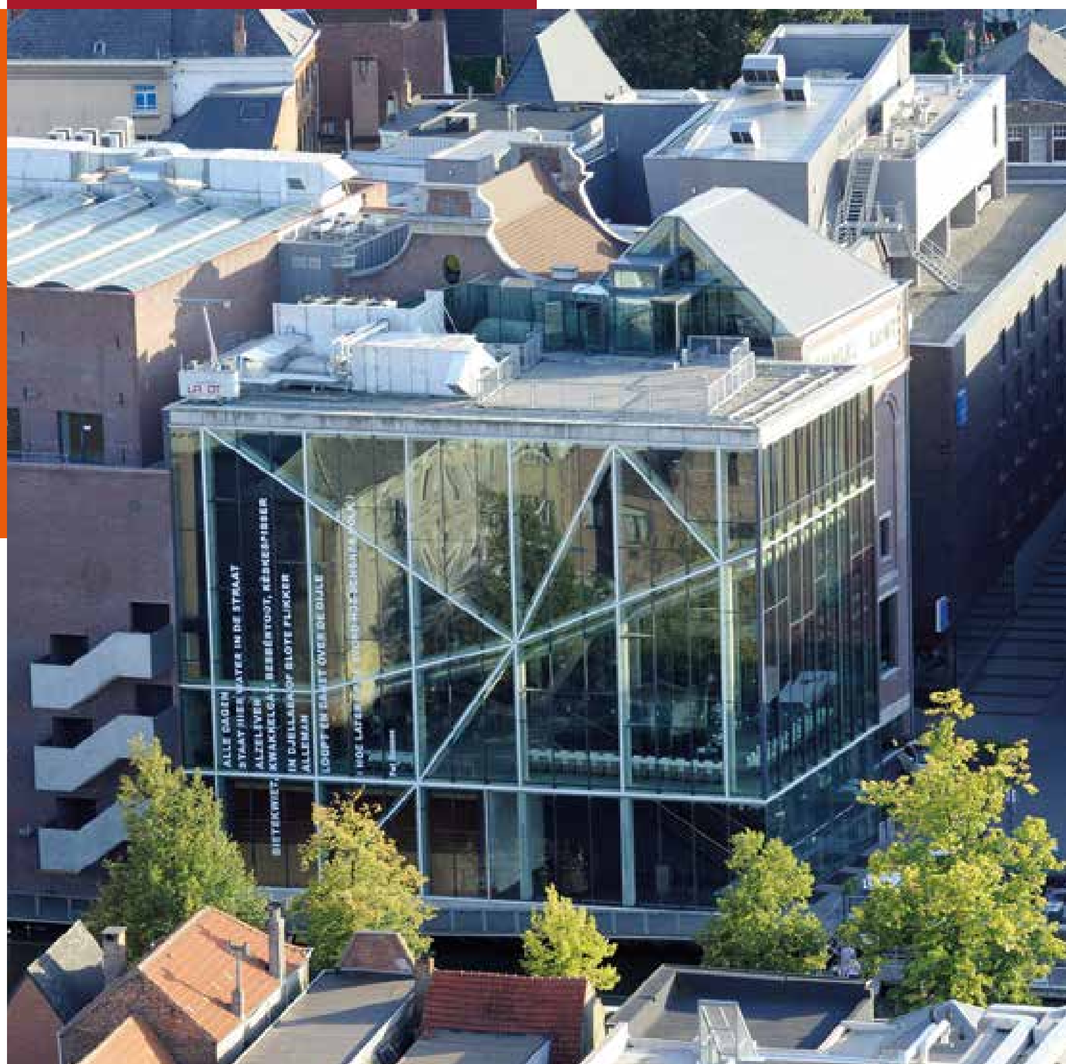
BSTH

Belgian Society on Thrombosis and Haemostasis

27TH ANNUAL MEETING

28-29 NOVEMBER 2019

LAMOT BELGIUM



LOCAL ORGANISERS
ALAIN GADISSEUR
INGE VANGENECHTEN

UZ ANTWERPEN

PROGRAM &
ABSTRACTS

WWW.BSTH2019.ORG

At Takeda, we know that every patient is unique; that's why we consider the individual needs of each patient.

It's personal

SUPPORTING THE BLEEDING DISORDER COMMUNITY

C-ANPROM/BE//0481 – Date of creation/review October 2019.

03

TABLE OF CONTENTS

Table of contents

General info	4
Welcome	5
Program	6-9
BSTH Board 2019	10
Exhibition rules	11
Registration fees	11
Floorplan	12
Educational I & II	13 / 20
State of the art I & II	15 / 21
Prof Gaston Baele Memorial Lecture	19
Sponsored Satellite Symposia	
- Novo Nordisk	14
- Sanofi Genzyme	18
- CSL Behring	20
Abstracts of Oral presentations	
- Basis Research	24
- Clinical / Laboratory	28
Abstracts of Poster presentations	34
List of sponsors	56

4

GENERAL INFO

ORGANISATION
Alain Gadisseur
Inge Vangenechten

UZ Antwerpen

WWW.BSTH2019.ORG

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

05

WELCOME TO THE 27TH ANNUAL MEETING

Dear participants,

I would like to welcome you to the BSTH Annual Meeting 2019, the 27th already, once again in the historic city of Mechelen. The city of Mechelen was at the peak of its power as the capital of the Burgundian Low Countries under Duke Charles the Bold in the 15th century , and especially under the regent Margaret of Austria in the 16th century.

It is safe to say that these last years Mechelen has been the "capital" of the Belgian haemostasis community during the BSTH Annual Meeting each November. A place where colleagues working in the field of haemostasis and thrombosis come together for presentations and discussions in their field, and at the same time enjoy a nice time together.

This year is a special year for the BSTH and for me personally. Firstly my term as president of the BSTH will come to an end this year. It has been an honour to have been at the head of the BSTH for these last 8 years and to work closely together with colleagues in the board of the BSTH, several of which will be leaving the board at the end of this year. We wish those friends well, and the same is of course true for the BSTH itself. This also means new faces will appear in the BSTH board, with new accents, ideas and initiatives. It is always good for a society to go for rejuvenation.

It is also the third time that the organization of the BSTH Annual Meeting has been entrusted to me, representing Antwerp University. To nobody's surprise, like on previous occasions (2005, 2012) the programme will have a definite Von Willebrand emphasis in the state-of-the-art sessions. However, this is balanced by some interesting other topics like platelet genomics, inhibitor immunology and coagulopathy of trauma.

We have tried to make the social programme on Thursday evening different from other years with a more informal feel, going for more of a food truck festival, be it an indoors one. Hopefully you will enjoy this and participate.

We are very thankful for the continued support from the industry, both as sponsors of the BSTH as organization and as sponsors of the BSTH Annual Meeting. This year we have reached the highest number of corporate BSTH members so far. The industry sponsored satellite symposia focus this year on haemophilia and Thrombotic Thrombocytopenic Purpura (TTP).

It is good to see the thrombosis and haemostasis community thriving and more and more Belgian researchers and clinicians giving presentations at international scientific meetings. It is also a mark of respect and appreciation from our colleagues in other national hemostasis and thrombosis societies that the European Congress on Thrombosis and Haemostasis (ECTH) will have its 4th bi-annual meeting in Gent in 2021 with part of the first day filled by the BSTH.

I hope you will all enjoy this 27th Annual Meeting of the Belgian Society on Thrombosis and Haemostasis.

Alain Gadisseur
President of the BSTH

DATE
28 - 29 NOVEMBER 2019

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

06

PROGRAM THURSDAY 28 NOVEMBER 2019

09:25 WELCOME

EDUCATIONAL I
Chairs: C. Orlando and P.Q. Le

09:30 Thrombogenetics implementation in Leuven
K. Freson, Belgium

10:15 **SPONSORED SATELLITE SYMPOSIUM I - NOVO NORDISK**

Welcome and introduction
Chair: C. Hermans, UCLouvain, Brussels, Belgium

N9-GP, a new extended half-life recombinant factor IX: PK and PD considerations
C. Hermans, UCLouvain, Brussels, Belgium

From clinical trials to real life clinical experience with N9-GP
C. Feistritzer, Medical University, Innsbruck, Austria

Q&A and summary
Moderated by C. Hermans

11:15 COFFEE BREAK

ORAL PRESENTATIONS: BASIC RESEARCH
Chairs: S. De Meyer and T. Vanassche

11:45 O01 A novel antithrombotic coating for blood-contacting medical devices
L. Musumeci, Liège

11:57 O02 Evaluation of the time course of Carboxypeptidase U (CPU, CPB2, TAFIa) generation and influence of AZD9684, a selective CPU inhibitor, in experimental rat stroke models.
J. Mertens, Antwerp

12:09 O03 Gene therapy for congenital thrombotic thrombocytopenic purpura using intramuscular electrotransfer DNA delivery
C. Dekimpe, Kortrijk

12:21 O04 From the antiplatelet drug ticagrelor to antibiotics : a study of structure-activity relationships
N. Jacques, Liège

12:33 O05 Inhibition of ADAMTS13 prevents the loss of high molecular weight von Willebrand factor multimers in an in vitro left ventricular assist device
S. Deconinck, Kortrijk

12:45 Paul Capel Prize Clinical & Laboratory

07

PROGRAM THURSDAY 28 NOVEMBER 2019

13:00 LUNCH

14:00 BSTH GENERAL ASSEMBLY

STATE OF THE ART I
Chairs: A. Demulder and K. Vanhoorelbeke

14:30 VWD Diagnostic Thresholds
J. O'Donnell, Ireland

15:00 Specificity of cardiovascular-disorders in von *Willebrand disease*
S. Susen, France

15:30 Evaluation of semi-automatic VWF multimer assay
I. Vangenechten, Belgium

16:00 BREAK

16:30 **SPONSORED SATELLITE SYMPOSIUM II - SANOFI GENZYME**
Chair: C. Lambert, Cliniques Universitaires Saint-Luc, Belgium

New paradigm for the management of acquired thrombotic thrombocytopenic purpura
P. Coppo, Saint-Antoine Hospital, Paris, France

17:30 **PROFESSOR GASTON BAELE MEMORIAL LECTURE**
Chair: K. Jochmans

Platelet biochemistry
Hans Deckmyn, Kortrijk, Belgium

18:00 CLOSURE OF DAY PROGRAMME

EVENING PROGRAM

WELCOME RECEPTION

Siemens Healthineers invites all participants On Thursday night at 18:00 hrs. to join the welcome reception.

DINNER

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

Preregistration is required.
Each participant including delegates of companies can register to join for a fixed price per person.

08

PROGRAM
FRIDAY
29 NOVEMBER 2019

08:55 WELCOME

ORAL PRESENTATIONS: CLINICAL & LABORATORY
Chairs: K. Devreese and K. Vandenbosch

09:00 O06 Histological analysis of a thrombectomy-resistant ischemic stroke thrombus: a case report
S. Staessens, Leuven

09:12 O07 Understanding the TTP literacy in patients with TTP
L.C. Velasquez Pereira, Kortrijk

09:24 O08 Comparison of INNOVANCE PFA P2Y with light transmission aggregometry to detect clopidogrel resistance
L. Heireman, Ghent

09:36 O09 Evaluation of the influence of statin therapy on procarboxypeptidase U (proCPU, TAFI, proCPB2) biology in patients eligible for statin therapy
K. Claesen, Antwerp

09:47 O10 Optimization of measurement of emicizumab, FVIII and anti-FVIII concentration in patients treated with emicizumab
L. Heireman, Ghent

10:00 **PAUL CAPEL PRIZE BASIC RESEARCH**

10:15 **SATELLITE SYMPOSIUM III - CSL BEHRING**
Chairs: A. Demulder, CHU Brugmann and V. Mondelaers, UZ Gent, Belgium

Who is carrying the burden
C. Hermans, UCLouvain, Brussels, Belgium

11:15 COFFEE BREAK

11:45 CSL Behring Encouragement Award

EDUCATIONAL II
Chairs: F. Mullier and J. Emmerechts

12:15 The coagulopathy of trauma
H. Schöchl, Austria

13:00 LUNCH

13:00 POSTER WALK

STATE OF THE ART II
Chairs: A. Gadisseur and C. Oury

14:00 Why and how do inhibitors develop in haemophilia A and B?
D. Matino, Canada

14:30 The potential use of nanobodies in VWD and hemophilia
P. Lenting, France

15:00 Acquired von Willebrand Syndrome
J. Eikenboom, the Netherlands

15:30 CLOSURE

15:35 RECEPTION

09

PROGRAM
FRIDAY
29 NOVEMBER 2019

10

BSTH BOARD 2019

THE PRESENT MEMBERS OF THE BSTH BOARD 2019 ARE:

ALAIN GADISSEUR
Universitair Ziekenhuis Antwerpen (UZA), Edegem
Alain.Gadisseur@uza.be

ANNE DEMULDER
CHU Brugmann, Brussels
anne.demulder@ulb.ac.be

KATRIEN DEVREESE
Universitair Ziekenhuis (UZ) Gent
katrien.devreese@uzgent.be

HANS DECKMYN
KU Leuven Kulak, Kortrijk
hans.deckmyn@kuleuven-kulak.be

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

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

KRISTIN JOCHMANS
UZ Brussel
Kristin.Jochmans@uzbrussel.be

CHRISTELLE ORLANDO
UZ Brussel
christelle.orlando@uzbrussel.be

CECILE OURY
GIGA-Cardiovascular Sciences, Université de Liège
cecile.oury@ulg.ac.be

MURIEL SPRYNGER
Centre Hospitalier Universitaire de Liège
msprynger@chu.ulg.ac.be

THOMAS VANASSCHE
KU Leuven
thomas.vanassche@kuleuven.be

KRISTEL VANDENBOSCH
Centre Hospitalier Universitaire de Liège
kvandenbosch@chu.ulg.ac.be

KAREN VANHOORELBEKE
KU Leuven Kulak, Kortrijk
karen.vanhoorelbeke@kuleuven-kulak.be

FRANÇOIS MULLIER
UC Louvain, Yvoir
francois.mullier@uclouvain.be

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

11

EXHIBITION RULES & REGISTRATION FEES

EXHIBITION RULES

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

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

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

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

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

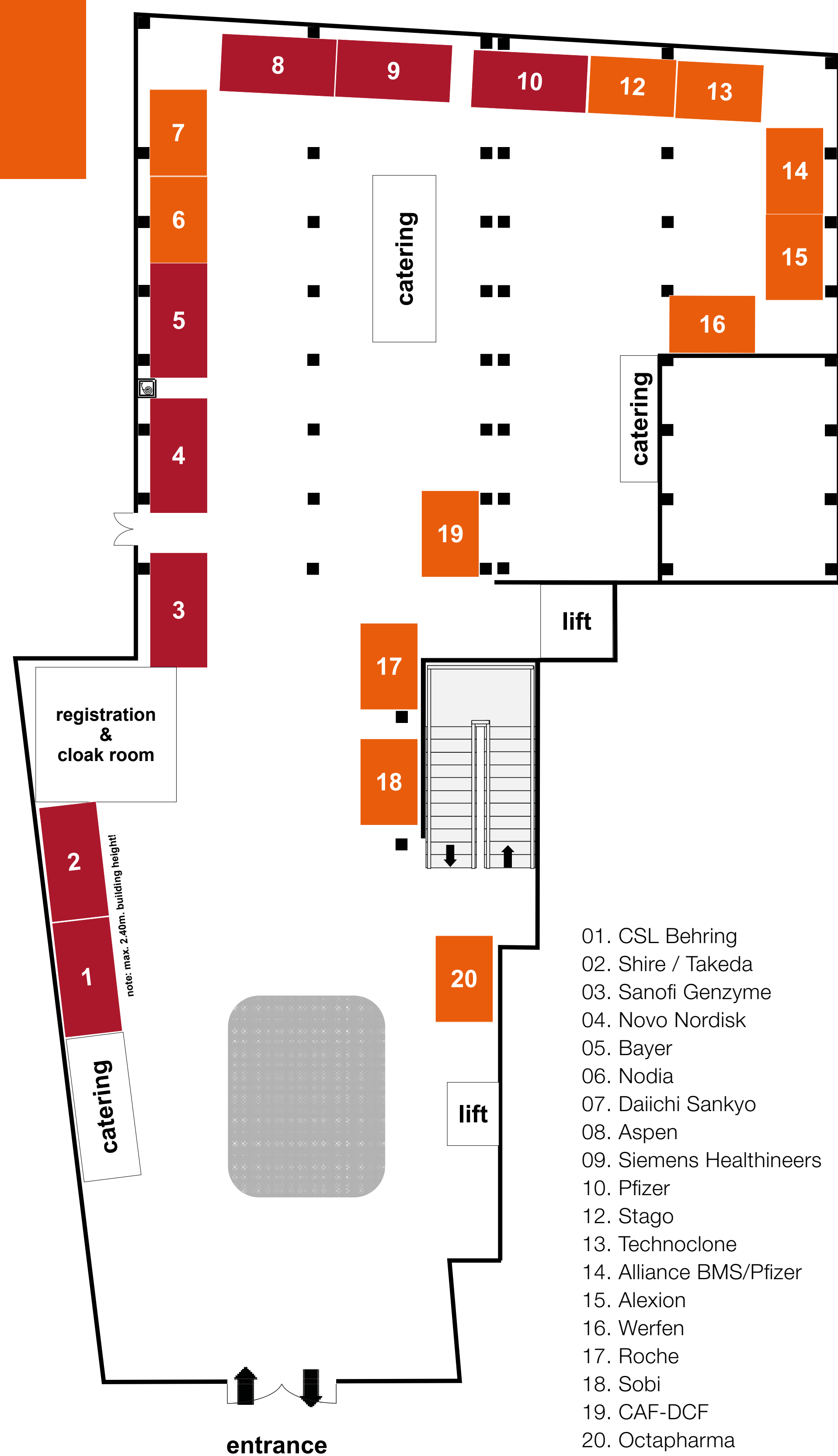
EXHIBITION OPENING HOURS
THU 28 NOV 2019 08:30-19:00
FRI 29 NOV 2019 08:30-16:30

REGISTRATION FEES

	EARLY-BIRD (UNTIL 1 OCT.)	REGULAR (FROM 1 OCT.)
MEMBER BSTH		
Regular (MD specialist, MSc specialist, PhD scientist)	100 EUR	140 EUR
MD trainee / PhD student	55 EUR	70 EUR
Nurse, paramedic, technician, data manager, student	25 EUR	40 EUR
NON MEMBER BSTH		
Regular (MD specialist, MSc specialist, PhD scientist)	165 EUR	225 EUR
MD trainee / PhD student	110 EUR	140 EUR
Nurse, paramedic, technician, data manager, student	70 EUR	85 EUR

12

FLOORPLAN



01. CSL Behring
02. Shire / Takeda
03. Sanofi Genzyme
04. Novo Nordisk
05. Bayer
06. Nodia
07. Daiichi Sankyo
08. Aspen
09. Siemens Healthineers
10. Pfizer
12. Stago
13. Technoclone
14. Alliance BMS/Pfizer
15. Alexion
16. Werfen
17. Roche
18. Sobi
19. CAF-DCF
20. Octapharma

13

EDUCATIONAL I

Thrombogenomics implementation in Leuven

K Freson
Center for Molecular and Vascular Biology, KULeuven, LEUVEN, Belgium

Between 2015 and 2018, the international study ThromboGenomics has developed and tested a targeted high-throughput sequencing (HTS) gene panel test for clinical diagnostics of patients with rare bleeding, thrombotic or platelet disorders (BTPD) (1,2). After the initial validation of this platform, 2396 index patients were sequenced and a mean diagnostic rate of 49.2% was reached for all thrombotic, coagulation, platelet count and function disorder patients while this rate dropped to 3.2% for patients with unexplained bleeding disorders that were characterized by normal haemostasis test results. Since beginning of 2019, a similar HTS test for BTPD has been implemented at the Center for Human Genetics (UZLeuven). This test screens the 99 diagnostic-grade genes that were recently curated by the Scientific and Standardization Committee (SSC) for Genetics in Thrombosis and Haemostasis (ISTH SSC-GinTH) (3). Upon inclusion, clinicians can opt for one of the three panels: 1. coagulation panel test for abnormal bleeding or thrombosis with aberrant test parameters, 2. platelet dysfunction panel test for inherited thrombocytopenia or known platelet dysfunctions and 3. the unexplained bleeding panel test but with evidence for Ehlers Danlos Syndrome or Rendu-Osler-Weber disease. Inclusion and exclusion criteria will be discussed. Detailed information about clinical phenotype, family history and laboratory test results are essential for correct interpretation of the genetic results. All data are analysed by a Multi-Disciplinary Team that typically have to discuss 2-4 potential candidate variants/patient. Genetic variants were classified using the ACMG criteria. After 6 months, about 80 patients were included by national recruitment and our diagnostic rate is similar to that observed in the ThromboGenomics study. The aim would be to provide results to clinicians within 4 months after inclusion. Some examples of positive reports and unexpected findings will be discussed in more detail. I will also touch on ethics and incidental findings when applying HTS for BTPDs.

References

Simeoni I, et al..Blood. 2016;127:2791
Downes K, et al Blood. 2019 [Epub ahead of print]
Megy K, et al J Thromb Haemost. 2019;17:1253

Kathleen Freson is Professor at the University of Leuven in the department of Cardiovascular Sciences and director of the Centre for Molecular and Vascular Biology. She has a background in bioscience engineering and her research focussed on genetic and biology studies using platelets to unravel new bleeding disorders but she studied also other mainly neurological diseases where the platelet was used as a model cell for the disease. She co-chaired the Multi-Disciplinary Team for the clinical high-throughput sequencing platform ‘ThromboGenomics’ and is co-PI of the UK NHIR-rare diseases project to whole genome sequence unknown Bleeding and Platelet Disorders for gene discovery. She is Chair of the ISTH Subcommittee ‘Genomics in Thrombosis and Haemostasis’ and co-chair of the NHI supported ClinGen working group for thrombosis and haemostasis.

14

NOVO NORDISK SATELLITE SYMPOSIUM I

N9-GP (Refixia®), a new extended half-life recombinant factor IX: PK and PD considerations

C. Hermans
Catholic University of Louvain, BRUSSELS, Belgium

Several development efforts have recently focused on extending the half-life of recombinant FIX by modifying its physiological and pharmacokinetic properties with the aim of reducing treatment burden and thereby potentially improving treatment compliance and clinical outcomes of patients with haemophilia B.
Approaches to prolong the half-life have included protein fusion (fusing human albumin or Ig to rFIX). Glycopegylation is a novel approach of half-life extension in haemophilia B. Refixia® (nonacog beta pegol; N9-GP) is an extended half-life factor IX molecule for replacement therapy that has been approved in the EU for the treatment and prophylaxis of bleeding in patients aged ≥ 12 years with haemophilia B.
This lecture will discuss the pharmacodynamic and pharmacokinetic data relevant to the use of Refixia® in this patient population.

Cedric Hermans currently heads the Division of Haematology, the Hemostasis and Thrombosis Unit as well as the Hemophilia Center of the Saint-Luc University Hospital in Brussels, Belgium. He was appointed Associate Professor at the Medical School of the Catholic University of Louvain in 2003, Full Professor in 2012 and Vice-Dean in 2015.
Professor Hermans has (co)-authored more than 220 original articles in international journals and is a member of several scientific societies and international advisory boards and collaborative research projects. He is past-president of EAHAD, member of the Board of Directors of the World Federation of Haemophilia and the Editor-in-Chief of the Haemophilia Journal.
His main research interests lie in the area of haemostasis and thrombosis, especially clinical studies on the treatment modalities and the wide spectrum of complications of haemophilia in both developed and developing countries, as well as new anticoagulants and the management of thrombosis.

