** Clinical Medicine Mannheim, University of Heidelberg, Department of Biometry and Statistics, Mannheim, Germany; **8** Vivantes Hospital Friedrichshain, Vascular Medicine and Haemostaseology, Berlin, Germany; **9** Universitätsklinikum Eppendorf, II. Medizinische Klinik und Poliklinik, Hamburg, Germany; **10** Medical University of Vienna, Department of Emergency Medicine, Vienna, Austria
DOI 10.1055/s-0044-1779224

Introduction With the widespread use of direct oral anticoagulants (DOACs), there is an urgent need for a rapid assay to exclude clinically relevant plasma levels. Some position papers have suggested measuring DOAC levels in patients presenting to emergency departments with serious bleeding, acute ischemic stroke or when major surgical interventions or other invasive procedures associated with high bleeding risk are needed. The levels must be determined by liquid chromatography tandem mass spectrometry (LC-MS/MS) or with specialized assays, such as calibrated chromogenic assays or viscoelastic methods. Specific threshold blood levels, which vary from ≤ 20 ng/mL to ≤ 100 ng/mL, have been proposed to guide medical decision for patients taking dabigatran or direct oral factor Xa (FXa) inhibitors. DOAC Dipstick (DOASENSE™, Heidelberg, Germany) is a point-of-care test that uses a disposable test strip to detect DOACs in urine and to differentiate between dabigatran and direct oral FXa inhibitors.

Method A systematic literature search was performed between 1993 and June 2023 to identify relevant studies in PubMed (MEDLINE) and Cochrane Library databases. Inclusion criteria were the determination of DOACs in urine samples of patients with atrial fibrillation and venous thromboembolism with rivaroxaban, apixaban, edoxaban and dabigatran compared to LC-MS/MS or calibrated chromogenic assay and reporting a plasma threshold of ≥ 30 ng/mL. Data were analyzed by simple pooling to determine DOAC Dipstick's sensitivity, negative predictive value (NPV), positive predictive value (PPV) and specificity. Unweighted mean values of these statistics with 95 % confidence intervals (CI) were calculated using Stata 17.0 confirmed by SAS version 4.5.

Results Of 1206 eligible studies, 5 clinical studies were eligible for inclusion in the pooled analysis. The sensitivity was > 97 %, (lower 95 % CI > 90 %) for both

DOAC classes. The NPV value for the direct oral FXa inhibitors was 86.6 % (95 % CI: 76 %– 93.7 %) and for dabigatran > 99 % (95 % CI: 98 % – 100 %). The NPV for the DOAC Dipstick was similar to the PPV for factor Xa inhibitors and more robust for thrombin inhibitors confirming the primary utility of a negative urine DOAC Dipstick pad result is to identify clinically relevant DOAC concentrations at a threshold of ≥ 30 ng/mL in the blood.

Conclusion Pooled analysis of five available studies, comparing DOAC Dipstick with LC-MS/MS or DOAC-calibrated chromogenic assays indicates an acceptably high negative predictive value for exclusion of DOACs requiring verification of a match with the patient's clinical picture. The DOAC Dipstick excludes clinically relevant blood concentrations of DOACs at a threshold of ≥ 30 ng/mL, which may support clinical decision-making in critical medical situations, such as excessive bleeding, prior to intravenous thrombolysis, or before urgent surgical procedures.

Conflict of Interest JH: general manager and founder of DOASENSE GmbH, Heidelberg, Germany. All other authors do not have to declare a conflict of interest.

T-18-11 Platelet phenotyping by immunofluorescence microscopy on the blood smears in patients with myeloproliferative neoplasms

Authors C. Zaninetti[1], L. Vater[1], L. Kaderali[2], L. Swensson[1], J. Fuhrmann[1], J. Wesche[1], C. Freyer[1], F. Heidel[3, 4], A. Greinacher[1]

Institutes **1** Universitätsmedizin Greifswald, Institut für Transfusionsmedizin, Greifswald, Germany; **2** Universitätsmedizin Greifswald, Institut für Bioinformatik, Greifswald, Germany; **3** Universitätsmedizin Greifswald, Innere Medizin C, Greifswald, Germany; **4** Medizinische Hochschule Hannover, Klinik für Hämatologie, Hämostaseologie, Onkologie und Stammzelltransplantation, Hannover, Germany

DOI 10.1055/s-0044-1779225

Introduction Myeloproliferative neoplasms (MPN) are clinically heterogeneous clonal disorders [1]. Alterations of platelet number or function have been reported in some forms. However, the platelet phenotype in MPN subjects has not been systematically assessed so far [2]. Immunofluorescence microscopy on the blood smear has been established and validated as a sensitive and specific screening tool for diverse inherited platelet disorders by recognition of specific morphologic alterations [3]. Using this method, we assessed platelet phenotypes in a cohort of well-characterized MPN subjects.

Method Peripheral blood smears were stained with May-Grünwald-Giemsa technique and a panel of 13 primary antibodies against many markers of platelet structures (granules, cytoskeleton, surface receptors). The slides were then assessed with standard light- and immunofluorescence microscopy upon staining with fluorescent-labelled secondary antibodies. The investigators performing the morphologic evaluation were blinded for clinical, genotypic and laboratory information regarding the individual patients. Morphologic changes were reported and assigned to the specific platelet structure. After uncovering patients' information, we searched for correlations between morphologic changes and clinical features.

Results We investigated 135 adult patients (55 % females) with confirmed MPN. The mean age was 63 (range 19 to 89). 45 (34 %) subjects had received a diagnosis of polycythaemia vera (PV), 41 (30 %) of essential thrombocythemia (ET), and 41 (30 %) of primary myelofibrosis. In 8 (6 %) patients the MPN was unclassifiable. In 9 PV- and 10 ET patients the disorder had evolved into a secondary myelofibrosis.

By immunofluorescence microscopy, we found in 82 (61 %) individuals a reduction of platelet alpha granule markers (von Willebrand factor, P-selectin, thrombospondin). In 98 (73 %) subjects, we observed aggregates consisting of the cytoskeletal protein non-muscular myosin IIA (NMMIIA) in erythrocytes, which also showed anisopoikilocytosis. The evidence of alpha granule deficiency significantly correlated with the initial diagnosis of PV. The presence of red blood cell NMMIIA aggregates showed a significant correlation with: i) the presence of bone marrow fibrosis; ii) the evolution of the disorder into a secondary myelofibrosis regardless the initially diagnosed MPN subtype; and iii) the development of splenomegaly. By light microscopy, a significant correlation was found between platelet anisocytosis and the diagnosis of ET.

A parallel abstract describes the method to detect NMMIIA aggregates in erythrocytes by flow cytometry.

Conclusion Morphological changes of platelets and red blood cells suggesting an MPN, and possibly its subtype, can be detected by immunofluorescence microscopy on the blood smear. Of note, aggregates of NMMIIA in the erythrocytes seem to associate with the grade of bone marrow fibrosis and might represent a novel early marker for transformation into secondary myelofibrosis.

Conflict of Interest The authors state they have no conflicts of interest to declare.

References

[1] Luque Paz D, Kralovics R, Skoda RC. Genetic basis and molecular profiling in myeloproliferative neoplasms. Blood. 2023; 141 (16): 1909–1921

[2] Nann D, Fend F. Synoptic Diagnostics of Myeloproliferative Neoplasms: Morphology and Molecular Genetics. Cancers (Basel) 2021; 13 (14): 3528

[3] Zaninetti C, Leinøe E, Lozano ML, Rossing M, Bastida JM, Zetterberg E, Rivera J, Greinacher A. Validation of immunofluorescence analysis of blood smears in patients with inherited platelet disorders. J Thromb Haemost 2023; 21 (4): 1010–1019

T-18-12 T-TAS HD-Chip in patients with myeloid neoplasia and thrombocytopenia

Authors K. Freitag[1], A. Siegemund[1], K. Metzeler[2], G.-N. Franke[2], S. Petros[1], U. Platzbecker[2], C. Pfrepper[1]

Institutes **1** University Hospital Leipzig, Division of Hemostaseology, Department of Hematology, Cellular Therapy, Hemostaseology and Infectiology, Leipzig, Germany; **2** University Hospital Leipzig, Division of Hematology and Cellular Therapy, Department of Hematology, Cellular Therapy, Hemostaseology and Infectiology, Leipzig, Germany

DOI 10.1055/s-0044-1779226

Introduction Thrombocytopenia is frequent in patients with hematologic malignancies. Platelet count is commonly used to assess the need for platelet transfusion. However, this approach does not consider platelet function and other coagulation parameters such as von Willebrand factor (VWF), which may influence bleeding tendency. T-TAS is a flow chamber based system to evaluate the hemostatic function in whole blood samples. The HD-chip of T-TAS was developed for the analysis of samples from patients with thrombocytopenia [1]. The aim of this study was to investigate the T-TAS HD-chip in thrombocytopenic patients with myeloid neoplasia.

Method Whole blood samples from patients with acute myeloid leukemia, myelodysplastic and myeloproliferative neoplasias and thrombocytopenia were analyzed at the Leipzig University Hospital. The area under the curve (AUC), occlusion start time (OST) and occlusion time (OT) of T-TAS were determined in patients with and without platelet transfusion. VWF activity and VWF antigen were measured and spearman correlation coefficient (ρ) between T-TAS and VWF parameters determined.

Results A total of 67 samples were collected from 28 patients before (n = 19), one hour after (n = 8) and 12 to 24 hours after platelet transfusion (n = 19), as well as 21 samples from patients without platelet transfusion. Median platelet count was 21 (range 3-63) Gpt/l. VWF antigen was determined in 57 and VWF activity in 47 samples, respectively.

Absence of occlusion in T-TAS was detected in samples with platelet counts < 21 Gpt/l, but 17 of 35 samples with platelet counts ≥ 21 Gpt/l showed occlusion, p < 0.001. From the 35 samples with platelet counts ≥ 21 Gpt/l, 19 were from patients receiving parenteral nutrition rich in lipids. Complete occlusion was seen in 5 (26.3 %) samples from patients with parenteral nutrition compared to 12 (75.0 %) in patients without parenteral nutrition, p = 0.007.

In samples with occlusion, there was no correlation between platelet count and AUC (ρ = 0.216), OST (ρ = -0,107) and OT (ρ = -0.306), but VWF antigen showed

a significant positive correlation with AUC (ρ = 0.685), a significant negative correlation with OST (ρ = -0.652) and OT (ρ = -0.718), while VWF activity was significantly correlated only with AUC (ρ = 0.661) and OT (ρ = -0.673).

Conclusion Our preliminary data suggest that occlusion in T-TAS HD-Chip in patients with myeloid neoplasia does not occur in patients with platelet counts < 21 Gpt/l. Parenteral nutrition seems to interfere with the occlusion of T-TAS HD chip. In patients with occlusion and a platelet count above 20 Gpt/l, VWF appears to have a greater impact on T-TAS than platelet count indicating that T-TAS reflects the interaction of von Willebrand factor with platelets in this cohort of patients.

Conflict of Interest The study was sponsored by an unrestricted grant from Fujimori Kogyo Co., Ltd. CP reports institutional grants and personal fees for lectures from Fujimori Kogyo Co., Ltd.

References

[1] Atari B, Ito T, Nagasato T et al. A modified microchip-based flow chamber system for evaluating thrombogenicity in patients with thrombocytopenia. Thromb J 2020; 18 (1): 3. doi:10.1186/s12959-020-00244-9

T-18-13 Evaluation of digital measuring device with an ADAMTS13 Activity Screening Test

Authors M. Griffiths, N. B. Binder

Institute Technoclone Herstellung von Diagnostika und Arzneimitteln GmbH, Vienna, Austria

DOI 10.1055/s-0044-1779227

Introduction The determination of von Willebrand Factor cleaving protease ADAMTS13 activity in plasma is an important diagnostic test to enable the differentiation of patients with thrombotic thrombocytopenic purpura (TTP) from those with other thrombotic microangiopathies (TMAs). It is critical to have a reliable ADAMTS13 Activity result within a quick reporting turn-around time to increase the possibility of a positive outcome of patient care, especially with TTP patients. The screening test has been described by Moore et al[1]. It is a rapid semi quantitative assay requiring no special laboratory equipment or highly skilled technician to perform the test. The operator determines the ADAMTS13 activity level by comparing the color (red) intensity of the 4 different levels (0, 0.1, 0.4 and 0.8 IU/mL) indicated on the color card verses the test well coloration. However, on relying in human interpretation from the colour card can potentially lead to variations in reported results. The aim wasto improve result interpretation by evaluating a small mobile measuring device to determine the colour intensity of the screen test. With a digital patient read out of the ADAMTS13 activity level, this should improve the consistency of result reporting, easy of use and confidence of patient results with the screening test [1].

Method To test the analytical performance of the measuring device in combination with the screening test, a three-way method comparison was performed. Patient and normal samples were assayed in a FRET assay, which was considered as the reference method and a Screening method with result interpretation using a colour card and a measuring device.

Results The repeatability using the measuring device for low sample and high sample was 2.4 & 0.4 % n = 10. Determining the colour intensity using the colour card (x3 operators) vs the measuring device value gave very comparable results. The calculated correlation betweenFRET vs Screening method/measuring device was $r^2 = 0.83$. The analytical performance of the FRET vs Screening method/device was calculated using a cut off value of ≤ 0.1 IU/mL and ≤ 20 respectively, resulting in a specificity of 89 % and sensitivity of 79 %.

Conclusion Our results showan excellent correlation between all 3 methods, suggesting the mobile measuring device would be an appropriate and reliable device for determining the ADAMTS13 Activity in the screening test. Therefore, patient results could be reported with confidence in timely manner aiding the rapid diagnosis TTP patients.

Conflict of Interest Both authors are employees of the affiliated company.

References

[1] Moore GW et al A multi-center evaluation of Technoscreen ADAMTS-13 activity assay as a screening tool for detecting deficiency of ADAMTS-13. J Thromb Haemost 2020; 18 (7): 1686–1694

T-18-14 LA detection with new taipan snake venom time and ecarin time reagents insensitive to warfarin, heparin and direct FXa inhibitors.

Authors M. Unterberger[1], G. W. Moore[2, 3], N. B. Binder[1]

Institutes **1** Technoclone Herstellung von Diagnostika und Arzneimitteln GmbH, Vienna, Austria; **2** Addenbrooke's Hospital, Specialist Haemostasis Unit, Cambridge, UK; **3** Middlesex University, Department of Natural Sciences, London, UK

DOI 10.1055/s-0044-1779228

Introduction Lupus anticoagulant (LA) detection by coagulation assays is compromised by other causes of elevated clotting times. Preponderance of thrombosis in the population warranting LA analysis leads to many requests for testing after initiation of anticoagulation, increasing the likelihood of false positive and negative interpretations in commonly used assays. Taipan snake venom time (TSVT) screening and ecarin time (ET) confirmatory assays are insensitive to vitamin K antagonist and direct factor Xa inhibitors, and all factor deficiencies except prothrombin.

Method TSVT/ET assays were performed on Ceveron s100 (Technoclone). TSVT ratio, ET ratio, % correction, and normalised screen/confirm ratio (NSCR) reference intervals (RI) were derived from 43 normal donors and calculated as ± 2 standard deviations of the mean. TSVT and ET ratios were derived using RI mean clotting time denominators. TSVT/ET analysis was undertaken on 16 plasmas from non-anticoagulated patients known to have LA, 1 normal and 1 positive control, 10 from warfarinised non-LA patients (INRs 1.90-4.69), 1 warfarinised patient with an LA (INR 4.1), and six non-LA plasmas containing anti Xa DOACs.

