BSTH

Belgian Society on Thrombosis and Haemostasis

29TH ANNUAL MEETING

24-25 NOVEMBER 2022

LAMOT BELGIUM



LOCAL ORGANIZER

SANDRINE HORMAN

UCLOUVAIN

PROGRAM & **ABSTRACTS**

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GENERAL INFO

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CONGRESS MANAGEMENT

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DATE
24 - 25 NOVEMBER 2022

VENUE
LAMOT congres- & erfgoedcentrum
Van Beethovenstraat 8 /10
2800 Mechelen, Belgium
015 294900

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WELCOME TO THE 29TH ANNUAL MEETING



Dear BSTH members,

Dear attendees,

On behalf of the BSTH Board, it is my great pleasure to welcome you to the 29th Edition of the BSTH annual meeting. After a virtual meeting in 2020 and the joint BSTH-ECTH meeting in Ghent in 2021, we are delighted to welcome you back in Lamot, Mechelen.

This year, Professor Sandrine Horman (UCLouvain), has prepared a great program on the theme “platelets in atherothrombosis and beyond”.
You will have the chance to attend two state of the art and two educational sessions on this theme, with internationally renowned speakers. In line with the BSTH mission to promote basic, laboratory and clinical research, eight talented young researchers have been selected to present their results in these areas of research. Two of them will be awarded the Paul Capel prize for the best presentations. One researcher, selected out of 13, will receive the CSL Behring encouragement award to conduct a research project in the field of thrombosis and haemostasis.

Satellite symposia by Novo Nordisk, Sobi and CSL Behring will let you know about major latest news in their specialized field. Finally, we will be honored to welcome Kristin Jochmans, Emeritus Professor of UZ Brussel and former President of the BSTH, who kindly accepted to give the Gaston Baele lecture in memory of a great man and a mentor.

We are grateful to all of you, and to our sponsors for making the BSTH a dynamic society at the forefront of clinical and research progress.

Cécile Oury, PhD
President of BSTH



08:30 **REGISTRATION, COFFEE, NETWORKING**

09:00 **WELCOME**
Cécile Oury

09:15 - 10:15 **STATE OF THE ART I: Future Directions in Platelet and Coagulation Research**
Chairs: Cécile Oury (ULiège), Sandrine Horman (UCLouvain, Brussels)

09:15 *Jonathan Douxfils (Université de Namur, Belgium)*
Global coagulation assays in the future of laboratory diagnostics of hemostasis and thrombosis – Interplay between platelets and coagulation

09:35 *Robert Ahrends (University of Vienna, Austria)*
Linking platelet lipidomics and functions: The way to novel diagnostic and therapeutic opportunities

09:55 *Claudia Tersteeg (KU Leuven Campus Kulak Kortrijk, Belgium)*
Newest insights and future research opportunities in the field of procoagulant platelets

10:15 – 11:15 **SPONSORED SATELLITE SYMPOSIUM I (Novo Nordisk)**
Chair: Cédric Hermans (Cliniques universitaires Saint-Luc, Brussels)

10:15 *Davide Matino (Hamilton, Canada)*
Clinical outcomes from Early use of N9-GP compared with previous treatment in patients with Haemophilia B

10:45 *Cedric Hermans (Cliniques universitaires Saint-Luc, Brussels, Belgium)*
Choosing optimal treatment using an integrative patient-oriented approach

11:15 **COFFEE BREAK**

11:45 - 12:45 **SELECTED ORAL PRESENTATIONS – BASIC RESEARCH**
Chairs: Karen Vanhoorelbeke (KU Leuven Kulak, Kortrijk), Simon Demeyer (KU Leuven Kulak, Kortrijk)

11:45 O1 *Kirsten Leeten (University of Liège)*
Ticagrelor prevents Staphylococcus aureus infective endocarditis by mitigating bacterial virulence

12:00 O2 *Sarah Vandelanotte (KU Leuven - Kulak Kortrijk)*
DNase-1 can overcome rt-PA resistance of platelet-rich ischemic stroke thrombi

12:15 O3 *Stijn Van Bruggen (KU Leuven)*
Deep vein thrombosis generates a systemic environment promoting release of neutrophil extracellular traps and detrimental cardiac remodeling

12:30 O4 *Julien De Poortere (UCLouvain, Brussels)*
α1AMP-activated protein kinase (α1AMPK) regulates hemostatic dysfunctions during septic shock

12:45 **PAUL CAPEL PRIZE - BASIC RESEARCH**

13:00 **LUNCH & POSTER WALK**

14:00 - 14:45 **EDUCATIONAL I**
Chairs: Katrien Devreese (UZ Ghent), Laure Gilis (CHC MontLégia, Liège)

Andrew Frelinger (Boston Children's Hospital, United States) (online)
Comprehensive phenotyping of human platelets by single-cell cytometry

14:45 - 15:45 **SPONSORED SATELLITE SYMPOSIUM II (Sobi)**
Chairs: Timothy Devos (UZ Leuven), Dries Deeren (AZ Delta, Roeselare)

14:45 *Nicole Straetmans (Cliniques universitaires Saint-Luc, Brussels, Belgium)*
PNH: current treatment options and remaining challenges

15:15 *Louis Terriou (CHRU de Lille - Hôpital Claude Huriez, France)*
Results of the C3 inhibitor Pegcetacoplan in PNH

15:45 **COFFEE BREAK**

16:15 **PROFESSOR GASTON BAELE MEMORIAL LECTURE**
Chairs: Christelle Orlando (UZ Brussels), Cécile Oury (ULiège)

Kristin Jochmans (UZ Brussels, Belgium)
Antithrombin, not just an old molecule

17:00 **BSTH GENERAL ASSEMBLY**

17:30 **CLOSURE OF DAY PROGRAMME**

EVENING PROGRAM

WELCOME RECEPTION

Bayer invites all participants on Thursday night at 17:30 - 18:30 hrs. to join the welcome reception.

DINNER

From 19:00hrs. you're invited to join our dinner in LAMOT.

Preregistration is required.

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PROGRAM
FRIDAY
25 NOVEMBER

08:30 **REGISTRATION, COFFEE, NETWORKING**

09:00 - 09:45 **EDUCATIONAL II**
Chairs: Kristel Vandenbosch (CHU Liège / ULiège), Phu Quoc Le (HUDERF, Brussels)

Katrien Devreese (UZ Ghent, Belgium)
Acquired TTP diagnosis

09:45 - 10:45 **SPONSORED SATELLITE SYMPOSIUM III: Gene therapy in hemophilia, from theory to reality (CSL Behring)**
Chairs: Peter Verhamme (UZ Leuven), Veerle Mondelaers (UZ Gent)

09:45 *Cedric Hermans (Cliniques universitaires Saint-Luc, Brussels, Belgium)*

10:15 *Michiel Coppens (University Medical Center Amsterdam, The Netherlands)*

10:45 **CSL BEHRING ENCOURAGEMENT AWARD**

11:00 **COFFEE BREAK**

11:30 - 12:30 **SELECTED ORAL PRESENTATIONS – CLINICAL AND LABORATORY**
Chairs: Christelle Orlando (UZ Brussels), Jan Emmerechts (AZ Sint-Jan, Brugge)

11:30 O5 *Charlotte Van Edom (UZ Leuven / KULeuven)*
Heparin monitoring in ImpellaTM-supported cardiogenic shock patients: parallel dual testing in critically ill

11:45 O6 *Christine Van Laer (UZ Leuven)*
Position of Next Generation Sequencing Testing in the Diagnostic Algorithm of Inherited Thrombophilia

12:00 O7 *Sandrine Bouteille (UZ Brussels)*
Genotype-phenotype correlation in Belgian patients with inherited Protein C deficiency

12:15 O8 *Matthias Engelen (UZ Leuven)*
Modulation of thromboinflammation in hospitalized COVID-19 patients with aprotinin, low molecular weight heparin, and anakinra: the DAWn-Antico study



12:30 **PAUL CAPEL PRIZE – CLINICAL AND LABORATORY**

12:45 **LUNCH & POSTER WALK**

14:00 - 15:00 **STATE OF THE ART II: In and Beyond Atherothrombosis**
Chairs: Thomas Vanassche (UZ Leuven / KU Leuven), Marie Moonen (CHU Liège / ULiège)

14:00 *Lina Badimon (Research Institute-Hospital Santa Creu i Sant Pau, Barcelona, Spain)*
Coronary artery disease: Progress in antiplatelet therapy

14:20 *Matthias Engelen (KU Leuven, Belgium)*
Antithrombotic therapy and Covid-19

14:40 *Marie-Astrid Van Dievoet (Cliniques universitaires Saint-Luc, Brussels, Belgium)*
Platelets in chronic liver disease

15:00 **CLOSING REMARKS**

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PROGRAM
FRIDAY
25 NOVEMBER



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BSTH BOARD 2022

THE PRESENT MEMBERS OF THE BSTH BOARD ARE:

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EXHIBITION RULES & REGISTRATION FEES

EXHIBITION RULES

At our meeting and exhibition at LAMOT certain restrictions are applicable.

FOOD AND BEVERAGE
It is not allowed to distribute prepared food or beverages at the booth or place any food cooking equipment.

MANNING OF STANDS
Exhibitors will be required to ensure that their stands are manned during the opening hours of the exhibition and must not dismantle their stands before the published closing time.

NOISE
Exhibitors may not use audible electronic, mechanical apparatus, or open audio systems that may be heard outside the exhibitor assigned space. Congress Care on behalf of BSTH and its organizers, reserves the right to require any exhibitor to discontinue any activity that may cause annoyance or interference with others.

SECURITY AND INSURANCE
BSTH and its organizers will not be held responsible for any accidents, loss or damage to exhibitors' goods and exhibitors are reminded that they should obtain their own insurance to cover this.

EXHIBITION OPENING HOURS

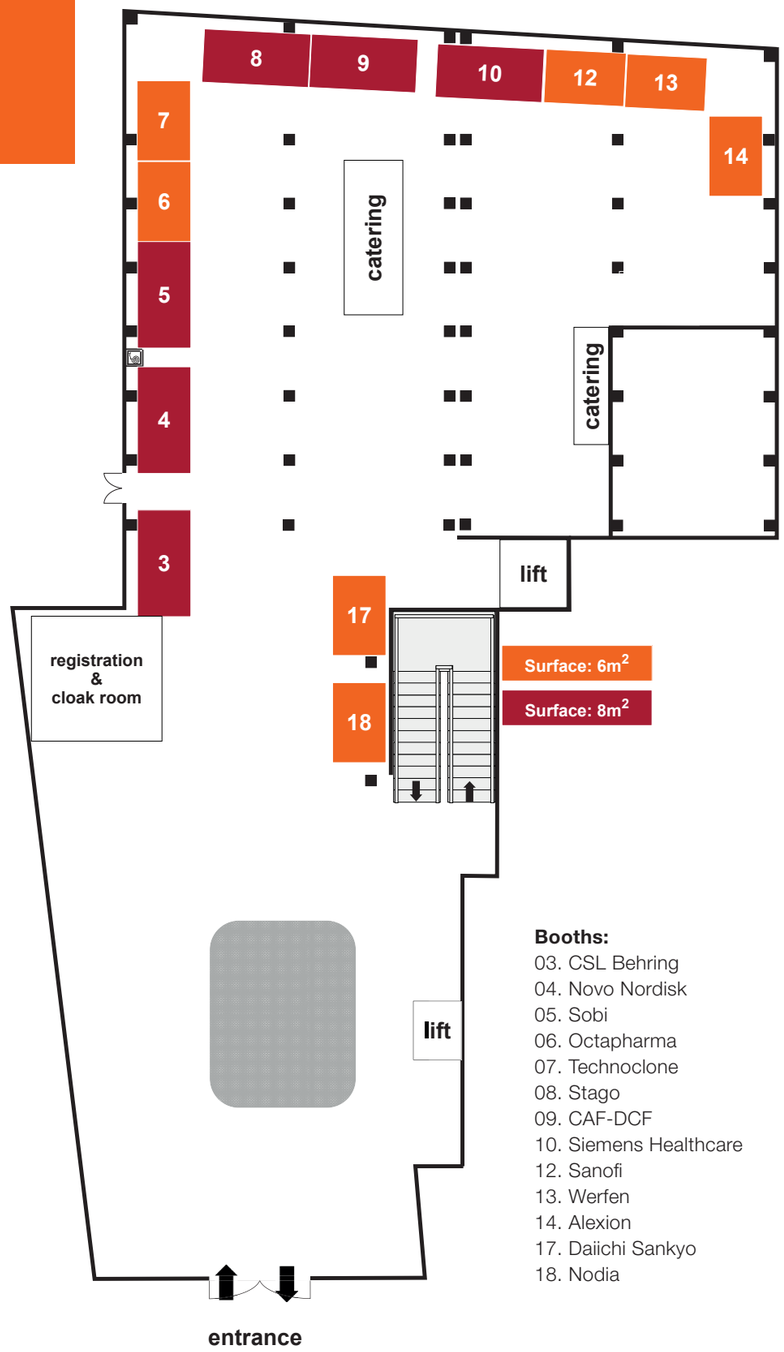
THU	24 NOV 2022	08:30-18:30
FRI	25 NOV 2022	08:30-15:00

REGISTRATION FEES

MEMBER BSTH		
Regular (MD specialist, MSc specialist, PhD scientist)	150	EUR
MD trainee / PhD student	80	EUR
Nurse, paramedic, technician, data manager, student	45	EUR
NON MEMBER BSTH		
Regular (MD specialist, MSc specialist, PhD scientist)	240	EUR
MD trainee / PhD student	150	EUR
Nurse, paramedic, technician, data manager, student	95	EUR

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FLOORPLAN



Booths:
03. CSL Behring
04. Novo Nordisk
05. Sobi
06. Octapharma
07. Technoclone
08. Stago
09. CAF-DCF
10. Siemens Healthcare
12. Sanofi
13. Werfen
14. Alexion
17. Daiichi Sankyo
18. Nodia

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STATE OF THE ART I

Newest insights and future research opportunities in the field of procoagulant platelets

Tersteeg C, KU Leuven Campus Kulak Kortrijk, Belgium

Upon vascular damage, platelets adhere to the injured vessel wall and become activated resulting in thrombus formation. Activated platelets can be differentiated into two groups according to their response towards agonists: the aggregating and the procoagulant platelets. Aggregating platelets have activated integrin αIIbß3 on their surface, capable of binding fibrinogen and hence important in platelet aggregate formation and clot retraction. Procoagulant platelets expose phosphatidylserine (PS) which is required for binding of coagulation factors to promote thrombin and fibrin generation, thereby stabilizing the clot to stop the bleeding.

