BSTH

Belgian Society on Thrombosis and Haemostasis

25TH ANNUAL MEETING

23-24 NOVEMBER 2017

LAMOT
BELGIUM

LOCAL ORGANISER

KATRIEN DEVREESE

GHENT UNIVERSITY HOSPITAL

LABORATORY MEDICINE

MEETING THEME:

THROMBOSIS AND BLEEDING IN WOMEN AND CHILDREN

PROGRAM & **ABSTRACTS**

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GENERAL INFO

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Con-txt

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DATE:
23 - 24 November 2017

VENUE:
Lamot Congres- & Erfgoedcentrum
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LAMOT
CONGRES- EN ERFGOEDCENTRUM
een uniek concept

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WELCOME TO THE 25TH ANNUAL MEETING

Dear participants,

Another year has passed and I would like to welcome you all to the 2017 Annual Meeting of the Belgian Society on Thrombosis. This is the 4th time the Annual Meeting will be held in Mechelen which is beginning to feel like home now. Many of you will know the venue of previous years.
The scientific organization of the 2017 Annual Meeting of the BSTH has been entrusted to the prof dr Katrien Devreese, head of the laboratory of clinical biology at the University Hospital Gent. She has put together a programme mostly dealing with thrombosis and Haemostasis in women and children, which are the topics for the two state-of-the art sessions, while the 3 satellite symposia organized by the pharmaceutical industry focus mostly upon the treatment of haemophilia. I am happy to give an educational session on the diagnostic of von Willebrand disease, while the second educational deals with functional interactions between factor V and tissue factor pathway inhibitor. It is not always easy to provide a programme that appeals to clinicians, clinical biologists and basic researchers at the same time, and I would like to that Katrien Devreese for her excellent work in putting together this annual meeting of the BSTH and the enormous work she does as both secretary and treasurer of the society.
We cordially invite all participants to the Networking Reception after the meeting on Thursday. Afterwards we have organized an evening programme in an authentic brewery in Mechelen.
The satellite symposia are evidence of the continued support that we enjoy from the pharmaceutical industry. Most companies active in the area of haemostasis and thrombosis are present at this meeting in one form or another, be it with a satellite symposium, and exhibition stand or through financial support. I would like to ask all participants to show appreciation for their support and visit the exhibition and interact with company representatives.
This year we celebrate the 25th anniversary of the Belgian Society on Thrombosis and Haemostasis (BSTH) and we have decided to mark this by the institution of the Annual Gaston Baele Memorial Lecture. Professor Gaston Baele (1939-2015) was one of the founders of the BSTH and the first BSTH president. Each year a speaker from the Belgian haemostasis and thrombosis community will be asked to give this lecture in recognition of the important work he or she has done during their career in the field of coagulation. The inaugural speech will be given by prof dr Jean Louis David, now retired from the University of Liège. Like Gaston Baele he was one of the founders of the BSTH.
I hope that we have another great Annual Meeting of the BSTH with interesting scientific topics, good interaction and a relaxed atmosphere.

Alain Gadissuer, MD, PhD
President of BSTH

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DAY PROGRAM THURSDAY 23RD NOVEMBER

08:30	Registration	
09:25	Welcome	
09:30	**EDUCATIONAL I:**	
CHAIR	K. DEVREESE AND A. DE MULDER	
	Diagnosis of von Willebrand Disease	A. Gadisseur (Antwerp)
10:15	**CSL BEHRING / SATELLITE SYMPOSIUM**	
	REACH HIGH IN HAEMOPHILIA A: AFSTYLA'S TOTAL OFFERING	
CHAIR	M. JACQUEMIN AND C. HERMANS	
	Afstyla®, rVIII-SingleChain in Haemophilia A	A. Veldman (Limburg)
	Laboratory monitoring of rVIII-SingleChain	R. Pruthi (Minnesota)
11:15	Break	
11:45	**ORAL PRESENTATIONS: CLINICAL & LABORATORY**	
CHAIR	C. ORLANDO AND J. EMMERECHTS	
	Inhibiting ADAMTS13 activity prevents the loss of high molecular weight VWF multimers in an in vitro left ventricular assist device	S. Deconinck (Kortrijk)
	Increased Acetyl-CoA carboxylase phosphorylation I state in platelets identify high risk patients with coronary artery disease: ACCTHEROMA trial	S. Kautbally (Brussels)
	The interaction of factor V & tissue factor pathway inhibitor in a myeloma patient with acquired factor V deficiency	E. De Maertelaere (Roeselare)
	Reversal of a Dabigatran overload with Idarucizumab: 5 mg may be a very insufficient dose	P. Cauchie (Lodelinsart)
	Cryopreservation impairs platelet function but generates procoagulant platelets	K.R. Six (Ghent)
12:45	Paul Capel Prize Clinical & Laboratory	
13:00	Lunch	

14:00 **BSTH GENERAL ASSEMBLY**

14:30 **STATE OF THE ART I: THROMBOSIS**

CHAIR K. VANHOORELBEKE AND K. VANDENBOSCH

Thrombotic thrombocytopenic purpura in pregnancy	A. Veyradier (Paris)
Direct oral anticoagulants and women	H. Cohen (London)
Risk factors for thrombosis in children	U. Nowak-Göttl (Kiel)

16:00 Break

16:30 **SOBI / SATELLITE SYMPOSIUM**

REAL WORLD EXPERIENCE WITH INITIATING AND CONTINUING TREATMENT WITH RFVIIIFC AND RFIXFC AND THEIR IMPACT ON LONG-TERM TREATMENT GOALS

CHAIR C. HERMANS

Experiences with rFVIIIFc and rFIXFc	K. Peerlinck (Leuven)

CHAIR A. GADISSEUR AND L. PHU QUO

Optimizing PK-guided Personalized Prophylaxis with rFVIIIFc and rFIXFc	K.J. Pasi (London)

CHAIR M.B. MAES AND K. DEVREESE

EHL assays and reactives, the Belgian situation	M. Jacquemin (Leuven)

17:30 **PROFESSOR GASTON BAELE MEMORIAL LECTURE** J.L. David (Liège)

CHAIR A. GADISSEUR

18:00 Closure of day program

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DAY PROGRAM THURSDAY 23RD NOVEMBER

EVENING PROGRAM

WELCOME RECEPTION

On Thursday night 18:00 all participants are invited to join the welcome reception.

DINNER PROGRAM

From 19:30 you're invited to join our exclusive program with a tour, tasting and walking dinner at the authentic brewery 'Het Anker'.

We will gather at 19h15, pre registration is required.