Results TSVT ratio, ET ratio, % correction and NSCR RIs all had Gaussian distributions, and upper limits for cut-offs were 1.12/1.09/10.9/1.11 respectively. 14/16 (87.5 %) non-anticoagulated LA-positive plasmas had elevated TSVT ratios (range 1.16-1.6) all with confirmed phospholipid dependence via elevated NSCRs (range 1.12-1.53). One of the normal TSVT ratio samples nonetheless returned an elevated NSCR of 1.14, indicating positivity with the integrated interpretive model. Normal control TSVT ratio was 1.06, and positive control TSVT ratio was 1.68 with NSCR of 1.31. All DOAC plasmas had normal TSVT ratios (range 0.90-1.09) and ET ratios (range 0.95-1.06) and no elevated NSCRs. 6/10 of non-LA warfarinised plasmas (INRs 1.90-2.26) had normal TSVT and ET ratios and NSCRs, whilst 4/10 (INRs 3.32-4.69) had elevated TSVT ratios (range 1.25-1.35) but correspondingly elevated ET ratios (range 1.28-1.39), and consequently, no false-positive interpretations as NSCRs were normal. The plasma from a warfarinised patient with an LA returned a TSVT ratio of 1.80 and NSCR of 1.16.

Conclusion Standard LA detection with dilute Russell's viper venom time and activated partial thromboplastin time is commonly compromised by anticoagulants, and affected by factor deficiencies to which each assay is sensitive. Although charcoal adsorbents can remove direct oral anticoagulants prior to analysis they have their own limitations, whilst TSVT/ET analysis offers a direct route to LA detection in many anticoagulated patients, and is affected by fewer factor deficiencies. TSVT screen has good LA-sensitivity, warranting consideration as first-line assay in appropriately anticoagulated patients before initiating other, less reliable strategies, to reduce anticoagulant interference, and can also be used in non-anticoagulated patients.

Conflict of Interest MU, NBB are employees of, GWM receives consulting honoraria from Tehcnoclone.

T-18-15 Follow-on products of pentosan polysulfate differ chemically from the original one and activate the contact system similar to the heparin falsification OSCS

Authors S. Alban[1], D. Lenhart[2], M.-T. Tischhöfer[1]
Institutes **1** Kiel University, Pharmaceutical Institute, Kiel, Germany; **2** bene pharmaChem GmbH & Co.KG, Research & Development, Geretsried, Germany; **3** Kiel University, Pharmaceutical Institute, Kiel, Germany
DOI 10.1055/s-0044-1779229

Introduction Pentosan polysulfate (PPS), a semi-synthetic, heparin-like polysaccharide with manifold therapeutic actions [1], is approved for treatment of bladder pain syndrome/interstitial cystitis in humans and treatment of musculoskeletal diseases in animals. PPS is produced by a complex procedure using beech wood as starting material and consists of a mixture of sulfated glucuronoxylans, whose structural composition can hardly be fully characterized by physicochemical analysis [2]. Meanwhile, several follow-on products of the originator PPS are offered.The question arises whether these are identical with the original PPS justifying an approval as generic drug or have to be considered biosimilars associated with specific requirements for approval [3].

The aim of this study was to investigate whether commercially available PPS products differ in physicochemical characteristics and biological effects from the original.

Method Five original PPS (O-PPS) preparations and 5 follow-on products (M-PPS) from different manufactures were analyzed using orthogonal analytical techniques including, inter alia, size exclusion chromatography with triple detection, NMR spectroscopy, and high-resolution mid-infrared spectroscopy in aqueous solution with chemometric evaluation. For functional analysis, we measured kallikrein generation in citrated plasma, FXII activation in buffer, and C5a generation in hirudin, citrated and EDTA plasma using heparin and oversulfated chondroitin sulfate (OSCS) as reference compounds.

Results The 5 M-PPS significantly differed in their composition and structure from the 5 O-PPS as well as among each other. The chemical differences were complex requiring the combination of various methods. The deviations in composition and structure are caused by multiple variables including (1) heterogeneities of the used biological starting material, (2) process-related changes of the polysaccharide structure, and (3) inhomogeneous sulfation.

In contrast to O-PPS and heparin, the 5 M-PPS caused strong kallikrein generation similar to OSCS. They induced FXII activation, partly even stronger than OSCS, which confirmed their contact system activating potency. In hirudin plasma, all test compounds inhibited C5a generation und thus complement activation. In citrated plasma, however, the OSCS and M-PPS did not inhibit, but increased C5a generation; the same was observed with M-PPS in EDTA plasma. Such C5a generation indicates stimulation of C5 cleavage independent of complement activation.

Conclusion The study revealed that the approval application for PPS follow-on products requires – as for other biosimilars – a stepwise comparison with the original PPS. This generally includes both highly sensitive, orthogonal physicochemical methods and conclusive biological assays as well as, if necessary, clinical studies [3]. Since heparin falsified with OSCS had caused severe adverse reactions [4, 5], the OSCS-like effects of the PPS follow-on products warrant further investigations on their safety.

Conflict of Interest D.L. is employee of bene pharmaChem GmbH, the manufacturer of original PPS.

References

[1] Smith M.M., Melrose J. Pentosan Polysulfate affords pleotropic protection to multiple cells and tissues. Pharmaceuticals (Basel, Switzerland) 2023; 16 (3): 437
[2] Alekseeva A., Raman R., Eisele G., Clark T., Fisher A., Lee S.L., Bertini S. In-depth structural characterization of pentosan polysulfate sodium complex drug using orthogonal analytical tools. Carbohydrate Polymers 2020; 234: Article 115913
[3] European Medicines Agency Biosimilars in the EU – Information guide for healthcare professionals. European Medicines Agency 2019
[4] Kishimoto T.K., Viswanathan K., Ganguly T., Elankumaran S., Smith S., Pelzer K., Sasisekharan R. Contaminated heparin associated with adverse clinical events and activation of the contact system. The New England Journal of Medicine 2008; 358 (23): 2457–2467
[5] Warkentin T.E., Greinacher A. Heparin-induced anaphylactic and anaphylactoid reactions: two distinct but overlapping syndromes. Expert Opinion on Drug Safety 2009; 8 (2): 129–144

T-18-16 Thrombin generation measured with ST Genesia in patients undergoing thrombophilia testing

Authors A. Okhrem[1], T. Siegemund[2], A. Siegemund[2, 1], S. Petros[1], H. Bönigk[2], C. Pfrepper[1]
Institutes **1** University Hospital Leipzig, Division of Hemostaseology, Department of Hematology, Cellular Therapy, Hemostaseology and Infectiology, Leipzig, Germany; **2** MVZ Limbach Magdeburg, Magdeburg, Germany
DOI 10.1055/s-0044-1779230

Introduction The thrombin generation (TG) assay is a global hemostatic assay to assess the tendency of plasma to form thrombin after the initiation of the coagulation cascade. The introduction of fully standardized TG assays supports its use in clinical routine. The aim of this study was to investigate TG with the fully automated ST Genesia system in a large cohort of patients undergoing thrombophilia testing.

Method A total of 689 individuals, who underwent thrombophilia screening between September 2020 and August 2021 at the outpatient coagulation center MVZ Limbach (Magdeburg, Germany), were included in the analysis. Thrombin generation was measured using The Thromboscreen assay on the ST Genesia system.

Results Females using combined oral contraceptives (COC) and pregnant women showed a significantly higher thrombin peak (TP) and endogenous thrombin potential (ETP) especially if thrombomodulin (TM) is added. Median TP and ETP measured with TM were 257 (IQR 149 – 324) and 1084 (IQR 620 – 1280) in women using COC, 267 (IQR 223 – 325) and 1161 [IQR 888 – 1390] in pregnant women compared to 103 (IQR 67 – 150) and 456 (IQR 301 – 661) in non-pregnant women not on COC, $p < 0.001$, respectively. Patients with antiphospholipid syndrome (APS) had a significantly prolonged lag time 2.6 (IQR 2.1 – 3.4) compared to patients without APS 2.4 (IQR 2.1 – 2.7) ($p = 0.002$) but TP and ETP were comparable.

After exclusion of patients with APS, pregnant females and females using COC, those with elevated plasma levels of factor (F) VIII, FIX and those with protein S deficiency had a significantly higher TP with and without TM than patients with normal levels of these parameters, while the other TG parameters showed different responses. Patients with Prothombin-G20210A-Mutation (PGM) had a significantly higher TP and ETP with and without TM compared to patients without thrombophilia. TP and ETP measured with TM but not without TM were higher in patients with Factor-V-Leiden Mutation (FVL) compared to the cohort without thrombophilia.

Conclusion TG measured with ST Genesia and the Thromboscreen application is very sensitive for the use of COC and pregnancy. In addition, patients with increased FVIII and FIX, protein S deficiency, PGM and FVL show elevated TG. These data provide the basis for further prospective studies with clinical outcome parameters to determine the predictive value of fully automated TG in patients at risk for thrombosis.

Conflict of Interest TS reports honoraria for lectures from SOBI, AS reports honoraria for lectures from SOBI, CP reports institutional grants for research and studies from Chugai/Roche, Takeda, Zacros, and LeoPharma, and honoraria for lectures or consultancy from Bayer, Biomarin, Chugai/Roche, CSL Behring, Novo Nordisk, Pfizer, BMS, SOBI, and Takeda.

T-18-17 Flow cytometry-based method to detect aggregates of non-muscle myosin IIA in erythrocytes

Authors M. Wolff[1], L. Swensson[1], J. Wesche[1], J. Klauke[1], T. Schöder[2], F. Perner[2], F. Heidel[3], A. Greinacher[1]

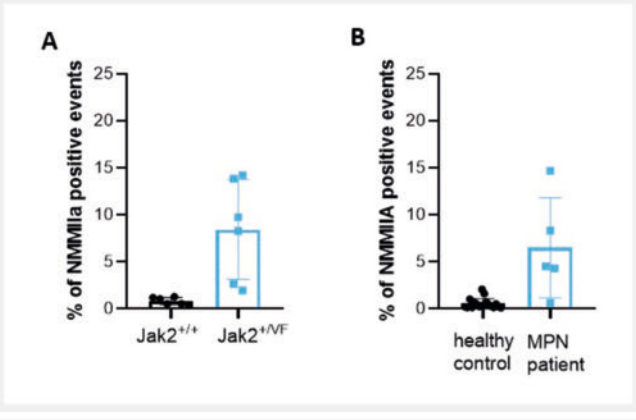
Institutes **1** Universitätsmedizin Greifswald, Institut für Transfusionsmedizin, Greifswald, Germany; **2** Universitätsmedizin Greifswald, Innere Medizin C, Greifswald, Germany; **3** Medizinische Hochschule Hannover, Klinik für Hämatologie, Hämostaseologie, Onkologie und Stammzelltransplantation, Hannover, Germany

DOI 10.1055/s-0044-1779231

Introduction Clonal hematopoiesis is caused by mutations of epigenetic regulators (e.g. TET2) or signaling proteins (e.g. JAK2). The clinical presentation is heterogeneous, ranging from clinically healthy individuals to myeloproliferative neoplasms (MPN). Clonal hematopoiesis is associated with a higher risk for cardiovascular events [1]. A parallel abstract describes the morphological changes of platelets and red cells in patients with MPN, that can be assessed by immunofluorescence microscopy on a blood smear. In this study and previous work [2], we showed that patients with disturbed erythropoiesis express aggregates of non-muscle myosin (NMMIIA) in erythrocytes. To facilitate the detection of these aggregates we developed a flow cytometry-based method.

Method We collected EDTA-anticoagulated whole blood from Jak2$^{+/VF}$ mice [3] and corresponding Jak2$^{+/+}$wildtype mice (WT) as well as samples from patients with genetically confirmed MPN and healthy controls. We prepared blood smears, fixed and stained them as described in [4] and screened them for aggregates of non-muscle myosin IIA in red blood cells. Simultaneously, the remaining whole blood samples were fixed, permeabilized and stained with anti-NMMIIA-AF647, anti-235a-PE and measured by flow cytometry. 235a-positive events (erythrocytes) were analyzed. A cut-off was created by measuring wild type mouse blood or healthy controls blood without NMMIIA-aggregates as assessed by immunofluorescence microscopy.

Results Approximately 10 times more NMMIIA-positive events were observed in erythrocytes of Jak2$^{+/VF}$ mice and patients with MPN compared to the corresponding controls. The mean percentage of NMMIIA-positive events in Jak2-mutant mice was 8.4 % vs 0.8 % in WT-mice. In patients with MPN and NMMIIA aggregates in immunofluorescence microscopy the mean percentage of NMMIIA-positive events was 6.5 % vs 0.5 % in healthy controls (▶ **Fig. 1**). Human blood samples that were stored in glycine buffered saline for 7 days provided comparable results to fresh samples.



▶ **Fig. 1 NMMIIA-aggregates in erythrocytes of mice [A] and humans [B] assessed by flow cytometry.** Jak2$^{+/+}$wildtype mice were compared to Jak2$^{+/VF}$ mice and healthy human controls were compared to patients with MPN-disease.

Conclusion We present a method to detect aggregates of non-muscle myosin IIA in erythrocytes by flow cytometry. The assay might be used as a fast and feasible screening test for impaired erythropoiesis in diseases such as MPN.

Conflict of Interest No conflict of interest

References

[1] Jaiswal S, Fontanillas P et al Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med 2014
[2] Vater L, Zaninetti C et al Aggregates of non-muscle myosin IIa in erythrocytes associate with GATA1-related thrombocytopenia. GTH abstracts 2023
[3] Mullally A, Lane SW et al. Physiological Jak2V617F expression causes a lethal myeloproliferative neoplasm with differential effects on hematopoietic stem and progenitor cells. Cancer Cell 2010
[4] Greinacher A, Pecci A et al Diagnosis of inherited platelet disorders on a blood smear: a tool to facilitate worldwide diagnosis of platelet disorders. J Thromb Haemost 2017; 15:

T-18-18 A method for real-time thrombin generation measurement in rat and porcine plasma

Authors S. Konrath[1], K. Klätschke[1], T. Renné[1, 2]

Institutes **1** University Medical Center Hamburg-Eppendorf, Institute of Clinical Chemistry and Laboratory Medicine, Hamburg, Germany; **2** Royal College of Surgeons in Ireland, Irish Centre for Vascular Biology, School of Pharmacy and Biomolecular Sciences, Dublin, Ireland; **3** Johannes Gutenberg University Medical Center, Center for Thrombosis and Hemostasis (CTH), Mainz, Germany

DOI 10.1055/s-0044-1779232

Introduction Large animals are predictive models for the analysis of thrombosis and haemostasis in patients. Real-time measurement of thrombin generation (TG) by calibrated automated thrombinography (CAT) is a highly sensitive and versatile method that allows comprehensive analysis of coagulation activating and inhibiting mechanisms in plasma samples.

Method TG measurements were based on the conversion of the thrombin-specific fluorogenic substrate Z-Gly-Gly-Arg-AMC measured at 390 nm emission and 460 nm excitation. Kaolin-stimulated TG was analysed in serial dilutions of porcine and rat plasma. $CaCl_2$ levels were also systematically tested in TG assays.

Results The factor XII contact activator kaolin induced TG in porcine and rat plasma, with short lag times < 1 sec using the established protocol for human plasma. Remarkably, baseline TG levels in unstimulated animal plasma were also high. Lowering the $CaCl_2$ concentration to 5 mM resulted in significant prolongation of lag times, allowing for a more accurate monitoring of TG in porcine and rat plasma. Following optimisation of the plasma volume, reaction temperature and $CaCl_2$ concentration used, we developed a CAT assay that allows the measurement of TG in porcine and rat plasma stimulated in a dose-dependent manner by different contact activators. The factor XII inhibitor infestin-4 fused to human albumin blocked contact activation-stimulated TG in porcine plasma. Inhibition of plasma kallikrein with aprotinin also reduced TG upon kaolin stimulation. Moreover, targeting factor Xa or thrombin with direct oral anticoagulants largely reduced TG driven by activated factor XII.

Conclusion We present a novel assay for the precise analysis of TG triggered by factor XII activation in rat and porcine plasma. Our method allows to measure coagulation in plasma samples of animals that have been exposed to medical devices such as stents and catheters, with relevance for patient safety.

