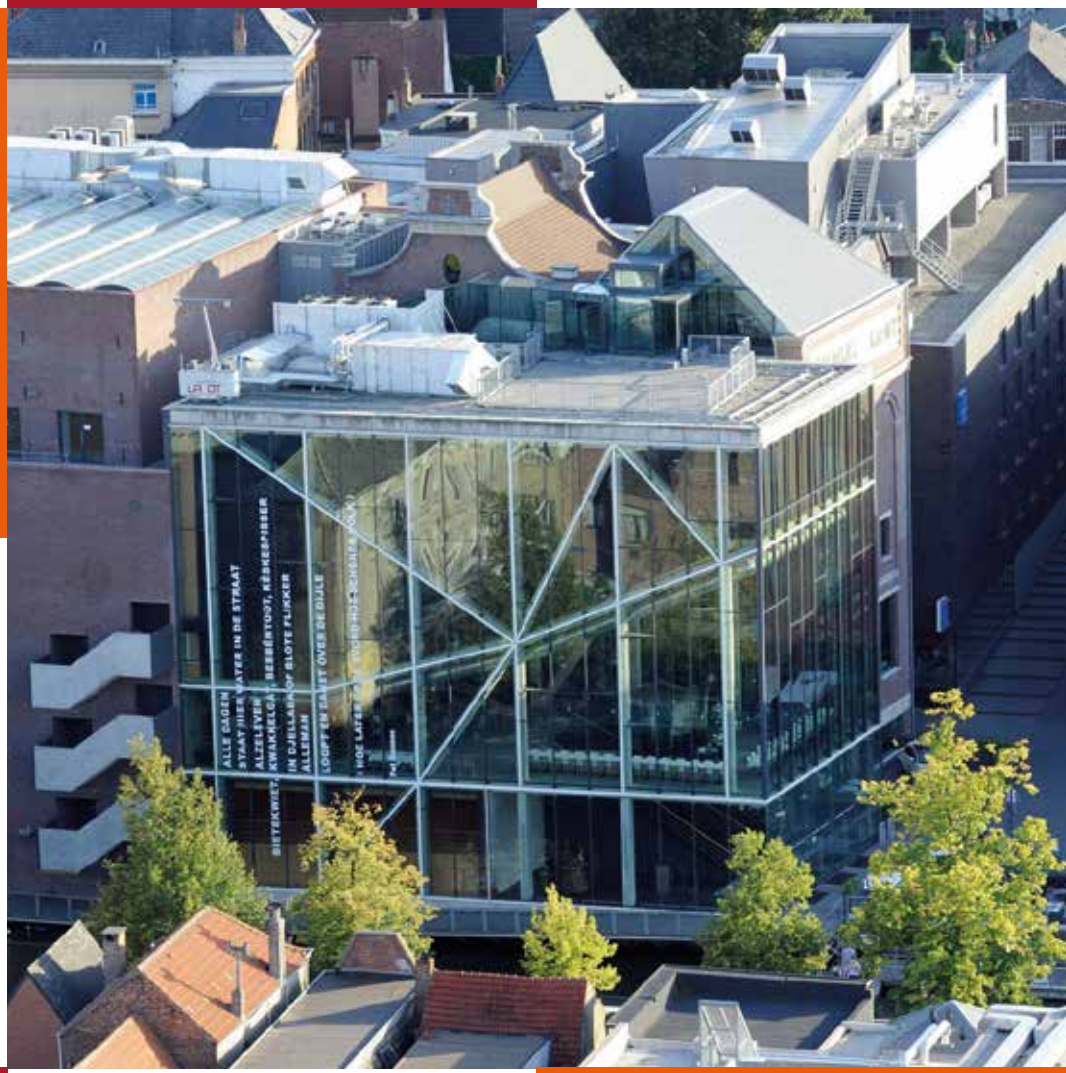
BSTH

Belgian Society on Thrombosis and Haemostasis

31ST ANNUAL MEETING

28-29 NOVEMBER 2024

LAMOT
MECHELEN
BELGIUM



LOCAL ORGANIZERS

SIMON DE MEYER
CLAUDIA TERSTEEG
THOMAS VANASSCHE

KU LEUVEN KULAK
UZ/KU LEUVEN

PROGRAM &
ABSTRACTS

WWW.BSTH2024.ORG



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WELCOME TO THE 31ST ANNUAL MEETING

Dear BSTH members,
Dear participants,

I would like to welcome you to the Annual Meeting of the Belgian Society on Thrombosis and Haemostasis (BSTH). This year marks the 31st edition. Once again, we are hosting our meeting at the Lamot Congress Center in Mechelen, which has been our host city for many years now.

It is a pleasure to see the growing interest in this meeting over the years, with participants coming from university hospitals, research centers, clinical labs, and institutions active in the fields of heart and vascular diseases, thrombosis, haemophilia, and other bleeding disorders.

This year, the scientific organization has been expertly handled by Thomas Vanassche (KU Leuven), Claudia Tersteeg (KU Leuven Campus KULAK), and Simon De Meyer (KU Leuven Campus KULAK). They have done a marvelous job putting together a great and varied program. The state-of-the-art lectures this year focus on different topics: thromboinflammation, the treatment of pulmonary embolism, the role of platelets in healing after myocardial infarction, and the molecular basis of platelet activation.

The educational sessions address rebalancing agents in haemophilia and genetics in venous thromboembolism.

In line with the BSTH mission to promote basic, laboratory, and clinical research, ten talented young researchers have been selected to present their work. Two of them will be awarded the Paul Capel Prize for the best presentations. Additionally, one researcher, selected from seven, will receive the CSL Behring Encouragement Award to conduct a research project in the field of thrombosis and haemostasis. We also kindly invite you to participate in the poster walks. Satellite symposia by Octapharma, Sobi, and CSL Behring will update you on the latest developments in their specialized fields.

Finally, we are honored to welcome Serge Motte, Emeritus Professor, who has kindly accepted the invitation to give the Gaston Baele Lecture in memory of a great man and mentor.

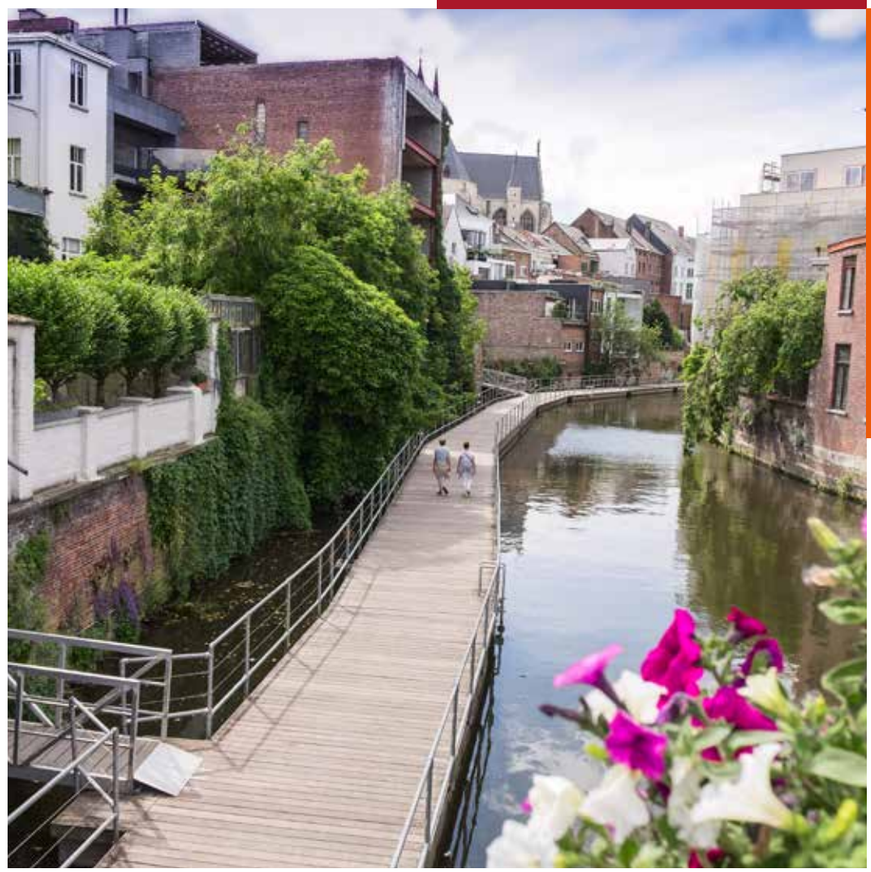
We are deeply grateful to all of you and to our sponsors for making BSTH a dynamic society at the forefront of clinical and research progress.

Along with the scientific program, I hope you find time to interact with colleagues and company representatives and enjoy the social program.

I hope you will all enjoy this 31st Annual Meeting of BSTH.

Katrien Devreese
President of BSTH





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GENERAL INFO

WWW.BSTH2024.ORG

CONGRESS MANAGEMENT
Congress care
PO Box 440
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The Netherlands
+31 (0)73 690 14 15
www.congresscare.com



DATES
28-29 NOVEMBER 2024

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

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BSTH BOARD 2024

THE PRESENT MEMBERS OF THE BSTH BOARD ARE:

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08:30 - 09:15 REGISTRATION, COFFEE, NETWORKING

09:15 WELCOME
Katrien Devreese

09:30 - 10:15 EDUCATIONAL I
Chairs: Thomas Vanassche, Phu Quoc Le

Rebalancing agents in Haemophilia
Quentin Van Thillo (Belgium)

10:15 - 11:15 SPONSORED SATELLITE SYMPOSIUM I – OCTAPHARMA
Breaking Tradition: The Role of FVIII in Haemostasis and Beyond

Welcome & Introduction
Cedric Hermans, BE

FVIII Modification: Impact on Platelet Binding and Mediated Signalling
Anja Strebel, CH

FVIII Endothelial Cell Mediated Signalling
Antonia Follenzi, IT

Generating Long-Term Data on Bone and Joint Health: The PROVE Study
Sander Botter, CH & Stephan Maack, CH

Q&A - Speakers and Audience

11:15 COFFEE BREAK

11:45 - 12:45 ORAL PRESENTATIONS CLINICAL AND LABORATORY
Chairs: Jan Emmerechts, Pierre Péters

11:45 O1 **Multi-Gene Panel for Thrombophilia Testing in Venous Thromboembolism**
Andreas Verstraete (UZ/KU Leuven)

11:57 O2 **Diagnostic performance of commercial antithrombin activity assays: do we get what we expect?**
Christelle Orlando (UZ Brussel)

12:09 O3 **Mendelian randomization implicates thromboembolism in the pathogenesis of long COVID**
Art Schuermans (KU Leuven)

12:21 O4 **Antithrombin deficiency: report of a rare case homozygous for p.Leu131Phe Heparin Binding Site mutation**
Sara Cherkaoui (Lhub-ulb)

12:33 O5 **In vitro testing of the anticoagulant capacity of rivaroxaban according to severity of liver disease**
Jill Vercruyssen (UZ Gent)

12:45 LUNCH BREAK AND POSTER WALK

13:45 - 14:15 BSTH GENERAL ASSEMBLY
Exclusively for members

14:15 - 15:15 STATE OF THE ART I – THROMBOINFLAMMATION/ IMMUNOTHROMBOSIS
Chairs: Simon De Meyer, Kristel Vandenbosch

14:15 **Cell damage contribution to platelet activation and immunothrombosis**
Julie Rayes (United Kingdom)

14:45 **Neutrophils in Thrombosis and Thrombolysis Resistance**
Benoit Ho-Tin-Noé (France)

15:15 - 16:15 SPONSORED SATELLITE SYMPOSIUM II – SOBI
Raising the bar in Haemophilia A care: The way towards normalized haemostasis

15:15 **The Clinical Perspective**
Cédric Hermans

15:40 **The Laboratory Perspective**
Marie-Astrid van Dievoet

16:05 **Q&A**

16:15 COFFEE BREAK

16:45 - 17:30 PROFESSOR GASTON BAELE MEMORIAL LECTURE
Chair: Katrien Devreese

What I would like to tell you about the BSTH
Serge Motte (Belgium)

17:30 - 17:45 PAUL CAPEL PRIZE – CLINICAL & LABORATORY
Chair: Katrien Devreese

17:45 CLOSURE & WELCOME RECEPTION

19:00 DINNER

EVENING PROGRAM

WELCOME RECEPTION

Novo Nordisk invites all participants on Thursday night at 17:45 - 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.

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PROGRAM
FRIDAY
29 NOVEMBER

08:30 - 09:00 REGISTRATION, COFFEE & NETWORKING

09:00 - 10:00 STATE OF THE ART II – NEW THERAPEUTIC OPTIONS IN INTERMEDIATE AND HIGH-RISK PE: WHAT, WHEN, AND HOW TO CHOOSE?
Chairs: Thomas Vanassche, Laure Gilis

09:00 **Trombusaspiration as a novel therapeutic option for (sub) massive PE**
Lawrence Bonne (Belgium)

09:30 **Interventional or conservative approach: how to treat (sub) massive PE patients in 2025?**
Menno Huisman (the Netherlands)

10:00 - 11:00 SPONSORED SATELLITE SYMPOSIUM III – CSL BEHRING
Empowering choices in hemophilia care with gene therapy in sight: a triad approach from patient, doctor and patient association
This symposium is intended for health care professionals only

10:00 **Welcome speech and introduction**
Cedric Hermans

10:05 **Empowering choices in hemophilia care with gene therapy in sight: the patient's association perspective**
Sarah De Witte

10:20 **Empowering choices in hemophilia care with gene therapy in sight: the doctor's approach**
Peter Verhamme

10:35 **Empowering choices in hemophilia care with gene therapy in sight: the patient's experience**

10:50 **Questions and answers and closing**

11:00 COFFEE BREAK

11:30 - 12:30 ORAL PRESENTATIONS BASIC SCIENCE
Chairs: Sandrine Horman, Christelle Orlando

11:30 **O6** **Targeting antigen-specific B cells in immune mediated thrombotic thrombocytopenic purpura using modular CAR T cells**
Sara Rovira Puig (KU Leuven Campus Kortrijk)

11:42 **O7** **imMKCL-based Glanzmann Thrombastenia models to study platelet formation and function**
Kato Ramaekers (KU Leuven)

11:54 **O8** **Conformational opening of ADAMTS13 does not necessarily indicate preactivation of its metalloprotease domain**
Quintijn Bonnez (KU Leuven Campus Kortrijk)



12:06 **O9** **An in-house ex-vivo NETs induction model for the evaluation of potential NETosis inhibitors**
Constant Gillot (Unamur)

12:18 **O10** **Sustainable polymers with improved biocompatibility: new materials for blood-contacting medical devices**
Sofia F. Melo (University of Liège)

12:30 - 12:45 CSL BEHRING ENCOURAGEMENT GRANT
Chair: Katrien Devreese

12:45 LUNCH BREAK AND POSTER WALK

13:45 - 14:30 EDUCATIONAL II
Chairs: Laurence Rozen, Sévérine de Bruijn

Genetics of VTE
Pierre-Emannuel Morange (France)

14:30 - 15:30 STATE OF THE ART III – PLATELETS
Chairs: Claudia Tersteeg, Cécile Oury

14:30 **Platelets in post-myocardial infarction healing**
Sandrine Horman (Belgium)

15:00 **The molecular basis of heterogeneous platelet activation**
Lorenzo Alberio (Switzerland)

15:30 - 15:45 PAUL CAPEL PRIZE – BASIC SCIENCE
Chair: Katrien Devreese

15:45 CLOSING REMARKS

16:00 CLOSURE

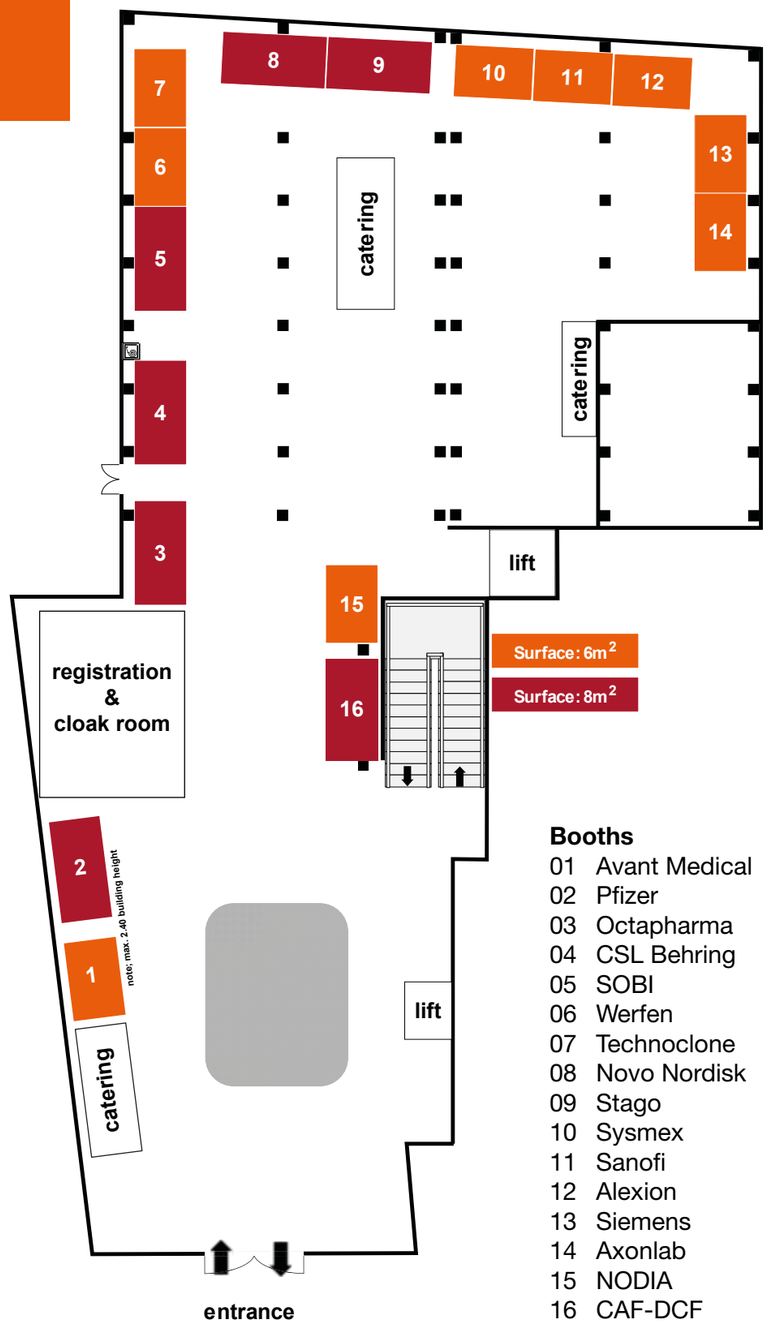
09

PROGRAM
FRIDAY
29 NOVEMBER



10

FLOORPLAN



Booths

01 Avant Medical
02 Pfizer
03 Octapharma
04 CSL Behring
05 SOBI
06 Werfen
07 Technoclone
08 Novo Nordisk
09 Stago
10 Sysmex
11 Sanofi
12 Alexion
13 Siemens
14 Axonlab
15 NODIA
16 CAF-DCF



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EXHIBITION RULES & REGISTRATION FEES

EXHIBITION RULES

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

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

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

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

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

EXHIBITION OPENING HOURS

THU 28 NOV 2024	08:30-18:30
FRI 29 NOV 2024	08:30-15:30

REGISTRATION FEES

MEMBER BSTH

Regular (MD specialist, MSc specialist, PhD scientist)	185 EUR
MD trainee / PhD student	95 EUR
Nurse, paramedic, technician, data manager, student	55 EUR

NON MEMBER BSTH

Regular (MD specialist, MSc specialist, PhD scientist)	285 EUR
MD trainee / PhD student	175 EUR
Nurse, paramedic, technician, data manager, student	110 EUR

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EDUCATIONAL I



Rebalancing agents in haemophilia

Quentin Van Thillo
UZ Leuven, Belgium

Thrombophilia is a well-known risk factor for venous thrombo-embolism in the general population. Common examples include deficiencies of anticoagulant proteins such as antithrombin deficiency, and deficiencies of proteins C and S. These aberrations are also encountered in haemophilia patients, in whom they generally lead to a more attenuated bleeding phenotype. This observation has led to the concept of rebalancing haemostasis, in which anticoagulant proteins are targeted in order to induce more thrombin generation in haemophilia patients. This way, the haemostatic deficit, that results from factor VIII or factor IX deficiency, can be at least partially corrected. Recent efforts have focused on targeting antithrombin, tissue factor pathway inhibitor (TFPI) and activated protein C.