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

For news visit our website bsth2017.com

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DAY PROGRAM FRIDAY 24TH NOVEMBER

08:55	Welcome	
09:00	**EDUCATIONAL II:**	
CHAIR	S. DE MEYER AND T. VANASSCHE	
	Functional interactions between FV and tissue factor pathway inhibitor	E. Castoldi (Maastricht)
09:45	**SHIRE / SATELLITE SYMPOSIUM** TAILORING TREATMENT TO THE INDIVIDUAL: REAL LIFE EXPERIENCE	
CHAIR	C HERMANS	
	PK-guided dose tailoring to Personalized Care: use in Real Life	M. Canaro (Palma de Mallorca)
	Extended Half-life Products in Hemophilia A: The Present and the Future of a New Treatment ERA	P. Turecek (Vienna)
	rpFVIII, a new approach for the treatment of patients with acquired hemophilia	P. Knöbl (Vienna)
10:45	Break	
11:15	**ORAL PRESENTATIONS: BASIC RESEARCH**	
CHAIR	H. DECKMYN AND C. OURY	
	An open conformation of ADAMTS13 is a hallmark of acute acquired thrombotic thrombocytopenic purpura	E. Roose (Kortijk)
	P2X1 ion channel regulates neutrophil NETosis in colitis	O. Wéra (Liege)
	Unraveling anti-spacer immunoprofiles of acquired TTP patients using anti-idiotypic antibodies	A.S. Schelpe (Kortrijk)
	Plasma of healthy donors contains specific anti-idiotypic antibodies that recognize two cloned human anti-ADAMTS13 autoantibodies	K. Kangro (Kortrijk)
	Phosphorylation of acetyl-CoA carboxylase by AMPK in platelets controls thromboxane generation, dense granules secretion and thrombus formation	S. Lepropre (Brussels)
12:15	Paul Capel Prize Basic Research	

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DAY PROGRAM FRIDAY 24TH NOVEMBER

12:25	**BSTH NEWS** CSL Behring Encouragement Award		
12:45	Lunch		
13:00	**POSTER WALK**		
14:00	**STATE OF THE ART II: BLEEDING**		
CHAIR	K. DEVREESE AND K. JOCHMANS		
	Bleeding in children	C. Van Geet (Leuven)	
	Bleeding in women	P. de Moerloose (Geneva)	
	Unexplained bleeding disorders	M. Makris (Sheffield)	
15:30	Poster presentation awards		
CHAIR	A. GADISSEUR		
15:35	Closure		
15:45	Reception		

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BSTH BOARD 2017

THE PRESENT MEMBERS OF THE BSTH BOARD 2017 ARE:

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MEMBERSHIP BENEFITS & FEES

MEMBERSHIP BENEFITS

The BSTH council has defined the membership benefits for the different categories of membership.

You will be able to register your membership of BSTH 2018 until 1 March 2018.

Standard members

- Access to member-only pages of the BSTH website
- Priority information on all BSTH activities
- Free admission to BSTH educational courses
- Reduced admission fees at BSTH Annual Scientific Meeting
- Eligibility for financial grants under auspices of the BSTH
- Eligibility for travel grants dispensed by the BSTH
- Eligibility for election to the BSTH Council
- Eligibility to participate in BSTH Council Committees
- Right to vote at the BSTH General Assembly

Associate members

- Access to member-only pages of the BSTH website
- Priority information on all BSTH activities
- Free admission to BSTH educational courses
- Reduced admission fees at BSTH Annual Scientific Meeting
- Eligibility for financial grants under auspices of the BSTH
- Eligibility for travel grants dispensed by the BSTH
- Eligibility to participate in BSTH Council Committees

Corporate members

- Acknowledgement as BSTH sponsor on the BHS website
- Hyperlink to company website on BSTH website
- Right to post announcements for scientific activities on the BSTH website (subject to approval by BSTH Council)
- Priority choice for booth space at the BSTH Annual Scientific Meeting
- Access to the BSTH address database for mailings for scientific activities (subject to approval by BSTH Council)
- Priority on proposals for satellite symposium during the BSTH Annual Scientific Meeting
- Free admission of 5 employees at BSTH Annual Meeting

At the end of the year all members are asked to renew their membership of the BSTH if they have not already done so at the occasion of the BSTH Annual Meeting.

ANNUAL MEETING FEES MEMBERS	Early registration	Registration	On site registration	Annual membership
MD specialist, MSc specialist, PhD scientist	€ 90	€ 100	€ 125	€ 50
MD trainee, PhD student	€ 50	€ 50	€ 65	€ 35
Nurse, paramedic, technician, data manager, student	€ 20	€ 25	€ 35	€ 25
Corporate	according to sponsorship or exhibitionbooth package			

ANNUAL MEETING FEES NON-MEMBERS			
MD specialist, MSc specialist, PhD scientist	€ 150	€ 175	€ 200
MD trainee, PhD student	€ 100	€ 115	€ 125
Nurse, paramedic, technician, data manager, student	€ 60	€ 65	€ 75

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EXHIBITION

EXHIBITION SCHEDULE

SET UP
WED 22 NOV 2017 19:00-22:00
THU 23 NOV 2017 07:00-08:30

OPENING HOURS
THU 23 NOV 2017 08:30-19:30
FRI 24 NOV 2017 08:30-16:30

DISMOUNT
FRI 24 NOV 2017 16:00-18:00

EXHIBITION RULES

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

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. Con-txt, 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.

COMPANY / PRODUCT PROFILE
A complete listing of all exhibitors and sponsors, including a 100-word entry will be included in the Final Program and Abstracts and is distributed to all attendees. You can submit your company / product profile by mail at bsth@con-txt.nl.

FAILURE TO OCCUPY SPACE
Exhibitors not occupying booth space by 8h30 on Thursday November 23, 2017, will forfeit their booth space without refund. The space may be resold or used by the BSTH.

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.

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FLOORPLAN

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STATE OF THE ART SPEAKERS

H. COHEN (LONDON)

Hannah Cohen is Professor of Haematology at University College London and Consultant Haematologist at University College London Hospitals, where she is Clinical Lead in anticoagulation and venous thromboembolism. She studied medicine at the University of Manchester, trained in haematology at the Middlesex and University College London Hospitals and was awarded an MD degree for studies on haemostasis in renal allograft recipients. Her clinical and research interests are anticoagulation and the pathogenesis and management of antiphospholipid syndrome. She established, in the first RCT in women with antiphospholipid antibodies and recurrent miscarriage, the regimen that has become standard treatment internationally. She led the RAPS (Rivaroxaban in Antiphospholipid Syndrome) trial and is leading the RISAPS (Rivaroxaban in Stroke Patients with APS) trial, both Arthritis Research UK funded. She has over 200 publications. As a Founder member and Steering Group Chair of the Serious Hazards of Transfusion (SHOT) UK confidential enquiry into transfusion risks, she established SHOT as an international gold standard in haemovigilance. She was Co-chair of the ISTH Women's Health Issues SSC 2015-2017 and appointed Co-chair of the Lupus anticoagulant/antiphospholipid antibodies SSC in 2017. She is a Founder member, Executive Committee member and UK Core Laboratory Director of APS ACTION and on the Scientific Committee responsible for organising the 16th (Manchester, 2019) International Congress on Antiphospholipid Antibodies.