Conflict of Interest The authors declare that there is no conflict of interest.

T-18-19 Thrombin generation with ST Genesia in patients with bleeding tendency

Authors J. D. Konkolewski[1], T. Siegemund[2], A. Siegemund[1, 2], S. Petros[1], H. Bönigk[2], C. Pfrepper[1]

Institutes **1** University Hospital Leipzig, Division of Hemostaseology, Department of Hematology, Cellular Therapy, Hemostaseology and Infectiology, Leipzig, Germany; **2** MVZ Limbach Magdeburg, Magdeburg, Germany

DOI 10.1055/s-0044-1779233

Introduction The thrombin generation (TG) assay is a global hemostatic assay that assesses the formation of thrombin in patients' plasma after stimulation

of the coagulation cascade. Several studies have investigated TG in patients with known bleeding disorders but the value of TG in the diagnosis of patients with unexplained bleeding tendency is still not well defined. The aim of this study was to evaluate TG for the diagnosis of patients with an unexplained bleeding tendency.

Method Patients visiting the outpatient coagulation center MVZ Limbach, Magdeburg, Germany between January 2020 and December 2022 with known bleeding disorders and patients examined due to an increased bleeding tendency were included. Plasma levels of clotting factors and other hemostatic-relevant parameters were measured for every patient. TG data were collected using the fully automated ST Genesia BleedScreen. ISTH-BAT score was used to assess the individual bleeding tendency.

Results A total of 1204 patients were identified. Known bleeding disorders were hemophilia A (HA, n = 35), hemophilia B (HB, n = 16), factor VII deficiency (n = 69), von Willebrand disease (n = 170), platelet function disorders (n = 129), other factor deficiencies (n = 46), and bleeding caused by anticoagulants (n = 39) while in 700 patients no underlying bleeding disorder could be identified.

In male HA patients with factor VIII activity below the reference range, there was a moderate but significant correlation (σ = 0.55-0.63) between FVIII and parameters of thrombin peak (TP), endogenous thrombin potential (ETP), and velocity index (VI). In HB patients there was a significant correlation only with the time to peak (TTP, σ = -0.77).

In the cohort of patients with bleeding tendency of unknown cause, women who were pregnant or used estrogen-containing contraceptives (ECC) had a significantly higher TP, ETP and VI with a shorter TTP.

For further analysis, pregnant women and women taking ECC were excluded. Of 590 patients with bleeding tendency of unknown cause, TG showed no pathological results in 339 patients, and no relation to the BAT score. In 128 patients, at least one TG parameter was found outside the normal range, mainly lag time (n = 112), followed by TTP (n = 38), TP (n = 35), ETP (n = 32), and VI (n = 28).

Conclusion TG shows moderate correlation in patients with hemophilia. In addition, it may help to detect some patients with unexplained bleeding tendency.

Conflict of Interest TS reports honoraria for lectures from Sobi, AS honoraria for lectures from Sobi, CP reports institutional grants for research and studies from Chugai/Roche, Takeda, Zacros, and LeoPharma, and honoraria for lectures or consultancy from Bayer, Biomarin, Chugai/Roche, CSL Behring, Novo Nordisk, Pfizer, BMS, SOBI, and Takeda.

T-19. Blood components and plasma concentrates

T-19-01 Erythropoietin and thrombopoietin release kinetics in clinical settings

Authors F. Wenzel[1], M. Hauser[1], L. Saemann[2, 1], L. Toll[1, 3]

Institutes **1** Furtwangen University, Faculty of Medical and Life Sciences, Villingen-Schwenningen, Germany; **2** University Hospital Halle (Saale), University of Halle, Department of Cardiac Surgery, Halle (Saale), Germany; **3** German Sport University Cologne, Institute of Biochemistry/ Center for Preventive Doping Research, Cologne, Germany

DOI 10.1055/s-0044-1779234

Introduction During bone marrow donation (BMD), peripheral blood stem cell apheresis (PBSC) and cardiac artery bypass grafting (CABG) alterations in erythrocytes and thrombocytes count could be observed. These alterations of blood cell count might have an influence on hematopoiesis regulating factors. Aim of this study was to compare erythropoietin (EPO) and thrombopoietin (TPO) concentrations, as well as hemoglobin concentration (HB) and platelet count (PLT) in BMD, PBSC and in CABG.

Method We collected blood samples from a total of 24 donors and patients. Quantification of EPO and TPO was performed via enzyme-linked immunosorbent assay (ELISA).

Results In BMD EPO concentration increased first one day after the operation, although HB was significantly decreased immediately after the intervention. PLT count significantly decreased after the operation. TPO progression appeared inverse to PLT count. In CABG patients, EPO release seemed to be dependent on kidney function.

Conclusion Stimulation for EPO release seems to be deferred in BMD and TPO concentration seems to have a maximum accumulation rate if baseline level of PLT´s is already low before PBSC. EPO and TPO concentrations could be a helpful marker in clinical settings for evaluation of blood cell regeneration.

Conflict of Interest There are no conflicts of interest.

T-19-02 Apoptosis Inhibition in Cold-stored Platelet Concentrates Preserves Thrombus Formation

Authors J. Kirschall[1], I. Marini[1, 2], L. Pelzl[1], A. Witzemann[1], N. Wolska[1], T. Bakchoul[1, 2]

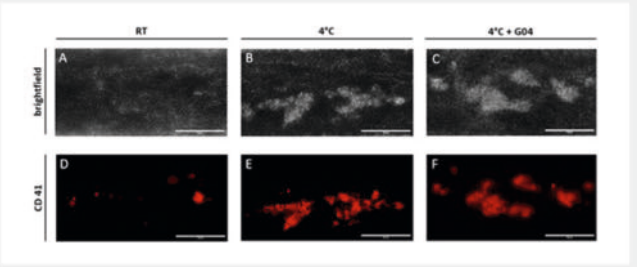
Institutes **1** Institute for Clinical and Experimental Transfusion Medicine, Medical Faculty, Tuebingen, Germany; **2** Center for Clinical Transfusion Medicine GmbH, Tuebingen, Germany

DOI 10.1055/s-0044-1779235

Introduction Transfusion of platelet concentrates (PCs) is an essential medical approach to treat or prevent bleeding in patients with impaired platelet function or after injury. Nevertheless, the standard storage of PCs at room-temperature (RT) is associated with an increased risk of bacterial contamination as well as reduced platelet functionality. We previously showed that cold-stored PCs have better functionality compared to RT-stored PCs but reduced *in vivo* circulation time due to cold-induced apoptosis. To investigate cold-stored PCs hemostatic functionality upon apoptosis inhibition, we established an *ex vivo* model simulating physiological blood flow to evaluate the contribution of cold-stored PCs to thrombus formation.

Method PCs were stored for 1, 4, 7 and 10 days at 4 °C or RT either with or without the apoptosis inhibitor G04 (RhoA GTPase inhibitor). Next, PCs were stained with CD41 antibody and incubated with TRAP6 (thrombin receptor-activating peptide 6) to stimulate thrombus formation. Platelet-depleted whole blood samples that mimic thrombocytopenic patients were spiked-in with PCs and applied to the *ex vivo* system under physiological shear flow. Afterwards, the resulting thrombi were imaged. Furthermore, viscoelastic measurements, impedance aggregometry and thrombin generation assay were performed.

Results Platelets from 24h storage PCs showed stable thrombus formation upon activation with TRAP6. Unstimulated platelets did not form any thrombi during the entire period of perfusion. To evaluate the effect of cold storage on platelet functions we analyzed thrombus formation of PCs stored at RT and 4 °C. Cold-stored platelets tend to form larger clots under flow conditions and maintain higher functionality *in vitro* compared to PCs stored at RT. In addition, incubation of cold-stored PCs with the apoptosis inhibitor G04 improved *ex vivo* thrombus formation compared to PCs stored without the inhibitor (▶ **Fig. 1**).



▶ **Fig. 1** Ex vivo thrombus formation of RT and 4 °C (with apoptosis inhibitor G04). Representative brightfield (upper panel) and immunofluorescence (lower panel) pictures of ex vivo thrombus formation. PCs, stored at RT (A and D), 4 °C (B and E) or 4 °C + G04 (C and F) were perfused through microfluidic channels, upon TRAP6 stimulation, for 5 minutes. Red signal: CD41 antibody. Scale bar: 200 µm.

Conclusion The results indicate that our *ex vivo* assay, which simulates PC transfusion in thrombocytopenic patients, is a suitable model to test the hemostatic functions of PCs under physiological flow conditions. Our results indicate that PCs stored at 4 °C show better thrombus formation ability upon stimulation by agonists compared to RT-stored PCs. Moreover, PCs ability to form clots under shear stress is still preserved after incubation with the apoptosis inhibitor.

Conflict of Interest The authors declare no conflict of interest. T. Bakchoul and I. Marini have a pending patent related to cold-storage of platelets.

T-19-03 Cold stored platelets –investigations of thrombus formation in a microfluidic system

Authors N. Wolska[1], J. Kirschall[1], I. Marini[1, 2], A. Witzemann[1], T. Bakchoul[1]
Institutes **1** Medical Faculty and University Hospital of Tuebingen, Instlinical and Experimental Transfusion Medicine, Tuebingen, Germany; **2** Medical Faculty and University Hospital of Tuebingen, Center for Clinical Transfusion Medicine, Tuebingen, Germany
DOI 10.1055/s-0044-1779236

Introduction Platelet concentrates (PCs) are used to treat or prevent bleeding in patients with impaired platelet function or after injury. Cold storage was shown to better preserve platelet function, over the current standard – room temperature (RT) storage *in vitro*. Our aim is to establish an *ex vivo* model to evaluate the contribution of PCs in thrombus formation and to investigate the effect of different storage conditions on platelet functions.

Method For the *ex vivo* modelling the microfluid channels were coated with collagen and blocked with human serum albumin. Next, PCs stored for 24h at RT were incubated with labelled with Calcein-AM. Platelet-depleted whole blood samples from healthy donors, that mimic thrombocytopenia, were spiked-in with PCs and recalcified. TRAP-6 (thrombin receptor-activating peptide 6) was added to initiate thrombus formation and reconstituted samples were immediately run through microchannels at physiological shear forces, and immunofluorescence snapshots were taken. Additionally, PCs were stored for 1, 4, 7 and 10 days either at RT or at 4 °C. Samples were then stained, recalcified and applied to the *ex vivo* system as well as other standard laboratory tests.

Results Platelets from PCs, after reconstitution, showed stable thrombus formed upon incubation with TRAP-6, while unstimulated PC did not form any thrombi (% surface area coverage, mean ± SD: 1.860 ± 8.844 vs. 10.70 ± 2.255 when activated, p = 0.0172). To evaluate the effect of cold storage on platelet functions we analyzed the extent of thrombus formation of PCs stored at RT and 4 °C upon florescent staining. Our results indicate that cold-stored platelets tend to form larger clots under flow conditions after TRAP-6 activation compared to PCs stored at RT (Figure. 1). This finding is also supported by standardized *in vitro* laboratory testing.

Conclusion The results indicate that our *ex vivo* assay, which simulates PC transfusion in thrombocytopenic patients, is suitable to test the hemostatic functions of PCs under physiological flow conditions. Moreover, it allows to investigate cold-induced effects on platelet functions during storage time and indicates improved preservation of platelet functionality in cold storage.

Conflict of Interest TB, MI – patent pending (cold storage of platelets)

T-19-04 Andexanet alfa versus 4-factor prothrombin complex concentrate for factor Xa inhibitor reversal in patients with intracranial hemorrhage; a systematic review and meta-analysis

Authors C. Chai-Adisaksopha[1], T. Tangsricharoen[2], T. Attachaipanich[1]
Institutes **1** chaing mai University, Department of Internal Medicine, chiang mai, Thailand; **2** Payao University, Department of Pediatrics, Payao, Germany
DOI 10.1055/s-0044-1779237

Introduction Andexanet alfa is a specific antidote for factor Xa inhibitors. However, its availability is restricted in various countries, leading to the utilization of 4-factor prothrombin complex concentrate (4F-PCC) as an alternative. This study aims to compare the efficacy of andexanet alfa versus 4F-PCC in patients with factor Xa inhibitor-associated intracranial hemorrhage (ICH).

Method We conducted a search of MEDLINE, SCOPUS, and CENTRAL databases, from inception to July 2023. We focused on prospective or retrospective cohort studies that included patients with ICH resulting from factor Xa inhibitor and who were treated with either andexanet alfa or 4F-PCC. Data extraction was carried out independently by two reviewers, and cross-checking was performed. We calculated the random-effects models to estimate the odds ratio (OR) along with corresponding 95 % confidence intervals (CIs). The main outcomes were the efficacy of hemostatic control, the occurrence of thrombotic complications, and all-cause mortality.

Results A total of 13 studies were included, involving patients with a median age ranging from 71 to 85 years. The primary indication for anticoagulation in these patients was atrial fibrillation, accounting for 73 % to 94 % of patients in each study. The reported sites ICH included intraparenchymal, subdural hemorrhage, and subarachnoid hemorrhage. Good or excellent hemostatic control was reported in 182 of 237 patients receiving andexanet alfa (76.8 %), whereas 166 of 241 patients receiving 4FPCC (68.9 %), OR 1.57 (1.00 to 2.45), I2 = 0 %. The overall mortality was 24.7 % in andexanet alfa group and 29.5 % in 4FPCC group (OR 0.66, 95 %CI 0.37 to 1.19, I2 = 46 %). Thromboembolic complication was reported 10.3 % in andexanet group and 10.9 % in 4FPCC group (OR 1.05, 95 %CI 0.55-1.99, I2 = 0 %).

Conclusion Andexanet alfa demonstrated significantly higher hemostatic control compared to 4F-PCC in patients with factor Xa inhibitor-associated ICH. Additionally, there was a numerical trend towards a lower risk of death in patients treated with andexanet alfa. The use of andexanet alfa did not result in an increased risk of thrombotic complications compared to 4F-PCC.

Conflict of Interest I have no conflcit of interest.

T-20. Vascular wall biology and disorders

T-20-01 Small intestinal villus vascularization is driven via an epithelial-to-endothelial PAR1 signaling axis

Authors M. Mimmler[1, 3], O. Dremova[1], A. Mann[1], F. Malinarich[1], N. Vergnolle[2], J.-P. Motta[2], I. Kalte[1], K. Mammadova[1], G. Pontarollo[1, 3], C. Reinhardt[1, 3]
Institutes **1** University Medical Center of the Johannes Gutenberg University Mainz, Center for Thrombosis and Hemostasis (CTH), Mainz, Germany; **2** University Toulouse III-Paul Sabatier, INSERM, Digestive Health Research Institute (IRSD), Toulouse, France; **3** These authors contributed equally, Mainz, Germany
DOI 10.1055/s-0044-1779238

Introduction Thrombin (*F2*) expression was recently reported in intestinal epithelial cells (IEC) impacting on microbial spatial organization and intestinal injuries. The thrombin receptor protease-activated receptor-1 (PAR1/F2r) is also expressed on IECs and in the context of inflammatory bowel disease, its activation is linked to disorders in gut mucosal healing. Interestingly, the gut microbiota can influence intestinal villus vascularization, required for nutrient harvest, in a tissue factor/PAR1-dependent fashion. Here, we aim to unravel the underlying epithelial-endothelial-crosstalk between epithelial thrombin/PAR1 and small intestinal villus vascularization.

Method Mice deficient for intestinal epithelial *F2* ($F2^{\Delta IEC}$) or *F2r* ($F2r^{\Delta IEC}$) and endothelial *F2r* ($F2r^{\Delta EC}$) were generated by crossing floxed *F2r* mice with a mouse line expressing Cre-recombinase under the control of the Villin- (IEC) or VE-Cadherin5-promotor (EC). Floxed mice that did not express Cre-recombinase were used as controls. Experimental groups were sex and age matched.