From clinical trials to real life clinical experience with N9-GP

C. Feistritzer
Medical University of Innsbruck, INNSBRUCK, Austria

Professor Clemens Feistritzer will review the data from the N9-GP paradigm clinical development programme. He will also share his real life clinical experience with the new extended half-life recombinant factor IX products including the use of these factors in the management of surgeries.

Professor Clemens Feistritzer is Professor of Hematology at the Medical University of Innsbruck and head of the Haemostasis and Thrombosis program. After graduation from the Medical University of Innsbruck in 2001 Clemens Feistritzer started his research in the Intensive Care Laboratory of Univ. Prof. Dr. Ch. Wiedermann in Innsbruck focusing on the role of endogenous anticoagulants in sepsis. He continued his work as a research fellow from 2004 to 2006 at the The Scripps Research Institute in La Jolla, California, working on the project “Protective Signaling by Protein C in Endothelial Cells”, which was also funded by the Austrian Science Fund.
After his return to Austria Clemens Feistritzer completed his residency at the Department of Internal Medicine at the Medical University of Innsbruck. Since 2012 he is staff member of the Department of Haematology and Oncology Innsbruck and Director of the Haemostasis and Thrombosis program. In 2016 he was promoted to the position of an Assistant Professor. During the last years he developed an out-patient clinic for patients with haemophilia and other coagulation disorders and participated in various clinical trials.

15

STATE OF THE ART I

VWD Diagnostic Thresholds
Low VWF and diagnostic thresholds

J. O'Donnell, Royal College of Surgeons in Ireland (RCSI), DUBLIN, Ireland

Partial quantitative deficiency of plasma von Willebrand factor (VWF) is responsible for the majority of cases of von Willebrand disease, the commonest inherited human bleeding disorder. International consensus guidelines recommend that patients with reduced plasma VWF:Ag levels and bleeding phenotypes should be considered in two distinct subsets. First, patients with marked reductions in plasma VWF levels (<30 IU/dL) usually have significant bleeding phenotypes and should be classified with ‘Type 1 VWD.’ In contrast, patients with intermediate reduced plasma VWF:Ag levels (in the range 30 – 50 IU/dL) should be considered in a separate category labeled ‘Low VWF levels.’ These patients with Low VWF commonly display variable bleeding phenotypes and often do not have VWF gene sequence variations. Since the pathophysiology underlying Low VWF levels remains largely undefined, diagnosis and management of these patients continues to pose significant difficulties.

To address these questions, the Low VWF Ireland Cohort (LoVIC) study has investigated more than 200 patients registered with Low VWF. Interestingly, despite their marginally reduced plasma VWF levels, ISTH BAT and Condensed MCMDM-1 VWD scores both confirmed significant bleeding phenotypes in the majority of LoVIC patients. For example, among female patients with Low VWF, 77% had ISTH BAT scores ≥6. Importantly, bleeding tendency did not correlate with plasma VWF levels within the 30-50 IU/dL range. Furthermore, bleeding phenotypes could not be explained by concurrent hemostatic defects. Plasma FVIII:C/VWF:Ag ratios were significantly increased in LoVIC patients compared to controls ($p<0.0001$). In contrast, increased plasma VWF:pp/VWF:Ag ratios >3 were observed in only 6% of the LoVIC cohort. Furthermore, platelet-VWF:Ag and platelet-VWF:CB levels were both significantly reduced compared to controls ($p<0.05$). In response to DDAVP, peak VWF levels exceeded 100 IU/dL in 88% of patients. Importantly, the DDAVP response was sustained (VWF:Ag and VWF:RCo both remained >100 IU/dL after 4 hours in 72% subjects). In conclusion, our novel data suggest that Low VWF levels can be associated with significant bleeding, and are predominantly due to reductions in VWF synthesis and/or constitutive secretion. Although enhanced VWF clearance may contribute to the pathophysiology in some individuals with Low VWF, the absolute reduction in VWF plasma half-life is usually mild and not sufficient to significantly impact upon the duration of DDAVP-induced VWF response.

Professor O’Donnell received his medical degree from Trinity College Dublin in 1990. After internship and medical residency in Australia, he completed clinical haematology training in the Hammersmith and Royal Free Hospitals in London. In 1998, he obtained a Medical Research Council Training Fellowship, and was awarded his PhD by Imperial College London in 2001. He subsequently completed a Post-Doctoral Fellowship in the Hammersmith Hospital, and was appointed as Senior Lecturer in Imperial College in 2004. He is a Fellow of both the Royal College of Physicians of Ireland, and the Royal College of Pathologists (UK). Following his return to Ireland in 2005, he became the first clinician scientist to receive the prestigious Science Foundation Ireland President of Ireland Young Investigator award. He is currently Professor of Vascular Biology in the Royal College of Surgeons in Ireland and Director of the recently established Irish Centre for Vascular Biology. The Haemostasis Research laboratory led by Prof. O'Donnell has been awarded more than £12 million in peer-reviewed grant funding since 2006. His research programme in Ireland has been funded by Science Foundation Ireland, the Health Research Board (HRB), the Irish Heart Foundation, the Wellcome Trust and the National Children's Research Centre (NCRC). Prof O'Donnell's laboratory has focussed on basic research related to different aspects of vascular biology in relation to clinical bleeding and thrombosis. He has published more than 130 publications in high impact peer reviewed journals (including the New England Journal of Medicine, Blood, ATVB and J Biol Chem). On the basis of this research, he has also been invited to give numerous lectures at the most prestigious national and international haematology meetings, including the American Society of Hematology Annual Meeting, the Gordon Conference in Hemostasis and the Congress of the

16

STATE OF THE ART I

Specificity of cardiovascular disorders in von Willebrand disease

S. Susen, Lille University Hospital and Inserm, LILLE, France

Cardiovascular disease represents a leading cause of morbidity and mortality in most developed countries. Despite the defect in von Willebrand factor (VWF), patients with von Willebrand disease (VWD) can develop atherosclerotic disease and thrombosis and they are exposed or develop age-related disorders including cardio-vascular disease or cancer. This raise the question of the management of the CV risk and the use of antithrombotics in these patients. It is still debated whether VWD patients with arterial thrombosis should benefit of the same recommendations than the general population and receive long-term antiplatelet or anticoagulant therapy.

The use of platelet inhibitory agents or anticoagulants in VWD patients may result in more bleeding complications especially from the gastrointestinal tract. On the other hand, VWD-patients for whom these drugs are not prescribed could be exposed to a higher risk of arterial re-thrombosis. Animal models have shown that VWF deficiency may have a protective effect against atherosclerosis and arterial thrombosis risk in different models relevant for coronary artery disease or stroke.

Several population-based studies have shown a significant but modest reduction in the risk of acute ischemic cardiovascular events in patients with VWD. However, the prevalence of co-morbidities that usually justify long-term prescription of antiplatelet or anticoagulant treatments remains significant in patients with Willebrand disease. The main limitation of the epidemiological studies is the absence of phenotypic and genotypic characterization of the VWD of the patients that are included. The underlying type of VWD could also modulate the risk of ischemic cardiovascular complications. The need for replacement therapies (VWF and desmopressin) could also increase the risk of acute events and mitigate the risk reduction even in severe patients. In-hospital medical and interventional management of acute events such as acute coronary syndromes appears to be well tolerated in a growing number of cases with a potential increase in procedure-related bleeding, but we have no data on the long-term follow-up of patients receiving antiplatelet therapy.

As the risk of ischemic cardiovascular events remains significant in VWD and the decision to initiate or not initiate antithrombotic therapy is made empirically on a case-by-case basis and it is still debated whether VWD patients with arterial atherosclerosis should receive long-term antiplatelet or anticoagulant therapy.
There is therefore a need for large population-based studies from international data registries including the genotype/phenotype characterization and the potential associated bleeding risk of the VWF defect, to assess the true prevalence of main comorbidities in VWD patients compared to control population and to investigate if their management is different according to the presence of VWD and its subtype and whether some subsets of VWD-patients should be managed differently.

Sophie Susen, MD, PhD, is a professor of haematology and director of the Department of Haemostasis and Transfusion at Lille University Hospital in Lille, France.
She has a special interest in von Willebrand disease and is the national coordinator for the French Reference Network for von Willebrand Disease and team leader for a research group at the French National Institute of Health and Medical Research (Inserm).
Prof Susen is a member of the board of the French Group on Thrombosis the Haemostasis and the Group on Perioperative Haemostasis and the SSC of ISTH on VWD

17

STATE OF THE ART I

Evaluation of semi-automatic VWF multimer assay

I. Vangenechten, UZA ANTWERPEN, Belgium

Von Willebrand factor (VWF) plays an essential role to manage normal hemostasis by binding to subendothelial collagen, Platelet GPIb and FVIII to prevent this latter from proteolytic degradation. Laboratory diagnosis and (sub)classification of von Willebrand disease (VWD) is based on a multistep process requiring the evaluation of von Willebrand Factor (VWF) multimeric distributions. VWF is a large and complex protein produced by endothelial cells. It circulates in plasma in different multimeric forms of different molecular weights with High molecular weight multimers having the greatest haemostatic capacity. Under high shear stress, VWF undergoes conformational changes resulting in converting of large multimers into small parts and production of cleavage products, which leads to typical multimeric patterns.
The first attempts of VWD classification were based on the multimeric patterns resulting in recognizing up to 50 different subtypes. Over time, importance of multimers in first line was further diminished. Different multimeric VWD types were lumped together into fewer and broader categories recognizing 6 types as it is in the current ISTH-SSC classification. An extended classification with additional type 2A subclassification into 2A/IIA, IIC, IID and IIE is used in Antwerp.
The widely used VWF multimer "gold standard" method is labor-intensive, not standardized technique, only performed in specialized laboratories. The first commercial semi-automated assay, HYDRAGEL VW multimer assay (Sebia), was developed. It has a ready to use kit, is performed on one single instrument which reduces workload, gives results within 1-day and has the opportunity of multimer quantification. In contrast with the "gold standard" method it is not able to evaluate the triplet structure. The semi-automated assay demonstrates overall comparable results with the "gold standard". Additionally to the quantitative normal reference ranges, significant pathological reference ranges can be defined for each VWD subtype.
Laboratories who riley on the three most common available assays (FVIII, VWF:Ag, VWF:GPIb) are only able to classify VWD restricted to his 3 main types. Addition of VWF:CB assay allows classification according to the ISTH-SSC classification as it is the same when using both multimeric methods. Only by using the multimeric analysis, type 2A can be classified into his subclasses. The semi-automated assay is able to classify all type 1, 2A/IIA, 2B, 2N and most 2A/IIE correctly compared with the final classification. Discordant results are observed within type 2M VWD with qualitative and quantitative loss of HMWM, where it was not seen with the "gold standard".
In conclusion the Hydragel is a suitable and quick technique with the possibility to standardize a qualitative technique. It has the opportunity to establish normal ranges and pathological reference ranges for different subtype of VWD, making it less observer dependent. It cannot completely replace the "gold standard" technique due to the lack of triplet structure evaluation, and adaptability, but it can by an useful addition to diagnose VWD as a screening tool of VWF multimers.

Inge Vangenechten graduated as a Medical Laboratory Technician and is head of the haemostasis research unit at the Antwerp University Hospital. In 2014 she started her PhD at the Antwerp University with studies on von Willebrand disease. Her research is focused on several population studies to characterize patients with von Willebrand Disease in the Czech Republic, Slovakia, Belgium etc. She has authored or co-authored several international peer-reviewed publications and has been invited for multiple oral presentations at national and international scientific meetings.

18

SANOFI GENZYME SATELLITE SYMPOSIUM II

New paradigm for the management of acquired thrombotic thrombocytopenic purpura

P. Coppo, Saint-Antoine Hospital, Paris, France

In recent years, major advances in the understanding of the pathophysiology of immune-mediated Thrombotic Thrombocytopenic Purpura (TTP), also called acquired TTP (aTTP), have advanced our approach on the management and treatment of patients suffering from this life-threatening disease. The ultimate goal is to address the significant unmet medical need in aTTP and to improve outcomes for patients.
In this educational session we will focus on:

- Faster diagnosis: how to rapidly identify the signs and symptoms of aTTP, and how to discriminate aTTP from other thrombotic microangiopathies;
- New treatment strategies: how to optimally use the standard of care together with new treatment options;
- Improved follow-up: what are the strategies and treatment options for patients at risk for relapse.

It is with great pleasure to announce that this educational session will be given by Professor Paul Coppo and moderated by Professor Catherine Lambert (Cliniques Universitaires Saint-Luc, Belgium)

Paul Coppo is Professor of Haematology and Doctor at the Saint-Antoine Hospital in Paris, France. His past and current research and medical activities focus on haemato-immunologic diseases. Paul Coppo is the Head of the French Reference Network for Thrombotic Microangiopathies (CNR-MAT), and leads the department of lymphoid malignancies. The CNR-MAT is a resource centre for the diagnosis and the management of TMAs. The work of his team are focused on genetic risk factors and immunomodulatory treatments in acquired thrombotic thrombocytopenic purpura.

Please join us to help defining the “New paradigm for the management of acquired Thrombotic Thrombocytopenic Purpura”.

19

PROFESSOR GASTON BAELE MEMORIAL LECTURE

Platelet biochemistry

H. Deckmyn
KU Leuven Kulak, KORTRIJK, Belgium

ADHESION: Upon injury of the blood vessel by e.g. atherosclerosis, several components of the subendothelium (e.g. collagen, fibronectin, laminin) become exposed, to which circulating platelets can adhere and be activated. In arterial thrombosis, adhering platelets have to withstand high shear forces induced by the fast flowing blood especially in stenotic areas, for which they rely on von Willebrand Factor (VWF). VWF is a large multimeric protein, present a.o. in plasma, that under normal conditions does not interact with its platelet receptor, glycoprotein (GP)Ib, part of the GPIb-IX-V complex. The cryptic GPIb binding site within the VWF-A1 domain becomes exposed, when VWF, bound to collagen in the damaged artery via its VWF-A3-domain, is stretched by the high shear forces. Platelets roll over immobilized VWF and by this are slowed down, which subsequently allows them to firmly adhere to the subendothelium through their two main collagen receptors: GPVI and integrin $\alpha2\beta1$ (or GPIa-IIa).

ACTIVATION: Cross-linked GPVI via its association with the the FcRγ chain, that contains an immunoreceptor tyrosine-based activation motif (ITAM), triggers intracellular signaling. This then again is controlled by inhibitory receptors, such as platelet endothelial cell adhesion molecule-1 (PECAM-1), G6B, and carcinoembryonic antigen-related cell adhesion molecule 1 and 2 (CEACAM), whose cytoplasmic domains bear immunoreceptor tyrosine-based inhibitory motifs (ITIMs). Tyrosine phosphorylation and activation of PLCγ2 results in the generation of IP3 and Ca2+-release from the dense tubular system and Ca2+ influx via the store-operated calcium channel Orai-1, into the cytosol. Elevated Ca2+ causes a.o. platelet shape change, thromboxane A2 (TXA2) generation, ADP secretion and presentation of a negatively charged phospholipid membrane onto which vitamin K-dependent coagulation factors can assemble, ultimately resulting in thrombin generation. These three agonists activate PLCβ via their respective G-protein coupled receptors (GPCR) TP, P2Y12/P2Y1 and the protease-activated receptors (PAR1 and PAR4) respectively, with more Ca2+ release, hence further activation. This self-amplifying process allows the recruitment of additional platelets for the formation of the haemostatic plug.

AGGREGATION: The key actor mediating the final aggregation is the integrin αIIbβ3 which is present on platelets in high numbers (40–80.000). αIIbβ3 itself is activated upon platelet stimulation involving proteins such as talin and kindlin3 that disrupt the interaction between the cytosolic tails of αIIb and β3. This causes a major conformational change in the extracellular part of αIIbβ3, which exposes the binding site for soluble symmetrical fibrinogen (or multimeric VWF) that will crosslink platelets, generating an aggregate.

LIMITATION: This self-amplifying system needs to be controlled and limited to the site of injury and indeed normally is stopped by inhibitors of platelet activation secreted by endothelial cells such as prostacyclin and NO, that stimulate the formation of platelet cAMP and cGMP respectively. The cyclic nucleotides cause sequestration of elevated Ca2+-levels from the cytosol and by this restrict platelet activation and aggregate formation to the site of damage.

After obtaining his PhD in Biochemistry in Leuven in 1980, Hans Deckmyn started research on platelets under the guidance of Jos Vermylen in the Center for Thrombosis and Vascular Research, where he stayed until 1992. In 1985-1987 he did a postdoc with Phil Majerus at the Washington University in St. Louis, Missouri. In 1992 he started his own research team in the Kulak, the Laboratory for Thrombosis Research, with focus on platelet adhesion to collagen via integrin $\alpha2\beta1$ and via von Willebrand factor/GPIb and especially on the development of inhibitory monoclonal antibodies as potential safer new antithrombotics. At present the team consists of some 25 members looking into platelet function using genetically modified mice, the role of ADAMTS13 in TTP (PI: K Vanhoorelbeke) and the structure of stroke thrombi (PI: SF De Meyer).

Hans Deckmyn published nearly 250 papers, some 30 of which in Blood, 15 book chapters, and was cited over 8300 times (WoS). Of the 4 patents that were issued, one was commercialized and is currently on the market as the IL GPIb:RCo test.
He was board member of the BSTH since 2002 and of the European Platelet Network (EUPLAN) for which he organised the meeting in 2018 in Bruges, executive officer of the European Thrombosis Research Organisation (ETRO) and member of the ISTH since 1988. He received a.o. the Boehringer-Ingelheim prize for Research on Thrombosis and Coagulation, the prize "Dr. en Mevr. Schamelhout-Koettlitz " and the Sidmar prize, both awarded by the Royal Academy for Medicine of Belgium. He is Doctor Honoris Causa at the University of Debrecen in Hungary and in 2017 he was elected “Most popular professor” by the Kulak students. He became emeritus professor in October 2018, but he still serves as the research coordinator of the KU Leuven campuses in Kortrijk and Bruges.

20

CSL BEHRING SATELLITE SYMPOSIUM III

Herbert Schöchl received his medical degree from the University of Innsbruck in 1984. He started with the anaesthesia training at the University Hospital of Salzburg. He has undertaken several clinical posts, including staff anaesthesiologist in both cardiac and vascular surgery at the University Hospital Salzburg. He spent 6 years as the vice-director of the emergency medical service in Salzburg and was also responsible for the rescue helicopter service for the area of Salzburg.
Since 1998 he is appointed to at the AUVA Trauma Hospital in Salzburg. Additionally he has a research contract at the AUVA Trauma Research Center, the "Ludwig Boltzmann Institute of experimental and clinical Traumatology" in Vienna.
His primary interest of research focuses on diagnoses and treatment strategies of trauma-induced coagulopathy. He developed hemostatic treatment algorithms based on visco-elastic test results.
He is the author of over 100 publications and reviewer for major journals. Moreover, he is founding member of the Austrian task force for "perioperative coagulation management" of the Austrian Society of Anesthesiology and Intensive Care Medicine.

Who is carrying the burden

C. Hermans, UCLouvain, Brussels, Belgium

Although it has been recognized that women are affected by bleeding disorders such as von Willebrand disease and haemophilia, the dominant clinical and research focus is predominantly on management of males with haemophilia A and B. This presentation describes the evolution of practice, unmet needs and options for both girls and women with haemophilia.

EDUCATIONAL II

The coagulopathy of trauma

H. Schöchl
Unfallkrankenhaus Salzburg, SALZBURG, Austria

Traumatic injuries are the fourth leading cause of mortality worldwide with approximately 50% of deaths due to exsanguination within 6 hours following trauma. [1] Exsanguination remains a significant cause of preventable mortality in trauma patients. Trauma-induced coagulopathy (TIC) is present in 25%–35% of severely injured patients on hospital admission and is associated with a higher incidence of bleeding, transfusion requirement, and multiorgan failure. Trauma patients with TIC have nearly a 4-fold higher mortality than those with comparable injury severity scores (ISS) but no TIC. [2] TIC could be renamed coagulopathic response to trauma because derangement of the coagulation system is not a uniform phenotype. [3] Following traumatic injury, sympathoadrenal activation, upregulated inflammatory/immune reactions, and coagulopathy lead to endothelial activation and injury. Proinflammatory mediators activate the coagulation system and are associated with a rise in plasma concentrations of chemokines and subsequent mobilization of granulocytes and monocytes.[4] Clot formation and lysis is critical to maintaining microvasculature blood flow and tissue perfusion which is altered by severe traumatic injury. TIC phenotypes range from prothrombotic profiles to bleeding disorders. Both thrombotic and bleeding phenotypes are associated with increased mortality [5] and are influenced by the extent and severity of tissue injury and degree of hemorrhagic shock. Blunt trauma without shock is associated with thrombotic phenotypes, while severe trauma with shock is associated with bleeding phenotypes and early reversal of shock and preservation of microcirculation are key to improving survival.

References

Pfeifer R, Teuben M, Andruszkow H, Barkatali BM, Pape HC. Mortality patterns in patients with multiple trauma: a systematic review of autopsy studies. PLoS One. 2016;11:e0148844.
Hess JR, Brohi K, Dutton RP, et al. The coagulopathy of trauma: a review of mechanisms. J Trauma. 2008;65:748–754.
Douqet P, Mora L, Levy J, Schöchl H. Pathophysiological response to trauma induced coagulopathy: a comprehensive review. Anest Analg 2019 in press
Darlington DN, Gonzales MD, Craig T, Dubick MA, Cap AP, Schwacha MG. Trauma-induced coagulopathy is associated with a complex inflammatory response in the rat. Shock. 2015;44(suppl 1):129–137.
Moore HB, Moore EE, Liras IN, et al. Targeting resuscitation to normalization of coagulating status: hyper and hypocoagulability after severe injury are both associated with increased mortality. Am J Surg. 2017;214:1041–1045.

Why and how do inhibitors develop in haemophilia A and B?

D. Matino
McMaster University, Hamilton, Canada

The immunogenicity of biopharmaceuticals, specifically the immune response after administration of proteins obtained by recombinant DNA technologies, can have serious and unpredictable patient outcomes with the potential to undermine treatment efficacy. This has been a prominent issue in the treatment of hemophilia patients. Several factors may contribute to the disruption of the host immunological tolerance to administered products. The complex interplay between the cellular and soluble mediators of immunity in response to FVIII and FIX administration has been the subject of a great number of studies and revealed a complex interplay between innate and adaptive immunity. The focus of this presentation is on the current understanding of the basic science of inhibitor development in hemophilia A and B and the key cellular interactions as well as potential clinical implications. Progresses in basic and translational science in the last two decades have greatly expanded our knowledge on tolerance and immunity to clotting factor concentrates. However, translating the results of fundamental studies into viable strategies to be used to prevent or eradicate inhibitors in the clinical setting has been challenging and still an area of active investigation.