In this presentation, the main characteristics of procoagulant platelets as well as the involved pathways will be explained. The events underlying the formation of procoagulant platelets include a complex cascade of signaling events. Key in this cascade is a high and sustained calcium influx which facilitates the formation of the mitochondrial permeability transition pore, leading to bioenergetic failure of the platelet. Procoagulant platelets are a key regulator of thrombus formation, and an imbalance in procoagulant platelet formation is linked to bleeding or thrombosis. Interestingly, in addition to their role in coagulation and thrombus stabilization, recent studies have demonstrated an important role for procoagulant platelets in thromboinflammation. Therefore, the potential to influence the formation of procoagulant platelets as a new antithrombotic strategy will be discussed.

Biography:
Claudia Tersteeg obtained her PhD at the University Medical Center Utrecht, the Netherlands, in 2013. From 2014 to 2017, she worked as a postdoctoral researcher in the Laboratory for Thrombosis Research at KU Leuven Campus Kulak Kortrijk. She continued her career at Ablynx where she was part of the team responsible for the fundamental research that helped achieving market approval for the Nanobody caplacizumab. She was appointed as an assistant professor in a tenure track at the Department of Cardiovascular Sciences at KU Leuven Campus Kulak Kortrijk in October 2019. Her research focuses on procoagulant platelets and their function in health and disease, with a specific interest in the mitochondrial regulation of procoagulant platelet formation, and their role in thomboinflammation.

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NOVO NORDISK SATELLITE SYMPOSIUM I

Clinical outcomes from Early use of N9-GP compared with previous treatment in patients with Haemophilia B

Matino D, Hamilton, Canada

Background: The Canadian Bleeding Disorders Registry (CBDR) captures data from 24 hemophilia treatment centers and patients directly. Nonacog beta pegol (N9-GP) was approved in Canada in 2018.
Objectives: To assess treatment outcomes following switching to N9-GP in a real-world setting.

Methods: CBDR data for Canadian male patients with hemophilia B receiving prophylactic N9-GP for ≥6 months as of March 31, 2021, were included.
To allow comparison with the previously used products, only patients for whom data were available in the CBDR for at least 6 months before the switch to N9-GP were included in this retrospective analysis.

Results: Forty-two patients were included in the analysis (total observation period:148.0 patient-years). The distribution of disease severity was 62% severe, 36% moderate, 2% mild, with 62% of patients previously receiving recombinant factor IX-Fc- fusion protein (rFIXFc) and 38% previously receiving standard half-life (SHL) recombinant factor IX (rFIX). During a median follow-up period of 2.3 years on N9-GP prophylaxis, 232 bleeds were reported in 30 patients, 29% of patients reported zero bleeds. The median overall annualized bleeding rate on N9-GP was 0.73 for patients switching from rFIXFc (previously 1.44) and 2.10 for patients switching from SHL rFIX (previously 6.06). Median total annualized factor consumption (IU/kg) was lower with N9-GP than with previous SHL rFIX (2152 vs 3018) and previous rFIXFc (1766 vs 2278).
Conclusions: Results from this first real-world study of N9-GP in patients with hemophilia B suggest optimal bleeding control with low factor consumption after switching to N9-GP, irrespective of the previous product

Biography:
Dr. Davide Matino is an Assistant Professor of Medicine at McMaster University in Hamilton, Ontario, Canada. Dr. Matino obtained his medical degree at the University of Perugia and completed his residency training at the University of Rome “Tor Vergata”. He completed his Clinical Fellowship in Bleeding disorders at McMaster University where he also received his MSc in Health Research Methodology. Prior to joining faculty at McMaster University in 2020 he was working at the University of Perugia as Assistant Professor in the Department of Experimental Medicine, leading a research project focused on the study of the immune response to FVIII in hemophilia, funded by the Italian Ministry of Health. He is actively engaged in the care of patients with bleeding disorders and his research is focused on inhibitor development in hemophilia A, personalized-prophylaxis regimens in hemophilia, and the treatment of hemophilia patients with inhibitors. Dr. Matino contributes to education in the field of bleeding disorders locally, nationally and internationally. He is an active clinical trial investigator involved in numerous trials in the field of thrombosis and haemostasis. Dr Matino also directs a basic research lab investigating coagulation factor VIII and factor IX and the molecular mechanisms of hemophilia A and B. His work has received several awards and has been presented both nationally and internationally at the International Society for Thrombosis and Haemostasis, American Society of Hematology, and other scientific meetings. He is an active member of the Association of Hemophilia Clinic Directors of Canada (AHCDC) and a member of the Local Organising Committee for the Montreal 2023 International Society on Thrombosis and Haemostasis (ISTH) scientific meeting.

Choosing optimal treatment using an integrative patient-oriented approach

Hermans C, Catholic University of Louvain, Brussels, Belgium

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NOVO NORDISK SATELLITE SYMPOSIUM I

The mainstay of hemophilia treatment is to prevent bleeding through regular long-term prophylaxis and to control acute breakthrough bleeds. Various treatment options including replacement and non-replacement therapies are currently available for prophylaxis. Treatment decision-making is a challenging and multifaceted process of identifying the most treatment appropriate option for each patient.
Shared treatment decision-making between clinicians and patients should consider the bleeding phenotype, the musculoskeletal status, the treatment adherence, venous access, and lifestyle. In parallel, patients' priorities, preferences, and goals must be explored and considered.
Algorithms identifying the most appropriate treatment option and tools evaluating patient’s preferences have recently been developed. They should assist in selecting a treatment for each patient that is as efficient as possible in preventing bleeds while also accounting for the patient's expectations and personal priorities.

Biography:
Cedric Hermans currently heads the Division of Haematology, the Hemostasis and Thrombosis Unit as well as the Hemophilia Center of the Saint-Luc University Hospital in Brussels, Belgium. He was appointed Associate Professor at the Medical School of the Catholic University of Louvain in 2003, Full Professor in 2012 and Vice-Dean in 2015.
Professor Hermans has (co)-authored more than 320 original articles in international journals and is a member of several scientific societies and international advisory boards and collaborative research projects.
He was president of EAHAD and is currently member of the Board of Directors of the World Federation of Haemophilia and the Editor-in-Chief of the Haemophilia Journal.
His main research interests lie in the area of haemostasis and thrombosis, especially clinical studies on the treatment modalities and the wide spectrum of complications of haemophilia in both developed and developing countries, as well as new anticoagulants and the management of thrombosis.

https://www.researchgate.net/profile/Cedric_Hermans/scores
https://dial.uclouvain.be/pr/boreal/en/search/site/cedric%20hermans

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EDUCATIONAL I

Comprehensive Phenotyping of Human Platelets by Single-cell Cytometry

Frelinger A L, Harvard Medical School, Boston Children's Hospital/Dana-Farber Cancer Institute, USA

Platelets play key roles in hemostasis, immunity, and inflammation, and tests of platelet phenotype and function are useful in studies of disease biology and pathology. Immunophenotyping of platelets by traditional flow cytometry has, independent of platelet count, identified markers associated with bleeding severity in patients with ITP and predicted increased bleeding in patients with severe hemophilia A. Our laboratory has explored the use of mass cytometry and spectral fluorescence cytometry with the aim of identifying platelet subsets which may serve as better markers of bleeding risk or have specialized roles in immunity and inflammation. Preliminary results using a 16 marker mass cytometry panel in individuals enrolled in the Framingham Heart Study revealed, after correction for large batch-to-batch variation, platelet subsets which differed in abundance by sex and aspirin use. To take advantage of the wider accessibility and lower cost compared to mass cytometry of spectral fluorescence flow cytometers, and the wider array of available fluorescent antibodies, we recently developed and validated a 16 color spectral cytometry panel including markers of procoagulant activity (annexin V) and immunity (TLR-9). Comprehensive immunophenotyping of platelets using these tools may provide mechanistic insights into disease processes and a means to predict disease progression and monitor therapeutic intervention.

Biography:

Dr. Andrew L. “Larry” Frelinger is Director of the Center for Platelet Research Studies and Staff Scientist at Boston Children’s Hospital, and Assistant Professor of Pediatrics at Harvard Medical School. Dr. Frelinger received his Ph.D. from Case Western Reserve University. Dr. Frelinger's experience spans basic, translational, and clinical studies of platelet function including conformational studies on platelet surface GPIIb-IIIa and development and clinical trials of platelet function monitoring devices and platelet-related therapeutic agents. Recent studies include the use of platelet biomarkers to monitor disease severity and therapeutic efficacy in multi-centered clinical trials and evaluation of mass cytometry to identify and enumerate platelet subtypes in health and disease.
Dr. Frelinger was a Co-Chair of the Platelet Physiology Scientific Subcommittee of the International Society on Thrombosis and Haemostasis where he authored the subcommittee’s position statement on monitoring of P2Y12 inhibitors. Dr. Frelinger is an Editorial Board Member on Scientific Reports, a Principal Editor for the journal Platelets, and Associate Editor for the 4th edition of the book Platelets.

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EDUCATIONAL II

Acquired TTP diagnosis

Devreese K, UZ Ghent, Belgium

Thrombotic thrombocytopenic purpura (TTP) is a rare and life-threatening thrombotic microangiopathy characterized by microangiopathic hemolytic anemia, severe thrombocytopenia, and organ ischemia linked to disseminated microthrombi. TTP is specifically related to a severe deficiency in ADAMTS13, the specific von Willebrand factor-cleaving protease. ADAMTS13 deficiency is most frequently acquired via ADAMTS13 autoantibodies, but rarely, it is inherited via mutations of the ADAMTS13 gene. The idiopathic autoimmune form of the disease is the most common.
The first acute episode of TTP usually occurs during adulthood, but TTP begins in childhood in the inherited forms. Rapid recognition of TTP is crucial to initiate appropriate treatment. Diagnosis relies on the combination of the clinical symptoms. the disturbed laboratory parameters and the measurement of ADAMTS13. Prompt identification/exclusion of TTP can be facilitated by rapid ADAMTS13 testing. Diagnostic assays are available to identify ADAMTS 13 activity. The most commonly utilized enzyme-linked immunosorbent (ELISA) assay takes several hours to perform and does not generally permit rapid testing. Nowadays, automated tests are available to measure ADAMTS13 activity. Also, acquired antibodies to the enzyme can be measured. Antibodies to ADAMTS 13 can be demonstrated in about 75% of cases of acquired TTP associated with ADAMTS 13 activity levels < 10%. These antibodies reduce circulating functional enzyme levels. Most autoantibodies are inhibitory and therefore can be detected and titrated in vitro using classical mixing studies. The use of recombinant ADAMTS 13 in an ELISA allows the rapid identification of autoantibodies, primarily IgG.
A long-term follow-up of patients with TTP is crucial to identify the occurrence of other autoimmune diseases, to control relapses, and to evaluate clinical sequelae. Long-term follow-up of patients with TTP, includes medical consultation, standard laboratory tests, and ADAMTS13 activity monitoring.

Biography:

Professor Katrien Devreese is professor in Hematology/Coagulation at the Ghent University (Belgium) and head of department Diagnostic Sciences at the Faculty of Medicine and Health Sciences of the Ghent University. She is head of department of Laboratory Medicine at the Ghent University Hospital.
She is past-chair and currently co-chair of the Lupus Anticoagulant/Antiphospholipid Antibodies subcommittee of the Scientific and Standardization Committee (SSC) of the International Society of Thrombosis and Haemostasis (ISTH). She is member of the ISTH Education Training subcommittee. She is executive board member and vice-president of scientific communications of the International Society for Laboratory Hematology. She is board member of the Belgian Society on Thrombosis and Haemostasis. She is associate editor of the International Journal of Laboratory Hematology.
Her research interests are focused on studies in the field of Antiphospholipid Syndrome. As active in the field of coagulation for many years, she also published in other domains in the field of coagulation.

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SOBI SATELLITE SYMPOSIUM II

PNH: current treatment options and remaining challenges & Results of the C3 inhibitor Pegcetacoplan in PNH

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare, acquired, life-threatening hematological disease characterized by complement-mediated hemolysis, thrombosis, bone marrow failure, and fatigue that impairs patients' quality of-life.
Standard-of-care terminal (SoC) complement C5 inhibitors have substantially improved outcomes for patients with PNH. Although these inhibitors effectively control intravascular hemolysis, they do not control extravascular hemolysis. To address this unmet medical need, there has been an increased attention to the development of proximal inhibitors, including a C3-inhibitor named pegcetacoplan.
Pegcetacoplan received EMA approval in December 2021 for the treatment of adult patients with paroxysmal nocturnal hemoglobinuria (PNH) who are anemic after treatment with a C5 inhibitor for at least 3 months.
During the satellite symposium, Prof. Straetmans will cover the pathophysiology of PNH, the current treatment options, the remaining unmet medical need and upcoming treatment options of this domain. Dr. Terriou will give us an overview of the most important outcomes of the PEGASUS trial, a study that showed pegcetacoplan's superiority to eculizumab for the primary endpoint of change from baseline in hemoglobin concentration at 16 weeks ($P < 0.001$) and which has shown that these high mean hemoglobin concentrations were maintained over 48 weeks.

Biographies:
Prof. Nicole Straetmans, MD, PhD, is head of Clinic at 'Cliniques Universitaires Saint-Luc (CUSL)' in Brussels and clinical professor since respectively 2016 and 2020.Previously she worked as associate head of clinic at Saint-Luc and has been a teaching professor at UCL.
She participated in the international protocols of LYSA, GRAALL, IFM, EORTC and HOVON and has been a member of the Belgian hematology society (BHS) and of various BHS subcommittees: transplantation, myeloproliferative syndromes, transfusion and acute leukemia.
She has been a member of the evaluation committee for accreditation in hematology at the ministry of health and was president of the transfusion committee of the Jolimont-Lobbes-Nivelles-Tubize Hospital Centers.
She is also a member of the PNH working group and responsible for PNH protocols at CUSL.

Dr. Louis Terriou is hematologist at 'Hôpital Claude Huriez – Centre hospitalier régional universitaire (CHRU) de Lille' in France since November 2012.
Previously, he was assistant senior clinician at the university hospital center (CHU) of Lille and he has been lecturer for the hematology course at the school of medicine from 2008-2021.
He has 14 years of experience in clinical trials as principal investigator or sub-investigator and has more than 120 publications since 2004.
He is an expert in the field of aplastic anemia, paroxysmal nocturnal hemoglobinuria (PNH) and immune cytopenia.
Since 2017, under the lead of Dr. Terriou, the hematology departement of Lille has been one of the 8 reference centers for bone marrow failure (aplasies médullaire) in France.