Hämostaseologie 2024; 44: S1–S125

Paraffin sections of the mid small intestine were stained by immunofluorescence for the endothelial cell marker CD31 to quantify the vascularized area in the small intestinal villus structures.
RNA extraction was performed on isolated IECs and whole small intestinal tissue, and converted to cDNA by reverse transcription. Gene expression was analyzed by qPCR using the delta delta Ct method.
Immunophenotyping of small intestinal lamina propia was conducted by flow cytometry. Samples were cleaned, Peyer's patches excised, IECs removed, and remaining tissue was digested to obtain single cell suspensions that were stained with fluorescent antibodies against different immune cell markers.
Results $F2^{\Delta IEC}$ and $F2r^{\Delta IEC}$ mice showed a decrease in vascular density of small intestinal villus structures. Expression of LYVE1, a marker for lymphatics, CD31, a panendothelial marker and eNOS, an endothelial enzyme involved in angiogenesis, was downregulated in the small intestine of $F2r^{\Delta IEC}$ mice. Additionally, this knockout altered the expression of several genes in the IEC compartment, including PDGF-B, which is involved in angiogenesis. In contrast, $F2r^{\Delta EC}$ mice did not show a decreased villus vascularization. Also, epithelial PAR1-deficiency did not affect the abundance of immune cell populations in the gut.
Conclusion Our results indicate that thrombin expression by the gut epithelium affects small intestinal villus vascularization. This is likely due to PAR1 signaling, since intestinal epithelial thrombin and PAR1-deficiency resulted in reduced vascularization. Taken together, our findings strengthen the hypothesis, that PAR1 signaling in intestinal epithelial cells is involved in vascular remodeling of villus capillaries.
Conflict of Interest The authors claim no conflict of interest.

T-20-02 The role of DUOX2 and intestinal epithelial TLR2 in villus vascularization

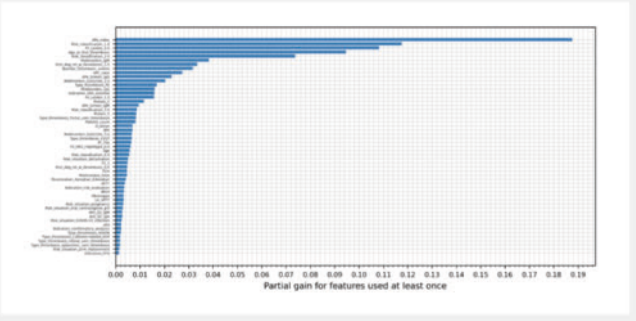
Authors N. B. Paeslack[1], A. Mann[1], F. Sommer[2], C. Reinhardt[1, 3]
Institutes **1** University Medical Center Mainz, Center for Thrombosis and Hemostasis (CTH), Mainz, Germany; **2** University of Kiel, Institute of Clinical Molecular Biology, Kiel, Germany; **3** German Center for Cardiovascular Research (DZHK), Partner Site RhineMain, Mainz, Germany
DOI 10.1055/s-0044-1779239
Introduction Recent research has highlighted the crucial role of the microbiota in shaping postnatal gut development and physiology. In the small intestine, the microbiota significantly influences the remodeling of villus capillary networks, which is essential for dietary-derived nutrient transport, through epithelial-to-endothelial crosstalk. The dual oxidase-2 (DUOX2) exerts microbicidal functions in the intestinal epithelium comprising an essential regulator of the microbiome-host interaction. Here, we provide insights on how DUOX2 and Toll-like receptor-2 (TLR2), which has been shown to impact intestinal epithelial cell renewal and angiogenesis, influence villus vascularization in the small intestine mediating microbiota-host interplay. Understanding these complex interactions offers insights into the intricate mechanisms behind gut morphogenesis and vascular network regulation.
Method In this study, we investigated two distinct mouse models: Mice with a global deficiency of DUOX2 as well as mice with an epithelial-specific deficiency of TLR2. To assess villus vessel density, immunofluorescence staining of vascular markers in the mid small intestine were performed. Additionally, quantitative real-time PCR (qRT-PCR) analyses were conducted to quantify the expression of vascular markers, *Duox2*, and its maturation factor *Duoxa2*. These experiments uncovered how the absence of TLR2 and DUOX2 impacts the regulation of vascular development in the gut, providing valuable information about the underlying mechanisms involved in this process. 16S sequencing analyses were performed for small intestinal content determining the microbial abundancies.
Results Mice lacking DUOX2 activity exhibited a notable reduction in vascularization in the small intestine, as well as an altered microbial community. This decrease in vascularization was accompanied by a decrease in *Duox2* and *Duoxa2* mRNA expression, indicating a critical role of these genes in regulating vascular development in the gut. Conversely, mice with an intestinal epithelial-specific deficiency of TLR2 displayed an increase in villus vascularization along with elevated expression levels of the vascular marker platelet and endothelial cell adhesion molecule 1 (*Pecam1*), while no alterations were found in the lymphatic vascular marker lymphatic vessel endothelial hyaluronan receptor 1 (*Lyve1*). Notably, this heightened vascularization was accompanied by an increase in *Duox2* expression in the small intestine, suggesting a complex interplay between TLR2, DUOX2, and vascular development in mucosal vascularization.
Conclusion We propose that small intestinal epithelial DUOX2, which is a known regulator of microbial colonization in the gut, is involved in the epithelial-to-endothelial crosstalk, influencing villus vascularization in a microbiota-dependent fashion. Further, we identified intestinal epithelial TLR2 as a potential modifier of vascular remodeling.
Conflict of Interest The authors declare no conflicts of interest in relation to this work.

T-21. Various Topics

T-21-01 Evaluation of an Explainable Tree-based AI Model for Optimizing Outpatient Thrombophilia Diagnosis and Thrombosis Risk Stratification

Authors H. L. McRae[1], F. Kahl[2], M. Kapsecker[2, 3], H. Rühl[1], S. M. Jonas[2], B. Pötzsch[1]
Institutes **1** University Hospital Bonn, Institute for Experimental Hematology and Transfusion Medicine, Bonn, Germany; **2** University Hospital Bonn, Institute for Digital Medicine, Bonn, Germany; **3** Technical University of Munich, Department of Computer Science, Munich, Germany
DOI 10.1055/s-0044-1779240
Introduction Thrombophilia diagnosis can be convoluted, involving clinical data analysis, specialized laboratory testing, and high-level decision-making. The diagnostic process can vary depending on individual differences in clinical practice, institutional guidelines, and resource availability, which may affect clinical outcomes. This in turn provides a potential opportunity for optimization using AI.
Method We retrospectively evaluated an AI-powered algorithm optimized to replicate the process of thrombophilia diagnosis. Electronic medical record (EMR) data were assessed from 256 patients who were referred for thrombophilia evaluation at our ambulatory hemostasis clinic from 11/2019 to 2/2023. Thrombophilia diagnosis was made based on a personal and/or family history of thrombosis as well as results of extensive thrombophilia testing. XGBoost, a gradient boosting algorithm for supervised learning, was used to perform a randomized search over a predefined set of tree parameters to find a well-performing configuration of the data using cross-validation. The dataset contained 12 clinical and 26 laboratory data parameters. The target variable consisted of the thrombophilia probability score (TPS) and thrombophilia risk factors (TRF). TPS were calculated based on clinical data from the following criteria: one point assigned each for a) spontaneous thrombotic event; b) mild risk situation; c) recurrent thrombosis; d) atypical thrombosis localization; e) age < 50 years at first thrombosis; f) family history of thrombosis and/or first degree relative with established thrombophilia. A score of 0 points corresponded to "unlikely thrombophilia"; 1-2 "thrombophilia cannot be excluded"; 3 "likely thrombophilia"; and 4 + "most likely thrombophilia". TRF were categorized by a scale of 0-3 indicating no (0), low (1), intermediate (2), and high risk (3), respectively.
Results 250 patients were included; 6 were excluded due to lack of sufficient data. The algorithm revealed decisive factors for the detection of thrombophilia and suggested modified thresholds (e.g. age at first thrombosis was used 20 times with a mean of 47.25 years and a standard deviation of 10.63) for an objective and standardized diagnostic procedure. The contribution of clinical and laboratory features in the overall algorithm performance varied consider-

ably (see ▶ **Fig. 1**). The dataset was divided into three subsets: 200 train, 25 validation, and 25 test data, ensuring a representative distribution across the evaluation process. In the evaluation, a misclassification of ± 1 was assumed to be no error. The model ultimately showed a sensitivity of 100 % and specificity of 100 % for TPS, and a sensitivity of 75 % and specificity of 98 % for TRF (▶ **Fig. 2**) for an error tolerance of 1 off.



▶ **Fig. 1** Gain metric showing the relative contribution of each clinical and laboratory feature to overall performance of the algorithm. Figure generated by means of the data provided by XGBoost.

Thrombophilia Probability Score	*Sensitivity*	*Specificity*	*Precision*
0: Thrombophilia Unlikely	1	1	1
1-2: Thrombophilia Cannot be Excluded	1	1	1
3: Thrombophilia Likely	1	1	1
4: Thrombophilia Most Likely	1	1	1
Overall Accuracy	100%	100%	100%
Thrombophilia Risk Factors	*Sensitivity*	*Specificity*	*Precision*
0: No Risk	1	1	1
1: Low Risk	1	0.92	0.33
2: Intermediate Risk	1	1	1
3: High Risk	0	1	-
Overall Accuracy	75%	98%	78%

▶ **Fig. 2** Accuracies for thrombophilia probability score and thrombophilia risk factors with an error tolerance of 1.

Conclusion Our results show the potential for tree-based models to optimize and standardize thrombophilia diagnosis and thrombosis risk stratification. Furthermore, medical professionals can gain helpful insights from our model due to its explainable nature. We expect that the algorithm will show significant sensitivity and specificity for the TRF when we repeat the tests with a larger data set (currently ongoing).

Conflict of Interest The authors have no conflicts of interest to disclose.

T-21-02 Gregor Johann Mendel – ways to the genome of the founder of genetics

Authors S. Pospisilova[1, 2], D. Fialova[3], F. Pardy[1], M. Doubek[1, 2], E. Drozdova[3]
Institutes **1** Masaryk Univerzity, CEITEC, Brno, Czech Republic; **2** Masaryk Univerzity, Faculty of Medicine, Brno, Czech Republic; **3** Masaryk Univerzity, Faculty of Science, Brno, Czech Republic
DOI 10.1055/s-0044-1779241

Introduction Gregor Johann Mendel – a multitalented scientist, considered the “father of modern genetics”, was born in 1822 and recent bicentennial anniversary of his birth provided a unique opportunity to remind the legacy of this great scientist. Mendel made the key discoveries in the Augustinian Abbey in Brno, Czech Republic, but a key role enabling his discoveries played his knowledge of mathematics, physics and statistics, which he obtained at the University of Vienna, Austria. Application of his interdisciplinary knowledge and tremendous experimental work involving a hybridization of about 28,000 pea plants led to a formulation of the generally accepted principles of heredity.

Method We have initiated a multidisciplinary project focused on the search for the Mendel´s genome. To find his biological material, we have performed an archeological research of the Augustinian tomb in Brno followed by the anthropological and genetic analysis of the found remains. In parallel, we have analysed Mendel´s personal belongings in the Abbey and succeeded to isolate his DNA, that we have used for an identification of the remains.

Results Using a comparison of mitochondrial DNAs isolated from the remains and from his personal belongings stored in the Abbey we have identified the remains of G.J.Mendel. Whole genome sequencing of DNA isolated from the teeth enabled us to obtain very exciting novel findings on Mendel´s personality and to study his genetic predispositions. We have identified a, autosomal dominant mutation that was probably responsible for his cardiological problems and early death. In addition, the genome analysis brought novel findings on Mendel’s blood group, physique, ancient ancestors, taste preferences and many other interesting details.

Conclusion We believe that the story of Mendel’s life and novel findings on his genetic predispositions and even so called "Mendelian disorders" have brought interesting information on this humble, but genious scientist, important not only for the Brno and Vienna, where he lived, studied and worked, but for the whole world.

Conflict of Interest Authors declare no conflict of interest.

T-21-03 Relevance of the vWF C4 domain for gain-of-function variants

Authors A. Mojzisch[1, 2], N. Remmert[2], S. Schneppenheim[3], V. Huck[1], S. W. Schneider[1], M. A. Brehm[2]
Institutes **1** University Medical-Center Hamburg-Eppendorf, Institute for Dermatology and Venerology, Hamburg, Germany; **2** University of Siegen, School of Life Sciences, Department of Digital Health Sciences and Biomedicine, Siegen, Germany; **3** MEDILYS Laborgesellschaft mbH, Haemostaseology, Hamburg, Germany
DOI 10.1055/s-0044-1779242

Introduction Von Willebrand Factor (vWF) is an essential protein in hemostasis. It consists of multiple domains and assembles into multimers, which circulate in peripheral blood stream. Upon exposure to increased shear forces at sites of vessel injury, vWF binding to platelets is induced via GPIbα and later stabilized by binding of vWF-C4-domian to GPIIb/IIIa. Dysfunction and loss of vWF can thus cause bleeding symptoms. However, hyperactivity caused by gain-of-function (GOF) variants has also been identified in recent years. We have focused on two published vWF variants in the C4 domain exhibiting either higher shear sensitivity (p.Phe2561Tyr) [1] or increased aggregate formation (p.Pro2555Arg) [2]. Both variants are assumed to affect structure and function of the vWF stem region, especially with respect to interaction with platelet GPIIb/IIIa. Hence, variants with homologous amino acid substitutions in other vWF C domains were investigated for their thrombotic potential.

Method Based on a sequence alignment of all vWF C domains [3], we identified matching positions for each variant. The positions were mutated by site-directed mutagenesis to either arginine or tyrosine. Additionally, amino acid substitutions listed in the SNP data bank ensemble were considered, resulting in the following five variants to be examined: p.Pro2302Arg/Thr in C1, p.Pro2373Arg/Ser in C2 and p.Phe2481Tyr in C3 domain. Additionally, we investigated amino acid substitution p.Tyr2631Phe in C5 as it may have a reverse effect to p.Phe2561Tyr. General hemostatic parameters such as secretion, multimerization and binding capacities to GPIbα, GPIIb/IIIa and collagen were measured. Moreover, activity of vWF variants was investigated under flow conditions by using light transmission aggregometry (LTA) and microfluidics.

Results For the p.Pro2373X variants, a faster running behavior was observed in multimer analysis pointing towards structural alterations. For most variants, secretion and binding parameters under static conditions were not significant-

Hämostaseologie 2024; 44: S1–S125

ly changed compared to wtvWF. In the LTA agglutination assay, four variants were less responsive to low Ristocetin concentration compared to wtvWF. For p.Pro2302Thr and p.Phe2481Tyr, increased vWF-platelet-complex formation was found only in 1 of 3 experiments.

Conclusion The aim of this study was to investigate, whether the GOF effects induced by p.Phe2561Tyr and p.Pro2555Arg also occur due to homologous amino acid exchanges in other C domains. Therefore, equivalent variants in C1, C2, C3 and C5 were analyzed. None of them exhibited a GOF effect as strong as the C4 variants. Rather, flow experiments show a reduced force responsiveness and lower platelet binding for some variants. The results indicate that despite of a high sequence identity among vWF C domains, homologous mutations in C domains other than C4 do not seems to induce the same GOF effects as C4 domain variants strengthening the hypothesis that the GOF arises from mutational effects on GPIIb/IIIa binding by vWF.

Conflict of Interest None.