21

STATE OF THE ART II

Dr. Davide Matino is Assistant Professor (Department of Medicine, Division of Hematology and Thromboembolism) at McMaster University, Hamilton, Canada. After obtaining his medical degree at the University of Perugia Medical School and his residency training at the University of Rome "Tor Vergata" in 2014 he completed a Clinical Fellowship in Bleeding disorders at McMaster University where he also received his MSc in Health Research Methodology, defending his thesis "Clinical application of the Web-Accessible Population Pharmacokinetic Service – Hemophilia (WAPPS-Hemo): proposal for a pilot study of a population pharmacokinetic approach to tailored prophylaxis in hemophilia". He then joined the University of Perugia as Assistant Professor in Pharmacology working on a research project focused on the study of the immune response to FVIII in hemophilia, funded through a Young Investigator Award from the Italian Ministry of Health.
His research is focused on inhibitor development in hemophilia, adaptive immunity and immunotolerance mechanisms, and the treatment of hemophilia patients with inhibitors.
He is actively involved in interventional and observational hemophilia clinical trials while continuing his translational and basic research activities on the mechanisms of inhibitor development at the Thrombosis and Atherosclerosis Research Institute (Department of Medicine, McMaster University).

22

STATE OF THE ART II

23

STATE OF THE ART II

The potential use of nanobodies in VWD and hemophilia

P. Lenting
INSERM, PARIS, France

Small single domain antibodies (sdAbs, also known as VHH or nanobodies) represent the variable domain of heavy chain-only antibodies that are found in member of camelid family members. Since their discovery in Belgium in the 1990s, these sdAbs have gained a lot of attention, and their research, diagnostic and therapeutic applications are widespread. In my presentation, I will discuss the use of sdAbs as potential therapeutic agents in the treatment of haemophilia and von Willebrand disease. These include the use of anti-von Willebrand factor (VWF) nanobodies to modify the properties of coagulation factor VIII (FVIII). Incorporating these nanobodies in the FVIII molecule increases the affinity for VWF 25-fold, to 13 pM. This high affinity increases the circulatory survival of FVIII 2-fold. Interestingly, this increased affinity is also associated with a strongly reduced immunogenicity of the FVIII protein. We have also used anti-VWF nanobodies fused to albumin-binding peptides to increase plasma levels of endogenous VWF following subcutaneous application, an approach that could potentially be developed for patients having quantitative reductions in VWF levels. Finally, we generated nanobodies against antithrombin, the main inhibitor in the coagulation cascade. Inhibitory anti-antithrombin nanobodies efficiently compensate for the absence of FVIII in in vitro thrombin generation assays. Also, these nanobodies markedly reduce the bleeding tendency in hemophilic mouse models, when given as protein therapy or as gene therapy. These are just three examples, and many more options are possible and currently being evaluated.

Peter Lenting obtained his PhD with honours at the medical faculty of the University of Amsterdam, the Netherlands in 1996. He was appointed associate-professor of haematology at the University Medical Center in Utrecht, the Netherlands in 2000. Between 2007 and 2009, he held a position of Director of Protein Discovery at Crucell Holland (Leiden, the Netherlands). In March 2009, he joined Inserm as Director of Research. His research activities are mainly focussed on the biology of the Factor VIII/von Willebrand factor (FVIII/VWF) complex and their related disorders haemophilia A and von Willebrand disease. He was an elected member of the council of the International Society of Thrombosis and Haemostasis (ISTH; 2012-2018), and was chair of the ISTH-WHO-liaison committee (2012-2018). Dr. Lenting was awarded the Prix Danièle Hermann (Foundation for Cardio-vascular Research, Institut de France) for his research activities in 2009. In 2018, he has co-founded Laelaps Therapeutics.

Acquired von Willebrand Syndrome

J. Eikenboom
Department of Internal Medicine, division of Thrombosis and Hemostasis, Leiden University Medical Center, LEIDEN, The Netherlands

Von Willebrand factor (VWF) plays a crucial role in platelet adhesion and aggregation. Congenital defects in VWF lead to the common inherited bleeding disorder von Willebrand disease (VWD). More rarely quantitative or qualitative defects in VWF are acquired, which is usually designated as acquired von Willebrand syndrome (AVWS). The clinical picture and laboratory phenotypes may be similar between VWD and AVWS. It can be difficult to distinguish between the two, however, making the correct diagnosis is important as treatment is different.
AVWS has been associated with several underlying disorders like autoimmune diseases, benign monoclonal gammopathies, lympho- and myeloproliferative disorders, excessive fibrinolysis and hypothyroidism. In recent years AVWS has been associated with cardiovascular disorders like aortic stenosis, left ventricular assist devices, and extracorporeal membrane oxygenation.
In AVWS the synthesis of VWF is usually normal, but several pathophysiologic mechanisms may result in quantitative and qualitative defects of circulating VWF. Clearance of VWF may be increased due to fast removal from the circulation by absorbance of VWF to malignant cells and by formation of immune complexes of VWF and auto-antibodies that are removed via the reticuloendothelial system. Another pathophysiologic mechanism is increased proteolysis of high molecular weight VWF multimers in conditions of high shear stress as in some cardiovascular disorders or as a result of proteases. Neutralizing antibodies that inhibit the specific function of the protein as seen in acquired haemophilia are not a common mechanism in AVWS.
The diagnostic workup of AVWS is similar to VWD. The assay of VWF propeptide (VWFpp) may indicate accelerated clearance when the VWFpp/VWF:Ag ratio is increased, however some mutations have been identified among congenital VWD that are also characterized by increased clearance. While neutralizing factor VIII inhibitors can be identified by mixing experiments, the antibodies in AVWS are usually non-neutralizing and cannot easily be measured with routine assays. Specialized ELISA based assays are required to identify the auto-antibodies that increase clearance. Finally, when the differential diagnosis between AVWS and VWD remain difficult, the diagnosis of AVWS can be confirmed by a test infusion of VWF concentrate and assessment of the clearance rate from the circulation. Treatment in AVWS depends on the underlying pathophysiologic mechanism.

Jeroen Eikenboom received his MD degree in 1988 at the Erasmus University Rotterdam, the Netherlands. He obtained his PhD at the Leiden University in 1994; the subject of his thesis was "The inheritance of von Willebrand disease". He was trained in internal medicine at the Leiden University Medical Center and registered as internist in 2001. Since then he sub-specialized in hematology and vascular medicine. He was appointed full professor of Internal Medicine/Hemostasis and Thrombosis in 2011. His clinical work is focused on bleeding disorders, thrombophilia and vascular medicine. His research is focused on the molecular genetics of von Willebrand disease, genotype-phenotype associations in von Willebrand disease, and the biochemical and cell biological aspects of synthesis, storage, secretion and clearance of von Willebrand factor. He has been (co)chairman of the Scientific Subcommittee on von Willebrand factor of the Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis (ISTH) from 1999-2013. He is member of the editorial board of the Journal of Thrombosis and Haemostasis and the British Journal of Haematology and he is associate editor of HemaSphere. He is board member of the Dutch Society of Internists Vascular Medicine.

ORAL PRESENTATIONS: BASIC RESEARCH

O01 **A novel antithrombotic coating for blood-contacting medical devices**
L. Musumec

O02 **Evaluation of the time course of Carboxypeptidase U (CPU, CPB2, TAFIa) generation and influence of AZD9684, a selective CPU inhibitor, in experimental rat stroke models**
J.C. Mertens

O03 **Gene therapy for congenital thrombotic thrombocytopenic purpura using intramuscular electrotransfer DNA delivery**
C. Dekimpe

O04 **From the antiplatelet drug ticagrelor to antibiotics: a study of structure-activity relationships**
N. Jacques

O05 **Inhibition of ADAMTS13 prevents the loss of high molecular weight von Willebrand factor multimers in an in vitro left ventricular assist device**
S. Deconinck

O01

BASIC RESEARCH

A novel antithrombotic coating for blood-contacting medical devices

L. Musumeci[1], A. Aqil[2], N. Jacques[1], C. Jérôme[2], P. Lancellotti[1], C. Oury[1]
[1]Cardiovascular Sciences, University of Liege, LIEGE, Belgium
[2]Chemistry, University of Liege, LIEGE, Belgium

BACKGROUND: Performance of blood-contacting medical devices is often hampered by clot formation due to contact of foreign materials with blood. Practically, there is a high medical need to produce more hemocompatible device surface to reduce thrombotic complication rates.

AIMS: Our study aimed at developing an antithrombotic coating forblood-contacting devices with the ultimate goal to improve device performance.

METHODS: Antiplatelet drug-loaded nanogels (100-200 nm) were formed by cross-linking oxidized polymer of methacrylamide bearing 3,4-dihydroxy-L-phenylalanine (polyDOPA) with polyallylamine hydrochloride (PAH) in the presence of ticagrelor. Coating was achieved by covalent grafting and layer-by-layer assembly of these nanogels onto any test materials pre-coated with polyDOPA covalently linked to PAH. Hydrophilic thiol end-functionalized poly(ethylene glycol) (PEG) (2,000 Da) was then grafted onto the top layer nanogels. The aptamer RB006, a factor IXa antagonist, was bound to the PEG chain. Coating antithrombotic efficacy was assessed by platelet adhesion and aggregation assays under static or flow conditions (cone-and-plate viscometer) as well as by contact activation testing (non-activated partial thromboplastin time).

RESULTS: Upon static incubation of human platelet-rich-plasma on polystyrene wells, surface coating efficiently inhibited platelet adhesion as compared to non-coated wells. Nanogels also conferred potent antiplatelet effect to coated surfaces under high shear rates (1,000 s-1), as shown by reduction of surface coverage by platelets below system detection limit levels, and recovery of baseline single platelet count in suspension. These antiplatelet effects were proportional to the number of nanogel layers. Very interestingly, grafting anti-factor IXa aptamer on the coating significantly reduced contact activation measured in plasma to a similar extent as the addition of corn trypsin inhibitor. Indeed, surface coating prolonged clotting time by about 2-fold as compared to non-coated surfaces.

CONCLUSION: Thus, we developed a novel antithrombotic polymer-based coating technology that could improve clinical performance of all devices in contact with blood.

O02

BASIC RESEARCH

Evaluation of the time course of Carboxypeptidase U (CPU, CPB2, TAFIa) generation and influence of AZD9684, a selective CPU inhibitor, in experimental rat stroke models

J.C. Mertens[1], W. Boisseau2, D. Leenaerts[1], L. Di Meglio[2], S. Loyau[2], A.M. Lambeir[1], C. Ducroux[2], M. Jandrot-Perrus[2], J.B. Michel[2], M. Mazhigi[2], D. Hendriks[1], J.P. Desilles[2]
[1]Laboratory of Medical Biochemistry, University of Antwerp, ANTWERP, Belgium
[2]Laboratory for Vascular Translational Sciences, Paris Diderot University, PARIS, France

BACKGROUND: Intravenous administration of recombinant tissue-type plasminogen activator (tPA) remains the only approved pharmacological treatment for acute ischemic stroke (AIS). However its benefit is limited due to the narrow treatment window, a limited recanalization rate of large vessel occlusions and the risk of hemorrhagic transformation. Novel pharmacological approaches are therefore urgently needed. The zymogen procarboxypeptidase U (proCPU, proCB2, TAFI) is activated by thrombin, the thrombin-thrombomodulin complex or plasmin into carboxypeptidase U (CPU, CPB2, TAFIa) which is a potent attenuator of fibrinolysis. Subsequently, inhibition of CPU is an interesting strategy to improve thrombolysis in AIS.

AIMS: In the current study, the time course of CPU activation and proCPU consumption were assessed in two experimental rat models of acute ischemic stroke (AIS). In addition the effect of the selective CPU inhibitor AZD9684 on CPU kinetics and AIS outcome were evaluated.

METHODS: Male Sprague-Dawley rats (320-400 g) were subjected either to an embolic (eMCAO; N=33) or to a transient middle cerebral artery occlusion (tMCAO; N=34) stroke model or to a sham procedure (N=9). Rats received recombinant tissue-type plasminogen activator (tPA; 10 mg/kg) or saline (0.9 % m/v NaCl) for one hour using a randomized treatment regime. Sham operated rats did not receive treatment. Blood was collected on citrated tubes (0.109 M) containing PPACK (5 µM) and aprotinin (130 µg/mL), inhibitors of thrombin and plasmin respectively. CPU activity and proCPU levels were assessed at five time points using CPU activity and proCPU assays that were modified and validated for use in rat plasma. The tMCAO stroke model was extended with two additional treatment groups, AZD9684 alone (9 mg/kg) and AZD9684 combined with tPA (9 mg/kg and 10 mg/kg respectively). All treatments were administered as a bolus (10 % of total dose) followed by a continuous infusion (90 % of total dose).

RESULTS: A clear activation of the proCPU/CPU system was observed after AIS induction, in both the eMCAO and tMCAO model, both in tPA-treated and untreated rats. Maximal CPU activities were observed shortly after treatment cessation and were higher in tPA-treated animals compared to the saline group (Mann-Whitney U; $P < 0.01$ and $P < 0.05$ for eMCAO and tMCAO respectively). Concomitant proCPU consumption over time was more pronounced in the tPA-treated rats ($P < 0.05$). The use of AZD9684 in the tMCAO model clearly suppressed the in vivo activity of functional CPU and reduced intracerebral fibrinogen deposition, suggesting a reduction of downstream microvascular thrombosis but without significant reduction on final AIS volume.

CONCLUSIONS: A pronounced activation of the proCPU/CPU system during AIS was observed in two different rat stroke models. Our findings suggests that CPU generation could reflect ongoing microvascular thrombosis and showed that selective inhibition of CPU with AZD9684 could reduce downstream microvascular thrombosis.

O03

BASIC RESEARCH

Gene therapy for congenital thrombotic thrombocytopenic purpura using intramuscular electrotransfer DNA delivery

C. Dekimpe[1], G. Vermeire[2], K. Hollevoet[2], N. Nick[3], S.F. De Meyer[1], P. Declerck[2], K. Vanhoorelbeke[1]
[1]Laboratory for Thrombosis Research, KU Leuven Campus Kulak Kortrijk, KORTRIJK, Belgium
[2]Laboratory for Therapeutic and Diagnostic antibodies, KU Leuven, LEUVEN, Belgium
[3]PharmAbs, the KU Leuven Antibody Center, KU Leuven, LEUVEN, Belgium

INTRODUCTION: Congenital thrombotic thrombocytopenic purpura (cTTP) is a rare and life-threatening microangiopathic disease caused by a severe von Willebrand factor-cleaving protease (ADAMTS13, A Disintegrin and Metalloprotease with

Thrombospondin Type 1 repeats, member 13) deficiency, because of bi-allelic mutations in the ADAMTS13 gene. Current disease management consists of therapeutic or prophylactic plasma infusions, but an urgent need for a life-long cure remains, as plasma therapy can have major complications. Therefore, the endogenous expression of ADAMTS13 following gene transfer would be an attractive alternative for plasma therapy. Recently, our group obtained long-term expression of transgene ADAMTS13, with prevention of the development of TTP signs in Adamts13-/- mice after non-viral hydrodynamic gene delivery. Clinically relevant DNA delivery is, however, still required to bring this approach closer to clinical translation.

AIM: We aimed at developing a clinically relevant gene therapy approach for cTTP. For this purpose, intramuscular electrotransfer was used to deliver the murine (m)ADAMTS13 gene to muscle cells of Adamts13-/- mice, since this technique is rapid, simple and drives high and long-term gene expression, as terminally differentiated muscle cells avoid DNA loss over time. In addition, this technique has already been used for human applications.

METHODS: The mADAMTS13 expression plasmid pCAG-mADAMTS13 was constructed using the In-Fusion® HD cloning kit. The construction of pCAG-mADAMTS13 and the ability of muscle cells to produce mADAMTS13, was evaluated by in vitro transfection of C2C12 (mouse myoblast cell line) cells using X-tremeGENETM HP Transfection reagent. Medium was harvested 7 days after transfection and mADAMTS13 antigen (Ag) was measured by ELISA. Next, pCAG-mADAMTS13 was delivered to the tibialis anterior (TA) muscle of Adamts13-/- mice using intramuscular electrotransfer. One hour after hyaluronidase injection, pCAG-mADAMTS13 (different doses: 1 (n=2) – 5 (n=2) – 10 (n=2) – 25 (n=2) – 60 (n=5) – 2x60 (n=4) µg pDNA) was injected, followed by electroporation (EP) using the NEPA21 Electroporator with CUY650P5 tweezer electrodes. Plasma samples were collected at day 3 – 7 – 10 – 14/15 after EP, and ADAMTS13 Ag and anti-ADAMTS13 antibodies were measured by ELISA. In addition, TA muscle lysates from 3 mice (60 µg/pDNA; 3-4 weeks post injection) were analysed in the ADAMTS13 Ag ELISA.

RESULTS: Successful construction of pCAG-mADAMTS13 and the ability of C2C12 cells to produce and secrete mADAMTS13 in vitro was demonstrated by detection of mADAMTS13 in medium from pCAG-mADAMTS13-transfected C2C12 cells, but not in non-transfected cells. In vivo, mADAMTS13 Ag was present in lysates from pCAG-mADAMTS13-EP, but not in the non-EP TA muscles. ADAMTS13 Ag, however, was not quantifiable in plasma after EP of pCAG-mADAMTS13. This could be explained by the strong anti-ADAMTS13 immune response, present in all mice from 14 days post injection.

CONCLUSIONS: In this study, we demonstrated functional in vivo mADAMTS13 gene transfer via intramuscular electroporation by detection of mADAMTS13 in pCAG-mADAMTS13-electroporated TA muscles lysates. However, in all mice, a strong anti-ADAMTS13 immune response cleared ADAMTS13 from plasma. To overcome this, we created immune-deficient Adamts13-/- mice to further investigate this methodology, since exogenous ADAMTS13 administration in humans in generally non-immunogenic

O04

BASIC RESEARCH

From the antiplatelet drug ticagrelor to antibiotics: a study of structure-activity relationships

N. Jacques, E. Goffin, L. Musumeci, B. Pirotte, P. Lancellotti, C. Oury
GIGA-Cardiovascular Sciences, Laboratory of Cardiology, University of Liège, and Department of Cardiology, University of Liège Hospital, CHU Sart-Tilman, LIÈGE, Belgium

BACKGROUND: Ticagrelor is a widely administered orally active antiplatelet drug belonging to 1,2,3-triazolo[4,5-d]pyrimidines, which acts by reversibly inhibiting the platelet P2Y12 receptor for ADP in a noncompetitive manner. Although ticagrelor does not need to be metabolized to be active in vivo, it is extensively metabolized to form a main metabolite known as AR-C124910, equally potent to inhibit P2Y12 receptors. Importantly, we recently discovered that ticagrelor and AR-C124910 exert bactericidal and anti-biofilm activity against gram-positive bacteria, including methicillin-resistant Staphylococcus aureus and vancomycin-resistant Enterococcus faecalis.

AIM: The present work aimed at establishing structure-activity relationships of a series of 1,2,3-triazolo[4,5-d]pyrimidines in order to identify structural determinants of antiplatelet and antibacterial activity and to assess the possibility of chemically dissociating the two properties.

METHODS: Ticagrelor (1S,2S,3R,5S)-3-[7-[(1R,2S)-2-(3,4-difluorophenyl)cyclopropylamino]-5-(propylthio)-3H-[1,2,3]-triazolo[4,5-d]pyrimidin-3-yl]-5-(2-hydroxyethoxy)-1,2-cyclopentanediol), five main metabolites, including AR-C124910 and AR-C133913, and eighteen 1,2,3-triazolo[4,5-d]pyrimidines lacking different chemical groups or bearing atom substitutions were synthetized. Molecule purity was assessed by NMR spectroscopy and elemental analysis. The antiplatelet activity of the test molecules was analysed by light transmission aggregometry upon platelet stimulation with ADP in citrated platelet-rich-plasma using doses equal to ticagrelor IC50. The antibacterial activity against methicillin-sensitive (MSSA, ATCC 6538) and methicillin-resistant (MRSA, ATCC BAA-1556) Staphylococcus aureus was determined by the broth microdilution method as recommended by EUCAST guidelines.

RESULTS: Only ticagrelor and AR-C124910 showed antibacterial activity against MSSA and MRSA. We found out that molecules lacking either the difluorphenylcyclopropyl group or the hydroxyethoxy cyclopentane-1,2-diol were all inactive against bacteria. Interestingly, such restriction did not apply to the antiplatelet activity. Indeed, seven of these molecules, devoid of antibacterial activity, still inhibited the ADP-induced platelet aggregation. Noteworthy, one of them, (1S,2R,3S,4R)-4-(7-(((1R,2S)-2-(3,4-difluorophenyl)cyclopropyl)amino)-5-(((S)-2-hydroxypropyl)thio)-3H-[1,2,3]triazolo[4,5-d]pyrimidin-3-yl)cyclopentane-1,2,3-triol, corresponding to a metabolite formed in vivo, was as potent as ticagrelor to inhibit platelets.

CONCLUSIONS: We identified molecules that preserved potent antiplatelet activity while being inactive against MSSA and MRSA. Thus, in vitro, the antiplatelet and antibacterial activity of 1,2,3-triazolo[4,5-d]pyrimidines are not necessarily linked. These molecules might help demonstrating ticagrelor antibacterial activity in vivo, independently of or in addition to its antiplatelet activity.

O05

BASIC RESEARCH

Inhibition of ADAMTS13 prevents the loss of high molecular weight von Willebrand factor multimers in an in vitro left ventricular assist device

S. Deconinck[1], A. Schelpe[1], E. Roose[1], S. Jacobs[2], C. Nix[3], S. Barth[3], H.B. Feys[4], I. Pareyn[1], C. Tersteeg[1], H. Deckmyn[1], B. Meyns[2], S.F. De Meyer[1], K. Vanhoorelbeke[1]
[1]Laboratory for Thrombosis Research, KU Leuven Campus Kulak Kortrijk, KORTRIJK, Belgium
[2]Department of Clinical Cardiac Surgery, KU Leuven, LEUVEN, Belgium
[3]Abiomed Europe GmbH, AACHEN, Germany
[4]Transfusion Research Center, Belgian Red Cross-Flanders, GHENT, Belgium

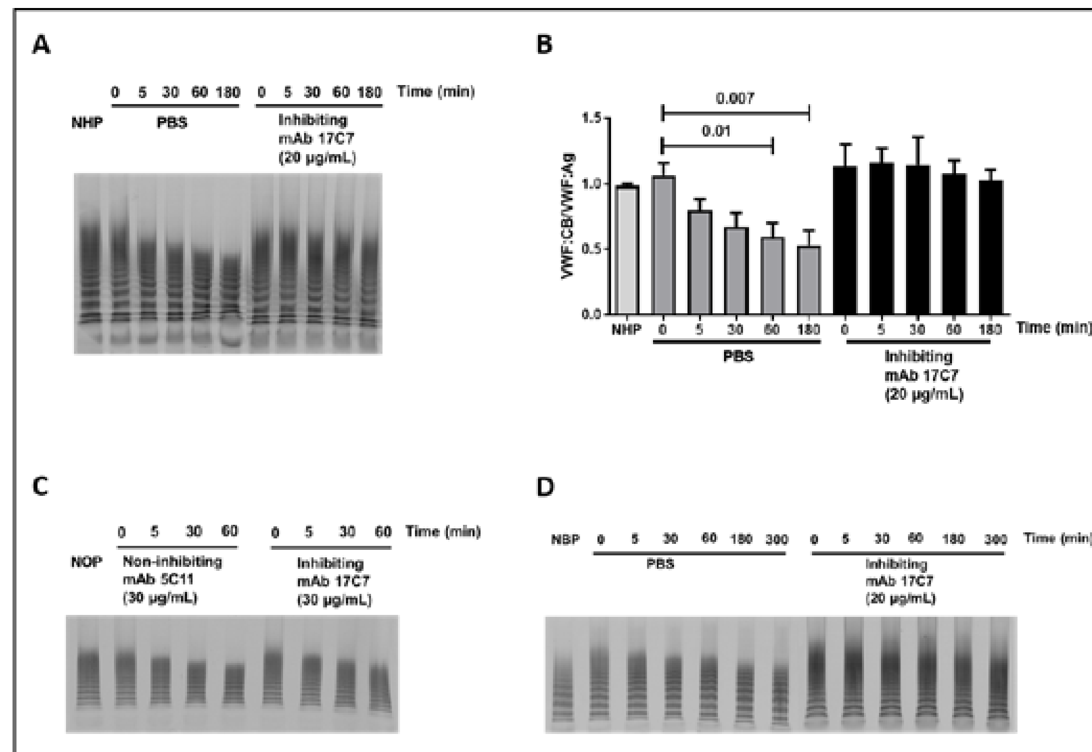
BACKGROUND: The bleeding diathesis observed in patients implanted with a left ventricular assist device (LVAD) is linked to the acquired von Willebrand syndrome as all patients have a loss of high molecular weight (HMW) VWF multimers. The loss of HMW VWF multimers might be explained by an increased shear-induced proteolysis of VWF by ADAMTS13. Hence, specifically blocking ADAMTS13 might be an efficient way to rescue the loss of HMW VWF multimers in LVAD patients.