A. VEYRADIER (PARIS)

Agnès Veyradier, MD, PHD, is professor of Hematology at University Paris-Diderot in Paris, France. Since 2006, she has been the head of the Hemostasis laboratory of Hospital Lariboisière and Saint Louis and the coordinator of the National Reference Center for von Willebrand disease and of the ADAMTS13/TTP laboratory of the National Reference Center for Thrombotic Microangiopathies.

Ulrike Nowak is Head of Thrombosis and Hemostasis Treatment Center, University Hospital Schleswig-Holstein, Campus Kiel & Lübeck and Deputy Director Institute of Clinical Chemistry UKSH, Kiel.
Ulrike is specialist in Pediatrics and professor in Appointment C3 and C3 Hemostasis. She has teaching or research experience in the field General Pathology and Pediatrics: Hematology & Oncology; Vascular accidents (venous & arterial thrombosis; ischaemic stroke) in neonates, children and adolescents with respect of inherited and acquired prothrombotic risk factors & genetics. She was involved in clinical trials including drug trials. Ulrike Nowak is member of many national medical and/or scientific societies/working groups. (DGKJ, GPOH,DGA,GTH). She also was an active member of ISTH: Chair/Co-Chair of the Scientific Standardisation Committee on "Pediatric & Perinatal Hemostasis (ISTH) 2000-2011.

U. NOWAK-GÖTTL (KIEL)

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STATE OF THE ART SPEAKERS

C. VAN GEET (LEUVEN)

Chris Van Geet, M.D., PhD, (born 1961) is vicerector for the Biomedical Sciences Group and University Development Cooperation. She holds a candidate's degree in psychology (1984) and a PhD in medicine, surgery, and obstetrics (1986). She became a paediatrician in 1993 and was certified as paediatric haematologist and oncologist in 2014. From 1999 to 2014 she was an FWO fellowship Clinical Investigator at the Centre for Molecular and Vascular Biology. Chris Van Geet has been a part-time full professor since 2005. From 2005 until august 2017 she was head of Paediatrics at University Hospitals Leuven; and as a paediatric haematologist she still continues to treat children with blood diseases and do research in the field. Development cooperation has been a constant factor in her life and work. Through her collaboration with partners in Congo and Benin, and in her role of Chair of the Interfaculty Council for Development Cooperation, she developed wide-ranging experience across different disciplines in the field.

P. DE MOERLOSE (GENEVA)

Philippe de Moerloose is Emeritus Professor of the Faculty of Medicine, Geneva, Switzerland where he was Head of the Haemostasis Unit and Adjunct Chef de Service for the Division of Angiology and Haemostasis at the Geneva University Hospital in Switzerland. He holds specialist degrees in Internal Medicine, Angiology as well as Haematology. He is an expert in bleeding and thrombotic disorders. Philippe de Moerloose is a recurrent recipient of national and international grants. He has authored or co-authored over 400 peer-reviewed publications, review articles and book chapters. Philippe de Moerloose is past President of the European Association for Haemophilia and Allied Disorders and is still active in research (especially fibrinogen disorders and antiphospholipid antibodies) and involved in projects for patients with bleeding disorders in several Sub-Saharan African countries.

Professor Michael Makris MD is Director of the Sheffield Haemophilia and Thrombosis Centre, Sheffield, UK. He trained in Medicine and Haematology at the Universities of Oxford, London and Sheffield in the UK. He is Professor of Haemostasis and Thrombosis at the University of Sheffield and since 1994 he has been an honorary consultant haematologist at The Sheffield Teaching Hospitals NHS Trust in Sheffield, UK. Professor Makris's research interests include the monitoring of adverse events in haemophilia, hepatitis C in haemophilia, thrombin generation assays in bleeding and thrombotic disorders and the genetics of inherited platelet disorders. He is one of two editors of the journal Haemophilia and is on the Editorial Board of the journals British Journal of Haematology, Journal of Thrombosis and Haemostasis and Blood Transfusion.

M. MAKRIS (SHEFFIELD)

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ABSTRACTS STATE OF THE ART LECTURES

THROMBOTIC THROMBOCYTOPENIC PURPURA IN PREGNANCY

A. VEYRADIER (PARIS)

Thrombotic thrombocytopenic purpura (TTP) is a thrombotic microangiopathy (TMA) which pathophysiology relies on a severe deficiency (either acquired via specific autoantibodies or inherited via genetic mutations) of ADAMTS13, the specific von Willebrand factor (VWF)-cleaving protease. TTP is characterized by a feminine predominance (sex ratio 2F/1M) and pregnancy is well established to be a precipitating factor for TTP boots. TTP is a very rare complication of pregnancy (about 1 case/100.000 pregnancies) but it is a life-threatening disease for both the mother and the fetus. Obstetrical TTP represents at least 20% of all TTPs occurring in child-bearing age women. Also, obstetrical TTP is characterized by a very high frequency of inherited TTP (~40%) when compared to adult-onset TTPs in general (~2% of inherited TTP).

The diagnosis of an obstetrical TTP is challenging because it mostly occurs in women with no antecedent of TTP and it has no specific clinical / biological symptoms except a severely deficient ADAMTS13. However, because of the severity of the prognosis in the absence of urgent treatment, any thrombocytopenia +/- hemolytic anemia in a pregnant woman with no alternative diagnosis to TMA, should be considered as TTP. In patients with inherited TTP, the first pregnancy is almost systematically associated with a TTP boot, mostly occurring during the second half of the pregnancy. Also, in obstetrical acquired TTP, the boot usually occurs after 20 weeks gestation and sometimes during the post-partum.

The management of an obstetrical TTP boot consists in a blood collection for ADAMTS13 investigation followed by an emergency first-line treatment by plasma exchange (PEX) that is usually efficient in both inherited and acquired TTP. PEX yields a maternal response rate of about 80% although the global stillbirth rate is likely to be close to 50%. In contrast to some other obstetrical TMAs, the fetal extraction in not sufficient to solve TTP.
The follow-up of a woman who recovered from an obstetrical TTP boot should include a complete ADAMTS13 investigation to distinguish between the inherited and the acquired form of TTP, in order to both estimate the risk for relapse and optimize prophylaxis indication during subsequent pregnancies. The relapse rate appears to be 100% in inherited TTP and about 20% in acquired TTP. Early prophylactic plasma infusion is thus indicated systematically in inherited TTP because it is clearly beneficial for both the mother and the fetus outcomes. In contrast, the optimal management is still debated in subsequent pregnancies of women with acquired TTP but their clinical and biological monitoring should be anyway very careful. Ideally, in women with an antecedent of acquired TTP, another pregnancy should be initiated only after a total biological remission (recovery of a normal ADAMTS13 activity, sometimes requiring an additional treatment with the immuno-modulator rituximab).
Many advances have been performed in the last 15 years in terms of diagnosis and treatment of obstetrical TTP. However, clear guidelines are still needed to optimize the management of subsequent pregnancies which may be significantly different as a function of the pathophysiology for ADAMTS13 severe deficiency.

