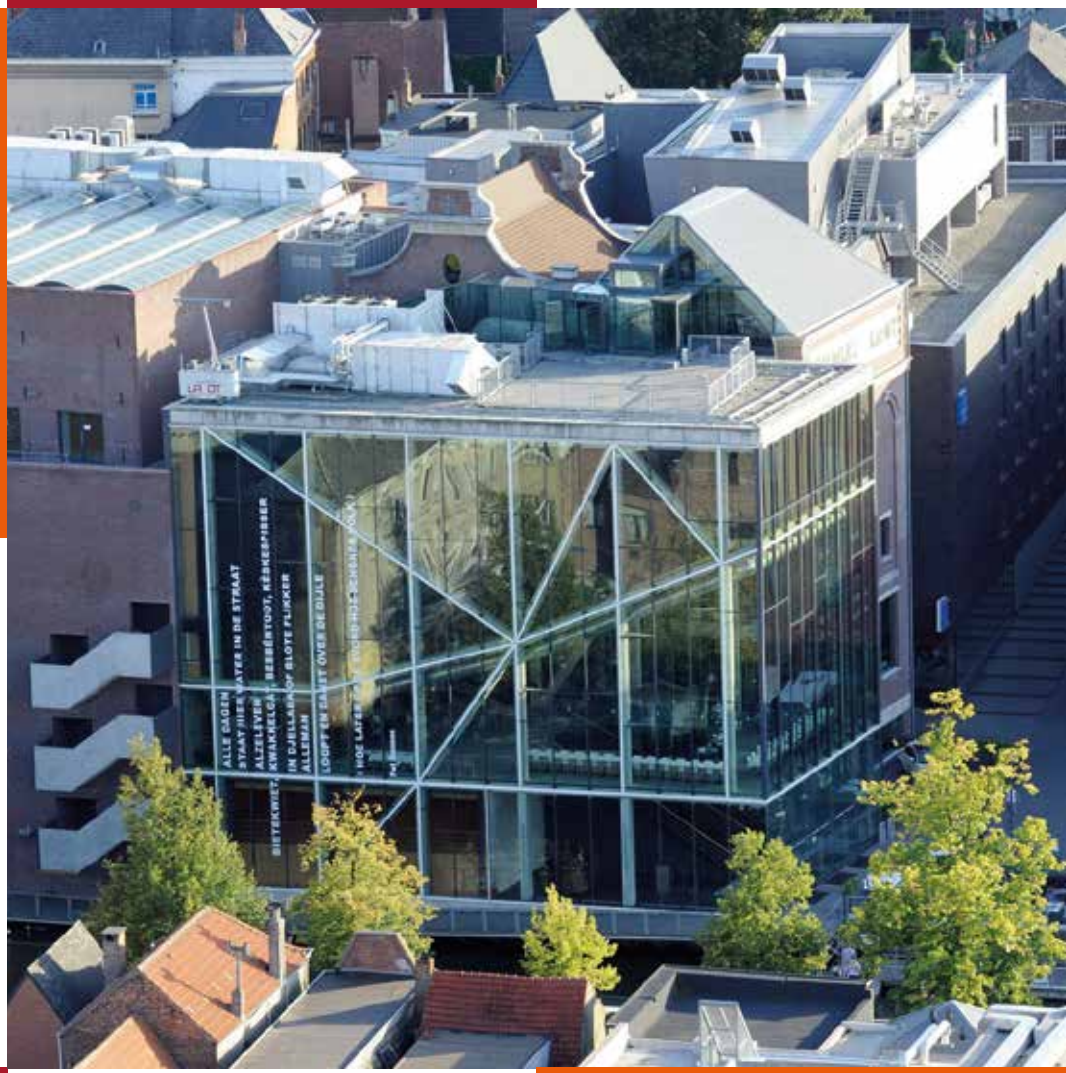
BSTH

Belgian Society on Thrombosis and Haemostasis

30TH ANNUAL MEETING

30 NOV
01 DEC
2023

LAMOT
BELGIUM



LOCAL ORGANIZERS

CHRISTELLE ORLANDO

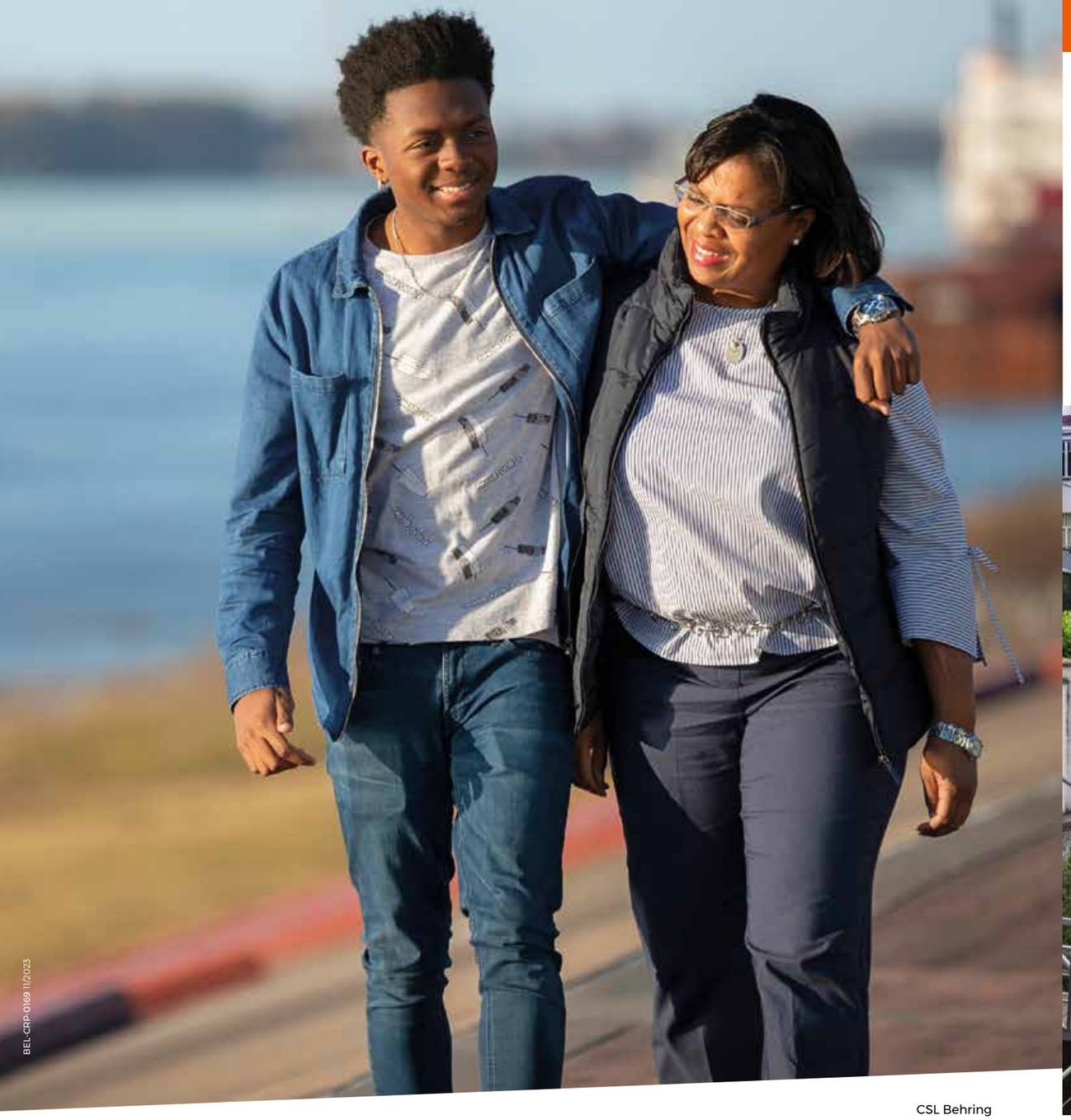
JAN EMMERECHTS

PROGRAM &
ABSTRACTS

WWW.BSTH2023.ORG

CSL Behring

Your partner in bleeding disorders



BEL-CRP-0169 11/2023

CSL Behring
Bedrijvenlaan 11 - B-2800 Mechelen
Tel. : +32 (0)15 28 89 20 - Fax : +32 (0)15 20 74 35
medinfobelgium@cslbehring.com - www.cslbehring.be

03

TABLE OF CONTENTS

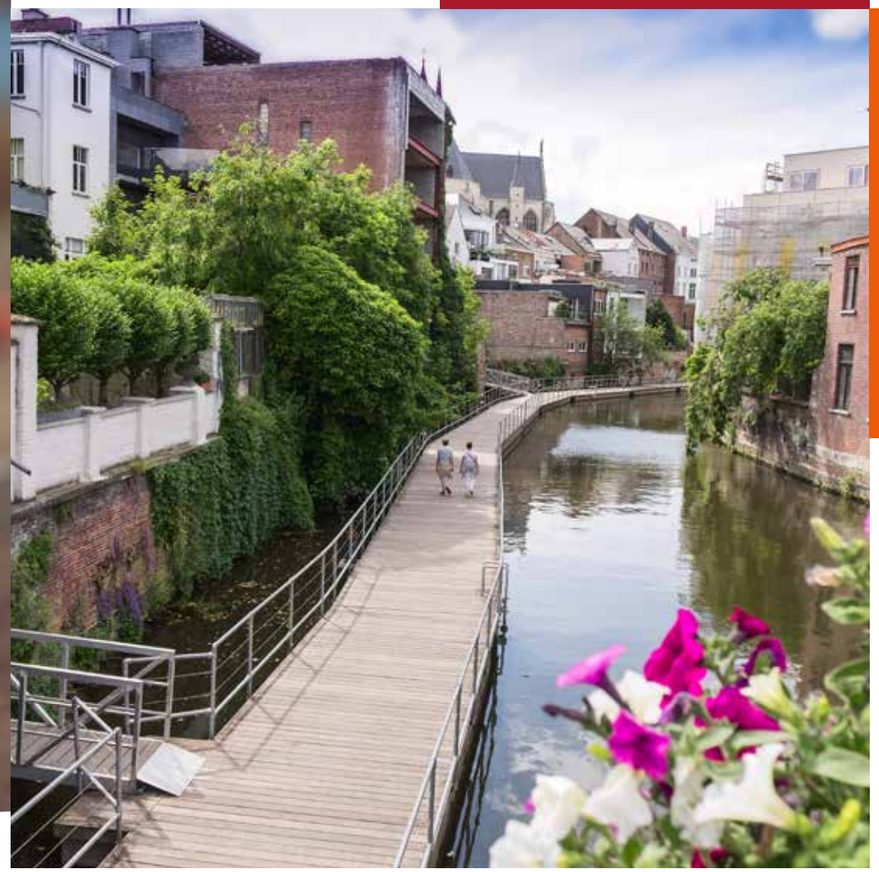
General info	4
Welcome	5
Program	6
BSTH Board 2023	10
Exhibition rules	11
Registration fees	11
Floorplan	12
State of the art I	13
Educational I	14
Educational II	15
Prof Gaston Baele Memorial Lecture	16
State of the art II	17
Educational III	20
State of the art III	20

Sponsored Satellite Symposia

- Pfizer	14
- Viatris	19

Abstracts of Oral presentations

- Basic Research	22
- Clinical / Laboratory	24
Abstracts of Poster presentations	29
List of sponsors	43



04

GENERAL INFO

WWW.BSTH2023.ORG

CONGRESS MANAGEMENT

Congress care
PO Box 440
5201 AK ‘s-Hertogenbosch
The Netherlands
+31 (0)73 690 14 15
www.congresscare.com

DATES
30 NOVEMBER -
1 DECEMBER 2023

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

05

WELCOME TO THE 30TH ANNUAL MEETING

Dear BSTH members,
Dear attendees,

On behalf of the BSTH Board, it is my great pleasure to welcome you to the 30th Edition of the BSTH annual meeting. This year is a special one, as it will mark the end of my term as President of the BSTH. I am therefore particularly grateful to our two local organizers, Christelle Orlando, scientific collaborator at the Hemostasis lab of the UZ Brussel and Secretary of the BSTH, and Jan Emmerechts, clinical biologist at AZ Sint-Jan Brugge, for having prepared a great and varied program.

During this meeting, several “where do we stand” topics on thrombosis will be addressed by internationally renowned speakers in three state-of-the-art and educational sessions. In line with the BSTH mission to promote basic, laboratory and clinical research, eight talented young researchers have been selected to present their work. Two of them will be awarded the Paul Capel prize for the best presentations. One researcher, selected out of seven, will receive the CSL Behring encouragement award to conduct a research project in the field of thrombosis and haemostasis.

Satellite symposia by Pfizer and Viatris will let you know about major latest news in their specialized field. Finally, we will be honored to welcome Paul Declerck, Emeritus Professor of KU Leuven, 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**

09:15 **WELCOME**
Cécile Oury

09:30 – 10:30 **STATE OF THE ART I: NEW APPLICATIONS FOR OLD TESTS**
Chairs: Katrien Devreese & Jan Emmerechts

09:30 **Update on viscoelastic testing**
Pieter De Kesel (Gent, Belgium)

10:00 **Update on platelet aggregation testing**
François Mullier (Namur, Belgium)

10:30 – 11:30 **SPONSORED SATELLITE SYMPOSIUM I - PFIZER**

Unmet need and therapeutic developments in Haemophilia
Cedric Hermans ((Université Catholique de Louvain Saint-Luc, Belgium)

11:30 **COFFEE BREAK**

12:00 – 13:00 **SELECTED ORAL PRESENTATIONS – BASIC RESEARCH**
Chairs: Cécile Oury & Simon Demeyer

12:00 **Analysis of the ultrastructural architecture of ischemic stroke thrombi using scanning electron microscopy: an observational study**
Sarah Vandelanotte (KU Leuven - KULAK)

12:15 **Septic shock is associated with a substantial change in the platelet lipidome**
Melanie Deschamps (UCL)

12:30 **Absence of mitochondrial deacetylase SIRT3 results in enhanced platelet apoptosis**
Jens Van Bael (KU Leuven - KULAK)

12:45 **Ticagrelor targets multiple lipids in the bacterial cytoplasmic membrane of multidrug resistant staphylococci**
Kirsten Leeten (ULiège)

13:00 **LUNCH AND POSTER WALK**

14:00 - 14:30 **BSTH GENERAL ASSEMBLY**
Only accessible for members of the BSTH

14:30 – 15:15 **EDUCATIONAL I**
Chair: Christelle Orlando

Congenital disorders of glycosylation
María Eugenia de La Morena-Barrio (Murcia, Spain)

15:15 – 16:00 **EDUCATIONAL II**
Chair: Sandrine Horman

Managing antithrombotic therapy in patients on mechanical circulatory support anno 2023: are we there yet?
Christophe Vandenbriele (London, UK)

16:00 **COFFEE BREAK**

16:30 – 17:15 **PROFESSOR GASTON BAELE MEMORIAL LECTURE**
Chair: Cécile Oury

Serendipity in the scientific career of an academic scientist
Paul Declerck (Leuven, Belgium)

17:15 **PAUL CAPEL PRIZE - BASIC RESEARCH**
Chair: Cécile Oury

17:30 **CLOSURE OF DAY PROGRAMME**

EVENING PROGRAM

WELCOME RECEPTION

Novo Nordisk 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 Salons van Dijck, Frederik de Merodestraat 33 in Mechelen.

Preregistration is required.

08

PROGRAM
FRIDAY
1 DECEMBER

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

09:00 – 10:00 **STATE OF THE ART II: UPDATE ON ANTICOAGULANT TREATMENT**
Chairs: Thomas Vanassche & Philip Maes

09:00 **Thrombophilia and anticoagulant treatment in pregnancy: what's new?**
Saskia Middeldorp (Nijmegen, The Netherlands)

09:30 **FXI inhibitors: where do we stand?**
Antonio Greco (Catania, Italy)

10:00 – 11:00 **SPONSORED SATELLITE SYMPOSIUM II – VIATRIS**
Chair: Harlinde Peperstraete (UZ Ghent)

10:00 **VTE prophylaxis in medically ill patients**
Thomas Vanassche (UZ Leuven)

10:20 **Arixtra in medically ill patients**
Alexander Cohen (Guy's and St Thomas' Hospitals and King's College London)

10:50 **Q&A**

11:00 **COFFEE BREAK**

11:30 – 12:30 **SELECTED ORAL PRESENTATIONS – CLINICAL AND LABORATORY**
Chairs: Phu Quoc Le & Kristel Vandenbosch

11:30 **European practices on antithrombotic management during percutaneous mechanical circulatory support in adults: An international survey of the ACVC of the ESC joint with the EuroELSO**
Charlotte Van Edom (KU Leuven)

11:45 **Correlation between ETP-based APC Resistance and the Relative Risk of Venous Thromboembolism in Women Using Combined Oral Contraceptives**
Laure Morimont (Qualiblood / Université Namur)

12:00 **Investigating phenotype – genotype relationships in patients with PROC or PROS1 variants**
Marthe Vanrentergem (UZ Leuven)

12:15 **The novel p.C1130S mutation is responsible for the complex phenotype in a Finnish family with VWD**
Bas Calcoen (KU Leuven / KULAK)

12:30 **CSL BEHRING ENCOURAGEMENT AWARD**

12:45 **LUNCH AND POSTER WALK**

13:45 – 14:30 **EDUCATIONAL III**
Chair: Jan Emmerechts

Bleeding and thrombosis in myeloproliferative disorders
Steffen Koschmieder (Aachen, Germany)

14:30 – 15:30 **STATE OF THE ART III: UPDATE ON DOAC**
Chairs: Christelle Orlando & Laure Gilis

14:30 **Treatment of thrombotic antiphospholipid syndrome: beyond vitamin-K antagonists**
Hannah Cohen (London, UK)

15:00 **When not to use DOACs**
Peter Verhamme (Leuven, Belgium)

15:30 **PAUL CAPEL PRIZE – CLINICAL AND LABORATORY**
Chair: Cécile Oury

15:45 **CLOSING REMARKS**

09

PROGRAM
FRIDAY
1 DECEMBER

10

BSTH BOARD 2023

THE PRESENT MEMBERS OF THE BSTH BOARD ARE:

CECILE OURY (PRESIDENT)
GIGA-Cardiovascular Sciences, Universite de Liege
cecile.oury@uliege.be

KATRIEN DEVREESE
UZ Gent
katrien.devreese@uzgent.be

SIMON DE MEYER
KU Leuven Kulak
simon.demeyer@kuleuven-kulak.be

JAN EMMERECHTS
AZ Sint-Jan, Campus Brugge
jan.emmerechts@azsintjan.be

LAURE GILIS
CHC Clinique St Joseph - Clinique Notre-Dame Hermalle
laure.gilis@chc.be

SANDRINE HORMAN
UCL
sandrine.horman@uclouvain.be

PHILIP MAES
UZA
Philip.maes@uza.be

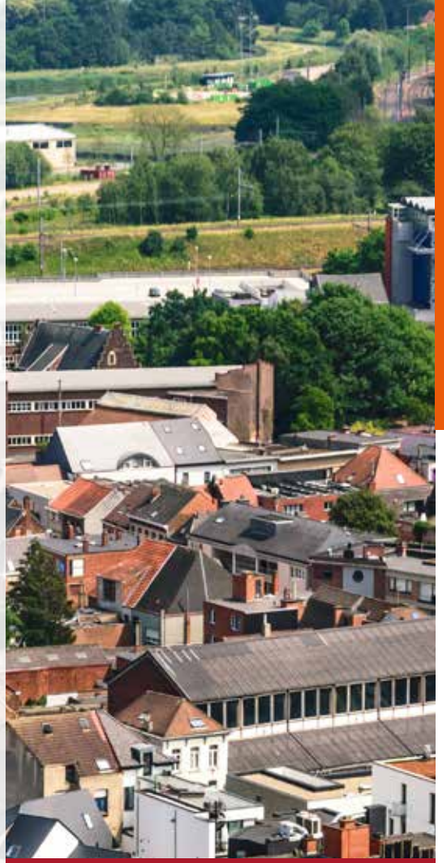
MARIE MOONEN
Centre Hospitalier Universitaire de Liege
m.moonen@chuliege.be

CHRISTELLE ORLANDO
UZ Brussel
christelle.orlando@uzbrussel.be

THOMAS VANASSCHE
KU Leuven
thomas.vanassche@uzleuven.be

KRISTEL VANDENBOSCH
Centre Hospitalier Universitaire de Liege
kvandenbosch@chuliege.be