AIM: To investigate if blocking ADAMTS13 using the inhibitory anti-ADAMTS13 monoclonal antibody (mAb) 17C7 can prevent the loss of HMW VWF multimers in an in vitro LVAD circuit using human, ovine and bovine blood.

METHODS: Citrate-anticoagulated human, ovine and bovine blood (750 mL) was circulated through an in vitro Impella CP® (heart pump used for short-term support) circuit in the prescence of the inhibitory mAb 17C7 (n=4) or the control mAb 5C11 (n=4) or PBS (n=4). Plasma samples were analysed for VWF multimers, VWF antigen (VWF:Ag) and VWF collagen binding activity (VWF:CB).

RESULTS: Blocking ADAMTS13 using the inhibitory mAb 17C7 prevented the loss of HMW VWF multimers and hence a decrease in VWF:CB/VWF:Ag in the in vitro Impella CP® system with human blood (Figure 1A and 1B) showing the therapeutic potential of 17C7 to rescue the loss of HMW VWF multimers in LVAD patients. However, blocking ovine ADAMTS13 with 17C7 did not prevent the loss of HMW VWF multimers in vitro (Figure 1C). In contrast, when using bovine blood in an in vitro Impella CP® system, the decrease in HMW VWF multimers could be prevented by adding 17C7 (Figure 1D), similar to what was observed with human blood.

CONCLUSION: Blocking ADAMTS13 is a promising therapeutic strategy to prevent the loss of HMW VWF multimers in LVAD patients. The loss of HMW VWF multimers is also ADAMTS13 dependent in calves, but not in sheep, making the calf the ideal preclinical animal model to study the in vivo effect of this novel therapy.



Inhibition of ADAMTS13 in an in vitro LVAD system using human, ovine and bovine blood.

ORAL PRESENTATIONS: CLINICAL & LABORATORY

O06 **Histological analysis of a thrombectomy-resistant ischemic stroke thrombus: a case report**
S. Staessens

O07 **Understanding the TTP literacy in patients with TTP**
L.C. Velasquez Pereira

O08 **Comparison of INNOVANCE PFA P2Y with light transmission aggregometry to detect clopidogrel resistance**
L. Heireman

O09 **Evaluation of the influence of statin therapy on procarboxypeptidase U (proCPU, TAFI, proCPB2) biology in patients eligible for statin therapy**
K. Claesen

O10 **Optimization of measurement of emicizumab, FVIII and anti-FVIII concentration in patients treated with emicizumab**
L. Heireman

O06

CLINICAL & LABORATORY

Histological analysis of a thrombectomy-resistant ischemic stroke thrombus: a case report

S. Staessens[1], O. François[2], L. Desender[3], T. Dewaele[2], P. Vanacker[4], R. Sciot[5], K. Vanhoorelbeke[1], T. Andersson[2], S.F. De Meyer[1]

[1]Laboratory for Thrombosis Research, KU Leuven campus Kulak, KORTRIJK, Belgium
[2]Department of Medical Imaging, Groeninge Hospital, KORTRIJK, Belgium
[3]Laboratory of Thrombosis Research, KU Leuven campus Kulak, KORTRIJK, Belgium
[4]Department of Neurology, Groeninge Hospital, KORTRIJK, Belgium
[5]Department of Pathology, University Hospitals Leuven, LEUVEN, Belgium

INTRODUCTION: Ischemic stroke is mostly caused by a thromboembolic occlusion of cerebral arteries. Since 2015, endovascular procedures have dramatically changed acute stroke treatment by mechanically removing the thrombus. One of the most important obstacles in this rapidly developing field is the fact that thrombi tend to differ in consistency and removability. Indeed, mechanical thrombectomy is unsuccessful in approximately 20% of the cases. Furthermore, multiple attempts to remove the thrombus are associated with worse clinical outcome.1 The reasons for this thrombectomy resistance are not well understood, but understanding thrombus composition might lead to novel insights on why certain thrombi are difficult to retrieve.

AIM: The aim of this study was to histologically analyze a thrombectomy-resistant stroke thrombus.

METHODS: Thrombus material was histologically analyzed using hematoxylin and eosin, martius scarlet blue staining (red blood cells (RBCs) and fibrin), Feulgen staining (DNA), von Kossa staining (calcifications) and immunohistochemical analysis of von Willebrand factor (VWF), platelets (GPIb), leukocytes (CD45) and neutrophil extracellular DNA traps (H3Cit; NETs).

RESULTS: A 63 year old female patient was admitted to the emergency department at the AZ Groeninge Hospital (Kortrijk, Belgium) with an occlusion of the right M1 segment of the middle cerebral artery. The etiology of the stroke was classified as cryptogenic, i.e. unknown. The patient was ineligible to receive intravenous thrombolysis, but fulfilled the requirements for thrombectomy. Strikingly, 11 attempts using various thrombectomy devices (4 attempts with EmboTrap II 5 x 33 mm, 4 attempts with Trevo 4 x 30 mm and 3 attempts with pure contact aspiration through an intermediate catheter – Sofia 5F) were required to eventually remove the thrombus. This rare case of achieving thrombus material from a nearly irretrievable thrombus allowed us to perform an in-depth histopathological study of the retrieved material. Histological analysis of the thrombus revealed an atypical structure. Approximately 40% of the thrombus consisted of platelet- and RBC-rich areas that are typically found in all stroke thrombi.2 The remaining 60%, however, consisted of an atypical structure with abundant amounts of extracellular DNA (exDNA) and VWF. The exDNA stained positive for the leukocyte marker and stained partially positive for H3Cit, indicating that the exDNA is most likely originating from leukocytes and to some extent from NETs. Of note, a von Kossa staining revealed abundant microcalcifications that colocalized with exDNA and VWF, suggesting an aged nature of the thrombus.

CONCLUSION: This case-report describes a unique case in which we were able to study composition of a stroke thrombus that was nearly completely thrombectomy-resistant. Interestingly, our study showed an atypical composition compared to the common structural features found in ischemic stroke thrombi. The bulk of the retrieved thrombus consisted of exDNA that colocalized with VWF and microcalcifications, which together could possibly explain the resistance to mechanical removal. Recent evidence indeed suggests that both NETs and microcalcifications could be associated with thrombectomy resistance.3,4 Our findings further our understanding on thrombus removability and are important in order to develop improved protocols and technologies that increase thrombectomy success rates, ultimately leading to better clinical outcome of thrombectomy-treated patients.

REFERENCES:

Luby, M. et al. Frequency of Blood-Brain Barrier Disruption Post-Endovascular Therapy and Multiple Thrombectomy Passes in Acute Ischemic Stroke Patients. Stroke 50, 2241–2244 (2019).
Staessens, S. et al. Structural analysis of ischemic stroke thrombi: histological indications for therapy resistance. Haematologica haematol.2019.219881 (2019). doi:10.3324/haematol.2019.219881
Ducroux, C. et al. Thrombus Neutrophil Extracellular Traps Content Impair tPA-Induced Thrombolysis in Acute Ischemic Stroke. Stroke 49, 754–757 (2018).
Dobrocky, T. et al. Thrombectomy of calcified emboli in stroke. Does histology of thrombi influence the effectiveness of thrombectomy? J. Neurointerv. Surg. 10, 345–350 (2018).

O07

CLINICAL & LABORATORY

Understanding the TTP literacy in patients with TTP

L.C. Velasquez Pereira[1], B. Ercig[2], K. Kangro[1], S. Malot[3], L. Galicier[3], P. Poullin[3], F. Provôt[3], K. Vanhoorelbeke[4], E. Azoulay[3], A. Veyradier[3], P. Coppo[3]

[1]Chemistry, KU Leuven Campus Kulak Kortrijk, KORTRIJK, Belgium
[2]Plasma Proteins, Sanquin-Academic Medical Center Landsteiner Laboratory, AMSTERDAM, Netherlands
[3]Centre de Référence des Microangiopathies Thrombotiques, AP-HP.6, PARIS, France
[4]KU Leuven Campus Kulak Kortrijk, KORTRIJK, Belgium

BACKGROUND/INTRODUCTION: Thrombotic thrombocytopenic purpura (TTP) is a thrombotic microangiopathy, which is characterized by a severe deficiency in the von Willebrand factor (VWF) cleavage protease ADAMTS13. Patients suffer from either the immune-mediated form of the disease (iTTP) or the congenital form (cTTP). Since after an acute TTP episode, patients

are at risk for relapse, a careful follow-up is needed. Adherence to the follow up by patients implies a good understanding of TTP. However, TTP literacy in patients is currently unknown.

AIM: To study TTP literacy in patients in order to identify factors associated with poor disease understanding.

METHODS/MATERIALS: An observational, analytical and prospective TTP literacy study was established. A questionnaire was developed focusing on sociodemographic factors, knowledge about TTP and patient's actions in an emergency setting. TTP literacy was scored using a scale range from 0 to 5. Scores below 3 and above 4 correspond to low and high TTP literacy, respectively, while a score, greater than or equal to 3 and less than 4, corresponds to an intermediate TTP literacy. A total of 120 TTP patients in remission from the French national registry for thrombotic microangiopathy (CNR-MAT), 119 iTTP patients and one cTTP, filled in the questionnaire. Association analysis was done by chi-square, one way ANOVA and multiple linear regression.

RESULTS: The TTP literacy score in TTP patients was low in 24%, intermediate in 39% and high in 37% of the patients. Low TTP literacy was associated with older age and low education level (high school and vocational high school). The main knowledge gaps in TTP literacy were as follows: the role of von Willebrand factor (VWF) in TTP was not known by 65% of the participants; 47% of participants did not consider themselves at risk for relapse; 36% of the participants recognized that TTP is a rare disease, however they were not aware of predisposing factors that lead to the development of TTP (like pregnancy); finally, 44% of the participants were aware that plasma exchange is the front line therapy in an acute episode, but few participants (8%) knew that it has to be administered daily. Regarding the attitude of participants in an emergency setting, participants did know the emergency action steps.

SUMMARY/CONCLUSION: It is required to understand TTP literacy in patients as well as design accessible, effective and comprehensive educational interventions in order to improve the treatment adherence, frequently follow-up the patients, and reduce associated morbidities in TTP patients. Our study shows that the educational material should pay special attention to the topics related to predisposing factors for TTP development, the role of VWF in TTP and frequency of plasma exchange in the acute phase of the disease; and that mainly age and education level influence the understanding of the disease.

O08

CLINICAL & LABORATORY

Comparison of INNOVANCE PFA P2Y with light transmission aggregometry to detect clopidogrel resistance

L. Heireman[1], T. Monteyne[1], D. Hemelsoet[2], K. Devreese[1]
[1]Coagulation Laboratory, Department of Laboratory Medicine, University Hospital Ghent, GHENT, Belgium
[2]Department of Neurology, Ghent University Hospital, GHENT, Belgium

INTRODUCTION: Clopidogrel is an irreversible antagonist of adenosine diphosphate (ADP)-induced aggregation by inhibiting the platelet membrane P2Y receptor. However, this prodrug is characterized by interindividual variability in pharmacokinetic and pharmacodynamic response. Platelet function tests are widely used to identify poor responders to the antiaggregant clopidogrel, who are exposed to a higher risk for cardiovascular events. Light transmission aggregometry (LTA) is still regarded as the golden standard method for detecting clopidogrel resistance, however, LTA is time consuming, requires large sample volumes and is not well standardized.

AIMS: The aim of the present study was to evaluate the inter-assay variability between LTA and the newly introduced INNOVANCE PFA P2Y test, requiring small sample volumes and less hands-on time, to accurately detect clopidogrel resistance.

MATERIALS/METHODS: In total, we included 107 patients chronically treated with clopidogrel 75 mg once daily for > 1 week for the secondary prevention of cardiovascular diseases or before undergoing intracranial stenting. LTA results of 115 samples obtained with the routinely used Chronolog 700 platelet aggregometer (Chronolog) (using Chrono-Par® ADP reagent) were compared to results of PFA P2Y performed on the INNOVANCE PFA-200® instrument (Siemens Healthcare). Responsiveness to clopidogrel was defined as a peak aggregation <50% and/or disaggregation >20% after adding 2.5 μM and/or 5 μM ADP. For PFA, we defined a PFA CT of >113 seconds as reference range for a clopidogrel effect (based on the manufacturer's recommended cut-off value of 106 and in-house imprecision). The correlation between LTA and INNOVANCE PFA P2Y was determined by the weighted kappa correlation coefficient (MedCalc).

RESULTS: For PFA P2Y, a clopidogrel effect was observed in 72% of the tested samples while aggregometry showed a clopidogrel effect in 85% of the cases. In total, 21 (18%) discrepancies were reported between both methods (Table 1). In most cases (18 or 16%), a false normal result was obtained with PFA P2Y, indicating clopidogrel resistance. In three cases (3%), a false abnormal result was found with PFA P2Y, potentially missing clopidogrel resistance. Discrepancies were almost equally observed in patients under clopidogrel monotherapy and clopidogrel and aspirin dual therapy (13/21 or 62%, as in the total study population). In more than half of the discrepant cases (62%), PFA P2Y did not correlate with LTA for both 2.5 and 5 μmol/L ADP agonists. The weighted kappa correlation coefficient was 0.469 (95% CI: 0.285 to 0.653), showing a moderate agreement between INNOVANCE PFA P2Y and LTA.

CONCLUSIONS: PFA P2Y could be used as a screening test, in combination with aggregometry if PFA P2Y result is normal. However, with this work-out, aggregometry has to performed in approximately 30% of the patients. In more than half of these patients, LTA will show an effect of clopidogrel. In addition, clopidogrel resistance will be missed in 18% of patients when applying PFA P2Y as screening tool. To conclude, a moderate comparability of the INNOVANCE PFA P2Y to LTA is observed. Overall, PFA P2Y is not sensitive enough to detect a clopidogrel response.

INNOVANCE PFA P2Y	LTA (Chronolog 700 platelet aggregometry)		
	No effect of clopidogrel	Effect of clopidogrel	Total
No effect of clopidogrel	14	18	32
Effect of clopidogrel	3	80	83
Total	17	98	115

Table 1: Overview of the comparison between LTA and INNOVANCE PFA P2Y to detect clopidogrel resistance

Evaluation of the influence of statin therapy on procarboxypeptidase U (proCPU, TAFI, proCPB2) biology in patients eligible for statin therapy

K. Claesen[1], J.C. Mertens[1], S. De Belder[2], J. Maes[2], J. Bosmans[3], H. Stoffelen[2], I. De Meester[1], D. Hendriks[1]
[1]Laboratory of Medical Biochemistry, University of Antwerp, WILRIJK, Belgium
[2]General practitioners' group Epione, EDEGEM, Belgium
[3]Departement of Cardiology, University Hospital Antwerp, ANTWERP, Belgium

O09

CLINICAL & LABORATORY

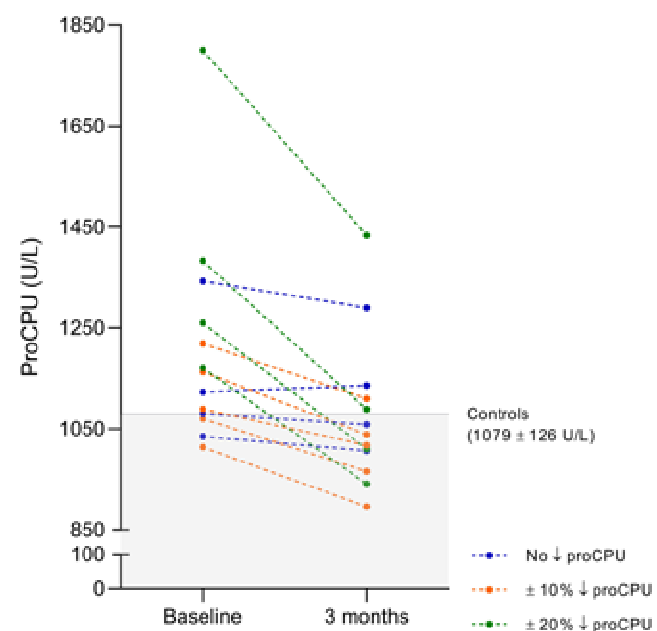
INTRODUCTION: Statins (hydroxymethyl-glutaryl-CoA-reductase inhibitors) are commonly used in hypercholesterolemic patients to lower cholesterol. In addition, statins appear to possess a range of non-lipid related pleiotropic effects, such as antithrombotic properties (e.g. influence on tissue factor, platelet aggregation, fibrinolysis). In this context the effect of statins on fibrinolysis and fibrinolytic parameters has been studied elaborately. One of the studied parameters is the antifibrinolytic enzyme carboxypeptidase U (CPU, TAFIa, CPB2), which is present in the circulation as its inactive precursor procarboxypeptidase U (proCPU, TAFI, proCPB2). However, up till now almost all studies evaluating the influence of statin therapy on the proCPU/CPU system were hampered by methodological limitations. Therefore, further investigation is required.

AIMS: In a proof of concept observational study, the influence of statin therapy on proCPU biology was evaluated in patients eligible for statin therapy before and after three months of therapy.

METHODS: Eligibility for statin therapy was assessed by the general practitioner based on the overall cardiovascular risk of a patient and according to the Systemic Coronary Risk Estimation (SCORE) chart of the European Society of Cardiology. In patients eligible for statin therapy, a serum and a citrated (0.109 M) plasma sample were collected before and after three months of statin therapy. Subjects who were not eligible for statin therapy served as controls from whom a single serum and citrated plasma sample were collected. Total cholesterol (TC) and low-density lipoprotein (LDL-C) were determined on the serum sample. The citrated plasma sample was used to study the proCPU biology. ProCPU levels were determined with an activity based proCPU assay using the selective and specific substrate Bz-o-cyano-Phe-Arg based on the method described by Heylen et al. Additionally clinical data of all included subjects were collected. Approval for the study was obtained by the local ethics committee (B300201837918) and all patients gave their informed consent.

RESULTS: Thirteen subjects eligible for statin therapy (25% male, median age 67 years (IQR 64–72)) were included. Ten subjects that were not eligible for statin therapy (50% male, median age 60 years (IQR 57–61)) served as controls. Baseline TC, LDL-C and proCPU levels were respectively 226 ± 20 mg/dL, 136 ± 19 mg/dL and 1211 ± 210 U/L for the statin-treated subjects, and 204 ± 48 mg/dL, 110 ± 28 mg/dL and 1079 ± 126 U/L for the controls. After three months, a significant decrease in TC (55 ± 28 mg/dL (24.3%); p=0.008) and LDL-C (57 ± 27 mg/dL (41.7%); p=0.008) were observed that coincided with a decrease in proCPU levels (136 ± 257 U/L (11.2%); p=0.002) in the subjects receiving statin therapy. It should be noted however that an important difference in proCPU decrease was observed between individual patients (Figure 1).

CONCLUSIONS: A 11.2% decrease in proCPU levels was observed after three months of statin therapy, suggesting an improvement in fibrinolysis and atherogenic tendencies upon statin therapy, but probably not to the same extent in all patients. Whether the observed phenomenon is of clinical relevance will be subject of a larger observational study.



Dot plot showing proCPU levels (U/L) of statin-treated subjects, before and after three months of statin therapy.

O10

CLINICAL & LABORATORY

Optimization of measurement of emicizumab, FVIII and anti-FVIII concentration in patients treated with emicizumab

L. Heireman, K. Devreese
Coagulation Laboratory, Department of Laboratory Medicine, University Hospital Ghent, GHENT, Belgium

INTRODUCTION: Hemlibra® contains emicizumab, a bispecific antibody that bridges factor IXa(FIX) and factor X(FX), allowing the coagulation cascade to continue in patients with haemophilia A. Emicizumab is approved as prophylactic treatment to prevent bleeding episodes in patients with FVIII inhibitors. It has been reported that activated partial thromboplastin time(aPTT) and one stage factor VIII(FVIII) assays are oversensitive to emicizumab.

AIMS: The first aim was to confirm that a one stage clotting assay(OSCA) and chromogenic assay(CA) modified with emicizumab calibrator is suited for quantification of emicizumab. Secondly, we evaluated a OSCA and CA for the measurement of FVIII and anti-FVIII concentrations in patient samples with emicizumab.

MATERIALS/METHODS: Both the OSCA(C.K. PREST® reagent, Diagnostica Stago; predilution 1/80) and CA(BIOPHEN™ FVIII:C reagent, HYPHEN BioMed; predilution 1/40) modified with emicizumab calibrator(r2 Diagnostics) were applied on STA-R EVO(Diagnostica Stago) for quantification of emicizumab(µg/mL) in a severe hemophilia patient before and during treatment with emicizumab, normal pooled plasma(NPP), FVIII deficient plasma and control samples(25µg/mL and 75µg/mL, r2 Diagnostics).
To explore potential interference of FVIII in the emicizumab assay, dilutions of NPP in FVIII deficient plasma(0-100% FVIII) were measured. FVIII and anti-FVIII (Bethesda assay) were measured with OSCA(C.K. PREST reagent) and CA(BIOPHEN™ FVIII:C and TriniCHROM Factor VIII:C reagent, Diagnostica Stago) in samples of the patient under emicizumab.

RESULTS: Coefficient of variation for emicizumab control samples(n=6) ranged from 1.8-2.1% for OSCA and 8.7-9.7% for CA. The limit of detection(LOD)(n=6) measured on FVIII deficient plasma with OSCA was <5µg/ml. Emicizumab levels(16-24µg/ml) in samples from the treated patient(n=3)(FVIII <0.3%) were similar(difference 2-17%) between both methods, and were undetected in samples before treatment(FVIII <0.3%-1.9%)(n=6). Repeated measurement with OSCA on NPP(n=6) resulted in a LOD of 20.7 µg/ml emicizumab. In a serial dilution series of FVIII, a positive linear correlation was observed between FVIII and emicizumab for both assays(figure 1). Interference was observed for FVIII concentrations >14% using OSCA and >0% using CA. In patients under emicizumab therapy, a large overestimation of FVIII was observed using the OSCA(121.0 to 175.8%) compared to the CA with BIOPHEN™ reagent(9.3-13.6%). CA with TriniCHROM reagent measured low FVIII(<1.6%) in this patient, as expected. In addition, anti-FVIII measured with CA Trinichrom in the patient samples containing emicizumab showed 2.8-3.5 BE similar as measured with the OSCA before treatment. Anti-FVIII measured with OSCA was underestimated(<0.5BE).