DIRECT ORAL ANTICOAGULANTS AND WOMEN

H. COHEN (LONDON)

Direct oral anticoagulants (DOACs) provide an effective, safe and convenient therapeutic alternative to vitamin K antagonists and are the standard of care for a wide range of indications. The use of DOACs in women merits special consideration mainly in two situations, the first of these being in relation to fertility, pregnancy, and lactation in the many women receiving DOACs in their reproductive years. The potential for reproductive toxicity of DOACs in humans, via maternal or paternal exposure, is undefined. Limited data do not indicate that DOAC exposure in pregnancy carries a high risk of embryopathy, however, more robust data are required.

The DOAC Summary of Product Characteristics recommend against their use in pregnancy and during breast-feeding, and International Society of Thrombosis and Haemostasis (ISTH) guidance provides comprehensive advice on the management of DOACs in women of childbearing potential. The second situation which merits special consideration in women is vaginal bleeding, particularly heavy menstrual bleeding (HMB), in the context of DOAC administration. Such bleeding, which may affect a woman's quality of life, is a common complication of oral anticoagulation and appears to occur more often with direct oral factor Xa inhibitors. Preventative strategies for HMB should focus on identification of women at risk, including detailed assessment of menstrual bleeding history. Most cases of HMB can be managed conservatively. Where there is a pre-existing history of HMB or unusually severe or recurrent HMB, further assessment including gynaecological review and investigation for an anatomical abnormality, is required.

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ABSTRACTS STATE OF THE ART LECTURES

RISK FACTORS FOR PEDIATRIC THROMBOSIS - UPDATE 2017

U. NOWAK-GÖTTL (KIEL)

Venous thrombosis (VTE) in children is gaining increased awareness, as advanced medical care has increased treatment intensity of hospitalized pediatric patients. The aim of this presentation is to summarize the data available and to discuss the controversial issue of thrombophilia (IT) screening. Underlying medical conditions occur in approximately 70% of pediatric VTE cases and differ with respect to pediatric age groups, i.e. neonates, toddlers, children and adolescents. Apart from provoking risk factors systematic reviews on pediatric VTE have shown significant associations between thrombosis and presence of protein C-, protein S- and antithrombin deficiency, factor 5 (F5: rs6025), factor 2 (F2: rs1799963), even more pronounced when combined IT were involved. Follow-up data for VTE recurrence in children suggest a recurrence rate between 3% in neonates and 21% in individuals with unprovoked VTE. The F2 mutation, protein C-, protein S-, and antithrombin deficiency did also play a significant role at VTE recurrence. Although we have learned more about the pathophysiology of VTE with the increased discovery of IT evidence is still lacking as to whether IT influence the clinical outcome in pediatric VTE. It still remains controversial as to whether children with VTE or offspring from thrombosis-prone families benefit from IT screening. Thus, IT testing in children should be individualized.

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ABSTRACTS STATE OF THE ART LECTURES

II. BLEEDING

BLEEDING IN CHILDREN

C. VAN GEET (LEUVEN)

VEERLE LABARQUE
KATHLEEN FRESON

Bleeding and bruising in children require specific attention to differentiate between physiological versus pathological bruising. Also the distinction between traumatic accidental versus non-accidental bleeding is necessary especially in infants and toddlers. In children with primary or secondary haemostatic defects the aim is to establish the severity of the defect by evaluating the amount of bleeding, the frequency of bleeding problems, the relation with (minor) trauma for its occurrence, the haematoma reabsorption time and the type of bleeding. The inclusion of pediatric-specific bleeding symptoms in a standardized Bleeding questionnaire is also useful. Haemostatic challenges, such as surgery, that the patient has eventually already been subjected to, will learn us a lot of the severity of the problem. However the younger the child, the more difficult the evaluation of the severity is. Indeed most of these young children did not encounter haemostatic challenges yet in their past. Additional information by a rigorous medical history of all family members is thus warranted. Based on these clinical features, the distinction can mostly be assumed between primary haemostatic problems (more petechiae and spontaneous mucosal bleeding) versus secondary haemostatic or coagulation disorders (hematoma with hard nidus, muscle and joint bleeding). The standard diagnostic approach comprises routine coagulation screening tests and when a platelet disorder is suspected, different laboratory investigations including platelet aggregation tests, ATP secretion, platelet adhesion by the platelet function analyzer (PFA100) and platelet morphology by electron microscopy. Platelets are easy accessible cells and different techniques are possible to study platelet function under basal and activated conditions. Defects in platelet aggregation, adhesion, signaling or secretion can result from mutations in platelet-specific genes leading to isolated thrombopathies or from mutations in widely expressed genes leading to broader clinical phenotype including a platelet defect. It is important to recognize how platelet research becomes an important tool to improve our knowledge in broad phenotype mendelian disorders.

Recently, also novel methods as next generation sequencing using gene panels as well as for whole exome/genome sequencing, epigenetic studies, proteomics and functional genetics using iPSC's or in zebrafish, led to the generation of novel insights in the complexity of genetic haemostatic disorders of which the pathology was known long before finding the responsible underlying genetic causative factor. To date 96 genes are already known to regulate bleeding, platelet and thrombotic disorders. The combination of standard platelet functional testing with these novel approaches will improve our insights in many diseases that are related to defect in platelets.

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ABSTRACTS STATE OF THE ART LECTURES

WOMEN AND BLEEDING

P. DE MOERLOOSE (GENEVA)

Women and bleeding is a very broad topic to deal with. In the general population menorrhagia, postpartum haemorrhages and miscarriages are major public health issues. In the presentation we will focus mainly on women with Inherited Bleeding Disorders (IBD).

Menorrhagia is often neglected. Bleeding can be very important and lead to anaemia; furthermore, affected women have a decreased quality of life. Various causes of menorrhagia exist but an underlying IBD should be suspected if menorrhagia start since menarche and if there is a family history or personal history of bleeding. Bleeding score assessment as well as pictorial blood assessment are useful to define better whether a woman has a true underlying disorder of haemostasis. All women with severe IBD (coagulation disorders as well as platelet disorders) are concerned but menorrhagia is a special call sign for type 1 von Willebrand disease (vWD). Indeed the prevalence of vWD in women with menorrhagia was reported to be 13% (95% CI 11-15%) in a systematic review of 11 studies totalling 988 women with menorrhagia. Various substitutive and non substitutive treatments will be discussed (e.g. contraceptive pill, levonorgestrel intrauterine devices, tranexamic acid, desmopressin). It is striking however to note the very limited number of randomized studies to provide strong guidelines.