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PROFESSOR GASTON BAELE MEMORIAL LECTURE

Antithrombin, not just an old molecule

Jochmans K, UZ Brussels, Belgium

Kristin Jochmans finished her medical training in 1982 at the ' Vrije Universiteit Brussel' (VUB). She obtained her certificate of specialist in Clinical Biology in 1988 at the VUB. Since then she has been working in the University Hospital in Brussels (UZ Brussel) in the department of Haematology. In 1999 she obtained the 'Boehringer Ingelheim Prize for Thrombosis and Haemostasis'. She became Doctor in Medical Sciences in 2000 (VUB) after defending her PhD thesis entitled 'Study of the clinical expression and the molecular genetic basis of inherited antithrombin deficiency'.
Her main interest goes to haemostasis and thrombophilia and more particular the study of the phenotype, the molecular basis and the clinical expression of inherited antithrombin and protein C deficiency
From 2000-2008 she was the President of the Belgian Society on Thrombosis and Haemostasis and member of the board till last year. She is active in several working groups on thrombosis and haemostasis.
She is Professor in Haemostasis at the Faculty of Medicine of the VUB and has been head of the Laboratory of Haematology/ Haemostasis at the Universitair Ziekenhuis Brussel. Actually she is still consultant in the hospital.

CSL BEHRING SATELLITE SYMPOSIUM III

Gene therapy in hemophilia, from theory to reality

Cedric Hermans currently heads the Division of Haematology, the Hemostasis and Thrombosis Unit as well as the Hemophilia Center of the Saint-Luc University Hospital in Brussels, Belgium. He was appointed Associate Professor at the Medical School of the Catholic University of Louvain in 2003, Full Professor in 2012 and Vice-Dean in 2015.
Professor Hermans has (co)-authored more than 320 original articles in international journals and is a member of several scientific societies and international advisory boards and collaborative research projects.
He was president of EAHAD and is currently member of the Board of Directors of the World Federation of Haemophilia and the Editor-in-Chief of the Haemophilia Journal.
His main research interests lie in the area of haemostasis and thrombosis, especially clinical studies on the treatment modalities and the wide spectrum of complications of haemophilia in both developed and developing countries, as well as new anticoagulants and the management of thrombosis.

Dr Michiel Coppens is 45 years old and works as an internist in vascular medicine and hemophilia at the Amsterdam University Medical Centers since 2013. He is actively involved in trials on gene therapy for hemophilia A and B. He was the first to treat a person with hemophilia with gene therapy in the Netherlands in 2015 and has dosed nine patients in multiple multiple trials. He is a member of the working group on Gene Therapy of the European Association for Haemophilia and Allied Disorders (EAHAD). He is an author on 113 peer-reviewed publications and has a Hirsch index of 26.

Antithrombotic therapy and COVID-19: an overview of the state-of-the-art and update of BSTH guidance

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COVID-19 is associated with an increased risk for thrombotic complications. The trials investigating the optimal thromboprophylactic dose are performed in challenging times and seemingly produce conflicting evidence. The burdensome circumstances, divergent endpoints, and different analytical approaches hamper comparison and extrapolation of available evidence. Most importantly, clinicians should provide thromboprophylaxis in hospitalized COVID-19 patients while (re)assessing bleeding and thrombotic risk frequently. The COVID-19 Thromboprophylaxis Working Group of the BSTH will present a first look into its updated guidance document. It aims to summarize the available evidence critically and to guide clinicians in providing the best possible thromboprophylaxis. In the Q&A session it is possible to comment on the updated guidance before the working group will finalize the document.

Biography:
Dr. Matthias Engelen graduated as a medical doctor in 2017 at the University Hospitals Leuven. In 2019 he started working on thromboinflammation and hemostasis at the Center for Molecular and Vascular Biology (CMVB) under the supervision of prof. Thomas Vanassche. During the COVID-19 pandemic, dr. Engelen and his supervisors mainly focused on the thrombotic and thromboinflammatory complications of COVID-19 to contribute to worldwide efforts to improve outcomes in a new widespread disease. Their research resulted in several original articles and (late-breaking) presentations at international congresses, as they studied in- and out-of-hospital thromboprophylaxis in COVID-19. They also investigated the modulation of thromboinflammation in moderate to severe COVID-19 in the multicentric randomized DAWn-AntiCo study and collaborated on several other randomized COVID-19 trials.

Platelets in chronic liver disease

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Chronic liver disease (CLD) was for a long time considered as a model of acquired hemorrhagic disease, reflected by, for example, prolonged prothrombin time. In the last 15 years, it has been acknowledged that CLD patients present complex alterations in the hemostatic system leading to so-called rebalanced hemostasis due to simultaneous alterations in pro-and anti-hemostatic drivers. This balance is however fragile and can tip towards both hemostasis-related bleeding and thrombosis. These complex hemostatic changes are caused by a reduced liver synthetic capacity, splenomegaly, chronic endothelial cell activation and consumption of platelets and coagulation factors by disseminated or localized activation of coagulation.
Thrombocytopenia is common and multifactorial in CLD patients. A low platelet count is an indicator of more advanced disease and is associated with poorer prognosis.
Several papers using static platelet function tests, platelet aggregation assays for example, indicate platelet hypofunction. It is however not clear if thrombocytopenia and platelet dysfunction also indicate defective primary hemostasis. Some papers report for example conserved capacity of cirrhotic platelets to adhere to the thrombogenic surface in flow based assays. Markedly elevated plasma von Willebrand factor levels are seen in CLD patients and enhance adherence to these thrombogenic surfaces. Elevated soluble platelet activation markers have also been reported. A difficulty met in studies on platelets, is their fragile and activatable nature. Platelets are very easily activated due to for example pre-analytical issues.
Likewise, a fairly preserved function of primary hemostasis is reflected by clinical observations. Liver transplantation is possible without pre-and peri-procedural correction of platelet count, although some studies report severe thrombocytopenia as a risk factor for procedure-related bleeding. Patients with chronic liver disease are also not protected from venous and arterial thrombosis.
In summary, growing evidence is in favor of a relatively balanced primary hemostasis in chronic liver disease. Studies on the cirrhotic platelet are still scarce and we believe that techniques as microfluidic assays and flow cytometry could possibly enhance our knowledge. In this regard, we set up a longitudinal study comprising decompensated pre-transplant children and adults using a multi-color flow cytometry assay to look at procoagulant platelets and priming effects (low and high concentrations of agonist). We also realize flow (Maastricht flow chamber) based assays in the pediatric cohort. Platelets studies are accompanied by coagulation and fibrinolysis tests to provide a more complete picture of hemostasis in these patients.
In this state of the art session, we will give you a short overview of rebalanced hemostasis and the current knowledge of primary hemostasis in CLD. Some preliminary data will also be presented.

Biography:
Marie-Astrid van Dievoet obtained a master's degree in Medicine from the University of Leuven, Belgium, which was followed by a specialization in Clinical Biology at the University Hospital Leuven. After obtaining her degree in clinical biology, she started at the laboratory department of Cliniques universitaires Saint-Luc where she supervises the coagulation laboratory. The clinical coagulation laboratory realizes both routine and specialized coagulation tests, including platelet function assays. Since Cliniques universitaires Saint-Luc is a hemophilia reference center, the laboratory is also specialized in diagnosis and follow-up of hemophilia patients.
In parallel of her clinical work, she started a PhD under the supervision of Prof. Xavier Stephenne, head of the pediatric gastroenterology department, on platelets in chronic liver disease. This is realized in collaboration with Prof. Sandrine Horman (UCL-IREC-CARD), Prof. Johan Heemskerk (Maastricht University), Prof Jonathan Douxfils (University of Namen) and Prof. Ton Lisman (UMCG).

O1

BASIC RESEARCH

Ticagrelor prevents Staphylococcus aureus infective endocarditis by mitigating bacterial virulence

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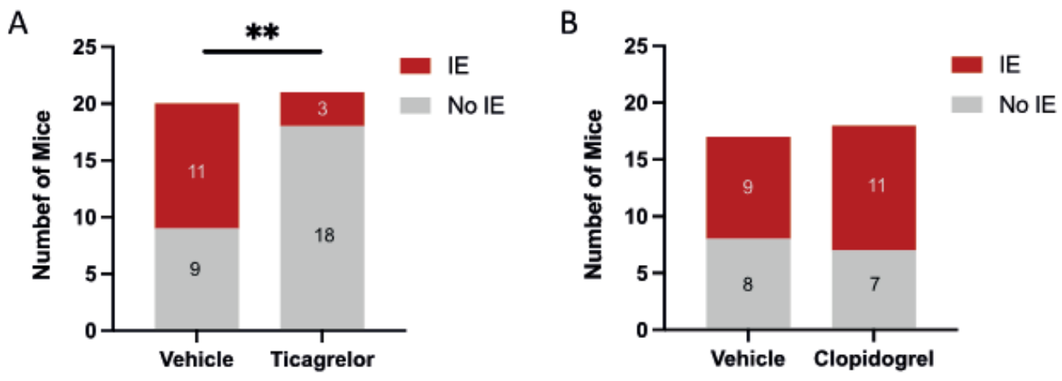
Background: Infective endocarditis is a deadly disease mainly caused by the gram-positive and highly virulent bacteria Staphylococcus aureus. The disease can affect up to 3% of patients with a prosthetic valve, and its prevalence is expected to rise due to steadily increasing number of heart valve implantations. Current therapy for these patients consists of antimicrobial therapy, having a low efficacy. Therefore, there is an urgent need to discover new strategies that could prevent this disease and reduce the associated increasing antibiotic resistance.

Aim: To assess the ability of the antiplatelet drug ticagrelor, which also displays antibacterial activity against gram-positive bacteria, to prevent Staphylococcus aureus Infective endocarditis.

Methods: We used a mouse model of infective endocarditis induced by a Staphylococcus aureus infective endocarditis clinical isolate. Ticagrelor and clopidogrel were administered prior to local histamine infusion on the aortic valve and infection by Staphylococcus aureus infective endocarditis clinical isolate. Infected vegetation presence was determined by Gram staining on heart sections after three days. The antibacterial effect of ticagrelor on key mechanisms of infective endocarditis was assessed in vitro.

Results: A single administration of ticagrelor at conventional antiplatelet dosage (3mg/kg) prior to infection significantly prevented the formation of infected vegetation, with infective endocarditis in only 14.3% of ticagrelor-treated mice (n=21) compared to 55 % of vehicle-treated mice (n=20). Interestingly, clopidogrel treatment failed to prevent disease development with infective endocarditis in 61.1% of the mice (n=18) which made it unlikely that solely the antiplatelet effect would explain infective endocarditis prevention by ticagrelor (Figure 1). Ticagrelor dosed at plasma levels achieved in patients (0.75ug/ml-1.25ug/ml) did not affect Staphylococcus aureus growth in liquid cultures but it caused drastic adherence defect on activated endothelial cells and extracellular components. Furthermore, we found that growing Staphylococcus aureus in the presence of ticagrelor altered the Staphylococcus aureus accessory gene regulator system, leading to reduced toxin production and toxin-induced platelet aggregation while preserving the ability of platelets to kill those bacteria.

Conclusion: Our study demonstrates unprecedented ability of ticagrelor to prevent infective endocarditis by directly mitigating bacterial virulence. Hence, clinical trials using ticagrelor as adjunct therapy to antibiotics in patients at risk for infective endocarditis are warranted.



O2

BASIC RESEARCH

DNase-1 can overcome rt-PA resistance of platelet-rich ischemic stroke thrombi

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Background: Recombinant tissue plasminogen activator (rt-PA) is the only approved drug that can be used to pharmacologically lyse the occluding thrombus in ischemic stroke patients. Due to several contraindications and due to therapy resistance, recanalization is achieved in only a small number of patients. The exact nature of this rt-PA resistance is not yet fully understood but it is thought that thrombus composition plays an important role. We previously showed that, besides fibrin, extracellular DNA and neutrophil extracellular traps (NETs) are a structural feature of ischemic stroke thrombi which could be important for thrombus stability, especially in platelet-rich thrombi.

Aim: To investigate the rt-PA resistance in ischemic stroke thrombi and to explore the additional thrombolytic effect of an adjuvant therapy of the DNA-degrading enzyme DNase-1 in combination with rt-PA.

Methods: A total of 51 thrombi retrieved from patients were divided into three treatment groups (1 µg/mL rt-PA, a combination of 1 µg/mL rt-PA and 100 U/mL DNase-1 or nothing). For each thrombus, one part was used for detailed histological analysis of seven thromboembolus components (red blood cells (RBC), fibrin, platelets, von Willebrand factor, leukocytes, intra- and extracellular DNA and NETs). The other part was subjected to lysis for 180 minutes, after which lysis efficiency was calculated as the percentage of residual thrombus weight compared to original weight.

Results: We observed that RBC-rich thrombi (>50% RBC content) were significantly more susceptible to rt-PA-mediated lysis compared to platelet-rich thrombi (<50% RBC) (residual weight after 180 minutes of 25.1%±19.1% and 55.4%±25.9% respectively). A strong positive correlation was found between the percentage of RBC and the lysis rate of the thrombus. Next, we wanted to test if addition of DNase-1 could increase lysis of ischemic stroke thrombi and more specifically the rt-PA resistant platelet-rich thrombi. Strikingly, combined use of rt-PA and DNase-1 significantly improved thrombolysis of platelet-rich thrombi (residual weight of 24.3%±18.4%), but had no additional effect on the rt-pa mediated lysis of RBC-rich thrombi (residual weight of 24.5%±7.3%)

Conclusion: This study showed that RBC-rich ischemic stroke thrombi are more susceptible to thrombolysis using standard rt-PA treatment and that combined use of rt-PA and DNase-1 can improve the thrombolysis of rt-PA resistant platelet-rich thrombi. These insights open new perspectives for the treatment of ischemic stroke patients and could potentially improve the thrombolysis of rt-PA resistant ischemic stroke thrombi.

O3

BASIC RESEARCH

Deep vein thrombosis generates a systemic environment promoting release of neutrophil extracellular traps and detrimental cardiac remodeling

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Introduction: Approximately 10-30% of people diagnosed with thrombosis die within one month. Evidently, this means that 70-90% of patients survive, but they are at risk of developing post-thrombotic syndrome. This systemic side effect of having had a thrombus leads to decline in quality of life and contributes to disability. The role of neutrophil extracellular traps (NETs), caused by the activation of peptidylarginine deiminase 4, in thrombus development has gained much interest in recent years, and was shown to be of major importance in thrombus development. However, the systemic influence of thrombus presence on neutrophil activation and NET release has not received the attention it needs.

Aims: Thrombus formation results in the disruption of normal homeostasis towards a state of inflammation, also termed thromboinflammation. This thrombus-induced inflammatory state is known to result in neutrophil activation at the site of the thrombus. We aimed to study if local inflammation due to thrombus formation can progress towards a systemic inflammatory state, eventually causing tissue damage and organ failure.