References

[1] Schneppenheim R, Hellermann N, Brehm MA, Klemm U, Obser T, Huck V, Schneider SW, Denis CV, Tischer A, Auton M, März W, Xu ER, Wilmanns M, Zotz RB 'The von Willebrand factor Tyr2561 allele is a gain-of-function variant and a risk factor for early myocardial infarction'. Blood 2019; 133 (4): 356–365

[2] Huck V, Chen PC, Xu ER, Tischer A, Klemm U, Aponte-Santamaría C, Mess C, Obser T, Kutzki F, König G, Denis CV, Gräter F, Wilmanns M, Auton M, Schneider SW, Schneppenheim R, Hennig J, Brehm MA. Gain-of-Function Variant p.Pro2555Arg of von Willebrand Factor Increases Aggregate Size through Altering Stem Dynamics. Thromb Haemost 2022; 122 (2): 226–239

[3] Zhou YF, Eng ET, Zhu J, Lu C, Walz T, Springer TA 'Sequence and structure relationships within von Willebrand factor'. Blood. 2012; 120 (2): 449–58

T-21-04 Changes in the Proteome of Platelets from Patients with Critical Progression of COVID-19

Authors M. Zimmermann[1], M. Wolny[1], S. Rozanova[2, 3], C. Knabbe[1], T. Flieder[1], A. Hohbein[1], K. Pfeiffer[2, 3], K. Barkovits[2, 3], K. Marcus[2, 3], I. Birschmann[1]

Institutes **1** Herz- und Diabeteszentrum NRW, Institute of Laboratory and Transfusion Medicine (ILTM), Bad Oeynhausen, Germany; **2** Ruhr-University Bochum, Medizinisches Proteom-Center, Medical Faculty, Bochum, Germany; **3** Ruhr-University Bochum, Medical Proteome Analysis, Center for Protein Diagnostics (ProDi), Bochum, Germany

DOI 10.1055/s-0044-1779243

Introduction Platelets are small, non-nucleated cell types that play a central role in hemostasis. In addition, they are important in supporting the immune system during virus infections. Overactivation of platelets has been reported for severe corona virus disease 2019 (COVID-19), resulting in the release of granules, exacerbating inflammation and contributing to a cytokine storm. Aim of this study was to comprehensively investigate the role of platelets in COVID-19 progression and to identify predictive biomarkers for disease outcome (Wolny et al., 2023) [1–2].

Method Mass spectrometry-based proteomic profiling of highly purified platelets from critically diseased COVID-19 patients with different outcomes (survivors and non-survivors) and age- and sex-matched controls was conducted. Potential biomarker candidates were further validated in both platelets and plasma using two independent methods including targeted mass spectrometry based on parallel reaction monitoring (PRM) and ELISA.

Results Platelets from severe COVID-19 patients showed significant differences in the abundance of proteins associated with protein folding, degranulation, cytokine and cell signaling. In addition, a number of proteins with isomerase activity were found to be more abundant in patient samples, apparently exerting an influence on platelet activity via the non-genomic properties of the glucocorticoid receptor (GR) and the nuclear factor κ-light-chain-enhancer of activated B cells (NFκB). Moreover, carbonic anhydrase 1 (CA-1) was identified as potential marker protein in platelets, showing a significant increase in COVID-19 patients.

Conclusion *Ingvild Birschmann received speaker's honoraria from Aspen Germany GmbH, Bristol-Myers Squibb/Pfizer, Siemens Healthcare and CSL Behring and reimbursement for congress traveling and accommodation from aspen and performed contract research for Siemens Healthcare. Ingvild Birschmann is a member of the advisory board of LFB biomedicaments, Siemens Healthcare and CSL Behring. Mathias Zimmermann, Monika Wolny, Andrea Hohbein, Tobias Flieder and Cornelius Knabbe have nothing to disclose.*

Conflict of Interest *Ingvild Birschmann received speaker's honoraria from Aspen Germany GmbH, Bristol-Myers Squibb/Pfizer, Siemens Healthcare and CSL Behring and reimbursement for congress traveling and accommodation from aspen and performed contract research for Siemens Healthcare. Ingvild Birschmann is a member of the advisory board of LFB biomedicaments, Siemens Healthcare and CSL Behring. Mathias Zimmermann, Monika Wolny, Andrea Hohbein, Tobias Flieder and Cornelius Knabbe have nothing to disclose.*

References

[1] Birschmann I., Mietner S., Dittrich M., Pfrang J., Dandekar T., Walter U. Use of functional highly purified human platelets for the identification of new proteins of the IPP signaling pathway. Thromb. Res. 2008; 122: 59–68

[2] Wolny M., Rozanova S., Knabbe C., Pfeiffer K., Barkovits K., Marcus K., Birschmann I. Changes in the Proteome of Platelets from Patients with Critical Progression of COVID-19. Cells 2023; 12: 2191. doi:10.3390/cells12172191

T-21-05 PAR4 activation promotes osteoclast formation and resorptive function

Authors T. Strini, S. Preinstorfer, H. Haidl, S. Tokic, S. Gallistl, A. Schlagenhauf

Institute Medical University of Graz, Department of General Pediatrics and Adolescent Medicine, Graz, Austria

DOI 10.1055/s-0044-1779244

Introduction Osteoclastogenesis involves several steps: proliferation of precursors, early differentiation into mononuclear cells, and fusion into multinucleated osteoclasts. RANKL (receptor activator of nuclear factor kB ligand), produced by osteoblasts, is the key regulator. Thrombin, the key enzyme of coagulation enhances the release of osteoclastogenic factors by osteoblasts but paradoxically impedes the initial stages of RANKL-induced osteoclast differentiation in precursor cells, independently of its proteolytic activity. In various other cell types, thrombin mediates intracellular signaling via protease-activated receptors 1 and 4 (PAR1/PAR4). A transient expression of PAR1 was observed in osteoclast precursor cells, while PAR4 expression has not yet been investigated. We aimed to assess the influence of PAR-activation on the differentiation of osteoclast precursors into mature osteoclasts.

Method Peripheral blood mononuclear cells (PBMCs) from whole blood were isolated followed by positive magnetic sorting for CD14 + monocytes. Monocytes were expanded to macrophages with 50 ng/ml recombinant macrophage colony-stimulating factor (rhM-CSF), and osteoclast differentiation was induced by adding 50 ng/ml RANKL. We tested the impact of PAR activation by adding peptides specifically activating PAR1 (PAR1-AP, 32 µM) or PAR4 (PAR4-AP, 50 µM) to the culture medium. Cells were fixed, stained for TRAP (tartrate-resistant acid phosphatase), and counterstained with hematoxylin. Tartrate-Resistant Acid Phosphatase in the conditioned medium (TRAcP) was quantified with a fluorometric assay. PAR4 expression was determined via Western blot analysis.

Results In our in-vitro differentiation assay, we observed an expedited osteoclast differentiation process upon the addition of PAR4-AP, as opposed to PAR1-AP. Preliminary data suggested that this acceleration manifests within 1-2 days of introducing RANKL, corresponding to an increase in TRAcP activity in the medium. Western blot analysis unveiled a robust expression of PAR4 in precursor cells treated with RANKL.

Conclusion Accelerated differentiation upon PAR4 activation of osteoclast precursor cells appears to contradict previous reports on the inhibiting activity of thrombin acting via PAR1 or independently of its proteolytic activity. PAR4 may serve as a regulator to fine-tune the action of thrombin. Additionally, Cathepsin K functions as a ligand for PAR4. As its secretion is a distinctive feature of osteoclast formation, this may potentially signify the presence of a positive feedback loop. Further functional studies employing physiological PAR4 ligands and antagonists are necessary to elucidate the precise role of PAR4 in osteoclastogenesis.

Conflict of Interest The authors declare no conflicts of interest.

T-21-06 *In vitro* generated megakaryocytes for the detection of human platelet antigen-specific alloantibodies

Authors G. Uzun[1, 2], J. Lucic[3], I. Marini[2], F. Rigoni[2], F. Lyshy[1], S. Nowak-Harnau[1], K. Althaus[1, 2], U. J. Sachs[4, 5], T. Bakchoul[1, 2]

Institutes **1** Centre for Clinical Transfusion Medicine, Tuebingen, Germany; **2** Medical Faculty and University Hospital of Tübingen, Institute for Clinical and Experimental Transfusion Medicine, Tuebingen, Germany; **3** Clinic for Anesthesiology LMU University Hospital Munich, Department of Transfusion Medicine, Cellular Therapeutics and Hemostaseology, Munich, Germany; **4** Justus-Liebig-University, Institute for Clinical Immunology, Transfusion Medicine and Hemostasis, Giessen, Germany; **5** University Hospital Giessen and Marburg, Campus Giessen, Department of Thrombosis and Haemostasis, Giessen, Germany

DOI 10.1055/s-0044-1779245

Introduction Serologic characterization of anti-human platelet antigen (HPA) alloantibodies is crucial in fetal neonatal alloimmune thrombocytopenia (FNAIT). The monoclonal antibody immobilization of platelet antigens (MAIPA) assay is accepted as the gold standard test for the detection of platelet antibodies, however, it requires fresh platelets from HPA-genotyped donors, which is challenging for some labs. Megakaryocytes express HPA epitopes and offer an alternative source for detecting anti-HPA antibodies. The objective of this study was to assess the efficacy of a novel assay called monoclonal antibody immobilization of megakaryocyte antigens (MAIMA) for detecting anti-HPA antibodies.

Method CD34 + cells were isolated from buffy coats of HPA-genotyped blood donors and were differentiated into megakaryocytes *in vitro*. The performance of the MAIMA assay was evaluated using WHO reference reagents for HPA-1a, HPA-3a, and HPA-5b, along with sera samples from patients who had well-characterized anti-HPA antibodies.

Results The WHO anti-HPA-1a reference reagent showed similar binding to megakaryocytes and platelets in MAIMA and MAIPA, respectively. On the other hand, OD values for the WHO anti-HPA-3a reference reagent were lower in MAIMA than in MAIPA. Anti-HPA-5b antibodies were not detectable in MAIMA. Patients' sera containing anti-HPA-1a antibodies were successfully detected in MAIMA in all clinical samples. Moreover, OD values in MAIPA and MAIMA showed high correlation ($r = 0.96$, $p < 0.001$). MAIMA was reactive for samples with anti-HPA-3a as well as anti-HPA-3b, however, OD values were lower compared to MAIPA. Interestingly, all patient samples with anti-HPA-5b antibodies were tested negative in MAIMA.

Conclusion *In vitro* generated megakaryocytes can be used to detect anti-HPA-1a alloantibodies. However, despite this potential, they may be less suitable for the detection of alloantibodies against other HPAs such as HPA-5b.

Conflict of Interest none reletad to this study

T-21-07 Insights into the differential role of GABARAPs proteins at the proteomic level

Authors M. Ibrahim, M. A. Jamil, J. Oldenburg, O. El-Maarri

Institute Universitätsklinikum Bonn, Institut für Experimentelle Hämatologie und Transfusionsmedizin (IHT), Bonn, Germany

DOI 10.1055/s-0044-1779472

Introduction Through the route of the conventional secretory pathway, FVIII is synthesized into the ER, folded and transported into the Golgi by interacting with chaperones and cargo proteins [1]. The mammalian family of ATG8 proteins, comprising GABARAP, GABARAPL1 and GABARAPL2, are currently being highly studied for their versatile roles in processes such as signaling, vesicular trafficking and autophagy [2]. We previously created CRISPR-cas9 knockouts of these genes and observed that GABARAP SKO decreased FVIII secretion (by 25 %), GABARAPL1 and GABARAPL2 SKO increased FVIII secretion (by 50-100 %). We also observed a cumulative effect upon performing the DKOs (GABARAP-GABARAPL1 by 35 %, GABARAP-GABARAPL2 by 95 %; GABARAPL1-L2 by 70 %) and TKOs (increase by 30 %). Therefore, despite their subtle differences, they appear to play distinct roles that may be hard to distinguish due to their high homology and to cellular vesicle complexity.

We aim to study the differential role played by these proteins, specifically by looking at the levels of differential interacting partner proteins in previously published Mass Spectrometry data.

Method We looked into proteomic analysis of previously published Mass Spectrometry data of immuno-precipitated proteins interacting with each of the GABARAPs, (https://doi.org/10.1038/s41467-021-23599-7[3]; https://doi.org/10.15252/embr.201948317[4]; https://doi.org/10.1038/s41467-019-12094-9[5]), obtained from PRIDE database (PXD024290, PXD015155, PXD014829, respectively).

Results We observed the expected common pathways as well as differential pathways enriched for each GABARAPs protein. Upon performing GO and KEGG analysis, some of the common notable processes observed were related to an energy role in the cell, namely Mitophagy, as well as processes related to cell division and homeostasis. We also looked into differential pathways related to each protein. GABARAP presented differential pathways that were related to cell division and cytoskeleton. GABARAPL1 role was shown to be implicated in some disease pathways, where identifying the underlying functions would need further analysis. GABARAPL2 differential interacting partners play a role in processes related to endosomal and lysosomal vesicular trafficking.

Conclusion This preliminary analysis further shows that the expected divergence in function of the GABARAPs is rooted in differential interaction partners, their cellular distributions and their bio-functions.

Conflict of Interest none

References

[1] Anelli T., Sitia R. Protein quality control in the early secretory pathway. The EMBO journal 2008; 27 (2): 315–327

[2] Chen J., Zhao H., Liu M., Chen L. A new perspective on the autophagic and non-autophagic functions of the GABARAP protein family: a potential therapeutic target for human diseases. Molecular and Cellular Biochemistry 2023; 1–27

[3] Yamamoto Y., Chino H., Tsukamoto S., Ode K.L., Ueda H.R., Mizushima N. NEK9 regulates primary cilia formation by acting as a selective autophagy adaptor for MYH9/myosin IIA. Nature Communications 2021; 12 (1): 3292

[4] Herhaus L., Bhaskara R.M., Lystad A.H., Gestal-Mato U., Covarrubias-Pinto A., Bonn F., Dikic I. TBK1-mediated phosphorylation of LC3C and GABARAP-L2 controls autophagosome shedding by ATG4 protease. EMBO reports 2020; 21 (1): e48317

[5] Holdgaard S.G., Cianfanelli V., Pupo E., Lambrughi M., Lubas M., Nielsen J.C., Cecconi F. Selective autophagy maintains centrosome integrity and accurate mitosis by turnover of centriolar satellites. Nature communications 2019; 10 (1): 4176

Index

Author Index

A
Aabrams V. S91
Abad-Franch L. S43
Abdelmageed A. S41
Adam E. S89
Agarwal S. S36, S79
Agis H. S27
Ahrends R. S11, S71
Akin I. S22
Alban S. S109
Alberio L. S63, S71, S76, S84, S88
Albert T. S39, S41, S101
Alecsi S. S62
Alesci R. S. S72
Aliotta A. S63, S71, S76, S84, S88
Alizadeh H. S49
Alrifai M. S86
Al Rifai R. S53
Althaus K. S16, S47, S48, S57, S85, S87, S93, S116
Altmüller J. S53
Alzahrani H. S81
Amano K. S43
Amiral J. S16
Amiri N. S62
Angchaisuksiri P. S79
Araqelyan S. S51
Arens J. S79
Armstrong R. S64
Arshad M. S48
Artru F. S88
Astermark J. S35, S81
Attachaipanich T. S112
Aumann V. S55
Avila L. S62
Ay C. S2, S4, S6, S7, S9, S23, S25, S26, S27, S29, S46, S54, S67, S68, S69, S76, S86, S91