Postpartum haemorrhage (PPH) is a leading cause of maternal mortality worldwide, most of the deaths occurring in low-income countries. IBD may increase the incidence and magnitude of bleeds cause by uterine atony, retained placenta and genital tract trauma. PPH usually is defined as a blood loss > 500 mL after vaginal delivery or > 1000 mL after caesarean section. However this definition is debatable and involves mainly for what happens within the first 24 hours which is not a good predictor of IBD. For women with IBD PPH is often delayed, therefore it is important to follow carefully these women for several weeks after delivery. Among the different treatments tranexamic acid is of particular interest as shown recently in the WOMAN trial (Lancet 2017;389:2105-16) and thus without significant adverse effects.

Miscarriage is relatively common in the general population, with around 15% of recognised pregnancies resulting in spontaneous abortions. Women with IBD may also be at increased risk of miscarriages, especially those with severe fibrinogen or factor XIII deficiencies who are usually not able to maintain a pregnancy without factors substitution. Miscarriages occur in the first weeks of pregnancy, indicating the crucial role of complete fibrin formation for placenta implantation and maintenance of pregnancy.

Women with IBD require specialised and individual care. Multidisciplinary team need to include gynaecologists, obstetricians, anaesthetists, paediatricians and haematologists to optimise the care of all women with IBD.

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ABSTRACTS STATE OF THE ART LECTURES

UNEXPLAINED BLEEDING DISORDERS

M. MAKRIS (SHEFFIELD)

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PROFESSOR GASTON BAELE MEMORIAL LECTURE

PROFESSOR GASTON BAELE MEMORIAL LECTURE

J-L DAVID (LIÈGE)

Jean-Louis David received his medical degree from the University of Liège (ULg) in1965.
At the end of his studentship he was involved in the field of platelets in the laboratory of Professor Jean Hugues (who was the first to report the specific adhesion of platelets to the collagen fibers).
He started his training in the department of internal medicine.
In 1967, he was a recipient of the scholarship « Claude Bernard « for his work on experimental thrombosis and he staid for one year (1967-68) in the Laboratory of haemostasis (Pr.Jacques Caen) in the department of haematology in the Hospital St Louis, Paris.

Afterwards, he completed his training of internal medicine, haematology and subsequently in nuclear medicine in ULg.
He developped quickly a consultation in thrombosis and haemostasis and carried on his clinical and laboratory activities in collaboration with different departments.
He was quickly be appointed as head of a thrombosis-haemostasis unit..

His research interests and publications were focused on :
the development of new antiaggregates, especially thienopyridines and new inhibitors of the Prostaglandins cascade ; the effects of oestrogens on the haemostatic profile ; the markers of in vivo prethrombotic states; the treatment of hemophilacs and of other bleeders.
He was appointed to teach the followers in internal and biological medicine and in intensive care, in belgian and in european inter-universities educational programs. He was invited by several pharmaceutical companies.to be consultant and investigator for several collaborative studies. He retired in 2005 but he is still appointed as consultant in the department of clinical haematology.

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EDUCATIONAL SPEAKERS

A. GADISSEUR (ANTWERP)

Professor Alain Gadisseur is a senior member of the clinical haematology department at the Antwerp University Hospital where he combines the positions of Director of the Haemophilia & von Willebrand Reference Centre with that of Director of Clinical Stem Cell Transplantation. He is Professor of Haemostasis at Antwerp University where he also holds the CSLBehring Chair in Von Willebrand Disease. His research is focused on von Willebrand Disease and, among others, he has initiated several population studies to characterise patients with von Willebrand Disease in Belgium, the Czech Republic and Slovakia with another project with London hospitals preparing to start. He is the current president of the Belgian Society on Thrombosis and Haemostasis.

Elisabetta Castoldi graduated in Biology from Ferrara University (Italy) in 1996. After obtaining her PhD on the molecular genetics of factor V under the supervision of Prof. Francesco Bernardi (2001), she moved to Maastricht University (The Netherlands) for biochemical training in Prof. Jan Rosing's laboratory. A VIDI grant from the Dutch Organisation for Scientific Research (2006) allowed her to start her own research line, which focusses on the molecular genetics and functional characterisation of inherited coagulation defects predisposing to bleeding or thrombosis.

E. CASTOLDI (MAASTRICHT)

I. DIAGNOSIS OF VON WILLEBRAND DISEASE

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ABSTRACTS EDUCATIONAL LECTURES

DIAGNOSIS OF VON WILLEBRAND DISEASE

A. GADISSEUR (ANTWERP)

Von Willebrand disease (VWD) is the most common inherited bleeding disorder, with a prevalence of symptomatic disease of 1in 10,000. Given the complexity of the disease, the ability to accurately and appropriately diagnose individuals with VWD continues to be an important and much discussed topic of interest.

VWD is traditionally classified into type 1 and type 3, which describe mild to moderate and severe quantitative deficiencies of VWF, respectively, and type 2, which is characterized by qualitative deficits in VWF. VWD affects individuals of all ethnic backgrounds, and the clinical symptoms can present at any age. Although VWD type 1 and 2 are generally inherited in an autosomal-dominant pattern, VWD type 3 is inherited in an autosomal-recessive pattern. Not all individuals that have mutations in VWF exhibit clinical symptoms, and many type 1 VWD patients do not have mutations in the VWF gene and genetic modifiers outside of the VWF gene may play significant roles in the modulation of VWF quantity, function, and multimer status.

When the diagnosis of VWD is made, the issue of classification and subtyping arises. The current ISTH classification is oversimplified and does not take into account the underlying molecular defects or pathologic mechanisms. Confirmatory tests like Factor VIII binding, multimer analysis, genetic typing are expensive and/or time consuming, and in many cases only available in specialised centres.

In this review, we highlight the current status of clinical testing and diagnostic classifications that are useful to the clinician while also underscoring the current limitations of the existing tests.

II. FUNCTIONAL INTERACTIONS BETWEEN FV AND TISSUE FACTOR PATHWAY INHIBITOR

FUNCTIONAL INTERACTIONS BETWEEN FACTOR V AND TISSUE FACTOR PATHWAY INHIBITOR

E. CASTOLDI (MAASTRICHT)

Tissue factor pathway inhibitor (TFPI) is a multivalent Kunitz-type protease inhibitor present on the endothelium, in plasma and in platelets. The plasma pool accounts for ~10% of all TFPI and consists entirely of the TFPIα isoform, which is found in the circulation with various degrees of C-terminal truncation. Only the full-length form, which circulates in complex with factor V (FV) and/or protein S, expresses full anticoagulant activity.

TFPIα is best known for its ability to inhibit tissue factor/factor VIIa and factor Xa (FXa) via its Kunitz domains 1 and 2, respectively. However, recent work has uncovered a role for full-length TFPIα in the regulation of FV activation and prothrombinase activity as well, which makes TFPIα an all-round regulator of the initiation of coagulation. In addition, protein S and FV have been shown to act as (synergistic) cofactors of TFPIα in the inhibition of FXa.