Methods: A mouse model of DVT, through inferior vena cava (IVC) stenosis, was adopted to study the systemic effects of thromboinflammation. Thrombus formation was initiated in either PAD4+/+ and PAD4-/- mice, after which systemic neutrophil activation was evaluated in vitro. After two weeks on the model, cardiac function, as well as tissue remodeling was evaluated through echocardiography and histology, respectively.

Results: After 14 days of thrombus development, blood neutrophils were isolated and stained for NET formation (colocalization of citrullinated histone H4, DNA and Ly6G). Neutrophils from mice which underwent DVT surgery released higher numbers of NETs in circulation, and showed increased priming towards activation as quantified through stimulation of neutrophils with the calcium ionophore ionomycin, as compared to sham surgery animals. These mice had decreased systolic cardiac function, evaluated by left ventricular ejection fraction (LVEF), as compared to sham surgery animals. Interestingly, when PAD4-/- mice underwent similar surgery, LVEF remained comparable to sham surgery mice after 14 days (Figure 1C). At 14 days, hearts were harvested and prepared for flow cytometric detection of infiltrated neutrophils. Increased numbers of cardiac infiltrated neutrophils were found in PAD4+/+ mice, 14 days after surgery, as compared to sham controls as well as to PAD4-/- mice. In addition, cardiomyocyte size increased significantly in the PAD4+/+ experimental group as compared to the PAD4+/+ sham controls and PAD4-/- animals.

Conclusion: The important role of PAD4 and NETs in thrombus formation has previously been established. However, the systemic role of activated neutrophils and NETs due to a venous thrombus has not yet been studied. Here we show that the presence of a DVT results in an increase of NETs in circulation, 14 days post thrombus development. NETs are known to be pro-inflammatory and cause damage to underlying tissue, and have been established as important mediators in cardiac damage in several disease models. Concordantly , the absence of NETS due to systemic PAD4 deletion resulted in less cardiac remodeling, thus protecting against systolic cardiac dysfunction due to the presence of a DVT.

O4

BASIC RESEARCH

α1AMP-activated protein kinase (α1AMPK) regulates hemostatic dysfunctions during septic shock

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Background: Sepsis is a pathological circumstance whose outcome is associated with organ dysfunction and death. Vascular hyperpermeability and hemostasis impairment both play a key role in the onset of organ failure. Our previous work highlighted the protective role of α1AMPK against sepsis-induced hyperpermeability. However, the impact of α1AMPK on hemostasis has never been investigated in this condition.

Aims: This study aims to characterize the interaction of α1AMPK with hemostasis during sepsis.

Methods: α1AMPK wild type (WT) and knockout (KO) mice were challenged with intraperitoneal injection of lipopolysaccharide (LPS) from Escherichia coli O55B5 (10mg/kg) for 18 hours. Plasmatic markers of coagulation, NETosis and platelet activation were measured by ELISA. To identify cell type responsible for the dysregulation, same analyses were conducted on endothelial-, leukocyte- or platelet- specific α1AMPK knockout mouse models.

Results: Hemostasis was altered after LPS administration, in both v1AMPK WT and KO mice. Thrombin generation, reflected by thrombin-antithrombin complex formation, was significantly increased in septic α1AMPK KO mice compared to septic WT mice. In contrast, fibrinolysis was more inhibited in α1AMPK KO than in WT mice, with a higher level of plasminogen activator inhibitor-1, while tissue plasminogen activator secretion was comparable. This exacerbated procoagulant phenotype in α1AMPK KO mice was associated with an increase of sCD62P in plasma, reflecting an overactivation of platelets under septic conditions. Septic α1AMPK KO mice also showed an increase in neutrophil elastase, and myeloperoxidase, suggesting a stronger activation of neutrophils compared to WT animals. Leukocyte specific α1AMPK KO mouse model did not recapitulate the phenotype observed in total α1AMPK KO mice after LPS administration. However, endothelial specific α1AMPK KO mice showed an increased platelet activation while platelet specific α1AMPK KO model prevented neutrophil activation.

Conclusions: Altogether, these results demonstrate that α1AMPK plays a crucial role in the regulation of hemostasis disturbances and NETosis during sepsis. While α1AMPK from leukocytes does not contribute to the septic damage, endothelial α1AMPK might be a brake for hemostatic dysregulation.

O5

CLINICAL & LABORATORY

Heparin monitoring in ImpellaTM-supported cardiogenic shock patients: parallel dual testing in critically ill

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Background/Introduction: Coagulopathic complications often jeopardize critically ill patients' outcome during mechanical circulatory support (MCS). Therefore, meticulous management of heparin therapy is mandatory to avoid complications in this patient population. The most convenient and most frequently used test in this setting is the APTT. However, the APTT can be prolonged or shortened due to various cofounding factors during critical illness (e.g., sepsis, liver failure, factor XI/XII consumption), unrelated to the anticoagulant effect of heparin. Alternatively, the anti-Xa assay directly measures heparin activity. Recently, an anti-Xa guided protocol for heparin titration with parallel APTT measurements in MCS-supported cardiogenic shock (CS) patients has been proposed.

Purpose: We aim to investigate the correlation between parallel anti-Xa and APTT-measurements in an anti-Xa guided heparin anticoagulated CS population during support with a left-sided ImpellaTM device.

Methods: Between April-2017 and June-2022, all CS patients with isolated left-sided ImpellaTM-support (>24h) and heparin anticoagulation monitored by anti-Xa with APTT in parallel were assessed at two high-volume tertiary institutions. Anti-Xa was considered in range between 0.2-0.3IU/mL or 0.3-0.5IU/mL based on the patient's profile (per center protocol, underlying atrial fibrillation or venous thromboembolism). In range APTT values were considered 40-60s and 60-80s respectively. Correlation between parallel anti-Xa and APTT values was assessed by a Pearson correlation model.

Results: Ninety-six ImpellaTM-patients (76 men, 79%) with a median age of 55 (IQR 46-65) were included. Median support was 6 days (IQR 4-12). A total of 2780 parallel samples was analyzed, showing an overall Pearson correlation coefficient of 0.54 (Fig. 1A). For samples with anti-Xa in range (0.2-0.3IU/mL or 0.3-0.5IU/mL) (N=1287), matching APPT was low in 540 (42%), in range in 478 (37%) and high in 269 (21%) samples (Fig. 1B).

Conclusions: We highlight a weak correlation between anti-Xa and APTT in an anti-Xa guided heparin anticoagulated CS population during left-sided ImpellaTM support. Heparin titration based on APTT alone would result in under- (21%) or overtitration (42%) in 63% of our cases, due to confounding factors influencing the APTT-assay in critically ill. Therefore, we strongly advocate a parallel anti-Xa/APTT anticoagulation monitoring strategy to adequately monitor the coagulation state of this critically ill population.

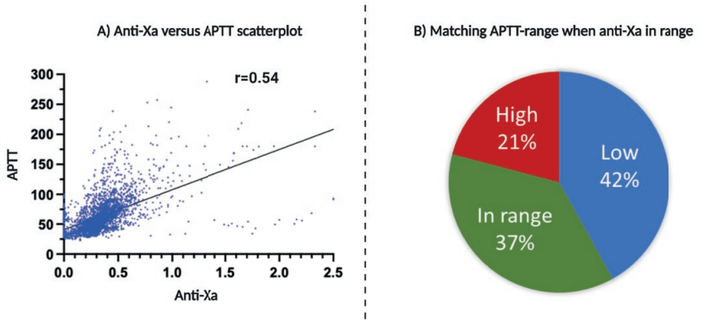


Figure 1:
Panel A: Scatterplot of anti-Xa (IU/mL) versus APTT (s) in 2780 matching samples in an anti-Xa guided heparin anticoagulated CS population, supported by a left-sided Impella™, shows a weak correlation (r=0.54).
Panel B: Pie-chart of matching APTT values when anti-Xa is in range (0.2-0.5IU/mL), indicating that APTT is low in 671 (35%), in range in 846 (45%) and high in 376 (20%) of the matching samples.

O6

CLINICAL & LABORATORY

Position of Next Generation Sequencing Testing in the Diagnostic Algorithm of Inherited Thrombophilia

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Background: Thrombosis is a multifactorial disease resulting from inherited and acquired risk factors. Different genes are known to elevate thrombophilia risk. Since 2019, we implemented the clinical use of next generation sequencing (NGS) for patients presenting with thrombosis at young age or evidence of a family history. The recommended laboratory diagnosis of thrombophilia involves plasma testing of natural anticoagulant proteins and genetic determination of presence of factor V Leiden (FVL) and F2 prothrombin, c.*97G>A, mutation [1].

Aims: We aimed to evaluate the additional value of NGS in the diagnostic algorithm of inherited thrombophilia.

Methods: Over a three-year period (Jan 2019 – Jan 2022) we performed genetic testing using whole exome sequencing of 30 (anti) coagulation factor genes for 172 patients with thrombotic phenotypes. We correlated the genetic results with available conventional laboratory thrombophilia screening and protein C, protein S and antithrombin plasma levels.

Results: The clinical application of NGS for inherited thrombophilia detected at least one genetic variant in known thrombotic genes in 63% of 172 patients. In total 155 variants were detected, of which 21% were pathogenic (PV) and 26% likely pathogenic variants (LPV) while 53% were variants of unknown significance (VUS). Most variants were detected in genes coding for protein C (PROC), protein S (PROS1) and antithrombin (SERPINC1) besides the known genetic thrombotic risk factors, FVL and F2 prothrombin variant. Multigene panel testing showed an added value for 31% (n=53) of the 172 patients in addition to conventional thrombophilia laboratory screening. (I) Nineteen of the 51 patients with a negative routine thrombophilia screening showed the presence of a genetic variant. (II) For 16 of 82 patients with positive plasma testing, we detected a second genetic variant in addition to the defect related to plasma testing. (III) In addition to the presence of the FVL or F2 prothrombin variant in 26 patients, 18 of these carried an additional genetic variant. (IV) For the last group without thrombophilia testing, 2 out of 13 showed the presence of a genetic variant. Normal levels of protein C, protein S and antithrombin could be measured in patients carrying (L)PV or VUS (figure 1). In total, 21 patients escaped the detection of having a thrombophilia risk using conventional laboratory plasma assays. The commonly detected PROS1 variant (p.Ser501Pro) was earlier described as the protein S Heerlen variant, which is associated with thrombosis and resulting in slightly decreased or low normal protein S levels [2]. Variants associated with normal levels require further functional studies to understand their effect on thrombosis.

Conclusion: For patients with suspicion of inherited thrombophilia or thrombosis at young age, extensive genetic testing for thrombosis related genes should be considered.

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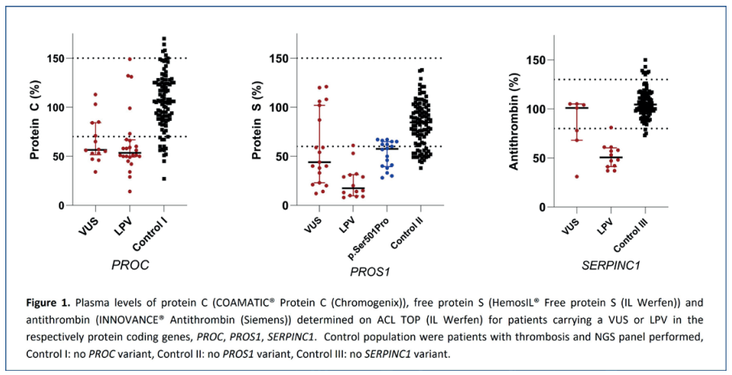


Figure 1. Plasma levels of protein C (COAMATIC® Protein C (Chromogenix)), free protein S (HemosIL® Free protein S (IL Werfen)) and antithrombin (INNOVANCE® Antithrombin (Siemens)) determined on ACL TOP (IL Werfen) for patients carrying a VUS or LPV in the respectively protein coding genes, *PROC*, *PROS1*, *SERPINC1*. Control population were patients with thrombosis and NGS panel performed, Control I: no *PROC* variant, Control II: no *PROS1* variant, Control III: no *SERPINC1* variant.

07

CLINICAL & LABORATORY

Genotype-phenotype correlation in Belgian patients with inherited Protein C deficiency

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Introduction: Protein C (PC) is a vitamin K-dependent protein that, upon activation, acts as a natural inhibitor of coagulation factors Va and VIIIa. Inherited PC deficiency is a rare, autosomal dominant disorder that predisposes to the development of venous thromboembolism (VTE). The prevalence of this deficiency varies between 3-5% in patients with a history of venous thrombosis.

Phenotypically, two types of PC deficiency can be distinguished. Type I deficiency is a quantitative deficiency characterised by an equal reduction in PC antigen and activity. Type II deficiency on the other hand is a qualitative deficiency with normal antigen levels but reduced PC activity. Molecular analysis of PROC, the gene encoding for PC, can be performed to determine the genotype of PC deficient patients.

Aim: The aim of our study was to evaluate the relationship between PC genotype, phenotype and clinical presentation in Belgian patients with inherited PC deficiency.

Methods: The study population consisted of 202 PC deficient patients recruited between January 2010 and May 2022. Protein C activity was measured with two commercial assays, based on a different measurement principle: a clotting-based method (HemosIL ProClot, Werfen) and a chromogenic method (Coamatic Protein C, Werfen). Protein C antigen was determined by ELISA (Asserachrom Protein C, Stago). Molecular analysis of the PC gene consisted of complete sequencing of all exons and intron-exon boundaries and multiplex ligation-dependent probe amplification.

Results: The majority of the patients in our study are female (62.9%). In approximately half of the PC deficient patients (51.5%) the first clinical manifestation was a VTE, with deep vein thrombosis being the most frequent presentation (58.6%). Women experienced their first thrombotic event approximately nine years earlier than men (32 and 41 years, respectively). In cases of provoked VTE, oestrogen-related risk factors were the most common.
Molecular analysis of PROC was carried out in 140 of the 141 index patients. A genetic defect was found in 88.6% (124/140) of these patients. These patients shared 57 different variants of whom 14 were previously unreported. All patients carried the variant in heterozygous state except for 1 homozygous and 1 compound heterozygous patient.
Of the 179 PC deficient patients with an available plasma sample, 89% (159/179) had a clear type I PC deficiency, 4% (8/179) had a type II deficiency while the remainder 7% (12/179) showed discrepant results (i.e. same genotype but different phenotype).

Conclusion: Our study is the first to report the genotype-phenotype correlation in Belgian patients with inherited PC deficiency. We showed that PC deficient women encounter their first thrombotic event approximately nine years earlier than men, probably due to exposure to female-specific risk factors for VTE. Fourteen novel variants in PROC were identified, leading to an important contribution to the mutation spectrum of PC deficiency.