PHU QUOC LE
Queen Fabiola University Children's Hospital, ULB
phuquoc.le@huderf.be



11

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

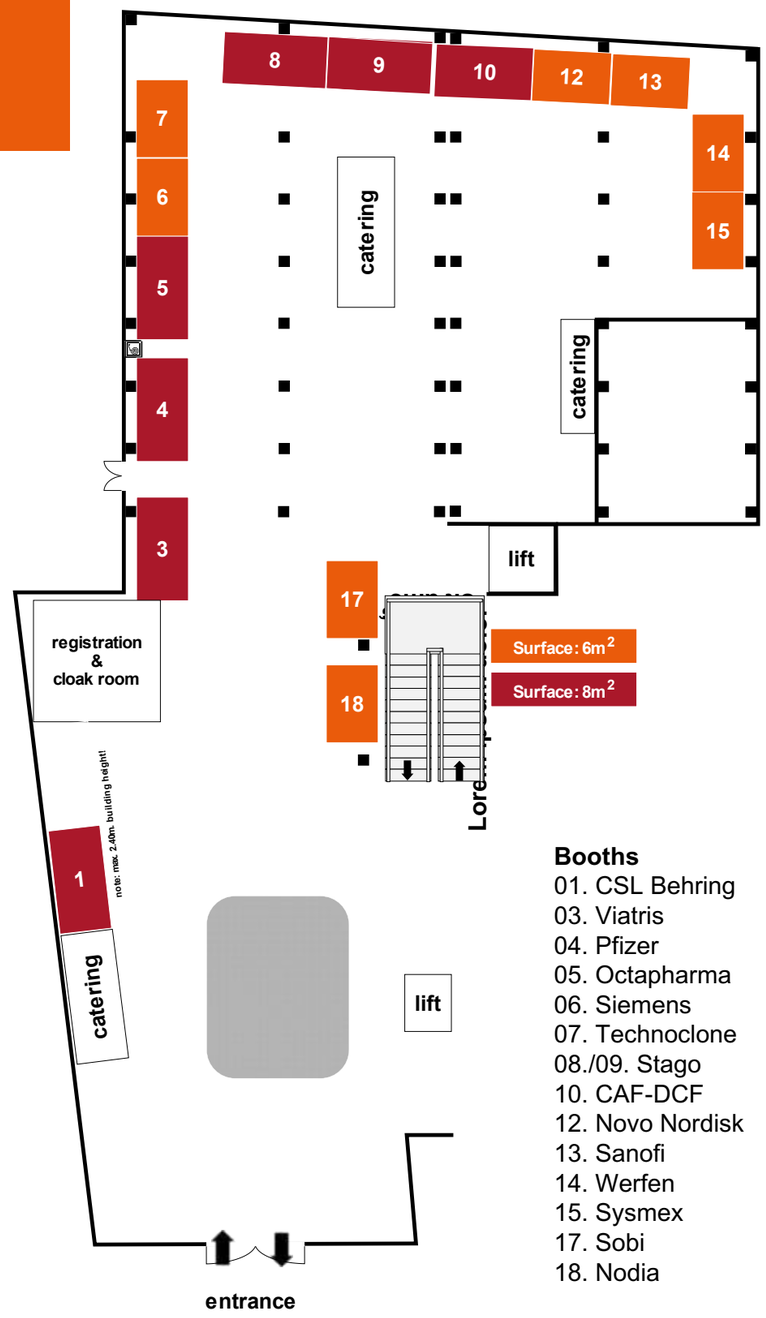
THU 30 NOV 2023	08:30-18:30
FRI 01 DEC 2023	08:30-15:30

REGISTRATION FEES

MEMBER BSTH		
Regular (MD specialist, MSc specialist, PhD scientist)	170	EUR
MD trainee / PhD student	90	EUR
Nurse, paramedic, technician, data manager, student	50	EUR
NON MEMBER BSTH		
Regular (MD specialist, MSc specialist, PhD scientist)	265	EUR
MD trainee / PhD student	165	EUR
Nurse, paramedic, technician, data manager, student	105	EUR

12

FLOORPLAN



Booths
01. CSL Behring
03. Viatris
04. Pfizer
05. Octapharma
06. Siemens
07. Technoclone
08./09. Stago
10. CAF-DCF
12. Novo Nordisk
13. Sanofi
14. Werfen
15. Sysmex
17. Sobi
18. Nodia

13

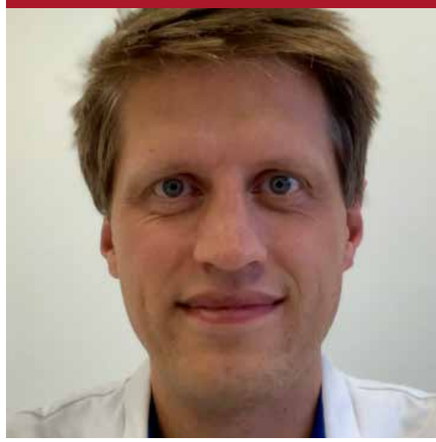
STATE OF THE ART I

Update on viscoelastic testing

De Kesel P
Department of Laboratory Medicine, Ghent University Hospital, Ghent, Belgium

Viscoelastic testing allows to evaluate coagulation dynamics in whole blood samples in real time at the point-of-care. In contrast to conventional coagulation tests, viscoelastic tests provide an integrated representation of secondary hemostasis, both visually and quantitatively, as they assess multiple factors contributing to clot formation, stabilization and lysis. In this presentation, an update on the technical evolution in the field of viscoelastic testing will be given, along with a discussion on recent clinical applications. Hitherto, the vast majority of studies on the clinical use of viscoelastic tests used mechanical viscoelastic techniques, including thromboelastography and rotational thromboelastometry. Recent methodological developments comprise cartridge-based platforms and resonance-based viscoelastic technologies. Concerning application in clinical practice, the existing evidence for and novel aspects of the use of viscoelastic tests in cardiac surgery, the field in which viscoelastic testing has been most extensively evaluated, will be discussed. Other areas in which viscoelastic tests has been used are major trauma and liver disease and transplantation, although high-level evidence is sometimes lacking. Additional recent applications include postpartum and subarachnoid hemorrhage, malignancy-associated coagulopathy and COVID-19.

Biography:
Pieter De Kesel graduated as Master in drug development/Pharmacist from Ghent University in 2009 and received a PhD in Pharmaceutical Sciences from the same university in 2015. Following a specialist training in laboratory medicine/clinical biology at Ghent University and Ghent University Hospital, he joined the Department of Laboratory Medicine of Ghent University Hospital in 2019, where he currently works as a clinical biologist in the coagulation laboratory headed by Prof. Dr. Katrien Devreese and in the clinical chemistry laboratory.



Update on platelet aggregation testing

Mullier F
Université Catholique De Louvain, CHU UCL Namur, Belgium

Platelet function testing is essential for the diagnosis of inherited platelet disorders (Glanzmann thrombasthenia, Bernard-Soulier Syndrome, Platelet type-von Willebrand disease (PT-VWD), 2B VWD (2B-VWD) and screening of platelet secretion disorders), confirmation of heparin-induced thrombocytopenia (HIT) while the laboratory monitoring of P2Y12 inhibitors is still debated. Many methods to test platelet function are available but standardization is often lacking and results of different platelet function tests are highly variable. Light transmission aggregometry has been the gold standard for over 60 years, with inherent challenges of working with live dynamic cells in specialized laboratories. In recent years, standardization efforts (preanalytical steps, agonists concentration, automatization, guidelines,….) have reduced the interlaboratory variability. This presentation will address the clinical applications of platelet aggregation testing (light transmission aggregometry, lumiaggregometry and whole blood aggregometry), the current pitfalls and the perspectives in the field (IVDR, use of 5B9 for improvement of HIT confirmation,…).

Biography:
Prof. François Mullier was granted his pharmaceutical degree in 2005, PhD in 2012 and complementary master in laboratory medicine also in 2012. He is head of department of laboratory medicine since 2019 and head of hemostasis hematology laboratory in CHU UCL NAMUR (Dinant/Godinne) since 2012. He is also Professor at the University catholique de Louvain and visiting professor at the Université de Namur. He is currently co-chair of the subcommittee Control of anticoagulation of the International Society of Thrombosis and Hemostasis (ISTH), member of the editorial board of Thrombosis Journal, member of the Hemostasis and Platelets committee of the International Society of Laboratory Hematology (ISLH), teacher in the European course of laboratory medicine organized by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) and member of organizing committee and teacher of the European course on antithrombotic management CAS-AM https://www.cas-am.eu/. He was a past co-chair of the subcommittee Platelet Immunology of the ISTH, Past Expert for the European Medicine Agency and European Society of Anesthesiology. His major laboratory interest is contributing to improvement to patient care. His research interests include appropriate prescription in laboratory medicine, diagnosis of bleeding of unknown origin (BUC), mild bleeding disorders (MBD), monitoring of antithrombotics and diagnosis/follow-up of hemostasis disorders in intensive care unit. He has authored/co-authored 202 peer-reviewed cited publications and have given 121 international lectures.

14

PFIZER SATELLITE SYMPOSIUM I

Unmet need and therapeutic developments in Haemophilia

Hermans C
Cliniques universitaires Saint-Luc, Catholic University of Louvain, Brussels, Belgium

The aim of this symposium is to explore the remaining challenges in haemophilia management, and the current and potential future haemophilia treatment landscape, including:
The various challenges faced by people living with haemophilia and their healthcare providers, including how these may change during different life stages
How these remaining unmet needs may evolve as the treatment landscape changes
How the haemophilia treatment landscape has evolved to date and potential future changes and beyond
The scientific principles underpinning the approaches currently under investigation for the treatment of haemophilia

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 330 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, associate member of the Belgian Royal Academy of Medicine 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.

EDUCATIONAL I

Congenital disorders of glycosylation: relevance in hemostasis and thrombosis

De la Morena-Barrio ME
University of Murcia, Spain

N-glycosylation is a key post-translational modification of many proteins, including all hemostatic proteins. The incorporation of a complex, heavy (2 KDa) and negatively charged N-glycan at certain asparagine residues located in a specific sequence (Asn-X-Ser/Thr), plays a key role in their folding, intracellular pathway, secretion, and interaction of proteins. Therefore, this post-translational modification may influence levels and function of these proteins. Accordingly, an aberrant N-glycosylation may have significant functional consequences with pathogenic relevance. In this session we will review the two types of N-glycosylation defects that may affect any protein: gene-specific or global defect, and we will focus, as a model, on a key endogenous anticoagulant: antithrombin. We will detail the heterogeneous functional and clinical effects of mutations in SERPINC1 deleting or generating N-glycosylation sequences in antithrombin. On the other hand, we will also show that up to 5% of cases with antithrombin deficiency, all without SERPINC1 gene defects, are explained by a global defect of glycosylation that affect many other hepatic proteins, and might be diagnosed as congenital disorders of glycosylation (CDG). Interestingly, the CDG patients diagnosed by their antithrombin deficiency and thrombotic events, include classical recessive CDGs (including new CDGs), many of them treatable by dietary interventions, since mannose supplementation or galactose removal might rebalance the hemostatic system allowing withdrawal of anticoagulant treatments. Furthermore, certain CDGs may result from a combination of genetic defects and environmental factors, such as alcohol intake. Lastly, we will review the impact of classical CDGs on the hemostatic system focusing on the search for potential markers of vascular events, which are relatively prevalent in these patients.

The session aims to join basic and clinical aspects related to N-glycosylation in the hemostatic system.

Biography:
María Eugenia de la Morena-Barrio is principal investigator and teacher at the University of Murcia, Spain, in the Experimental Hematology and Oncology Group. Graduated in Biochemistry in 2008 and received an International PhD in Medicine from the University of Murcia in 2013, awarded with an extraordinary doctoral prize and recognized as the best thesis by the Robles Chillida Foundation. In 2012, she completed a training period at the Catholic University of Leuven, Belgium under the supervision of Dr. Freson and Dr. Jaeken on congenital disorders of glycosylation. Author of 83 articles, h-index of 19, i10-index of 37, with a total of 981 citations. Holds 3 patents and founded a technological-based company in 2020 specializing in genetic sequencing of long reads. Member of the research team in 25 competitive projects, principal investigator of 4 of them. Supervised of three doctoral theses. Selected in 2020 by international societies EHA/ASH for the Translational Research Training in Hematology (TRTH) international leadership program. Serves as associate editor for 2 journals, guest editor for 2 journals, and reviewer for 12 JCR journals. Member of the Spanish Network on Rare Diseases (CIBERER), the Spanish Society of Thrombosis and Hemostasis (SETH), the European Hematology Association (EHA), the American Society of Hematology (ASH), and the International Society of Thrombosis and Haemostasis (ISTH). Specializes in hemostasis and in congenital disorders of glycosylation; focusing on molecular bases and mechanisms involved in thrombotic diseases (antithrombin deficiency) and hemorrhagic disorders (congenital Factor XI deficiency).
Holds 35 research awards, including recognition from the Royal Academy of Medicine and the Royal Spanish Foundation for Hemophilia Victoria Eugenia.

Managing antithrombotic therapy in patients on mechanical circulatory support anno 2023: are we there yet?

Vandenbriele C
Royal Brompton & Harefield Hospitals, Guy's and St Thomas' NHS Foundation Trust, Imperial College, London, United Kingdom

The utilization of mechanical support devices (ECMO, Impella, etc.) has witnessed a significant global surge in critically ill cardiogenic shock patients. Post the COVID-19 pandemic, there has been a remarkable increase in the adoption of these devices. However, despite the widespread use, several studies indicate that the high mortality rate of cardiogenic shock (approximately 50%) has scarcely diminished since the introduction of these expensive devices. This persistent mortality rate is largely attributed to a high incidence of complications, notably bleeding, hemolysis, and thrombotic events.

Effectively managing mandatory anticoagulant therapy with these devices (for thrombosis and bleeding prevention) and addressing complications such as bleeding and hemolysis are crucial to reduce morbidity and mortality. Despite significant advancements in equipment in recent years and minimal device failures, knowledge regarding anticoagulation management remains limited but pivotal.

This research focuses on fundamental questions within this patient population: determining the most suitable anticoagulant for critically ill cardiogenic shock patients, identifying optimal monitoring tests or strategies for unfractionated heparin, establishing the most appropriate anticoagulation target, addressing antiplatelet therapy in mechanical circulatory support, defining major bleeding in cardiogenic shock patients, etc.

This educational lecture highlights the anticoagulation protocol developed in collaboration with multiple MCS centers, discusses the evidence and pitfalls of unfractionated heparin tests through real-world cases, reviews available data on various anticoagulants in this patient group, introduces a new bleeding score for the ICU, and provides an overview of the prevention and treatment of hemolysis and bleeding in critically ill patients. Finally, the lecture offers insights into ongoing and upcoming research projects aimed at advancing this intriguing field where even the most basic questions remain unanswered.

Biography:
Christophe Vandenbriele trained as a cardiologist and intensivist at UZ Leuven and the renowned royal Brompton hospital in London. His special interest lies in supporting critically ill cardiogenic shock patients with mechanical support devices and more specifically in managing anticoagulation in this population. He is a board member of the acute cardiovascular care association (ACVC) and the thrombosis working group of the ESC. He currently works as a cardiologist intensivist at Harefield hospital, part of the Royal Brompton & Harefield, Guy's & St Thomas' NHS Foundation Trust in London and conducts research within the Heart and Lung institute at Imperial College, London.

15

EDUCATIONAL II

16

PROFESSOR GASTON BAELE MEMORIAL LECTURE

Serendipity in the scientific career of an academic scientist

Declerck P
KU Leuven, Linden, Belgium

Introduction
Starting scientists often experience stress regarding decisions to be made and how this may affect their career. In many cases they want, at the very beginning of their putative career, to predict their future career path until the end. The question can be raised whether it is worth spending energy to even consider worrying about a career path.

Methods
Retrospective analysis of a career path to identifying the different turning points that marked the scientific career. Start date October 1980, end date September 2023. Number of years 43. Number of careers 1.

Results
The analysis identified at least five turning points that marked the scientific career. The observations revealed that there was no correlation nor any relevant association between the subject of the initial project of October 1980 and the final outcome at September 2023. No parameters could be identified during the career path that had a major predictive value for the career outcomes.
The study illustrates that even starting from protoplast fusions to generate new antibiotics followed by studies on the electrochemistry of nitroimidazoles one may end up in the area of thrombosis and haemostasis through monoclonal antibody-based and structural studies on antifibrinolytic proteins.

Conclusion
The data illustrate that a starting scientist should not aim at predicting her/his long term career path. Decisions should be guided by intrinsic motivation in combination with opportunities and daring to take on challenges. Serendipity is an important characteristic for an exciting professional career.
More studies may be needed to extrapolate these finding to other careers.

Biography:
Professor Paul J. Declerck, PharmD, PhD, obtained his Ph.D. in Pharmaceutical Sciences from the KU Leuven (Belgium) in 1984. After a post-doctoral training in the Laboratory of Biochemical Cytology (Prof. Dr. C. de Duve) at the Rockefeller University in New York he joined in 1986 the Center for Molecular and Vascular Biology (Prof. D. Collen) at the KU Leuven. In 1991 he was appointed professor of Pharmaceutical Biotechnology at the Faculty of Pharmaceutical Sciences. He became full professor in 1997 and emeritus professor in 2023. He was Research Director of the Laboratory for Therapeutic and Diagnostic Antibodies at the Department of Pharmaceutical and Pharmacological Sciences (KU Leuven). His research focused on structure-function relationships of (recombinant) proteins and on the development of monoclonal antibodies for research, diagnostic and therapeutic purposes. He has expertise in the area of recombinant proteins, monoclonal antibody technology, biotechnology, drug development, structure-function relationship in proteins, biosimilars, immunoassays, immunogenicity of biologicals.
Prof. Declerck has given numerous invited lectures at international meetings and has authored more than 310 scientific papers in peer-reviewed journals.
He served as Dean of the faculty of Pharmaceutical Sciences of the KU Leuven and member of various international scientific advisory boards. He is member of the Commission of Medicines for human use of the Belgian Federal Agency for Medicines and Health Products, and Member of the Working Party on Gene Therapy Products (European Pharmacopoeia Commission, European Directorate for the Quality of Medicines).

17

STATE OF THE ART II

Thrombophilia and anticoagulant treatment in pregnancy: what's new?

Middeldorp S
Radboudumc, Nijmegen, the Netherlands

Venous thromboembolism (VTE) during pregnancy and the postpartum period is a main cause of maternal mortality, and always associated with significant morbidity. Use of therapeutic anticoagulation during pregnancy, around delivery and in the postpartum period is associated with the burden of parenteral treatment, an increased risk of bleeding, and high costs. Women with pregnancy-related VTE suffer long-term consequences, such as postthrombotic syndrome, the need to avoid oral contraceptives, and the need for prevention during subsequent pregnancies.

Thrombophilia not only is associated with VTE, but, depending on the type of thrombophilia also with recurrent miscarriage and placenta-mediated pregnancy complications, such as preeclampsia and HELLP syndrome.

The optimal prevention of pregnancy-related VTE and the use of low-molecular-weight heparin to prevent recurrent miscarriage in specific populations was mainly based on very weak evidence. Saskia Middeldorp will discuss the results of the recently published international ALIFE2 en Highlow randomized controlled trials, focused on how these trials may impact clinical practice.