Variations in the levels of plasma TFPIα have been associated with the risk of venous thrombosis and bleeding. In particular, an ~10-fold increase in plasma TFPIα levels has been recently shown to underlie the previously unexplained East Texas and Amsterdam bleeding disorders. Interestingly, the mutations responsible for these disorders reside in the F5 gene (rather than in the TFPI gene) and act by up-reguating FV splicing isoforms (FV-short) that bind TFPIα with high affinity.

This lecture will review these novel findings with an emphasis on the functional significance of the FV-TFPIα interaction.

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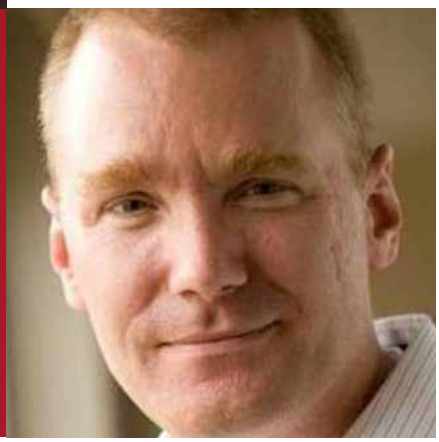
CSL BEHRING SATELLITE SYMPOSIUM SPEAKERS



A. VELDMAN (LIMBURG)

As a board certified paediatrician, neonatologist and paediatric cardiologist, Alex Veldman has more than 15 years of experience in managing critically ill newborns and children in large university hospitals. In addition, he worked in the pharmaceutical industry as Senior Director for Global Clinical R&D and later Therapeutic Area Head in clinical R&D at CSL Behring for 6 years. Currently, Alex is Head of Paediatrics at St. Vincenz Hospital in Limburg, Germany. In addition, he is holding an academic position as Associate Professor in the Faculty of Medicine, Dept. of Paediatrics at Monash University in Melbourne.

Alex Veldman is been held as an internationally reputable specialist for paediatric and neonatal critical care medicine and haemophilia. His research is focused on new therapies in haemophilia and other rare diseases in neonates and children. In 2009, Dr Veldman's group together with Professor Guenter Schwarz from Cologne, Germany, pioneered a treatment for a previously fatal metabolic disease, Molybdenum Cofactor Deficiency.

R. PRUTHI (MINNESOTA)

Rajiv K Pruthi, M.D, born in Nairobi, Kenya, obtained his Medical degree in India.

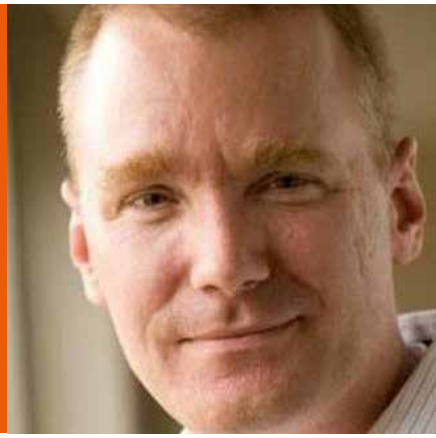
Residency in Internal Medicine in Milwaukee, WI and Hematology/Oncology Fellowship: Mayo Clinic, Rochester. Research fellowship in molecular biology (2 years) at Mayo Clinic in Molecular Biology and Genetics; mentor Dr. Steve S. Sommer, MD, PhD.

Current position: Consultant, Department of Internal Medicine and Hematology, Director Comprehensive Hemophilia Center, Co-Director Special Coagulation and Molecular Hematopathology Laboratories, Department of Laboratory Medicine and Pathology Mayo Clinic.Academic Rank: Associate Professor, Mayo Medical School.

REACH HIGH IN HAEMOPHILIA A: AFSTYLA'S TOTAL OFFERING

AFSTYLA®, rVIII-SINGLECHAIN IN HAEMOPHILIA A

A. VELDMAN (LIMBURG)



The innovative recombinant FVIII molecule with a single chain design, Afstyla® (rVIII-SingleChain), was developed to improve molecular stability and to have an improved binding to VWF[1] for treatment of haemophilia A. Once activated, Afstyla® has an amino acid sequence identical to that of endogenous FVIIIa [2]. The affinity of Afstyla® for VWF is 3-fold higher than that of full-length rFVIII [2]. Affinity, the clinical study program in 173 adult and adolescent and 84 pediatric patients with severe hemophilia A investigated the efficacy and safety of Afstyla® in previously treated patients[3, 4]. An extension study, including PUPs is ongoing (NCT02172950). The clinical study program reflects real-life clinical use, since the dose and infusion frequency could be chosen by the investigator. In both adults/adolescents and children the median AsBR was 0.0 during prophylactic treatment[3, 4]. In on demand treatment 93.5% and 96.3% of bleeds were effectively controlled with ≤ 2 infusions in adults/adolescents and children, respectively. In the surgical setting, hemostatic efficacy of Afstyla® was rated as excellent or good in 100% of surgeries[3](5). More than 1 in 3 adult or adolescent patients and 46% of pediatric patients were treated on a prophylactic regimen of 2 infusions per week (with median AsBR of 0.0).During the clinical studies, Afstyla® showed a good safety profile[3, 4].

References:

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2. Schmidbauer, S., et al., Physicochemical characterisation of rVIII-SingleChain, a novel recombinant single-chain factor VIII. Thromb Res, 2015. 136(2): p. 388-95.
3. Mahlangu, J., et al., Efficacy and safety of rVIII-SingleChain: results of a phase I/III multicenter clinical trial in severe hemophilia A. Blood, 2016.
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5. Abdul Karim F et al. rVIII-SingleChain in Surgical Prophylaxis: Efficacy and safety in 35 surgeries. Res Pract Thromb Haemost 2017;1 (suppl 1): 1-1451, PB965

R. PRUTHI (MINNESOTA)

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ABSTRACTS SATELLITE SYMPOSIUM CSL BEHRING

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SOBI SATELLITE SYMPOSIUM SPEAKERS

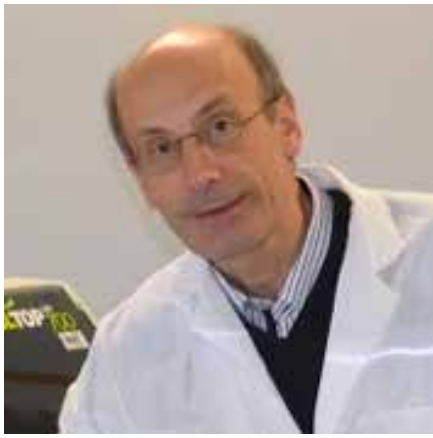
K.J. PASI (LONDON)

K John Pasi MB ChB PhD FRCP FRCPath FRCPCH, Professor of Haemostasis and Thrombosis, Barts and the London School of Medicine and Dentistry, Queen Mary University of London. Honorary Consultant Haematologist, the Royal London Hospital, Barts Health NHS Trust, London, UK.