Modulation of thromboinflammation in hospitalized COVID-19 patients with aprotinin, low molecular weight heparin, and anakinra: the DAWn-Antico study

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08

CLINICAL & LABORATORY

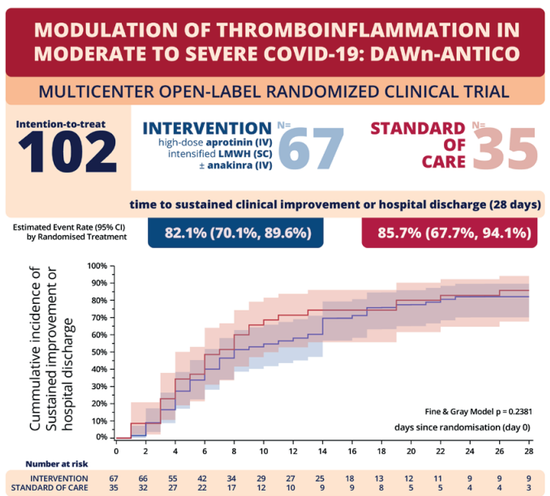
Background: Thromboinflammation in severe COVID-19 is associated with disease severity and outcome. The kallikrein pathway is suggested to mediate thromboinflammation in COVID-19 by activating inflammatory pathways and contact-mediated coagulation.

Aims: The DAWn-antico study investigates if a multistep multitarget modulation of the thromboinflammatory response improves outcomes in hospitalized patients with severe COVID-19.

Methods: This multicenter open-label randomized clinical trial was performed in three Belgian hospitals (EudraCT 2020-001739-28). Using a centralized web-based randomization, patients hospitalized with COVID-19 were randomized to receive standard of care (SOC) or SOC plus study intervention according to a 1:2 allocation scheme stratified by study site with randomly selected block sizes of six or nine. The study intervention consisted of aprotinin (2.000.000 IE IV four times per day) combined with low molecular weight heparin (LMWH; SC 50 IU/kg twice daily at the ward, 75 IU/kg twice daily at intensive care). Additionally, patients with predefined hyperinflammation received the interleukin-1 receptor antagonist anakinra (100 mg IV four times per day). In this open label study, patients, clinicians, and study personnel were aware of the assigned treatment. The primary outcome was time to a sustained 2-point improvement on the 7-point WHO ordinal scale for clinical status, or discharge.

Results: Between June 24th, 2020, and February 1st, 2021, 105 patients were randomized to intervention (N=69) or SOC (N=36). Ultimately, 102 patients were included in the full analysis set (intervention N=67 vs. SOC N=35). Twenty-five patients from the intervention group (37%) received anakinra. The mean age of the study population was 58 years (SD 13) and 75% of patients were male. Patients had elevated D-dimer (median 665 µg/L, IQR 460-1090 µg/L) and C-reactive protein (median 65 mg/L, IQR 41-112 mg/L) at admission. After 28 days, the intervention did not affect the time to sustained clinical improvement or hospital discharge (primary endpoint obtained in intervention n=52 (82%) vs. SOC n=30 (86%), HR 0.77 [CI 0.50; 1.19], p=0.24). Twenty-eight-day mortality was not different between intervention (n=3, 4.6%) and SOC (n=2, 5.7%) (HR 0.82, [CI 0.14; 4.94], p=0.83). Except for D-dimer on day 3, there was no significant C-reactive protein or D-dimer reduction. There was one treatment-related adverse event in the intervention group (hematuria, 1.49%). There was one pulmonary embolism in the intervention group (1.49%) and one other thrombotic event in the SOC group (2.86%). There were no major bleedings.

Conclusions: In hospitalized COVID-19 patients, modulation of thromboinflammation with high-dose aprotinin and LMWH with or without anakinra did not improve clinical nor biochemical outcome in patients with moderate to severe COVID-19.



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P01

ABSTRACTS POSTERS

Dual antiplatelet therapy is associated with high α-tubulin acetylation in circulating platelets from coronary artery disease patients

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Introduction: Platelet inhibition is the main treatment strategy to prevent atherothrombosis. Despite dual antiplatelet therapy (DAPT) combining aspirin and a $P2Y_{12}$ receptor inhibitor, high on-treatment platelet reactivity persists in some patients due to poor response to treatment and is associated with ischemic risk. It remains unknown if circulating platelets in high-risk patients have distinct morphological characteristics which could contribute in their pro-thrombotic potential.

Aims: Knowing the key role of α-tubulin acetylation in regulating platelet shape change, we investigated if this post-translational modification could differ according to antiplatelet therapy and on-treatment platelet reactivity.

Methods: Platelets were isolated from arterial blood samples of 240 patients admitted for coronary angiography and acetyl α-tubulin levels were evaluated by immunoblotting. Platelet reactivity was assessed in whole blood using multiplate analysis. 141 (59%) patients were taking aspirin among which 32 (13%) were treated with an additional $P2Y_{12}$ inhibitor. High on-treatment platelet reactivity was detected in 8 out of 32 DAPT-treated patients. Participants provided written informed consent and the study was approved by the institutional ethics committee.

Results: Platelet α-tubulin acetylation was significantly increased in DAPT-treated patients ($p<0.001$). The minority of non-treated patients (11.4%) exhibited high α-tubulin acetylation level (third acetyl α-tubulin tertile). In contrast, high level of α-tubulin acetylation was observed in most patients on DAPT achieving low platelet reactivity (83.3%). Interestingly, the proportion of patients with high acetyl α-tubulin level (37.5%) was drastically decreased among patients with high on-treatment platelet reactivity. Multivariate logistic regression further supported that DAPT achieving platelet inhibition is independently associated with a 27-fold increase in the likelihood of being in the highest acetyl α-tubulin tertile ($p<0.001$).

Summary: We revealed high platelet α-tubulin acetylation as a potential marker of platelet inhibition in response to dual antiplatelet therapy. Since α-tubulin acetylation is a hallmark of stable microtubules, its increase could contribute to maintaining resting morphology of circulating platelets.

P02

ABSTRACTS
POSTERS

Association between thromboembolus composition and first pass recanalisation after thrombectomy in ischemic stroke

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Background and aims: When performing thrombectomy on ischemic stroke patients, achieving good recanalisation in the first pass (first pass effect, FPE) is associated with a good clinical outcome. Achieving FPE has therefore become an important objective of the procedure. The reasons why multiple attempts are sometimes needed are not fully understood, but the composition of the thromboembolus is considered to be an important factor. The aim of this study was to investigate the link between thromboembolus composition and FPE.

Methods: In total, 497 stroke thromboemboli from the AZ Groeninge Hospital (Kortrijk, Belgium) and CHU Lille (Lille, France) were analysed histologically for seven components: red blood cells (RBC), fibrin, von Willebrand factor, platelets, leukocytes, citrullinated histone 3 (marker for neutrophil extracellular traps) and DNA (intra- and extracellular). FPE was defined as obtaining a modified Thrombolysis in Cerebral Ischemia (mTICI) score of 2c/3 after the first pass. Thromboembolus histology was compared between cases in which an FPE was achieved and cases in which a mTICI score of 2c/3 was achieved after multiple passes.

Results: Good recanalization (mTICI 2c/3) was obtained in 348 patients: 208 with an FPE and 140 needing multiple passes. Patients in which no good recanalization was obtained (n= 149) were excluded for this analysis. Interestingly, thromboemboli from FPE cases contained significantly more RBC (median (IQR): 44.74% (29.53%-58.13%) versus 39.85% (24.99%-52.88%), p=0.02) compared with patients in which multiple passes were needed. In addition, achieving an FPE was associated with better clinical outcome indicated by a lower modified ranking score at 90 days (median (IQR): 2 (1-3) vs 3 (1-5), p=0.0003).

Conclusions: Achieving an FPE is more likely in patients with RBC-richer thromboemboli and achieving an FPE results in better clinical outcome. Future research and technical improvements are needed to improve FPE-rates in RBC-poor thromboemboli.

P03

ABSTRACTS
POSTERS

Quantification of extracellular DNA in ischemic stroke thrombi reveals a correlation with stroke etiology and thrombectomy procedural success

De Wilde M[1], Staessens S[1], Desender L[1], Vandelanotte S[1], François O[2], Dewaele T[2], Vanacker P[3,4], Tersteeg C[1], Vanhoorelbeke K[1], Andersson T[2,5], De Meyer S[1]

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Background: Removal of occluding thrombi in ischemic stroke patients is not always successful. The mechanisms of therapy resistance are poorly understood, but thrombus composition is likely a major contributor. Recently, the organization of DNA in ischemic stroke thrombi was described, but a good quantification method only assessing extracellular DNA (exDNA) and not including nuclear DNA was lacking.

Aims: To establish a standardized method for quantification of exDNA in ischemic stroke thrombi and to correlate exDNA content with stroke etiology, clinical outcome, thrombectomy procedural parameters and other thrombus characteristics.

Methods: Thrombi (n=262) were collected from acute ischemic stroke patients treated with mechanical thrombectomy at AZ Groeninge Hospital in Kortrijk, Belgium. Consecutive thrombus sections were stained with Feulgen's reaction and haematoxylin-eosin, after which DNA content was determined via color-based segmentation analysis. To determine exDNA content, the amount of nuclear DNA was subtracted from the total DNA in thrombi. The quantitative results for exDNA were then correlated with patient and procedural parameters.

Results: Thrombus exDNA content was determined via an in-house standardized quantification method. The amount of exDNA ranged from 0 to 66.5% of the total thrombus area. ExDNA content was positively correlated with the amount of leukocytes, platelets, fibrin and citrullinated histones and inversely correlated with the amount of erythrocytes. Interestingly, exDNA was significantly more abundant in thrombi of cardioembolic origin (11.2%±11.7%) compared to large artery atherosclerosis thrombi (6.9%±7.9%, p=0.0159) or thrombi from other known etiologies (4.9%±4.0%, p=0.0108). Older thrombi contained significantly more exDNA (14.2%±11.7%) compared to fresher thrombi (6.2%±9.4%, p<00001). No associations were found between exDNA content and stroke severity, clinical outcome, length of thrombectomy procedure, revascularization status and number of passes performed during thrombectomy. However, thrombi from thrombectomy procedures in which only one pass was needed to achieve good recanalization contained significantly less exDNA (8.2%±9.5%) than thrombi for which multiple passes were needed (11.0%±10.1%, p=0.0479).

Conclusion: ExDNA is a typical structural feature of platelet-rich stroke thrombi and cardioembolic origin is associated with high exDNA content. A 'first pass recanalization' is associated with lower amounts of exDNA, suggesting that exDNA renders thrombi more resistant to thrombectomy.

P04

ABSTRACTS
POSTERS

Role of platelet GARP in post-myocardial infarction fibrosis

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Introduction: After myocardial infarction (MI), cardiac fibroblasts differentiate into myofibroblasts. Myodifferentiation leads to excessive extracellular matrix proteins secretion within the myocardium, resulting in fibrosis which holds a prominent role in adverse left ventricular (LV) remodeling. TGFβ1 is the main mediator of myodifferentiation and is abundantly produced by platelets. Moreover, platelets are involved in TGFβ1 activation via the Glycoprotein A Repetitions Predominant (GARP) present on their surface. Although platelets are known to infiltrate the infarcted myocardium, their role as a source of active TGFβ1 in post-MI cardiac remodeling has never been investigated.

Aims: The current proposal sought to determine the contribution of platelet GARP protein in the regulation of fibrosis and LV remodeling after MI.

Methods: We generated a new Cre transgenic mouse strain that allows megakaryocyte/platelet specific invalidation of GARP (GpIba-Cre x GARPfl/fl). The impact of GARP deficiency on platelet function was evaluated in vitro by flow cytometry using thrombin and CRP as agonists. Serum production of total and active TGFβ1 was assessed by ELISA. pGARP WT and pGARP KO mice underwent MI via permanent ligation of left anterior descending coronary artery (LAD). Immune cells infiltration was evaluated by immunohistochemistry in the hearts of pGARP WT and KO mice. Cardiac fibroblast proliferation and myodifferentiation was assessed by immunofluorescence with PCNA or αSMA antibodies. Cicatricial and interstitial fibrosis was evaluated at 7- and 14-days using sirius red staining or RT-qPCR. Cardiac function was measured by echocardiography.

Results: GARP exposure at platelet surface increased upon thrombin and CRP stimulation. However, GARP did not influence platelet activation as platelets without GARP displayed normal agonist induced granule secretion. As expected, the generation of active TGFβ1 was drastically impaired in the serum of platelet GARP KO mice. Following MI, immunochemistry data show that platelets were detectable within the myocardium between 1- and 3-days, compared to the sham-operated mice. Platelet infiltration was similar between pGARP WT and KO infarcted hearts. Surprisingly, the deletion of GARP had no impact on cardiac fibroblast proliferation or myodifferentiation or on the extent of cicatricial and interstitial fibrosis. However, collagen composition differed, as an increase in collagen III mRNA content was measured in pGARP KO infarcts, at 7 days post-MI. We also revealed an increased content in M2 macrophages at 3 days post-infarction, suggesting an alteration of the inflammatory response, susceptible to impact the healing process. In line with these results, preliminary echocardiography data highlights the presence of LV pseudoaneurysm and increased LV dilation in the pGARP KO infarcted hearts.

Conclusions: Our data confirms that GARP is not required for platelet activation but is crucial for TGFα activation. The early and transient platelet infiltration into the infarcted myocardium seems to be associated with a modulation of the inflammatory process, which might impact the healing process, alter scar maturation and foster adverse LV remodeling.

P05

ABSTRACTS
POSTERS

Linking platelet lipidome and inflammatory response in septic patients

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Background: Sepsis has been recognized as a global health priority by the WHO and is characterized by major endothelial dysfunction, microvascular alterations, and coagulopathy. Cellular and molecular mechanisms involved in its pathophysiology are complex and not yet fully understood. Platelets play an important role as their activation promotes thrombo-inflammation and influences the inflammatory response via cytokines secretion. Platelets also produce lipids, some of which are also inflammatory mediators. Previously, we observed that platelet acetyl-CoA carboxylase 1 (ACC1), responsible for de novo lipid synthesis, is phosphorylated and thus inhibited by AMP-activated protein kinase (AMPK) upon thrombin stimulation, which leads to changes in the platelet lipidome. The role of AMPK-ACC signaling in the regulation of the platelet lipidome during sepsis, and its potential impact on inflammation, has never been investigated. A comprehensive analysis of intraplatelet lipids and of their regulation might offer novel diagnostic or therapeutic options.

Aim: To investigate whether changes in ACC1 phosphorylation can influence the platelet lipidome and the inflammatory response of patients during sepsis.