Biography:
Saskia Middeldorp is Professor of Medicine and Head of the Department of Internal Medicine of the Radboud University Medical Center in Nijmegen, The Netherlands. Prior to her transfer to Nijmegen in January 2021, she has been a professor of Medicine at Amsterdam University Medical Centers for over 10 years, leading the clinical thrombosis and haemostasis research lines of the Department of Vascular Medicine. Since January 2023, Saskia Middeldorp is one of the 4 Research Domain Leaders in the Radboudumc Research Institution for Medical Innovation.
Her present research focuses on several aspects of hereditary and acquired thrombophilia, women's issues in thrombosis and haemostasis, and the clinical evaluation of new anticoagulants and antidotes. She is principal investigator of practice-changing trials such as the investigator-initiated ALIFE, ALIFE2 and Highlow randomized controlled trials.
Saskia Middeldorp is elected council member of the International Society of Thrombosis and Haemostasis, immediate past chair of the INVENT-VTE network, Associate Editor of the Journal of Thrombosis and Haemostasis and Editorial Board Member of Blood.
In December 2016, she held the Ham-Wasserman Lecture and received the accompanying award in recognition of pioneering work in inherited thrombophilia at the annual American Society of Hematology (ASH) meeting in San Diego. In October 2021, she received the Heijmans van den Bergh penning, a once-per-five year honorary distinction of the Netherlands Internal Medicine Society for her achievements in terms of science, education, governance and being a role model for young internists.
She is chair of the Thrombophilia Chapter of ASH VTE Guidelines (2023). Saskia Middeldorp has co-authored the Pregnancy chapter of the ASH pregnancy guideline in 2018 as well as the 9th Edition Antithrombotic Guidelines of the American College of Chest Physicians (ACCP) in 2012.
Saskia has supervised and still supervises numerous PhD students and has co-authored over 400 peer-reviewed papers and several book chapters, and reviews manuscripts for international scientific journals.

18

STATE OF THE ART II

FXI inhibitors: where do we stand?

Greco A
University Of Catania, Catania, Italy

Therapeutic anticoagulation is necessary in various scenarios to prevent or treat venous and arterial thromboembolism across different fields of medicine. Although available anticoagulant drugs have different mechanisms of action, both parenteral and oral formulations share the common principle of impeding or halting crucial steps in the coagulation cascade. However, this approach inevitably leads to an increased risk of bleeding complications. Hemorrhagic events not only directly affect patient prognosis but also indirectly hinder the adoption of an effective antithrombotic strategy (i.e., patients are more prone to disrupt the antithrombotic therapy).
To address this issue, researchers have recently explored the inhibition of factor XI (FXI) as a potential strategy to separate the pharmacological effects of anticoagulant therapy from its adverse events (i.e., bleeding). This concept stems from the observation that FXI has a differential effect in physiological hemostasis and pathological thrombosis: it plays a significant role in thrombus amplification but only plays a secondary role in the final consolidation of clots during hemostasis. Consequently, multiple agents have been developed to target different stages of FXI inhibition, including suppression of biosynthesis, prevention of zymogen activation, and disruption of the biological action of the active form. These agents encompass antisense oligonucleotides, monoclonal antibodies, small synthetic molecules, natural peptides, and aptamers.
Phase 2 studies conducted on various classes of FXI inhibitors in orthopedic surgery have demonstrated dose-dependent reductions in thrombotic complications without a corresponding increase in bleeding compared to low-molecular-weight heparin. Similarly, the FXI inhibitor asundexian exhibited lower bleeding rates compared to the activated factor X inhibitor apixaban in patients with atrial fibrillation, although its therapeutic effect on stroke prevention has yet to be established. Furthermore, FXI inhibition holds potential benefits for patients with conditions such as end-stage renal disease, noncardioembolic stroke, or acute myocardial infarction, as evidenced by other phase 2 studies conducted in these populations.
Nevertheless, the balance between thromboprophylaxis and bleeding achieved by FXI inhibition requires confirmation through large-scale phase 3 clinical trials with sufficient power to evaluate clinical endpoints. Numerous ongoing or planned trials aim to define the role of FXI inhibitors in clinical practice and determine the most suitable FXI inhibitor for each specific clinical indication. These trials will provide valuable insights into the rationale, pharmacology, and future perspectives of FXI-inhibiting drugs.

Biography:
Dr. Antonio Greco is Cardiologist, currently working as consultant in the Cardiology Department of the A.O.U. Policlinico "G. Rodolico - San Marco", University of Catania (Catania, Italy). Currently, he is also attending the third year of a PhD course in Translational Biomedicine at the University of Catania. In 2014, he graduated in Medicine and Surgery at the University of Catania, where he also completed the specialization course in Cardiovascular Diseases in 2020. In 2021, he completed an advanced university course on biomedical statistics applied to research in the human field at the University of Palermo (Italy). He worked as an Assistant Researcher at the University of Catania between 2021 and 2022, was Director of a medical Contract Research Organization in 2022, and collaborated as a Scientific Consultant for the European Commission.
Research activities of Dr. Greco mainly focuses on antithrombotic therapy, atherosclerotic cardiovascular disease, risk stratification and personalized medicine. He participated into several clinical trials and research projects in the field of cardiology as a member of the Steering Committee or the Clinical Event Adjudication Committee, Data Manager or Local Investigator. Dr. Greco is also collaborating with scientific Journals in the field of Medicine or Cardiology, acting as an Editorial Board Member and as a Reviewer. He published more than 40 articles in peer reviewed Journals and has an H-index of 10. He is also member of a number of national and international Societies in the field of cardiology, including the European Society of Cardiology, and he is part of working groups on thrombosis. He was invited as a speaker and involved in the organization of national and international cardiology conferences.

19

VIATRIS SATELLITE SYMPOSIUM II

VTE prophylaxis in medically ill patients

Venous thromboembolism (VTE), encompassing deep vein thrombosis (DVT) and pulmonary embolism (PE), is a significant cause of morbidity and mortality in hospitalized patients. Medically ill patients, particularly those with acute medical conditions such as congestive heart failure, respiratory disease, and infectious or inflammatory disorders, are at an even higher risk of VTE. Despite the known benefits of VTE prophylaxis, underutilization remains a persistent challenge, contributing to preventable adverse outcomes.
The symposium will highlight the importance of VTE prophylaxis in medically ill patients, emphasizing the potential for significant morbidity and mortality reduction. Risk factors for the development of VTE in this population and the benefits of various VTE prophylaxis modalities, including low-molecular-weight heparins (LMWHs) and fondaparinux (Arixtra®) will be addressed. A more in-depth overview of the clinical evidence supporting the efficacy, safety and convenience of fondaparinux (Arixtra®) will provide further information to discuss its place and added value in the current clinical setting.
We invite you to embrace the opportunity to enhance your knowledge, strengthen your clinical practice, and contribute to the discussion about prevention of VTE, a leading cause of preventable morbidity and mortality.

Biographies:
Prof. dr. Thomas Vanassche is a cardiologist and assistant professor at University Hospitals Leuven (UZ Leuven) and KU Leuven. He is a specialist in vascular medicine and prevention, thrombosis and hemostasis, hypertension, and cardiac intensive care. He is also the vice-president of the Belgian Society for Thrombosis and Haemostasis (BSTH).
Prof. dr. Vanassche has a particular interest in the prevention of venous thromboembolism (VTE). He has conducted research on the effectiveness of different VTE prophylaxis strategies and has published his findings in leading medical journals. He is also a passionate advocate for patient education and empowerment. He believes that patients should be informed about their risk of VTE and should be involved in making decisions about their VTE prophylaxis.
Prof. dr. Vanassche is a respected member of the cardiovascular community and is committed to improving the care of patients with cardiovascular disease.

Prof. dr. Alexander Cohen is a vascular physician and epidemiologist with a distinguished career spanning over three decades. He is currently affiliated with Guy's and St Thomas' Hospitals and King's College London, where he holds the position of Consultant Physician and Epidemiologist in Vascular Medicine.
Dr. Cohen's research interests lie in the epidemiology, prevention, and treatment of venous thromboembolism (VTE). He has made significant contributions to our understanding of the risk factors for VTE and has developed novel approaches to VTE prevention. Dr. Cohen is a prolific author, having published over 450 peer-reviewed articles in leading medical journals. He is also an active member of several professional societies, including the European Society of Cardiology, the Royal Australasian College of Physicians, and the Royal College of Physicians.
Dr. Cohen is a true pioneer in his field and continues to make significant contributions to our understanding and treatment of VTE

Dr. Harlinde Peperstraete is a staff member in intensive care at UZ Ghent.
She obtained her degree in medicine and anaesthesia from the Catholic University of Leuven. She further specialised in intensive care for congenital heart defect patients at Necker-Enfants Malade in Paris and obtained her intensive care certification, after additional training at Ghent University.
She has a special interest extracorporeal life support, in coagulation problems in the critically ill patient and in quality of life after a severe intensive care admission. She is a PhD candidate where the thesis deals with "ECMO Intensive Care Programme: optimisation of clinical and technical aspects and optimisation of training for caregivers."

20

EDUCATIONAL III

Bleeding and Thrombosis in Myeloproliferative Neoplasms (MPN)

Koschmieder S
RWTH Aachen University / Center for Integrated Oncology Aachen Bonn Cologne Düsseldorf (CIO ABCD), Aachen, Germany

Patients with Myeloproliferative Neoplasms (MPN) suffer from a variety of clinical signs and symptoms, including bone pain, generalized pruritus, night sweats, abdominal discomfort, weight loss, and fatigue. Their survival may be significantly compromised by progression of the underlying disease to myelofibrosis and acute leukemia, but also by vascular complications, such as thrombosis and thromboembolism as well as severe hemorrhoage. The relative risk of venous and arterial thrombosis is increased by up to 4- and 13-fold, respectively. And recently, it has become clear that not only polycythemia vera (PV) and essential thrombocythemia (ET) pose such risks, but also primary myelofibrosis (PMF). Prevention of these vascular complications, both by primary and secondary prevention strategies, are at the focus of current treatment algoritms for patients with MPN. Here, I will discuss recent advances in our understanding of MPN disease pathogenesis and its link to vascular complications, novel diagnostic tools and parameters (e.g. CHIP, VAF), and up-to-date treatments for patients with MPN.

Biography:
Professor Dr. med. Steffen Koschmieder is board-certified in Internal Medicine, Hematology/Oncology, and Hemostaseology. He studied Medicine in Bochum, Mainz, Dijon, Houston, and Salt Lake City, received his specialty training in Frankfurt and Münster, and was a postdoctoral researcher in Prof. Daniel Tenen´s laboratory at the Harvard Institutes of Medicine in Boston. He is currently Professor for Translational Hematology and Oncology and Attending Physician at RWTH Aachen University in Germany. His clinical and preclinical research focuses on Myeloproliferative Neoplasms (MPN) and disorders of blood coagulation, and he has published over 200 scientific publications. He is Research Coordinator of the federally funded Clinical Research Unit 344 on MPN and Myelofibrosis in Aachen and co-speaker of the German Study Group (GSG-MPN) and the GSG-MPN bioregistry. He has coauthored the current “onkopedia” guidelines for PMF, PV and ET of the German Society for Hematology and Medical Oncology (DGHO), the DGHO recommendations for thrombosis and bleeding management in MPN, as well as the revised treatment recommendations for MPN by the European LeukemiaNet, and he is a member of the Guidelines Committee of the European Haematology Association and Chairman of the Working Party for Hemostasis of DGHO. In addition, he serves as Associate Editor of “HemaSphere”.

STATE OF THE ART III

Treatment of thrombotic antiphospholipid syndrome: beyond vitamin-K antagonists

Cohen H
University College London Hospitals and University College London, United Kingdom

The mainstay of treatment for thrombotic antiphospholipid syndrome (APS) is lifelong anticoagulation with warfarin or an alternative vitamin-K antagonist (VKA).

The RAPS (Rivaroxaban in APS) randomised controlled trial (RCT) of rivaroxaban versus warfarin reported that rivaroxaban could be an effective and safe alternative in patients with APS and venous thromboembolism (VTE) requiring standard-intensity anticoagulation. Subsequent direct oral anticoagulant (DOAC) RCTs all included a heterogenous patient population with respect to thrombotic phenotype (venous and/or arterial thrombosis, the latter excluded in RAPS). Meta-analyses of DOAC RCTs in APS patients found a significantly higher risk of subsequent arterial thrombosis during treatment with DOACs compared to warfarin, although the risk of subsequent VTE was not increased. In accordance with the European Medicines Agency recommendations, adopted by regulatory agencies worldwide, current guidance recommends: a) consideration of continuation of the DOAC in single or double antiphospholipid antibody (aPL) positive non-“high risk” APS patients who have been on a DOAC as standard of care treatment following a first episode of VTE; and b) against the use of DOACs in APS patients with arterial thrombosis and/or triple-aPL positivity. All the DOAC RCTs in APS had used standard (or lower, in ASTRO-APS) intensity DOAC doses. The RISAPS (Rivaroxaban in Stroke Patients with APS) RCT is a proof of principle trial of: a) non-inferiority in the efficacy of high-intensity rivaroxaban compared with high-intensity warfarin; and b) absence of safety signals. Novel approaches to anticoagulation, such as factor XI/XIa inhibitors, are being studied in other patient populations.

Some patients with APS experience breakthrough thrombosis despite seemingly adequate anticoagulation with a VKA, i.e. anticoagulant-refractory thrombotic APS. In this regard, point-of-care (POC) INRs should be interpreted with caution in APS patients. Initial treatment options following a thrombotic event while on standard-intensity VKA (target INR range 2.0-3.0) include intensification of VKA anticoagulation (i.e., high-intensity VKA, target INR range 3.0-4.0); addition of low dose aspirin; or a switch to low-molecular-weight heparin (LMWH). Fondaparinux may be effective where high-intensity/escalated high-intensity LMWH has failed. Vascular options, such as vasodilators, epidermal skin grafting, digital sympathectomy and hyperbaric oxygen, may be useful.

The importance of optimisation of traditional cardiovascular risk factors, such as hypertension and hyperlipidaemia, is highlighted by the adjusted Global AntiphosPholipid Syndrome Score (aGAPSS), which includes hypertension and hyperlipidaemia in addition to the three criteria aPL (lupus anticoagulant, anticardiolipin and anti-ß2 glycoprotein I antibodies), with higher scores associated with recurrent arterial thrombosis.

The multiple mechanisms involved in the generation of the thrombotic phenotype in APS suggest that anticoagulation alone may not control thrombosis, thus, other modalities merit consideration. These include adjunctive treatment with hydroxychloroquine, statins, and vitamin D. Therapeutic agents that may have potential utility for patients with thrombotic APS include those that target autoreactive B cells, plasma cells, neonatal Fc receptors or complement activation.

Appropriately designed and powered studies, which capture the clinical and laboratory heterogeneity of APS, could eventually provide sufficient data for evidence-based management. The development of suitable outcome measures, including a disease activity index and an optimal damage index, would facilitate these studies.

Biography:
Hannah Cohen (MD FRCPath) is a consultant haematologist at University College London Hospitals, where she leads the haematology service for antiphospholipid syndrome, and professor of haematology at University College London. Her main research focus is the pathophysiology, diagnosis and management of antiphospholipid syndrome. She is chair of the International Society on Thrombosis and Haemostasis Scientific and Standardization Committee Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibodies and a founder member/co-chair of APS ACTION (AntiPhospholipid Syndrome Alliance For Clinical Trials and InternatiOnal Networking).

When not to use DOACs?

Verhamme P
UZ Leuven, Belgium

DOACs have become the antithrombotic therapy of choice for the treatment and (secondary) prevention of venous thromboembolism and the prevention of stroke in patients with atrial fibrillation.
However, some patients with particular characteristics have been ignored during clinical studies whereas for other patient groups, vitamin K antagonists remain the standard antithrombotic therapy.

Biography:
Peter Verhamme is an internist with a focus on vascular medicine, thrombosis and haemostasis at UZ Leuven and also investigator at the Center for Molecular and Vascular Biology at KU Leuven.

21

STATE OF THE ART III

O1

BASIC RESEARCH

Analysis of the ultrastructural architecture of ischemic stroke thrombi using scanning electron microscopy: an observational study

Vandelanotte S[1], François O[2], Vanhoorne A[2], Tersteeg C[1], Vanhoorelbeke K[1], Vanacker P[3,4,5], Andersson T[2,6], De Meyer S[1]
[1]KU Leuven, Campus Kulak, Kortrijk, Belgium, [2]Department of Medical Imaging, AZ Groeninge, Kortrijk, Belgium, [3]Department of Neurology, AZ Groeninge, Kortrijk, Belgium, [4]Department of Neurology, University Hospitals Antwerp, Antwerp, Belgium, [5]Department of Translational Neuroscience, University of Antwerp, Antwerp, Belgium, [6]Departments of Neuroradiology, Karolinska University Hospital, and Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden

Introduction: The mainstay of acute ischemic stroke (AIS) therapy is timely recanalization using either pharmacological thrombolysis using recombinant tissue plasminogen activator (rt-PA), mechanical thrombectomy (MT), or a combination of both. However, due to several contraindications and therapy resistance, rt-PA treatment is only successful in a few patients. Despite advancements in revascularization rates with MT, there remains a subset of thrombi that display resistance to removal. The precise mechanisms underlying this rt-PA or MT resistance is not completely understood but it is thought that thrombus composition could play an important role.

Aims: The aim of this study was to investigate the ultrastructural organisation of AIS thrombi using scanning electron microscopy (SEM) and standard histological analysis.

Methods: Thrombi from AIS patients were retrieved via MT at AZ Groeninge Hospital in Kortrijk. One part of the thrombus was used for histological analysis of seven thrombus components (red blood cells (RBC), fibrin, platelets, von Willebrand factor (VWF), leukocytes and intra- and extracellular DNA). The other part was cut in thrombus pieces of 2 to 3 mm and prepared for SEM imaging. Thrombus pieces were aligned to either look at the interior or at the exterior of the thrombus. Finally, thrombus sections (5 µM) of paraffin embedded thrombi with different composition determined via standard histological analysis, were rehydrated and processed again for SEM imaging. Before imaging, all samples were sputter-coated with platinum (3-5 nm).

Results: SEM imaging of AIS thrombi revealed different ultrastructural characteristics of ischemic stroke thrombi. Thrombi showed the presence of a highly compacted outer layer. This dense outer layer varied in thickness from 1.3 µm to 10 µm and formed a physical barrier between the interior and the exterior of the thrombus. The interior of the thrombi is primarily comprised of three distinct patterns. First, we observed RBC-rich regions characterized by densely packed and deformed RBCs known as polyhedrocytes that are intermixed with thin fibrin fibers of 0.1 to 0.3 µm tick. Secondly, some areas presented as highly compacted structures that lacks RBCs and looked similar to the outer layer of the thrombus. These regions aligned with platelet-rich areas confirmed via classical histology. Finally, some areas presented with a branched fibrin network containing thick fibrin fiber bundles measuring up to 5 µm in thickness, comprised of multiple smaller fibrin fibers. These tick fibrin bundles are surrounded by platelet aggregates. The smaller branches of this fibrin network are mixed with lose and intact RBCs. Investigation of 5 µm thrombus sections of different thrombus types revealed that the organised fiber networks are mostly located at the interplay between the dense RBC-rich regions and the compacted platelet regions.

Conclusions: Our SEM study, in combination with histology, revealed different architectural features that further enhance our understanding on of the heterogeneity of AIS thrombi.

O2

BASIC RESEARCH

Septic shock is associated with a substantial change in the platelet lipidome

Dechamps M[1,2], de Cartier E[1], De Poortere J[1], Robaux V[1], Des Rosiers C[3], Forest A[3], Ambroise J[1], Bertrand L[1], Beauloye C[1,2], Horman S[1]
[1]Uclouvain, Brussels, Belgium, [2]Cliniques Universitaires St Luc, Brussels, Belgium, [3]Montreal Heart Institute Research Center, Montreal, Canada

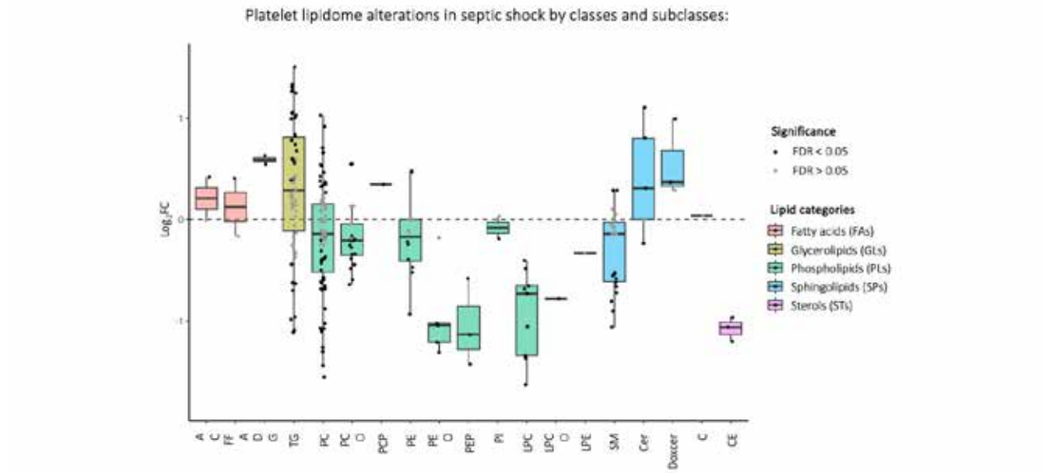
Background: According to the WHO, sepsis is responsible for one in five deaths worldwide. The syndrome is defined as life-threatening organ dysfunction caused by an impaired host immune response to infection. Sepsis is characterized by major endothelial dysfunction, microvascular alterations, and coagulopathy. In addition to their involvement in pathological hemostatic processes, platelets are key players in sepsis as they promote immunothrombosis, notably by generating cytokines and lipid mediators of inflammation. It is increasingly recognized that the composition of the platelet lipidome is critical to their function. Sepsis is associated with major perturbations in cell signaling and metabolism, the impact of which on lipid metabolism has already been demonstrated in various cells. However, the lipidomic profile of platelets during sepsis has never been studied.