John Pasi is Professor of Haemostasis and Thrombosis at The Royal London Hospital, Barts and the London School of Medicine and Dentistry and Haemophilia Centre Director. His clinical practice spans both adults and children and covers all aspects of haemostasis and thrombosis. His research interests cover many aspects of inherited and acquired bleeding and clotting disorders, particularly new bioengineered therapies for haemophilia, novel therapies for haemophilia including gene therapy and RNAi technologies as well as the optimization of currently available therapies. He is closely involved in the design and development of clinical trials for new therapies and evolving phase 1-4 programmes. In addition, he has a major interest in the development of robust and harmonised outcome measures for haemophilia including application to research. He has also been involved in the development of national policy and guideline formulation across a broad range of haemostatic and thrombotic disorders and the development of novel models for haematology training initiatives such as residential academies and preceptorships for both haemostasis and thrombosis. He currently chairs the London Haemophilia Clinical Advisory Group and Commissioning Forum, is a member of NHS England Clinical Reference Group (GRG) for Inherited Bleeding Disorders and leads the link between the CRG and NIHR on research.

K. PEERLINCK (LEUVEN)

Dr Kathelijne Peerlinck is professor at the center for Molecular and Vascular Biology of the KULeuven, Belgium, staff member at the division of Heart and Vascular disorders of the University Hospitals Leuven and director of the Haemophilia Comprehensive Care Center of the University Hospitals.
Dr Peerlinck received her medical degree at the University of Gent (Belgium) and specialised in Internal Medicine and Haematology in Gent (Belgium) and Leiden (The Netherlands). She obtained her PhD at the KULeuven with studies on von Willebrand's disease and Haemophilia.
Dr Peerlinck's clinical and research interests include haemophilia, with special interest in mechanisms and treatment of inhibitors in haemophilia and evaluation of treatment in patients with coagulation disorders and a special focus of management of cardiovascular disorders in these patients.



Marc Jacquemin is responsible for the Hemostasis Laboratory of the University Hospitals Gasthuisberg in Leuven. He is also carrying out research in the Center for Molecular and Vascular Biology in KULeuven. He obtained his medical degree and specialized in Clinical Biology at the Catholic University of Louvain, Belgium. He also obtained a PhD at the Catholic University of Louvain for his work on human monoclonal anti-Factor VIII antibodies. His research is focused on treatment of inhibitors in hemophilia A patients and on the mechanisms regulating FVIII activity.

M. JACQUEMIN (LEUVEN)

REAL WORLD EXPERIENCE WITH INITIATING AND CONTINUING TREATMENT WITH rFVIIIFC AND rFIXFC AND THEIR IMPACT ON LONG-TERM TREATMENT GOALS

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ABSTRACTS SATELLITE SYMPOSIUM SOBI

Clinical experience with extended half-life products in patients with hemophilia

Extended half-life factor VIII and IX products are currently being introduced into routine clinical practice in Belgium. Our current practice when switching a patient to an extended half-life product will be illustrated based on clinical case histories.
Before switching it is important to discuss with the patient opportunities, realistic expectations and possible adverse reactions. Discussions will differ markedly for EHL-factor VIII and EHL-factor IX. When switching to an EHL-factor concentrate individual pharmacokinetic data should be obtained if possible to be able to optimize the new EHL-factor concentrate regimen. After switching to an EHL-factor concentrate patients should be closely followed up.
The regimen needs to be adjusted over time based on the pattern of break-through bleeds and measured trough levels. In patients who experience no bleeding episodes after switching a reduction in dose or frequency may be considered.

EXPERIENCES WITH rFVIIIFC AND rFIXFC

K. PEERLINCK (LEUVEN)

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ABSTRACTS SATELLITE SYMPOSIUM SOBI

OPTIMIZING PK-GUIDED PERSONALIZED PROPHYLAXIS WITH rFVIIIFC AND rFIXFC

K.J. PASI (LONDON)

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Historically treaters have adopted a broad 'one size fits all' approach to prophylaxis with a standard regimes for twice to three times weekly prophylaxis for either factor VIII or factor IX deficiency to maintain a trough level greater than 1 iu/dL. However, this basic approach does not take into account the variability that we see in haemophilia. Such variability importantly includes levels of activity, individual's personal half-life and clearance of factor concentrates, personal wishes and circumstances. As such a 'one size fits' all approach will not necessarily be the optimal treatment regime for patient. Considering these issues, it is quite clear one single approach to prophylaxis cannot address the needs of all our patients with severe haemophilia optimally. This has led to the increasing view that prophylactic treatments need to be tailored or personalised to the individual bearing in mind not only personal circumstances, but also individual pharmacokinetic handling of factor concentrates. Furthermore, we can see from first principles that extended half-life therapies will not only potentially magnify such variations and can further highlighted the benefits of personalised therapy. Allied to this is the evolving concept that a trough level of 1 IU/dL is not optimal.

A common approach to addressing these issues is clinically based and relies upon changing treatment regimes in response to observed patterns bleeding clinical response to treatment. However, a more evidence-based approach to personalising treatment entails determining treatment dose and frequency based on knowing how that individual handles factor VIII and IX pharmacokinetically. Pharmacokinetic analysis can be performed two ways, using either traditional individual PK analysis using multiple time points or using a Bayesian population pharmacokinetic approach using fewer time points. An important programme development for population modelling that is not product specific has been provided by McMaster University (WAPPS, https://www.wapps-hemo.org/) which is freely accessible to participate in.

The advent of extended half-life therapies has provided still greater opportunities for both extending interval and raising trough levels. In such settings the principle of personalised approach, allows us to increasingly achieve and maximise the benefits of the new generation therapy. Individualising therapy and personalising the approach using PK clearly is a rational way forward for the effective management of prophylactic regimes for people with severe haemophilia. By closely involving our patients we will be able to achieve for them a better outcome and improved patient concordance with the treatment regimes. Our experience has taught us that 'one-size fits' all approach is clearly not ideal and that in practical terms by personalising prophylaxis using PK guided dosing with extended half-life therapies we can see both considerable improvements in the quality of care that we provide for our patients as well as being cost-effective.