Antithrombin can be targeted by fitusiran, which suppresses the production of antithrombin in the liver. Lower antithrombin levels lead to a reduced inhibition of factor X and thrombin, resulting in more thrombin generation. Fitusiran resulted in a decrease of total bleeds in patients with haemophilia A or B above 12 years with inhibitors (ATLAS-INH), and in patients without inhibitors compared to either on-demand treatment (ATLAS-A/B) or prophylaxis (ATLAS-PPX). These trials were temporarily put on hold due to several thrombotic events, but these could be well managed eventually with a revised dosing scheme and altered bleeding management plan. Second, TFPI represents an interesting target. Inhibition of TFPI has a theoretically lower risk of thrombosis because of the continued presence of anticoagulants further downstream in the coagulation cascade. Two monoclonal antibodies have been tested in phase 3 trials: concizumab and marstacimab. Concizumab daily injections have demonstrated a lower bleeding rate in the explorer 7 (patients with inhibitors) and explorer 8 trials (patients without inhibitors). Weekly injection of marstacimab has demonstrated a similar effect on the bleeding rate of patients with haemophilia. Finally, activated protein C and protein S can be targeted. Several studies in preclinical mouse models of haemophilia have shown a protective effect against hemarthrosis using these strategies. The clinically most advanced product is SerpinPC, inhibiting activated protein C, but the AP-0101 trial was recently put on hold.

Rebalancing agents potentially offer different advantages, such as the ease of subcutaneous administration, and the potential use in patients with inhibitors. Especially for patients with haemophilia B and inhibitors, there are currently no good prophylactic treatments available. However, several concerns need to be addressed before their further application into the clinic. There is no routinely available assay to compare the haemostatic effect of these agents compared to other treatments. Furthermore, the management of bleeds and surgery is also not yet fully established. The main task will be to determine the place of these rebalancing agents in the treatment landscape of haemophilia, which is becoming increasingly competitive with plenty of good treatment options already available. Despite these hurdles, rebalancing agents are an exciting class of drugs that further enable us to control the coagulation cascade, and they open a new avenue for treating not only haemophilia, but also rare coagulation disorders, inherited platelet disorders and other underappreciated bleeding conditions.

Biography
Quentin Van Thillo was trained as a haematologist at the University Hospitals of Leuven and Ghent. He obtained his PhD at VIB - KU Leuven on the cooperation of oncogenic transcription factors in acute lymphoblastic leukaemia. He is currently leading the Haemophilia Treatment Centre in Leuven.

EDUCATIONAL II



Genetic Basis of Venous Thrombosis

Pierre-Emannuel Morange
Aix-marseille University, France

Venous thromboembolism (VT), comprising deep vein thrombosis (DVT) and pulmonary embolism (PE), affects roughly 0.2% of the population annually and is associated with significant mortality. This multifactorial disorder arises from a complex interplay of genetic and environmental factors such as hormonal therapy, pregnancy, immobility, and obesity. Family history notably increases VT risk, and classic inherited thrombophilias—such as deficiencies in antithrombin (AT), protein C (PC), protein S (PS), and mutations like factor V Leiden and prothrombin G20210A—underscore its genetic basis.

Genomic advances, particularly genome-wide association studies (GWAS), have identified novel genetic loci linked to VT. An initial GWAS led by our team, analyzing over 317,000 single nucleotide polymorphisms (SNPs), highlighted the important role of the ABO blood group in VT risk. Further meta-analyses, including a large-scale 2022 INVENT Consortium study with 81,669 cases and over 1.4 million controls, have expanded the number of loci implicated to 85, including for example SLC44A2, which influences neutrophil-driven mechanisms in VT.

Polygenic risk scores (PRS) represent a significant step forward in stratifying VT risk by integrating multiple genetic variants. High PRS scores correlate with increased VT risk, providing a foundation for personalized risk assessment, particularly in populations such as women taking hormonal contraceptives. This approach offers opportunities for tailored preventive strategies.

The search for rare variants using next-generation sequencing techniques, such as whole-exome sequencing (WES), has led to the identification of new associations, including rare variants in the STAB2 gene, which are linked to von Willebrand factor levels and VT risk. These findings underscore the necessity of expanding genetic screening to encompass a broader array of variants, thereby refining our understanding of thrombophilia's complex genetic landscape.

In summary, the genetic architecture of thrombophilia is intricate and continuously evolving. Ongoing research aims to unravel the role of both common and rare variants. The convergence of GWAS, PRS methodologies, and next-generation sequencing holds promise for enhancing risk prediction, guiding personalized treatment, and ultimately improving VT management and prevention.

Biography
Pr. Pierre-Emmanuel Morange is the Head of the Hematology Laboratory at Timone University Hospital, part of the Assistance Publique-Hôpitaux de Marseille (APHM), where he also serves as Deputy Head of the Medical Biology Department. A Professor of Medicine with a Drate in Molecular Biology, Pr. Morange has been a key figure in clinical diagnostics, heading both hematology departments at Timone and CHU Nord in Marseille.

He is a leading researcher affiliated with INSERM and the INRAE Center for Cardiovascular and Nutrition Research, contributing extensively to venous thrombosis genetics and predictive diagnostics. With over 330 publications and numerous awards, including recognition from the International Society for Thrombosis and Hemostasis and Fondation de France, Pr. Morange plays a pivotal role in international research consortia and coordinates significant national and European research projects.

Additionally, Pr. Morange is deeply committed to education, overseeing specialized programs in medical biology and genetics at Aix-Marseille University while supervising numerous master's and PhD theses.

What I would like to tell you about the BSTH

Serge Motte
Hôpital Erasme, Ulb, Belgium

The presentation will briefly discuss my clinical career by highlighting the interest of the interdisciplinary approach in clinical practice and how I came to be particularly interested in the treatment of thrombosis and the management of anticoagulant treatments. The BSTH is a typical multidisciplinary scientific society offering a forum for discussion and collaboration between researchers, clinical laboratory physicians and biomedical scientists and clinicians in the fields of basic research, clinical and medical biology research and clinical practice. This is a unique opportunity for interdisciplinary collaboration to promote research. The Society has evolved a lot and the presentation will come back to the key points of evolution. The role of working groups whose objective is to propose consensus documents for clinical practice will be discussed.

Biography
Dr Motte qualified as a medical dr in 1982, went on to complete his post-graduate training in Internal Medicine in 1987, and was awarded his PhD in 1997, all at the Université Libre de Bruxelles. He has an executive master's degree in management in health care institutions from the Université Libre de Bruxelles.

He was from 2002 to 2021 Head of the Thrombosis and Antithrombotic Treatment Clinic, Department of Vascular Diseases, Erasme University hospital, Université Libre de Bruxelles, Brussels. He is an honorary professor at the Faculty of Medicine and at the School of Public Health of the Université Libre de Bruxelles.

Dr Motte was President of the Belgian Working Group on Angiology from 2005 to 2008 and of the Belgian Society on Thrombosis and Haemostasis from 2008 to 2012. He was Chairman of the Thrombosis Guideline Group from 2003 to 2009.
His major research interests include the epidemiology, prevention, treatment, and economic burden of venous thromboembolism. Dr Motte has authored and co-authored over 50 publications on a variety of subjects.

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PROFESSOR GASTON BAELE MEMORIAL LECTURE



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STATE OF THE ART I



Cell damage contribution to platelet activation and immunothrombosis

Julie Rayes
University of Birmingham, UK

Platelets are essential components of the haemostatic system, playing a crucial role in limiting blood loss at sites of vascular injury and modulating the inflammatory response in both sterile and infectious conditions. Upon activation, platelets release pro-thrombotic and inflammatory mediators, promoting interactions with endothelial cells and innate immune cells, which can exacerbate the inflammatory response and contribute to organ dysfunction. Inflammatory and infectious conditions trigger the release of damage-associated molecular patterns (DAMPs) from injured or activated cells, which drive platelet activation and contribute to immunothrombosis. Elevated levels of these DAMPs are associated with thrombosis, inflammation, and organ damage.

Among these danger signals, heme—released from damaged red blood cells and muscle—is a potent driver of thrombo-inflammation and organ damage. Heme activates both endothelial cells and platelets, promoting thrombosis through a mechanism that is resistant to clinically approved anti-platelet drugs. However, heme-induced platelet activation can be inhibited by targeting downstream signalling pathways of the immune receptors CLEC-2 and GPVI on platelets. While heme is pro-thrombotic, it also impairs the activation of the coagulation cascade. In septic mice, heme injection destabilizes platelet aggregates and increases the risk of pulmonary embolism.

In addition to its direct effects on platelets, heme-activated platelets facilitate the formation of neutrophil extracellular traps (NETs). These NETs release potent DAMPs, such as histones, free DNA, and S100A8/A9, which further amplify platelet activation and thrombo-inflammation. These DAMPs act through different receptors on platelets, leading to the formation of distinct platelet populations with varying functional characteristics. Notably, S100A8/A9 alters platelet behaviour, inducing the formation of a pro-coagulant platelet population that supports coagulation while maintaining low aggregation capacity, a phenomenon resistant to anti-platelet drugs. Reducing innate immune cell activation improves thrombocytopenia and reduces coagulopathy in septic mice.

In conclusion, DAMPs released during cellular damage trigger platelet activation and support inflammation-driven thrombosis. Early intervention with low-dose anti-inflammatory drugs may provide a promising strategy to limit thrombo-inflammation while minimizing the risk of bleeding in acute injuries and infections.

Biography
Julie Rayes holds a PhD in thrombosis and hemostasis from the University of Paris VII Denis Diderot in France. She is an Associate Professor in the Cardiovascular Sciences department at the University of Birmingham in the UK and holds a British Heart Foundation Intermediate Fellowship. Her research focuses on understanding the mechanisms by which cell damage, through the release of damage-associated molecular patterns, drives platelet activation and dysfunction, as well as their contribution to immunothrombosis and thrombo-inflammation in acute infections (e.g., sepsis) and chronic inflammation (e.g., sickle cell disease).



Neutrophils in Thrombosis and Thrombolysis Resistance

Benoit Ho-Tin-Noé
Inserm Unit 1144, France

Neutrophils and neutrophil extracellular traps are now recognized as integral players of thrombotic diseases. We will discuss how recognition of the contribution of neutrophils and NETs to thrombogenic and hypofibrinolytic states offers new therapeutic opportunities for improving current and future thrombolytic therapies, as well as to reduce ischemia-reperfusion injury in ischemic stroke.

Biography
After completing a PhD in Hemostasis and Vascular Biology (Paris Diderot University) during which he studied the mechanisms of cellular plasminogen activation, Benoit Ho-Tin-Noé joined the laboratory of Dr Denisa Wagner in 2006, where he worked for 3 years as a postdral fellow. There, he initiated a series of studies on the role of platelets in the maintenance of vascular integrity in inflamed organs and solid tumors. He was then recruited in 2009 as a tenured researcher by the French National Institute of Health & Medical Research (INSERM) to work in a cardiovascular research academic laboratory in Paris (The Laboratory for Vascular Translational Science). After 3 years as a junior investigator, he founded his own research group in 2012. In 2023, his group joined the Opten Laboratory (Therapeutic Optimization in Neurology) at the Paris Faculty of Pharmacy. His research focuses on studying how thromboinflammation contributes to the pathophysiology of thrombotic diseases and on optimizing the efficacy and safety of thrombolytic and antithrombotic therapies.

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STATE OF THE ART II



Trombusaspiration as a novel therapeutic option for (sub)massive PE

Lawrence Bonne
UZ Leuven, Belgium

Thrombus aspiration has emerged as a minimally invasive treatment option for patients with acute pulmonary embolism, more specifically in those with high-risk or intermediate-high-risk classifications. Large bore aspiration systems have demonstrated the potential for safe, rapid clot removal and hemodynamic stabilization in a minimally invasive manner. While current evidence is predominantly derived from observational studies focusing on safety, several randomized controlled trials are underway to provide robust validation of the technique's efficacy. The outcomes of these highly anticipated studies are crucial for establishing the definitive value of thrombus aspiration as a therapeutic modality.

Biography
Lawrence Bonne obtained his Bachelor's and Master's degrees in Medicine from the University of Leuven (K.U. Leuven). He further pursued a Master in Specialist Medicine (Radiology), with residency training at University Hospitals Leuven and Onze Lieve Vrouw-Hospital Aalst, focusing on interventional radiology.

Professionally, he has been working in vascular and interventional radiology at University Hospitals Leuven (UZ Leuven) since 2016, with intervals of fellowship experiences, including interventional oncology at The Royal Marsden NHS Foundation Trust in London and interventional neuroradiology at ZOL Genk.

Treatment of acute intermediate risk pulmonary embolism with anticoagulants

Menno Huisman
Leiden University Medical Center, the Netherlands

According to the 2019 European Society of Cardiology guidelines of pulmonary embolism (PE) treatment with oral anticoagulants (preceded or not with LMWH or fondaparinux over UFH) is recommended for patients with acute intermediate risk PE (class 1A evidence). Primary reperfusion treatment with thrombolysis is not recommended in these patients (class IIIB evidence). Recently, there have been reports of registries and single cohort studies evaluating the efficacy and safety of other ways of reperfusion therapy, including (ultrasound guided) catheter directed thrombolysis or catheter thrombectomy. It remains to be demonstrated whether these interventional techniques will prove superior and safe in terms of clinically relevant outcomes.

Biography
Menno Huisman, MD, PhD, FESC, is internist and registrar in cardiovascular medicine and professor of Medicine in the Department of Medicine – Thrombosis and Hemostasis at the Leiden University Medical Center, Leiden the Netherlands.

He obtained his medical degree at the University of Amsterdam and a PhD degree with the subject of objective diagnosis of deep-vein thrombosis. He completed a residency in internal medicine, followed by completion of a 2-year fellowship in hematology, at the Academic Medical Center in Amsterdam. He has raised the subspecialty of Vascular Medicine in the Netherlands. Dr Huisman's main research areas are in clinical research, particularly in the areas of diagnosis and treatment of venous and arterial thromboembolism.

He has raised the multidisciplinary EXPERT-PE network in Europe – www.expert-pe.org - and the LUMC based multidisciplinary EXPERT-PE team and is the European representative in the Board of Directors of the United States based PERT organisation – www.pertconsortium.org. He is member of the European Society of Cardiology Guidelines Committee on diagnosis and treatment of pulmonary embolism and has been member of the updated VTE guidelines of the American College of Chest Physicians.



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STATE OF THE ART III



Platelets in post-myocardial infarction healing

Sandrine Horman
UC Louvain, Belgium

Following myocardial infarction (MI), necrotic cardiomyocytes initiate an inflammatory response critical for infarct healing and ventricular remodeling, marked by inflammatory cell infiltration and upregulation of inflammatory genes. Transforming growth factor-beta (TGF-beta) plays a central role in modulating this inflammation and supporting cardiac repair post-MI. Early in this process, platelets infiltrate the infarcted myocardium and release substantial amounts of TGF-beta1, while also expressing Glycoprotein A Repetitions Predominant (GARP) on their surface, underscoring their capacity for both production and activation of TGF-beta1. In this study, we used a mouse model with permanent coronary artery ligation to explore the role of platelet GARP in TGF-beta1 activation and subsequent modulation of post-MI processes. Platelet-specific GARP knockout (pKO) mice exhibited increased rates of myocardial rupture and pronounced left ventricular dilation post-MI compared to wild-type mice. Analysis of pKO hearts revealed enhanced endothelial activation, increased neutrophil and macrophage infiltration, and elevated production of pro-inflammatory cytokines, which collectively delayed inflammation resolution and impaired healing. These findings underscore the critical role of the platelet GARP/TGF-beta1 pathway in managing the inflammatory phase and promoting inflammation resolution during myocardial healing. They also caution against GARP inhibition therapies, particularly during the acute phase of ischemic events.

Biography
Sandrine Horman is a Professor and FNRS Researcher at the University of Louvain Medical School (UCLouvain, Belgium). She holds a PhD in Biological Sciences from the Free University of Brussels (ULB) and spent seven years at the de Duve Institute (UCLouvain), where she investigated AMPK targets in cardiovascular health. Since 2008, she has led a research team as a principal investigator within the Pole of Cardiovascular Research at the Institute of Experimental and Clinical Research. Her work focuses on uncovering molecular mechanisms that influence the roles of platelets in thrombotic and thrombo-inflammatory processes, with a particular emphasis on the pathophysiology of sepsis, post-infarction cardiac remodeling, and heart failure.



The molecular basis of heterogeneous platelet activation

Lorenzo Alberio
University Hospital of Lausanne (CHUV), Switzerland

During the lecture I will present the current model of heterogeneous platelet activation. I will recapitulate key mechanisms responsible for the differentiation of procoagulant platelets from the aggregating ones. Finally, I will describe the approach we are currently employing to identify the signaling events preceding and those regulating the dichotomous platelet response.

Biography
Lorenzo Alberio is Full Professor of Haematology at the Faculty of Biology and Medicine of the University of Lausanne (UNIL) and Head Physician in the Service and Central Haematology Laboratory at the University Hospital of Lausanne (CHUV), Switzerland. He carries the academic responsibility for the Clinical Division of General Haematology and Haemostasias and for the Haematology Diagnostic Laboratory.