Aim: The aim of this study is to investigate the platelet lipidome of septic patients. The additional objective of this analysis is to explore the potential association between altered lipids and platelet reactivity, as well as to identify new potential biomarkers of sepsis.

Method: Platelets were isolated from 48 septic and 48 control patients with similar age, sex, and comorbidities. Lipidomic analysis was carried out by untargeted liquid chromatography–mass spectrometry (QTOF). Inflammatory biomarkers of the patients were measured by enzyme-linked immunoassay (ELISA).

Results: The lipidomic analysis identified 224 species and showed significant changes in the lipid composition of platelets during sepsis. Platelets from patients with septic shock showed increased levels of diacyl and triacylglycerols, as well as ceramides and deoxyceramides. A concordant decrease in sphingomyelin species was also observed. Excessive ceramide formation has been associated with multiple disorders, including atherosclerosis and cardiovascular disease. Regarding phospholipids, patients showed a reduction in lysophospholipids as well as alterations in the composition of fatty acid chains. An increase in short and (un)saturated fatty acid chains was observed and associated with a substantial reduction in phosphatidylcholines and phosphatidylethanolamines containing long polyunsaturated fatty acid chains (ω3 and ω6). The latter are key phospholipids for the generation of pro- and anti-inflammatory lipid mediators.

Conclusions: Our data reveal that critical changes in the platelet lipidome occur during sepsis. Upregulated lipids are mainly glycerolipids and ceramides, while lysophospholipids are drastically reduced. These changes as well as alterations in the composition of the fatty acyl chains of phospholipids might play a role in the pathophysiology of the disease.



Platelet lipid box plots, comparing patients with septic shock to matched controls
Abbreviations: AC = Acylcarnitine, FFA = Free fatty-acid, DG = Diacylglycerol, TG = Triacylglycerol, PC = Phosphatidylcholine, PCO = Plasmanyl phosphatidylcholine, PCP = Phosphatidylcholine plasmalogen, PE = Phosphatidylethanolamine, PEO = Plasmanyl phosphatidylethanolamine, PEP = Phosphatidylethanolamine plasmalogen, PI = Phosphatidylinositol, LPC = Lysophosphatidylcholine, LPCO = Plasmanyl lysophosphatidylcholine, LPE = Lysophosphatidylethanolamine, SM = Sphingomyelin, Cer = Ceramide, Doxcer = Deoxyceramide, C = Cholesterol, CE = Cholesterol ester, FDR = False discovery rate

O3

BASIC RESEARCH

Absence of mitochondrial deacetylase SIRT3 results in enhanced platelet apoptosis

Van Bael J[1], Vangheluwe J[1], Delmote A[1], Pareyn I[1], De Meyer S[1], Vanhoorelbeke K[1], Tersteeg C[1]
[1]KU Leuven, Kortrijk, Belgium

Background: Sirtuin 3 (SIRT3) is a nicotinamide adenine dinucleotide (NAD)-dependent deacetylase and considered as the primary deacetylase in mitochondria. In nucleated cells, it plays a crucial role in maintaining mitochondrial homeostasis by interacting with at least 84 mitochondrial proteins. Among others, SIRT3 regulates mitochondrial function and biosynthetic pathways, including cell death, through reversible lysine deacetylation. While SIRT3 is present in murine and human platelets, its exact role in platelet function remains unclear.

Aim: Examine the role of SIRT3 in platelet activation, mitochondrial function and apoptosis.

Methods: Sirt3fl/fl;gpIb-Cre+/- (SIRT3plt-/-) and Sirt3fl/fl;gpIb-Cre-/- (SIRT3plt+/+) mice were generated by crossing male gpIb-Cre+/- mice with female Sirt3fl/fl mice. Absence of SIRT3 in platelets was confirmed via western blot. Total blood count was performed using an automated cell counter. H2DCFDA was used to determine cellular reactive oxygen species (ROS) levels. Thiazole orange (TO) was used to identify reticulated platelets. To induce apoptosis, platelets were incubated with 10 µM ABT-737. To activate platelets, platelets were stimulated with CRP-XL or thrombin. AnnexinV, anti-CD62P and anti-activated CD41/61 were used to detect respectively surface phosphatidylserine (PS) exposure and platelet activation.

Results: Platelets of SIRT3plt-/- mice were larger (mean platelet volume in SIRT3plt-/- 6.37fL ± 0.21, and 5.59fL ± 0.14 in SIRT3plt+/+), demonstrated higher GPVI expression levels (MFI anti-GPVI in SIRT3plt-/- 45,957.0 ± 6195.2 and 39,464.4 ± 2935.8, in SIRT3plt+/+) and had increased levels of cellular ROS (MFI H2DCFDA in SIRT3plt-/- 537.2 ± 63.5, and 403.5 ± 53.7 in SIRT3plt+/+). Additionally, more reticulated platelets were observed in SIRT3 KO mice (MFI TO in SIRT3plt-/- 53,847.3 ± 19,490.3, and 36,315.1 ± 8,018.0 in SIRT3plt+/+). While platelet activation was similar between the two genotypes, increased platelet apoptosis was observed in SIRT3plt-/- platelets after incubation with ABT-737 for 120 minutes (65.8% ± 4.0 PS+ platelets in SIRT3plt-/- and 56.3% ± 5.2 PS+ platelet in SIRT3plt+/+).

Conclusions: Our results demonstrate an increase in younger platelets in mice deficient in platelet SIRT3, observed by an increase in platelet size, increased GPVI expression, elevated cellular ROS levels and increased reticulated platelets. In addition, SIRT3plt-/- were more susceptible to apoptosis upon stimulation with ABT-737. Therefore, our findings suggest that SIRT3 plays a role in regulating platelet apoptosis. Experiments are ongoing to support our findings, including analysis of Bcl-XL, Bax, p53, caspase-3, cytochrome C and mitochondrial respiration.

04

BASIC RESEARCH

Ticagrelor targets multiple lipids in the bacterial cytoplasmic membrane of multidrug resistant staphylococci

Leeten K[1], Westermann L[2], Bornikoel J[2], Schneider D[2], Straetner J[2], Henriksen C[3], Musumeci L[1], Jacques N[1], Sánchez-López E[4], Giera M[4], Frees D[3], Brötz-Oesterhelt H[2], Lancellotti P[1], Oury C[1]
[1]Laboratory of cardiology, Liège, Belgium, [2]Interfaculty Institute of Microbiology and Infection Medicine Tübingen (IMIT), University of Tübingen, Tübingen, Germany, [3]Department of Veterinary and Animal Sciences, Faculty of Health and Medical sciences, University of Copenhagen, Copenhagen, Denmark, [4]Leiden university medical centre, University of Leiden, Leiden, The Netherlands

Background/Introduction: Infection with multidrug resistant bacteria poses a major health care problem which urges the need for novel treatment options. In addition to its potent antiplatelet properties, we found that the P2Y12 antagonist ticagrelor has antibacterial activity against Gram-positive bacteria, including methicillin resistant Staphylococcus aureus (MRSA) and Staphylococcus epidermidis (MRSE). Our findings were corroborated by several studies in cardiovascular patients who had a lower risk of Staphylococcus aureus bacteremia when receiving ticagrelor instead of clopidogrel.

Aims: To assess the mode of action of ticagrelor on multidrug resistant staphylococci.

Methods/Materials: To get insights into the mechanism, we first used Bacillus subtilis as a model of Gram-positive bacteria to conduct an antibiotic stress induced bioreporter assay. We then generated ticagrelor-resistant MRSE and MRSA clones in vitro, by exposing bacteria to subinhibitory drug concentrations. Phenotypic changes of MRSA treated with ticagrelor were analyzed by super-resolution microscopy.

Results: The bioreporter assay in Bacillus subtilis revealed a subinhibitory concentration of ticagrelor to induce biochemical pathways essential for maintaining the integrity of the bacterial cytoplasmic membrane. Furthermore, this concentration of ticagrelor caused membrane depolarization in Bacillus subtilis and MRSA without altering bacterial growth and survival. At bactericidal concentration (20 µg/ml), ticagrelor affected membrane integrity, as shown by propidium iodide incorporation as well as lipid aggregation. Whole genome sequencing of in vitro generated MRSE mutants revealed a mutation in the cdsA gene which encodes for the phosphatidate cytidylyltransferase enzyme. This enzyme catalyzes the synthesis of cytidine diphosphate-diacylglycerol, an essential intermediate of membrane phosphatidylglycerol (PG) and cardiolipin (CL). In vitro generated MRSA clones showed mutations in genes encoding for ClpP, ClpX and YjbH, which are part of the ClpXP proteolytic machinery, a major player in bacterial virulence and biofilm formation. Importantly, high-throughput lipidomics indicated lower CL and 14:0 fatty acyl chain-bearing PG content in ticagrelor-resistant MRSA mutants. The addition of exogeneous CL or PG to Staphylococcus aureus and Bacillus subtilis cultures quenched the antibacterial properties of ticagrelor. We subsequently assessed the mechanism of ticagrelor compared to the membrane active antibiotic daptomycin. Interestingly, only PG could inhibit daptomycin activity against MRSA and no cross-resistance was observed between ticagrelor and daptomycin.

Summary/Conclusions: Our study demonstrates that ticagrelor targets multiple lipids in the cytoplasmic membrane of Gram-positive bacteria, thereby retaining activity against multidrug resistant staphylococci including daptomycin resistant strains.

05

CLINICAL & LABORATORY

European practices on antithrombotic management during percutaneous mechanical circulatory support in adults: An international survey of the ACVC of the ESC joint with the EuroELSO

Van Edom C[1,2], Castelein T[3], Chieffo A[4], Dauwe D[5], Gorog D[6,7], Gramegna M[8], Hermans G[9], Huber K[10], Leonardi S[11], Lubnow M[12], Mebazaa A[13,14], Meyns B[1,15], Mueller T[12], Ott S[16], Pappalardo F[17], Price S[6,18], Schaubroeck H[19], Schrage B[20], Swol J[21], Tavazzi G[22], Vanassche T[1,2], Van der Linden L[24], Vercaemst L[15], Vranckx P[25], Vandenbriele C[1,18]
[1]Department of Cardiovascular Sciences, University of Leuven, Leuven, Belgium, [2]Department of Cardiovascular Diseases, University Hospitals Leuven, Leuven, Belgium, [3]Cardiovascular Center, Onze-Lieve-Vrouwziekenhuis, Aalst, Belgium, [4]Interventional Cardiology Unit, San Raffaele Scientific Institute, Milan, Italy, [5]Department of Intensive Care Medicine, University Hospitals Leuven, Leuven, Belgium, [6]National Heart & Lung Institute, Imperial College, London, United Kingdom, [7]Postgraduate Medical School, University of Hertfordshire, Hertfordshire, United Kingdom, [8]Cardiac Intensive Care Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy, [9]Medical Intensive Care Unit, Department of General Internal Medicine, University Hospitals Leuven, Leuven, Belgium, [10]3rd Department of Medicine, Cardiology and Intensive Care Medicine, Wilhelminenhospital and Sigmund Freud University, Medical Faculty, Vienna, Austria, [11]Department of Medical Sciences and Infective Disease, University of Pavia and Fondazione, IRCCS Policlinico San Matteo, Pavia, Italy, [12]Department of Internal Medicine II, University Hospital Regensburg, Regensburg, Germany, [13]Inserm UMR-S 942, Cardiovascular Markers in Stress Conditions (MASCOT), Paris, France, [14]Department of Anesthesiology and Critical Care, Hôpitaux Universitaires Saint Louis-Lariboisière, Assistance Publique des Hopitaux de Paris, Paris, France, [15]Department of Cardiac Surgery, University Hospitals Leuven, Leuven, Belgium, [16]Department of Cardiac Anesthesiology and Intensive Care Medicine, Charité - Universitätsmedizin Berlin, Berlin, Germany, [17]Department of Anesthesia & Intensive Care, Azienda Ospedaliera SS. Antonio e Biagio e Cesare Arrigo, Alessandria, Italy, [18]Department of Critical Care, Royal Brompton & Harefield Hospitals, Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom, [19]Department of Intensive Care Medicine, Ghent University Hospital, Ghent University, Ghent, Belgium, [20]Department of Cardiology, University Heart and Vascular Center Hamburg, Hamburg, Germany, [21]Department of Respiratory Medicine, Paracelsus Medical University Nuremberg, Nuremberg, Germany, [22]Department of Clinical-Surgical, Diagnostic and Paediatric Sciences, University of Pavia, Pavia, Italy, [23]Intensive Care Unit, Fondazione Policlinico San Matteo IRCCS, Pavia, Italy, [24]Pharmacy Department, University Hospitals Leuven, Leuven, Belgium, [25]Department of Cardiology and Critical Care Medicine, Hartcentrum Hasselt, Jessa Ziekenhuis, and the Faculty of Medicine and Life Sciences, Hasselt University, Hasselt, Belgium

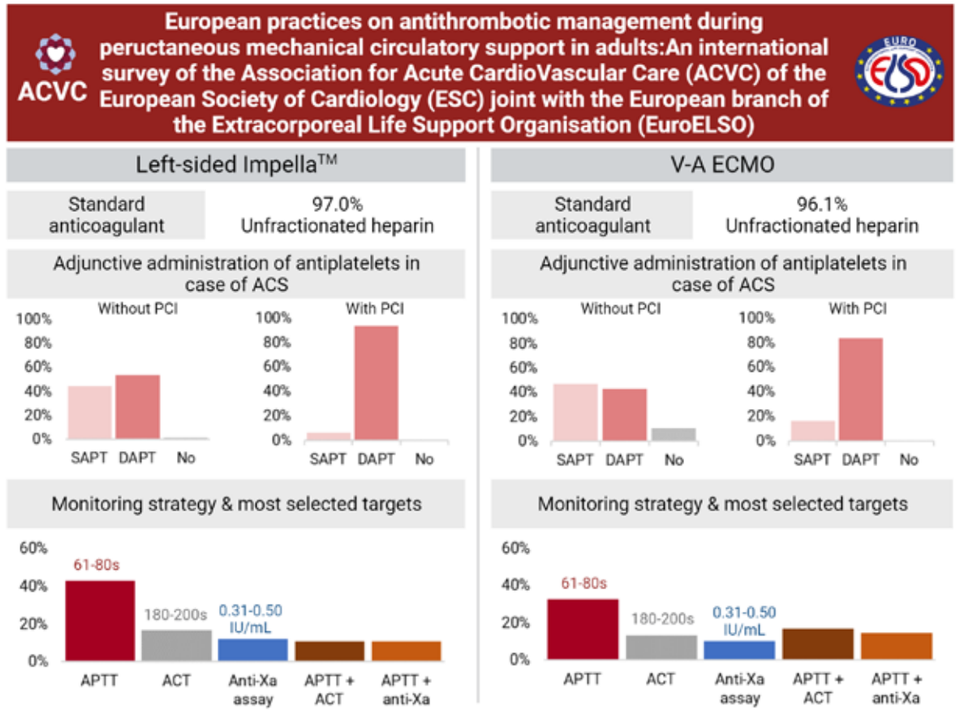
Introduction: Coagulopathic complications strongly impair the outcome of patients on percutaneous mechanical circulatory support (pMCS) with veno-arterial extracorporeal membrane oxygenation (V-A ECMO) and/or left-sided microaxial flow pumps such as the Impella™. Evidently, antithrombotic practices are an important determinant of the bleeding and thrombotic risk, but its management is heterogeneous and relies mostly on local experience rather than evidence.

Aims: This survey aims to give an overview of the current European practices in antithrombotic management in adults on pMCS, as a first crucial step to design future trials, standardize practice and improve outcome.

Methods: This online cross-sectional survey was distributed via a digital newsletter and social media platforms through the Association of Acute Cardiovascular Care (ACVC) and the European chapter of the Extracorporeal Life Support Organization (EuroELSO). The survey was accessible between 17.04.2023 and 23.05.2023. The target population were European clinicians involved in adult critical care and pMCS-management. However, since we also received completed surveys from non-European countries, we report on non-European practices as well.

Results: We included responses from 105 different units from 26 European countries and 79 units from non-European countries. 72.4% of the European responders have an institutional anticoagulation protocol installed, similar to 75.6% in the non-European group. Typical thresholds for transfusion or administration of blood products without acute bleeding as indication vary substantially among centres and are frequently not predefined. Unfractionated heparin is the predominantly used anticoagulant in both the European group (Impella™: 97.0% and V-A ECMO: 96.1%) and the non-European group (Impella™: 96.6% and V-A ECMO: 93.6%). In Europe, argatroban is the preferred alternative in case of heparin-induced thrombocytopenia (Impella™: 52.3% and V-A ECMO: 50.7%), whereas this is bivalirudin in the non-European group (Impella™: 65.5% and V-A ECMO: 56.5%). In the European group, 10.8% and 14.5% of the anticoagulation protocols rely on anti-factor-Xa assay with activated partial thromboplastin time (APTT) in parallel for Impella™ and V-A ECMO, respectively. Similarly, in the non-European group, this parallel approach is used in 13.8% (Impella™) and 10.6% (V-A ECMO). Up to 43.1% (Impella™) and 32.9% (V-A ECMO) of the European respondents rely on a monitoring strategy with APTT alone. This is comparable to the non-European group, where 44.8% (Impella™) and 48.9% (V-A ECMO) of the responders use an APTT-based protocol. Anticoagulant targets for heparin vary significantly between institutions, both in the European as well as in the non-European group. 54.0% and 42.7% of the European survey participants administer dual antiplatelet therapy (DAPT) during Impella™ and V-A ECMO support after acute coronary syndrome without percutaneous coronary intervention (PCI), increasing to 93.7% and 84.0% after recent PCI, respectively. A trend towards less frequent usage of DAPT is seen in the non-European group (Impella™ without/after PCI: 51.7%/79.3% and V-A ECMO without/after PCI: 36.2%/82.6%).

Conclusions: Large heterogeneity in antithrombotic practices is observed across the world. Consequently, device-associated coagulopathic complications may largely vary due to non-standardized management and heterogeneity between institutions. This survey underpins the importance of urgent prospective trials investigating important basic questions concerning antithrombotic therapy during pMCS (anticoagulation targets, monitoring strategy, concomitant use of DAPT among others).



O6

CLINICAL & LABORATORY

Correlation between ETP-based APC Resistance and the Relative Risk of Venous Thromboembolism in Women Using Combined Oral Contraceptives

Morimont L[1,2], Donis N[1], Foidart J[3,4], Douxfils J[1,2]
[1]Qualiblood, Research Department, Namur , Belgium, [2]University of Namur, Department of Pharmacy, Clinical Pharmacology Research Group, Namur Research Institute for Life Sciences (NARILIS), Namur, Belgium, [3]Estetra SRL, An affiliate's Company of Mithra Pharmaceuticals, Liège, Belgium, [4]University of Liège, Liège, Belgium

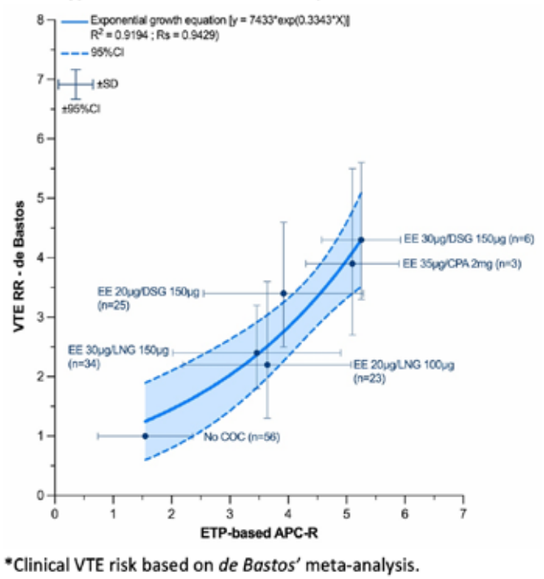
Background: The International Society on Thrombosis and Hemostasis (ISTH) Scientific and Standardization Committee (SSC) supports the potential use of the endogenous thrombin potential-based activated protein C resistance (ETP-based APC-R) to assess acquired APC resistance. The implementation of this assay into routine clinical practice requires this test to be recognized as a surrogate biomarker for venous thromboembolism (VTE) risk assessment.