B
Bachofner A. S96
Baer C. S26
Baidildinova G. S2
Bajorat T. P. S90
Bakchoul T. S15, S16, S20, S47, S48, S57, S85, S87, S93, S111, S112, S116
Barco S. S4
Barki S. S76
Barkovits K. S115
Batty P. S31
Bauer A. T. S27
Bauer M. S32, S94
Baulain U. S55
Becattini C. S106
Becker-Gotot J. S41
Becker K. S45
Beckmann L. S7, S29, S30, S78
Beck R. S90
Beer-Hammer S. S3
Beer N. S92
Behrens F. S64
Beitzen-Heineke A. S7, S78
Bereczky Z. S49
Berghoff A. S25, S26, S29
Berkemeier A.-C. S39
Bernar A. S32
Bertaggia Calderara D. S63, S71, S76, S84, S88
Betticher C. S63
Beutel M. S2
Bevot A. S87
Beyer-Westendorf J. S3, S66, S97
Bikdeli B. S6
Binder N. B. S103, S108
Birschmann I. S18, S115
Biswas A. S44
Blahakova I. S34
Blinc A. S10
Boc A. S10
Boc V. S10
Boda Z. S49
Boeckelmann D. S61
Bogić A. S104
Boiffin B. S46
Boiti F. S45
Bokemeyer C. S7, S29, S30, S78
Bomke B. S60
Bönigk H. S104, S109, S110
Borel Derlon A. S46
Borst O. S71
Bosch A. S62
Boucher A.-Y. S95
Boujataoui S. S12
Bouzabia B. S3
Bozic Mijovski M. S21
Bradáčová P. S74
Brandao L. S62
Brauer M. S94
Brehm M. S22
Brehm M. A. S48, S114
Brinkkötter P. T. S83
Brodt G. S54, S60, S65
Broome C. S17
Brugger B. A. S98, S99
Brühl M. S32, S49
Bucio García J. A. S3
Bunting S. S79
Buri M. S50
Bury L. S12
Busse J. S5
Bystricka L. S41, S43

C
Caletka P. S24
Castaman G. S83
Ceppi F. S63
Chai-Adisaksopha C. S94, S112
Chang J. S5
Chang T. S50, S79
Chatterjee M. S3
Chen Z. S73
Christoph K. S45
Church R. S83
Cibis C. L. S89
Cimen T. S11
Coen Herak D. S94, S104
Collins P. S50
Coppens M. S35
Crockett L. S79
Crodel C. S26
Croteau S. E. S79
Cuker A. S81, S106
Cvirn G. S11, S98, S99

D
Dahlen B. S2
Danelyan S. S51
Daoud A. S56
Dasenbrook B. S35
Daxboeck C. S98, S99
de Groot P. G. S13, S17
de Hart G. S81
de Jonckheere B. S71
de Laat B. S13, S17, S29, S91, S93
Dempfle C. E. S72
Dereuddre-Bosquet N. S79
Derksen J. S22
De Simone I. S93
Detering C. S55
de Vos-Geelen J. S29
Dhalluin C. S50
Dishkelov A. S77
Divivier M. S13
Divjak L. P. S94
Dixer B. S76
Donners A.A.M. T. S101
Doubek M. S34, S114
Douxfils J. S95
Drayss M. S75
Drbohlavova E. S52
Dreier L. S87
Dreier T. S68, S69
Dreisbach E. S48
Dremova O. S12, S112
Drozdova E. S114
Dumont J. S41
Dürschmied D. S22

E
Eichelberger B. S69
Eichinger B. S69
Eichler H. S45, S58, S86
Ekhlasi-Hundrieser M. S55
El Amrani M. S101
El Hazzouri S. S57
Eller K. S83
El-Maarri O. S53, S57, S116
Englert H. S13
Englisch C. S25, S26, S29
Erlacher M. S61
Ernemann U. S87
Ernst T. S26
Escuriola-Ettingshausen C. S40, S42, S43
Etscheid M. S92
Eyster E. S79

F
Faizy T. D. S7
Fehse B. S30
Feichtinger J. S98
Feriel J. S100
Fialova D. S114
Fiehler J. S7
Fijnvandraat K. S50
Filip J. S58
Fillitz M. S76, S91
Fischer L. S55
Fischer R. S60
Fleck E. S60, S64
Flieder T. S18, S115
Flottmann F. S7
Fluger I. S24
Fong S. S31, S79
Forstner-Bergauer B. S67
Forstner D. S98, S99
Fovet C.-M. S79
Fraga M. S84, S88
Francisco I. S6
Franke C. S105
Franke G.-N. S107
Frazer-Abel A. S81
Freitag K. S105, S107
Frenzel L. S81
Freyer C. S107
Friedman K. D. S70
Fritzer-Szekeres M. S29
Frühwald M. S66
Fuchs T. S13
Fuhrmann J. S19, S102, S107
Fuiman J. S81
Fu X. S3

G
Galante N. S35, S59, S83
Gallistl S. S115
Gao Z. S6, S9
Gasper S. S101
Gassler N. S60
Gatzke F. S87, S89, S99
Gauster M. S98, S99
Gebetsberger J. S32
Gebhart J. S9, S67, S68, S69, S76, S91
Geisen C. S60, S64
George J. N. S70
George L. S79
Georgiev H. S61
Gerber B. S20
Gerritzen H. S41
Gesell-Salazar M. S78
Giermasz A. S37, S59
Gieswinkel A. S2
Girbl T. S11
Gisslinger H. S27
Glonnegger H. S61
Göbel J. S13
Goldmann G. S38, S39, S49
Gomez E. S59
Gomez F. J. S63
Göncziné Kiss J. S49
Gorodezki D. S64
Gorzelanny C. S27
Gosselin R. S106
Goudemand J. S46
Graf L. S20
Greinacher A. S19, S20, S78, S102, S107, S110
Griesheimer A. S52
Griffiths M. S108
Grimmig K. M. S103
Groeneveld D. S79
Gross C. S11
Grützmann M. S22
Guettler J. S98, S99
Gupta S. S79, S81
Gutensohn K. S55

H
Haack L. S35
Haag M. S3
Habermann B. S56
Hackenbroch C. S11
Haegele F. S45
Hagedorn N. S54, S55
Hahn G. S66
Haidl H. S115
Hajek R. S24
Halimeh S. S51, S56, S58, S62, S66
Halm-Heinrich I. S55
Hambardzumyan L. S51
Hamedani N. S. S101
Hammer S. S48, S57, S93
Handtke S. S78
Handyside B. S79
Hanžek I. S94
Harenberg J. S95, S106
Harpell L. S31
Harris S. S31, S37, S79
Hart C. S86
Haslacher H. S67, S68
Hauser M. S111
Hecking C. S72
Heemskerk J. W. S13, S17
Heidel F. S107, S110
Heine A. S41
Heinrichs D. S56
Heinze G. S4
Heller C. S60
Hemkemeyer S. A. S19
Hennersdorf F. S87
Henriet C. S46
Henschler R. S105
Henshaw J. S36
Herbst K. S39
Herkner H. S106
Hermans-Borgmeyer I. S13
Hermans-Borgmeyer U. S13
Hermans C. S35, S36, S59
Hernández-Blasco L. S6
Herrmann J. S24
Hertrampf E. S99
Hetjens S. S106
Heuer L. S57, S93
Hickey K. S105
Hiemann R. S102
Hilberg T. S32, S33, S49
Hmida J. S49
Hochhaus A. S26
Hock B. S79, S81
Hoffmann U. S22
Hofmann K. M. S94
Hofmann U. S3
Hohbein A. S18, S115
Hohensinner P. S23, S46
Holm K. B. S52
Holstein K. S58, S78, S86
Holzapfel J. S66
Holze J. S12
Holzer U. S64
Holzhauer S. S43, S55
Hölz J. S65
Hopp M.-T. S12, S39, S103
Horneff S. S39
Hrachovinova I. S52, S70
Hsu F. S9
Huck V. S114
Huseynov A. S22
Hüsing A. S46
Huskens D. S13, S17, S93
Hynst J. S34

I
Ibrahim M. S116
Icheva V. S64, S87
Imhof D. S12, S39, S103
Ismail A. M. S31

J
Jaeger S. S79
Jamil M. A. S53, S116
Jansen C. S5
Jarvis Brunsvig K. S62
Jaworski K. S79
Jiang S. S73
Jia X. S73
Jiménez D. S6
Jiménez-Yuste V. S50
Jock A. S87
Jonas S. M. S113
Jug B. S21
Juhl D. S90
Jurado-Mestre B. S41
Juranek S. S40, S65
Jurcevic S. S77
Jurk K. S2, S6
Just B. S75

K
Kaderali L. S102, S107
Kager G. S11
Kahl F. S113
Kaider A. S69
Kaiser K. S55
Kalte I. S112
Kalus M. S40
Kampmann P. S35, S59
Kanse S. S92
Kanthack L. S54
Kapsecker M. S113
Kargl J. S98
Katsarou O. S81
Kausche L. E. S89
Kavakli K. S50, S81
Kazmi R. S35, S83
Kejžar N. S10
Kenet G. S50, S79
Kentouche K. S54, S55, S60, S65
Kern J. S87
Key N. S35
Khachatryan H. S51
Khan U. S17
Khattak N. S31
Khuu M. P. S12
Kieninger S. S47, S57
Kiialainen A. S50
Kimmich N. S96
Kim S. S54
Kiouptsi K. S6, S9
Kirschall J. S111, S112
Kiss C. S49
Kitchen S. S105
Kittel M. S22
Kjaer M. S64
Kjeldsen-Kragh J. S64
Klamroth R. S35, S40, S43, S55, S75, S81, S86, S106
Klätschke K. S110
Klauke J. S19, S110
Klauser-Braun R. S23
Klein C. S39, S101
Klemm U. S105
Klok F. A. S4
Kluft C. S15
Knabbe C. S18, S115
Knöbl P. S83
Knöbl P. N. S70
Knoebl P. S86
Knoefler R. S45, S54, S55, S66
Knowles L. M. S45
Koehler U. S61
Köhm M. S64
Kolde H.-J. S105
Kollotzek F. S71
Königsbrügge O. S2, S23, S54
Königs C. S41, S43
Konings J. S13, S17
Konkolewski J. D. S110
Konrath S. S19, S110
Körber S. S60
Kortgen A. S94
Kostenchak-Svystak O. S96
Kosuta D. S21
Kotov V. S41
Kowalski A. S90
Kraemmer D. S4, S46, S54
Krahforst A. S51
Kralova K. S17
Krammer-Steiner B. S35, S43, S55
Krappinger J. S98
Krause M. S60
Kraus S. S75
Krauth M. T. S27
Kremer-Hovinga J. A. S20, S70, S96
Kremers R.D. L. S13, S17
Kristina M. S24
Krüger P.-C. S65
Kügler S. S90
Kühne L. S83
Kühnöl C. S54, S55
Kupper N. S98, S99
Kürschner M. S45
Kurts C. S41
Kuta P. S78

L
Laane E. S91
Lackner K. L. S2
Lackner M. S3

Hämostaseologie 2024; 44: S1–S125

Lämmerhofer M. S3
Lämmle B. S70
Lamprecht M. S11
Langer F. S7, S29, S30, S40, S42, S78, S106
Lapisatepun W. S94
Laschke M. W. S45
Lattmann K. S90
Lau K. S81
Ledinski G. S11
Leebeek F. S35
Le Grand R. S79
Lehle M. S50
Lehner S. S55
Lehr C. S29, S30
Lemmel E. S63
Lemons R. S35, S59
Lenhart D. S109
le Quellec S. S35, S59, S83
Letscher H. S79
Levy G. S79
Lickert S. S75
Liedl P. S103
Likavcova P. S34
Lillicrap D. S31
Linhoff T. S40, S43
Liu X. S27
Lohse J. S45, S66
Long B. S79, S81
Lonsky V. S24
Lorenz M. S23
Lotz-Havla A. S65
Lucas S. S83
Lucic J. S116
Lurz E. S65
Luterán J. S100
Lux L. S78
Lyshy F. S85, S116
Lyssy F. S98, S99

M
MacDougall A. S79
Mäder J. S30
Mahlangu J. S37
Mailer R. K. S19
Maisonnasse P. S79
Male C. S67
Malinarich F. S112
Mammadova K. S112
Mancuso M. E. S50
Mann A. S112, S113
Marcus K. S115
Marini F. S12
Marini I. S111, S112, S116
Marquardt N. S38, S39
Marten S. S3, S97
Marton I. S49
Matino D. S81
Matsumoto M. S70
Matthey Guirao E. S63
McCrae K. S17
McKay J. S81
McRae H. L. S14, S113
Mdawar-Bailly É. S84, S88
Meggendorfer M. S26
Mehic D. S67, S68, S69, S76, S91
Meijer K. S35, S83
Meixner-Götz L. S11
Menard A. S31
Mendez A. S20
Menger M. D. S45
Menig-Benzig L.-S. S3
Mentzel H.-J. S54, S65
Metternich S. S78
Metzeler K. S107
Meunier S. S43
Meybohm P. S24
Miesbach W. S35, S36, S42, S43, S52, S58, S79, S86, S87, S89, S99
Mihalic Z. S98
Milanov P. S52
Miloš M. S94, S104
Mimmler M. S112
Mingozzi F. S79
Mo A. S31
Moehnle P. S86
Moik F. S25, S26, S29
Mojzisch A. S22, S114
Möller R. S55
Monahan P. S35, S59, S83
Monreal M. S6
Moore G. W. S103, S108
Moormeier J. S79
Moradpour D. S84, S88
Morath O. S26
Morimont L. S95
Motta J.-P. S112
Mott K. S75
Mowen K. A. S13
Mühlfeld C. S55
Müller J. S5, S14, S39, S101, S103
Münzel T. S2

N
Nagae C. S41
Nagler M. S20
Naue C. S3, S97
Nefischer L. S98
Neill G. S43
Nemes L. S49
Neu A. S87
Neuper L. S98, S99
Newman V. S36
Niekrens C. S40
Niggli M. S50
Nilius H. S20
Niprapan P. S94
Nonn O. S99
Nopp S. S2, S4, S6, S7, S29
Nordmeyer J. S64
Nossent A. Y. S2
Novakovic M. S21
Nowak-Göttl U. S90
Nowak-Harnau S. S116
Nüsgen N. S53, S57

O
Obermayer-Pietsch B. S99
O'Connell N. S35, S83
Ohsawa H. S105
Okhrem A. S109
Oldenburg J. S5, S14, S38, S39, S40, S41, S43, S44, S49, S50, S53, S57, S58, S60, S86, S101, S103, S116
Olivieri M. S40, S43, S65, S66
Ondruschka B. S19
O'Neill C. S79
Osmond D. S36, S37
Otero R. S6
Ozelo M. S37

P
Pabinger I. S2, S4, S9, S23, S25, S26, S29, S46, S51, S54, S67, S68, S69, S76, S91, S96, S106
Paeslack N. B. S113
Panholzer B. S90
Panova-Noeva M. S2
Pardy F. S114
Patki K. S64
Pavlova A. S51, S60
Pelicon K. S10
Pellequer J. L. S44
Pelzl L. S16, S111
Pender A. S31
Pennacchio F. A. S75
Pereira Portela C. S71, S76, S84, S88
Peris L. S6
Perner F. S110
Petek K. S10
Petersen B. S55
Petros S. S107, S109, S110
Petrosyan L. S51
Peyrer-Heimstaett S. S103
Peyvandi F. S36, S50
Pezeshkpoor B. S39, S51, S60
Pfarrer C. S55
Pfeiffer K. S115
Pfeiffer N. S2
Pfrepper C. S42, S43, S55, S86, S104, S105, S107, S109, S110
Pilch J. S45
Pipanmekaporn T. S94
Pipe S. S35, S36, S59
Pipe S. W. S50
Platzbecker U. S107
Poets C. F. S64
Poli S. S87
Pontarollo G. S112
Pospisilova S. S34, S114
Pötzsch B. S5, S14, S39, S101, S113
Preinstorfer S. S115
Preisler B. S60
Prell T. S65
Preusser M. S25, S26, S29
Procházková D. S74
Proquitte H. S60, S65
Provaznikova D. S52
Puzii O. S96