He studied medicine at the University of Bern and completed his clinical training in Internal Medicine and Haematology in the Italian and German speaking parts of Switzerland (Lugano, Locarno, and Bern). He performed research in the USA (Health Science Center, Oklahoma City), France (Établissement Français du Sang, Strasbourg), and Switzerland (Theodor Kocher Institute, Bern) and established his own research group in Lausanne (https://www.chuv.ch/fr/hematologie/hem-home/recherche/our-research-leaders/pr-lorenzo-alberio-1). He is considered one of the founding fathers of the field of procoagulant platelets. His other scientific interests are devoted to the diagnostic approach and treatment of heparin-induced thrombocytopenia and to the clinical usefulness of global coagulation assays in various conditions, such as bleeding disorders, liver cirrhosis, and haematologic malignancies. His research is supported among others by the Swiss National Science Foundation. He is the recipient of national (e.g., Ellerman Prize) and international (e.g., Lukas Schönlein Prize) scientific awards.

He has been a member of the board of the Society of Thrombosis and Haemostasis Research (GTH) and vice-chairman of the Swiss Haemophilia Network (SHN). Currently, he is a board member of the Swiss Society of Haematology (SGH-SSH) and the chairman of the Expert Committee of the Swiss Association of Laboratory Medicine (FAMH).

Together with Prof. Manuela Albisetti he is the President of the next Scientific Meeting of the Society on Thrombosis and Haemostasis research (www.gth2025.com).

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NOTES

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SPONSORED SATELLITE SYMPOSIUM I - OCTAPHARMA

Breaking Tradition: The Role of FVIII in Haemostasis and Beyond

The 2024 BSTH Symposium titled, “Breaking Tradition: The Role of FVIII in Haemostasis and Beyond,” will take place on 28th November in Mechelen, Belgium during the Belgian Society on Thrombosis and Haemostasis (BSTH) Annual Meeting. The symposium, sponsored by Octapharma, is chaired by Professor Cedric Hermans.

The symposium features presentations from several esteemed speakers, including Prof. Antonia Follenzi, Ms. Anja Strebel, and Dr Sander Botter. The agenda includes a series of talks that will cover various aspects of Factor VIII, with focus on the newly researched roles that FVIII mediates in haemostasis and joint health. The symposium will begin with an introduction by Prof. Hermans, who will provide an overview of FVIII products and their continued importance in the age of non-factor therapies, as well as new roles of FVIII that are rapidly gaining interest.

Ms. Strebel will then discuss the emerging role of FVIII in mediating the function of platelets, and the impact of FVIII modification on platelet binding and clot formation. She will also present in vitro binding data of platelets with FVIII products. Next, Prof. Follenzi will present new findings on the interactions between FVIII and endothelial cells. She will expand on FVIII-endothelial cell mediated signalling, discuss the observed attenuation of endothelial cell function in patients with haemophilia A, and provide data on endothelial cell gene regulation.

Dr. Botter will then discuss the PROVE study, which focuses on generating long-term data on bone and joint health in haemophilia A patients. FVIII is hypothesized to play a key role in maintaining bone health in these patients, and long-term data from individuals receiving both FVIII and non-factor FVIII products for prophylaxis is currently lacking. He will provide background information on FVIII’s emerging role in joint and bone health in haemophilia A, then detail the study’s aim, design, and target endpoints. The symposium will conclude with a discussion led by Prof. Hermans, where all speakers will participate.

Overall, the audience will learn about the latest research and developments regarding the multifaceted role of FVIII in haemostasis, particularly its non-procoagulant roles which are now gaining more attention. It is hoped that the audience will understand the impact of FVIII modifications on these various physiological functions, and how such modifications may influence the long-term effectiveness in patients receiving different FVIII or non-FVIII treatments for prolonged periods. Overall, they will gain insights into the ongoing relevance of FVIII in both current and future therapeutic approaches.



Biographies

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 Full Professor at the Medical School of the Catholic University of Louvain in 2012 and Vice-Dean in 2015.
Professor Hermans has (co)-authored more than 370 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.



Anja Strebel is a PhD student in the Laboratory of Applied Mechanobiology, at ETH Zürich Switzerland. She has been working under the supervision of Prof. Dr. Viola Vogel and Prof. Dr. Bernd Wollscheid since November 2023. Prior to this she completed a MSc in Biomedical Engineering and a BSc in Health Sciences and Technology. Her current research focuses on the central role of recombinant FVIII-platelet interaction in Hemophilia A, specifically exploring its effects in the context of the mechanobiology of blood clot formation, which influences hemostasis and shapes clinical outcomes. By investigating the mechanism underlying this interaction, Anja and her team aim to improve the efficiency of Hemophilia A treatment.



Antonia Follenzi is a full professor of Histology, Cell and Gene Therapy at the School of Medicine of the University of Piemonte Orientale in Novara, Italy. She graduated in Medicine at the University of Torino, Italy in 1992 and received the PhD in Human Oncology in 2001. During this period, she spent one year in Israel at the Weizmann Institute of Science working in the lab of Prof. Yarden. As a post-doc working with Prof. L. Naldini, developed novel lentiviral vectors (LV) for gene therapy applications. She exploited LV injection in mice for targeted expression in the hepatocytes, being able to reduce immune responses after LV delivery. In collaboration with Prof. S. Gupta at Einstein College in New York, demonstrated that liver sinusoidal endothelial cells (LSEC) can repopulate the liver, secrete coagulation FVIII and correct the bleeding phenotype in hemophilic mice. She implemented the technology of generating iPSCs from healthy and hemophilic hematopoietic cells and differentiating them to endothelial cells that after LV correction were able to secrete FVIII and once transplanted correct the bleeding phenotype in hemophilic mice. One of her focus is optimizing gene transfer for hemophilia A by using novel promoters to restrict FVIII expression in endothelial cells that contribute to the immune tolerance of Factor VIII. Recently, her group demonstrated the differences in the transcriptomic and proteomic profile between healthy and hemophilic ECs unveiling the extra-coagulative roles of FVIII.
She is a member of the European Society of Gene and Cell Therapy (ESGCT), of the American Society of Gene and Cell Therapy (ASGCT) and of the International Society of Thrombosis and Haemostasis (ISTH) and Società Italiana per gli Studi dell’emostasi e della trombosi (SISET). She received the ESGCT Young Investigator Award in 2008 in Bruges (Belgium) and in 2010 the CSL-Behring Prof Heimburger Award for her coagulation studies. She is the owner of several national and international grants and has published 150 scientific articles in international and indexed journals.



Sander Botter is the Platform Director of the Swiss Centre for Musculoskeletal Biobanking, at the Balgrist Campus in Zürich, Switzerland. Dr. Botter completed his PhD in dynamic subchondral bone changes at the Erasmus Medical Centre in the Netherlands, followed by a post-doc conducted in collaboration with the life science company DNage B.V. This expanded his research expertise to include premature aging bone phenotypes, studied with in vivo micro-computed tomography and microarrays. This work was published in Nature. Sander then moved to the Balgrist University Hospital in Zürich Switzerland, which is recognised worldwide as a highly specialised, leading centre of excellence for assessing, treating and following up on all types of musculoskeletal injuries and spinal cord injuries. He held the position of Scientific Researcher for 5 years prior to becoming Platform Director. As Director at the Balgrist Campus, Dr. Botter is now responsible for setting up and running a SERI-financed, certified, open biobank/technology platform infrastructure, helping to establish a network between research groups, physicians and industrial partners in the field of (pre-) clinical musculoskeletal research, to find answers for unsolved clinical musculo-skeletal research questions. Sander Botter has authored 58 scientific publications to date and has been cited over 1,500 times. He is also currently a member of the Swiss Group for Clinical Cancer Research (SAKK) Patient Advisory Board.

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SPONSORED SATELLITE SYMPOSIUM II – SOBI

Raising the bar in Haemophilia A care: The way towards normalized haemostasis

Despite treatment advances, haemophilia still impacts everyday life. Life-threatening bleeds, joint bleeds and subclinical bleeds still occur, which have long-term physical and quality of life (QoL) implications.[1-4] Today, people with haemophilia A (PwHA) are only able to achieve factor activity levels in the non-haemophilia range (≥40%) with frequent dosing of FVIII replacement therapy, meaning that they continue to make trade-offs between treatment burden and predictable protection, impacting their life choices.
First Prof. Cédric Hermans (*Cliniques universitaires Saint-Luc, Bruxelles*) will elaborate on target FVIII levels and treatment outcomes during prophylaxis, and how these are being re-evaluated as more data suggest that higher sustained factor levels provide improved protection from all bleeds and preserve long-term joint health.[5-7] He will explain that the introduction of new innovative treatments shifts expectations closer to the goal of achieving optimal outcomes, health equity and a "*haemophilia-free mind*".[8,9]
Next Dr. Marie-Astrid van Dievoet (*Cliniques universitaires Saint-Luc, Bruxelles*) will bring the perspective from the lab on efanescog alfa becoming available to patients.
As for every FVIII product, the structural and chemical modifications of efanescog alfa influence its behaviour in FVIII activity assays. Hence, to reliably monitor patients treated with this new drug, it is important to assess its performance in these assays.
Dr. van Dievoet will present the results from a comparative assay field study by Steven Pipe et al.[10] that evaluated the performance of one-stage clotting assays (OSAs) and chromogenic substrate assays (CSAs) in the measurement of FVIII activity of efanescog alfa in plasma samples using a variety of OSA reagents, CSA kits and analyzers in clinical haemostasis laboratories in multiple countries, including Belgium.

References:
1. Olivieri M, et al. Haemophilia. 2012;18:369–374; 2. Gooding R, et al. J Blood Med. 2021;12:209–220; 3. Gualtierotti R, et al. J Thromb Haemost. 2021;19:2112–2121; 4. Warren BB, et al. Blood Adv. 2020;4(11):2451–2459; 5. den Uijl I, et al. Haemophilia. 2011;17(1):41–44; 6. Soucie J, et al. Blood Adv. 2018;2(16):2136–2144; 7. Chowdary P, et al. Thromb Haemost. 2020;120(5):728–736; 8. Skinner MW, et al. Haemophilia. 2020;26(1):17–24; 9. Krumb E and Hermans C. Res Pract Thromb Haemost. 2021;5(5):e12567; 10. Pipe S, et al. Haemophilia 2024;30:214–223.

NP-37908, November 2024



Biographies
Cédric Hermans obtained his Medical degree at the Université Catholique de Louvain, which was followed by a specialization in Internal Medicine and a Drate in Biomedical Sciences (Toxicology). He obtained his Certificate of competence in haematology in 2004 and became Head of the Adult Haematology Department at the Cliniques Universitaires Saint-Luc in 2006.
As from 2012 he holds the position of Ordinary Academic-Clinical Professor at the Université Catholique de Louvain.
Professor Hermans specialises in blood coagulation disorders, in particular haemophilia and other inherited bleeding disorders. He also specialises in the diagnosis and treatment of venous and arterial thrombotic diseases. His scientific interests include haemophilia and von Willebrand disease, thrombotic diseases and various rare diseases (angioedema, Osler Rendu disease, etc.).
Professor Hermans has published over 330 scientific articles in various international medical journals. He is Editor-in-Chief of the international journal Haemophilia (WILEY), Editor-in-Chief of Louvain Médical and Associate Editor of Therapeutic Advances in Hematology.
He is a former President of EAHAD. He is currently a member of the Board of Directors of the World Federation of Haemophilia (WFH), and a member of several international scientific societies (EAHAD, ISTH, ASH, etc.) as well as numerous international expert groups.
He is a Fellow of the Royal Colleges of Physicians (London and Edinburgh) and an Associate Member of the Académie Royale de Médecine de Belgique.



Marie-Astrid van Dievoet obtained a master's degree in Medicine from the University of Leuven, Belgium, which was followed by a specialization in Clinical Biology at the University Hospital Leuven. After obtaining her degree in clinical biology, she started at the laboratory department of Cliniques universitaires Saint-Luc where she supervises the coagulation laboratory. The clinical coagulation laboratory realizes both routine and specialized coagulation tests. Since Cliniques universitaires Saint-Luc is a hemophilia reference center, the laboratory is also specialized in diagnosis and follow-up of hemophilia patients. In parallel of her clinical work, she started a PhD under the supervision of Prof. Xavier Stephenne, head of the pediatric gastroenterology and hepatology department, on platelets in chronic liver disease.

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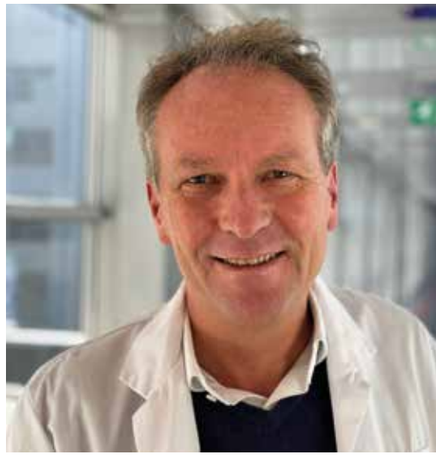
SPONSORED SATELLITE SYMPOSIUM III – CSL BEHRING

Empowering choices in hemophilia care with gene therapy in sight: a triad approach from patient, doctor and patient association

Hemophilia B, also known as Christmas disease, is a hereditary bleeding disorder caused by mutations in the gene for blood coagulation factor IX (FIX) on the X chromosome. The severity of hemophilia B is categorized based on residual FIX activity, with severe cases having less than 1% of normal FIX levels. Traditional treatment for severe hemophilia B involves coagulation factor replacement therapy, where FIX protein concentrates are infused to manage bleeding episodes and prevent spontaneous hemorrhages. The cornerstone of hemophilia B treatment has been the replacement of the deficient FIX protein.
This therapy involves on-demand infusions of FIX concentrates during bleeding episodes and regular prophylactic infusions to maintain FIX activity above 1% of normal levels. However, the short half-life of FIX (18-20 hours) necessitates frequent intravenous infusions, typically twice a week, which can significantly impact the quality of life.
To address the burden of frequent infusions, extended half-life (EHL) FIX products have been developed. These products use technologies to prolong the half-life of FIX. EHL-FIX products can increase the half-life up to 4.8 times compared to standard FIX, reducing the frequency of infusions to once every 7 to 14 days. This advancement has significantly improved the convenience of prophylactic treatment.
In addition to factor replacement therapies, non-factor therapies have emerged as promising alternatives. These therapies aim to rebalance the coagulation system without directly replacing the missing FIX. Gene therapy represents a transformative approach to treating hemophilia B. Given the monogenic nature of the disease, hemophilia B is an ideal candidate for gene therapy, which aims to introduce a functional copy of the FIX gene into the patient's cells. Adeno-associated viruses (AAVs) are commonly used as vectors to deliver the FIX gene to target cells, such as liver cells, which then produce FIX protein. Early clinical trials using AAV-mediated gene therapy have shown promising results, with most patients achieving sustained FIX activity levels sufficient to reduce or eliminate the need for regular FIX infusions. The ongoing development of gene therapy continues to refine the delivery methods and enhance the safety and efficacy of this treatment.
In conclusion, the treatment landscape for hemophilia B has evolved significantly with advancements in coagulation factor replacement therapy, the development of extended half-life FIX products, the introduction of non-factor therapies, and the promising potential of gene therapy. These innovations offer hope for improved management and quality of life for individuals with hemophilia B.



Biographies
Peter Verhamme is Associate professor at the KU Leuven, staff member vascular medicine and haemostasis, department of cardiovascular diseases and professor of Medicine in Centre of Molecular and Vascular Biology at the University Hospitals Leuven. His areas of focus are thrombosis and haemostasis, venous thromboembolism, vascular medicine and prevention. Professor Verhamme is author and co-author of more than 400 publications in the broad field of vascular biology and vascular medicine: from fundamental studies to clinical development, large phase III trials and guidelines.



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 Full Professor at the Medical School of the Catholic University of Louvain in 2012 and Vice-Dean in 2015.
Professor Hermans has (co)-authored more than 360 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.

Sarah De Witte is the president of the patient organisation AHVH. Sarah studied social work and holds a master's degree in criminology and law. She works as a legal expert and is the mother of a son suffering from haemophilia A. The AHVH has always provided the necessary support, information and answers to her questions and supported her family through contact with fellow patients. Sharing experiences with others as a parent and someone with a blood clotting disorder is extremely valuable. She had already been active as a volunteer for several years and is president since 2023 also on a voluntary basis.
The AHVH connects patients with coagulation disorders (and parents, partners, friends, caregivers, sympathizers, etc.) so they can share their stories and tips. Camps where children learn how to self-puncture and play safe sports are organized. There are also camps for teenagers and those over 18. For questions, the AHVH is always available by email and phone. The patient organisation hosts an annual symposium and keeps members informed through newsletters, social media and the website. The patient association offers unlimited home-based education. In addition, the AHVH has developed the be-Coag card that provides access to an online patient record in case of an emergency or for a consultation.



O1

CLINICAL & LABORATORY

Multi-Gene Panel for Thrombophilia Testing in Venous Thromboembolism

Verstraete A[1,2], De Vera M[3], Van Laer C[2,3], Van Thillo Q[1], Baert S[4], Kint C[4], Labarque V[2,5], Van Geet C[2,5], Jacquemin M[2,3], Verhamme P[1,2], Freson K[2], Vanassche T[1,2]

[1]Department of Cardiovascular Diseases, University Hospitals Leuven, Leuven, Belgium, [2]Center for Molecular and Vascular Biology, Department of Cardiovascular Sciences, KU Leuven, Leuven, Belgium, [3]Clinical Department of Laboratory Medicine, University Hospitals Leuven, Leuven, Belgium, [4]Center for Human Genetics, University Hospitals Leuven, Leuven, Belgium, [5]Department of Pediatric Hemato-Oncology, University Hospitals Leuven, Leuven, Belgium

Background: Conventional tests for inherited thrombophilia focus on the five most-established inherited thrombophilias; i.e. deficiencies in natural anticoagulants - antithrombin, protein C, and protein S – as well as gain-of-function variants in coagulation factors, specifically the common factor V Leiden and prothrombin G20210A variants. These tests identify thrombophilia in only about 40% of tested patients with venous thromboembolism (VTE) and their therapeutic value is limited. Therefore, guidelines recommend against their routine use in clinical practice. Over the past decade, next-generation sequencing has been increasingly used in research to detect rare variants in multiple coagulation-related genes, yet its clinical value for VTE remains unknown.