Aim: This study aims to evaluate acquired APC resistance induced by combined oral contraceptives (COCs), using the ETP-based APC-R assay and to refine an existing VTE prediction model.

Method: ETP-based APC-R was assessed on 197 plasma samples. Values from non-COC (n=56) and COC users (ethinylestradiol (EE) with levonorgestrel (n=23 for EE 20µg, n=34 for EE 30µg), desogestrel (n=25 for EE 20µg, n=6 for EE 30µg) or cyproterone acetate (n=6)) were used to build the VTE prediction model. Relative risks (RR) of VTE associated with these COCs were extracted from published epidemiological studies. The model performance was challenged by estimating VTE RR of 3 other COCs, ethinylestradiol/dienogest (n=11), estradiol/nomegestrol acetate (n=5) and estetrol/drospirenone (n=34), based on their ETP-based APC-R values.

Results: The model showed a Spearman's rank correlation of 0.94 (Figure 1). Based on this model, RR estimates were 3.55 for ethinylestradiol/dienogest, 1.66 for estradiol/nomegestrol acetate and 1.59 for estetrol/drospirenone versus non-COC users.
Conclusion: The model predicted RR estimates concordant with recently published post marketing surveillance data comparing COC VTE risk versus non-COC users. The lower predicted risk for estetrol/drospirenone fits with results from clinical studies showing a low impact of this new combination on hemostasis parameters. These findings support that ETP-based APC-R could become a surrogate biomarker for estimating the VTE risk of a particular COC, which represents the main cause of acquired APC resistance in a young population.

Figure 1: Correlation between endogenous thrombin potential ETP-based activated protein C resistance (ETP-based APC-R) and the relative risk of venous thromboembolism depending on the type of combined oral contraceptive*



*Clinical VTE risk based on *de Bastos'* meta-analysis.

O7

CLINICAL & LABORATORY

Investigating phenotype – genotype relationships in patients with PROC or PROS1 variants

Vanrenterghem M[1], Van Laer C[1,2], Jacquemin M[1,2], Labarque V[2,3], Verhamme P[2,4], Vanassche T[2,4], Baert S[5], Freson K[2]
[1]Department of Laboratory Medicine, University Hospitals Leuven, Leuven, Belgium, [2]Department of Cardiovascular Sciences, Centre for Molecular and Vascular Biology, University of Leuven, Leuven, Belgium, [3]Department of Pediatric Hemato-Oncology, University Hospitals Leuven, Leuven, Belgium, [4]Vascular Medicine and Hemostasis, University Hospitals Leuven, Leuven, Belgium, [5]Center for Human Genetics, University Hospitals Leuven, Leuven, Belgium

Introduction: Thrombophilia is an increased tendency for venous thrombosis caused by inherited and/or acquired conditions. Laboratory thrombophilia screening includes plasma level determination of natural anticoagulants including protein C, protein S and antithrombin. Protein C is commonly measured with a chromogenic assay, but some specific deficiencies (type 2b) can be missed.[1] Clotting-based assays are capable to detect this subtype but are not routinely implemented because of the reduced specificity. Laboratory diagnosis of protein S deficiency is challenging, due to complex physiological interactions. Free protein S antigen assay is the first-choice assay, but type II defects will be missed, and a following clot-based assay must be performed.[2] Since 2019, a multigene panel screening is used for inherited thrombophilia that includes genes coding for protein C (PROC) and protein S (PROS1).

Aim: We studied the performance of different laboratory assays for protein C and S. We evaluated these assays for phenotype – genotype correlation in 32 patients known with PROC and PROS1 variants after applying a multigene test.

Methods/Materials: The performance evaluation included a correlation study (n=85) between a chromogenic protein C assay (Werfen) or a free protein S antigen assay (Werfen) with three different clot-based assays for protein C or S (Werfen, Hyphen Biomed, Stago) on ACL-TOP. We analysed citrate plasma samples of patients with (likely) pathogenic variants ((L)PV) or variants of unknown significance (VUS). Mean values of three different clot-based assays are used for evaluation.

Results: All reagent kits showed a good correlation. Some differences in deficiency classification related to use of different reference ranges were observed. Additionally, 32 samples from patients with PROS1 (n=20) or PROC (n=12) variants were evaluated (figure 1). Three unrelated patients known with the LPV PROC variant p.Arg42His had normal protein C plasma levels with a chromogenic test while deficiency could be detected with a clot-based assay. For such samples, a two-step approach should be performed for laboratory diagnosis. We studied 5 unrelated patients with VUS in PROC of which 2 presented with normal values for protein C and 3 with decreased values in the chromogenic and clot-based assays. Family segregation studies are required to proof pathogenicity. Of 13 patients with a VUS in PROS1, a deficiency was found using the antigen and/or clot-based method in 9 patients (69%). For 7 patients with the known and relatively common pathogenic PROS1 Heerlen variant (p.Ser501Pro), protein S deficiency could be detected with the free antigen method while 3 out of the 7 patients were also abnormal for the clot-based method.

Conclusion: The assessment of laboratory- and assay specific reference values are important for clot-based assays. A two-step approach using different assays is only recommended for some patients with strong suspicion of inherited thrombophilia due to the presence of very specific genetic variants. Genetic testing could add additional information to plasma thrombophilia testing, especially for patients with normal protein C and S levels but larger studies are needed.

References:

[1]. Cooper, P. C. et al., JTH, (2020)
[2]. Marlar, R. A., et al., JTH, (2021)

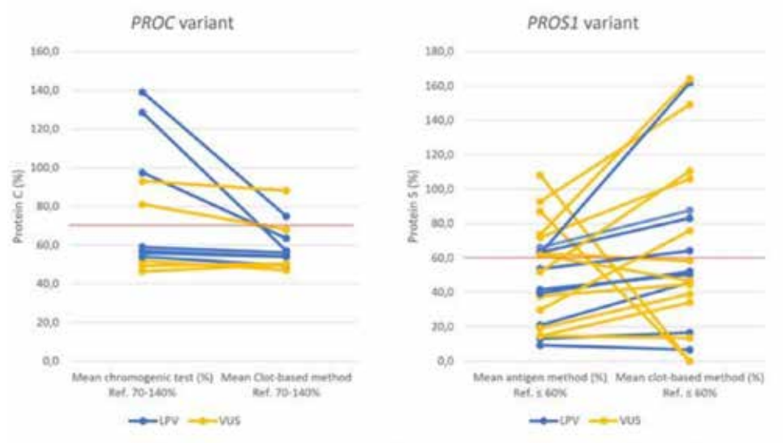


Figure 1: (Left), Protein C plasma levels (%) from patients with a known *PROC* variant subclassified as LPV/VUS measured with a chromogenic test and a clot-based method. **(Right):** Protein S plasma levels (%) from patients with a known *PROS1* variant, subclassified as LPV/VUS measured with a free antigen method and a clot-based test. Abbreviations: LPV, likely pathogen variant; VUS, variant of unknown significance. Red line indicates lower reference limit.

O8

CLINICAL & LABORATORY

The novel p.C1130S mutation is responsible for the complex phenotype in a Finnish family with VWD

Calcoen B[1], Portier I[1], Pareyn I[1], Vandenbulcke A[1], Lehtinen E[2], Lassila R[2,3], Tersteeg C[1], Vanhoorelbeke K[1], De Meyer S[1], Szanto T[2,3]

[1]Laboratory For Thrombosis Research, KU Leuven Campus Kulak, Kortrijk, Belgium, [2]Coagulation Disorders Unit, Department of Hematology, Comprehensive Cancer Center, Helsinki University Hospital, Helsinki, Finland, [3]Research Program Unit in Systems Oncology, Faculty of Medicine, Helsinki University, Helsinki, Finland

Introduction: Von Willebrand factor (VWF) is a multimeric plasma glycoprotein with an essential role during primary haemostasis. Both a shortage or a dysfunction of VWF can lead to von Willebrand disease (VWD), the most common inherited bleeding disorder. VWF deficits in VWD patients are mainly caused by genetic mutations in the VWF gene and can be classified into the quantitative VWD type 1 (i.e. partial deficit) and 3 (i.e. virtual absence) or VWD type 2, which encompasses all functional VWF defects. Correct classification is important as it influences the therapeutic strategy, but this is not always straightforward due to overlap in phenotypic elements between the different VWD types and the heterogeneous molecular basis of VWD.

Aim: Our aim was to perform an in-depth analysis of the complex VWD phenotype that was observed in an index patient and his children who present with mild-to-moderate bleedings. We aimed at identifying the responsible genetic mutations in the VWF gene and to elucidate their effect on VWF function.

Materials and Methods: First, a phenotypic assessment of all the family members was performed. This included haemostatic screening tests as well as assessing the complete VWF status. The latter includes determination of VWF antigen (VWF:Ag), VWF platelet binding (VWF:GPIbM), VWF collagen binding (VWF:CB), VWF binding to FVIII (VWF:FVIIIB) and VWF multimers. In addition, genetic analysis of exons 2-52 of the VWF gene was performed on the index patient and his children. Site-directed mutagenesis was used to construct expression plasmids of the mutant VWF, which were used in transfection experiments in Chinese hamster ovary (CHO) K1 cells to study the impact of the VWF mutant on VWF production or secretion.

Results: The index patient is a 52-year old male who was recalled together with his four children as part of a comprehensive re-evaluation of historical VWD diagnosis made in the Helsinki Coagulation Disorders Unit. Both the index patient and his children displayed low VWF:Ag (< 30 IU/dL) with a normal platelet binding activity (VWF GPIbM/Ag ratio ≥ 0.7). VWF multimers were normal but the VWF CB/Ag ratio of all family members was < 0.7. Moreover, VWF:FVIIIB was borderline in all children and was undetectable in the father. Genetic analysis revealed that the index patient was compound heterozygous for the common type 2N mutation c.2561G>A (p.R854Q), and for the novel c.3388T>A mutation (p.C1130S). Genetic analysis of the four children showed that all were heterozygous for the c.3388T>A mutation (p.C1130S). Interestingly, transient transfection of CHO K1 cells with mutant VWF plasmids revealed that the newly identified p.C1130S VWF mutant shows significantly impaired VWF production or secretion, showing that this mutation is causative for the low VWF:Ag seen in the patients.

Conclusion: We identified a novel c.3388T>A mutation (p.C1130S) in all members of a Finnish family with a mild VWD phenotype. We showed that this mutation was causative for the low VWF:Ag, which alone results in a mild type 1 in the children and in combination with the p.R854Q mutation in a mixed type 1/2N phenotype in the index case.

P01

ABSTRACTS POSTERS

Hemostatic imbalance associated with tamoxifen in estrogen receptor positive breast cancer patients: an observational study

Didembourg M[1], Reda S[2], Oldenburg J[2], Rühl H[2], Douxfils J[1,3], Morimont L[1,3]

[1]University Of Namur, Department of Pharmacy, Namur Thrombosis and Hemostasis Center (NTHC), Namur Research Institute for Life Sciences (NARILIS), Namur, Belgium, [2]University Hospital Bonn, Institute of Experimental Hematology and Transfusion Medicine, Bonn, Germany, [3]QUALIblood s.a., Research and Development Department, Namur, Belgium

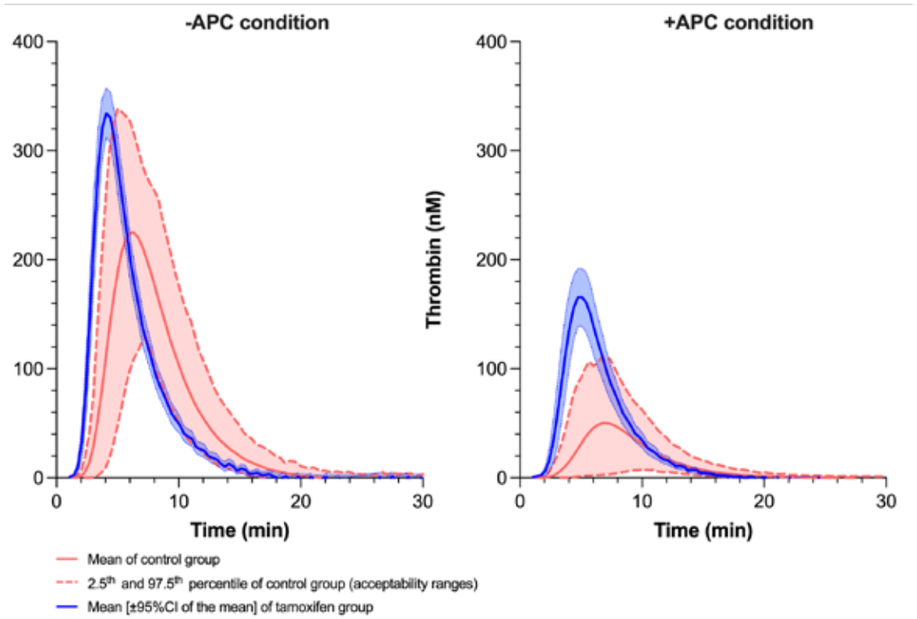
Background: Breast cancer is the most prevalent cancer in women worldwide and estrogen receptor (ER)-positive breast cancer accounts for approximately 75% of all breast cancers. Tamoxifen, a selective estrogen receptor modulator (SERM) is the standard adjuvant treatment. Although better tolerated than aromatase inhibitors, tamoxifen increases the risk of venous thromboembolism (VTE) 1.4-fold. Compared to non-users of adjuvant therapy, this risk is 5-fold higher.

Aim: To assess the hemostatic imbalance induced by tamoxifen in adjuvant treatment of ER-positive breast cancer.

Method: The study protocol was in accordance with the Declaration of Helsinki and approved by the Institutional Review Board and Ethics committee of the Medical Faculty of the University of Bonn. Twenty-five patients in remission from ER-positive breast cancer under adjuvant therapy with tamoxifen for at least 4 weeks without interruption were included. One hundred thirty one age- and BMI-matched healthy controls were included to define reference ranges (2.5th and 97.5th percentiles) of thrombin generation parameters. Thrombin generation was performed in absence and in presence of exogenously added activated protein C (APC) to permit the calculation of the normalized APC sensitivity ratio (nAPCsr), a marker of APC resistance.

Results: Both in absence and in presence of APC, all TG parameters but the endogenous thrombin potential (ETP) (-APC) and the Lag time (LT) (+APC) were significantly impacted by tamoxifen (p-value <0.001). In absence of APC, regardless of thrombin generation parameters, at least 50% of results were out of the reference ranges, except for the ETP which lays above the upper reference limit in only two individuals. In presence of APC regarding the LT, 4 individuals were below the lower reference value. The most impacted parameter was the peak height with 52% (-APC) and 80% (+APC) of results above the upper reference range limit, respectively. The nAPCsr was significantly higher in tamoxifen users (mean ± standard deviation [ranges] = 3.18 ± 0.91 [1.17-4.90]) compared to the control group (2.19 ± 0.92 [0.47-3.97] (p-value<0.0001)).

Conclusion: This observational study showed that patients in remission from ER-positive breast cancer taking tamoxifen as adjuvant therapy had an altered thrombin generation, depicting a shift towards a hypercoagulable state as well as an acquired APC resistance. Moreover, this is the first study using the validated ETP-based APC resistance assay in tamoxifen-treated patients. Finally, these data support our hypothesis that the ETP-based APC resistance assay is able to detect the level of APC resistance induced by hormonal therapies and could therefore serve as a biomarker for the estimation of VTE risk associated with hormonal therapies. Nevertheless, further clinical and scientific research is required to reduce the risk of VTE in tamoxifen-treated patients.



P02

ABSTRACTS
POSTERS

Structural dynamics in the ADAMTS13 CUB domains perturb the ADAMTS13 global latency

Bonnez Q[1,2], Legan E[2], Pareyn I[1], Tersteeg C[1], De Meyer S[1], Li R[2], Vanhoorelbeke K[1]
[1]KU Leuven Campus Kulak Kortrijk, Kortrijk, Belgium, [2]Aflac Cancer and Blood Disorder Center, Children's Healthcare of Atlanta, Emory University School of Medicine, Atlanta, USA

Background: Over the last decade, the structure/function relationship of ADAMTS13 has been thoroughly investigated. Resolving the CUB1-2 crystal structure, docking simulations and enzyme kinetics characterization revealed ADAMTS13 global latency to be controlled by various Spacer-CUB interactions. Anti-ADAMTS13 antibodies (e.g. 17G2) are described to disrupt global latency in vitro as ADAMTS13 becomes allosterically activated and cryptic epitopes become exposed. To date, the molecular mechanism by which Spacer-CUB uncoupling perturbs global latency remains elusive.

Aim: To explore structural dynamics in ADAMTS13 CUB1 and CUB2 domains upon 17G2 antibody-mediated Spacer-CUB disruption using Hydrogen/Deuterium eXchange Mass Spectrometry (HDX-MS).

Methods: Triplicate HDX-MS analysis was performed using the truncated ADAMTS13 variant T2C2, representing the ADAMTS13 C-terminal tail including both CUB1 and CUB2 domains. In absence of 17G2 antibody, undeuterated T2C2 was analyzed as well as T2C2 following deuterium exchange for 10, 100, 500 and 1000 seconds. Also, the T2C2 variant was complexed with an equimolar concentration of 17G2 antibody and identical deuterium exchange timepoints were evaluated. To validate the 17G2 binding epitope, single point mutations were introduced into the CUB1 domain of full-length ADAMTS13 and mutants were screened in ELISA for abolished 17G2 binding and conformational rearrangements.

Results: HDX-MS analysis in presence and absence of 17G2 resulted in a total of 202 unique peptides that fully cover the ADAMTS13 CUB1-2 domains with an average amino acid redundancy of 9.52. Relative fractional deuterium uptake was mapped onto the CUB1-2 crystal structure (PDB ID: 7B01), which identified two loops in CUB1 that could contain the conformational epitope of the 17G2 antibody. Introducing mutations in these loops, confirmed they contained the 17G2 epitope as in five out of six mutants significantly reduced or fully abolished 17G2 binding was observed. As expected, these mutants did not have an altered overall ADAMTS13 conformation and maintained global latency, as demonstrated in ELISA. Additional differences in HDX rate, besides the ones in the two loops comprising the 17G2 epitope, revealed structural dynamics in CUB1, CUB2 and the CUB1-CUB2 interface induced by 17G2 binding, whereas minimal difference in HDX rate was observed at the suggested Spacer-CUB interaction site. Whether these regions are involved in perturbing global latency remains to be determined.

Conclusions: HDX-MS revealed structural rearrangements in both ADAMTS13 CUB1 and CUB2 domains. In agreement with the confirmed binding epitope, ADAMTS13 CUB1 mutants, with fully abolished 17G2 binding, maintained global latency after 17G2 incubation suggesting that the epitope region itself is only poorly involved in global latency perturbance. Other structural rearrangements within the ADAMTS13 CUB1-2 domains could explain how global latency is perturbed. Indeed, HDX rates at the suggested Spacer-CUB interaction site predominantly remained unaffected, suggesting that 17G2 binding does not directly induce uncoupling of the Spacer-CUB interaction. Interestingly, HDX rate differences were also induced by 17G2 at various CUB1 and CUB2 regions as well as at the CUB1-CUB2 interface. This observation suggests that these regions could be involved in antibody-mediated Spacer-CUB uncoupling.