Methods: A total of 48 septic shock and 48 control patients have been included. Blood sampling and platelets isolation have been done within the first 24 hours of the diagnosis. In parallel, mice injected intraperitoneally with lipopolysaccharides were used as a model of endotoxemia. Platelets ACC1 phosphorylation/expression levels were measured by western blot. Lipidomics analysis was carried out by untargeted liquid chromatography–mass spectrometry or on the commercial Lipidyzer platform. Plasma factors were measured by Enzyme-linked immunosorbent assays (ELISA).

Results: Platelet ACC1 expression and phosphorylation were increased both in septic patients and in septic mice. Lipidomics analysis highlighted significant changes of lipids in human and murine platelets during sepsis, with a substantial reduction in phosphatidylcholines and phosphatidylethanolamines containing long polyunsaturated fatty acid chains (ω3 and ω6), which are key phospholipids for the generation of pro- and anti-inflammatory lipid mediators. Our preliminary data obtained in humans and mice, suggest that elevated ACC1 phosphorylation in sepsis is associated with a higher content of these phospholipids as well as a plasma reduction in myeloid inflammatory parameters.

Summary/Conclusions: Our data reveal that critical changes in the platelet lipidome occur during sepsis and may contribute to the pathophysiology of the disease. Results also suggest that ACC1 can influence the platelet lipid content and/or composition.

P06

ABSTRACTS
POSTERS

Evaluating the dose regimen of intravenous unfractionated heparin and low molecular weight heparin in critically ill patients and critically ill COVID-19 patients using anti-Xa levels

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Introduction: COVID-19 patients are predisposed to thromboembolic events, presumedly due to an intricate interaction of dysregulation of the renin-angiotensin-aldosterone system and a dysregulated immune response (1-3). Given the higher risk for thromboembolic events, our institution adjusted its anticoagulant dose regimen for hospitalized critically ill COVID-19 patients.

Aims: We evaluated the dose regimen of our new anticoagulation protocol in critically ill COVID-19 patients using anti-Xa assays. We calculated the percentage of COVID-19 patients with anti-Xa values outside of the desired range and studied the time to reach adequate anti-Xa levels.

Methods: In this retrospective observational single center study, we examined all patients admitted to the intensive care unit between 01/2020 and 09/2021 who received UFH or LMWH (enoxaparin) and were monitored using anti-Xa assays (N=813). For VTE prophylaxis using LMWH, the dose regimen was 0.5mg/kg once or twice daily for non-COVID-19 and COVID-19 critically ill patients, respectively. In both groups the therapeutic LMWH dose was 1mg/kg twice daily. The initial dosage of UFH for both groups was 8U/kg/h and 12U/kg/h for VTE prophylaxis and treatment, respectively. Anti-Xa assays were measured every 6 hours in UFH and 4 hours after the third dose of LMWH. We defined the expected time to reach adequate anti-Xa was 52 hours for LMWH once daily, 28 hours for LWMH twice daily and 24 hours for UFH. Anti-Xa targets were 0.1-0.3IU/mL and 0.2-0.4IU/mL for VTE prophylaxis with UFH and LMWH respectively and 0.3-0.7IU/mL and 0.6-1IU/mL for VTE treatment with UFH and LMWH, respectively.

Results: For VTE prophylaxis with LMWH, 38.5% [CI 32.6-45.4%] (87/226) of the adjusted regimen group in critically ill COVID-19 patients had anti-Xa levels within range in the expected timeframe as compared to 12.5% [CI 8.8-17.7%] (28/224) of the non-COVID-19 group. However, the study population comprised a considerable number of competing events (switch to therapeutic dose, switch to UFH, missing anti-Xa values at target time, discrepancies in LMWH administration, discharge or death). Treating those as censoring events would result in target anti-Xa levels in 40.8% [CI 34.6-48.0%] of COVID-19 patients and 16.8% [CI 12.0-23.7%] of critically ill patients without COVID-19. In the LMWH prophylaxis group of the COVID-19 patients 24.3% had an anti-Xa level above the desired range.

Conclusions: For VTE prophylaxis with LMWH, adequate anti-Xa levels were attained in a higher fraction of patients using the adjusted dose regimen in the COVID-19 group, as compared to VTE prophylaxis in the non-COVID-19 group. The overall percentage of patients (critically ill and critically ill COVID-19) that reached adequate anti-Xa levels using our current thromboembolic prophylactic and therapeutic protocols was lower than a priori projected, favoring higher dosage of anticoagulants in critically ill patients with and without COVID-19. The incidence of supratherapeutic dosing in COVID-19 patients was not negligible.

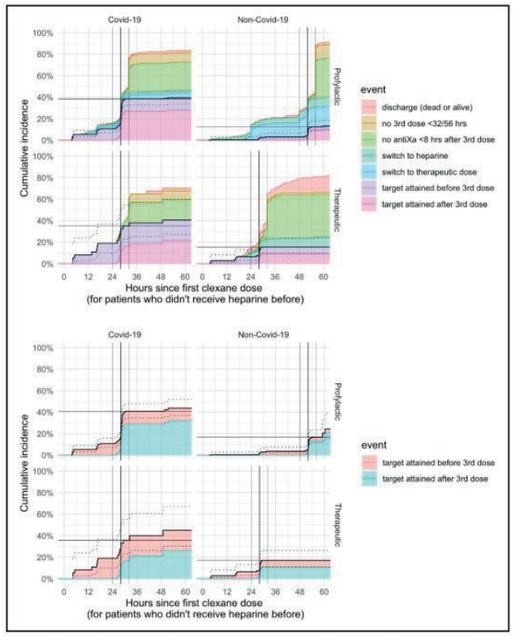


Figure 1: *Time to reach adequate anti-Xa values since first clexane dose*

Above: all included COVID-19 patients and non-COVID-19 patients
Below: adapted population, treating competing events as censoring events

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Neutrophil Extracellular Traps in a Mouse Model of Ischemic Stroke

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P07

ABSTRACTS
POSTERS

Background: Thromboinflammatory processes modulate the complex pathophysiology of cerebral ischemia reperfusion (I/R) injury in ischemic stroke patients, but the exact underlying mechanisms remain poorly understood. Emerging evidence indicates that neutrophil extracellular traps (NETs) might play an important role in the thromboinflammatory cascade. In addition, the link between von Willebrand factor (VWF) and neutrophil recruitment in the ischemic brain might play a role in thromboinflammation, possibly by the formation of NETs.

Aims: To study NET formation in a murine model of cerebral I/R injury in ischemic stroke and to explore the potential promotion of NETosis by VWF and the VWF-cleaving protease ADAMTS13.
Methods: The filament-induced transient middle cerebral artery occlusion (tMCAO) model was used to induce 60 minutes of focal cerebral ischemia in wild-type (WT), littermate Vwf-knockout (KO) and Adamts13-KO mice after which reperfusion was allowed. At different timepoints post-ischemia, NETs (Ly6G+, H3Cit+, DNA+) were identified in the ischemic mouse brain using quantitative immunofluorescence microscopy.

Results: NETs could be identified in the ipsilateral brain hemisphere. Interestingly, quantification of NETs at different timepoints, revealed that NETs can already be detected at 6 hours after the start of ischemia. Their presence peaked around 24 hours post-ischemia and NETs started to disappear again 48 hours post-ischemia. Remarkably, NETs were predominantly localized within the brain vasculature post-ischemia and to a lesser extent in the brain parenchyma, suggesting that NETs might play a role in secondary microthrombosis. Strikingly, NET formation was significantly decreased in Vwf-KO mice and significantly increased in Adamts13-KO mice compared to littermate WT mice 24 hours post-ischemia, indicating a possible role for VWF in promoting NETosis in the ischemic brain.

Conclusion: This study identified the spatiotemporal profile of NET formation in a mouse model of cerebral I/R injury in ischemic stroke. NETs, potentially in combination with VWF, might be an attractive target for the development of novel therapeutic strategies in stroke treatment.

P08

ABSTRACTS
POSTERS

Small-molecule cyclophilin inhibitors as a potential therapeutic application to limit thrombosis

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Background: Cyclophilins (CyP) are involved in various pathways regulating platelet function. CyPA is a Ca2+ regulator and is involved in integrin αIIbβ3 bidirectional signaling, while CyPD mediates opening of the mitochondrial permeability transition pore resulting in procoagulant platelet formation. Procoagulant platelets acts as a scaffold for the prothrombinase complex, catalyzing fibrin formation. Platelet CyP has therefore been suggested as a therapeutic target to limit thrombosis. Currently, many studies utilize the immunosuppressant Cyclosporin A (CsA) or derivatives. However, these have several disadvantages including side-effects unrelated to CyP inhibition. CsA was shown to enhance platelet aggregation, which was suggested to be responsible for the thrombotic complications observed in CsA treated patients. Therefore, potent CyP inhibitors unrelated to CsA are needed.

Aim: Test the effect of novel non-peptidic small-molecule CyP inhibitors (SMCypIs) on platelet activation, aggregation and procoagulant platelet formation, in comparison with CsA and its analogue Alisporivir.

Methods: Prior to the experiment, either whole blood, platelet rich plasma (PRP) or washed human platelets were incubated with 4 µM CsA, 4 µM Alisporivir, 125 µM SMCypI, or vehicle control. Procoagulant platelet formation was measured using flow cytometry, by analyzing AnnexinV binding and P-selectin expression, as well as tetramethylrhodamine methyl ester staining demonstrating an intact mitochondrial membrane potential. Additionally, P-selectin expression and activated integrin αIIbβ3 were measured via flow cytometry. In a modified activated Partial Thromboplastin Time (aPTT) assay, resting or activated platelets were added to citrated plasma together with allegic acid and the time to form a fibrin clot was measured upon addition of $CaCl_2$. Fibrin formation under flow was visualized by perfusing recalcified whole blood in the presence of tPA-Alexa488 over a Horm collagen coated surface. Fibrin formation was quantified by measuring the mean fluorescent intensity (MFI) of the Alexa488 signal. Platelet aggregation was measured using light transmission aggregometry.

Results: Firstly, addition of each SMCypI, Alisporivir or CsA resulted in a significant decrease in AnnexinV+/P-selectin+ platelets upon dual-agonist activation. Similarly, dual-agonist induced loss of mitochondrial membrane potential was prevented. No effect was observed on P-selectin expression and integrin αIIbβ3 activation after ADP, CRP-XL or thrombin stimulation.
Secondly, SMCypIs, Alisporivir and CsA reduced the procoagulant platelet function. Using a modified aPTT assay, a faster clotting time was observed when platelets were activated with dual agonists, compared to non-activated platelets. Addition of SMCypIs, Alisporivir, or CsA did not affect the clotting time of resting platelets, but significantly delayed the clotting time when platelets were activated with dual agonists. CsA reduced fibrin formation when perfusing recalcified whole blood over a Horm collagen coated surface. Experiments with the SMCypIs are currently ongoing.
Lastly, SMCypIs did not affect platelet aggregation, while CsA and Alisporivir increased both ADP and Horm collagen induced platelet aggregation.

Conclusions: Similarly to CsA, SMCypIs and Alisporivir reduced procoagulant platelet formation, resulting in a decreased procoagulant activity. SMCypIs did not affect platelet aggregation, while CsA and Alisporivir increased ADP and Horm collagen induced aggregation. Therefore, SMCypIs form a promising therapeutic approach to limit thrombosis.

P09

ABSTRACTS
POSTERS

High-dimensional analysis of circulating blood cells reveals distinct granulocyte activation and NETosis profiles in COVID-19

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Background: Coronavirus disease 19 (COVID-19) represents a perfect storm for immunothrombosis leading to severe disease, with release of neutrophil extracellular traps (NETs) being an important contributor now frequently described both in blood and in tissues. NETs contribute to thrombosis by trapping platelets and activating the coagulation cascade, as well as endotheliopathy. NET formation is an active process initiated by factors such as activated platelets and requiring histone modifying enzymes including peptidylarginine deiminease 4.

Aims: We aimed to characterize early NETosis in circulating neutrophils in the context of neutrophil activation, immunothrombosis, and the broader immune response in COVID-19, particularly in severe disease involving ICU stays.

Methods: We designed a custom panel of 42 antibodies to stain peripheral blood collected from COVID-19 patients in ICU at UZ Leuven during the COVID-19 Delta-wave. This panel comprised cell-specific epitopes, surface activation markers, intracellular staining for NET-associated cell biological changes, and platelets. Samples were run using cytometry by time-of-flight (CyTOF) on a Helios mass cytometer and analyzed using FlowJo software with FlowSOM clustering and visualisation.

Results: Morphometric analysis showed cell biological changes common to NET formation. Nonbiased FlowSOM analysis was performed and identified 14 distinct populations of granulocytes. Five of these populations were present only in COVID-19 patients, with high levels of CD11b, CD177, and CD16. Ex vivo stimulation of healthy donor granulocytes toward NETosis shifted the expression profile toward that of COVID-19 patient granulocytes.

Conclusions: High dimensional profiling of circulating blood cells identifies distinct granulocyte populations undergoing extracellular trap formation in COVID-19, highlighting the heterogeneity of neutrophil responses.

P10

ABSTRACTS
POSTERS

Neutrophil peptidylarginine deiminase 4 plays a systemic role in obesity-induced chronic inflammation in mice

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Introduction: Obesity is a major problem in our current society, with a multi-origin complex pathology that is difficult to treat and easy to acquire unnoticeably. Its occurrence is taking on pandemic proportions, and is only expected to continue rising. Levels of circulating neutrophil extracellular traps (NETs) were found to be higher in morbidly obese patients as compared to lean subjects. Essential in the process of NET formation is the activation and nuclear translocation of peptidylarginine deiminase 4 (PAD4).
Aims: We aimed to understand how NETs contribute to the detrimental effects which are associated with a prolonged state of (morbid) obesity.

Methods: In order to study the effect of obesity onset and progression, an animal model of diet-induced obesity was adopted. Six-week-old mice on a C57BL6/J background were fed a high fat containing diet (HFD – 60kcal% fat) for a period of 10 weeks. In order to investigate the effect of diet-induced NET formation on systemic organs, a neutrophil-selective PAD4 knockout mice (Ne-PAD4-/-) was evaluated in parallel to WT control mice. Weight gain was followed on a weekly basis. In addition, neutrophil physiology was evaluated through in vitro stimulation of isolated neutrophils. Ten weeks post dietary change, cardiac function was evaluated using echocardiography.