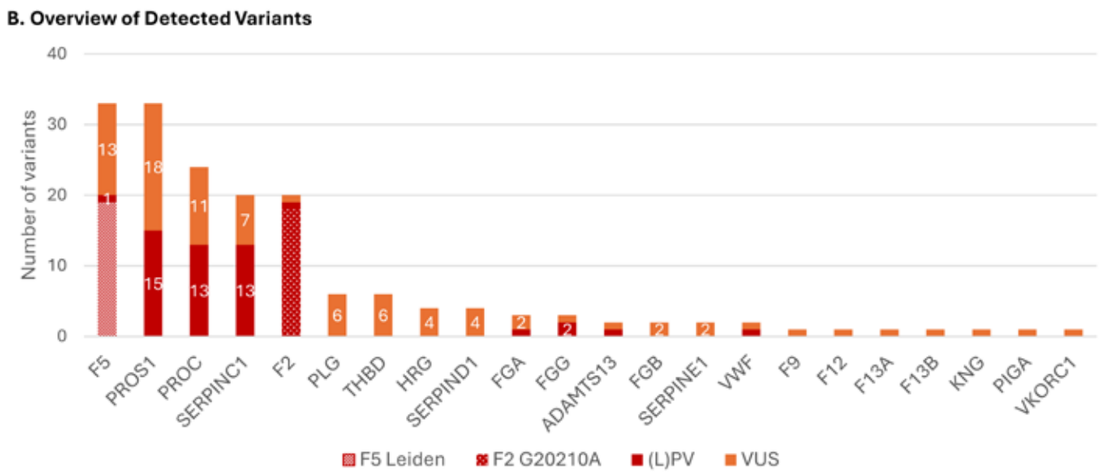
Aims: We aimed to evaluate the use of a multi-gene coagulation panel in the clinical context of VTE and its complementarity to conventional thrombophilia testing. Additionally, we wanted to identify clinical characteristics associated with a positive gene panel result.

Methods: We conducted a single-center retrospective study at the University Hospitals of Leuven, Belgium, and included all VTE patients who underwent testing with a multi-gene panel of 31 diagnostic-grade genes between January 2019 and December 2023. We reported the genetic findings from the multi-gene panel and compared these results with conventional thrombophilia tests. Additionally, we analyzed the impact of various clinical characteristics on the multi-gene panel results.

Results: We included 194 VTE patients who underwent multi-gene panel testing for the investigation of VTE. The mean age at first VTE occurrence was 33 years (+/- 13). Deep vein thrombosis and/or pulmonary embolism were the most common VTE manifestations (n=156, 80%). Nearly half of the patients (n=88, 45%) experienced more than one VTE event prior to multi-gene testing, and 95 patients (49%) had a family history of VTE.

The multi-gene panel identified 171 (anti)coagulation gene variants in 123 VTE patients, resulting in an overall detection rate of 63% (Figure A and B). In 76 patients (39%), at least one (likely) pathogenic variant ((L)PV) was reported, while 47 cases (24%) carried only variants of unknown significance (VUS). Overall, half of the detected variants were classified as (L)PV (figure C). Thirty-six (19%) patients had variants in multiple genes. Interestingly, 22 out of 37 (60%) FVL and/or prothrombin G20210A carriers also harbored additional genetic variants (Figure D). Among the 185 patients with available conventional test results, the multi-gene panel detected non-compatible genetic variants ((L)PV or VUS) in 76 patients (41%), which would remain undetected through conventional tests. Strictly concordant genetic findings were observed in 92 cases (50%). Lastly, in a multivariate logistic regression model – accounting for sex, age at first VTE occurrence, type of VTE history, family history of VTE, and presence of VTE risk factors – family history of VTE was the only characteristic significantly associated with a positive multi-gene panel result (p = 0.008).

Conclusion: The use of the multi-gene coagulation panel for VTE enhances the clinical diagnosis by revealing previously undetected (L)PV and can improve understanding of VTE pathogenesis through the detection of VUS that require additional studies.



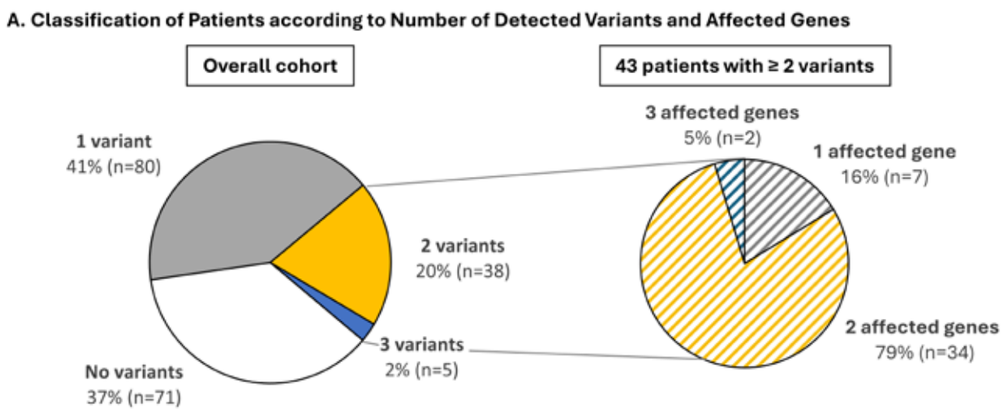


Figure. Results of the multi-gene panel test in 194 index patients with venous thromboembolism.

A: 194 index patients categorized according to the number of reported variants, ranging from none to 3 genetic variants. Sub-chart of 43 index patients with ≥ 2 genetic variants, categorized according to the number of genes in which a variant was found B: Occurrence of all detected genetic variants in the curated diagnostic-grade coagulation related genes. C: Classification of 171 non-unique identified variants as VUS, LPV and PV. D: Overview of additional genetic findings in 19 *F5* Leiden carriers and 18 *F2* G20210A carriers. In approximately 60% of carriers, additional genetic variants were identified.

O2

CLINICAL & LABORATORY

Diagnostic performance of commercial antithrombin activity assays: do we get what we expect?

Orlando C[1], Drèze C[1], Evenepoel A[1], Seneca S[2], Jochmans K[1]
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Background: Hereditary antithrombin (AT) deficiency is a rare autosomal dominant disorder that predisposes to recurrent venous thromboembolism. Diagnosis is based on the measurement of reduced plasma AT activity. However, not all commercial AT activity assays are equally suited for diagnosis.

Aims: To compare the commonly used AT activity assays in their ability to diagnose inherited antithrombin deficiency, with a focus on type II AT deficiencies.

Methods: For the diagnostic performance study, we used samples from genetically confirmed AT deficient subjects (n = 76) and healthy volunteers (n = 23). For the method comparison, samples from patients with request for antithrombin activity for diagnostic work-up (n = 152) were used. Antithrombin activity was measured with five commercial assays (Innovance® Antithrombin, Berichrom® AT, Biophen® AT anti-(h-Xa LRT), HemosIL® Liquid AT and sthem® AT L) on three coagulation analyzers (ACL TOP750 from Werfen, CN6000 from Sysmex and sthemO 301 from Stago).

Results:
Diagnostic performance
The diagnostic sensitivity of the assays varied widely, from 36.8% to 100%. The assay with the best sensitivity (100%) towards all investigated variants was the sthemO® AT L assay. However, this assay showed the poorest specificity: 56.5%. All reagent/analyzer combinations correctly identified variants causing type I AT deficiencies and only 1 variant causing type II Heparin Binding Site deficiency: p.Arg79Cys. The Berichrom® AT and HemosIL® Liquid AT showed the lowest sensitivity: 39.5% and 36.8% respectively. Both assays are almost completely insensitive for the p.Arg45Trp, p.Pro73Leu, p.Arg79His and p.Thr147Ala variants. Their sensitivity towards p.Leu131Phe and p.Thr250Ile is poor, 40% and 25% respectively.
For Biophen® AT anti-(h)-Xa LRT), diagnostic sensitivity differed largely depending on the platform on which the assay was performed: 55.3% on CN6000 compared to 94.7% on ACL TOP750. The major difference between both analyzers is the incubation time of sample with FXa.

Method comparison
Agreement between the assays performed on ACL TOP750 and CN6000 was good. The best agreement was observed for assays performed on CN6000. The results obtained on sthemO 301 are not comparable to any of the other assays, with both proportional and systematic bias. Results were interpreted based on the reference ranges provided by the manufacturer. The highest proportion of abnormal AT activity values was observed for sthemO® AT L (27.8%), due to the lower limit of the reference range of 88%. Seventeen samples with at least 1 AT activity value <75% were subjected to genetic analysis of SERPINC1. A pathological variant was identified in three patients.

Conclusions: Our study was the first to report diagnostic performance data for assays performed on sthemO 301 and CN6000. For one assay, sensitivity differed largely depending on the platform on which it is performed. Comparison of assay characteristics suggests that incubation time of sample and FXa is a crucial driver of the sensitivity towards type II AT variants.
Diagnostic sensitivity varied widely between assays. Nevertheless, assays with poor performance are still widely used. Laboratories should be aware of the limitations of these assays, the importance of adequate reference ranges and that type II antithrombin deficiencies might go undiagnosed.

O3

CLINICAL & LABORATORY

Mendelian randomization implicates thromboembolism in the pathogenesis of long COVID

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[1]KU Leuven, Leuven, Belgium, [2]Broad Institute of MIT and Harvard, Cambridge, USA

Background: Around 5% of individuals infected with SARS-CoV-2 experience long-term symptoms, known as “long COVID”. The biological basis of long COVID, including a potential role for thrombosis and coagulation, remains unclear.

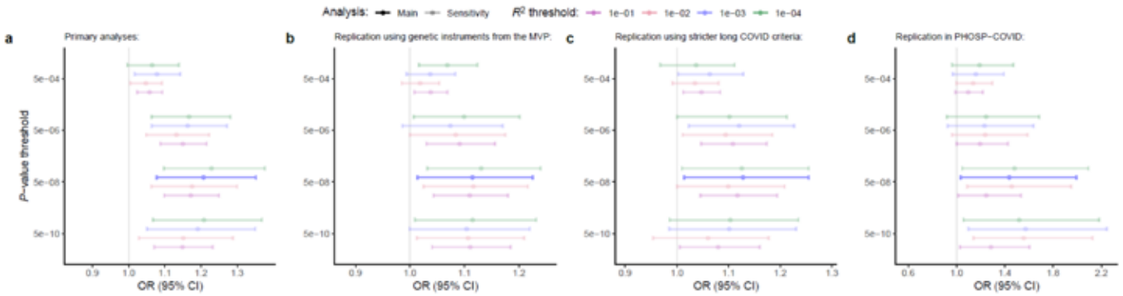
Aim: The present study aimed to test whether genetic predisposition to thromboembolism is associated with a higher risk of long COVID.

Methods: The present study used a Mendelian randomization (MR) approach, which can be used to estimate the association of genetic predisposition to an exposure of interest (e.g., venous thromboembolism) with an outcome of interest (e.g., long COVID). Genetic variants associated with venous thromboembolism were obtained from FinnGen (release 10; N=412,181) and combined into one statistical entity (i.e., a genetic instrument). The association of genetic liability to venous thromboembolism with long COVID was tested in the Long COVID Host Genetics Initiative (N=997,600). Primary analyses tested the association of genetic predisposition to venous thromboembolism with long COVID using genome-wide significant (P<5e-8), uncorrelated (R^2<0.001) variants using the inverse-variance-weighted method. Sensitivity analyses tested for horizontal pleiotropy using pleiotropy-robust methods and explored different instruments constructed using a range of P-value (P<5e-4/5e-6/5e-8/5e-10) and R^2 (R^2<0.1/0.01/0.001/0.0001) thresholds. Replication analyses were performed with external datasets for venous thromboembolism (Million Veterans Program; N=190,266) and long COVID (PHOSP-COVID; N=1,097). Downstream analyses were performed to identify molecular mediators using gene-specific genetic instruments for venous thromboembolism, circulating protein data, and gene expression data from multiple tissues.

Results: Primary analyses revealed that each unit increase in the log-odds of venous thromboembolism was associated with 1.21-fold odds of developing long COVID (95% CI: 1.08-1.35; P=1.2e-3). This association was robust to sensitivity analyses using pleiotropy-robust methods and genetic instruments constructed using different P-value and R^2 thresholds (Figure 1). Replication analyses using external datasets for venous thromboembolism and long COVID confirmed these results (Figure 1). Downstream analyses showed that genetic variants associated with venous thromboembolism near F2R (i.e., the gene encoding the proteinase-activated receptor 1 [PAR-1]) were associated with the greatest risk of long COVID among all coagulation-related genes (OR, 4.50 [95% CI, 1.12-18.16]; P=3.5e-2). Genetically predicted circulating protein levels and multi-tissue expression levels for PAR-1 were robustly associated with long COVID, supporting a mediating role for this platelet- and endothelial cell-derived protein in the association of venous thromboembolism with long COVID.

Conclusions: This study provides human genetic evidence suggesting a role for thromboembolism, and in particular thrombin receptor signaling, in long COVID. Future clinical trials targeting thrombosis-related mediators (e.g., PAR-1) to prevent long COVID may be warranted.

Figure 1. Association of genetic predisposition to venous thromboembolism with long COVID in sensitivity and replication analyses.



Sensitivity analyses tested the association of genetic predisposition to venous thromboembolism with long COVID using the inverse-variance weighted method with genetic instruments constructed using a range of *P*-value (*y*-axis) and linkage disequilibrium R^2 (different colors as indicated) thresholds to construct genetic instruments. The *P*-value threshold determines the minimal association strength for genetic variants included in each genetic instrument, whereas the linkage disequilibrium R^2 thresholds determines the maximal allowed correlation between genetic variants in each genetic instrument. In primary analyses, associations were calculated using data from *FinnGen* and a broad control definition for long COVID from the *Long COVID Host Genetics Initiative*. Replication analyses used genetic instruments constructed from the *Million Veterans Program* (MVP; “Replication using genetic instruments from the MVP”), an alternative—stricter—control definition (“Replication using stricter long COVID criteria”), or data from an external cohort with long COVID ascertainment (“Replication in PHOSP-COVID”). CI indicates confidence interval; OR, odds ratio.

Antithrombin deficiency: report of a rare case homozygous for p.Leu131Phe Heparin Binding Site mutation

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O4

CLINICAL & LABORATORY

Introduction: Antithrombin (AT) is a 58 200 Da glycoprotein serine protease inhibitor (serpin), produced by the liver. AT is a major inhibitor of thrombin, FXa, and other coagulation factors such as FVIIa, FIXa and FXIa, and FXIIa.
Congenital AT deficiency is a rare autosomal dominant thrombophilia that occurs secondary to mutations in the SERPINC1 gene. Most deficient individuals are heterozygotes. Homozygosity is very rare and is associated with severe thrombotic disease. Antithrombin deficiency can be subdivided into type I (quantitative) and type II (qualitative) defects. The prevalence of AT deficiency in the general population is estimated between 1 in 500 and 1 in 5,000 individuals.
Aim: We report a case of a major AT deficiency type II affecting the heparin binding site (HBS) in a 6-week-old baby.

Methods: AT assays
AT activity in platelet poor citrated plasma was measured with a chromogenic assay (Innovance® Antithrombin) on a CS-5100 analyzer (Siemens Diagnostic, Munich, Germany). Antithrombin antigen was measured by rocket immunoelectrophoresis. The normal range for AT is 80-120%.
DNA studies
PCR amplification and sequencing of the coding regions of the 7 exons and parts of the flanking introns of the SERPINC1 gene were performed.

Case report: We report the case of a 6-week-old boy admitted to the emergency department for cough, nasal congestion, and respiratory difficulties requiring hospitalization in the intensive care unit for continuous positive airway pressure therapy. The mother had an AT deficiency (AT 55%) identified during pregnancy, investigated due to a previous pregnancy loss at 33+6 weeks, with no thromboembolic events reported.
The parents are consanguineous of Romanian origin . The father is asymptomatic.
The boy’s results showed very low AT heparin cofactor activity (12%, tested twice in two different samples). His antithrombin antigen level was 59%.
The DNA results revealed a homozygous variant c.391C>T, p.Leu131Phe that confirms the diagnosis of AT deficiency type II HBS. The mother’s genetic analysis showed the same heterozygous pathogenic variant. The father and other family members have not yet been tested. Thus far, the patient (9 months old) is asymptomatic, did not receive anticoagulant treatment and is being monitored closely with evaluations every four months.

Conclusion: The p.Leu131Phe variant was originally described by Olds et al. in 1992 and is also known as the Budapest III variant. The variant originates from a founder effect in the Balkan region. Homozygous AT deficiency is typically considered incompatible with life, except in cases of homozygous HBS mutations. Therefore, the survival and asymptomatic presentation of this newborn represents a very rare case, underscoring the

importance of documenting such rare events. A recent study identified a high prevalence of inferior vena cava anomalies in patients homozygous for the Budapest III mutation.
Homozygous AT type II HBS deficiency is a rare and severe thrombotic condition associated with arterial and venous thrombosis at a young age and complications during pregnancy. It may require anticoagulant prophylaxis depending on individual AT levels. There is currently a need for recommendations for both prophylaxis and therapeutic treatment for the management of this condition.

O5

BASIC SCIENCE

In vitro testing of the anticoagulant capacity of rivaroxaban according to severity of liver disease

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Introduction: Bleeding issues have been considered as a dominant clinical problem in chronic liver disease for many years. This old dogma is being dispelled in favor of the newly emerging concept that the blood coagulation in these patients is rebalanced, meaning that there is a parallel reduction of both procoagulant and anticoagulant factors. These observations suggest that patients with chronic liver disease are not naturally “auto-anticoagulated”. Therefore, they should be considered for specific antithrombotic prophylaxis. There are limited data on the use of direct oral anticoagulants (DOACs) in cirrhotic patients since they have been systematically excluded from clinical trials.

Aims: This study aims to evaluate the anticoagulant effect of the antiXa DOAC rivaroxaban in patients with Child-Pugh A, B and C cirrhosis.

Materials and methods: The first part of the study consisted of a dilution series of FX deficient plasma with normal pooled plasma (NPP) and the second part included samples from cirrhotic patients (n= 30) according to their Child-Pugh score (n=10 in each subgroup A, B, C). Samples were spiked with rivaroxaban (final concentrations: 0, 67 and 300 ng/mL). Thrombin generation assay was analyzed using ST Genesia (Stago) with DrugScreen reagent. Comparisons between the different Child-Pugh stage patient groups were performed using analysis of variance (ANOVA) or the Kruskal-Wallis test.

Results: For the dilution series, as FX levels decreased, lag time (LT) increased and peak height (PH) and endogenous thrombin potential (ETP) decreased, at baseline and more pronounced at higher concentrations of rivaroxaban, most clearly for LT and PH, indicating a correlation between FX concentration and rivaroxaban’s anticoagulant effect. In cirrhotic patients lower FX levels were observed related to the Child-Pugh score. A higher rivaroxaban concentration let to increased LT and decreased PH in the overall patient population and subgroups, depending on the rivaroxaban concentration. However, the anticoagulant effect (percentual change in PH) of rivaroxaban was less pronounced in patients with severe liver disease (Child-Pugh C).

Conclusion: The anticoagulant effect of rivaroxaban in cirrhotic patients varies based on the severity of liver disease and FX levels. These findings raise the question whether dose-adjustments of rivaroxaban and monitoring may be clinically useful in cirrhotic patients.