P03

ABSTRACTS
POSTERS

Evaluation of Sivelestat as a NETosis inhibitor using an in-house in-vitro model

Gillot C[1], Favresse J[1,2], Herzog M[3], Kelly T[4], Dogné J[1], Douxfils J[1,5]
[1]Department of Pharmacy, Namur Research Institute for Life Sciences, Namur Thrombosis and Hemostasis Center, University of Namur, Namur, Belgium, [2]Department of Laboratory Medicine, Clinique St-Luc Bouge, Namur, Belgium, [3]Belgian Volition SPRL, Isnes, Belgium, [4]Volition America & Volition Veterinary Diagnostic Development, Austin, Texas, United States of America, [5]Qualiblood s.a., Research and Development Department, Namur, Belgium

Background: NETosis is a form of neutrophil death leading to the release of extracellular chromatin and the assembling of proteins primed by an initial pathogenic stimulus. Under certain specific conditions, neutrophils can exhibit a double-edged activity. This event has been implicated in COVID-19 among other conditions. Neutrophil extracellular traps (NETs) are involved in the pathogenesis of thrombosis and disseminated intravascular coagulation by promoting a pro-inflammatory and a procoagulant state leading to multiorgan failure.

Aims: The aim of this study is to evaluate the performance of a novel NETosis monitoring assay to detect the behavior and the potency of potential NETosis inhibitors.

Methods/Materials: We developed an in vitro model to evaluate the NETs formation and the impact of several drugs on the NETosis process. Neutrophils were freshly extracted from whole blood of one healthy donor. Tumor necrosis factor alpha (TNF-alpha) and interleukin 8 (IL-8) were used to trigger NETosis in extracted neutrophils. NETs monitoring was performed using Sytox Green to measure DNA release and neutrophile elastase (NE) release was assessed using a specific fluorescent substrate. Measurement were performed on a SpectraMax 3iD plate reader. Reading were done every 5 minutes for 5 hours. Sivelestat, a NE inhibitor, was used at 10-, 100-, and 1000-nM to assess NETosis inhibition in TNF-alpha and IL-8 induced neutrophils. Negative control was performed using neutrophil without inducer and inhibitor. Positive control was performed using neutrophils and inducer without inhibitor. Results were express as the percentage of DNA or NE released as function of time after induction. Time to onset the NETosis, time to cross 50% of release (T50) and maximum NETs release were computed.

Results: Siveletsat demonstrated similar results on DNA release following TNF-alpha and IL-8 induction. Time to onset ranged from 40 min for the IL-8 inducer to 75 min for the TNF-alpha. T50 increased with sivelestat concentration, from 166.2 min to 218.5 min for TNF-alpha, and from 167.8 min to 247.6 min for IL-8. (Figure 1a, 1b) Regarding NE release, sivelestat, in presence TNF-alpha as inducer, increased time to onset from 40 min to 100 min. T50 was reduced with 195.2 min for 10 nM and 231.7 min for 100 nM, the 1000 nM concentration didn't cross the 50% of NE release. (Figure 1c)

Conclusion: We successfully developed an in vitro model to analyze the NETosis which provided a tool to assess the impact of different drugs on this immune mechanism. In this study we demonstrated a strong impact of siveletstat on NETosis with two different fluorescent markers. We observed a time dependent modification of NEtosis which was dose-dependent. Moreover, we observed a stronger impact on NE release than on DNA, which coincides with the mechanism of action of this inhibitor. This in vitro model could be used as a monitoring tool to assess NETosis and help in the development of NETosis inhibitors.

Figure 1.A

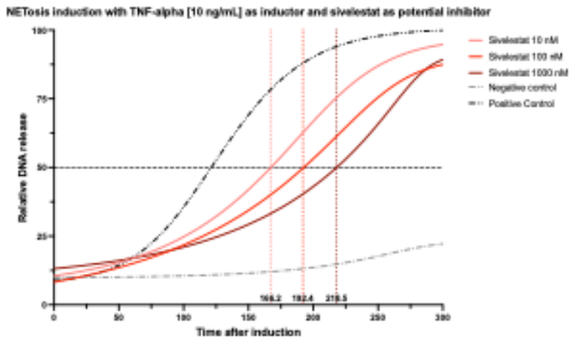


Figure 1.B

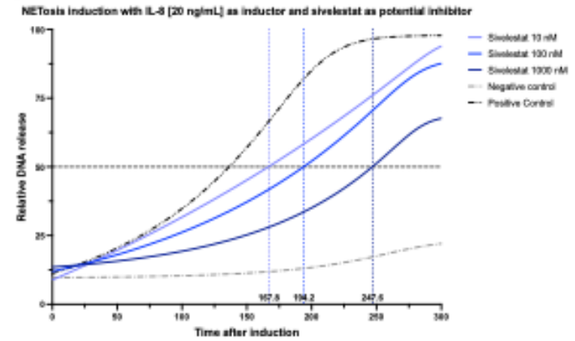
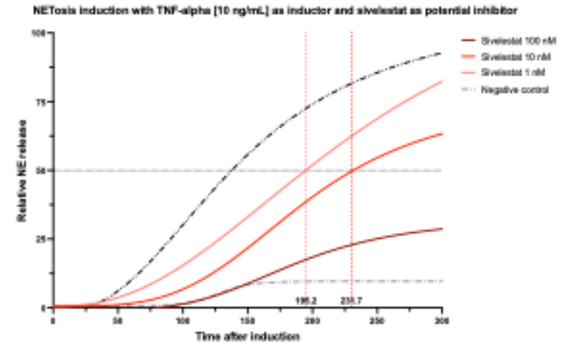


Figure 1.C



P04

ABSTRACTS
POSTERS

Exploring hypercoagulability in long COVID: Unraveling the endothelial dysfunction through the VWF-ADAMTS13 axis

Muys M[1], Demulder A[1], Rozen L[1]
[1]Laboratory of Hematology, LHUB-ULB, Université Libre de Bruxelles ULB, Brussels, Belgium

Introduction: A significant portion of SARS-CoV-2–infected individuals may suffer multi-organ manifestations that extend beyond the acute phase of the disease known as “long COVID” or post-acute sequelae of SARS-CoV-2 infection (PASC). Lingering symptoms such as fatigue, muscle weakness, neuro-cognitive symptoms, dyspnea, may persist several months after an infection with SARS-CoV-2.
While COVID-19 itself has been associated with a hypercoagulable state during the acute infection, evidence suggests that hypercoagulability can persist into the long COVID phase, further complicating the health outcomes of affected individuals.
In addition to the hypercoagulable state observed, persistent endotheliopathy appears to play a key role. Hence, it seems interesting to investigate the coagulation disorders as well as endothelial health.

Aims: This study aims to evaluate the coagulation disorders in patients diagnosed with PASC, through the analysis of thrombin generation test using thrombomodulin. Thrombomodulin, a membrane protein expressed on the surface of endothelial cells is essential for the proper functioning of the protein C anticoagulant system. In addition to thrombomodulin, VWF antigen and ADAMTS-13 activity will be used to assess endothelial health.

Methods: Plasma samples from patients with PASC recruited since October 2020 by the clinical research unit of the Brugmann University Hospital were analyzed. Thrombin generation test was performed on the St-Genesia® (Stago) using the Thromboscreen kit ; VWF antigen was determined on CS-2500 (Siemens) and ADAMTS-13 activity on BioTek ElX808.

Results: Ninety patients were included. Thrombin generation was significantly higher in patients with PASC compared to healthy subjects, albeit to a lesser extent when compared to those with acute COVID-19.
Thrombin generation testing with addition of thrombomodulin showed significantly reduced inhibition of thrombin generation compared to healthy subjects, potentially reflecting endotheliopathy. Conversely, we did not observe statistically significant differences in VWF:Ag levels and ADAMTS13 activity. Overall VWF:Ag to ADAMTS13 activity ratio was not increased, nevertheless 11% of patients with PASC presented an abnormal VWF/ADAMTS13 ratio ≥ 1.5.

Conclusions: Patients with PASC/long COVID show a pathological hemostatic profile characterized by a hypercoagulable state. Our findings suggest a dysfunctional protein C anticoagulant system, possibly due to an impaired thrombomodulin function.
Our study did not reveal VWF/ADAMTS-13 axis imbalance for all patients. Whether patients with an abnormal VWF/ADAMTS-13 ratio is correlated to a more severe disease warrants further exploration.

P05

ABSTRACTS
POSTERS

Innovative Hemocompatible Non-Isocyanate Polyurethanes for 3D Printing of Blood-Contacting Medical Devices

Ferreira Melo S[1], Pierrard A[2], Detrembleur C[2], Jérôme C[2], Lancellotti P[1,3], Oury C[1]
[1]University of Liège - GIGA, Belgium, [2]University of Liège - CERM, Belgium, [3]University of Liège Hospital - Department of Cardiology, Heart Valve Clinic, Belgium

Background: Polyurethanes (PUs) have been a popular choice for blood-contacting medical devices, given their appropriate mechanical properties and reported bio-stability. However, in vitro and in vivo evaluation of PUs revealed drawbacks, including thrombogenicity, infection or calcification. Non-isocyanate polyurethanes (NIPUs) recently emerged as more sustainable and customizable alternatives, and might represent new choices for biomaterials.

Aims: To synthesize, test the mechanical and hemo/biocompatibility properties, and 3D print new “green” NIPUs, to be used in the manufacturing of blood-contacting devices, such as intravenous/arterial catheters or prosthetic heart valves.

Methods: UV-crosslinked NIPUs derived from polypropylene glycol (PPG) were synthesized using different polythiols (SH2, SH3 or SH4), and tested mechanically and biologically (in vitro). Mechanical properties were tested in a dynamic mechanical analyzer (DMA) and the Young’s modulus (E), stress at break (σB) and elongation at break (εB) were determined. Hemolysis tests were performed with washed red blood cells, activation of the coagulation cascade was evaluated using platelet-poor plasma, and platelet adhesion was quantified by lactate dehydrogenase activity upon incubation with platelet-rich plasma. Cytotoxicity was investigated using human fibroblasts, by evaluating cells morphology and metabolic activity. The printability of the synthesized NIPUs was initially tested through time sweep rheometry experiments, to confirm compatibility with digital-light processing (DLP), a UV-mediated 3D printing technique. After that, several printing parameters were adjusted and optimized.

Results: DMA revealed E values between 1.8 and 16.0 MPa, σB between 0.5 and 1.1 MPa and εB between 21.8 and 108.6%, comparable to the values found for human blood vessels or native valve leaflets. None of the synthesized NIPUs showed hemolytic effects, always inducing less than 2% of hemolysis, similarly to medical grade PU. NIPUs did not activate the contact phase of coagulation: clotting times after incubation with NIPUs were longer than with PU. Platelet adhesion on NIPUs surface was similar to PU, except for SH4-based NIPU, in which more platelets were found. Upon indirect (via extracts) and direct contact with all the produced NIPUs, human fibroblasts kept their normal metabolic activity and shape. Altogether, data confirm low thrombogenicity and absence of toxicity induced by the synthesized NIPUs, which could outperform PU. After confirmation of in vitro hemo/biocompatibility, the formulations with SH2, SH3 and SH4 were used to test 3D printability, revealing gel points of 11 s, 7 s and 5 s, respectively, compatible with the use of DLP. Several square-shaped objects were printed, and the correlation between theoretical and experimentally obtained X-Y dimensions show that the printed objects reproduce with very high fidelity the computer-aided design (CAD) model. The limit of resolution found was close to 200 µm.

Summary: The NIPU networks exhibited remarkable mechanical properties, suggesting great potential for their use in several biomedical applications, such as synthetic vascular grafts or heart valve prostheses. Adding to a cost-effective and environmental-friendly production, synthesized NIPUs displayed overall improved hemo/biocompatibility. These NIPUs therefore hold promise for their use in the manufacturing of blood-contacting devices, with the advantage of being suitable for 3D printing.

P06

ABSTRACTS
POSTERS

Glycocalicin is released and retained in a retracted in vitro thrombus

Debaene C[1,2], Six K[1], Compernolle V[1,2,3], Feys H[1,2]
[1]Transfusion Research Center, Belgian Red Cross Flanders , Ghent, Belgium, [2]Faculty of Medicine and Health Sciences, Ghent University, Ghent, Belgium, [3]Blood services, Belgian Red Cross Flanders, Mechelen, Belgium

Introduction: GPIbα is a component of the GPIb-IX-V complex on platelets and is important for adhesion to von Willebrand factor (vWF) in conditions of elevated shear stress. Following strong and sustained activation of platelets, a disintegrin and metalloproteinase 17 (ADAM17) enzyme cleaves the large extracellular domain of GPIbα thereby releasing its soluble ectodomain called glycocalicin. We recently demonstrated that the cleavage (also called shedding) of GPIbα is much slower than the kinetics of the main platelet contributions during primary hemostasis. Furthermore, GPIbα shedding is controlled by strict intracellular containment of both the enzyme and the substrate. GPIbα receptors present on the platelet surface, important for adhesion to the vessel wall, are therefore insensitive to shedding.

Aim: This study aimed to investigate shedding of the GPIbα receptor and the spatial distribution of released glycocalicin during in vitro thrombus formation.

Methods: Thrombus formation was induced by the addition of 15 mM CaCl2 to platelet-rich plasma containing 500,000 PLT/µL. After coagulation and retraction (± 100 minutes at 37°C), serum surrounding the thrombus was collected followed by washing and mechanical homogenization of the thrombus. Paired samples with metalloproteinase inhibitors were included as a control. Platelet-poor plasma (PPP), serum and clot samples were interrogated for glycocalicin content using SDS-PAGE western blotting and an in-house developed ELISA.

Results: The serum surrounding the retracted thrombus contained 12.3 nM glycocalicin at endpoint (100 minutes) which was not significantly different from the initial concentration in PPP (10.8 nM, p = 0.671, n= 14). This observation suggests no release of glycocalicin from the outer platelet shell of the thrombus nor from free-floating platelets. The latter were in fact very rare at endpoint because undetectable by standard blood counting (detection limit: 104 platelets per µL) and extremely low by flow cytometry (<200 CD61+ events/µL). Glycocalicin content inside thrombi was 3.4-fold higher compared to thrombi formed in the presence of ADAM17 inhibitors (p=0.011, n=10). Remarkably, the average thrombus contained 81.1 nM of glycocalicin which is more then 10-fold higher than the median glycocalicin concentration in plasma of healthy blood donors determined as 6.5 nM [5.1-7.7 nM] (median [IQR]; n=407).

Conclusion: GPIbα is shed from platelets during coagulation initiated by Ca2+ addition. Additional stimulation with platelet agonists was not required to cause shedding in this in vitro model. No significant difference in glycocalicin levels is observed between PPP and serum, indicating that glycocalicin is not released into the surrounding serum. Instead, glycocalicin remains trapped inside the thrombus at supraphysiologic concentrations.

P07

ABSTRACTS
POSTERS

Evaluation of the automated HemosIL®CL HIT-IgG chemiluminescence immunoassay on the new ACL TOP®970 coagulation analyzer

Ameye S[1], Cauwelier B[1], Devos H[1], Devreese K[2], Emmerechts J[1]
[1]Hematology Laboratory, AZ Sint-Jan Hospital, Bruges, Belgium, [2]Coagulation Laboratory, Ghent University Hospital, Ghent, Belgium

Introduction: Heparin-induced thrombocytopenia (HIT) is a life-threatening adverse reaction to heparin that must be identified quickly to determine appropriate anticoagulant therapy strategies. Widely available PF4-dependent enzyme immunoassays (EIAs) have high sensitivity but poor specificity for HIT, and positive results require confirmation by a functional platelet activation assay (fPAA) to indicate PF4/heparin antibodies without platelet activating characteristics.
EIAs can be performed either by labour-intensive manual or semi-automated ELISA, or by fully automated chemiluminescence immunoassays (CLIA).

Aim: To evaluate the fully automated, cartridge-based HemosIL®CL HIT-IgG analysis by the CLIA device that is integrated in the new ACL TOP®970 coagulation analyzer (Werfen, Bedford, USA).

Methods: We evaluated within- (n=3x10) and between-run (n=2x10) imprecision using patient samples and internal quality control material and trueness using external quality control material (n=3) on the ACL TOP®970 and correlated results of patient samples with our current manual ELISA assay (PF4 Enhanced®, Immucor, Waukesha, USA, n=26: 7 negative and 19 positive with ELISA) . Reference values, sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) were determined by ROC-curve analysis (n=65). As gold standard

for the latter analysis, we either used the measurement of a reference population of diseased controls without HIT (no recent heparin treatment, no thrombocytopenia, n=41) considered as 'negative', or routine clinical samples considered as either 'negative' (n=9) or 'positive' (n=15) based on ELISA results with (if positive) or without (if negative) fPAA confirmation.

Results: Maximum CV for within- and between run was 5.7%. Maximum bias for external quality control material was 12,3% with correct clinical interpretation for all 3 samples. Using the company-defined cut-off for the ACL TOP of 1.00 U/mL resulted in a kappa agreement of 0.538 with ELISA and 0.909 with fPAA, a sensitivity of 87%, specificity of 100%, NPV of 96% and a PPV of 100%. As EIA's are used as screening assays, sensitivity should reach 100%. Therefore, cut-off was lowered based on ROC-curve analysis to 0.60 resulting in a kappa agreement of 0.906 with ELISA and 0.879 with fPAA, a sensitivity of 100%, specificity of 94%, NPV of 100% and a PPV of 83%.

Conclusion: The fully automated HemosIL®CL HIT-IgG analysis on ACL TOP®970 demonstrated good analytical performance in our sample cohort. However, lowering the company-defined cut-off of 1.00 IU/mL to 0.60 IU/mL was necessary to ensure maximum sensitivity. Results <0.60 IU/mL will therefore be reported as 'negative', 0.60-1.00 as 'weakly positive' and >1.00 as 'positive' in our hospital. Confirmation by fPAA of all weakly positive or positive results on ACL TOP®970 is still necessary to ensure specificity for PF4/heparin antibodies with platelet activating characteristics.

P08

ABSTRACTS POSTERS

Accuracy of Direct Oral Anticoagulants (DOACs) Dipstick urine test from patients treated peri-operatively with DOACs compared to ultra-high-performance liquid chromatography coupled to tandem mass spectrometry

Vassart J[1], Boucher A[1], Morimont L[1,2], Tafur A[3], Walenga J[4], Harenberg J[5,6], Douxfils J[1,2,7]
[1]University of Namur, Namur Research Institute for Life Sciences (NARILIS), Faculty of Medicine, Department of Pharmacy, Clinical Pharmacology Research Group, Namur, Belgium, [2]QUALIblood s.a., Research and Development Department, Namur, Belgium, [3]NorthShore University Health System, Department of Medicine, Evanston, United States, [4]Loyola University Medical Center, Cardiovascular Research Institute, Department of Pathology and Laboratory Medicine, Maywood, United States, [5]Heidelberg University, Medical Faculty Mannheim, Heidelberg, Germany, [6]DOASENSE GmbH, Heidelberg, Germany, [7]CHU Clermont-Ferrand, Hôpital Estaing, Department of Biological Hematology, Clermont-Ferrand, France

Background: In the peri-operative context, there is an unmet need for a rapid and accurate method to exclude clinically significant levels of DOACs in plasma. The DOACs Dipstick is a test strip designed to detect qualitatively oral direct factor Xa (FXA) or oral direct factor IIa (FIIa) inhibitors in urine.

Aims: We aimed to assess the accuracy of the FXa inhibitors pad of the DOACs Dipstick in patients who interrupted their anticoagulation before surgery.

Methods: Citrated plasma and urine were collected from 47 patients treated with apixaban (n=33) and rivaroxaban (n=14) who interrupted their treatment 1 day before a low-bleeding-risk and 3 days before a high-bleeding-risk scheduled surgical procedure. Plasma and urine concentrations of DOACs were quantified by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS). These results were compared to the DOACs Dipstick urine results with plasma cut-offs set at >30 ng/mL and >50 ng/mL. FXA pads of DOACs Dipstick were evaluated visually as positive or negative (DOACs present or absent), according to the color visible after 10 minutes. The optimal plasma cut-off value of UHPLC-MS/MS-determined concertation of FXA inhibitors versus DOACs Dipstick was computed as the one corresponding to the highest Youden index.