Results: Neutrophil activation status, evaluated through the formation of NETs in response to calcium ionophore ionomycin (4 µM) stimulation, was initially reduced after 3 days of HFD feeding. At later timepoints (5- and 10-weeks post dietary change), neutrophils isolated from HFD-fed mice showed increased levels of NET formation in response to ionomycin stimulation in comparison to neutrophils from control diet-fed mice. Therefore, increased fat intake, and obesity development has a significant effect on neutrophils and NETs. When Ne-PAD4-/- mice were put on a HFD, a clear distinction in weight gain could be observed after 7 weeks on the diet. Ne-PAD4-/- mice showed a reduction in weight gain, while WT mice had a constant gain of body weight over the 10-week experimental period. Finally, after a 10-week period of high fat intake, cardiac function of both WT and Ne-PAD4-/- mice was evaluated through echocardiography. Interestingly, in WT mice, diastolic function (evaluated through E/A ratio) decreased as compared to baseline values (E/A ratio prior to dietary change), while Ne-PAD4-/- mice showed an E/A ratio comparable to baseline levels.

Conclusion: From the above study, it is clear that neutrophil PAD4 has a systemic impact during settings of prolonged high fat intake and subsequent obesity development. Obesity has been well established as having an influence on immune system composition and compliance, an observation which yet again was clear in severe COVID-19, where NETs have been shown to be of major importance. We now demonstrate the importance of systemic NET release under metabolic challenge, and how it can contribute to cardiac deterioration at a young age due to obesity-induced alteration in the innate immune system.

P11

ABSTRACTS
POSTERS

Polymeric Prosthetic Heart Valve for Life: Creative Chemistry for a Sustainable Future

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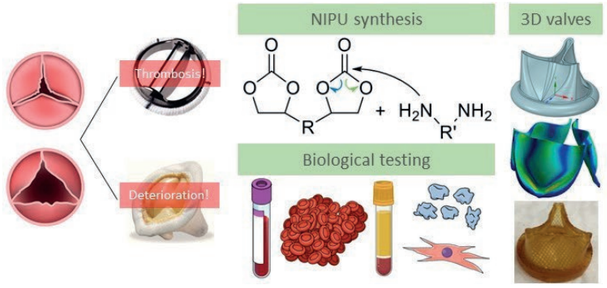
Background: Valvular heart diseases affect about 13% of the elder population (>75), which represents more than 100 million people worldwide. The only available treatment is heart valve replacement, either with mechanical or biological prostheses. The ideal prosthesis does not exist. Mechanical prostheses provide long-term durability, but inevitably bring along high risk of thromboembolism. While biological prostheses provide better hemocompatibility, they are prone to degeneration. Our team currently assesses new synthetic polymers as potential biomaterials for prosthetic valves.

Aims: Our goal is to obtain polymers with improved mechanical properties and hemo/biocompatibility compared to available medical grade polyurethane (PU). Our team focuses on the development and testing of innovative non-isocyanate polyurethanes (NIPUs), which represent the “green” alternative to PU since their production is free of toxic diisocyanates.

Methods: The in vitro hemocompatibility of the produced NIPUs was assessed using different blood components from human whole blood. Hemolysis tests were performed in the presence of washed red blood cells, the activation of the coagulation cascade was evaluated using platelet-poor plasma, and platelet adhesion was quantified upon incubation with platelet-rich plasma. Cytotoxic effects towards primary human fibroblasts were investigated through microscopic evaluation of cell morphology. Tri-leaflet prosthetic heart valves were produced by injection molding, acquiring the 3D structure previously designed by computational fluid dynamic simulation to comply with strict hemodynamic requirements.

Results: The different synthetized NIPUs did not show hemolytic effects (hemolysis rates <2%), the plasma incubated with NIPUs experienced longer clotting times than the plasma incubated with PU (meaning no contact activation), and NIPUs surfaces promoted less platelet adhesion than PU surfaces, indicating low thrombogenicity of our polymers. Upon indirect contact with PU/NIPU, human fibroblasts displayed normal morphology (spindle-like shape). The 3D valves tested in the pulse duplicator equipment (Vivitro labs) showed low regurgitation (<10%), normal transaortic flow, and acceptable effective orifice areas (>0.85cm2).

Conclusion: In conclusion, we set up the basis for future development of a cost effective, environmentally friendly, mechanically competent, and hemocompatible prosthetic heart valve made of synthetic NIPUs.



	Control	NIPU 1	NIPU 2
Hemolysis	<2%	<2%	<2%
Clotting time	↓	↑	↑
Platelet adhesion	↑	↓	↓
Cell morphology	fusiform	fusiform	fusiform

Red arrows refer to comparisons with NIPUs. Green arrows refer to comparisons with PU.

	Control	NIPU 1	NIPU 2
Regurge	<10%	<10%	<10%
Transaortic flow	normal	normal	normal
EOA	>0.85 cm²	>0.85 cm²	>0.85 cm²

Development and validation of a fiber optic – surface plasmon resonance-based assay to measure von Willebrand factor antigen levels

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Background: Von Willebrand disease (VWD) is the most common inherited bleeding disorder with a general prevalence up to 1%. VWD is caused by mutations that lead to a quantitative (type 1 or type 3 VWD) or qualitative defect (type 2 VWD) of von Willebrand factor (VWF), a glycoprotein that is crucial for normal hemostasis. Currently, diagnosing VWD is based on multiple, specialized and labor-intensive laboratory assays that are not always readily available.

Aims: Our aim was to develop and validate an assay that measures VWF antigen levels (VWF:Ag) via fiber optic – surface plasmon resonance (FO-SPR), a technology that allows easy and fast determination of antigen concentrations via a single plasma dip of an optical fiber in plasma samples.

Methods: First, an FO-SPR-based assay to detect VWF:Ag was developed. In short, gold-coated optical fibers were functionalized to detect VWF:Ag. To do so, EDC/NHS chemistry was used to activate the carboxyl-SAM layer on these fibers, allowing binding of the monoclonal anti-VWF antibody 6D1. After binding of 6D1, blocking conditions were optimized to guarantee the specificity of the functionalized fibers. Next, the functionalized fibers were dipped in diluted plasma to capture VWF within that plasma. Finally, bound VWF was detected as an SPR signal using biotinylated polyclonal anti-VWF antibodies and anti-biotin coated gold nanoparticles.
Second, a methodological optimization of the FO-SPR-based VWF:Ag assay was performed. This included the determination of the optimal conditions of each assay step, establishing a robust dose-response curve.
Third, method agreement with our in-house ELISA was assessed by measuring 90 samples originating from healthy donors (n=30) and VWD patients (type 1: n=30, type 2: n=29, type 3: n=1). Finally, clinical performance of the FO-SPR-based VWF:Ag assay was determined based on results by an in-hospital validated VWF:Ag assay.

Results: Using optimized assay conditions, a reproducible calibration curve could be established (R^2 = 0.975) using the newly developed FO-SPR-based assay (n=3, CV <20%). Using the established standard curve, VWF:Ag levels were determined in 30 healthy donor plasma samples and 60 VWD plasma samples. VWF:Ag levels determined by FO-SPR showed a good correlation with the VWF:Ag of these samples measured via our in-house ELISA (Spearman rho: 0.891, p<0.0001). Furthermore, FO-SPR-based determination of VWF:Ag also correlated well with the previously established VWF:Ag levels determined in the hospital for the VWD samples (Spearman rho: 0.770, p<0.0001). Additionally, the performance of the FO-SPR assay to detect samples with a clinically relevant shortage in VWF:Ag concentration (VWF:Ag <30 UI/dL) compared to results obtained by an in-hospital validated method showed excellent sensitivity with good specificity of 100% (95% CI: 74 – 100%) and 71.05% (95% CI: 60-81%), respectively.

Conclusions: We were able to develop a FO-SPR-based assay that reliably measures VWF:Ag levels. We are currently developing and validation assays to measure VWF function, such as collagen and platelet binding. Taken together, if further validated, these FO-SPR assays could be used simultaneously to determine a complete VWF status in one single run and would allow easier standardization of VWF parameter determination between different laboratories.

Pain relief with eptacog beta in hemophilia patients with inhibitors

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Introduction: Bleeding episodes (BEs) into joints and muscles cause acute pain that can be reduced by quick and efficient treatment. Eptacog beta (LFB) is a new recombinant coagulation factor VIIa approved by the EMA in patients (≥12 years of age) with hemophilia A or B with inhibitors (PwHABI) for the treatment of BEs and for the prevention of bleeding in those undergoing surgery or invasive procedures. We herewith describe the impact of eptacog beta treatment on pain relief across two different age groups using data from the pivotal clinical trials.

Aim: To assess the evolution of pain after the treatment of BEs with eptacog beta.

Methods: Eptacog beta pain score data originated from two pivotal Phase 3 clinical trials of similar design that evaluated the efficacy and safety of eptacog beta on BE in PwHABI, including one (PERSEPT1) conducted in adults/adolescents and the other one (PERSEPT2) in children. Subjects received initial doses of eptacog beta 75 or 225µg/kg followed by 75µg/kg dose at predefined intervals determined by clinical response. Pain was assessed by the subject, guardian, or caregiver using a visual analog scale (VAS). Successful pain relief was defined by a VAS pain score at 12 hours after eptacog beta administration being lower to that recorded prior to eptacog beta administration. Descriptive statistics were applied for pain analysis.

R**esults:** Of the 52 subjects evaluated, 27 were ≥12 years old with a mean (±SD) age of 31.3 (±12.35) years and 25 <12 years old with a mean (±SD) age of 4.9 (±3.28) years. Majority of the 1017 treated BEs were successfully treated at 12 hours and at 24 hours in both age groups. The proportion of BEs with successful pain relief was approximately 90% in both groups, irrespective of BE severity. From baseline until 24 hours after eptacog beta administration, the VAS pain score consistently decreased at all time points in both age groups: mean (±SD) VAS pain score of 40.8 (±25.6) at baseline to 2.8 (±8.2) at 24 hours in the ≥12 year-group and from 50.6 (±25.1) to 6.7 (±14.9) in the <12 year-group, respectively. The median time to pain relief was 3 hours in the older group and above 5 hours in the younger. Concomitant analgesic requirement was less common among older vs younger patients (29.6% vs 48%, respectively).

Discussion/Conclusion: Eptacog beta resulted in clinically relevant pain reduction and bleed resolution across all age groups in PwHABI. A higher analgesic requirement and longer time to pain relief were observed in younger patients, possibly due to difficulty in assessing pain in this younger patient population.

P14

ABSTRACTS
POSTERS

STA Coag Expert rules for Lupus Anticoagulant diagnosis (Stago): data analysis from one year use in routine practice

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Introduction: Lupus anticoagulant (LA) testing requires a three-step (screening, mixing, confirmation) procedure with two test systems: diluted Russell's viper venom time (dRVVT) and activated partial thromboplastin time (aPTT). This step-wise procedure can be controlled manually or automatically by a middleware. In 2020, we introduced the automatic algorithm "STA Coag Expert rules for Lupus Anticoagulant diagnosis" (Stago, France) integrated into the CoagOne (Stago) middleware. This algorithm results in a final "positive" or "negative" conclusion. This algorithm was based on the 2009 ISTH SSC guidelines. Predefined cut-offs and rules from the algorithm were not adapted. After technical validation on the CoagOne middleware all results are directly send to the LIS. LA testing was performed on STAR MAX analyser with Stago LA-reagents.

Aims: We used all data of all LA tests of the year 2021, i.e. one year after introduction of het STA Coag Expert rules for LA testing. Data analysis was performed to evaluate the LA-positivity rate and the results of LA conclusions.

Method: Data were extracted from the LIS (LAB400, Cegeka, Belgium) and were statistically processed by MedCalc 19.4.1.

Results: We obtained data from 439 patients tested for LA. Median age was 45y (min-max: 4-89y). The algorithm concluded 401 (91%) samples as LA negative and 38 (9%) samples as LA positive. All positive LA's were checked manually (independent interpretation of the results by a trained clinical biologist) and clinically (evaluation of the patients' medical record: DOAC, VKA, CRP, aCL, B2GPI, previous results etc). Of all positive samples, 16 were positive in both pathways, 2 in dRVVT and 13 in aPTT pathway only. 7/38 (18%) LA positive results were adapted from "positive" to "inconclusive" because of possible interference of DOAC of VKA, resulting in a final positivity of 7% (31/439).

Conclusions: The STA Coag Expert rules for Lupus Anticoagulant diagnosis performs well. It is an easy tool to work out LA testing in a standardized way. Results are easily transferred to the LIS. Positive conclusions must be interpreted carefully, matched to important interfering parameters and adapted if necessary. The algorithm should be adapted to the 2020 ISTH SSC updated recommendations and guidelines for LA testing.

P15

ABSTRACTS
POSTERS

Corticosteroids do not alter coagulopathy in critical COVID-19

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Background: Severe Acute Respiratory Syndrome Coronavirus 2 (SARS CoV 2) may induce severe forms of Coronavirus disease 2019 (COVID-19). The infection triggers a progressive systemic inflammatory reaction, an endothelial cell activation and subsequently an activation of the coagulation system in a phenomenon called "immunothrombosis" and involving the Neutrophil Extracellular Traps (NETs) formation. Accordingly, severe forms of COVID-19 can develop a coagulopathy called COVID-19 associated coagulopathy (CAC), leading to thrombosis and increasing mortality rate.

Several anti-inflammatory or anticoagulant medications have been explored to treat severe COVID-19 but only low-dose corticosteroids (CS) have been shown to improve survival.

Aims: This study aimed to compare CAC in patients admitted to the ICU for critical forms of COVID-19 treated or not with CS.

Methods/Materials: Population
Patients were those admitted to the ICU for moderate or severe ARDS due to SARS-Cov-2 and were included within the first five days of admission. Patients of the first wave of COVID-19 pandemic were compared with patients of the second wave, the latter having been routinely treated with CS.

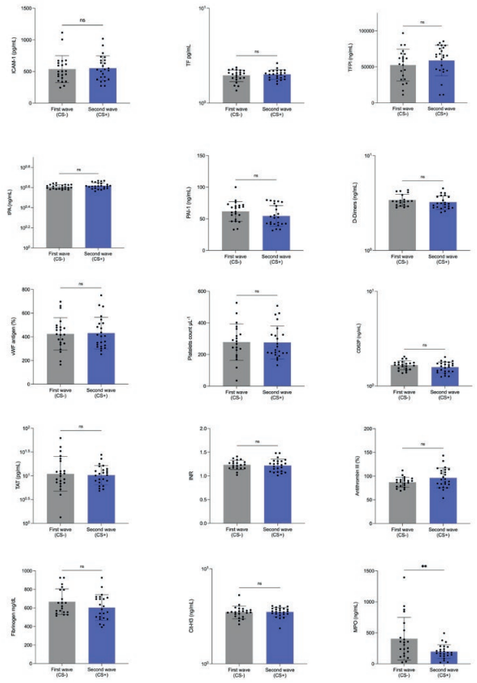
Clinical outcomes: Patients baseline characteristics and clinical outcomes were compared. Disseminated intravascular coagulation (DIC) and sepsis-induced coagulopathy (SIC) were diagnosed using the International Society of Thrombosis and Haemostasis scoring at inclusion.