O6

BASIC SCIENCE

Targeting antigen-specific B cells in immune mediated thrombotic thrombocytopenic purpura using modular CAR T cells

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Background: Treatment of refractory immune-mediated thrombotic thrombocytopenic purpura (iTTP) patients is still an unmet clinical need. Chimeric Antigen Receptor T (CAR T) cell therapy, where CD19 expressing B cells were targeted, has been successfully explored for treating refractory systemic lupus erythematosus. However, this approach targets both the pathogenic and non-pathogenic B cells. Moreover, conventional CAR T cell therapy, in the absence of a regulatory mechanism, has been associated with severe and live-threating adverse events.
A modular variant of CAR, such as the Universal CAR (UniCAR), would be ideally suited to enhance the safety of CAR T cell therapy. To this aim, UniCAR T cells express a universal single-chain Fv (scFv) receptor that binds to a target module (TM). The TM consists of a human La-derived peptide, recognized by the CAR, linked to a protein that binds to the target B cell. Infusing the TM into the patient allows precise control of UniCAR T cell activity. Additionally, if the TM contains the autoantigen (ADAMTS13 in the case of iTTP), only pathogenic B cells will be targeted, preserving the rest of the immune system.

Aim: To test the efficacy of the UniCAR T system in killing hybridoma cells expressing an anti-ADAMTS13 antibody.

Methods: Human T cells were transduced with lentiviral vectors expressing the UniCAR construct. MDTCS (the N-terminal part of ADAMTS13) was linked to the human La-derived peptide and the resulting lentiviral vectors were used to transduce fibroblast 3T3 cells. The TM was purified using Ni2+-NTA affinity column chromatography. Cytotoxicity of the UniCAR T system against the anti-spacer 15D1 hybridoma target cell line was assessed via a flow cytometry viability assay. UniCAR T cells (effector cells) were incubated at different ratios with the target cells in the presence of different concentrations of TM. As a negative control (unspecific killing), UniCAR T cells were incubated with the hybridoma target cells in the absence of TM. Killing of the target cells was evaluated after 24h.

Results: In a first set of experiments, the generated UniCAR T cells were co-cultured with the 15D1 hybridoma target cells using 4 different concentrations of TM: 0.5, 2.5, 5 and 25 nM. For an E:T ratio of 1:1, 15.8%; 18.9%; 19.0% and 25.7% of killing of the hybridoma target cells was observed in the respective concentrations(n=3). In contrast, in the absence of the TM an unspecific background killing of only 4.0% was observed. At a higher E:T ratio of 5:1, a specific hybridoma cell lysis of 31.5%; 46.2%; 47.1% and 50.7% was determined in the presence of the same TM concentrations(n=3). These percentages were significantly higher than the ones observed with the unspecific killing (14.0%).

Conclusion: Our study shows that the UniCAR T cell strategy can be used to specifically kill hybridoma cells that express the 15D1 anti-ADAMTS13 antibody on their surface. The efficacy of the system is currently being assessed towards human Nalm-6 cells expressing a human anti-ADAMTS13 antibody on their surface. Finally, the efficacy of this therapy will also be studied in a mouse model of iTTP.

imMKCL-based Glanzmann Thrombastenia models to study platelet formation and function

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Introduction: The main limitation of next generation sequencing studies for inherited platelet disorders (IPD) remains the detection of numerous variants of unknown significance (VUS) and variants in novel genes with unknown roles in platelets and megakaryocytes (MK). A cell-based model that allows gene/variant characterisation is required that mimics MK/platelet formation and function defects found in IPD patients.

Aims: This research aims to develop such a model using genetically modified immortalized megakaryocyte cell lines (imMCKLs) (Nakamura et al., cell stem cell, 2014). This is a proof-of-concept study for Glanzmann Thrombasthenia (GT), a well-known IPD caused by integrin α2bß3 defects. We have studied MK/platelet formation and function from ITGB3-/- imMKCLs.

O7

BASIC SCIENCE

Methods: MKs and platelets from four ITGB3-/- imMKCLs were produced in static, turbulent (shaking flasks) and 3D (silk bone marrow model, Di Buduo et al., blood, 2015) conditions and characterised using flow cytometry, immunoblotting, and imaging.

Results: GT MK showed absent expression of α2bß3 and binding to fluorescent-labelled fibrinogen (Fig.1A). These MK showed normal differentiation, polyploidisation, and expression of GPIb/IX/V in static cultures. However, the GT clones form significantly less proplatelets than WT imMKCL (Fig.1B). Platelet formation from imMKCLs could only be studied in turbulent and 3D conditions (Fig.1C). GT imMKCLs seem to produce less platelets in turbulent (63±19 %) and 3D (60±25 %) conditions (p<0.01). As expected, there is an absent PAC1 binding in GT platelets formed in turbulent conditions (p=0.0170, Fig.1D).

Conclusions: This study shows that genetically modified imMKCL can serve as a disease model to study IPD. We observed a reduction in platelet production in our ITGB3-/- model compared to WT, which was not described in GT patients previously. However, in 7 out of 12 of our GT patients, a platelet count in the lower normal range (<180 G/L) is observed (Figure 1E). This study demonstrates that in-depth characterization of diseases in the lab using a reliable cell model can enhance our understanding of specific diseases, which might otherwise be overlooked.

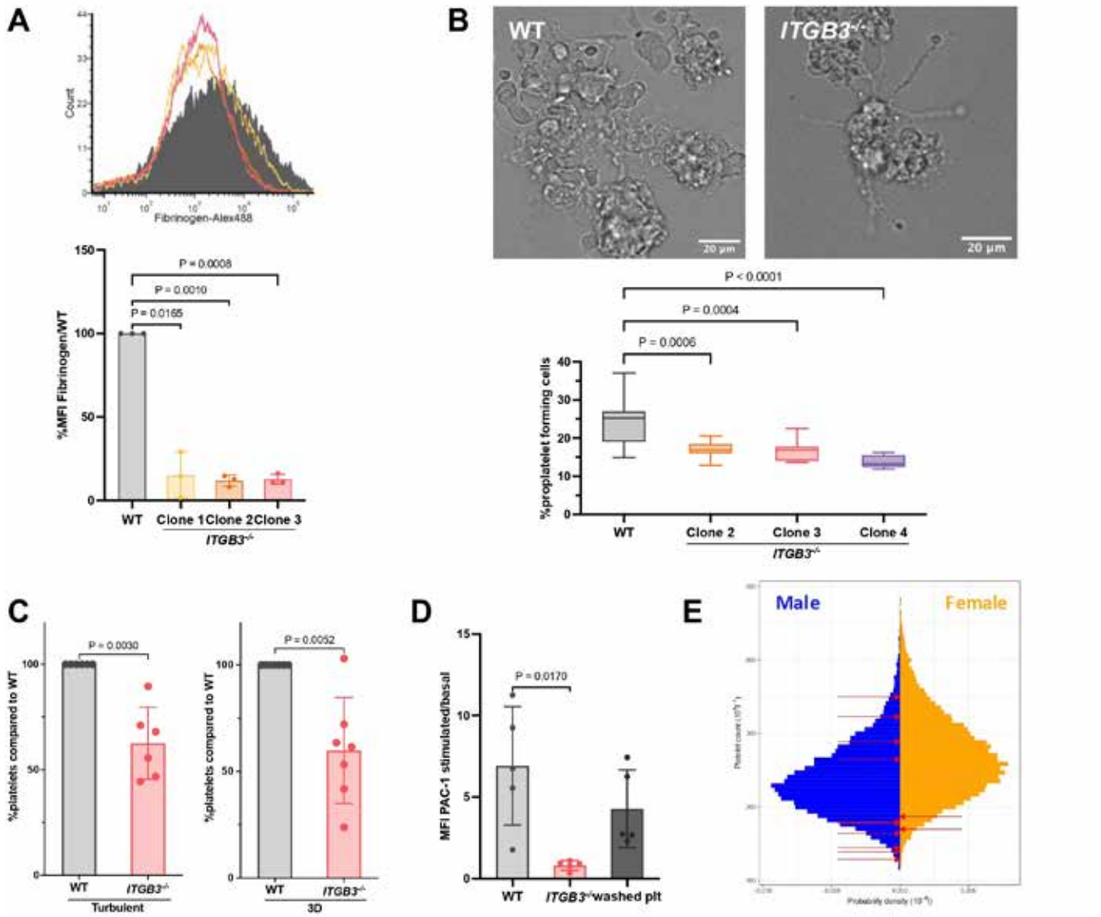


Figure 1. Characterisation of our Glanzmann Thrombasthenia (GT) imMKCL disease model using static, turbulent and 3D culture conditions. A) Representative example of fibrinogen binding experiment for WT and GT megakaryocytes (MK) (top) and quantification of three experiments (bottom) GT MK showed no binding to fibrinogen compared to WT imMKCL. B) representative examples of proplatelets from WT and ITGB3-/- imMKCL (top) and proplatelet formation assessment of day 5 differentiated WT and ITGB3-/- imMKCL of three blinded experiments (bottom). Less proplatelets were formed in the GT clones. C) GT megakaryocytes (MK) form less platelets in shaker flasks (turbulent, left) and silk bone marrow model (3D, right) conditions compared to WT imMKCL. D) GT platelets formed in turbulent conditions show absent PAC-1 binding compared to WT platelets. E) A gender-stratified histogram of platelet count including 48,345 measurements from donors in the INTERVAL randomized controlled trial (Moore et al., Trials, 2014). Red arrows indicate the mean platelet count of the 12 GT patients in our database. All statistical analyses were performed using paired t-test or ANOVA (groups).

O8

BASIC SCIENCE

Conformational opening of ADAMTS13 does not necessarily indicate preactivation of its metalloprotease domain

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Background/Introduction: ADAMTS13 exists in a closed conformation, maintained by non-covalent interactions between the Spacer and CUB domains. Additionally, the active site cleft within the metalloprotease (MP) domain is blocked in this autoinhibited conformation. In our previous work, we demonstrated that certain monoclonal antibodies (mAbs) targeting the ADAMTS13 Spacer and CUB1 domains not only disrupt this Spacer-CUB interaction, but also preactivate the MP domain. Hereto, we used our mAbs 1C4 (anti-Spacer) and 6A6 (anti-MP) which specifically recognize epitopes that only become accessible upon allosteric ADAMTS13 activation. However, ADAMTS13 is a large, multi-domain enzyme and it remained unknown how targeting other domains than the Spacer and CUB domains affect its conformation and preactivation. Understanding these effects is essential to better comprehend the ADAMTS13 allosteric regulation.

Aims: To assess the effect of mAbs targeting each individual ADAMTS13 domain on its conformation, preactivation and proteolytic function.

Methods/Materials: A panel of 46 mAbs was used to target each ADAMTS13 domain. In ELISA, their ability to conformationally open plasma ADAMTS13 (via 1C4 exposure) and to preactivate its MP domain (via 6A6 exposure) was verified. A subset of these mAbs was functionally characterized using a VWF96 proteolysis ELISA to assess mAb-induced changes in catalytic efficiency. Michaelis-Menten kinetics were applied to evaluate how these mAbs affected VWF substrate turnover and binding.

Results: When targeting each individual ADAMTS13 domain, mAbs successfully disrupted the Spacer-CUB interaction, leading to the conformational opening of ADAMTS13 as indicated by the exposure of the 1C4 Spacer epitope. After this opening, mAbs against most domains also preactivated the MP domain, making the 6A6 epitope accessible. Unexpectedly, when targeting the D, T1, T7 and CUB2 domains, a subset of mAbs could open ADAMTS13 but did not preactivate the MP domain, as the 6A6 epitope remained inaccessible. Interestingly, VWF96 proteolysis assays revealed that some anti-D domain mAbs, which exposed the 1C4 Spacer epitope but not the 6A6 MP epitope, inhibited the ADAMTS13 catalytic efficiency, as indicated by a significant decrease in substrate turnover. Alternatively, anti-CUB2 mAbs that induced exposure of the 1C4 Spacer epitope but not of the 6A6 MP epitope, enhanced proteolytic activity as demonstrated by increased VWF turnover in Michaelis-Menten kinetic analyses.

Summary/Conclusions: We investigated how mAbs targeting each ADAMTS13 domain affects its conformation, preactivation and proteolytic activity. Using mAbs targeting each ADAMTS13 domain individually, the closed, autoinhibited conformation of plasma ADAMTS13 was successfully opened. Unlike previously studied anti-Spacer and anti-CUB1 mAbs, a subset of mAbs against the D, T1, T7 or CUB2 domains could conformationally open ADAMTS13 without preactivation of its MP domain. Interestingly, this subset included mAbs that either activated (anti-CUB2) or inhibited (anti-D) ADAMTS13 proteolytic function, as reflected by corresponding changes in VWF substrate turnover. Thereby, our data correspond to the idea that upon mAb-induced conformational ADAMTS13 opening, diverse molecular mechanisms can not only conformationally preactivate, but also may conformationally restrain the ADAMTS13 MP domain to diminish its enzymatic function. Thereby, the molecular mechanism for ADAMTS13 opening is linked to, but differs from the mechanism that allosterically regulates the MP domain.

O9

BASIC SCIENCE

An in-house ex-vivo NETs induction model for the evaluation of potential NETosis inhibitors

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Background: NETosis is a type of neutrophil cell death that contributes to the development of thrombosis and disseminated intravascular coagulation by inducing a pro-inflammatory and procoagulant environment, ultimately resulting in multiorgan failure.

Aims: This study aims to evaluate the capacity of a novel NETosis monitoring assay to detect the potency of compounds known to be NETosis inhibitors.

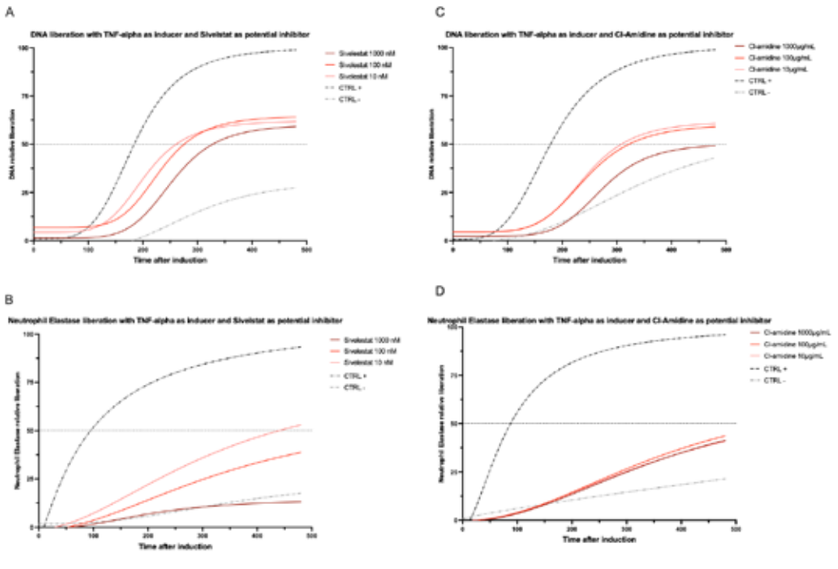
Methods: We developed an ex-vivo model to evaluate the NETosis and the impact of two potential NETosis inhibitors, sivelestat and Cl-amidine on the NETosis process. Neutrophils were extracted from whole blood of a healthy donor. Tumor necrosis factor-alpha was used to trigger NETosis. The detection of NETs was carried out using specific fluorescent markers that target DNA and neutrophil elastase (NE). Results were expressed as the percentage of DNA/NE released as a function of time. Time to cross 50% release (T50) and area under the curve (AUC), representing total NETosis potential, were computed.

Results: We used two different fluorescent tags in this study, and we could observe two different liberation kinetics. Regarding the DNA release using the sytox green reagent, there is a clear sigmoid kinetic model (Figure 1.A;1.C) while the release of NE using the MeOSuc-Ala-Ala-Pro-Val peptide, is more described by first order association kinetics model (Figure 1.B;1.D). For the DNA release, the three sivelestat concentration tested cross the 50% of DNA relative liberation ranging from 263.0 min (95% CI: 229.7 – 296.3 min) for the 10 nM solution to 335.3 min (95% CI: 298.8 – 371.1 min) for the 1000 nM solution (Figure 1.A). Compared to sivelestat, a dose-dependent effect is not observed with cl-amidine, suggesting some mechanism of action rapidly reach their maximal effect on NETosis inhibition (Figure 1). For the DNA release, two of the three cl-amidine concentrations tested cross the 50% of DNA relative liberation ranging from 325,8 min (95% CI: 261,1 – 390,4 min) for the 10 µg/mL solution to 335,3 min (95% CI: 262,4 –

420,6 min) for the 100 µg/mL solution (Figure 1.C). The highest concentration of cl-amidine don't cross the 50% of DNA relative liberation and the T50 is 472,5 min (95% CI: 454,2 – 490,8 min). For the NE release, none of the cl-amidine concentration crosses the 50% of NE relative liberation (Figure 1.D). AUCs of each inhibitor concentrations were significantly different from the AUC of the positive control.

Conclusion: We successfully developed an ex-vivo model to analyze the NETosis. In this study we analyzed the impact of sivelestat and cl-amidine as two potential NETosis inhibitors. We demonstrated a strong impact of sivelestat and cl-amidine on NETosis with two different fluorescent markers. Results show that sivelestat and cl-amidine partially inhibits the NETosis process for the three concentrations we analyzed. Both the initiation and the amplitude of NETosis are reduced with the sivelestat in a dose-dependent manner. 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)** *Evolution of the DNA release as function of time using TNF-alpha as inducer and sivelestat as potential inhibitor.* **(B)** *Evolution of the NE release as function of time using TNF-alpha as inducer and sivelestat as potential inhibitor.* **(C)** *Evolution of the DNA release as function of time using TNF-alpha as inducer and cl-amidine as potential inhibitor.* **(D)** *Evolution of the NE release as function of time using TNF-alpha as inducer and cl-amidine as potential inhibitor.*



O10

BASIC SCIENCE

Sustainable polymers with improved biocompatibility: new materials for blood-contacting medical devices

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Nowadays, the only effective treatment for most patients with heart valve disease is valve replacement by implantation of mechanical or biological prostheses. However, mechanical valves represent high risk of thromboembolism, and biological prostheses are prone to early degeneration. The emergence of a non-thrombogenic, durable, polymeric heart valve therefore appears as a promising solution. Polyurethanes (PUs) have adjustable mechanical properties which make them suitable for a wide range of applications, including in the biomedical field. Historically, these PUs have been synthesized from toxic isocyanates, representing an environmental and public health threat. This has encouraged the search for safer and more environmental-friendly synthetic routes, leading today to the production of non-isocyanate polyurethanes (NIPUs).

One of the mail goals of our project was therefore to develop new NIPU-based prosthetic heart valves, together with other NIPU-based blood-contacting medical devices. For that, different routes for NIPUs synthesis were explored, as well as diverse processing techniques. In vitro and in vivo testing were perfomed on these novel NIPUs, representing the first ever full characterization of NIPUs in terms of biological interactions.