Q
Quehenberger P. S91

R
Radovska A. S52
Ragni M. V. S79
Raheja P. S35, S83
Ramaraje Urs S. U. S44
Rangaswamy C. S13
Ranieri E. S96
Ransmann P. S32, S49
Rapp S. S2
Rashvand S. S77
Rasko J. E. S79
Rast J. S68, S69, S76, S91
Rathod D. C. S12
Rauchfuß S. S87, S89, S99
Raum M. S65
Razon L. S31
Reda S. S5, S14
Regenhardt J. S29, S30
Reinhardt C. S6, S9, S12, S112, S113
Reinsberger K. S61
Reitsma S. E. S91
Relouzat F. S79
Remmert N. S114
Renné T. S13, S19, S78, S110
Repesse Y. S46
Richter H. S33
Riecken K. S30
Riedl J. S27
Rigoni F. S16, S116
Ritgen M. S90
Rizwam Alam M. S76
Rizzi M. S63
Robinson T. S37
Rodewald A.-K. S96
Roest M. S13, S17, S29, S93
Roggenbuck D. S102
Roggenbuck J. S102
Rogić D. S104
Rolling C. S29, S30, S78
Rösch A. S47, S82

Röth A. S17
Roth S. S52, S87, S89, S99
Rozanova S. S115
Rubenzucker S. S11
Rühl H. S5, S14, S39, S101, S113
Rupon J. S81
Rupprecht H. S12
Rüsing L. Z. S27

S

Sachs U. J. S40, S116
Saemann L. S111
Säemann M. S2, S23
Salaj P. S52
Samelson-Jones B. J. S79
Santagostino E. S41
Santavy P. S24
Sargsyan N. S51
Schaeffeler E. S3
Schäfer S.M. G. S64
Scheeler S. S79
Scheibler F. S42
Schepperle C. S42
Schilling K. S26, S55, S86
Schimmer R. S96
Schirmer M. S32
Schlagenhauf A. S11, S115
Schlager O. S4
Schlammadinger Á. S49
Schleußner E. S60
Schmaldienst S. S23
Schmidt A. S20, S32, S33
Schmitt C. S50
Schmitt C. A. S89
Schmitz J. S18
Schmoldt D. S47, S82
Schmutz F. S11
Schnaidt C. S15
Schneider S. W. S27, S114
Schneppenheim R. S70
Schneppenheim S. S105, S114
Schober S. S64
Schöder T. S110
Scholz R. S102
Scholz U. S40, S55, S100
Schötz S. L. S41
Schramm T. S9, S67, S76, S91
Schraner M. S70
Schreieck J. S48
Schrenk G. S103
Schrottmaier W. S9
Schulenkorf A. S30
Schulte J. H. S64
Schulze H. S24, S75
Schuster G. S89
Schwab M. S3
Schwarz N. S5, S14
Schwarz S. L. S78
Sedighi A. S9
Seifried E. S64
Seitel T. S79
Selçuk K. S75
Selmeczi A. S49
Severin K. S58
Shafer F. S17
Shapiro A. S81
Shehwar D. S76
Sheridan D. S64
Sidonio R. S36
Siebert M. S56, S62
Siecke A. S55
Siegemund A. S104, S107, S109, S110
Siegemund T. S104, S109, S110
Sigel C. E. S102
Sigl-Kraetzig M. S33
Simonikova M. S52
Simonitsch-Klupp I. S27
Singh S. S44
Sinitsa E. S20
Slavík L. S24, S74
Smejkal P. S52
Smitka M. S66
Sommer F. S12, S113
Sondergaard Jensen J. S64
Sparber-Sauer M. S61
Spielvogel C. S6
Stadler N. S66
Stähler R. S42
Stano Kozubik K. S34
Starzer A. S26
Stegner D. S11
Steil L. S78
Steinbrunn T. S75
Steiner D. S4, S7, S25, S26, S29
Stichel D. S45
Stika J. S34
Strahm B. S61
Strassburg C. P. S5
Strauch K. S2
Strauss A. C. S32, S49
Strauss M. S57
Strebel A. S75
Streif W. S32
Strini T. S115
Stubert J. S35
Studt J.-D. S20, S96
Sucker C. S60
Sullivan S. S79
Sumera A. J. S52
Sun P. S81
Sun S. S13, S17
Susen S. S46
Sutter T. S96
Svozilova H. S34
Swensson L. S19, S107, S110
Swieringa F. S13, S17
Szegedi I. S49

T

Tafur A. S95
Tamamyan G. S51
Tan C. W. S37
Tangsricharoen T. S112
Teitel J. S79
ten Cate H. S2, S29
ten Cate V. S2
Teulon J. M. S44
Thiele T. S78
Thom K. S67
Tiebel O. S45
Tiede A. S86
Tischhöfer M.-T. S109
Tittl L. S3, S97
Tokic S. S115
Tolios A. S69
Toll L. S111
Tomasits J. S89
Tomičić Nikić M. S94
Tomić Mahečić T. S94
Tomschi F. S32, S33, S49
Tosetto A. S43
Tran H. S79
Trappl M. S46
Trautmann-Grill K. S55, S86
Trinchero A. S96
Trivedi T. S79
Tsakiris D. A. S20
Tsiflikas I. S64
Tsiountsioura M. S11

U

Ueda Y. S17
Ugurlar D. S39
Ujčič K. S98, S99
Úlehlová J. S24, S74
Ullrychová J. S74
Unterberger M. S108
Urbanus R. T. S13
Ursu C. E. S91
Uzun G. S16, S85, S87, S116

V

Vaide I. S91
Vaidya S. M. S103
van der Valk P. S35, S83
van Luin M. S101
Vardumyan E. S51
Vassart J. S95
Vater L. S107
Verbruggen B. S103
Vergnolle N. S112
Verhamme P. S35, S83
Vettermann C. S79, S81
Veuthey L. S71, S76, S84
Vezendi K. S49
Villalpando I. S79
Vincelli J. S62
Vishnu P. S20
Visweshwar N. S35
Vogel V. S75
Vogler T. S33
Voigtländer M. S30, S78
Völker L. A. S83
von Bargen K. S18
von Depka Prondzinski M. S40, S55
von Drygalski A. S41, S83
von Krogh A. S. S70
Vospernik L. S27
Vostatek R. S23, S46
Vrzalova Z. S34

W

Waginger M. S65
Walenga J. S95
Walter C. S64
Wang J.-D. S37, S81
Wang Y. S27
Wardęcki M. S17
Weber B. S4, S7
Weik K. S42
Weindl G. S12
Weiss C. S106
Weiss J. S52
Weiss L. J. S24, S75
Weißmann M. V. S72
Wekerle M. S57
Well L. S7
Wenning S. S43, S58
Wenzel F. S111
Wernitznig S. S98, S99
Wesche J. S102, S107, S110
Weyand A. S43
Wieland I. S45
Wild P. S. S2
Willems R. A.L. S29
Willemze A. S41, S43
Winterborn A. S31
Wirtz D. S49
Witzemann A. S16, S111, S112
Wolberg A. S. S91, S93
Wolff M. S19, S78, S110
Wollenhaupt C. S60
Wollermann L. S19
Wolny M. S115
Wolska N. S16, S47, S87, S111, S112
Wolter C. S45
Wolter M. S61
Wuillemin W. A. S20
Wynn T. S79

Hämostaseologie 2024; 44: S1–S125

X
Xia R. S73

Y
Yadegari H. S51
Yates B. S31, S79
Yoo R. S17
Yoshimi-Nöllke A. S61
Young G. S35, S50
Yu H. S37

Z
Zaninetti C. S102, S107
Zapotocka E. S52
Zappek J. S56
Zellner M. S9
Zermatten M. G. S84, S88
Zieger B. S61, S90
Ziegler T. S11
Ziemele B. M. S52
Zimmermann M. S18, S115
Zimmermann R. S55
Zlamal J. S15, S16, S47, S87
Zombori M. S49
Zou J. S13, S17
Zou Y. S62
Zuscich O. S24

Keyword Index

(VERITAS-Pro) questionnaire: 123

A

AAV
SOA-20-OC-04 186
AAV Gene Therapy
SOA-20-OC-05 187
AAV Vector
BLEED-OC-02 76
AAV: 85, 87
AAV5: 190
Accuracy: 227
acquired bleeding disorder: 204
acquired hemophilia A: 211
acquired hemophilia: 204, 205
Acquired Hemophilia: 121
Acquired von Willebrand diseas: 214
Actin cytoskeleton: 114
Acute lymphoblastic Leukaemia
SOA-13-OC-06 149
acute-phase proteins: 277
Acylcarnitines
Joint Sym-01-OC-04 8
adamts13
SOA-03-OC-04 199
ADAMTS13 activity: 249
ADAMTS13: 247, 249, 257
adenosine diphosphate
THROMB-OC-03 7
adenovirus vector vaccine: 203
Adherence to Prophylactic Treatment: 123
adherence: 89
Adolescents: 155
adults: 105
Aging: 109, 171
alloimmunization
SOA-12-OC-04 152
andexanet alfa: 268
Angiodysplasia: 129
angiogenesis: 272
Animal Model: 129
Anti-ADAMTS13 antibodies: 249
anticoagulant: 225, 258
anticoagulation
SOA-14-OC-04 201
Anticoagulation
Joint Sym-01-OC-04 8
SOA-10-OC-04 9
anticoagulation: 51, 113
Anticoagulation: 16, 73
anti-FVIII antibody: 92
antiphospholip syndrom: 260
Antiphospholipid Autoantibody
SOA-08-OC-05 35
Antithrombin: 252
anxiety: 17
Apoptosis inhibition: 266
arterial thromboembolism
SOA-19-OC-04 63
arthropathy: 106, 128
Artificial intelligence
SOA-12-OC-05 245
Artificial Intelligence
SOA-12-OC-06 274
artreal fibrilation: 113
atherosclerosis: 28
Atomic Force Microscopy (AFM): 104
Atomic force microscopy: 53
atresia VCI: 154
atrial fibrillation
THROMB-OC-03 7
Atrial fibrillation
SOA-10-OC-04 9
autoimmune hemolytic anemia: 41
autoimmune thyroiditis: 175
Automated immunofluorescence microscopy
SOA-12-OC-05 245

B

B cells: 96
bacteria: 183
balance
SOA-18-OC-05 78
BAY 94-9027: 135
BDUC: 163
BENEGENE-2: 192
Bioimage analysis
SOA-12-OC-05 245
biological aging
SOA-14-OC-05 56
Biomarkers: 109
biosimilars: 259
bleeding
SOA-04-OC-05 82
SOA-14-OC-05 56
Bleeding
SOA-10-OC-04 9
SOA-19-OC-05 67
bleeding disorder: 184
bleeding disorders of unknown cause
SOA-04-OC-04 161
bleeding episodes: 108
Bleeding of unknown cause: 263
bleeding risk: 71
bleeding: 85, 106, 113, 121, 145, 211, 226, 268
Bleeding: 87
BleedScreen: 263
Blood cell dependent coagulation: 70
Blood platelets
SOA-08-OC-05 35
Blood smears: 255
bone density: 128
bone marrow transplantation: 266

C

C5a generation: 259
CAD
Joint Sym-01-OC-04 8
Cancer and hemostasis
SOA-19-OC-03 61
Cancer associated thrombosis: 70, 73
cancer patients: 71
cancer-associated thrombosis
SOA-02-OC-03 66
SOA-02-OC-04 64
caplacizumab
SOA-03-OC-04 199
caplacizumab: 230
cardiogenic shock: 51
cardiopulmonary bypass - cell salvage - platelet-rich plasma: 57
carrier: 132
causal variant: 122
CD41 and CD61: 20
cerebral vein and sinus thrombosis: 155
Cerebral venous sinus thrombosis: 207
Children
SOA-13-OC-06 149
children: 105
Cirrhosis decompensation: 208
clot growth
SOA-10-OC-03 36
clot waveform analysis: 250
clotting time: 134
coagulation activation: 234
Coagulation analysis: 93
coagulation disorders
Joint Sym-02-OC-05 81
coagulation factor VIII: 91
coagulation factors
SOA-18-OC-03 77
Coagulation testing: 262
coagulopathy: 153
cold storge: 267
Cold-storage: 266
Complement Activation: 190
Complement Inhibitor: 16
complement: 41
Congenital amegakaryocytic thrombocytopenia: 143
Consensus recommendation: 204
contact system activation: 259
contraception: 260
coronary artery disease
Joint Sym-01-OC-05 50
covid vaccination: 175
COVID-19: 209, 277
Cryo-EM: 91
CVST: 155
CXCR7
Joint Sym-01-OC-04 8
cytokines
SOA-18-OC-03 77

D

daratumumab: 211
d-dimer: 234
demographics: 115
depression: 170
diagnostic
SOA-05-OC-05 246
diagnostic assays: 248
Diagnostics: 262
dietary supplements: 26
Direct oral anticoagulants: 254
Dirloctocogene Samoparvovec
SOA-20-OC-06 188
disease burden: 132
DOAC
SOA-14-OC-04 201
DOAC Dipstick: 227
DOAC Stop: 252
DOAC: 252
dual x-ray apsorptiometry: 116
DUOX2: 272
Durability
SOA-20-OC-04 186
DVT
Joint Sym-01-OC-04 8

E

ECMO: 213
Efanesoctocog Alfa: 97, 101
effectiveness: 94, 102
Effectiveness: 135
electronic patient file: 194
ELISA: 92
Emicizumab
SOA-05-OC-06 243
emicizumab: 121, 130, 250
Emicizumab: 118
endothelial cell permeability: 114
endothelial cells: 38
Endothelium
THROMB-OC-02 33
Endovascular procedures
Joint Sym-01-OC-06 23
end-stage kidney disease
SOA-14-OC-05 56

Hämostaseologie 2024; 44: S1–S125

epidemiology: 130
Epigenetics
SOA-20-OC-04 186
epinephrine
THROMB-OC-03 7
epithelial-endothelial-crosstalk
THROMB-OC-04 271
erythrocytes: 261
erythropoietin: 266
etranacogene dezaparvovec
BLEED-OC-01 83
etranacogene dezaparvovec: 137, 196
Ex vivo: 266
exercise training
Joint Sym-01-OC-05 50
Experimental animal model: 262
Expression profiling: 125
Extended Half Life: 135
extended half-life concentrates: 130
extracellular vesicles
SOA-02-OC-03 66

F

F XIII: 226
F2r
THROMB-OC-04 271
F8: 122
factor concentrate: 268
factor replacement: 89
Factor VIII activity: 134
factor VIII: 177, 250
Factor VIII: 97, 101, 112, 135
Factor XI: 145
Factor XII: 45, 262
Factor XIII: 209
Fat embolism syndrome: 45
fatigue: 170
FcyRIIA
SOA-08-OC-05 35
fetal and neonatal alloimmune thrombocytopenia
SOA-12-OC-04 152
Fibrin clot formation
SOA-13-OC-06 149
Fibrin: 219
Fibrinolysis
SOA-09-OC-03 217
Fibrinolysis: 220, 221
Fidanacogene elaparvovec: 192
flow-cytometry: 261
FNAIT
SOA-12-OC-04 152
FNAIT: 279
frailty: 211
FSAP: 219
functional limitations
SOA-01-OC-03 11
FVIII
BLEED-OC-0276
SOA-05-OC-05 246
SOA-20-OC-06 188
FVIII interference
SOA-05-OC-06 243
FVIII neutralization
SOA-05-OC-06 243
FVIII: 104, 111, 118, 125, 280
FVIII-vWF complex: 104
FXI inhibition: 73

G

GABARAP: 280
gain-of-function: 276
gene therapy
BLEED-OC-01 83
Gene therapy
SOA-20-OC-06 188
Gene Therapy
BLEED-OC-02 76
gene therapy: 85, 137, 192, 196
Gene Therapy: 87, 194
genetics
Joint Sym-02-OC-04 142
Joint Sym-02-OC-06 275
genome
Joint Sym-02-OC-06 275
GEPHARD: 138
glycoprotein IIb/IIIa complex: 20
GPIIbIIIa: 44
GPVI deficiency: 175
growth differentiation factor 15: 71
GSAV: 111
GSDMD
Joint Sym-03-OC-05 31
gut
THROMB-OC-04 271