Measurement of biomarkers: Soluble biomarkers of endothelial and platelet activation, coagulopathy and NETosis were determined by enzyme-linked immunosorbent assay (ELISA) or suspension array sandwich immunoassays.

Results: Patients baseline characteristics
Overall, 22 patients were included during the first wave and 24 during the second wave. They were no significant differences in the mean age and main comorbidities between the two groups. Patients included during the second wave had a more severe respiratory failure. None of the patients met the criteria for SIC nor DIC.

Clinical outcome: Mean ventilation duration and ICU length of stay were longer for the patients during the second wave. 30-day mortality rate was 27% for the first wave patients and 37% for the second wave patients.
Endothelial activation, coagulopathy and NETosis
The study showed no difference in the level of plasmatic endothelial and coagulopathy biomarkers (ICAM-1, TF, TFPI, tPA, PAI-1, D-Dimers, VWF, TAT, Fibrinogen) between the two groups. INR was slightly prolonged in both groups (1.23 ± 0.10 and 1.22 ± 0.13) and they were no ATIII consumption (87 ± 11% and 96 ± 21%). Platelet count was on average in the normal range in both group (278.000/µL ± 115.000/µL and 277.000/µL ± 104.000/µL) with similar level of CD62P (50 ± 22 and 43 ± 21 ng/mL) as a biomarker of platelet activation. As plasmatic biomarkers of NETosis, levels of Cit-H3 were similar but MPO was reduced in the CS treated group. Changes in plasmatic biomarkers in the second wave patients are described in Figure 1.

Summary/Conclusion: In conclusion, treatment of critical COVID-19 patients with CS has little impact on the endothelial activation, NETosis formation and COVID-19-induced coagulopathy with persistent very elevated level of circulating TF.



Distribution of VWD type 2 patients in a Belgian cohort of 250 patients and characterization of their phenotypes

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Introduction: Von Willebrand disease (VWD) is the most common inherited bleeding disorder with an estimated incidence of 1% in the general population. Von Willebrand factor (VWF) is a multimeric plasma glycoprotein essential for primary and secondary haemostasis. VWD is subdivided into a partial (type 1) or total (type 3) quantitative deficiency, and a qualitative deficiency (type 2). Symptomatology can occur at any age and is very heterogeneous, especially in type 2, ranging from no symptoms to very severe manifestations. There is therefore a great disparity in the use of haemostatic treatments.

Objective: To reassess all patients diagnosed with VWD, in order to estimate the proportion of the different types. As well as to determine the clinical phenotype of VWD2 patients and to evaluate the frequency of VWF-CDP use in spontaneous bleeding or in short-term prophylaxis during surgical procedures.

Method: Patients over 16 years of age followed up and treated for type 2 VWD at the University Clinics of Saint-Luc over the period 2000-2022 were included in this retrospective study.

Result: 242 patients with VWD were included in the study, of which 91 patients (36% of the cohort) with VWD2 were identified. Clinical data were collected in 78 patients (50 females and 28 males) with VWD2. The mean age was 46 years, and the age of diagnosis was 20 years. The patients were subclassified as type 2A in 24%, type 2B in 13%, type 2M in 56.5% and type 2N in 6.5%. The most common symptoms were epistaxis (44%), prolonged bleeding (41%), gum bleeding (30%), haematoma (10%), gastro-intestinal tract bleeding (8%) and haemarthrosis (3%). A VWF-CDP was used on 104 occasions (44 outside our institute), with an average of 60 IU/kg/d, 11.000 IU/intervention and 610.000 IU in total. Long-term secondary prophylaxis was initiated in 2 patients. A minority of patients consulted for uncontrolled spontaneous bleeding, representing 31 out of 259 consultations.

Conclusion: Our study describes a cohort with an overrepresentation of VWD2 patients, with a majority of subtype 2M. This could be the explanation for the less severe phenotype compared to the literature, characterized by a mild symptomatology and the use of VWF-CDP mainly for surgical prophylaxis.

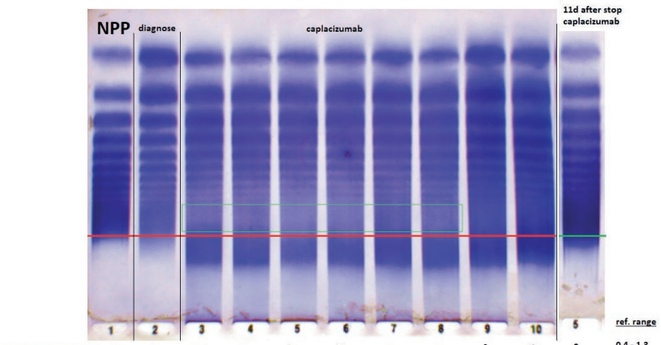
Persistent absence of ADAMTS13 activity despite clinical remission following a de novo COVID-19 vaccine-associated iTTP

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Introduction: Immune-mediated thrombotic thrombocytopenic purpura (iTTP) is a rare but potentially life-threatening thrombotic microangiopathy, resulting from an autoantibody-mediated severe deficiency of ADAMTS13, and characterized by hemolytic anemia, consumptive thrombocytopenia, and platelet-rich microthrombi causing organ failure. In June 2021, we published the first case of a de novo iTTP event after the nucleoside modified mRNA BNT162b2 anti-COVID-19 vaccine (Pfizer-BioNTech). Clinical features of this vaccine-associated iTTP episode were comparable to pre-pandemic iTTP and the patient obtained an excellent clinical outcome to standard of care treatment. However, although achieving a stable clinical remission, a rather atypical biochemical course was noted.

Case Report: At diagnosis, in addition to ADAMTS13 enzyme activity and autoantibody titer, multimer analysis was performed using the semiautomatic assay HYDRAGEL VW multimer assay (H5/11VWM, Sebia). Multimer analysis showed a reduced amount (27%; ref. range > 40.2%) of high-molecular-weight multimers (HMWM). According to the ISTH guidelines the patient was treated with plasma exchange (PEX) in combination with high-dose corticosteroids. Rituximab was associated on day 8 because of an impending biochemical exacerbation, reasulting in clinical remission. However, ADAMTS13 activity still remained undetectable with a high autoantibody titer. On day 18 caplacizumab was started to reduce the risk of recurrence (Figure 1). During the use of caplacizumab, HMWM were still reduced (30%), but now also ultra-large molecular-weight multimers (ULHMWM) were observed which were not present at diagnosis. Nonetheless, on day 30, the patient had persistent absence of ADAMTS13 activity and high anti-ADAMTS13 antibodies wherefore an additional 4 doses of rituximab were given. After 8 doses of rituximab in total, caplacizumab and corticosteroids could be discontinued after 3 and 4 months of treatment respectively since the autoantibody titer started to decline. Subsequently, low dose acetylsalicylic acid was started. It is of interest that after stopping caplacizumab, no more ULHMWM were observed and a normal amount of HMWM (51.7%) was achieved (Figure 2). A low level of ADAMTS13 activity (1%) was detected for the first time at month 10 after the initial event, while the autoantibody titer normalised. Currently, 16 months after diagnosis, the ADAMTS13 activity level is recovering gradually, however, partial ADAMTS13 remission is still not obtained (18%). Interestingly, the patient is doing clinically well and qualitatively normal VWF multimers, with a characteristic triplet band distribution, are detected reflecting proteolytic degradation of the high molecular weight multimers.

Discussion: Despite using standard treatment guidelines, our patient still has a persistent low ADAMTS13 activity (<20%) without affecting the clinic outcome or multimer analysis. The most intriguing question now is which of these two biochemical parameters can best be used for the follow-up of our patient and which of these will predict best a possible relapse; the ADAMTS13 activity or the VWF multimers? Finally, this case makes us consider whether the current use of aspirin and/or previous use of caplacizumab is sufficient to compensate for the missing ADAMTS13 activity. By observing multimer analysis, we believe that the ULHMWM, which were eliminated at the time of the acute iTTP event and consequently causing symptoms, are blocked by using caplacizumab.



													ref. range
ADAMTS-13 Ac	U/mL	-	0	0	0	0	0	0	0	0	0	0	0.4 - 1.3
ADAMTS-13 Ag	U/mL	-	0.07	0.79	-	-	-	-	-	-	-	0.84	0.41 - 1.41
ADAMTS-13 Inh	BU	0	107	42	42	42	-	33	-	-	24	24	0
VWF:Ag	%	111	119	141	143	148	139	150	145	235	228	332	60 - 130
HMWM	%	45.4	27.6	31.6	33.3	30.5	30.3	35.9	33.7	32.4	38.3	51.7	>40.2
ULHMWM		-	-	+	+	+	+	+	+	+	+	-	

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ABSTRACTS
POSTERS

Absence of effect of emicizumab on D-dimer concentrations in adult patients with severe hemophilia A

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Background: The D-dimer (DD) assay is an essential biological test for the diagnosis and monitoring of thrombotic conditions. DD testing is usually not performed as part of the routine laboratory management of patients with hemophilia (PWH). There is an increasing concern about the risk of thrombotic complications in PWH, which is likely related to age, cardiovascular risk factors, invasive thrombogenic procedures, over-correction of Factor VIII (FVIII) or FIX, or administration of new therapeutic agents mimicking FVIII or rebalancing coagulation.

Objective: This retrospective study sought to assess the basal DD levels in PWH treated prophylactically with FVIII, and to evaluate potential changes after switching to emicizumab

Method: Patients over 18 years of age treated with emicizumab within a single center over the period 2017-2022 were included in the study.

Result: DD levels were measured in 40 adult PWH (37 severe/ three moderate / two with FVIII inhibitor) with a median age of 46 years, before and at least 3 months after emicizumab initiation. No significant changes were revealed, with DD median values of 257 ng/mL before and 250 ng/mL after the switch (p=0.9).

Conclusion: Most adult PWH on prophylaxis using FVIII display DD levels within the normal range, which remain unchanged after switching to emicizumab.

TABLE 1- Patients characteristics

Sex (n)	Males (%)	25 (32)
	Females (%)	50 (68)
Age (years)	Mean (+/- SD)	46 (18)
	Median [range]	43 [16-93]
Diagnostic age (years)	Mean (+/- SD)	20 (18)
	Median [range]	14,5 [1-79]
Family (n)		18
Type (n)	1 (%)	148 (63)
	2 (%)	84 (36)
	2A (%)	19 (8)
	2B (%)	10 (4)
	2M (%)	44 (19)
	2N (%)	5 (2)
	Unclassifiable (%)	6 (3)
	3 (%)	3 (1)
Blood group (n)	O (%)	36 (46)
	Non-O (%)	32 (45)
	Missing (%)	10 (9)
Diagnostic circumstances (n)	Bleeding (%)	19 (24)
	Post-op bleeding (%)	12 (15)
	Pre-op assessment(%)	6 (8)
	Coincidence (%)	4 (5)
	Family assessment (%)	37 (47)
Anti-platelet agent /anticoagulant treatment	n (%)	5 (6)
Infectious disease (n)	HIV (%)	1 (1)
	HCV (%)	5 (6)
Thrombotic event (n)		2 (3)

Table 2: FVIII, fibrinogen, CRP, and D-dimer, before and after initiating emicizumab prophylaxis

	Pre-emicizumab (40 patients)	Post-emicizumab (40 patients)	p-value
FVIII (normal range : 50-150 IU/mL)			
Median [range]	3 [1-47]	29 [5-48]	**0.01**
Patients above normal range (> 150 IU/mL)	0	0	
Fibrinogen (normal range : 150-450 mg/dL)			
Median [range]	375,5 [267-578]	373,5 [171-678]	0.8
Patients above normal range (> 450 mg/dL)	3	4	
C-reactive protein (normal range : < 5 mg/dL)			
Median [range]	1 [1-114]	1 [1-10,4]	0.6
Patient above normal range (> 5mg/L)	5	4	
D-Dimer (normal range : < 500 ng/mL)			
Median [range]	257 [250-2876]	250 [250-6205]	0.9
Patient above normal range (> 500ng/mL)	9	5	

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ABSTRACTS
POSTERS

Investigation of platelet function in patients on antiplatelet agents targeting the P2Y12 receptor

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Introduction: Dual antiplatelet aggregation is the standard therapy for patients undergoing coronary stenting or suffering from an acute coronary syndrome. Despite this dual therapy, cardiovascular accidents can still occur. In this context, resistance to Clopidrogrel is the most studied. Clopidogrel is a prodrug. The metabolic pathways leading to the formation of the active metabolite are subject to genetic polymorphism causing individual response variability. Among available antiplatelets, Prasugrel and Ticagrelor are the most effective, but at the cost of a more frequent risk of bleeding. All target the P2Y12 receptor. Prasugrel is also a prodrug but has a better bioavailability, while Ticagrelor acts directly.

Aim of the study: Comparison of different laboratory tests targeting the P2Y12 receptor in order to identify the method with the best cost/benefit ratio to meet the requests for monitoring antiplatelet agents targeting the P2Y12 receptor, and in particular the Clopidogrel resistance.

Material and methods: 4 tests were evaluated using citrated blood samples of healthy volunteers. 2 LTA type methods (Light Transmission Aggregometer with ADP as agonist on Platelet-rich Plasma): Chronolog (Avant Medical) and CS-2500 (Siemens), and 2 POCT type methods (on whole blood) : PFA-200 P2Y12 cartridge (Siemens) and Verify Now P2Y (Werfen). For each technique, usual values at percentile 5-95 were determined and validated using internal controls. Repeatability, stability over time, influence of pneumatic transport were assessed, as well as reactivity of PFA P2Y12 and Verify Now P2Y to Ticagrecol spiked samples of healthy volunteers.

Results: Usual ranges were: for ADP aggregation determined on CS-2500 and Chronolog 75- 91% (n=18), for PFA P2Y12: 41-111 sec (n=23), for Verify Now P2Y is 164-272 PRU (n=23). Repeatability showed a CV of less than 5% for PFA and Verify now. Test stability on Verify Now and CS-2500 was 4 hours and 6 hours on PFA-200. Pneumatic transport did not affect the results, regardless of the test performed. PFA and Verify Now showed good reactivity to Ticagrelor spiked samples with a significant inter-individual variability, with no correlation between the two methods.

Conclusion: All tests showed good performance. As expected, POCT tests were the easiest to perform and saved valuable technician time compared to LTA techniques. Cost/benefit ratio was the best for the Verify Now, mainly due to the price of the cartridges, which is much lower than those of the PFA P2Y12. However, in a laboratory that already uses a PFA routinely, this device may be sufficient for occasional monitoring requests. An optimal number of requests must be evaluated to justify the investment in a Verify now.

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