In a first approach, thermally crosslinked NIPU networks were synthesized, tested in vitro, and used to produce aortic heart valves. Injection molded NIPU valves were produced and tested in a pulse duplicator, showing compliant hydrodynamic performances, comparable to bioprostheses used in clinics. Subsequently to this work, other NIPUs, more adequate for easily adaptable manufacturing techniques (e.g. 3D printing), were explored. Given the unique viscous properties of some of these NIPUs, formulations for light-based 3D printing were successfully developed and objects were printed with a resolution down to the micrometer scale. Finally, a more recent class of NIPUs was synthesized, aiming to use heat-based additive manufacturing to process such biomaterials. A new thermoplastic elastomer was produced, processed by different manufacturing techniques, and evaluated in vitro and in vivo, after subcutaneous implantation in rabbits. Regarding the hemocompatibility of NIPU, the ensemble of the three classes of NIPUs developed showed no hemolytic effects, very low platelet adhesion on the polymeric surfaces, and longer clotting times than medical grade PU (control).

All the developed NIPUs showed remarkable hemo/biocompatibility in vitro, key for blood-contacting applications.

P01

ABSTRACTS
POSTERS

Novel cryptic epitopes in ADAMTS13 indicate a resemblant open conformation in immune-mediated thrombotic thrombocytopenic purpura

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Background/Introduction: Plasma ADAMTS13 (pADAMTS13) is secreted in a closed, autoinhibited conformation, where its CUB domains interact with its spacer domain. Recent structure predictions suggest that in the closed state, additional domains besides the spacer and CUB domains possibly engage in interdomain interactions. In immune-mediated thrombotic thrombocytopenic purpura (iTTP), patient IgG autoantibodies trigger ADAMTS13 to adopt an open conformation, which is specifically detected by our monoclonal antibody (mAb) 1C4 that recognizes a cryptic spacer epitope. This open ADAMTS13 conformation has gained clinical relevance as a biomarker for diagnosing acute and subclinical iTTP and monitoring an increased relapse risk in remission patients. However, it remains unclear whether iTTP patients display one resemblant open or different open ADAMTS13 conformations.

Aims: To identify additional mAbs that recognize cryptic epitopes in pADAMTS13 and investigate whether iTTP patients present one resemblant or different open conformations.

Methods/Materials: Anti-ADAMTS13 mAbs were screened in ELISA to identify those that bind cryptic epitopes. Cryptic epitopes were defined as those not accessible in closed pADAMTS13 that expose after conformational opening using our anti-CUB1 mAb 17G2. ADAMTS13 antigen and conformation were evaluated in 25 healthy donors (HDs) and 25 acute iTTP patients using in-house ELISA assays. Total IgGs were purified from iTTP patients and added in ELISA to closed pADAMTS13 from HDs.

Results: In addition to our anti-spacer (1C4) and anti-metalloprotease (6A6) mAbs, four mAbs were identified that recognized previously unidentified cryptic epitopes in the ADAMTS13 disintegrin-like (1D5), seventh (9C12) and eight (19H4) thrombospondin type-1 repeat and CUB1 (10D2) domains. Screening of HDs using each mAb defined a cut-off for distinguishing the open and closed pADAMTS13 conformations. Nearly all HDs showed a natively closed pADAMTS13 conformation with all cryptic epitopes concealed. Upon 17G2 preincubation, pADAMTS13 opened, exposing all cryptic epitopes. Three HDs exhibited a partially open conformation as both 6A6 and 9C12 epitopes were exposed, while the 1C4, 1D5, 19H4 and 10D2 epitopes remained concealed. Interestingly, all iTTP patients exhibited a resemblant open pADAMTS13 conformation with all cryptic epitopes being exposed, both with and without 17G2 preincubation. Moreover, incubation of closed pADAMTS13 from HDs with the total IgG fraction of an iTTP patient induced the same resemblant open conformation with all cryptic epitopes exposed.

Summary/Conclusions
We identified mAbs that recognize previously unknown cryptic epitopes in various ADAMTS13 domains which become exposed upon mAb-induced conformational opening. This finding supports the existence of a compact, closed conformation of ADAMTS13 where multiple domains likely interact with each other. Nearly all HDs presented this natively closed conformation, while all iTTP patients exhibited a resemblant open conformation. We also confirmed that pathological iTTP patient autoantibodies can induce this resemblant open ADAMTS13 conformation. Our findings thus highlight that native, closed ADAMTS13 likely circulates in a compact, folded conformation and that iTTP patients have a resemblant open conformation during acute phase.

P02

ABSTRACTS
POSTERS

Development and in vitro evaluation of a novel UniCAR T cell therapy targeting B and plasma cells for autoimmune disease treatment

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Background/Introduction: Chimeric antigen receptor (CAR) T cell therapy has demonstrated to be very effective in treating B cell cancers and has become a standard of care. Recently, CAR T cell therapies targeting B cells in refractory autoimmune disease patients, like systemic lupus erythematosus (SLE) have also been explored. While this approach holds promise, it carries risks, such as cytokine release syndrome (CRS). Therefore, switchable adaptor platforms are being investigated. One such alternative is the Universal CAR T (UniCAR T) platform. In this system, a target module (TM) acts as a bridge between the UniCAR T cell and the target cell. The TM is composed of a single-chain variable fragment (scFv), that recognizes the target cell, coupled to a human La-derived peptide. This epitope in the TM is recognized by another scFv expressed on the UniCAR T cell. This interaction leads to the destruction of target cells. This design allows the therapy to be switched on or off depending on the presence or absence of the TM. Whether this therapy is successful to treat refractory immune-mediated thrombotic thrombocytopenic purpura (iTTP) is currently not known.

Aims: In this study, we aimed to use the UniCAR T adaptor platform to study in vitro destruction of cells expressing the CD19 B cell marker and the CD38 plasma cell marker.

Methods/Materials: Stable 3T3 fibroblast cell lines were transduced with lentiviral vectors expressing anti-CD19 and anti-CD38 TMs. TMs were purified via Ni2+-NTA affinity column chromatography. The quality of the TMs was evaluated through SDS-PAGE and Western Blot analysis. T cells from different healthy donors were transduced using lentiviral vectors encoding the CAR construct. Nalm-6 cells were used as target cells as they express both CD19 and CD38. These target cells were co-incubated with UniCAR T cells, with and without the corresponding TM, to assess the cytotoxic efficacy of this therapy. Additionally, an in-house cell-based ELISA was used to measure the binding affinity of both TMs to their respective targets.

Results: After successfully generating UniCAR T cells and both anti-CD19 and anti-CD38 TMs, the cytotoxic potential of the UniCAR T cells was evaluated. Both anti-CD19 and anti-CD38 TM bound with high affinity to the Nalm-6 target cells (Kd of 5.08 nM and 6.07 nM respectively). Incubation of UniCAR T cells together with the target cells in a 2:1 ratio in the presence of 5 pmol of either the anti-CD19 or the anti-CD38 TM led to 74% and 56% killing of the target cells respectively. This killing was specific as the incubation of UniCAR T cells and the target cells without the TMs led to a background killing of the target cells of 14%.

Summary/Conclusion: We here showed that the UniCAR T adaptor platform is ideally suited to kill CD19 and CD38 expressing cells. We are now investigating the efficacy of the platform to selectively kill anti-ADAMTS13 Ab producing cells in vitro and in vivo in a mouse model for iTTP.

P03

ABSTRACTS POSTERS

Asundexian and milvexian: Pharmacodynamic insights from coagulation assays

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Introduction: Over the last few years, factor eleven (FXI) inhibitors have been investigated in arterial and venous thromboembolic diseases. However, the sudden interruption of the OCEANIC-AF Phase 3 trial (NCT05643573) comparing asundexian to apixaban in atrial fibrillation has raised concerns regarding the ability of asundexian, but also other molecules such as milvexian, to prevent thromboembolism in this indication.

Aims: We aimed to investigate the impact of therapeutically plausible concentrations of asundexian and milvexian on different coagulation assays to compare the pharmacodynamic profiles of these anticoagulants.

Materials and methods: Normal pooled plasma (Namur Exchange Biobank, Namur, Belgium) was spiked with different solutions of asundexian and milvexian (dilution factor=20) to yield final concentration ranges of 0-2000 ng/mL (asundexian) and 0-5000 ng/mL (milvexian). Thrombin Generation Assay (TGA) was performed on a Calibrated Automated Thrombogram (CAT). Each plasma solution was tested in the presence of four different reagents: 1 pM Tissue factor (TF), 4 µM phospholipids (PL) (PPP-Reagent Low); 5 pM TF, 4 µM PL (PPP-Reagent); 20 pM TF, 4 µM PL (PPP-Reagent High) and a mixture of ellagic acid 0.42 µM and purified phosphatides (Actin FS®). Thrombin generation was triggered by adding FluCa Reagent (fluorogenic substrate, buffer, calcium chloride). aPTT (activated partial thromboplastin time) and prothrombin time (PT) assays were performed on an ACL-TOP 700 (HemosIL® reagents) and a STA R MAX (other reagents). The aPTT was realized with four different reagents: HemosIL® SynthASil (activator= silica), STA®-C.K. Prest (activator=kaolin), STA® PTT Automate 5 (activator=silica) and Actin FS® (activator= ellagic acid). Two prothrombin time (PT) assays were measured with the following reagents: HemosIL®RecombiPlasTin 2G (human recombinant tissue factor) and STA®-NeoPTimal 5 (rabbit brain tissue factor). Statistical analyses were performed with the GraphPad Prism software version 10.3.1 (Boston, Massachusetts USA). The relations between the concentration of inhibitors and the parameters of the different assays were described by one-phase decay equations and inhibitory concentrations 50 (IC50) or double clotting times were computed.

Results: In the aPTT assay and TGA milvexian globally showed lower IC50 and two-fold clotting times than asundexian. Both molecules had no impact on the PT assays. (detailed results in Table 1).

Conclusions: These results indicate that milvexian might be a more potent inhibitor of intrinsic pathway-dependent coagulation and that asundexian would require higher plasma concentrations to exert similar effects on coagulation assays.

Table 1. Pharmacodynamic parameters from the aPTT, PT and TGA assays performed in normal pooled plasma spiked with asundexian (0-2000 ng/mL) or milvexian (0-5000 ng/mL)

Test	Analyzer	Reagent	Parameter (ng/mL)	asundexian	milvexian
Thrombin Generation Assay	Calibrated Automated Thrombogram (CAT)	PPP-Reagent Low	2x Lag time (ng/mL) [95% CI]	n.c. (>2000)	n.c. (>5000)
		PPP-Reagent		n.c. (>2000)	n.c. (>5000)
		PPP-Reagent High		n.c. (>2000)	n.c. (>5000)
		Actin FS*		n.c. (>2000)	n.c. (>5000)
		PPP-Reagent Low	IC_{50} ETP (ng/mL) [95% CI]	n.c. (>2000)	643.8 [558.2 – 742.4]
		PPP-Reagent		n.c. (>2000)	n.c. (>5000)
		PPP-Reagent High		n.c. (>2000)	n.c. (>5000)
		Actin FS*		n.c. (>2000)	n.c. (>5000)
		PPP-Reagent Low	IC_{50} Peak (ng/mL) [95% CI]	1876.0 [1598.1 – >2000]	419.4 [360.4 – 489.9]
		PPP-Reagent		n.c. (>2000)	1893.8 [1633.4 – 2188.7]
		PPP-Reagent High		n.c. (>2000)	n.c. (>5000)
		Actin FS*		n.c. (>2000)	486.0 [435.1 – 542.8]
		PPP-Reagent Low	2x Time to peak (ng/mL) [95% CI]	n.c. (>2000)	n.c. (>5000)
		PPP-Reagent		n.c. (>2000)	n.c. (>5000)
		PPP-Reagent High		n.c. (>2000)	n.c. (>5000)
		Actin FS*		n.c. (>2000)	599.8 [580.3 – 620.2]
		PPP-Reagent Low	IC_{50} mVRI (ng/mL) [95% CI]	1428.4 [1053.6 – 1855.3]	342.0 [287.3 – 409.8]
		PPP-Reagent		n.c. (>2000)	1008.6 [833.6 – 1225.5]
		PPP-Reagent High		n.c. (>2000)	3791.9 [3558.8 – 4227.5]
		Actin FS*		1435.3 [1214.9 – 1743.4]	262.8 [228.9 – 303.3]
aPTT	ACL-TOP 700*	HemosIL* SynthASil	2x aPTT [95% CI]	n.c. (>2000)	1570.9 [1478.7 – 1669.4]
	STA R MAX*	STA*-C.K. Prest		1806.4 [1607.4 – >2000]	648.8 [583.2 – 786.8]
		STA* PTT Automate 5		n.c. (>2000)	1173 [924.3 – 1521.9]
		Actin FS*		1291.6 [1098.4 – 1473.2]	608.8 [531.2 – 701.0]
PT	ACL-TOP 700*	HemosIL* RecombiPlasTin 2G	2x PT [95% CI]	n.c. (>2000)	n.c. (>5000)
	STA R MAX*	STA*-NeoPTimal 5		n.c. (>2000)	n.c. (>5000)

aPTT, activated partial thromboplastin time; PT, prothrombin time; TGA, thrombin generation assay; PPP, platelet poor plasma; 95%CI, 95% confidence interval; 2x, two-fold; IC_{50}, inhibitory concentration 50; ETP, endogenous thrombin potential; mVRI, mean velocity rate index; n.c., not calculable as the value of the parameter corresponding to 50% of inhibition or the two-fold clotting time does not appear in the range of tested concentrations (no possible interpolation)
The TGA results in the presence of tissue factor trigger reagents (PPP-Reagents) were retrieved from Vassart, Douxfils et al, *Thrombosis research, 236*, 236–239. https://doi.org/10.1016/j.thromres.2024.03.001. All the other results have not been published yet. The commercial names of the reagents and coagulometers were included to ensure scientific transparency and conciseness.

Acquired von Willebrand syndrome secondary to monoclonal gammopathy: a single center case series

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P04

ABSTRACTS POSTERS

Background: Acquired von Willebrand syndrome (AVWS) is a rare bleeding disorder with a similar bleeding phenotype to congenital von Willebrand disease (VWD). AVWS can arise in patients with monoclonal gammopathy. Management of the bleeding phenotype in this subset of patients can be particularly challenging (1). To this day, clear recommendations on how to handle AVWS secondary to monoclonal gammopathy are lacking.

Aims: Our aim was to characterize the clinical and laboratory findings in AVWS secondary to monoclonal gammopathy at our center.

Methods: We retrospectively analyzed the data of adult patients who were diagnosed at University Hospitals Leuven between 2002 and 2023. All laboratory analyses were performed following routine clinical practice.

Results: Seven patients with a monoclonal gammopathy were diagnosed with AVWS. Most patients had a monoclonal gammopathy of unknown significance (MGUS) according to IMWG (2) at diagnosis (n = 6) with either monoclonal IgG (n = 5) or IgM (n = 1). One patient had a Waldenström macroglobulinemia at diagnosis. Two patients progressed to smoldering myeloma and multiple myeloma, respectively. Patients with IgG MGUS generally responded well to intravenous immunoglobulins (IVIG) (Figure 1). Notably, one patient who did not respond, also had likely pathogenic (3) heterozygous mutations in the von Willebrand factor (VWF) and fibrinogen gamma chain (FGG) genes. The IgM patients were treated with either VWF/FVIII concentrate alone, or a combination of VWF/FVIII concentrate, rituximab and methylprednisolone due to a lack of clinical benefit of IVIG. The patients with Waldenström's and multiple myeloma were treated accordingly.

Conclusion: ematologists should think of the association between monoclonal gammopathy and AVWS. As suggested in the literature (4–6), we confirm the effect of IVIG in IgG-associated AVWS. In case of poor response, sequencing of the VWF gene might be insightful. For IgM-associated AVWS, we observed better results administering VWF/FVIII concentrate or a combination of therapies.

References

1. Franchini M, Mannucci PM. Acquired von Willebrand syndrome: Focused for hematologists. Haematologica. 2020;105(8). doi:10.3324/haematol.2020.255117
2. Kyle RA, Durie BGM, Rajkumar S V., et al. Monoclonal gammopathy of undetermined significance (MGUS) and smoldering (asymptomatic) multiple myeloma: IMWG consensus perspectives risk factors for progression and guidelines for monitoring and management. Leukemia. 2010;24(6). doi:10.1038/leu.2010.60
3. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5). doi:10.1038/gim.2015.30
4. Mannucci PM, Lombardi R, Bader R, et al. Studies of the pathophysiology of acquired von Willebrand's disease in seven patients with lymphoproliferative disorders or benign monoclonal gammopathies. Blood. 1984;64(3). doi:10.1182/blood.v64.3.614.614
5. Federici AB, Stabile F, Castaman G, Canciani MT, Mannucci PM. Treatment of acquired von Willebrand syndrome in patients with monoclonal gammopathy of uncertain significance: Comparison of three different therapeutic approaches. Blood. 1998;92(8). doi:10.1182/blood.v92.8.2707.420k25_2707_2711
6. Abou-Ismail MY, Rodgers GM, Bray PF, Lim MY. Acquired von Willebrand syndrome in monoclonal gammopathy – A scoping review on hemostatic management. Res Pract Thromb Haemost. 2021;5(2). doi:10.1002/rth2.12481

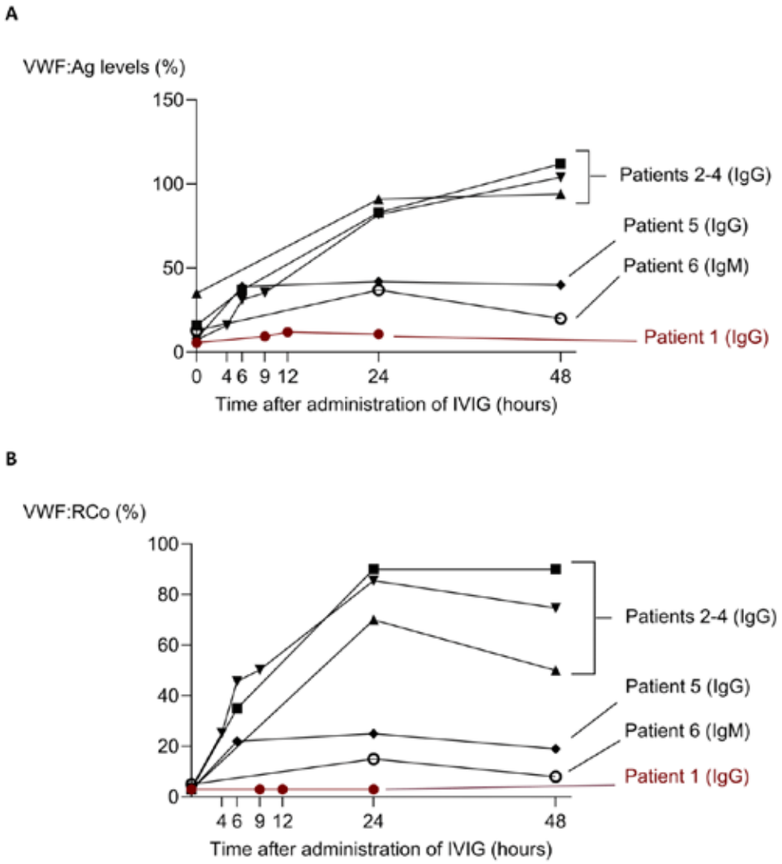


Figure 1: The VWF:Ag levels (A) and VWF:RCo (B) increased after administration of IVIG in the majority of cases with IgG MGUS. Three out of five patients with IgG MGUS (patient 2-4) responded to IVIG (VWF:Ag and VWF:RCo > 50%). Out of the two other cases, one had an additional *VWF* mutation (patient 1, indicated in red). The patient with IgM MGUS (patient 6) did not respond. IVIG: intravenous immunoglobulins.