H

Haemophila A: 101
haemophilia A: 94, 122, 177
haemophilia B: 102
haemophilia severities: 116
haemophilia: 89, 105, 132
Haemophilia: 112, 123, 131, 135
haemophilic arthropathy
SOA-18-OC-05 78
haemostasis: 177
heat inactivation
SOA-05-OC-06 243
heavy mestrual bleeding
SOA-04-OC-04 161
hemarthrosis: 91
Hematopoietic stem cell transplantation: 176
heme: 27, 91
hemodialysis
SOA-14-OC-05 56
hemoglobin: 248
hemolysis: 248
hemophilia
BLEED-OC-01 83
Hemophilia
SOA-20-OC-04 186
SOA-20-OC-05 187
Hemophilia A
BLEED-OC-02 76
SOA-18-OC-03 77
SOA-20-OC-06 188
hemophilia A: 92, 96, 99
Hemophilia A: 85, 97, 104, 118, 194
hemophilia B
BLEED-OC-01 83
hemophilia b: 137, 196
hemophilia B: 192
Hemophilia B: 194
hemophilia: 113, 128, 130, 134, 137, 263
Hemophilia: 106, 109, 126, 138
hemophilic arthropathy: 89
Hemorrhage
Joint Sym-01-OC-06 23
hemorrhage: 91
hemostasis: 247
heparan sulfate
SOA-02-OC-03 66
Heparin Binding Site Defect: 252
Heparin: 38
Heparin-induced thrombocytopenia
THROMB-OC-01 48
heparinoids
SOA-10-OC-03 36
hepatectomy: 225
Hepatitis B: 196
hepatitis C: 196
Hepatocellular carcinoma: 73
hereditary haemophilia
Joint Sym-02-OC-05 81
hereditary thrombophilia
Joint Sym-02-OC-05 81
high intensity interval training
Joint Sym-01-OC-05 50
histology: 236
HIV: 137
Hmophilia A: 87
HOPE-B
BLEED-OC-01 83
HPA-1a
SOA-12-OC-04 152
hTTP
SOA-03-OC-06 166
Hub & Spoke: 194
Human platelet antigen: 279
human platelet antigen-1a
SOA-12-OC-04 152
Hypercoagulability: 208
hypersomnia: 170
hypogranular thrombocytes: 175

I

ICI therapy
SOA-02-OC-04 64
Immune Modulation
SOA-20-OC-05 187
immune response: 183
Immune Thrombocytopenia
SOA-09-OC-03 217
immune thrombocytopenia: 180
Immune thrombocytopenia: 170
immune thrombosis: 38
Immunofluorescence microscopy: 255
immunofluorescence: 44
Immunogenicity
SOA-20-OC-05 187
immunological characterization: 107
Immunothrombosis
Joint Sym-03-OC-05 31
Incremental recovery: 93
Infants: 118
infection: 96
Inflammation
SOA-13-OC-05 147
inflammation: 106
inheritance
Joint Sym-02-OC-06 275
Inherited platelet disorders
SOA-12-OC-05 245
inherited platelet disorders: 144
inherited thrombophilia: 239
Inhibitor
SOA-05-OC-05 246
inhibitor: 107
inhibitors: 92, 96, 121
inSight Joint Health: 89
intracranial bleeding: 268
Intravital microscopy
Joint Sym-03-OC-06 25
intrinsic coagulation
SOA-10-OC-03 36
intron: 122
IPA analysis: 125
iron deficiency anemia
SOA-04-OC-04 161
iron defiency
SOA-04-OC-04 161
Isotype: 190
ISTH-BAT: 263
ITP: 279
iTTP: 230
IVIG
SOA-08-OC-05 35

J

JAK2: 261
Joint Health: 97
Joint: 131

K
Kidney Failure
SOA-08-OC-065

L
labile heme: 248
laboratory: 279
large platelets: 183
Leukotrienes
Joint Sym-03-OC-06 25
Lipid metabolism
BLEED-OC-05 168
Lipidomics
Joint Sym-03-OC-06 25
Lipids: 45
Lipoprotein oxidation: 26
liver cirrhosis
SOA-14-OC-04 201
Liver cirrhosis: 208
liver endothelial cell: 28
Liver transplant surgery: 226
LMWH: 212, 231
long term observation: 205
long term prophylaxis: 108
Lonoctocog alfa: 93
Low VWF: 120
LSEC: 125
Luminex-based assay: 92
Lupus anticoagulant: 20
lupus antigoagulans: 258
LVAD: 213
Lysis Time: 221

M
Machine learning
SOA-12-OC-05 245
Machine Learning
SOA-01-OC-04 15
Mass spectrometry
BLEED-OC-05 168
mass spectrometry: 277
Mass Spectrometry: 280
MCMDM-1 VWD Bleeding Questionnaire: 184
MD2: 27
Mediation analysis
SOA-13-OC-05 147
Megakaryopoiesis
BLEED-OC-05 168
melanoma
SOA-02-OC-03 66
Mendel
Joint Sym-02-OC-06 275
mesenteric ischemia-reperfusion injury.: 19
microbiome: 28, 272
microfluidic system: 267
microfluidics: 38, 247
Microfluidics: 251
microRNAs
SOA-08-OC-06 5
microscopy: 44
mild hemophilia a: 107
mild to moderate bleeding disorders
SOA-04-OC-04 161
miRNA: 125
miscarriage: 234
mitochondria calcium: 179
mitochondria: 179
Mitochondrial calcium uniporter: 179
Mitochondrial DNA: 109
MK kinase signaling
BLEED-OC-05 168
moderate intensity continuous training
Joint Sym-01-OC-05 50
monocytes
SOA-19-OC-06 69
Monogenic Disease
BLEED-OC-02 76
mortality: 209
MPL-gene: 143
MPN: 261
Multiple coagulation factor deficiencies
SOA-05-OC-04 140
Multiple Myeloma
SOA-19-OC-05 67
mutation
Joint Sym-02-OC-04 142
Mutation: 120
myeloablation: 176
myeloid neoplasia: 256
Myeloproliferative neoplasms
SOA-08-OC-04 13
Myeloproliferative neoplasms: 255
Myeloproliferative Neoplasms: 214

N
Natural anticoagulants
THROMB-OC-02 33
Neutrophil Extracellular Traps
Joint Sym-03-OC-05 31
neutrophil extracellular traps: 19
Next Generation Sequencing
SOA-05-OC-04 140
next-generation sequencing
SOA-19-OC-04 63
NGS
Joint Sym-02-OC-06 275
NGS: 122, 143
NMMIIA: 261

O
obstructive sleeping apnea syndrome: 170
Octocog alfa: 93
one-stage assay: 250
osteoblast
SOA-18-OC-03 77
osteochondral damage
SOA-18-OC-04 80
osteoclast
SOA-18-OC-03 77
Osteoclastogenesis: 278
osteodensitometry: 128
outcome: 205
Outcomes
THROMB-OC-01 48
overall haemostatic potential
Joint Sym-01-OC-05 50
oversulfated chondroitin sulfate: 259

P
PAD4
Joint Sym-03-OC-05 31
Paediatrics: 138
PAI-1: 220
Pancreatic cancer: 70
PAR1
THROMB-OC-04 271
Parathyroid Hormone: 126
parenteral nutrition: 256
Paroxysmal Nocturnal Hemoglobinuria: 16
patient registry: 115
patient-centered outcomes
SOA-01-OC-03 11
patient-reported outcomes: 17
PCC: 268
pd1
SOA-19-OC-06 69
pdl1
SOA-19-OC-06 69
pediatric: 108
Pediatrics
SOA-13-OC-05 147
pentosan polysulfate: 259
perioperative: 225
Perioperative: 227
peripheral apheresis: 266
Peripheral arterial disease
Joint Sym-01-OC-06 23
Persistence
SOA-10-OC-04 9
PF4: 207
PFA-100: 163
PFA200 pathology: 175
Phosphatidylserin: 112
phosphoproteome
THROMB-OC-05 167
Physical activity: 116
Placenta Thromboembolism: 236
plasma exchange
SOA-03-OC-04 199
plasma: 254
Plasma: 221
Plasmapheresis
SOA-20-OC-05 187
Plasmin Generation
SOA-09-OC-03 217
Plasmin: 220
Plasminogen
Joint Sym-02-OC-04 142
Plasminogen: 219, 220
platelet activation: 235
platelet aggregation: 26
Platelet aggregation: 251
Platelet concentrates: 267
platelet disorders
SOA-03-OC-04 199
platelet dysfunction: 176
platelet factor 4: 203
platelet function analyzer: 184
platelet function defects: 163
Platelet: 171
platelet-leukocyte-aggregates: 43
Platelet-Released Factors: 238
platelets
SOA-19-OC-06 69
THROMB-OC-03 7
platelets: 44, 177
Platelets: 20, 238, 255, 266, 277
Platelets-Platelet factor 4-antibodies-thrombosis
SOA-09-OC-04 37
POC: 226
point of care methods: 254
point-of-care: 134
Point-of-care: 227
Polyphosphates: 45
pooled analysis: 254
portal vein thrombosis: 225
postpartum hemorrhage
SOA-04-OC-05 82
Prediction
SOA-01-OC-04 15
predisposition: 144
Preeclampsia
SOA-03-OC-06 166
Pre-existing Immunity: 190
pregnancy
SOA-03-OC-05 141
SOA-04-OC-05 82
Pregnancy
SOA-03-OC-06 166
pregnancy: 230, 231, 234, 235, 239, 260
Pregnancy: 212, 238
pregnancy-specific glycoproteins: 235
primary haemostasis: 105
primary immune thrombocytopenia: 180
primary myelofibrosis
SOA-19-OC-04 63
Procoagulant platelets
THROMB-OC-05 167
procoagulant platelets: 176
Procoagulant platelets: 179
Procoagulant Platelets: 112
Product Classes: 111
prognostic factors: 205
Prophylaxis

Hämostaseologie 2024; 44: S1–S125

SOA-03-OC-06 166
prophylaxis: 94, 99, 130, 204
Prophylaxis: 97, 101
Proplatelet formation
BLEED-OC-05 168
proprioception
SOA-18-OC-05 78
Prospective cohort
THROMB-OC-01 48
Protease-activated receptor 4: 278
Protein C
SOA-08-OC-04 13
Protein C deficiency
THROMB-OC-02 33
Protein C pathway
THROMB-OC-02 33
proteomics: 277
Prothrombin G20210A: 231
prothrombotic potential
SOA-14-OC-04 201
Pulmonary Arterial Hypertension
SOA-01-OC-04 15
Pulmonary embolism
SOA-01-OC-04 15
Pulmonary embolism: 45
PUPs: 138
PVFS scale
SOA-01-OC-03 11

Q
Quality Improvement
SOA-12-OC-06 274
quality of life: 17, 132
quantification: 248

R
r ADAMTS13
SOA-03-OC-05 141
Radiosynoviorthesis: 131
rare disease
SOA-18-OC-04 80
rare disease: 153
Reactivation
SOA-20-OC-04 186
real-word: 102
Recurrence
SOA-13-OC-05 147
recurrence: 205
recurrent pregnancy loss: 236
Red blood cell: 255
Reference range: 251
reversible inhibitors
SOA-10-OC-03 36
Risk assessment
Joint Sym-01-OC-06 23
Risk assessment model
SOA-19-OC-05 67
risk of falling
SOA-18-OC-05 78
Rivaroxaban
SOA-10-OC-04 9
rivaroxaban: 155
rIX-FP: 102
RNA-Sequence Analysis
SOA-08-OC-06 5
RUNX1: 144
rVIII-SingleChain: 94

S
S. aureus: 183
SARS-CoV-2: 203
Screening: 257
secondary myelofibrosis
SOA-19-OC-04 63
secondary thrombosis prophylaxis: 155
Serotype Positivity: 190
shared decision making: 99
shear stress: 43, 247
signaling: 27
Sinus Thrombosis: 16
small intestinal vascularization: 272
small platelets: 183
sphingolipids: 28
Spike-in: 221
Splanchnic vein thrombosis
SOA-08-OC-04 13
Stability evaluation: 249
stem cell: 266
Steroid Hormones: 238
strength
SOA-18-OC-05 78
stroke: 113
struma nodosa: 145
surgery: 108, 225
sutimlimab: 41
synovitis
SOA-18-OC-04 80

T
TAFI: 219
Taipan: 258
Talin-1: 171
telomere length
SOA-14-OC-05 56
Telomere: 109
tether: 44
thrombelastography: 134
Thrombelastography: 221
thrombelastometry: 26
Thrombin
SOA-08-OC-04 13
thrombin generation
SOA-14-OC-04 201
Thrombin generation
SOA-13-OC-06 149
thrombin generation: 26, 239, 260, 263
Thrombin generation: 70, 208, 262
Thrombin: 278
Thrombingeneration: 112
thrombmodulin: 153
thrombocytopathy: 144
thrombocytopenia: 38, 51, 144, 256
thromboembolic events: 41, 180
Thromboembolism
SOA-08-OC-06 5
thromboembolism: 234
Thrombo-inflammation
Joint Sym-03-OC-06 25
thrombolysis: 219
Thrombophilia
SOA-12-OC-06 274
THROMB-OC-02 33
Thrombophilia Diagnosis
SOA-12-OC-06 274
thrombophilia: 236, 260
thrombopoietin: 266
Thromboprophylaxis
SOA-19-OC-05 67
Thromboprophylaxis: 212
Thrombosis
SOA-08-OC-05 35
SOA-13-OC-06 149
Thrombosis Risk
SOA-12-OC-06 274
thrombosis: 20, 154, 176, 180
Thrombosis: 16, 53, 208
thrombotic thrombocytopenic pupura
SOA-03-OC-04 199
thrombotic thrombocytopenic purpura
SOA-03-OC-05 141
Thrombotic thrombocytopenic purpura: 249
thrombus formation: 267
TLR: 272
TLR4: 27
tolerability: 94, 102
Toll-like receptor (TLR) 2 signaling: 19
tPA: 220
transaldolase 1: 153
transcriptomics: 28
Treatment of Bleeds: 101
treatment: 115, 177
Treatment: 138
Treg: 96
tRNAs
SOA-08-OC-06 5
trophoblast: 235
Trophoblast: 238
trophoblast-derived proteins: 235
T-TAS: 251, 256
TTP: 231, 257
Type 1 VWD: 120

U
Ubiquitination: 171
UHPLC-MS/MS: 227
ultrasound
SOA-18-OC-04 80
Ultrasound: 131
urine: 254

V
Validation study
Joint Sym-01-OC-06 23
variant classification
Joint Sym-02-OC-05 81
variant of the norm: 154
Vascular biology
Joint Sym-03-OC-05 31
vascularization
THROMB-OC-04 271
VE-Cadherin: 114
venous thromboembolism
SOA-01-OC-03 11
SOA-02-OC-04 64
SOA-19-OC-04 63
THROMB-OC-03 7
Venous thromboembolism
SOA-01-OC-04 15
SOA-13-OC-05 147
SOA-19-OC-05 67
venous thromboembolism: 17
Venous thromboembolism: 70
Vitamin D: 126
VITT: 203, 207
Von Willebrand disease
SOA-04-OC-05 82
von Willebrand disease: 114, 115, 163
von Willebrand Disease: 108, 129
Von Willebrand disease: 120
von willebrand factor
SOA-02-OC-03 66
Von Willebrand factor
SOA-04-OC-05 82
von Willebrand Factor (vWF): 276
von Willebrand factor multimer assay: 214
von Willebrand factor: 184, 256
von Willebrand Factor: 53, 120
Von Willebrand Factor: 247
von-Willebrand-disease: 213
VTE: 212
VWF: 114

W
Whole blood hemostasis: 251
Wound healing: 106