CONCLUSIONS: The OSCA(C.K. PREST® reagent) modified with emicizumab calibrator is more accurate compared to CA(BIOPHEN™ reagent) for the quantification of emicizumab. Prior denaturation of FVIII is necessary in samples that contain native FVIII due to interference with both emicizumab assays. The CA(TriniCHROM reagent) is the preferred method for the quantification of FVIII and anti-FVIII in patients treated with emicizumab, explained by the use of bovine instead of human coagulation factors in this assay on which emicizumab is not active.

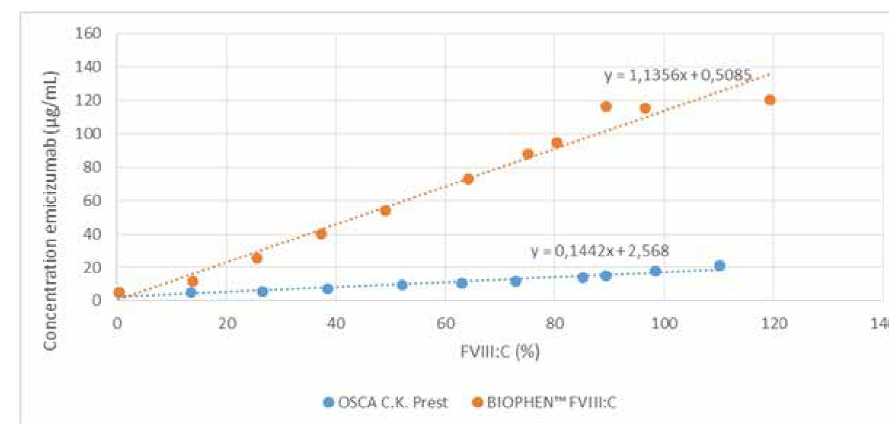


Figure 1: Results of emicizumab concentration in serial dilution series with FVIII (0-100%).

ABSTRACTS POSTERS
P01 **Assessing aPTT reagent sensitivity to lupus anticoagulant**
K. Ver Elst
P02 **Analysis of von Willebrand Disease in the Slovak Republic (Bratislava): The BRA-VWD study; an update**
I. Vangenechten
P03 **Clot waveform analysis as an added value to optical clot detection in aPTT measurements**
K. Eeckhout
P04 **Assessment of Second and Third Generation Oral Contraceptives APC Resistance by a Newly Validated ETP-Based APC Resistance Assay: A Pilot Study**
L. Morimont
P05 **Assessment of thrombomodulin resistance in women using second generation, third generation and progestin-only contraceptives**
L. Morimont
P06 **Validation of an original ETP-based APC resistance assay for the evaluation of prothrombotic states**
L. Morimont
P07 **Proposition of a new scale for the harmonization and normalization of the nAPCsr: a scientific and regulatory need for clinical studies comparison**
L. Morimont
P08 **Comparison of the TEG5000 with other thromboelastographic and -metric analysers**
T. Cammaert
P09 **Resolving anti-FXa DOAC interference on a FXa based method for Antithrombin measurement by using activated carbon**
T. Cammaert
P10 **Determining the optimal method for FVIII:C and anti-FVIII quantification in a patient treated with Adynovi® who developed anti-FVIII during treatment**
L. Heireman
P11 **Method comparison of rotational thromboelastometry (ROTEM®) sigma and delta devices**
L. Heireman
P12 **A lot of variables to consider when monitoring Hemophilia B patients on extended half-life (EHL) products?**
I. Vangenechten
P13 **Reduction of preoperative waiting time before semi-urgent surgery in patients on P2Y12 inhibitors using multiple electrode aggregometry: a retrospective study**
C. Dupuis
P14 **Evaluation of the automated and standardized analyzer St-Genesia in order to measure thrombin generation in the clinical lab**
L. Rozen
P15 **An ultra-high-performance liquid chromatography coupled with a tandem mass spectrometry method for the quantification of edoxaban: The importance to measure active metabolite**
R. Siriez
P16 **Assessment of low plasma concentration of apixaban in the periprocedural setting**
H. Godeaux
P17 **An unusual cause of abnormal APTT: prekallikrein (Fletcher factor) deficiency**
P. Cauchie
P18 **Validation of the haemolysis, icterus and lipaemia preanalytical module on Stago STA R Max analysers**
H. Paridaens
P19 **Evaluation of DOAC Remove® for the measurement of thrombophilia screening parameters in patients treated with direct anti-Xa oral anticoagulants**
A. Evenepoel
P20 **Functional characterization of antithrombin p.Thr147Ala: not just another SNP?**
C. Orlando
P21 **Platelets and aortic valve calcification: insights from a rabbit model and from patients with severe aortic stenosis**
N. Donis
P22 **Neutrophils and neutrophil extracellular traps in inflammation-induced Staphylococcus aureus endocarditis**
S. Meyers
P23 **Hyperactive SRC kinase results in thrombocytopenia: studies in patients and inducible Pluripotent Stem Cells**
L. De Kock
P24 **Pharmacological ACC inhibition decreases thrombin-induced platelet aggregation by a mechanism independent of lipid content**
M. Octave

Assessing aPTT reagent sensitivity to lupus anticoagulant

K. Ver Elst[1], S. Jacobs[2], S. Vermeiren[1], S. Weekx[1]
[1]Laboratory of Hematology, GZA Hospitals, WILRIJK, Belgium
[2]Quality Management, GZA Hospitals, ANTWERP, Belgium

INTRODUCTION: The activated partial thromboplastin time (APTT) assay is a very commonly performed coagulation test. The test may be requested for screening of factor deficiencies in the contact factor (intrinsic) pathway, monitoring of unfractionated heparin or lupus anticoagulant (LA) screening. APTT reagents differ significantly in their sensitivity to LA, largely due to differences in phospholipid type and concentration, but may also be influenced by the activator type. A good working knowledge of the laboratory's APTT reagent sensitivity to prolongation by LA has practical applications as to how unexpectedly long APTTs is handled, management of UFH therapy, the need for mixing tests and their interpretation. APTT is sometimes used as part of a LA test panel using sensitive and insensitive reagents.

AIM: LA sensitivity of the APTT reagent SynthASiL® (HemosIL, Werfen, USA) was performed on retrospective laboratory data from the laboratory information system (LIS).

MATERIALS AND METHODS: APTT and LA test results were exported from the LIS to Excel. Data from 480 patients (median age 48yr, min-max 1-88yr) and 3 External Quality samples from ECAT were collected. LA testing was performed following guidelines for lupus detection. LA testing was performed by dRVVT Screen® (dRVVT-S) and Confirm® reagents and Silica Clotting Time Screen® (SCT-S) and Confirm® on ACL TOP 500® (HemosIL, Werfen, USA). Mixing test (1:1) was performed by a commercial normal pool plasma (VisuCon-F, Affinity Biologicals Inc, Canada). LA conclusion was made after interpretation by the local LA Interpretation flowchart. After interpretation, a final positive LA conclusion can be made if one or both pathways are positive. To determine the sensitivity of the APTT reagent, APTT was compared to dRVVT-S and SCT-S ratio in 457 patients, after exclusion of 23 patients with other conclusions (10 DOAC, 10 LMWH and 3 factor deficiency).

RESULTS AND DISCUSSION: Although an overlap is seen, the difference in APTT ratio between LA positive (n=33) and negative patients (n=424) is statistically significant, confirming the discriminating ability of the reagent. In LA positive patients, 26/33 patients had an abnormal APTT. Ratios were abnormal in 21/33 dRVVT-S and 29/33 SCT-S. From the 33 LA positive patients, 4 were positive only in the dRVVT pathway, 12 only in the SCT pathway and 17 in both pathways. A significant difference in APTT is seen between LA positive patients in one pathway (median 1.31; n=20) and both pathways (median 1.98; n=13). APTT is more often false positive in LA negative patients than is dRVVT-S or SCT-S. APTT is less often false normal in LA positive patients than is dRVVT-S but more often than SCT-S. Diagnostic specificity, sensitivity, negative predictive value (NPV) and positive predictive value (PPV) are shown in Table 1. APTT ratios were normal (true negative) in 85.6% of LA negative patients and abnormal (true positive) in 78.8% of LA positive patients.

CONCLUSION: Sensitivity of APTT reagent SynthASiL to lupus anticoagulant of varied strengths is 78,8%.

%	APTT	dRVVT	SCT
Specificity	85,6	95,5	97,6
Sensitivity	78,8	69,7	97,0
NPV	98,1	97,6	99,8
PPV	29,9	54,8	76,2

P02

Analysis of von Willebrand Disease in the Slovak Republic (Bratislava): The BRA-VWD study; an update

I. Vangenechten[1], A. Batarova[2], A. Gadisseur[1]
[1]Department of Hematology, UZ Antwerp, EDEGEM, Belgium
[2]Department of Hematology & Blood Transfusion, Bratislava University Hospital, BRATISLAVA, Slovak Republic

BACKGROUND: Von Willebrand Disease (VWD) is an inherited bleeding disorder caused by a quantitative (type 1 and 3) or qualitative (type 2) defect of von Willebrand factor (VWF). The current ISTH classification based on measurement of VWF:Ag, VWF:RCo, Ristocetin Induced Platelet Aggregation (RIPA) and VWF multimers leaves a heterogeneity of laboratory phenotyping making diagnosing difficult.

AIMS: A cross-sectional, family based VWD study in a collaboration between the Bratislava University Hospital (Slovak Republic) and the Antwerp University Hospital (Belgium).

METHODS: Blood samples from 180 patients (130 families) were collected from patients with suspected or known VWD with one of the following characteristics: VWF:Ag<35%, VWF:Ac/VWF:Ag<0.6, VWF:CB/VWF:Ag<0.6, FVIII:c/VWF:A <0.5, or positive low concentration RIPA, and grouped in families. From each family the proband was included in the study together with a at least one affected sibling or parent. Complete laboratory analysis was established using all available VWD assays including VWF multimers and genetic analysis.

RESULTS: The current (sub)classification results indicate that type 1 VWD represents around half of the patients (54.4%), type 2 28.7% and type 3 16.9%. Within type 2 the majority of the cases are type 2A with 20.8%, with smaller numbers for type 2B (6.2%), type 2M (16.2%), and type 2N (3.1%). All cases of type 3 were based on the presence of (often asymptomatic) type 1 VWD in both parents. Molecular work is ongoing and has so far yielded causal mutations in the VWF gene in 123/180 patient samples through direct DNA sequencing, with 52 different mutations. Fifty per cent of all cases was represented by five individual mutations, p.Pro812ArgfsX31 (type 1, "Aland mutation") in 11.7% of cases, p.Arg1315Cys (type 2M) and p.Arg924Gln (type 1) in 3.9%, p.Arg1374His (type 2A/IIA) and p.Trp1144Gly (type 2A/IIE) in 3.3%. Currently 12 different new causal mutations, not included in the EAHAD data base on VWF mutations, have been found in 22 patients and are awaiting expression studies, but they have been confirmed by 2 genetic prediction programs. MLPA (Multiplex ligation-dependent probe amplification) is ongoing and has so far yielded 6 cases of large deletions, all of exon 1-3.

CONCLUSION: This study is the first characterisation of VWD in Bratislava (Slovak Republic). These data are important for understanding of phenotype-genotype relationship in VWD. The full laboratory and multimeric analysis for all samples are finished. Further molecular analysis is ongoing.

P03

Clot waveform analysis as an added value to optical clot detection in aPTT measurements

K. Eeckhout, J. Van Den Bossche, K. Deiteren, R. Malfait, M.B. Maes
Laboratory of Hematology, Antwerp University Hospital, EDEGEM, Belgium

BACKGROUND/INTRODUCTION: In our laboratory, analysis of the routine coagulation parameter aPTT is performed on the automated coagulation analyzer Sysmex CS-5100 (Siemens Healthcare Diagnostics, Germany) using optical clot detection. A limitation of optical clot detection is the inability to produce correct results in samples with an early reaction error (ERE). These samples are reanalyzed with the benchtop analyzer STart (Stago Diagnostica, The Netherlands) using mechanical clot detection. However, this is a practical inconvenient and time-consuming manual method. Here we introduce Clot Wave Analysis (CWA) on the Sysmex CS-5100 as a possible solution for samples with ERE. [1]

AIMS: The aim of this study was to evaluate the CWA protocol on the Sysmex CS-5100 as a fast and less labor-intensive alternative method for determination of aPTT in samples in which the routine coagulation analyzer cannot produce a result due to ERE.

METHODS/ MATERIALS: 105 Citrated platelet poor plasma samples (81 with ERE and 24 without ERE) were analyzed (fresh or after freezing at -80°C) for aPTT on the Sysmex CS-5100 with the standard aPTT protocol, the CWA protocol, followed by the Stago STart mechanical method. For all methods, same aPTT reagent (Actin FS (Siemens)), same protocol and incubation times were used. Inter- and intra-assay precision of the different protocols were measured by analyzing different control levels 5 times at 5 consecutive days (Siemens control N, Siemens Citrol 2, a normal pooled plasma (NPP) and a pooled plasma with an elevated aPTT (EPP)). Statistical analysis was performed using Medcalc statistical Software version 17.5.5 (Ostend, Belgium).

RESULTS: With the CWA method we obtained aPTT results for all tested samples. Excellent correlation was found with the mechanical STart method. Results of the correlation between the different methods by Passing and Bablok analysis and Bland-Altman can be found in table 1A. The CWA protocol produced results that were highly interchangeable with the routine APTT protocol, making the CWA protocol a reliable method for routine laboratory practice.
Results of inter- and intra- assay precision for aPTT of the standard CS-5100, STart and CWA protocol can be found in table 1B. As could be expected, the CV% for the aPTT generated by the automated software-based CWA protocol were significantly lower than that of the manual STart protocol at all control levels, and comparable with that of the standard aPTT protocol on the Sysmex CS-5100. CWA even reached the desirable analytical performance specifications (CV% < 1.4). [2]

SUMMARY/CONCLUSIONS: Since early reaction errors (ERE) are most often seen in samples from critically ill patients from the intensive care unit, it is especially important that the lab can provide a quick and accurate aPTT result in all patients. Our study shows that the clot wave analysis (CWA) protocol on the CS-5100 is a reliable, highly reproducible, quick and easily implemented alternative for samples in which the routine coagulation analyzer cannot produce an aPTT result due to ERE.

REFERENCES:
1. *Dade Behring Hemostasis. Troubleshouting Guide for Sysmex CA Analyzers.*
2. *Minchinela J, Ricós C, Perich C, Fernández-Calle P, Alvarez V, Domenech M, et al. Biological variation database and quality specifications for imprecision, bias and total error (desirable and minimum). The 2014 update. http://www.westgard.com/biodatabase-2014-update.htm (Accessed September 2019).*

Table 1. Overview statistical results evaluation CWA protocol on the Sysmex CS-5100.

Table 1A. Comparison between aPTT measured with STart and the CWA protocol on CS-5100 .

Sample	Sample size (n)	Measurement range STart (sec)	Measurement range CWA (sec)	Pearson correlation coefficient r (p-value)	Passing & Bablok intercept (95% CI)	Passing & Bablok slope (95% CI)	Bland-Altman mean difference (-1,96 SD to + 1,96 SD) (sec)
Samples with ERE	81	16.9 – 182.5	16.9 – 138.7	0.98 (<0.0001)	0.25 (-1.01 to 1.18)	1.01 (0.98 to 1.04)	0.15 (-12,74 to 13.05)
Samples without ERE	24	21.3 – 62.3	21.7 – 61.9	0.99 (<0.0001)	-1.70 (-4.11 to 0.86)	1.11 (1.05 to 1.19)	2.44 (-1.40 to 6.20)

Table 1B. Intra-/inter- assay precision (CV%) for aPTT of the standard CS-5100, STart and CWA protocol on CS-5100.

	Intra- assay precision (CV%)			Inter- assay precision (CV%)		
Control material (n=25)	CS-5100	CWA	STart	CS-5100	CWA	STart
Control N	0,6	0,4	5,9	1,0	0,8	8,9
Ci-Trol 2	0,4	0,3	4,8	0,5	0,3	5,7
NPP	0,5	0,4	1,7	1,6	1,4	3,1
EPP	1,0	1,0	3,1	1,1	1,1	5,1

Table 1. Overview statistical results evaluation CWA protocol on the Sysmex CS-5100.

P04

Assessment of Second and Third Generation Oral Contraceptives APC Resistance by a Newly Validated ETP-Based APC Resistance Assay: A Pilot Study

L. Morimont[1], J. Evrard[2], A.S. Delvigne[1], C. Bouvy[1], J.M. Dogné[2], J. Douxfils[1]
[1]QUALIblood, NAMUR, Belgium
[2]Pharmacy Department, UNamur, NAMUR, Belgium

BACKGROUND: The ETP-based APC resistance has been identified as a marker of the prothrombotic state observed in women taking combined hormonal contraceptives (COC). Currently, it is based on home-made method which lacks standardization and validation impeding study-to-study comparison.

AIMS: The aim of this study is to assess the sensitivity of a newly developed and validated ETP-based APC resistance assay in plasmas from healthy volunteers and from women using COC.

METHODS: A total of 37 volunteers (FV Leiden negative) aged from 18-35 years were enrolled (approval by the ethical committee of the CHU UCL Namur, Yvoir, Belgium) and stratified into several subgroups (men, women not using hormonal contraception, women using second-generation COC and women using third-generation COC). Sub-populations have been compared based on APC resistance values (expressed in inhibition percentage of the ETP).

RESULTS: The demographic characteristics (age and BMI) of the four groups were well matched. Women not using COC exhibited a significantly decreased percentage of ETP inhibition ($P < 0.05$) compared to men. Women using third generation COC were significantly less responsive to APC than women not using COC ($P < 0.05$). A trend towards a significant difference between second and third COC generation is observed but our pilot study was not sufficiently powered and was not designed for this endpoint.

CONCLUSIONS: This newly validated APC resistance test is sensitive to differentiate men from women not using hormonal contraception, and can also differentiate different levels of APC resistance based on the type of COC. The availability of a universal assay for evaluating ETP-based APC resistance will allow study-to-study comparison in order to properly assess the safety of new COC in development. The implementation of this validated assay in routine can also identify subjects with higher absolute risk at baseline, before use of any hormonal treatment.

P05

Assessment of thrombomodulin resistance in women using second generation, third generation and progestin-only contraceptives

L. Morimont[1], J. Evrard[2], A.S. Delvigne[1], C. Bouvy[1], J.M. Dogné[2], J. Douxfils[1]
[1]QUALIblood, NAMUR, Belgium
[2]Pharmacy Department, UNamur, NAMUR, Belgium

BACKGROUND: Identification of an APC resistance using home-made ETP-based APC resistance assay is well described but few of past studies consider the involvement of thrombomodulin, which physiologically, promotes protein C activation.

AIMS: This study evaluates the response to thrombomodulin in plasmas from male and female healthy volunteers and from women using hormonal contraceptives using a commercially available kit for the assessment of thrombin generation in presence or absence of thrombomodulin.

METHOD: This study includes 43 volunteers aged from 18-35 years (approval by the ethical committee of the CHU UCL Namur, Yvoir, Belgium) which have been stratified into several subgroups (men, women not using hormonal contraception, women using second-generation combined oral contraceptive (COC), women using third-generation COC and women using progestin-only contraceptive). Thrombomodulin resistance is expressed as inhibition percentages of the ETP between the condition minus and plus thrombomodulin.

RESULTS: Women not using hormonal contraception are significantly less sensitive to thrombomodulin than men ($P < 0.05$) and compared to women using hormonal contraceptives, this depends on the type of contraceptive. A significant difference is observed between women using second and women using third generation COC with women not using hormonal contraception ($P < 0.05$). The use of progestin-only contraceptive does not affect the sensitivity to thrombomodulin. Nonetheless, the negative point of this method results in the large variability within subgroups (e.g. CV of 47% in women using second-generation COC).

CONCLUSION: The thrombomodulin resistance is an interesting assay able to discriminate women depending on the type of oral contraceptive. However, the high variability observed in specific subgroups warrants further investigation on the possible confounding variables. An improvement of the method is mandatory in order to address the issue of the important inter-individual variation observed and provide more reliable tools for risk stratification.

Validation of an original ETP-based APC resistance assay for the evaluation of prothrombotic states

P06

L. Morimont[1], J. Evrard[2], A.S. Delvigne[1], C. Bouvy[1], J.M. Dogné[2], J. Douxfils[1]
[1]QUALIblood, NAMUR, Belgium
[2]Pharmacy Department, UNamur, NAMUR, Belgium

BACKGROUND: The activated protein C resistance assay based on the endogenous thrombin potential (ETP-based APCr assay) is recommended in guidance from medicines regulatory authorities (e.g. EMA and FDA) for the investigation of steroid contraceptives. The results are usually “normalized” with a reference plasma to provide the “normalized APC sensitivity ratio” (nAPCsr). However, the methods described in the literature are home-made and mostly without standardization of the method, the reagents, the reference plasma and the quality controls.

AIMS: The present study aims at validating the analytical procedure of an ETP-based APCr assay according to the regulatory standard ICHQ2R1.

METHOD: Three quality controls representing plasmas with different levels of coagulation and one reference plasma were used. The method targets a 90% inhibition of the ETP in healthy donors in presence of APC compared to the same condition in absence of APC. As the pool of healthy donors is not produced at large scale, specific algorithms are applied to the commercial reference plasma to correlate with the pool.

RESULTS: Repeatability (intra- and inter-run) and intermediate precision passed the acceptance criteria (<10% of standard deviation). The assay demonstrated a curvilinear dose-response to protein S and APC concentrations ($R2>0.99$). Analysis of plasma samples from 50 healthy individuals (22 women not taking COC and 28 men, no FV Leiden carrier) confirmed the validity of the tests with a mean inhibition percentage of 89% (95% CI: 87-91). Investigations in women taking COC confirmed the good sensitivity of the assay.

CONCLUSION: This study is the first describing the validation of ETP-based APCr assay according to regulatory standards. It provides the stakeholders, the regulatory bodies and the physicians with a reproducible, sensitive and validated assay. This will allow study-to-study comparison as well as perspectives for the establishment of specific thresholds to reflect the prothrombotic state in the individual patient.

Proposition of a new scale for the harmonization and normalization of the nAPCsr: a scientific and regulatory need for clinical studies comparison

P07

L. Morimont[1], J. Evrard[2], A.S. Delvigne[1], C. Bouvy[1], J.M. Dogné[2], J. Douxfils[1]
[1]QUALIblood, NAMUR, Belgium
[2]Pharmacy Department, UNamur, NAMUR, Belgium

BACKGROUND: The evaluation of the activated protein C resistance based on the endogenous thrombin potential (ETP-based APCr assay) is recommended during the development of steroid contraceptives. Results are usually expressed as “normalized APC sensitivity ratio” (nAPCsr) which is the ratio of the patient’s sample

on a reference plasma regarding the inhibitory sensitivity of the ETP towards exogenous APC. The reference plasma should achieve around 10% residual ETP in the presence of exogenous APC. Because of the inter-assay variability, achieving exactly 10% residual ETP is difficult and can significantly affect the theoretical 0 to 10 scale of nAPCsr. In addition, the use of homemade reference plasma makes the nAPCsr difficult to compare between studies.