P05

ABSTRACTS
POSTERS

Current practices for Lupus Anticoagulant in Belgian laboratories and comparison with the updated 2020 International Society on Thrombosis and Haemostasis Scientific and Standardization Committee guideline

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Background/Aims: Our study aimed to investigate the practices in measurement of Lupus anticoagulant (LA) in Belgian laboratories and to verify these findings against the 2020 International Society on Thrombosis and Haemostasis Scientific and Standardization Committee (ISTH-SSC) Guideline (J Thromb Haemost. 2020;18:2828–39).

Methods: A survey, interrogating pre- to post-analytical aspects of LA analysis, was distributed by Sciensano to all Belgian laboratories (n=111) certified for autoimmune, coagulation and haematology testing.

Results: Fifty-eight percent (%) of the Belgian laboratories participated. Sixty-six percent of the responders performed LA analysis, of which 12% were private, 69% hospital and 19% university laboratories. Seventy-eight percent of the laboratories performed LA analysis on (double-centrifuged) thrombocyte-free citrated plasma. The procedure to obtain thrombocyte-free plasma was variable. Only 19% performed LA testing in parallel with other antiphospholipid (aPL) tests (anticardiolipin and anti-ß2 glycoprotein I antibodies). Additional testing of prothrombin time, activated partial thromboplastin time (aPTT) and thrombin time was only performed in 36% of laboratories. Anti-Xa assays were only rarely (7%) performed and independently of heparin use. For the LA-specific assays, most laboratories (90%) performed 2 test panels: the dilute Russell's viper venom time (dRVVT) and aPTT, of which 79% was based on silica activation. For the dRVVT and aPTT panel, respectively, 82% and 78% of the laboratories performed the mixing and confirmation test only if the screening test was prolonged. Analytically, both panels were mostly determined on Sysmex, Werfen or Stago devices using a variety of reagents. Usually, commercial lyophilised (56%) or frozen (44%) normal pooled plasma (NPP) (83%) was used for mixing tests, mostly (98%) in a 1:1 NPP:patient plasma ratio. NPP was seldomly (12%) prepared in-house, using a variable number of donors. dRVVT and aPTT panels were interpreted using a normalized clotting time ratio for the screening (resp. 77% and 80%), mixing (resp. 76% and 70%) and confirmation step (resp. 80% and 54%). For the final interpretation, the normalized ratio of screening over confirming was mostly used (resp. 80% and 54%). Both manufacturers' (resp. 50% and 53%) and own study data (resp. 38% and 41%) were used as cut-off values to interpret dRVVT and aPTT panel results. LA was reported with a final conclusion in 61% of laboratories. Only 34% reported an analytical result for every step per panel. 88% of laboratories added an additional comment to the test results, generally after a positive result (94%). All laboratories applied a barring period, after an initial positive result, usually of 12 weeks (88%). LA detection in patients receiving direct oral anticoagulants was done by 66% (27/41) of laboratories, usually after absorption of the DOAC with active charcoal (74%). LA testing for vitamin K antagonist and heparin-treated patients is done by 54%, regardless of international normalized ratio (64%) or anti-FXa results (82%).

Conclusions: Our survey revealed variability in LA analysis performed by Belgian laboratories, specifically in the pre- and postanalytical phase. The two test panel and the three-step method are well applied. However, additional efforts are required to further implement the 2020 ISTH-SSC guidelines, harmonizing LA analysis practices.

P06

ABSTRACTS
POSTERS

Development of a microfluidic assay to study intrathrombus leukocyte crawling

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Introduction: During thrombus formation, interactions between leukocytes and platelets form the basis of thromboinflammation, the complex interplay between coagulation and inflammation. Leukocytes are recruited to the thrombus by release of chemoattractants from platelets leading subsequently to leukocyte adhesion, integrin activation and eventually migration through the thrombus. Microfluidic parallel-plate flow chambers are used to mimic these physiological processes in vitro by mimicking blood vessels and allowing precise control over shear forces. Current microfluidics assays studying thromboinflammation are limited in time (≤ 30 minutes).

Aim: We aimed to develop a microfluidic assay to study intrathrombus leukocyte migration over a longer time period to study platelet-leukocyte interactions in process beyond hemostasis.

Methods: A glass coverslip was coated with microspots of fibrillar collagen type I and recombinant human tissue factor. Venous whole blood was collected from healthy human volunteers into sodium citrate vacutainers containing corn trypsin inhibitor (5µM) to prevent coagulation by contact activation. In the first phase (30 minutes), stimulating thrombus formation, the blood was recalcified with 10mM $CaCl_2$ and perfused through the parallel-plate flow chamber

at a shear rate of 1000s-1. During the following 30 minutes, the flow conditions were the same but dabigatran (0.5mM) was added to the perfused blood to limit excessive coagulation and obstruction of the microchannel. From minute 60 onwards, the shear rate was reduced to 100s-1 to support leukocyte adhesion, rolling and crawling. Visualization of leukocytes was by labeling with DRAQ5 (10µM). Images were acquired on a ZEISS Axio Observer Z1 and analyzed with ImageJ using the TrackMate plugin.

Results: Firm platelet adhesion was observed during the first minutes of flow and resulted in initiation of coagulation and eventually a stable thrombus was formed. In this first thrombus forming phase, the number of leukocytes attached to the thrombus was close to zero. Only upon decreasing the shear rate, a significant number of leukocyte tethered onto the thrombus followed by firm adhesion and eventually intrathrombus crawling. Tracking of the leukocyte movement onto the thrombus was performed for six biological repeats and various parameters were determined in a reproducible manner. Parameters that could be analyzed from this set-up are track displacement, track mean speed and confinement ratio, amongst others.

Conclusion: A novel microfluidic assay was developed to allow long-term investigation of leukocyte mi-gration into thrombi in vitro. The assay will be further used to provide insights into key cell-cell interactions between platelets and leukocytes during thromboinflammation over a long time period.

P07

ABSTRACTS
POSTERS

Asundexian and milvexian: What impact on laboratory coagulation assays?

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Introduction: In recent years, factor eleven (FXI) inhibitors have emerged as promising anticoagulants. Among them, the small molecules asundexian and milvexian are currently being investigated in Phase 3 clinical trials. However, their overall effects on coagulation assays remain largely unknown and will be needed to interpret correctly laboratory results once these drugs are on the market.

Aims: We aimed to assess the impact of therapeutic plasma concentrations of asundexian and milvexian on different coagulation assays.

Materials and methods: Normal pooled plasma (Namur Exchange Biobank, Namur, Belgium) was spiked with different solutions of asundexian and milvexian to yield final concentration ranges of 0-2000 ng/mL (asundexian) and 0-5000 ng/mL (milvexian). The following tests were performed on these samples using an ACL-Top 700 analyzer: fibrinogen quantitation (Clauss method, HemosIL® Fibrinogen-C) and PT-derived method, HemosIL®RecombiPlasTin 2G), an aPTT-based protein C assay using Protac® as protein C activator (HemosIL® ProClot), a latex particle-based agglutination assay for free protein S determination (HemosIL® Free PS), one-stage aPTT-based FXI, FXII, FIX , FVIII assays and one-stage PT-based FII, FV, FX, FVII assays (HemosIL® Factor Deficient plasmas), dilute Russel's viper venom assays (HemosIL® dRVVT Screen and Confirm). Statistical analyses, including the one-way ANOVA test, were performed using the GraphPad Prism software version 10.3.1 (Boston, Massachusetts USA).

Results: Asundexian and milvexian slightly impacted the PT-derived fibrinogen quantitation, the protein S assay, the one-stage PT-based FV, and FVII assays (P>0.05, Table 1.). Both inhibitors showed a more pronounced impact on the protein C assay (P>0.05). Asundexian and milvexian exerted a negligible effect on the dRVVT assays (variable statistical significance for the different concentrations of inhibitors). The impact of milvexian on the Clauss fibrinogen assay, the 1-stage PT-based FII and FX assays was slightly more pronounced than that of asundexian and statistically significant for some concentrations of milvexian. Milvexian and, to a lesser extent, asundexian reduced the activity of FXI, FXII, FIX, and FVIII in the 1-stage aPTT-based assays. However, this effect was only statistically significant for the highest concentrations of milvexian (2000-5000 ng/mL, Table 1.).

Conclusions: Although further investigation in patients treated with asundexian and milvexian is required to generate coagulation testing recommendations, one should consider the possible impact of milvexian and asundexian, on some common coagulation assays.

Table 1. Results of the coagulation assays in normal pooled plasma spiked with asundexian (0-2000 ng/mL) or milvexian (0 to 5000 ng/mL)

Coagulation assay	Reagent(s)	Method	Inhibitor	Variation from baseline (%), [inhibitor] = 2000 ng/mL	Statistical significance
Fibrinogen (Clauss method)	HemosIL® Fibrinogen-C	Chronometric	asundexian	↓4.4%	ns
			milvexian	↓7.1%	**100-250 and 2000 ng/mL *500 ng/mL ns 1000 ng/mL ***5000 ng/mL
Fibrinogen (PT-derived)	HemosIL® RecombiPlasTin 2G	Chronometric	asundexian	↑1.8%	ns
			milvexian	↑2.6%	ns
Protein C	HemosIL® ProClot	Chronometric	asundexian	↑7.0%	ns
			milvexian	↑6.0%	ns
Protein S	HemosIL® Free Protein S	Immuno-turbidimetric	asundexian	↑2.6%	ns
			milvexian	↑2.0%	ns
FXI (1-stage aPTT-based assay)	HemosIL®FXI Deficient Plasma HemosIL® SynthASil	Chronometric	asundexian	↓14.6%	ns
			milvexian	↓42.5%	**2000 ng/mL ****5000 ng/mL
FXII (1-stage aPTT-based assay)	HemosIL®FXII Deficient Plasma HemosIL® SynthASil	Chronometric	asundexian	↓20.8%	ns
			milvexian	↓61.8%	**2000 ng/mL ***5000 ng/mL
FIX (1-stage aPTT-based assay)	HemosIL®FIX Deficient Plasma HemosIL® SynthASil	Chronometric	asundexian	↓16.9%	ns
			milvexian	↓46.0%	*2000 ng/mL ***5000 ng/mL
FVIII (1-stage aPTT-based assay)	HemosIL®FVIII Deficient Plasma HemosIL® SynthASil	Chronometric	asundexian	↓16.7%	ns
			milvexian	↓40.4%	*2000 ng/mL ***5000 ng/mL
FII (1-stage PT-based assay)	HemosIL®FII Deficient Plasma HemosIL® RecombiPlasTin 2G	Chronometric	asundexian	↓3.8%	ns
			milvexian	↓8.4%	*2000 ng/mL **5000 ng/mL
FV (1-stage PT-based assay)	HemosIL®FV Deficient Plasma HemosIL® RecombiPlasTin 2G	Chronometric	asundexian	↓1.3%	ns
			milvexian	↓2.5%	ns
FX (1-stage PT-based assay)	HemosIL®FX Deficient Plasma HemosIL® RecombiPlasTin 2G	Chronometric	asundexian	↓5.3%	ns
			milvexian	↓7.2%	*5000 ng/mL
FVII (1-stage PT-based assay)	HemosIL®FVII Deficient Plasma HemosIL® RecombiPlasTin 2G	Chronometric	asundexian	↓2.5%	ns
			milvexian	↓2.6%	ns
dRVVT (low [phospholipids])	HemosIL® dRVVT Screen	Chronometric	asundexian	0%	***100-500 ng/mL ns 1000-2000 ng/mL
			milvexian	↓2.8%	*100 and 2000 ng/mL ns 250 ng/mL ***500-1000 ng/mL **5000 ng/mL
dRVVT (high [phospholipids])	HemosIL® dRVVT Confirm	Chronometric	asundexian	↑2.1%	ns
			milvexian	↑2.1%	**5000 ng/mL

[phospholipids], concentration of phospholipids. Statistical significance was assessed using one-way ANOVA with Tukey's multiple comparison test. The degree of significance refers to the comparison of the results for a specific concentration of inhibitor with the baseline value of the assay (normal pooled plasma spiked with phosphate buffer saline, dilution factor=20). ns= not statistically significant. *:P-value <0.05. **: P-value <0.01. ***:P-value<0.001. ****:P-value <0.0001. The variation from baseline value of each test is given for a concentration of 2000 ng/mL of each inhibitor. The commercial names of the reagents and coagulometers were included to ensure scientific transparency and conciseness.

Correlation of anti-Xa activity and activated partial thromboplastin time in patients on unfractionated heparin therapy

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P08

ABSTRACTS
POSTERS

Introduction: Activated partial thromboplastin time (APTT) has long been the method of choice to monitor the anticoagulant effect of unfractionated heparin (UFH). Recent studies advise monitoring UFH therapy by the anti-Xa assay in parallel with APTT. As anti-Xa activity reflects the concentration of heparin in the blood, it is less prone to fluctuations caused by others factors (e.g. inflammation or factor depletion) as APTT. However, the anti-Xa assay is not accessible in every routine laboratory and APTT and anti-Xa levels do not always correlate, especially in critically ill patients. International guidelines for monitoring UFH anticoagulation are lacking.

Aims: We aimed to evaluate the correlation between anti-Xa activity and APTT for patients receiving UFH in the Antwerp University Hospital. Moreover, we looked into the effects of possible confounding factors (fibrinogen, antithrombin, C-reactive protein (CRP), total, direct and indirect bilirubin and free hemoglobin) and compared patients on extracorporeal membrane oxygenation (ECMO) with patients not on ECMO.

Methods: We performed a retrospective analysis of 40 patients who received UFH at the Antwerp University Hospital. Anti-Xa activity and APTT were both measured on the Sysmex CN6000 analyzer with respectively the Innovance Anti-Xa assay and the Dade Actin FS Activated PTT reagent (Siemens Healthineers). Pearson correlation between anti-Xa and APTT was assessed using SPSS. To test for possible effects of confounding factors, one-way MANOVA was carried out between groups based on the therapeutic targets. For this analysis, we used a therapeutic target of 0.3-0.7 IU/mL for anti-Xa, as described in recent studies, which corresponds with an APTT of 60-100 s.

Results: The correlation between anti-Xa and APTT was moderate for the entire dataset, r = 0.575 (p<0.001, N=1489). Interestingly, the interindividual correlations ranged widely from no correlation to very strong correlations (r = 0.8-1.0). The correlation for patients not on ECMO was stronger (r=0.598, p<0.001), than for patients on ECMO (r=0.436, p<0.001). APTT and anti-Xa were discordant in 30.5% of all samples. Only 10.7% of all samples had an APTT and anti-Xa level between the therapeutic target. Most samples were under the therapeutic target for both methods (52.5%), 30% of the samples were in the therapeutic range for anti-Xa, but had an APTT <60 s and 3.5% of the samples were in the therapeutic range for APTT, but had an anti-Xa level <0.3 IU/mL. For most patients on ECMO a therapeutic target of anti-Xa between 0.2-0.4 IU/mL is used in our hospital, which probably explains the large group of samples under the predefined therapeutic target. One-way MANOVA showed differences in CRP, fibrinogen and antithrombin between groups based on the therapeutic targets.

Conclusions: Although anti-Xa and APTT are correlated, their correlation seems to be dependent on different factors. Firstly, we see interindividual differences in the strength of the correlation. Also the use of ECMO seems to have an impact. Moreover, inflammatory parameters like CRP and fibrinogen have an effect on this correlation, as well as antithrombin. These issues will have to be taken into account when international guidelines for monitoring UFH anticoagulation are compiled.

P09

ABSTRACTS
POSTERS

Unraveling in vitro megakaryopoiesis

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Introduction: Megakaryopoiesis is the process by which hematopoietic stem and progenitor cells (HSPCs) differentiate into megakaryocytes (MKs) that ultimately yield platelets in a subsequent process termed thrombopoiesis. In adult healthy humans, these processes primarily take place in the bone marrow where MKs produce 10^11 platelets per day at steady state. Mimicking this process in vitro has been a major challenge. Although feasible, it is inefficient typically yielding 10 to 50 fold fewer platelets per generated MK compared to in vivo.

Aims: This study was designed to characterize the various developmental stages of MK differentiation in vitro. By gaining a better understanding of in vitro megakaryopoiesis, this research aims to ultimately enhance the efficiency of the in vitro platelet production.

Methodology: CD34+ cell enrichment is achieved using anti-CD34 magnetic beads (EasySep from Stemcell Technologies). Subsequently, cells are FACS sorted to obtain a CD34+CD38+/-Lin- HPSC population with >95% purity. Lineage markers, including CD71, CD123 and CD61, are used to minimize outgrowth of non-megakaryocyte lineage-committed cells. Next, the cells are expanded and differentiated towards megakaryocytes according to an established culturing protocol. Flow cytometry was used for kinetically phenotyping the differentiated MKs.

Results: To unravel the differentiation pathway of MKs in vitro, CD235a+CD71high cells biased towards erythroid development, were excluded. In the CD235a-CD71dim cell population a clear cellular trajectory was identified based on the expression of two early MK markers, CD123 and CD41. First, HSPCs acquire CD123 before upregulating CD41. Subsequently, these CD123+CD41+ MK precursors lose expression of CD123 and gain higher CD41 expression. Their further developmental trajectory was determined by analyzing the expression of CD61 and CD42b on day 12. The CD123+CD41+ population first acquires CD61 but still lacks CD42b expression. Subsequently, CD42b expression is upregulated in the more mature CD123-CD41+ population. Interestingly, donor-dependent differentiation trajectories were identified. The first trajectory follows the indirect trajectory via a CD123+CD41+ subpopulation as described above. Contrasting, some donors follow a direct trajectory (via CD123-CD41dim subpopulation) towards CD41 expressing MKs, without the expression of CD123. Finally, a combinatorial trajectory where both subpopulations, CD123+CD41+ & CD123-CD41dim, are present was observed as well.

Conclusion: The preliminary data from this study enhances our basic understanding of MK development in vitro. Here, donor-dependent in vitro differentiation trajectories of HSPC towards MKs were identified. Notably, all donors ultimately achieve similar levels of maturation. Future work will involve an in-depth characterization of the identified subpopulations using bulk RNA analysis. The role of key transcription factors in MK development will be explored by CRISPR/Cas9-mediated deletion experiments.