Results: The relation between plasma and urine concentrations of DOACs measured by UHPLC-MS/MS was high and depicted by the following equation: y=16.80x- 21.38 (R2=0.83, r=0.91, Figure 1A). Mean drug concentrations of paired plasma samples of positive pads were 48.2 ng/mL [27.6 – 68.7] (95% confidence interval), and 6.1 ng/mL [3.8 – 8.4] of negative pads (Figure 1B). At the cut-off plasma value of >30 ng/mL, DOACs Dipstick exhibited a sensitivity of 100.0% [73.5 – 100.0], a specificity of 71.4% [53.7 – 85.4], a positive predictive value (PPV) of 38.2% [26.8 – 51.1], a negative predictive value (NPV) of 100.0% [86.3 – 100.0] and an accuracy of 75.7% [61.0 – 87.0]. At >50 ng/mL threshold, the values were: sensitivity 100.0% [63.1 – 100.0], specificity 64.1% [47.2 – 78.8], PPV 7.9% [5.4 – 11.6], NPV 100.0% [86.3 – 100.0] and accuracy 65.2% [49.9 – 78.5]. The optimal cut-off was calculated as >17.7 ng/mL plasma, corresponding to a Youden Index of 77.8 (sensitivity 81.8% [61.5 – 92.7], specificity 96.0% [80.5 – 99.6]) and the area under the receiver-operating curve was 0.92 [0.82 – 1.0].

Conclusions: Negative DOACs Dipstick reliably excluded clinically significant levels of DOACs in plasma. If DOACs Dipstick generates positive results a second testing method may be needed to ensure that the concentration of DOAC in plasma is well above the clinical decision-making thresholds. However, additional testing may depend on the clinical picture of the patient and the availability of other methods.

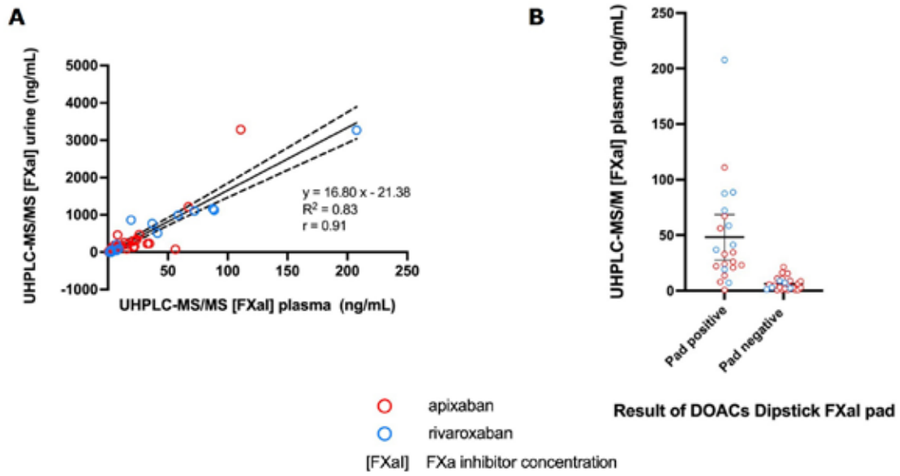


Figure 1A. Relation between plasma and urine concentrations of FXa inhibitors (ng/mL) measured by UHPLC-MS/MS (N=47)
Figure 1B. Comparison of plasma concentrations of FXa inhibitors (ng/mL) measured by UHPLC-MS/MS associated with DOACs Dipstick positive and negative FXa inhibitors pad (N=47)

P09

ABSTRACTS POSTERS

Thromboembolic event after liver transplantation: to screen the donor for thrombophilia or not?

de Bruijn S[1], Vangenechten I[2,3], Maes M[3], Gadisseur A[1,2]
[1]Department of Hematology, Antwerp University Hospital, Antwerp, Belgium, [2]Hemostasis and Thrombosis Research Center, Antwerp University Hospital, Antwerp, Belgium, [3]Department of Clinical Biology, Antwerp University Hospital, Antwerp, Belgium

Introduction: Liver transplant recipients are at risk of venous thromboembolism (VTE) resulting in significant morbidity and cost. Sometimes, heritable thrombophilia can be acquired during liver transplantation (LTX) influencing clinical outcomes. Predicting thrombosis risk and initiating prophylaxis in high-risk situations could potentially prevent VTE after LTX. Nevertheless screening of candidate donors and recipients for prothrombotic tendency remains controversial and is not standard practice.

Case Report: A 62-year-old Caucasian female presented to the emergency department with extensive deep venous thrombosis from the left calf vein to the femoral bifurcation. Three months earlier she had undergone a LTX from a heart-beating donor due to an unresectable neoplasm. Additional CT angiography showed associated bilateral peripheral pulmonary emboli. The patient was initially started on therapeutic low-molecular-weight heparin and subsequently switched to apixaban 5 mg bid. A new duplex scan after six months of treatment showed residual thrombosis at the level of the femoral vein. Thrombophilia screening was performed after stopping apixaban, revealing activated protein C resistance (APCR) (normalised ratio of 0.44; cut off 0,7) using ProC® Global assay (Siemens Healthineers). No factor V Leiden (FVL, Arg506Gln) mutation could be identified by genetic analysis. Given the current specific amplification method, false-negative genetic result seems highly unlikely but can occur with sample misidentification. A false-positive result appears extremely improbable as well, since ProC® Global assay has a sensitivity of 98% for FVL and a specificity of 99%. Other FV gene mutations, such as FV Cambridge (Arg306Thr), show limited to even no APCR. Therefore, most likely this FVL phenotype-genotype discrepancy can be explained by acquired donor APCR. As it involved an unprovoked VTE in association with APC resistance, extended treatment with prophylactic apixaban was started.

Discussion: The question raised by this case is whether this VTE event could have been prevented if the donor had been screened for thrombophilia? APCR is the most common hereditary risk factor in Caucasian population for VTE and 90-95% of these cases are caused by FVL mutation (R506Q). FVL is a moderate risk factor for VTE and most patients who acquire this thrombophilia through LTX will not be diagnosed until a first thromboembolic event occurs. Besides the technical difficulties of thrombophilia screening in deceased donors, the nature and extent of such screens are not standardized. Furthermore, liver donors are nowadays not excluded based on thrombosis-susceptible polymorphisms, irrespective if they have a personal history of thrombosis. However, thrombophilia screening could improve the graft -and patient survival by adjusting the VTE prophylaxis strategy postoperatively. We believe that pharmacological thromboprophylaxis should be prolonged posttransplant after hospital discharge according to the individual risk of thrombosis.

P10

ABSTRACTS
POSTERS

Evaluation of the AcuStar HIT-IgG[PF4-H] kit on AcuStar for detection of antibodies against PF4-heparin complex

Cornette M[1], Devreese K[1]
[1]Ghent University, Ghent, Belgium

Background/Introduction: Heparine induced thrombocytopenia (HIT) is a severe complication of heparin therapy caused by antibodies against platelet factor 4 (PF4)-heparin complex that induce platelet activation, bringing the patient in a prothrombotic state. Presence of these antibodies can be detected with an immunoassay. In our hospital, a manual performed enzyme immunoassay (ELISA) is used as first step. Since this is a labor-intensive test, an automated alternative would benefit the turnaround time (TAT). A positive immunoassay should be followed by a functional test, such as flowcytometry, to check platelet activating ability of the detected PF4-heparin complex antibodies.

Aims: Evaluation of the AcuStar HIT-IgG[PF4-H] kit on AcuStar (Werfen, Barcelona, Spain), an automated chemiluminescence immunoassay (CLIA) for detection of antibodies against PF4-heparin complex in the context of HIT.

Methods/Materials: 99 samples from patients with clinical suspicion of HIT were analyzed in parallel with ELISA (ZYMUTESTTM HIA IgG, Hyphen Biomed, Neuville-sur-Oise, France), CLIA (AcuStar HIT-IgG[PF4-H] kit on AcuStar, Werfen) and an in-house flowcytometric method (heparin-induced CD62p-selectin expression) that was considered as reference method. Based on the latter method, 15/99 patient samples were positive for platelet activating PF4-heparin complex antibodies. ELISA was considered positive when the obtained optical density (OD) of the sample exceeded the run dependent cut-off OD. A result of ≥1 U/mL for CLIA on AcuStar was considered positive. Flowcytometry was interpreted based on the CD62p expression percentage at different concentrations of heparin (0.0 U/mL, 0.3 U/mL and 100.0 U/mL UFH) measured on at least 4 healthy donors.

Results: ELISA assay showed a sensitivity and negative predictive value (NPV) of 100%, specificity of 81% and positive predictive value (PPV) of 48%. CLIA on AcuStar showed a sensitivity of 87% (two false negatives), NPV of 97%, specificity of 89% and PPV of 59%. Results are shown in table 1. The two false negative results by CLIA were considered in more detail: both samples showed a positive result with ELISA and flowcytometry but a negative result with CLIA on AcuStar. Since the obtained titers for both samples were very low (0.24 U/mL; 0.00 U/mL), lowering the cut-off value to enhance sensitivity would not lead to a different interpretation.

Summary/Conclusions: Two samples positive for platelet activating PF4-heparin complex antibodies were missed with CLIA on AcuStar. Therefore, in our laboratory setting the CLIA test was considered unacceptable for detection of PF4-heparin complex antibodies in routine setting.

Table 1: ELISA (ZYMUTEST™ HIA IgG, Hyphen Biomed) and CLIA (AcuStar HIT-IgG[PF4-H] kit on AcuStar, Werfen) sensitivity, specificity, NPV and PPV compared to flowcytometry for the detection of PF4-heparin complex antibodies.

			Flowcytometry				Flowcytometry	
	ELISA		negative	positive	CLIA		negative	positive
		negative	68	0		negative	75	2
		positive	16	15		positive	9	13
Sensitivity	100%				87%			
Specificity	81%				89%			
NPV	100%				97%			
PPV	48%				59%			

ELISA: ZYMUTEST™ HIA IgG, Hyphen Biomed; CLIA: AcuStar HIT-IgG[PF4-H] kit on AcuStar, Werfen; NPV: negative predictive value; PPV: positive predictive value

P11

ABSTRACTS
POSTERS

Leukoreduction filters from blood establishments are a valuable source of leukocytes for research purposes

Six K[1], Vertongen S[1], Seghers S[2], De Bleser D[2], Compernolle V[1,2,3,4], Feys H[1,3]
[1]Transfusion Research Center - Belgian Red Cross Flanders, Ghent, Belgium, [2]Transfusion Innovation Center - Belgian Red Cross Flanders, Ghent, Belgium, [3]Faculty of Medicine and health Sciences - Ghent University, Ghent, Belgium, [4]Blood Services - Belgian Red Cross Flanders, Mechelen, Belgium

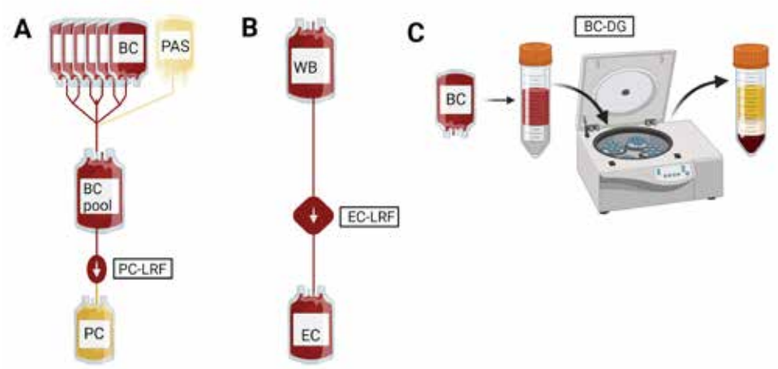
Background: Peripheral blood leukocytes are used for a variety of research purposes and are generally obtained from buffy coats by density gradient centrifugation. Leukoreduction filters are used by blood establishments to restrain leukocytes from blood components in order to avoid transfusion reactions. Leukoreduction filters are currently discarded but could be a valuable alternative source of leukocytes for investigational work in Belgium.

Aim: This study aimed to investigate if two types of leukoreduction filters (LRF) are a valuable source of leukocytes for research. PC-LRF and EC-LRF are obtained from the production chain of two different blood components. Leukocytes from both PC-LRF and EC-LRF were compared to the gold standard, i.e. leukocytes isolated from buffy coats by density gradient centrifugation (BC-DG).

Methods: Leukocytes were isolated from the leukoreduction filters by flushing the filter in the reverse filter direction using a saline buffer filled syringe. The isolated cells were compared to leukocytes isolated from BC-DG. The yield and purity was investigated using a hematology analyzer. The leukocyte differential composition was further investigated using flow cytometry.

Results: A significantly higher number of leukocytes was isolated from PC-LRF compared to BC-DG, with respectively 1,049 ± 40 x 10^6 and 632 ± 66 x 10^6 cells (P<0.0001). On the other hand, the number of leukocytes isolated from EC-LRF was lower than from BC-DG with 78 ± 9 x 10^6 cells. Samples isolated from PC-LRF and EC-LRF contained 12.9 ± 1.5 x 10^9 and 94.6 ± 2.2 x 10^9 remaining platelets, respectively, while this was only 0.7 ± 0.1 x 10^9 for BC-DG. Also the number of red blood cells in the LRF samples was significantly higher compared to BC-DG (P<0.0001). The leukocyte composition of BC-DG and PC-LRF was comparable with approximately 45% T-cells, 25% granulocytes and both monocytes and B-cells around 15%. In contrast, the leukocyte population isolated from EC-LRF consisted of more than 80% granulocytes and was thus significantly different from the two other samples. The turn-around-time for leukocyte isolation by laboratory staff is only 20 minutes for both PC-LRF and EC-LRF while this is 240 minutes for BC-DG.

Conclusion: Leukoreduction filters, currently a waste product of the blood component production chain, could be a valuable source of leukocytes for scientific purposes. A significantly higher leukocyte number, with comparable cell type composition, could be isolated from PC-LRF compared to buffy coats. The number of leukocytes isolated from EC-LRF is limited but has a unique composition with ≥ 80% granulocytes. An additional advantage of the leukoreduction filters is the tenfold faster isolation time compared to the current density gradient isolation from buffy coats. An additional purification step to remove contaminating red blood cells and/or platelets could be necessary depending on the downstream application. Altogether, the data show that leukoreduction filters could be a promising alternative to buffy coats as leukocyte source for research.



Reassessment of dextran sulfate in anti-Xa assay for unfractionated heparin laboratory monitoring

Hardy M, Cabo J, Deliège A, Douxfils J, Gouin-Thibault I, Lecompte T, Mullier F
Université Catholique De Louvain, CHU UCL Namur, Belgium

Introduction: Anti-Xa assays are used for unfractionated heparin (UFH) monitoring. Dextran sulfate (DS) is used in some assays to overcome the artefactual preanalytical release of platelet factor 4. However, the practical implications of this test modification have been little studied.

Methods: We studied factor Xa inhibition, using an assay without DS (Stago Liquid anti-Xa), in normal pool plasma spiked with various concentrations of UFH (up to 1IU/mL) in presence of increasing concentrations of DS (up to 2,560µg/mL). We also investigated the effect of DS on factor Xa inhibition measured after the addition of UFH and heparin antagonists (protamine, polybrene). Eventually, we compared the anti-Xa levels measured using the assay without DS to those measured with an assay containing DS (Biophen Heparin LRT).

Results: DS per se had a detectable anti-Xa effect. Factor Xa inhibition in UFH-spiked plasma linearly increased with increasing concentrations of added DS with a plateau at about 160µg/mL DS, at which the apparent anti-Xa level had almost doubled. In presence of heparin antagonists, the addition of DS increased anti-Xa levels, corresponding to the dissociation of the UFH-antagonists complexes in vitro. With the anti-Xa assay containing DS, UFH inhibition was not detected.

Conclusion: In the presence of high concentrations of DS, factor Xa inhibition was much higher than predicted from added UFH amounts, presumably related to the greater availability of UFH for interaction with antithrombin. While the relevance of measuring this 'masked' heparin has not been demonstrated, the presence of DS renders the result inaccurate in the presence of protamine or polybrene.

P12

ABSTRACTS
POSTERS

P13

ABSTRACTS
POSTERS

Investigation of the prevalence and clinically relevant causes of shortened aPTT in a clinical diagnostic laboratory

Maes J[1], Cauwelier B[1], Devos H[1], Emmerechts J[1]
[1]Department of Laboratory Medicine, Diagnostic Hematology, AZ Sint-Jan, Bruges, Belgium

Introduction: Although shortened activated partial thromboplastin times (aPTTs) are generally considered to be laboratory artefacts due to preanalytical problems, there are several studies suggesting that a shortened aPTT may reflect a hypercoagulable state, potentially associated with increased thrombotic risk.

Aims: The aim of this study was to determine the causes and clinical relevance of a shortened aPTT based on literature search and analysis of routine clinical samples.

Methods: First, a literature search was performed to investigate causes and clinical relevance of a shortened aPTT.
Second, currently used reference values in our hospital (25,1-36,5 sec) were verified for ruling out falsely shortened aPTT values. Therefore, samples from 52 healthy volunteers (83% female) aged 21-61 years were analyzed on ACL TOP 750 (Werfen, Bedford, USA) and reference values for aPTT were calculated using the robust method recommended by CLSI.
Third, the prevalence and causes of a shortened aPTT were determined in our hospital. Therefore, a query was run on aPTT measurements between February 2022 and September 2023. 41 samples of patients having repeatedly shortened aPTTs were collected and coagulation factors (FII, FV, FVII, FVIII, FIX, FX, FXI and FXII) were determined. Medical records of 64 patients with shortened aPTT were reviewed looking for possible causes in the patient's condition.

Results: According to literature, elevated FVIII is the most frequent cause of a shortened aPTT when preanalytical issues are excluded. Associations of shortened aPTT are found with venous thromboembolism (VTE), myocardial infarction, pregnancy, cancer, hyperthyroidism and diabetes mellitus. Increased FVIII is an independent risk factor for primary and recurrent VTE. However, there is yet insufficient evidence in the literature to justify initiation of therapy or performing additional thrombophilia testing or measurement of coagulation factors in patients with a repeatedly shortened aPTT.
Our results confirmed currently used reference values on ACL TOP (25,1-36,5 sec). The prevalence of shortened aPTT in our hospital was 11.2% (median 23.8s) and the departments with the highest prevalence were obstetrics (26.8%), maternity (25.6%), hematology (23.2%) and gynecology (20.3%). FVIII level was elevated in most samples (34/41, 83%) (range of FVIII 96.2-355.6%), followed by FIX (32/41, 78%), FV (29/41, 71%), FII (17/41, 42%), FXI (16/41, 39%), FXII (16/41, 39%), FX (12/41, 29%), fibrinogen (only measured when requested by the physician) (6/25, 24%) and FVII (8/41, 20%). A significant correlation was found between the aPTT and FVIII (correlation coefficient: -0.465; $p<0.05$) but not between FVIII and CRP. In 50/64 patients (78%), a possible cause for shortened aPTT, as described in literature, could be found in the medical records. 8/64 (13%) patients with shortened aPTT were admitted because of arterial or venous thrombosis.

Conclusions: A shortened aPTT is frequently found in routine clinical samples, often as the result of an elevated FVIII level. A shortened aPTT may prompt increased vigilance for other thrombotic risk factors or conditions, but current literature evidence does not justify therapeutic actions or further diagnostic investigations.

P14

ABSTRACTS
POSTERS

Low thrombin generation in menopausal women using Estetrol (E4)

Donis N[1], Douxfils J[1,2], Didembourg M[2], Bouvy C[1], Brisbois C[1], Jost M[3], Taziaux M[3], Gaspard U[4], Foidart J[3,4], Morimont L[1,2]
[1]QUALIblood s.a., Namur, Belgium, [2]Department of Pharmacy, Namur Research for Life Sciences, Namur Thrombosis and Hemostasis Center, University of Namur, Namur, Belgium, [3]Estetra SRL, An affiliate's Company of Mithra Pharmaceuticals, Liège, Belgium, [4]Department of Obstetrics and Gynecology, University of Liège, Liège, Belgium

Background: The risk of developing venous thromboembolism remains a matter of concern for menopausal women on hormonal therapy. Estetrol (E4), classified as the first Native Estrogen with Selective Tissue activity (NEST), represents a novel estrogen of interest for the relief of vasomotor symptoms. In postmenopausal women, E4 has been reported to have a low impact on hemostasis parameters and activated protein C resistance. Nevertheless, these investigations were carried on individual parameters precluding an overview of the impact of E4 on the entire coagulation process. To capture the contributive effect of the changes observed in some coagulation parameters, a global coagulation test like the thrombin generation assay (TGA) is required.