AIMS: The aim of this study was to compare the nAPCsr with nAPCsr10, a newly proposed method for calculation of APC resistance.

METHOD: Seven hundred ninety individual plasmas were analysed to compare nAPCsr and nAPCsr10. These values were measured following our validated protocol of the ETP-based APCr assay. A commercially reference plasma and three levels of quality controls were used to validate each experiment.

RESULTS: The Spearman correlation between nAPCsr and nAPCsr10had a coefficient (rs) of 0.9896 (95% CI 0.9880 to 0.9910; p-value<0.0001) showing that the pairing was significantly effective. Linear regression showed the following equation y = 0.9334*x + 0.02630 (r2 = 0.97). When differences (nAPCsr10-nAPCsr), either expressed in absolute values or as a percentage of nAPCsr10, were plotted against nAPCsr10, the mean difference equalled to 0.18 (95% CI -0.43 to 0.79] in absolute values and 5.64% (95% CI -10.10% to 21.39%] when expressed as a percentage of nAPCsr10.

CONCLUSION: This is the first study presenting a new scale for harmonization and normalization of the nAPCsr. Results show a better reproducibility with the nAPCsr10. It avoids the additional variability and the unharmonized scale brought by the use of a reference plasma. Instead, this reference plasma serves as an additional level of control targeting the desired 10% of residual activity. This adapted method of the evaluation of APC based on the ETP provides the pharmaceutical industry, the regulatory bodies and the health care professionals with more reproducible and harmonized estimates. This definitively helps in study-to-study comparison. Finally, it could be the basis for the establishment of thresholds to assess patient's thrombotic state according to his resistance to APC.

P08

Comparison of the TEG5000 with other thromboelastographic and -metric analysers

T. Cammaert, E. Bailleul
Department of Laboratory Medicine, Hematology Laboratory, OLVZ Aalst, GHENT, Belgium

INTRODUCTION: The benefits of blood clotting analysers, based on thromboelastography or thromboelastometry are : reduction in transfusion of blood products, a substantiated choice of product, improvement in morbidity of patients with bleeding and cost savings.
In the OLVZ hospital of Aalst, we evaluated five devices and compared them to the actual method with TEG5000® (Haemonetics Corporation, Niles, IL, USA).

AIM: The primary aim of this study was to log similarities and differences between the use and possibilities of the instruments. We tested imprecision and compared the tests in actual use for routine samples by using reagents of the intrinsic pathway, either with or without a heparinase.

METHODS: (Dis)advantages were listed. The interrun precision was tested on two levels of quality controls (QC) for parameters comparable to R (reaction time) and MA (maximal amplitude) of TEG5000®. The correlation was calculated between these parameters and its comparable parameter on the tested devices for routine samples and discrepancies were registrated.

RESULTS: Five devices were tested. All devices use citrated whole blood, in contrast to our TEG5000® method, which uses a fresh whole blood sample. Two are semi-automatic and require pipetting of reagents and samples: Clotpro® (Enicor, Munich, Germany) and Rotem Delta® (Werfen, Bedford, MA, USA). The first offers six measuring channels, the latter only four. There is a free choice of tests and different patients can be tested at once. The other three analysers use cartridges, including 4 fixed tests at once on the same sample. TEG6s® (Haemonetics Corporation, Niles, IL, USA) still needs a pipetting step, Rotem Sigma® (Werfen, Bedford, MA, USA), and Quantra® (Hemosonics, Charlottesville, VA, USA) don't. Analysis on Quantra® stops automatically after ca. 15 minutes in contrast to other analysers. Therefore hyperfibrinolysis is not detectable an extra test should be performed. Quantra® also introduced a different way of displaying. Test reagent activators differed according to the manufacturer.
QCs were run daily if possible during 14 days. Most companies provided lyophilised materials, the QCs of Quantra® consisted of frozen RBCs fixed in a plasma matrix. Rotem Sigma® showed the best reproducibility. Coefficients of variation (CV) are overall higher on the pipetting systems.
Quantra® showed a good correlation for all parameters and tests and performed as reported in literature. Conversely TEG6s® did not meet the criteria of the company's leaflet. For the other devices, there were no published data to compare with. Most discrepancies in test outcomes were observed between TEG5000® and Clotpro®. This might be due to a limited number of samples, differences in characteristics of the activators, no published reference range to compare to and measuring principles.

CONCLUSIONS: The examined analysers differ in several ways. Cartridge-based systems are user-friendly, perform a better precision, but are generally more expensive because of the four fixed tests in one cartridge. Furthermore they can only examine one patient at a time, causing the need of several devices. Semi-automatic devices with pipetting steps offer more flexibility but require skilled staff. The measurements of Quantra® are time-restricted, which rules out the detection of hyperfibrinolysis in one test. Its display also differs from the other methods. Depending on a hospital's need, its preference might be varying.

P09

Resolving anti-FXa DOAC interference on a FXa based method for Antithrombin measurement by using activated carbon

T. Cammaert
OLVZ Aalst, GHENT, Belgium

INTRODUCTION: In a previous publication (Frans et al., J. Thromb. Haemost. Aug 2019; DOI:10.1111/jth.14488) we successfully proved that activated carbon (AC) can be used to resolve DOAC interference on routine laboratory coagulation tests and lupus anticoagulant assays.
Antithrombin (AT) deficiency is associated with thrombophilia. AT activity can be tested by a FIIa or FXa based chromogenic assay. Results of the test based on measurement of remaining FXa can be overestimated in the presence of anti-FXa inhibitor drugs. This could result in false normal AT results.

AIM: We neutralised anti-FXa DOAC activity (rivaroxaban, apixaban and edoxaban) by using AC to examine the influence of DOAC on the test results of our FXa based AT measurement method and to see whether we could obtain more accurate results this way.

METHODS: We included 36 patients taking oral anti-Xa drugs (rivaroxaban (n = 14), apixaban (n = 12), and edoxaban (n = 10)), 8 controls taking the anti-FIIa drug dabigatran, and 24 patients not treated with a DOAC. Daily routine samples were selected. Citrated plasma was stored at -80°C and thawed at 37°C before analysis.

The anti-Xa activity (Liquid Anti-Xa®, Werfen, Bedford, MA, USA) and anti-II activity (DTI®, Werfen) of DOACs in citrated plasma were performed for the respective DOAC before and after addition of 20 mg/mL AC (Norit Carbomix®, KELA Pharma, Sint-Niklaas, Belgium), as well as the AT activity (Liquid antithrombin®, Werfen). After addition of 20 mg/mL of AC, samples and supernatant respectively underwent twice a centrifugal cycle of 2000 x g of 5 minutes.

To determine whether addition of AC interferes on AT assays and whether overestimation of the result was reduced by using AC, the following was done. First, the influence of AC on the AT measurement was tested on the normal routine samples by calculating the difference in AT value before and after adding AC. And second, differences between DOAC concentrations and between AT measurements before and after AC were calculated on the DOAC samples.

RESULTS: All 24 normal samples showed a slightly increased activity after manipulation with AC (3,5% with a 95% confidence interval [2,2%; 4,9%]). This increase is significant according to the non-parametric Wilcoxon Signed-Ranks Test (p<0,001), yet clinically unimportant. The same applies to the dabigatran control group.
As observed before, all DOAC concentrations were significantly reduced, after addition of AC. However some anti-Xa levels remained, though minimally, higher than the LoQ (20 ng/ml and 15 ng/ml for apixaban).
As a result of using AC, there were average decreases in measured AT activities of 10,5 % (apixaban; p<0,01), 16,6 % (rivaroxaban; p ≤0.001) and 5,7 % (edoxaban; p= 0,217). For every FXa inhibitor, we demonstrated linear correlations between the difference in antithrombin activities and difference in DOAC concentrations before and after adding AC.
We found no clinical important difference in AT activity in a subgroup with DOAC levels below 100 ng/ml, which are expected trough levels. On the contrary, we saw a non-significant increase of 2% AT activity [-4%; 8%].

CONCLUSIONS: AC removes selectively DOAC anti-Xa interference on our AT FXa-based assay. The interference is linear to the concentration of anti-FXa DOAC in the sample. The AT assay might be correctly interpreted during DOAC trough levels as no interference was noted. However further investigation is warranted as our database was limited.

P10

Determining the optimal method for FVIII:C and anti-FVIII quantification in a patient treated with Adynovi® who developed anti-FVIII during treatment

L. Heireman, K. Devreese
Coagulation Laboratory, Department of Laboratory Medicine, University Hospital Ghent, GHENT, Belgium

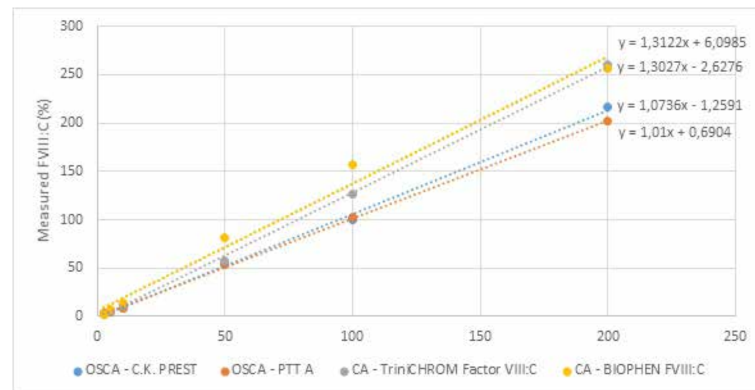
INTRODUCTION: Adynovi® (Takeda) contains rurioctocog alfa pegol, a novel recombinant factor VIII (rFVIII) indicated for the treatment of haemophilia A. Since Adynovi® structurally strongly differs from other rFVIII proteins, the optimal assay for measurement of this pegylated FVIII must be determined.

AIMS: In this study, we compared two one stage clotting assays (OSCA) with two chromogenic assays (CA) for the FVIII activity measurement in patients treated with Adynovi®. Secondly, we investigated the Bethesda assay for the quantification of anti-FVIII inhibitor against Adynovi®.

MATERIALS/METHODS: Adynovi® was diluted in FVIII deficient plasma to obtain end concentrations of 2.5%, 5%, 10%, 50%, 100% and 200%. FVIII:C concentration was measured in the dilution series and samples of a patient treated with Adynovi® therapy (N=9). Four different methods were tested, including OSCA with C.K. PREST® and PTT A reagents (both Diagnostica Stago), and CA with TriniCHROM Factor VIII:C (Diagnostica Stago) and BIOPHEN™ FVIII:C (HYPHEN BioMed) on STA-R EVO (Stago Diagnostica). In addition, anti-FVIII inhibitor against Adynovi® was measured in samples (N=2) of a patient chronically treated with Adynovi® who developed anti-FVIII (1.3-6.2 Bethesda units). The classical Bethesda assay was applied using both 100% Unicalibrator (Stago Diagnostica) and 100% Adynovi® as reference plasma.

RESULTS: For FVIII:C measured with OSCA, an acceptable recovery of -13 to 9% was observed for C.K. PREST and -11 to 8% for PTT A reagent for Adynovi® concentrations 5-200%. In contrast, we observed a large positive deviation from the expected value with both CA (14-30% with TriniCHROM Factor VIII:C reagent and 28-63% with BIOPHEN™ FVIII:C reagent). In Adynovi® patient samples, we also report a large difference between OSCA and CA (10-150% for TriniCHROM Factor VIII:C and 22-110% for BIOPHEN™ FVIII:C reagent). For quantification of anti-FVIII, only a small difference of 0.3% was observed between the application of 100% unicalibrator and 100% Adynovi® as reference for the Bethesda assay.

CONCLUSIONS: The OSCA with aPTT reagent (C.K. PREST® or PTT A) is the preferred method to monitor FVIII:C in patients treated with Adynovi®. The Bethesda assay yields comparable results for quantification of anti-FVIII inhibitor against Adynovi® using 100% Unicalibrator and 100% Adynovi® as reference plasma.



P11

Method comparison of rotational thromboelastometry (ROTEM®) sigma and delta devices

L. Heireman, K. Devreese
Coagulation Laboratory, Department of Laboratory Medicine, University Hospital Ghent, GHENT, Belgium

INTRODUCTION: Thromboelastometry (ROTEM®) is widely applied to assess the entire clotting process and guide management in bleeding patients. ROTEM® sigma with the same pin and cup technology (rotational thromboelastometry) as ROTEM® delta is the new generation of instruments that is fully automated. The liquid reagents of ROTEM® delta are replaced by cartridges that contain lyophilized reagents and also sample handling is automated.

AIMS: We aimed to determine whether ROTEM® sigma and ROTEM® delta produce similar patient results by comparison of different ROTEM® parameters.

MATERIALS/METHODS: Citrated whole blood was drawn from 30 patients with increased bleeding risk. The ROTEM® tests INTEM, EXTEM, FIBTEM and APTEM were performed on both ROTEM® sigma and delta (Werfen). All assays were performed within 4 hours after blood collection. The parameters clotting time (CT) (INTEM and EXTEM), clotting formation time (CFT) (INTEM and EXTEM), maximal clot firmness (MCF) (INTEM, EXTEM, FIBTEM) and maximal lysis (APTEM) were compared between both ROTEM® devices using Passing Bablok regression. If the 95% CI of the slope and intercept includes the values 1 and 0 respectively, there is no statistically significant difference. After checking the ROTEM sigma specific manufacturer's reference ranges according to CLSI guidelines, we tested whether the same reference range (from ROTEM delta or sigma) can be used for both devices.

RESULTS: Twenty-three patients showed a normal thromboelastogram. Of the seven patients with abnormal ROTEM® pattern, four showed a prolonged CT EXTEM. Other less frequent observed abnormalities were a prolonged CT INTEM, CFT INTEM and decreased MCF (all channels). Passing-Bablok regression showed only a statistically significant difference between both ROTEM® devices for CT EXTEM (y = 1.50 (95% CI 1.07 to 3.25)x - 31.50 (95%CI -142.50 to -3.47)) and MCF EXTEM (y = 1.00 (95% CI 1.00 to 1.20)x – 2.00 (95%CI -14.90 to -2.00)). Of the four patients with prolonged CT EXTEM on ROTEM® delta, one patient showed normal results on ROTEM® sigma (independent of whether ROTEM® delta or sigma reference range is applied). For MCF EXTEM, no differences in interpretation were observed between both devices. However, five samples with prolonged CT INTEM values on ROTEM® sigma tested normal on ROTEM® delta when applying device specific reference ranges. This discrepancy can be explained by the lower upper limit of the reference range applied for ROTEM® sigma (204s) compared to ROTEM® delta (240s). When applying the reference range of ROTEM sigma on ROTEM delta results, three falsely prolonged CT INTEM values are observed. If the reference ranges of ROTEM delta are used for both devices, no discrepancies in interpretation of CT INTEM are found but instead we observe four falsely decreased results for MCF FIBTEM.

CONCLUSIONS: Results for MCF (FIBTEM and INTEM), ML (APTEM), CFT (INTEM and EXTEM) and CT (INTEM) were similar between ROTEM® sigma and delta. For CT and MCF EXTEM, a statistically significant difference was observed between both methods without clinical significance. However, clinical interpretation differed between both methods for CT INTEM when applying device specific reference ranges. Finally, reference values of both devices cannot be used interchangeably.

P12

A lot of variables to consider when monitoring Hemophilia B patients on extended half-life (EHL) products?

I. Vangenechten[1], K. Decloedt[2], M.B. Maes[2], A. Gadisseur[1]
[1]Department of Hematology, UZ Antwerp, EDEGEM, Belgium
[2]Department of Laboratory Medicine, UZ Antwerp, EDEGEM, Belgium

BACKGROUND: aPTT based One-Stage Clotting assay (OSA) is routinely used for measuring clotting factors. Chromogenic Substrate assay (CSA) has been proposed as the most accurate assay for novel extended half-life (EHL) clotting factor products. The use of EHL recombinant FIX fusion protein preparations, rIX-FP (CSL Behring) and recombinant factor IX Fc domain fusion protein, rFIXFc (Sobi) have important laboratory issues.

AIMS: Identification of most appropriate FIX assay to monitor patients under EHL-product treatment by comparing CSA and OSA.

METHODS: FIX measurement on rIX-FP and rFIXFc spiked samples. CSA: BiophenTMFIX (Hyphen Biomed) and OSA: Silica, Kaolin and Ellagic acid based aPTT (Stago, Siemens and Werfen, respectively) using three immune-depleted FIX plasmas. Performed on STA®-Compact Max (Stago) and Sysmex®CS-5100 (Siemens).

RESULTS: As suspected, across all aPTT reagents, rIX-FP and rIX-FP ≥0.05U/mL were more divergent. The rIX-FP activity was consistently overestimated (>30%) when using CSA; OSA activities varied widely depending on used aPTT reagent. HemosIL®SynthAFax overestimated rIX-FP activity across all three deficient FIX plasmas and both analysers, while an overall limited underestimation (<30%) was observed with silica based aPTT reagents STA®-PTTa and TriniCLOTTM. Results obtained with OSA using Pathromtin®SL were most comparable with the assigned target value, across all three deficient plasmas. Using Dade®Actin®FS major rIX-FP activity differences were seen between different deficient FIX plasmas which were also analyser dependent. The FIX activity was underestimated to almost a half of the target value when using the Siemens and Werfen FIX deficient plasma, while this was not the case for Stago deficient plasma on STA-Compact Max. In contract with rIX-FP spiked samples, CSA seemed appropriate for FIX-Fc activity measurement. When evaluating OSA, silica containing reagents (Dade®Actin®FS, HemosIL®SynthAFax) overestimated rFIXFc activity levels across all three deficient FIX plasmas by >30%. A limited overall underestimation (<30%) was seen for OSA using Pathromtin®SL and STA®-PTTa depending on deficient plasma and analyser. In our experiments; results obtained with TriniCLOTTMAutomated aPTT were those which were most in line with the assigned target value. In general, both coagulation analysers obtained comparable results with minor tendency of Sysmex®CS-5100 analyser to measure lower FIX activities compared with STA®-Compact Max, which can be explained by fact that different calibrator plasmas were used. Normalisation of FIX activity against NPP resulted in more similar results. Conclusion: Our study confirmed differences between FIX activity of EHL FIX products measured using OSA or CSA, as well as between OSA results generated using different aPTT reagents. It also demonstrate that FIX activity levels not only dependent on aPTT reagent used in OSA but also on type of deficient FIX plasma and analyser used. In contrast to monitoring FVIII EHL products, CSA may not always be the most preferable assay. It is clinically important that each laboratory verifies his own working protocol considering the method , CSA or OSA, aPTT reagent, deficient plasma and analyser used to monitor EHL FIX activity levels, and this is specific for each different EHL product that is used for patient treatments.

P13

Reduction of preoperative waiting time before semi-urgent surgery in patients on P2Y12 inhibitors using multiple electrode aggregometry: a retrospective study

C. Dupuis
Cliniques universitaires Saint-Luc, WOLUWÉ-SAINT-LAMBERT, Belgium

BACKGROUND: P2Y12 inhibitors discontinuation is essential before surgery to avoid bleeding complications. Based on pharmacokinetics data, 5 or 7 days of discontinuation are theoretically recommended before surgery (1). However, as platelet function recovery is highly variable among individuals after discontinuation of these drugs (2), a more individualized strategy based on platelet function tests (PFT) has been proposed to reduce the preoperative waiting time in these patients (3).

AIMS: The aim of this study is to determine if preoperative platelet function analysis using ADP-multiple electrode aggregometry (MEA, Multiplate®) is associated with a reduction of preoperative delay in patients on P2Y12 inhibitors scheduled for semi-urgent surgery.

METHODS: 28 patients under P2Y12 inhibitors having benefited from preoperative platelet function analysis using ADP-MEA have been analyzed retrospectively. Primary outcome was defined as the delay between last P2Y12 inhibitor intake and incision in patients for whom a threshold of 19 AUC has been reached before surgery. The occurrence of postoperative Thrombolysis in Myocardial Infarction (TIMI) bleeding, blood transfusion and recovery of platelet function after were also examined.

RESULTS: The majority of patients underwent a CABG (61%). Median time between last P2Y12 inhibitor intake and surgery was 2.9 days (IQR 1.3 - 4.0). Out of the 28 study patients, 20 have been operated with a MEA ≥ 19, and the preoperative delay was reduced by 1.46 days in these patients, by comparison with theoretical recommendations. However, platelet function recovery was highly variable among individuals (Fig. 1), highlighting the opportunity to use PFT in this indication. The 19 AUC ADP-MEA threshold was significantly associated with perioperative platelet transfusion (p=0.03), but not with packed red blood cells transfusion (p=0.099). Two patients who underwent intracranial surgery experienced TIMI major bleeding despite an ADP-MEA value > 19 AUC, suggesting that a more conservative threshold is probably necessary in this situation.

CONCLUSION: Preoperative platelet function analysis using ADP-MEA before semi-urgent surgery in patients on P2Y12 inhibitors is associated with a reduction in preoperative waiting time. However, the 19 AUC threshold used in this study seems insufficient to prevent major bleeding after intracranial surgery.

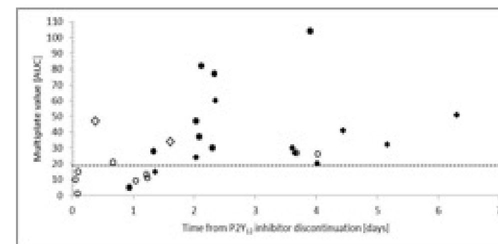


Fig. 1: Last preoperative ADP-MEA value according to time until P2Y12 inhibitor discontinuation.

P14

Evaluation of the automated and standardized analyzer St-Genesia in order to measure thrombin generation in the clinical lab

L. Rozen, C. Noel, M. Faklan, M. El Ouaaliti, B. Cantinieaux, A. Demulder
Laboratory of Hematology, LHUB-ULB, BRUSSEL, Belgium

BACKGROUND: Thrombin generation test (TGT) is a global coagulation test that reflects the dynamic equilibrium between the procoagulant and anticoagulant components of the coagulation network. Although promising in exploring disruption of the coagulation balance in both bleeding and thrombotic disorders, its lack of standardization limits its implementation in clinical lab.
AIM: The aim of our study was to evaluate a new fully automated TGT automate, St-Genesia (Stago) in comparison with the CAT method (Calibrated Automated Thrombogram).

MATERIAL AND METHODS: Two St-Genesia reagents were evaluated (BleedscreenTM and ThromboscreenTM). Determination of precision, stability, practicability and usual reference values according to age were established on quality control, individual or pooled citrated platelet-poor plasmas obtained after a double centrifugation at 1900 g for 15 min. Comparison between fresh and freezed samples was performed on 10 samples. Comparison between CAT method and St-Genesia was performed on 40 samples. If TGT was not performed within 4 hours, plasma were stored at – 80° C until analysis and then thawed at 37° C for 5 min.
All TGT parameters (ETP, Peak, velocity, lag time, time to peak) were analyzed.
Results are normalized toward a reference plasma.