P10

ABSTRACTS
POSTERS

Activated platelets release two isoforms of glycocalicin with different isoelectric points

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Introduction: GPIbα is a unique and abundant receptor on platelets essential for adhesion to extracellular matrix proteins at high shear rates. The soluble GPIbα ectodomain called glycocalicin can be cleaved from the membrane surface in a process called proteolysis, catalyzed by the metalloproteinase ADAM17. GPIbα is proteolyzed from platelets at a constitutive but slow rate in circulation or when stored ex vivo. The rate of proteolysis can be significantly increased during strong and sustained platelet activation.

Aim: We aimed to purify significant quantities of glycocalicin from the supernatant of strong and sustained activated platelets to further unravel its biological role.

Method: Platelets, obtained from buffy coat pooled platelet concentrates, are washed before stimulation with calcium ionophore for 180 minutes. Glycocalicin in the activated platelet supernatant is primarily purified by wheat germ agglutinin (WGA) agarose. A second purification step is done by anion exchange chromatography at pH 7.4 using a DEAE column. Consecutive chromatography steps are conducted with AKTATM Start chromatography system (Cytiva). Total protein concentration is determined with a NanoDrop spectrophotometer or a bicinchonic assay (BCA) kit. Glycocalicin levels are determined using an-in house glycocalicin ELISA. Sample purity is checked by silver staining.

Results: Initially, the platelet supernatant of activated platelets from one platelet concentrate contains 357 ± 104 µg glycocalicin which is approximately 0.1% of the total protein mass. Following dialysis and WGA agarose chromatography, the purity of glycocalicin in the protein sample increased to 15% with only limited elution of glycocalicin in the unbound flow through fraction. In the final anion exchange chromatography step, purity further increased to more than 90% but the yield in the elution fractions was only 14 ± 6 µg per platelet concentrate which corresponds to 20%. Of note, the majority of glycocalicin eluted in the flow through or unbound fraction during anion exchange chromatography (73 ± 33 µg).

Conclusion: We are able to purify glycocalicin from the supernatant of activated platelets. Two isoforms of glycocalicin are in the sample at physiological pH, one negatively charged(pI < 7.4) retrieved from the elution fractions and one positively charged isoform (pI ≥ 7.4) in the flowthrough fractions during DEAE chromatography. The ratio of acid vs. neutral/basic isoform is 1:5.

Lupus Anticoagulant and coagulation testing: Why reagent choice matters

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P11

ABSTRACTS
POSTERS

Introduction: In recent years, considerable efforts have been made to improve the quality and reliability of lupus anticoagulant (LA) testing, a key laboratory marker for diagnosing antiphospholipid syndrome (APS). While it is commonly known that LA testing is sensitive to preanalytical and analytical factors such as reagent choice and the use of anticoagulants, the impact of LA on other coagulation tests like prothrombin time (PT)/international normalized ratio (INR) is less well known. LA can artificially elevate INR by interfering with phospholipid-dependent coagulation factors, leading to an overestimation of anticoagulation in patients on vitamin K antagonists (VKA), a common treatment for many LA-positive patients.

Case Report: A 83-year-old woman with a history of low-grade B-cell non-Hodgkin lymphoma (Ann Arbor stage IVA, currently untreated) presented at a hematology consultation of the Antwerp University Hospital with an unexplained prolonged activated partial thromboplastin time (APTT) of 89.8 s (Dade Actin FS Activated PTT reagent (Siemens Healthineers); normal range: 23-31 s) and an INR of 2.11 (Dade Innovin PT reagent (Siemens Healthineers); normal range: 0.9-1.2). Both APTT and PT were normal in 2019. The patient had no symptoms of excessive bleeding and was not on anticoagulation therapy. Given her asymptomatic presentation and previously normal coagulation tests, a circulating anticoagulant such as LA was suspected. Laboratory investigations confirmed a strongly positive LA, with elevated anticardiolipin IgM and anti-beta2-glycoprotein I IgM levels. However, without a history of thromboembolic or obstetric events, a diagnosis of APS was not confirmed.
Due to the unexplained prolonged PT at presentation, vitamin K-dependent coagulation factors were also tested and returned abnormal: FII: 22% [normal range: 87-153%], FVII: 3% [normal range: 58-198%], and FX: 8% [normal range: 77-189%]) using Dade Innovin ; FIX levels were normal using Dade Actin FS . FV was undetectable (<3% [normal range: 77-148%]). Reassessment of the coagulation factors with another PT reagent (STA-Neoplastine (Stago Diagnostica)) normalized the factor levels (FII: 60%, FV: 76%, FVII: 103%, FX: 91%). Determination of factor levels with Dade Innovin on a dilution series of the patients plasma confirmed the presence of an inhibitor. These results suggest analytical interference by the LA antibodies.

Discussion: LA significantly affects many thromboplastin reagents, leading to greater INR variability in LA-positive patients compared to those without LA, complicating anticoagulant management with VKA. Since LA antibodies are heterogeneous, their impact on INR can vary depending on the reagent used. Previous studies have shown that Dade Innovin is particularly sensitive to LA, though this effect is sporadic. In our case, a spontaneously elevated INR of >2 was observed, even in the absence of anticoagulant therapy. This elevation was associated with reduced PT-related coagulation factors, which could easily lead to misinterpretation by clinicians unfamiliar with the impact of LA on PT reagents. This case highlights the critical importance of understanding the properties of coagulation reagents when interpreting results in LA-positive patients to prevent misdiagnosis or inappropriate treatment.

Lower reporting of venous thromboembolisms events with estetrol-drospirenone compared to estradiol and ethinylestradiol containing pills: A disproportionality analysis of the Eudravigilance database

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P12

ABSTRACTS
POSTERS

Background: The safety of combined oral contraceptives (COCs), particularly those containing synthetic estrogens like ethinylestradiol (EE), has been closely monitored due to their increased venous thromboembolism (VTE) risk. Epidemiological studies classify VTE risk based on COC components, but such phase 4 studies for newer COCs may take 5 to 7 years. Pharmacovigilance data analysis can accelerate the identification of drug-related safety signals or reassure on the safety profile. This study evaluates the VTE risk of newer COC formulations with natural estrogens, such as estradiol (E2) and estetrol (E4), using data from the EudraVigilance (EV) database.

Methods: We conducted a disproportionality reporting rate (DPRR) analysis of VTE events associated with various COC formulations by extracting individual case reports from EV database up to July 28th 2024. The study compared the proportionality reporting rate (PRR) between natural estrogen-based COCs (E2 and E4) and conventional synthetic estrogen-based COCs (EE), with a comparison to EE-levonorgestrel (EE-LNG).

Results: The analysis revealed that COCs containing natural estrogens exhibited significantly lower PRRs for thrombotic events compared to EE-based COCs. Specifically, E4-drospirenone (E4-DRSP) showed the lowest PRR (0.12) similar to progestin-only pills. EE-drospirenone (EE-DRSP) had the highest PRR (2.25), suggesting an increased thrombotic risk.

Conclusion: The study supports the safer thrombotic profile of natural estrogen-based COCs, particularly E2 and E4 formulations, over synthetic estrogen-based COCs containing EE. These findings advocate for the preferential prescribing of COCs with natural estrogens to mitigate thrombotic risks, aligning with a shift towards safer contraceptive options in clinical practice.

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ABSTRACTS POSTERS

Results of a 2023 survey on the current practices on antiphospholipid antibody testing with focus on immunoassays in Belgian laboratories

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Background/Aim: Antiphospholipid syndrome (APS) diagnosis relies on the detection of antiphospholipid (aPL) antibodies, including lupus anticoagulant (LA), anticardiolipin (aCL) IgG/IgM, and anti-beta2-glycoprotein I (aß2GPI) IgG/IgM. We aimed to evaluate the routine practices of aCL and aß2GPI testing in Belgian laboratories.

Methods: A survey interrogating aPL diagnostics was distributed by Sciensano to all Belgian laboratories (n=111) certified for autoimmune, coagulation and haematology testing.

Results: Fifty-eight percent (%) of Belgian laboratories [22% primary care, 64% secondary care, 14% tertiary care] responded. Most laboratories (63%) offered aCL and aß2GPI independently and upon request.
When aPL testing was done, at least aCL IgG testing was performed (77%). The total panel of IgG/M aCL/aß2GPI testing was analyzed in-house by 42% of the responders, and 36% also offered LA testing. Seventy-eight percent of the university hospitals performed all four aPL immunoassays. If a laboratory did not perform the full aPL panel, tests were, upon request, outsourced under controlled conditions.
aCL and aß2GPI tests were mainly performed in autoimmunity departments (77%) using serum samples. Fluorescence enzyme immunoassay (FEIA) and chemiluminescence immunoassay (CLIA) were the most used assay methods. Results were mainly reported quantitatively (70%). Eighteen percent of the laboratories add semi-quantitative interpretations. Within the same assay methodology, the units and cutoff values varied significantly. Post-analytically, 43% of respondents indicated the use of a barring period, after a first positive result (95%) and to a lesser extent after a negative result (14%) or a second positive result (5%). Only 38% of laboratories added a comment suggesting repeat testing after a positive result.

Conclusion: The survey highlights the need for aCL and aß2GPI harmonisation in testing practice and between assays, directly impacting the accuracy of APS diagnosis across the country.

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ABSTRACTS POSTERS

Algorithms using CS-5100 and Siemens reagents for the detection of a lupus anticoagulant

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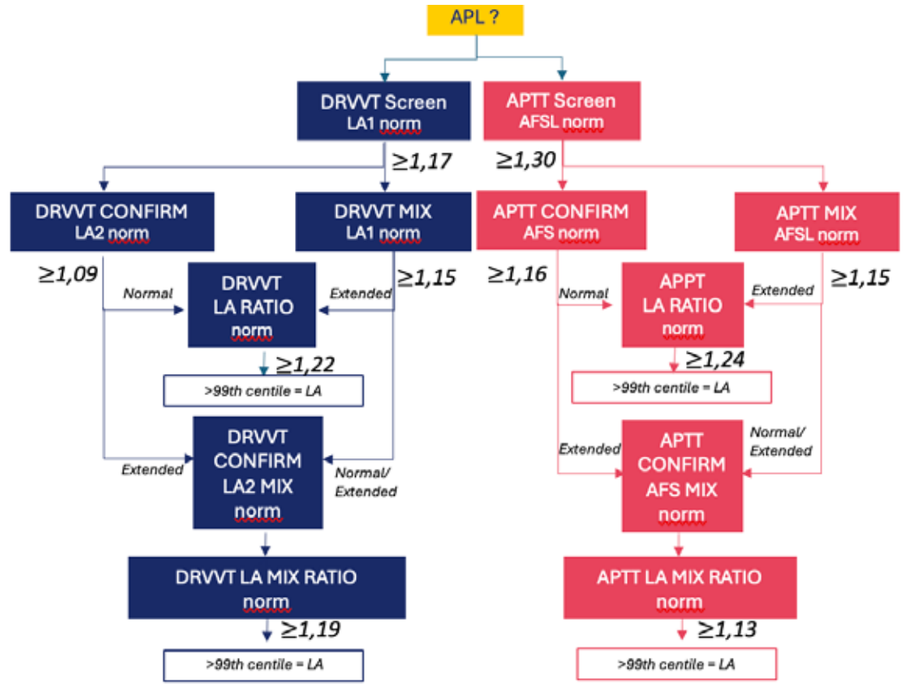
Antiphospholipid syndrome is a challenging diagnosis requiring a thrombotic event or a pregnancy related morbidity with persistently positive laboratory criteria such as lupus anticoagulant, anti-cardiolipin IgG/IgM and anti-ß2glycoproteine I IgG/IgM.
Lupus anticoagulants are autoantibodies directed against phospholipid-binding plasma proteins inducing paradoxically a hypercoagulable state in vivo but that extends phospholipids-based test such as APTT and DRVVT.

The aim of this study was to establish a clear flow chart and cut-off using Siemens CS 5100 instruments and reagents based on the guidance from the scientific and standardization committee for lupus anticoagulant of the ISTH.

Fresh blood drawn on 0.109M citrate from 120 healthy subjects were double centrifuged and plasma was frozen within 4h. NPP normalized “screen, mix and confirm” APTT and DRVVT were measured, and 99th centiles were taken as cut-off. Flow chart and cut-off were then confronted with 15 positive LA samples from a reference lab in Leuven Belgium using competing reagents. APTT was measured using the ellagic acid-based APTT reagents from Siemens with soy and rabbit phospholipids. Actin FSL® (lupus sensitive) is poor in phospholipids and was the screening reagent. Actin FS ® (factor sensitive), which is our routine APTT reagent, is rich in phospholipids, insensitive to lupus and was the confirmation reagent. A mixing test was realized by a 1:1 dilution of patient with normal pool plasma in first instance with the screening and confirmation test according to guidance. Screen, mix and confirm of the diluted russel viper venom times (DRVVT) were obtained with LA1 and LA2 reagents. Lupus ratio (screen/confirm) was the final readout for both APTT and DRVVT paths. Lupus Mix ratio (screen mix / confirm mix) was used to conclude if the confirmation test was still extended. Samples were judged positive if one of the paths (APTT or DRVVT) were positive.

DRVVT is the most sensitive test with a positive APTT LA ratio in 66,6% and a positive DRVVT LA ratio in 93,3% of the 15 LA positive samples. The reference lab found 60% of positive sample in the APTT path and 100% of the sample in the DRVVT path. Our algorithm found one more sample positive in the APTT path meaning that our cut off and reagents are sensitive for LA detection.
One sample is DRVVT negative in our hand and could be a false positive (just above the cut-off) of the reference lab.

Conclusion: Siemens reagents on the CS 5100 are sensitive and specific tests for lupus detection. Cut-off and flow chart have been hereby established for sensitive LA detection and have proved efficient compared to reference lab using competing reagents. Noteworthy false positive should be avoided due to potential lifelong treatment and those cut off seems to be specific enough in routine practice.



A hereditary thrombocytopenia responding to thrombopoietin-mimetics

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We describe here a patient with hereditary thrombocytopenia, initially diagnosed as autoimmune purpura and successfully treated with thrombopoietin mimetic.

Macrothrombocytopenia is detected at the age of 10 years in the preoperative evaluation for cryptorchidism. It fluctuates between 30 to 60,000 /mm³ without treatment and is considered as probably autoimmune. The patient presents with a minor hemorrhagic diathesis characterized mainly by ecchymoses. In 2021, in a preoperative context, a corticosteroid treatment induces only a transient platelet increase. Thereafter a treatment with the thrombopoietin mimetic eltrombopag induces a platelet increase up to 100.000/mm³ and the resolution of the hemorragic diathesis. Under this treatment, this increase has remained stable to this day.

The absence of normal platelet count in history, the very constant nature of thrombocytopenia and a notion of an analogous phenomenon in a second-degree relative induce a diagnosis re-evaluation.

P15

ABSTRACTS POSTERS

Platelet function tests reveal a clear CD41a (GP IIb-IIIa) deficiency at 24.385 sites/cell (normal value of 37.000 – 65.000) with a very low CD41/CD42 ratio of 0.44 (normal value 0.89 - 1.81). Platelet aggregation shows hypoaggregability to various agonists except ristocetin. We also note the absence or a reduction of secretion to all the inducers tested.

Genetic analysis shows X-linked problem. The patient is hemizygous for the mutation c.652G>A (p.Asp2l8Asn) in the GATA1 gene (OMIM*305371). This gene controls the expression of a large number of genes in many cell lines and is essential, among other things, for the differentiation of erythrocytes and megakaryocytesis. This mutation is associated with thrombocytopenia with or without dyserythropoietic anemia. It has already been described with substantiated functional data (Hermans et al 2014, Bastida et al. 2022) and is likely pathogenic.

Eltrombopag has already been successfully tested in various inherited thrombocytopenia (Zaninetti et al 2020) but data remain scares, mainly for long term treatment. A misdiagnosis allows us to demonstrate a 3 years efficiency of a thrombopoietin-mimetic in this congenital thrombocytopenia, both on platelet count and clinical haemorrhagic diathesis.

P16

ABSTRACTS
POSTERS

Routine hemostasis parameters on the CN6000®: analytical validation and method comparison with ACL TOP750

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Background: The CN6000 (Sysmex, Japan) is a high-throughput hemostasis analyzer capable to preform coagulation and immuno-turbidimetric assays, as well as light transmission aggregometry. Both Siemens (Marburg, Germany) and Hyphen Biomed (Asnières, France) reagents can be used on this platform.

Aims: Firstly, the Hyphen Biomed reagents were analytically validated on the CN6000. Secondly, we performed a method comparison study between Hyphen Biomed reagents and Siemens reagents on the CN6000 and Werfen reagents on the ACL TOP750 (Werfen, Bedford, USA).

Materials & Methods: We analytically validated the PT-Phen™ LRT, CEPHEN™ and FIBRIPHEN™ LRT. Currently, there is no D-dimer assay in the Hyphen Biomed portfolio. The imprecision of these assays was assessed by following the CLSI 15-A3 protocol, while for the linearity the CLSI EP06-A was used. Acceptance criteria originate either form the EFLM database or the respective insert. On the ACL TOP750, following reagents were used: HemosILL® Recombiplastin 2G (n = 153), HemosIL® APTT-SP (liquid) (n = 183), HemosIL® SynthASil (n = 74), HemosIL® Q.F.A. Thrombin (n = 100), HemosIL® D-dimer HS500 (n = 80). Siemens reagents on CN6000 were Dade® Innovin®, Dade® Actin FS®, Dade® Thrombin and Innovance® D-dimer. Passing-bablok analysis was used as statistical method for the comparison study. If available in the EFLM database, the test specific total allowable error was used, otherwise an arbitrary allowable error of 15% was chosen.

Results: The imprecision and linearity for all the tested parameters meets the predetermined criteria. The Hyphen Biomed reagents show acceptable and robust results for the analytical validation as can be seen in table 1.
Method comparison revealed discordances between the reagents. However, further splitting the results into specific categories, considering the clinical impact of the results, leads to more comparable results with none or minor biases This was observed for parameters such as the aPTT. Persisting difference could be ascribed the inherent difference in reagent characteristics, such as factor sensitivity.

Conclusion: The novel CN6000 is a robust and easy to use analyzer. Validation showed good analytical characteristics of the Hyphen Biomed assays. These results are in line with other literature finds. Initially, method comparison revealed discordances between the tested kits. Yet, upon closer inspection most of these discordances were not significant.

Analytical Validation			
	PT-Phen™ LRT	CEPHEN™	FIBRIPHEN™ LRT
Between day imprecision	Level 1: 0.26% Level 2: 0.19%	Level 1: 0.15% Level 2: 0.60%	Level 1: 2.53% Level 2: 2.57%
Whitin day imprecision	Level 1: 0.54% Level 2: 0.34%	Level 1: 0.23% Level 2: 0.68%	Level 1: 1.69% Level 2: 3.19%
Total imprecision	Level 1: 0.48% Level 2: 0.30%	Level 1: 0.21% Level 2: 0.61%	Level 1: 1.51% Level 2: 2.85%
Linearity	R^2 = 0.98 y = 8.824 + 1.003x	N.A.	R^2 = 0.99 y = -0.0342 + 1.007x

Table 1: Results of the analytical validation of the Hyphen Biomed assays

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