Aims: To assess the global effect of E4 in postmenopausal women on coagulation, using TGA.

Materials & Methods: Patients: This was a multicenter randomized placebo-controlled, dose-finding study in postmenopausal women (NCT0283431). A total of 168 postmenopausal women (40-65 years of age) were included for TGA analysis.
Interventions: Placebo (n=31) or E4 at a dose of 2.5 mg (n=42), 5 mg (n=29), 10 mg (n=34) or 15 mg (n=32) was administered daily for 12 weeks.
Main Outcome Measures: Thrombograms and TGA parameters (lag time; peak; time to peak; endogenous thrombin potential [ETP] and mean velocity rate index [mVRI]) were extracted for each subject at baseline and after 12 weeks of treatment. TGA reference ranges were determined based on baseline values (2.5th-97.5th percentile of the entire cohort population [n=168]). Ordinary one-way ANOVA with Tukey's multiple comparisons tests were performed to compare TGA parameters at baseline and after 12 weeks of treatment.

Results: After 12 weeks of treatment, no clinically relevant changes from baseline were observed for both placebo and treatment groups as mean thrombograms and TGA parameters (±95%CI of the mean) remained within reference ranges (i.e., 2.5th-97.5th percentile of all baseline thrombograms [n=168]). Furthermore, even if significant changes were observed for the peak, lag time, time to peak and mVRI in the E4 15 mg group after 12 weeks of treatment compared to baseline, these changes were not significant compared to the placebo group.

Conclusions: These results suggest that E4 has no clinically relevant impact on a global coagulation test like TGA, which demonstrates the neutral profile of this native estrogen on coagulation.

P15

ABSTRACTS
POSTERS

Comparison of rotational thromboelastometry during cardiopulmonary bypass versus after protamine administration: A prospective, observational single center study

Bockstael B[1], Bailleul E[1], Van Vaerenbergh G[1], Bouchez S[1]
[1]OLVziekenhuis Aalst, Aalst, Belgium

Background: Rotational thromboelastometry (ROTEM Sigma® - Werfen) is a whole blood point-of-test used to assess the patient's coagulation status in order to reduce blood loss by targeting the deficits in the underlying coagulopathy. EXTEM and FIBTEM contain the heparin inhibitor polybrene that inactivates up to 5 IU/ ml unfractionated heparin (UFH) while HEPTEM contains heparinase that eliminates up to 7 IU/ ml UFH. In theory, this enables the use and interpretation of these assays even under high UFH concentrations, such as on cardiopulmonary bypass (CPB). Performing ROTEM analysis during CPB might allow the anesthesiologist to anticipate faster to the need for specific transfusion products.

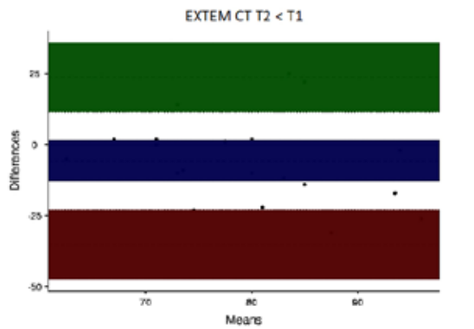
Aims: The goal of this study was to validate ROTEM Sigma ®analysis in the presence of very high heparin concentrations during CPB.

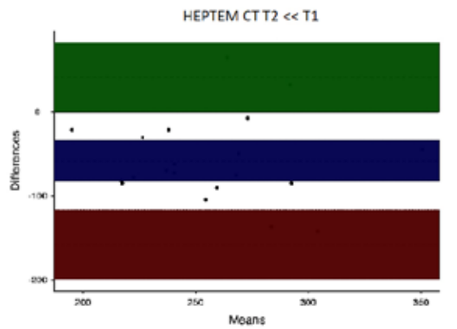
Methods and Materials: This is a prospective, observational trial in twenty patients undergoing cardiac surgery, receiving a UFH dose of 400 IU/kg.
ROTEM Sigma ®analysis was performed at the end of CPB (T1) and 10 min after protamine administration (T2). The following tests were performed: EXTEM, FIBTEM, INTEM, and HEPTEM. Anti-Xa activity (U/mL – Liquid anti-FXa HemosIL®Werfen ACLTOP350), platelet counts (#/µL – Sysmex XN-3000) and Fibrinogen (mg/dL – Q.F.A. Thrombin HemosIL®Werfen ACLTOP350) were determined at T1 and T2.

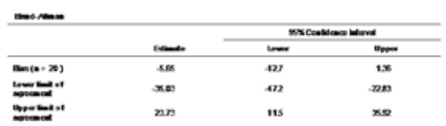
Results: CT EXTEM, CT FIBTEM,EXTEM A5, FIBTEM A5 and HEPTEM A5 differed non-significantly after protamine administration versus on CPB: respectively -5,65 s, -4,85 s, +1,15 mm+0.1 mm and +0,5 mm. CT HEPTEM differed significantly: -58,5 s ($p < 0.0001$) shorter after protamine administration compared to on CPB.
Anti-FXa activity levels on CPB varied between 4.76-8.56 U/mL (median 6.6 U/mL), while anti-FXa levels post protamine varied between 0.03-1.21 U/mL (median 0.3 U/mL).
The correlation between the ratio CT HEPTEM/CT INTEM and anti-Xa levels for T2was low (Pearson's r 0,022). The correlation between A5 EXTEM and platelet count per µL was good (resp. Pearson's r = 0.75 and 0.86) for both groups, the correlation between A5 FIBTEM and Fibrinogen (mg/dL) was also good for both groups (resp. Pearson's r= 0.90 and 0.84).

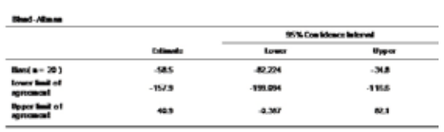
Conclusion: CT HEPTEM coagulation time is statistically significantly prolonged during high UFH concentration on CPB while changes in EXTEM, FIBTEM values are considered not statistically significant. This does suggest that either the UFH concentration exceeds the heparinase level present in the HEPTEM test or that neutralization of UFH was achieved to a greater extent with the use of polybrene in the EXTEM and FIBTEM tests. Literature suggests that polybrene seems to be a more appropriate agent to neutralize UFH 2,3. Caution is warranted when interpreting ROTEM Sigma ® data during CPB versus after protamine administration. A5 EXTEM and A5 FIBTEM remain reliable parameters for respectively platelet count and Fibrinogen in both settings and can be useful for predicting thrombocytopenia and hypofibrinogenemia.

1. Scala E, et al. Massive Hemorrhage: The Role of Whole Blood Viscoelastic Assays. Hämostaseologie 2020;40:515–23.
2. Božič-Mijovski M, et al. Ex vivo neutralization of unfractionated heparin for assessing overall haemostatic potential in patient plasma. Thromb Res 2015;135:1042–4.









P17

ABSTRACTS
POSTERS

A concise evaluation of TECHNOSCREEN® ADAMTS13 activity assay before implementation as a screening tool for detecting deficiency of ADAMTS13

Cornette M[1], Devreese K[1]
[1]Ghent University, Ghent, Belgium

Background/Introduction: ADAMTS13 (a desintegrin and metalloprotease with a thrombospondin type 1 motif, member 13) deficiency results in an accumulation of ultra large von Willebrand factor (VWF) multimers leading to thrombotic thrombocytopenic purpura (TTP), a rare life-threatening disease that requires rapid diagnosis for adequate treatment. Quantitative testing of ADAMT13 activity is labor-intensive, time-consuming and requires specific instruments and expertise. Turnaround time could be significantly reduced by a rapid test, that is also accessible to laboratories that are not equipped for quantitative testing.

Aims: Evaluation of the TECHNOSCREEN® ADAMTS13 (Technoclone, Vienna, Austria), a semiquantitative flow through assay that determines ADAMTS13 activity levels in human citrated plasma, for early exclusion of TTP.

Methods/Materials: 39 samples from patients with clinical suspicion of TTP (ADAMTS13 activity range 0-89 IU/dL) were analysed in parallel with ELISA ((TECHNOZYM® ADAMTS13 Activity ELISA Kit, Technoclone) and TECHNOSCREEN®. The TECHNOSCREEN® assay was evaluated by three blinded readers. ADAMTS13 activity of <10 IU/dL was considered positive for TTP, activity of ≥10 IU/dL was considered negative for TTP.

Results: For all three readers, a sensitivity and negative predictive value (NPV) of 100% compared to ELISA was obtained. Specificity and positive predictive value (PPV) varied between readers: 26% to 79% and 42% to 71%, respectively. At first, a batch related difference in VWF substrate was suggested as the cause of the varying specificity and PPV. Experiments with different batches of the TECHNOSCREEN® assay on the plasma of five healthy volunteers before and after denaturation, showed that specificity and PPV were not significantly different from the initial findings on patient samples. Based on our study results, varying specificity and PPV are rather related to different inter-reader interpretation than to batch to batch differences. An optical reader device could lead to more harmonization.
Also, including a normal and abnormal sample in parallel with the patient sample provides a point of comparison for color development on the membrane of the device, instead of the kit included color card. Furthermore, we would advise the manufacturer to consider negative or positive TTP result interpretation instead of using gradations, since a dilution series did not show clear color gradations on the TECHNOSCREEN® assay for ADAMTS13 concentrations ≥10 IU/dL.
For ten out of 39 (26%) samples, flow-through issues prevented result interpretation. These issues remained present after standard centrifugation, but were solved after ultracentrifugation of the plasma. Based on our experiences, the TECHNOSCREEN® assay can be performed on fresh samples without prior ultracentrifugation. Denaturated and frozen samples however, should first be ultracentrifuged.

Summary/Conclusions: Keeping pre-analytical handling in mind the TECHNOSCREEN® assay could be used to rule out TTP when indicating an ADAMTS13 activity of ≥10 IU/dL, considering the sensitivity and NPV of 100%. Although the assay is simple to perform, the interpretation is difficult, but can be facilitated by including a positive and negative patient sample for comparison, what increases the reagent cost per patient. Nevertheless, diagnosis of TTP should not be made based on this test and results should be confirmed with a quantitative method.

P18

ABSTRACTS
POSTERS

Clinical Study : Pregnancy Induced Thrombosis

Didembourg M[1], David C[2], Morimont L[1,2], De Gottal E[3], Vandervinne S[4], Douxfils J[1,2]
[1]University Of Namur, Department of Pharmacy, Namur Thrombosis and Hemostasis Center (NTHC), Namur Research Institute for Life Sciences (NARILIS), Namur, Belgium, [2]QUALIblood s.a., Research and Development Department, Namur, Belgium, [3]Centre Hospitalier Régional de Huy, Obstetrics and Gynaecology department, Huy, Belgium, [4]Centre Hospitalier Régional de Huy, Laboratory of Clinical Biology, Huy, Belgium

In developed countries, venous thromboembolic event (VTE) is the leading cause of death during pregnancy and postpartum. When compared to non-pregnant women, the risk of VTE is 5-fold higher during pregnancy and 20-fold higher during the postpartum.

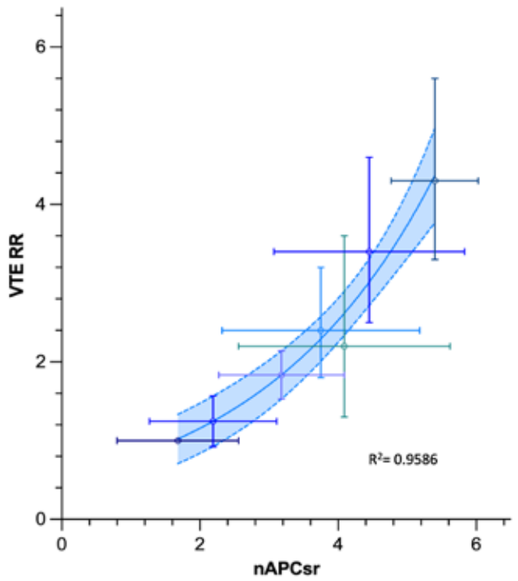
The pathogenesis of VTE during pregnancy is multifactorial. Well known, coagulation factors rise during pregnancy, along with a reduction of the fibrinolysis process. Moreover, physical changes related to the position of the foetus, the immobilization as well as the vascular trauma related to delivery can explain the increased VTE risk observed. Additional risk factors further impact the VTE risk, such as Body mass index (BMI), age, familial thrombotic history, and thrombophilia.
However, despite different markers of thrombosis, none of them is efficient to prevent or precisely evaluate the risk. It is of both clinical and societal utility to develop an efficient tool to assess the hemostatic equilibrium of pregnant women to ensure a better prevention and monitoring of newly introduced medication.

To develop a clinical decision tool allowing health professionals to generate a global score reflecting the thrombotic risk and ensuring personalized therapeutic choice. Also, to improve our knowledge of physiology during pregnancy, especially to optimize risk threshold for initiating patient-oriented additional management.

This study is a single-center, prospective study to assess the evolution of laboratory markers of thrombosis during and after pregnancy. The anticipated sample size is 150 pregnant women and 150 non-pregnant women, enrolled over 15 months. The study has been approved by the ethical committee of the CHR Huy (N°035-10/2022) and is conducted according to the Declaration of Helsinki.
Demographic characteristics and traditional thrombotic risk factors will be tabulated such as age, BMI, previous thromboembolic events, thrombophilia, extended immobilization, anticoagulation, previous parity, …. Besides the usual clinical parameters evaluated, the activated partial prothrombin time (aPTT), the international normalized ratio (INR) and the fibrinemia, will be quantified at each visit. In addition, thrombin generation assay, Fibwave assay and Endogenous Thrombin Potential-based Activated Protein C resistance assay (ETP-based APCr assay) will be performed. Assessment of other biomarkers such as Prothrombin Fragment1+2, t-PA, PAI-1, TAFI, TAT, Protein C and Protein S will also be measured.

The study started on the 25th of May 2023. To this date, 12 pregnant patients have been included. According to the literature, we are expecting pro-coagulation parameters to increase, along with a decrease in fibrinolytic factors, according to trimester. We plan to set in parallel classic coagulation test, ETP-based APCr assay and outcomes that occurred during pregnancy or post-partum. Moreover, we would like to show the evolution of the nAPCsr and its relation to the estimated relative risk of VTE. To do so, at the end of the study and according to trimester, we will implement nAPCsr results in our risk prediction model.

We hope this study will allow to establish normal reference ranges for pregnant women regarding different parameters reflecting the haemostasis. Moreover, this study will show the importance and ability of nAPCsr to reflect the thrombotic risk and to ensure a good and personalized therapeutic follow-up.



Unreliable D-dimer dilution results: consequences for algorithms using high D-Dimer results in the prediction of clot volume in extracorporeal membrane oxygenation

Blomme S[1], Bailleul E[1]
[1]Department of Laboratory Medicine OLVZ Aalst, Aalst, Belgium

Introduction: Thrombosis inside the membrane oxygenator is a critical complication during venovenous extracorporeal membrane oxygenation (ECMO).1,2 In several Belgian centers, algorithms are used to predict clot formation and therefore the need for oxygenator exchange, and within these algorithms, D-dimer levels are included. Although no references about cut-off values are available, some centers use a D-Dimer concentration cut-off of 20000 µg/L which exceeds the analyzer calibration curves. Therefore, automatic dilution needs to be performed by our analyzer when the concentration reaches the limit of detection. Aim was to investigate whether this D-dimer dilution testing is reliable.

Methods/Materials: Citrate plasma samples with different D-dimer concentrations (n=24) were diluted by three different methods: automatic 1:10 dilution by the analyzer (ACLTOP550® – Werfen) using Factor Diluent reagent, manual 1:10 dilution using Factor Diluent reagent, and manual 1:10 dilution using normal citrate plasma (with D-dimer concentration of 300 µg/L). D-Dimer testing was performed on the ACL TOP550® automated analyzer using HemosIL D-Dimer HS®, a latex-enhanced turbidimetric immunoassay (Werfen).

P19

ABSTRACTS
POSTERS

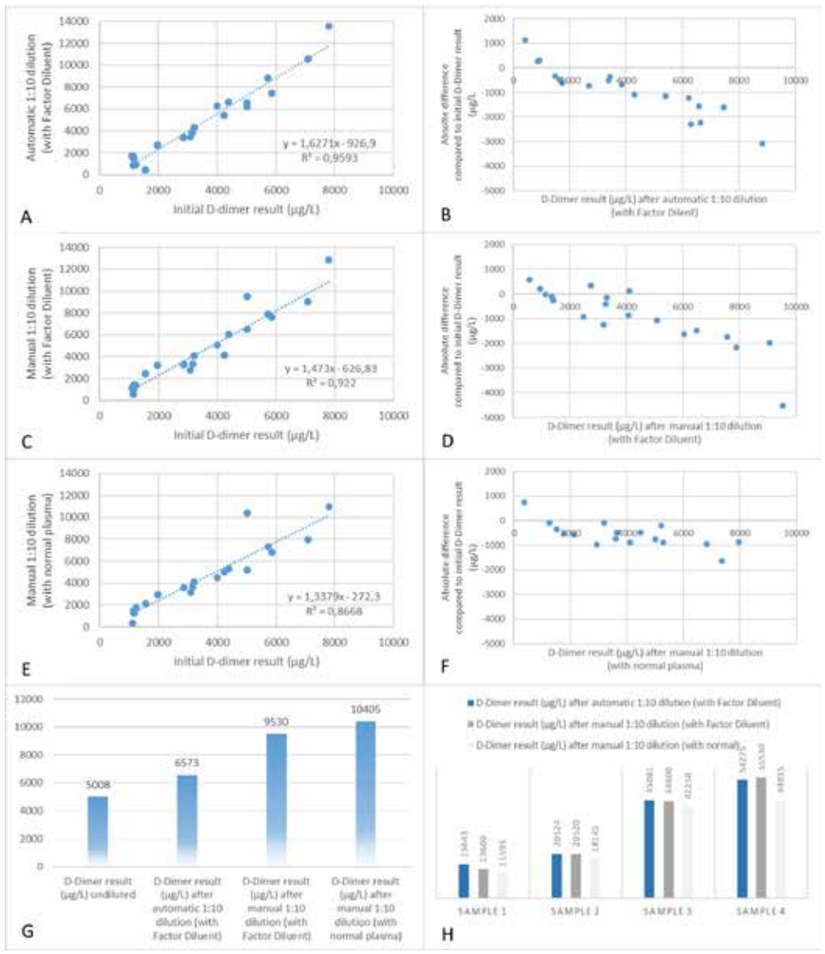
Results: For 20 samples, D-Dimer concentration was within the range of the calibration curve and could be analyzed undiluted by the analyzer. D-Dimer results after 1:10 dilution and recalculation of these samples did not correlate well with the original non-diluted D-dimer results with R^2 values < 0.975 for all three dilution methods (Fig 1 A-C-E). Bland Altman curves showed high absolute differences between undiluted and diluted results for some samples (Fig 1 B-D-F) with variable results of one sample shown in Fig 1 G. For 4 samples, D-Dimer concentrations were too high and could not be analyzed undiluted by the analyzer (reported as >7000 µg/L). 1:10 dilution of these samples showed variable results between the three methods, with one sample reaching a value >20000µg/L diluting with Factor Diluent compared to a value <20000µg/L diluting with normal plasma (Fig 1 H). For this sample, another decision could have been made considering oxygenator exchange in ECMO, but is not the only factor in the decision making.

Conclusions: Dilution of citrate plasma samples for the determination of high D-Dimers values do not always show reliable results. There is no difference between the methods of dilution. This should be taken into account when using specific cut-offs for oxygenator exchange in ECMO patients.

References:

1 Dornia C., Philipp A., et al. D-Dimers are a predictor of clot volume inside membrane oxygenators during extracorporeal membrane oxygenation. 2015. https://doi.org/10.1111/aor.12460.
2 Lubnow M., Philipp A., et al. D-dimers as an early marker for oxygenator exchange in extracorporeal membrane oxygenation. 2014. DOI:10.1016/jcrc.2013.12.008

Figure 1: D-Dimer dilution results



SATELLITE SYMPOSIUM SPONSORS

SPONSORS

octapharma

Convinced of the interest of

FVIII-based therapy in the treatment of Haemophilia A

&

A 1:1 VWF/FVIII ratio in concentrates used for the treatment of VWD