RESULTS: For Bleedscreen TM, within run variation showed a coefficient variation (CV) for ETP of 1.3% for QC norm and 3.1% for QC low (n=11) ; between-run CV was 4.2% for QC norm and 3.3% for QC low (n=10).
Similar results were obtained with ThromboscreenTM : CV within run were 3.2% for QC norm, 4.9% for QC low and 1.8% for QC high (n=11) ; between-run CV were 3.5% for QC norm, 5.8% for QC low and 3.5% for QC high (n=10). No statistical difference was observed between results obtained from fresh samples and after freezing-thawing.
Results obtained for selected patients showed differences between CAT method and St-Genesia. We determined reference ranges on St-Genesia according to age for ETP for both type of reagents (Table 1).
During the validation process numerous technical problems have occurred, blocking the automate and slowing test execution.

CONCLUSION: Automation, normalization of results with a reference plasma, and a strict control of temperature throughout the assay, improves standardization and could possibly be part of the hemostasis panel test in the clinical lab.
The differences between CAT method and St-Genesia are probably due to difference in tissue factor and phospholipids concentrations between CAT and St-Genesia reagents. This highlights the need for reference ranges on device. However the technical performance of the device still requires several technical improvements that are ongoing by the firm. More tests are needed to get a better idea of the add-value of St-Genesia in bleeding or thrombotic disorders.

ETP (nM*min) (median and range	0.5 – 6 years	7 – 11 years	12 – 16 years	17 – 25 years	26 – 50 years	>50 years
Bleedscreen™ (nM*min)	791.8 (404.5 – 1167)	810.5 (383 – 1330)	846.7 (555.4 – 1249)	1204 (696.3 – 1770)	1196 (560.7 – 2143)	986.8 (675.3 – 1983)
Thromboscreen™ TM- (nM*min)	939.8 (170.1 - 1337)	1038 (910.5 - 1265)	1029 (632.2 - 1313)	1405 (688.7 - 2461)	1383 (803.1 - 2854)	1277 (417.3 - 2613)
Thromboscreen™ TM + (nM*min)	569.8 (236.4 - 1157)	526.9 (354.3 – 746.1)	652.2 (133.7 – 873.7)	749.5 (263.8 – 2107)	795.2 (312.8 - 2105)	600.3 (104.8 - 1357)
% reduction in presence of TM	42.2 (12.6 -59.4)	51.3 (29 - 67)	35.3 (23.7 – 78.8)	36.9 (14.4 – 70.4)	42.5 (12.5 – 75.2)	53.5 (16 – 79.2)

Table 1 – Usual range for ETP (median and range)

P15

An ultra-high-performance liquid chromatography coupled with a tandem mass spectrometry method for the quantification of edoxaban: The importance to measure active metabolite

R. Siriez[1], J. Evrard[1], J. Laloy[2], J.M. Dogné[1], J. Douxfils[1]
[1]Department of Pharmacy, Namur Thrombosis and Hemostasis Center (NTHC), UNamur, NAMUR, Belgium
[2]Department of Pharmacy, Namur Nanosafety Center (NNC), UNamur, NAMUR, Belgium

BACKGROUND: Although DOACs do not require regular measurements of their blood concentrations, some clinical situation may require an assessment of their concentration such as the detection of drug accumulation in case of acute renal or hepatic failure, recurrence of a stroke, occurrence of bleedings, planification of urgent invasive procedures, undocumented or multiple drug interactions or in case of multiple interfering factors. Among the factor Xa inhibitors, edoxaban is the only compound for which some of the metabolites (edoxaban-M4, -M6 and -M8 metabolites - sorted by relative occurrence in patients) are reported to be pharmacologically actives. Therefore, the contribution of these metabolites could potentially interfere with chromogenic assays usually used for the estimation of edoxaban concentration. However, considering their respective IC50towards human factor Xa, these metabolites would inhibit factor Xa at different degree. The scientific literature contains many articles about liquid chromatography measurement of edoxaban, few of them provide and discuss the simultaneous quantification of edoxaban and its M4 metabolite.

AIMS: To develop a validated UHPLC-MS/MS method to quantify simultaneously edoxaban and edoxaban-M4 metabolite in human plasma.

METHODS: Electrospray ionization and chromatographic separation were optimized for the simultaneous dosage of edoxaban and edoxaban-M4 metabolite. The method was validated according to the requirements of regulatory guidelines for bioanalytical method validation provided by the EMA and the FDA.

RESULTS: The total run time was 6 minutes. The method was validated for calibration curves, precision, accuracy, carry-over, selectivity, matrix effect and short-time stability.

CONCLUSIONS: This validated UHPLC-MS/MS method allows the quantification of edoxaban and its active M-4 metabolite. The interest of synchronously measuring edoxaban and edoxaban-M4 metabolite is to obtain complementary information about the inhibitory effect of this active metabolite in chronometric or chromogenic assays. This is of great importance to understand the relevance of pharmacokinetic interaction in treated patients. Although edoxaban has usually low concentrations of active metabolites, which should not contribute significantly to the anticoagulant activity, the measurement of the M4-metabolite method is interesting in case of drug interactions (e.g. co-treatment with quinidine, verapamil, ketoconazole, rifampicin, phenytoin, carbamazepine…). Indeed, concomitant prescriptions of edoxaban and carbamazepine or rifampicin, some first-intention treatments, is frequent. As these drugs are inducers of CYP3A4, the metabolite to parent compound ratio is increased (Figure 1). In lights of the differences in terms of inhibitory activity between edoxaban and the M4-metabolite, the estimations of edoxaban concentration by chromogenic anti-Xa assays may be disturbed. Specifically, this estimation relies on the global anti-Xa activity of the plasma sample. The different inhibitory activities of edoxaban and its metabolites may introduce a bias in the estimation of the edoxaban equivalent concentrations given by the anti-Xa assay. Therefore, patients are at risk of having inadequate control of coagulation supporting the need to measure the most representative edoxaban metabolite concomitantly to the parent compound.

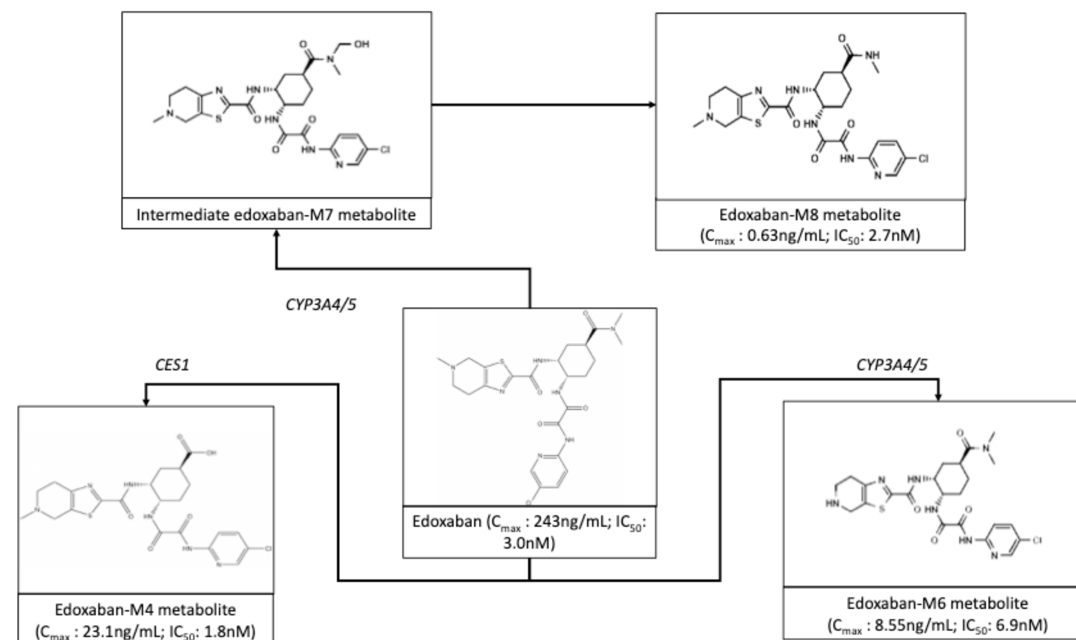


Figure 1: Postulated edoxaban metabolism for active metabolites

P16

Assessment of low plasma concentration of apixaban in the periprocedural setting

H. Godeaux+, S. Lessire*, A-S. Dincq*, R. Siriez†, L. Pochet†, A-L. Sennesael‡, O. Vornicu*, M. Hardy*, O. Deceuninck‡‡, J. Douxfils†, F. Mullier§
+Université catholique de Louvain, CHU UCL Namur, Department of Anesthesiology YVOIR, Belgium
*Université catholique de Louvain, CHU UCL Namur, Namur Thrombosis and Hemostasis Center (NTHC), NAmur Research Institute for LIfe Sciences (NARILIS), Department of Anesthesiology YVOIR, Belgium
†University of Namur, Namur Thrombosis and Hemostasis Center (NTHC), NAmur Research Institute for LIfeSciences (NARILIS), Department of Pharmacy, NAMUR, Belgium
‡Université catholique de Louvain, CHU UCL Namur, Namur Thrombosis and Hemostasis Center (NTHC), NAmur Research Institute for LIfeSciences (NARILIS), Department of Pharmacy, YVOIR, Belgium
‡‡Université catholique de Louvain, CHU UCL Namur, Department of Cardiology, YVOIR, Belgium
§Université catholique de Louvain, CHU UCL Namur, Namur Thrombosis and Hemostasis Center (NTHC), NAmur Research Institute for LIfe Sciences (NARILIS), Haematology Laboratory, YVOIR, Belgium

INTRODUCTION: Estimation of residual apixaban plasma concentrations may be requested in the management of emergencies. In this clinical setting, different anti-Xa assays may be used, but their performance for different ranges of apixaban plasma concentration should be evaluated. Furthermore, the interference of these assays with low molecular weight heparin (LMWH) are useful to assess as patients may be switched from apixaban to LMWH (eg fasting days) and may undergo urgently an invasive procedure at high bleeding risk during this timeframe.

AIMS: This study aims at assessing the performance of three specific anti-Xa assays calibrated with apixaban on real-life samples with very low apixaban plasma concentrations and on treatment ranges, with and without interference of LMWH.

MATERIAL AND METHODS: The performance of the STA®-Liquid Anti-Xaassay (STA®LAX) and the low and normal procedures of the Biophen®DirectFactor XaInhibitors (DiXal) assay was tested on 134 blood samples, collected from patients on apixaban, wherefrom 74 patients received LMWH after apixaban arrest. The results were compared to the LC-MS/MS measurements.

RESULTS: The Biophen®DiXal, Biophen®DiXal LOW and STA®LAX showed very good correlation with LC-MS/MS measurements in patients not bridged with LMWH (Spearman r 0.95, 0.99 and 0.98, respectively). Their limit of quantitation was measured at 46, 14

and 4 ng/ml, respectively. The Bland-Altman test measured mean bias (SD) at 5.6 (13.1), -2.5 (5.0) and -0.8 (6.1) ng/ml, respectively. The Spearman r of the Biophen®DiXal decreased to 0.64 in presence of very low apixaban concentrations (<30ng/ml). The Spearman r of the Biophen®DiXal LOW and STA®LAX decreased to 0.39 and 0.26, respectively, in presence of LMWH.

CONCLUSIONS: The accuracy of the low methodologies (Biophen®DiXal LOW and STA®LAX) is slightly improved for very low apixaban plasma concentrations, compared with the normal procedure of Biophen®DiXal. The interference of LMWH on the low methodologies is measurable, however less important than previously reported interference of LMWH on rivaroxaban calibrated specific anti-Xa assays.

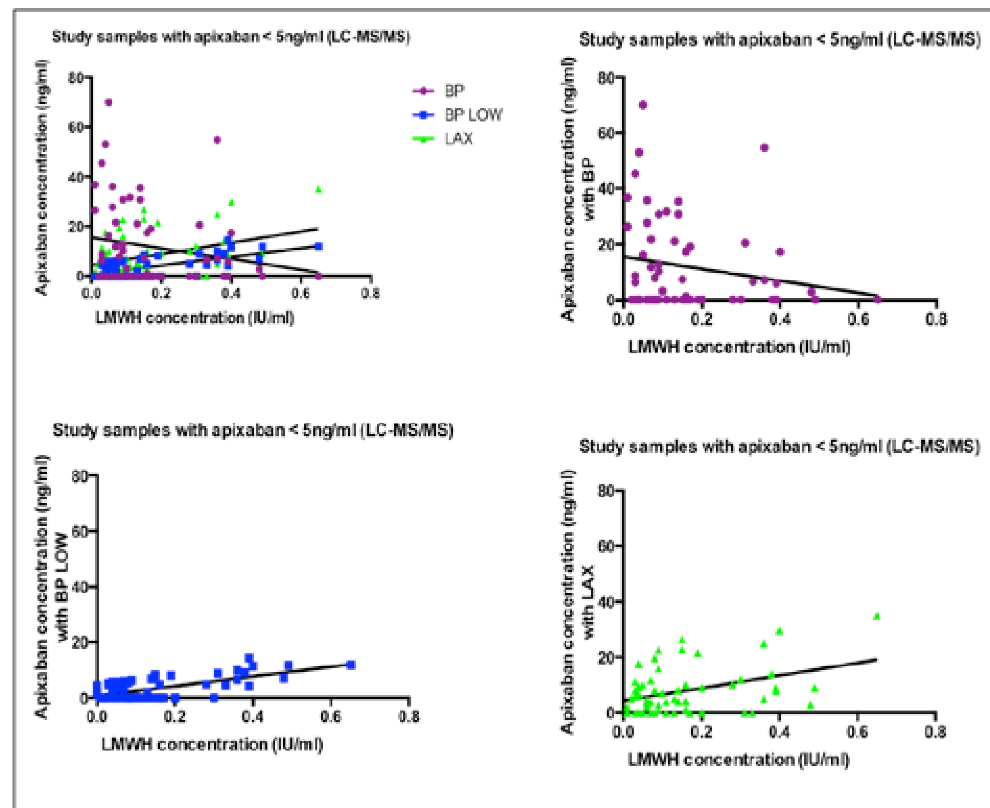


Figure 1. Interference of residual LMWH on the performance of three chromogenic assays calibrated for apixaban
Measurements of apixaban concentrations with three specific chromogenic anti-Xa assays in study samples containing max 5ng/ml of apixaban measured with LC-MS/MS.
BP: normal procedure of the Biophen®DiXal assay
BP LOW: low procedure of Biophen®DiXal assay
LAX: STA®LAX

P17

An unusual cause of abnormal APTT: prekallikrein (Fletcher factor) deficiency

P. Cauchie[1], E. Saccomando[2], A. Tamigniau[2]
[1]Clinical laboratory and haemostasis consultation, CHU de Charleroi, CHARLEROI, Belgium
[2]Clinical laboratory, CHU de Charleroi, CHARLEROI, Belgium

The presence of a very prolonged APTT was detected two years ago in an adult female during a preoperative assessment. Anamnestic records showed no bleeding tendency (Tosetto bleeding score = 0) or other associated pathology. The coagulation factors VIII, IX, XI and XII were all strictly normal. However, the complete correction of the mixing studies and the strong discrepancy between two silica-activated APTT reagents and an ellagic acid-activated reagent suggested a contact phase deficiency. So no further investigations were initially made.
Since 1/04/2019 the dosage of contact phase factors is reimbursed for the exploration of a prolonged APTT with no other coagulation factors deficiency (diagnostic rule 144). The assay was performed this summer at the KUL laboratory and revealed a complete prekallikrein (PK) deficiency.
The congenital PK deficiency is a rare condition with a relatively few published cases. This may be due to the low sensitivity of ellagic acid-activated reagent and the patients lack of symptomatology. Occasional bleeding or thromboses have been reported in a few patients but this was only due to the presence of associated risk factors.

Quick PT: STA Neoplastine R (Stago)	82%	70 – 120
APTT 1 : STA PTT-A (Stago)	160 sec	28 – 40
APTT 2 : PTT-LA (Stago)	129 sec	30 – 42
APTT 3 : Actin FS (Siemens)	35.6 sec	23.0 – 33.0
HMW kininogen	92%	70 – 120
Prekallikrein	< 2%	70 – 120

Table 1

Diagnosis is based on the presence of
1) A prolongation of APTT and normal prothrombin time and thrombin time
2) The correction of the APTT by the addition of normal plasma
3) The difference between ellagic and silicate activated reagents
4) The shortening of the APTT with prolonged incubation times, but this may also be seen with lupus anticoagulant (Robert A, Thromb Haemost 1994)
It is important to arrive at an accurate diagnosis to reassure the patients and avoid unnecessary repetition of analyses

P18

Validation of the haemolysis, icterus and lipaemia preanalytical module on Stago STA R Max analysers

H. Paridaens[1], C. Rosseels[2]
[1]Laboratory of Hematology, CHR Citadelle, LIÈGE, Belgium
[2]Laboratory of Hematology, Clinique Saint-Luc, BOUGE, Belgium

BACKGROUND/INTRODUCTION: Haemolysis, icterus and lipaemia (HIL) may affect haemostasis tests results. Therefore, an accurate evaluation of those parameters is mandatory to ensure an appropriate diagnosis.

AIMS: We assessed the performance of the HIL preanalytical module of our two STA R Max coagulation analysers by comparing them to our laboratory chemical analyzer (COBAS 700, Roche, Switzerland). We also compared one STA R Max analyser to the other regarding the HIL preanalytical module.

METHODS/MATERIALS: Blood samples were collected in 0.109 M/3.2% trisodium citrate anticoagulant (BD VacutainerTM Plus, Becton Dickinson, Franklin Lakes, NJ, USA) in the proportion 1 volume of citrate to 9 volumes of blood and processed in accordance with the GEHT (Groupes d'Etudes sur l'Hémostase et la Thrombose) guidelines on preanalytical steps for coagulation samples. Few samples were collected from serum (BD Vacutainer Serum Separator Tubes (SST II Advance)). Samples for which an activated partial thromboblastin time (APTT), prothrombin time (PT), fibrinogen or D-dimers was performed were selected based on their HIL index. All the coagulation assays were performed using a STA R Max analyser (Stago, Asnières sur Seine, France). The levels of haemolysis, icterus and lipaemia were compared with the measurement of hemoglobin, bilirubin and triglycerides concentration using the serum index analysis on a COBAS 700 (Roche, Switzerland) which were in a second time converted into the semi-quantitative HIL index of Stago.

The STA R Max coagulation analyzer is equipped with a preanalytical check module (Expert Preanalytical Check). This module is able to detect hemolysis, icterius and lipemia expressed as indices providing an objective assessment of the interferent based on absorbance spectrum of the samples at different wavelengths (hemolysis 415 nm and 582 nm, icterius at 467 nm and lipemia at 700 nm). HIL measurements on STA R Max do not require any additional reagent.

RESULTS: Most samples were citrate tubes (126/135). 9 serum were selected to assess lipemia and icterus indexes which were not found in our daily clinical coagulation samples. Tough, the HIL module index of the STA R Max analysers is validated for the citrate tubes only. Concordance for those samples between both our STA R Max analysers and the COBAS was excellent (see Table 1.A&B&C)

A sample with a very high lipemia index (6 on STA R Max corresponding to a triglycerides concentration of 2253 mg/dl) had an underestimation of its hemolysis index with the STA R Max analysers suggesting that an overlap of wavelengths due to this hyper lipemic sample could occur with the optical measurement of hemolysis on the HIL module.

SUMMARY/CONCLUSIONS: The preanalytical module of the STA R Max analyser can ensure an accurate evaluation of haemolysis, icterus and lipaemia and then a reliable representation of the preanalytical phase. Although a more throughout evaluation of this module with external quality control samples has still to be achieved.

A. Concordance between STA R Max 2678 – Cobas 700		
	Concordance	% of concordance
Haemolysis	120	96
Icterus	119	95.2
Lipaemia	125	100
B. Concordance between STA R Max 2687 – Cobas 700		
	Concordance	% of concordance
Haemolysis	120	96
Icterus	118	94.4
Lipaemia	124	99.2
C. Concordance between STA R Max 2678 and STA R Max 2687 (serum and citrate samples)		
	Concordance	% of concordance
Haemolysis	132	98.5
Icterus	133	99.2
Lipaemia	132	98.5

Table 1. Concordance of the STA R Max's HIL module and our chemical analyser (A&B). Concordance of the two STA R Max's HIL module (C)

P19

Evaluation of DOAC Remove® for the measurement of thrombophilia screening parameters in patients treated with direct anti-Xa oral anticoagulants

A. Evenepoel, C. Orlando, K. Jochmans
Hematology Laboratory, UZ Brussel, BRUSSELS, Belgium

BACKGROUND: The use of direct oral anticoagulant (DOAC) treatment is on the rise. DOACs can cause interferences with laboratory coagulation parameters, in particular on those used for thrombophilia screening.

AIM: This study aims to evaluate the use of DOAC Remove® to abolish the interference of anti-Xa DOACs with routine coagulation and thrombophilia parameters in patient plasma samples.

MATERIAL & METHODS: We collected samples from patients treated with a direct anti-Xa inhibitor from which a non-DOAC sample ('non-DOAC') was available. In total 28 sample pairs were collected (rivaroxaban: n = 14; apixaban: n = 8; edoxaban: n = 6). APTT, PT, fibrinogen, antithrombin activity (anti-Xa assay), APC-resistance and DOAC concentrations (anti-Xa assay) were determined on ACL TOP Family instruments (Werfen, USA). Protein C activity (both chromogenic and clot-based assays) and free protein S antigen were measured on the ACL Elite Pro (Werfen, USA). Samples containing DOACs were measured before ('DOAC') and after DOAC Remove® treatment ('DOAC Remove'). Statistical analysis was performed taking the results of the 'DOAC' sample as reference.

RESULTS: All the samples contained their specific DOAC, each in a broad concentration range. For rivaroxaban, we observed a significant effect of the DOAC on aPTT, PT, protein C clotting-based, antithrombin and APC resistance. The PT was the only parameter that was significantly affected by apixaban. For edoxaban, there seemed to be no significant influence of this DOAC on the evaluated parameters. Treatment of the DOAC sample with DOAC-Remove® significantly reduced the rivaroxaban and apixaban concentration. All affected parameters normalized to the value of the non-DOAC sample.

CONCLUSION: Rivaroxaban was the direct anti-Xa inhibitor that interfered most with the measurement of the investigated parameters. These results are in accordance with previous reports [1]. The presence of apixaban only influenced the PT. The affected parameters all return to their 'non-DOAC' values when DOAC Remove® is used. In our samples series, none of the investigated parameters were affected by edoxaban but the minimum concentration of edoxaban needed to observe any effect on these parameters was reported to be minimally 200ng/mL [2]. However, none of the samples in our study contained such a concentration. Our study is limited by the relatively small number of samples in each DOAC category, so we can mainly conclude that DOAC Remove® nullifies the effects of rivaroxaban in a clinical setting with patient samples. More data are required to make solid conclusions for all anti-Xa DOACs.

[1] J. Favresse et al., "Evaluation of the DOAC-Stop® Procedure to Overcome the Effect of DOACs on Several Thrombophilia Screening Tests," TH Open, vol. 02, no. 02, pp. e202–e209, Apr. 2018.
[2] R. Gosselin, R. P. Grant, and D. M. Adcock, "Comparison of the effect of the anti-Xa direct oral anticoagulants apixaban, edoxaban, and rivaroxaban on coagulation assays," Int. J. Lab. Hematol., vol. 38, no. 5, pp. 505–513, Oct. 2016.

		Rivaroxaban (n = 14)			Apixaban (n = 8)			Edoxaban (n = 6)		
		Non-DOAC	DOAC	DOAC Remove	Non-DOAC	DOAC	DOAC Remove	Non-DOAC	DOAC	DOAC Remove
DOAC concentration (ng/mL)	Median		191,2	0,3 *		96	8 *		48	11
	Range		8,3-451,9	0-5.0		16-336	0-28		23-175	7-14
aPTT (s)	Median	31,1 *	39,0	33,2 *	27,3	29,4	33,7	34,6	33,6	37,0
	Range	24,2-39,3	32,6-62,3	28,0-38,8	25,5-45,8	28,5-41,7	29,3-39,2	27,2-42,8	26,4-41,5	27,4-42,6
PT (INR)	Median	1,0 *	1,6	1,0 *	1,2	1,2	1,1 *	1,1	1,3	1,11
	Range	1,0-1,4	1,1-3,2	0,9-1,3	1,0 – 1,3	1,1 -1,4	0,9 -1,3	1,0-1,2	1,2-1,6	1,1-1,2
Fibrinogen (mg/dL)	Median	462	348	399	372 *	327	310	368	403	362
	Range	229-749	253-661	256-661	223 - 496	234 - 725	232 - 661	344-404	262-624	244-474
PC chrom. (%)	Median	101	108	104	90	89	98	94	100	105
	Range	78-156	74-158	70-152	44-107	73 - 114	79 - 115	87-111	85-120	93-124
PC Clot. (%)	Median	90	130	95 *	80	87	78	93	94	82
	Range	72-179	86-221	56-155	46 -117	52 -120	52- 107	88-101	77-122	72-99
Free PS antigen (%)	Median	88	94	88	93	97	97	105	107	111
	Range	57-108	54-112	45-107	53-117	71-119	82 -119	93 -109	72-118	73-116
Antithrombin (%)	Median	102 *	144	103 *	105	116	104	103	114	108
	Range	62-118	67-170	62-136	74 -125	101-149	77 -125	86-119	112-117	102-121
APC – Ratio	Median	2,97 *	3,51	3,03	3,09	3,08	3,24	2,92	3,16	3,24
	Range	1,78 -3,19	1,37-4,56	1,86-3,26	1,78-3,70	1,70-3,43	1,76-3,37	2,87-3,14	2,89-3,68	3,06-3,31
APC – Normalized ratio	Median	1,02	1,14	1,04 *	1,00	1,04	1,07	0,94	1,03	1,05
	Range	0,58-1,14	0,49-1,48	0,61-1,18	0,58-1,04	0,55-1,20	0,57-1,10	0,93-1,02	0,94-1,07	0,99-1,07

** P-value < 0,05.*

EVALUATION OF DOAC REMOVE® FOR THE MEASUREMENT OF THROMBOPHILIA SCREENING PARAMETERS IN PATIENTS TREATED WITH DIRECT ANTI-Xa ORAL ANTICOAGULANTS

Median and range values of the measured parameters

P20

Functional characterization of antithrombin p.Thr147Ala: not just another SNP?

C. Orlando[1], M.E. De la Morena-Barrio[2], I. Pareyn[3], K. Vanhoorelbeke[3], J. Corral[2], K. Jochmans[1]
[1]Department of Haematology, Vrije Universiteit Brussel (VUB), Universitair Ziekenhuis Brussel, BRUSSELS, Belgium
[2]Servicio de Hematología y Oncología Médica, Centro Regional de Hemodonación, Universidad de Murcia, IMIB-Arrixaca, CIBERER, MURCIA, Spain
[3]Laboratory for Thrombosis Research, IRF Life Sciences, KU Leuven Campus Kulak Kortrijk, KORTRIJK, Belgium

BACKGROUND: Hereditary antithrombin (AT) deficiency is a rare autosomal dominant disorder characterised by decreased AT activity in plasma and predisposition to recurrent venous thromboembolism (VTE). Hereditary AT deficiency is caused by mutations in the SERPINC1 gene.

AIM: We investigated a variant in the SERPINC1 gene, p.Thr147Ala, in ten patients from African origin. Eight of the ten patients experienced VTE, stroke or obstetric complications. This variant is known as single nucleotide polymorphism rs2227606 with minor allele frequency of 0.52% in Africans and absent in Europeans. In silico prediction tools for pathogenicity render conflicting results.

METHODS: Plasma AT activity was measured with three different chromogenic methods (two anti-Xa and one anti-IIa). Antigen levels were determined by rocket immunoelectrophoresis while heparin affinity was evaluated by crossed immunoelectrophoresis (CIE). Thermal stability was assessed by incubation of plasma for 24h at 40°C. Recombinant AT molecules were constructed by site-directed mutagenesis and expressed in HEK-293T cells. Secreted AT was purified and studied with functional assays. Structural modelling of the variant was performed using UCSF Chimera software.

RESULTS: AT anti-Xa activity was reduced with one commercial anti-Xa based assay while anti-IIa activity and antigen levels were normal. When shortening the incubation time, we observed a reduction of anti-Xa activity in patient plasma but not in controls. Heating of plasma also induced a more pronounced reduction of the anticoagulant activity in patients (90.1±8.7% vs. 63.8±15.1%) compared to controls (103.2±3.9% vs. 91.3±5.3%) On CIE under high ionic strength conditions, an increase of AT fractions with reduced heparin affinity was seen while this was not the case in physiological conditions. The purified recombinant p.Thr147Ala protein displayed anti-Xa activity of 61.6±1.6% of the wild type recombinant AT ($p<0.05$). Structural modelling revealed that residue Thr147 forms three hydrogen (H) bonds that are all abolished when mutated to Ala. The H-bond with Arg49 is of specific interest as this residue is known to directly interact with heparin.

CONCLUSIONS: Antithrombin activity in our patients was only reduced when measured with one commercial assay. Combined with normal antigen levels, these results suggest a Type II AT deficiency. Our study provides further evidence of the limitations of commercially available methods to diagnose AT deficiency, especially for mutations affecting the heparin binding site (HBS). Data of the structural modelling suggest a possible effect on heparin binding as H-bonds with residues of the heparin binding site are abolished. This hypothesis is supported by the incubation time-dependency of the AT activity in plasma of affected individuals, a characteristic previously observed in other Type II HBS mutations. Recombinant expression of the mutated protein confirmed the pathogenicity of this variant.Interestingly, all patients were from Black African ancestry. Type II AT mutations are known to cluster in specific regions, like p.Phe131Leu (Budapest III) in the Balkan and p.Ala416Ser (Cambridge II) in Spain and Scotland. A possible founder effect could however not be investigated.
Altogether, our data suggest that the p.Thr147Ala mutation causes a Type II HBS deficiency and is restricted to patients from Black African origin.

P21

Platelets and aortic valve calcification: insights from a rabbit model and from patients with severe aortic stenosis

N. Donis[1], C. D'Emal[1], A. Hulin[1], M. Gustin[1], R.E. Dulgheru[2], P. Lancellotti[2], C. Oury[1]
[1]GIGA - Cardiovascular Sciences, University of Liège, LIÈGE, Belgium
[2]Department of Cardiology, Heart Valve Clinic, University of Liège Hospital, LIÈGE, Belgium

BACKGROUND: Calcific aortic stenosis (CAS) is the most frequent valvular heart disease in industrialized countries. CAS is characterized by progressive aortic valve remodelling and calcification, which leads to valvular dysfunction and subsequent cardiac impairment. The extent of aortic valve calcification accurately predicts AS severity and prognosis. A recent preclinical study proposed a role for activated platelets in valvular calcification through transforming growth factor-b (TGF-b) or autotaxin-lysophosphatidic acid (LPA). Platelet parameters may therefore represent new circulating biomarkers of CAS progression and outcome.

AIM: To perform a longitudinal analysis of platelet markers in a new rabbit model of aorta and aortic valve calcification and study their correlation with aortic valve calcium score in patients with severe AS.

METHODS: Twelve-week old male New Zealand White rabbits were fed for 16 weeks (W) with a palm oil-enriched diet (5% palm oil) supplemented with vitamin D2 (25.000U/day/2.5kg) during the first two weeks. Computed tomography (CT) was performed at baseline, W4, W8, W12 and W16 to analyze the appearance of macrocalcifications. Blood samples were collected at the same time points to study the evolution of platelet count. ADP closure time (CT-ADP) was measured in whole blood on a PFA-200. After 16 weeks, the heart and the aorta were collected and fixed for histological analyses of tissue structure (hematoxylin-eosin staining) and calcification (alizarin red staining). In parallel, platelet count, number of activated platelets (CD62P+), CT-ADP, plasma levels of autotaxin, LPA and TGF-b, and CT aortic valve calcium score (CT-AVC) were studied in 36 patients with severe AS undergoing transcatheter aortic valve implantation (TAVI).

RESULTS: After 16 weeks, all rabbits fed with the palm-oil enriched diet exhibited massive aortic wall calcification, characterized by the presence of large calcification nodules in the aortic media. These aortic macrocalcifications were detectable in vivo by CT in 5 rabbits out of 6. Calcification nodules were also detected in the aortic valve fibrosa of 2 rabbits out of 6 via histological alizarin red staining of explanted hearts. Platelet count decreased as early as after 8 weeks of diet (489 K/µl vs 373K/µl, P=0.048) and kept decreasing progressively along with calcification development. No changes in CT-ADP values were observed over time. In AS patients, as expected, CT-AVC correlated well with peak aortic jet velocity (r=0.49, P=0.0054). Interestingly, CT-AVC was inversely correlated to platelet count (r=-0.52, P=0.0012) and to the number of circulating CD62P+ platelets (r=-0.45, P=0.0073). Although CT-ADP values were above normal values for AS patients, CT-ADP did not correlate with CT-AVC. Finally, in agreement with a role for activated platelets as a major source of TGF-b, levels of this cytokine showed good correlation with the number of CD62P+ platelets (r=0.46, P=0.0053).

CONCLUSIONS: Platelet consumption is associated with aortic valve calcification both in our rabbit model and in AS patients. These findings support a role for platelets in this process, possibly via TGF-b. Hence, platelet count might help assessing AS severity and provide prognostic information. This warrants further investigations.

P22

Neutrophils and neutrophil extracellular traps in inflammation-induced Staphylococcus aureus endocarditis

S. Meyers[1], L. Liesenborghs[1], M. Lox[2], S.F. De Meyers[3], T. Vanassche[1], P. Verhamme[1], K. Martinod1
[1]Center for molecular and vascular biology (CMVB), KU Leuven, LEUVEN, Belgium
[2]Center for molecular and vascular biology (CMVB), Center for molecular and vascular biology (CMVB), KU Leuven, LEUVEN, Belgium
[3]Laboratory for Thrombosis Research, KU Leuven Campus Kulak Kortrijk, KORTRIJK, Belgium

BACKGROUND/INTRODUCTION: In infective endocarditis (IE), the interplay between coagulation and Staphylococcus aureus (S. aureus) is crucial. IE is characterized by a vegetation, containing bacteria, platelets and fibrin. Previously, we have shown that S. aureus adheres to cardiac valves via platelets and von Willebrand factor. However, the process of progression from initial bacterial adhesion to a complex infected thrombus remains unclear, in particular how bacteria bypass the immune system and thrive in the host environment, remains unclear. Neutrophil extracellular traps (NETs) lie at this interface between host defense and thrombosis.

AIMS: We aimed to determine the role of neutrophils and NETs in IE progression using a novel mouse model.

METHODS: We experimentally induced endocarditis in mice using the S. aureus USA300 strain. We locally stimulated the endothelium of the aortic valve with catheter-administered histamine, followed by immediate infusion of 10^6 bacteria, resulting in IE lesions that originate on intact, inflamed heart valves. After one day we determined the development of IE on the aortic valves with Gram staining. We also investigated the presence of NETs after one day in 10 mice by immunofluorescence staining for citrullinated histone H3 (H3Cit), extracellular DNA, and myeloperoxidase (MPO). Of these 10 mice, 6 mice developed endocarditis and 3 mice developed a sterile lesion. In a separate set of experiments, we investigated the role of neutrophils in S. aureus Newman-induced IE development by injecting a neutrophil-depleting or control antibody 24h before surgery. In these experiments, bacteria were intravenously injected prior to histamine infusion and mice survived for three days.

RESULTS: After one day, H3Cit was detectable in all mice with endocarditis (6/6) and sterile lesions (3/3). H3Cit was not present in the mouse without endocarditis (0/1). More specifically, six mice with endocarditis and three with a sterile lesion had H3Cit+ neutrophils within thrombi, indicating early NETosis. Two mice with endocarditis and one with a sterile lesion had an extracellular H3Cit staining pattern within the thrombus. These extracellular H3Cit-positive regions were associated with DNA and myeloperoxidase, indicating the presence of a network of NETs (Figure 1). When we depleted neutrophils, mice developed significantly more endocarditis (7/16 vs. 1/15, p=0.03).

CONCLUSIONS: Neutrophils seem to protect the host against endocarditis, as neutrophil depletion promoted endocarditis vegetations. However, neutrophils also seem to play a role in endocarditis vegetation growth through NET formation. A better understanding of the role of neutrophils in growing vegetations may offer novel therapeutic insights in IE.

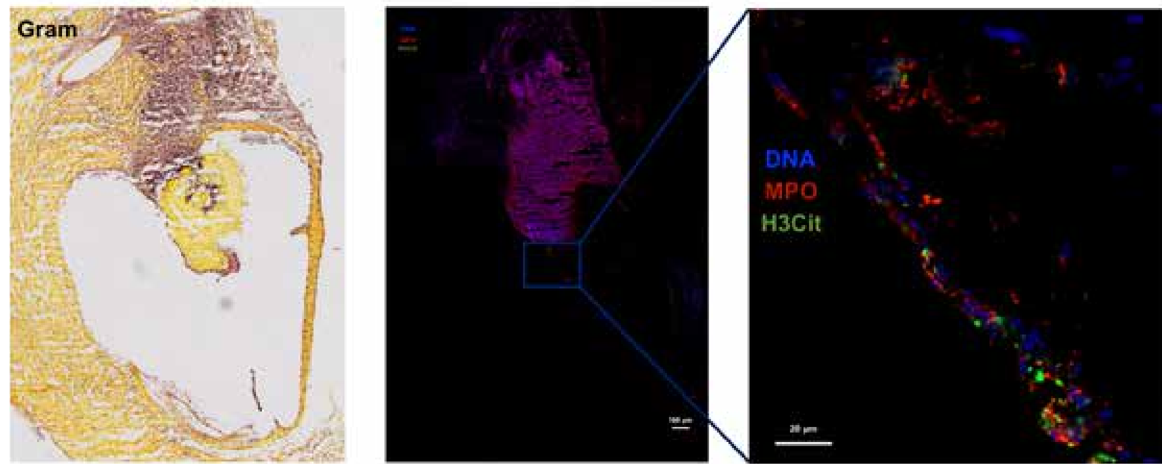


Figure 1: The presence of NETs in an endocarditis lesion

P23

Hyperactive SRC kinase results in thrombocytopenia: studies in patients and inducible Pluripotent Stem Cells

L. De Kock, C. Thys, C. Van Geet, K. Freson
Department of Cardiovascular Sciences, Centre for Molecular and Vascular Biology (KULeuven), LEUVEN, Belgium

BACKGROUND: SRC is a non-receptor tyrosine kinase protein that phosphorylates specific tyrosine residues in other proteins upon activation. A germline heterozygous gain-of-function E527K variant in SRC was previously found to cause thrombocytopenia, myelofibrosis, bleeding, bone pathologies, premature edentulism and mild facial dysmorphia in nine patients of a single pedigree1. We recently also reported a girl with syndromic thrombocytopenia due to the same SRC E527K variant that occured de novo2.

AIMS: Our aim is to delineate this novel clinical syndrome via detailed platelet function studies and to unravel SRC kinase activity during megakaryopoiesis.

METHODS: Bloodsmear analysis, platelet aggregations, P-selectin expression, SRC activation and tyrosine phosphorylation were performed for both families. Inducible Pluripotent Stem Cells (IPSCs) developed from a patient's skin fibroblasts are being used for forward programming to megakaryocytes (MK) and functional studies. CRISPR/Cas is used to correct the phenotype of these SRC-E527K deficient IPSCs as confirmed by immunofluorescence studies. CD34+ hematopoietic stem cells (HSCs) isolated from blood of healthy controls were transduced with lentiviral SRC-WT and SRC-E527K, differentiated to MKs and subjected to RNAseq.

RESULTS: Blood smear analysis showed thrombocytopenia with large platelets and a paucity of α-granules. Platelet aggregations specifically showed mainly reduced responses to collagen. Expression of α-granule membrane marker P-selectin after activation with GP6 agonists convulxin and collagen was significantly reduced. Western blot analysis showed significantly higher active SRC and increased overall tyrosine phosphorylation levels in platelets from E527K patients. Immunofluorescence staining for total and active SRC confirmed the SRC-E527K IPSC phenotype correction introduced by CRISPR/Cas technology via depletion of the mutant allele. Forward programming of control, patient and patient-corrected IPSCs generated 44,1%, 23% and 42,5% CD41/42+ MKs at differentiation day 26, respectively. RNA sequencing of MKs derived from differentiated CD34+ HSCs overexpressed with SRC-WT or SRC-E527K showed 691 significant differentially expressed genes (44% upregulated and 56% downregulated). Pathway analysis shows that upregulated genes are most involved in hematopoietic cell lineage, cytokine-cytokine receptor interaction and ECM-receptor interaction, while downregulated genes are mostly involved in platelet activation (including collagen GP6; p= 3,542E-29), gap junction and chemokine signalling pathway. Genes in these pathways will be validated in MKs generated from control, patient and patient-corrected IPSCs.

SUMMARY: This study strengthens our previous finding that hyperactive SRC kinase results in mild platelet dysfunction and thrombocytopenia with hypogranular platelets and further expands the clinical description of this syndrome to improve early recognition. Additional studies are needed to characterize the exact function of hyperactive SRC in platelet formation. Therefore, SRC-E527K deficient IPSCs together with control and patient-corrected IPSCs will be differentiated towards MKs for RNA sequencing and phosphoproteomics studies.

[1]Turro E et al. (2016) A dominant gain-of-function mutation in universal tyrosine kinase SRC causes a syndrome with thrombocytopenia, myelofibrosis, bleeding and bone pathologies. Science Translational Medicine doi: 10.1126/scitranslmed.aad7666
[2]De Kock L et al. (2019) De novo variant in tyrosine kinase SRC causes thrombocytopenia: case report of a second family. PLATELETS https://doi.org/10.1080/09537104.2019.1628197.

P24

Pharmacological ACC inhibition decreases thrombin-induced platelet aggregation by a mechanism independent of lipid content

M. Octave[1], L. Pirotton[1], S. Lepropre[1], A. Ginion[1], V. Robaux[1], S. Kautbally[1], V.M. Darley-Usmar[2], J. Ambroise[3], B. Guigas[4], M. Foretz[5], L. Betrand[1], C. Beauloye[1], S. Horman1
[1]UCLouvain, IREC, Pôle de recherche cardiovasculaire, BRUSSELS, Belgium
[2]University of Alabama at Birmingham, BIRMINGHAM, USA
[3]UCLouvain, IREC, Centre de technologies moléculaires appliquées, BRUSSELS, Belgium
[4]Leiden University Medical Center, LEIDEN, the Netherlands
[5]Université Paris Descartes, Institut Cochin, INSERM, PARIS, France

BACKGROUND: Acetyl-CoA carboxylase (ACC) is the first committed enzyme of the fatty acid biosynthesis pathway, while its phosphorylation on serine 79 by AMPK inhibits its activity. We have recently demonstrated that the overexpression of a constitutively active form of ACC (not phosphorylatable by AMPK) in platelets leads to an increase in the phospholipid content (especially plasmalogen phosphatidylethanolamines), which stimulates platelets and thrombus formation. Based on these data, we believe that ACC inhibition or invalidation might be a counter-regulatory mechanism limiting endogenous lipogenesis and platelet reactivity in certain pathological circumstances.

AIM: Our study aims to assess the impact of pharmacological ACC1 inhibition on platelet functions, de novo platelet lipogenesis and lipid content.

METHODS: Platelets were treated with the long-chain fatty acid analog 5-(tetradecyloxy)-2-furoic acid (TOFA, 30µM) or the active site-directed CP640.186 (CP, 60µM), two ACC inhibitors, for 2 hours before thrombin stimulation. Lipogenesis was measured via 14C-acetate incorporation into fatty acids. Platelet functions were assessed by aggregometry. Lipidomics analysis was carried out on the commercial Lipidyzer platform.

RESULTS: Both ACC inhibitors decreased lipogenesis and thrombin-induced platelet aggregation. TOFA, but not CP, drastically decreased mitochondrial oxygen consumption rate, an effect associated with an increased reactive oxygen species production and AMPK activation. Surprisingly, the quantitative lipidomics analyses showed that a 2 hours preincubation with the compounds did not affect global platelet lipid content. However, the short-term inhibition of ACC1 (and potential acetyl-coA accumulation) with CP was sufficient to modify the level of acetylated proteins such as tubulin, a key player in the regulation of platelet shape change and aggregation.

CONCLUSIONS: Given the effect of TOFA on mitochondrial respiration, oxidative stress and AMPK activation, CP seems more appropriate to investigate the impact of ACC inhibition in platelets. A short-term acute treatment with CP inhibits thrombin-induced aggregation by a mechanism which does not depend on lipid content but might involve changes in protein acetylation.

MAJOR SPONSORS

SPONSORS

NOTES

Roche

ReDEFINE HAEMOPHILIA

#MEMEOPHILIA

#Haemophilia can affect far more than blood, but sometimes it's not that easy to explain to others what it's really like to live with haemophilia. Discover how patients express themselves using memes.

www.redefinehaemophilia.com

DON'T WORRY HE JUST INFUSED

MY FACE WHEN I TELL THE DOCTOR I'VE BEEN SKIPPING INFUSIONS

BUT IF YOU TAKE MEDICINE WHY DO YOU STILL BLEED?

WHEN THE DENTIST TRIES TO EXPLAIN TO YOU HOW HAEMOPHILIA WORKS

WHEN YOU HAVE TO EXPLAIN A SEXUALLY CAUSED BLEED TO YOUR PARENTS

PACKING FOR A WEEKEND-AWAY WITH HAEM GOT ME LIKE

SLEEPING COMFORTABLY AFTER TREATING A DEVELOPING BLEED BEFORE BEDTIME

WHEN YOU HIT THE VEIN FIRST TRY

UNEXPECTED BLEED

NO OFFICER THEY ARE FOR MY HAEMOPHILIA

WHEN YOU HIT A VEIN FIRST TIME

R.E.: Dr. Christine Lenaerts - BE/HAEM/1019/0033b - created on 12/11/2019

See aTTP

Because acquired Thrombotic Thrombocytopenic Purpura (aTTP) is a medical emergency, diagnosing and starting treatment early may help guard against the high risk of early, preventable deaths in aTTP[1].

Join our satellite symposium on Thursday at 16h30

1. Scully M et al., Br J Haematol. 2012; 158(3):323–35

Sanofi Belgium/GZBE.CVD.19.11.0254