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ABSTRACTS SATELLITE SYMPOSIUM SOBI

ASSAYS AND REAGENTS FOR THE MEASUREMENT OF MODIFIED FVIII AND FIX WITH EXTENDED HALF-LIVES, THE BELGIAN SITUATION

M. JACQUEMIN (LEUVEN)

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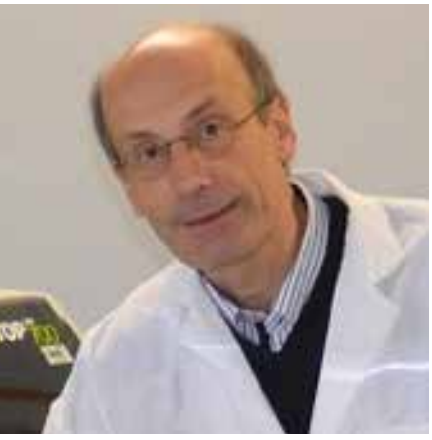
The monitoring of treatment with modified FVIII and FIX with extended half-lives is important to ensure patients' safety.

However, the measurement of the activity of these novel products represents a challenge for the laboratories carrying out the assays and for the clinicians analysing the data. Indeed, major differences between the results generated with chromogenic methods and one-stage clotting assays based on activated partial thromboplastin time have been reported. In additions, the results generated with different activators of the one-stage clotting assay may also differ widely.

Numerous international studies have therefore been organized to identify the appropriate methods for monitoring patients receiving the novel concentrates. Studies carried out by the Belgian Institute of Public Health also contributed to the evaluation of the different factor assays available in the country.

Based on these data, we made an inventory of the interactions between all the factor assay reagents available in Belgium and the FVIII and FIX with extended half-lives. According to an international consensus, an assay was considered as appropriate to measure a given product when it provides results diverging by no more than 30% of the target value defined either on the basis of the labelled potency of the concentrate or determined with the assay(s) used during the clinical studies which demonstrated the clinical efficacy of the molecule.

The international and Belgian studies focussed on rFVIIIFc and rFIXFc have also been reviewed in details. With the exception of kaolin based-assays, which are not recommended for the measurement of rFIXFc, all the assays used in Belgium provide acceptable values of rFVIIIFc and rFIXFc in the normal range, allowing an adequate monitoring of the patients treated with these products.



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SHIRE SATELLITE SYMPOSIUM SPEAKERS

P. TURECEK (VIENNA)

Hon.Prof (FH) Univ.-Doz. Dr. Peter Turecek, Senior Director, Global Medical Affairs,Scientific and Technical Expert, Hematology, Baxalta Innovations GmbH, part of Shire. Since joining R&D in the pharmaceutical industry in 1989, Dr Turecek has held management positions in Immuno, ÖIH, Baxter, Baxalta and Shire, in areas of vaccines, recombinant proteins, downstream processing, plasma fractionation, as well as diagnostics and preclinical product characterization. His work resulted in 815 patents in 75 patent families, and in more than 130 peer-reviewed papers and book articles. Dr Turecek holds a doctorate in biochemistry, a magister pharmaciae degree, and a diploma in business administration. He is an Associate Professor at the University of Vienna. There and at other universities he lectures as guest professor of pharmacology and toxicology, pharmaceutical- and protein-biotechnology, and quality and regulatory affairs issues of the biopharmaceutical industry. Dr Turecek's fields of expertise in pharmaceutical biotechnology in general include in particular blood derivatives, plasma products, down-stream processing, protein purification and characterization, pathogen inactivation, and protein-modification and -engineering. He represents biopharma to international regulatory authorities and to scientific and public organizations and is a frequent speaker at international scientific and academic conferences. He is chairman of the Standards Committee "Haemostaseology" of DIN and of the Blood Products Committee of BIOSafe Special Biologics Expert Working Group, and is assigned by the Federal Republic of Austria as a permanent specialist to Group of Experts Nr. 6B on Human Plasma and Plasma Products and Nr. 6 on Biological and Biotechnological Products in the European Pharmacopoeia.

M. CANARO (PALMA DE MALLORCA)

Dr. Mariana Isabel Canaro Hirnyk, MD
Hemostasia y Trombosis, Servicio de Hematologia
Hospital Universitario Son Espases, Palma de Mallorca, Spain

Dr. Mariana Canaro is a specialist physician for the Hematology and Hemotherapy Department of the Son Espases Hospital (Palma de Mallorca, Spain). She has been practicing medicine for 30 years and has a wide experience as a hemophilia treater. Dr. Canaro is author of more than 20 peer reviewed papers and communications to congresses. She has participated in many clinical trials as a principal investigator. Also she collaborates with national and regional hemophilia patients organizations.

P. KNÖBL (VIENNA)

Prof. Paul N. Knöbl, MD, Medical University of Vienna, Department of Medicine 1, Div. Hematology and Hemostasis. Professor Paul Knöbl is Professor of Medicine (MD) and Senior Physician at the Medical University of Vienna Division of Hematology and Hemostasis. He is Specialized in Internal Medicine, Hemato-Oncology and Intensive Care Medicine. His Scientific work includes blood coagulation, fibrinolysis, endothelial cell function, hemophilia, hematologic malignancies, TTP. Professor Knöbl has been involved in more than 170 publications of scientific articles and book chapters. He has been invited for multiple invited lectures and lots of oral presentations on national and international scientific meetings. He is Member of the numerous Societies among which the International Society on Thrombosis and Hemostasis, the Society of Critical Care Medicine Austrian Society of Internal Medicine, the Austrian Society of Hematology... His experience in clinical research is considerable and include multiple domains asAnti TFPI for Hemophilia, Recombinant ADAMTS13 for congenital TTP, leukemia.

PK-GUIDED DOSE TAILORING TO PERSONALIZED CARE: USE IN REAL LIFE

M. CANARO
(PALMA DE MALLORCA)

The administration of factor VIII is the optimal treatment for patients with severe haemophilia A to prevent life-threatening bleeding, progressive joint damage and chronic pain. It also improves activity, school attendance and quality of life. Unfortunately, despite prophylaxis breakthrough bleeding still occurs. Even in clinical trials, >50% of patients on prophylaxis failed to achieve an annual bleeding rate (ABR) of zero.

The personalized approach to haemophilia treatment is based on the fact that each patient is unique, and it is currently the best alternative therapy to achieve “zero” bleeds, preserve normal joint status and enjoy a quality of life (QoL) comparable to healthy subjects.

Prophylaxis is expensive, and the optimal dose will depend on the individual patient characteristics (weight, body mass index, bleeding phenotype, activity, lifestyle, joint health, and treatment adherence) but also on the treatment attributes (efficacy, safety, dosing intervals, peak and trough levels, time spent at specific levels, and population pharmacokinetics).

Pharmacokinetics (PK) is highly variable between patients and changes with age, the more time spent with FVIII levels < 1%, the higher the risk of bleeding. Conventional measurement of PK (ISTH guidelines) requires 8 plasma samples collected at 0, 30‘, 60’, 3h, 6h, 12h, 24h, 48h after FVIII infusion. Therefore, this method is very difficult to perform in the haemophilia patient population.

Online PK medical devices create individual PK profiles using models to estimate PK curves and tailor prophylaxis in patients treated with human coagulation factor VIII. This allows making individual PK available to a larger number of haemophilia patients.

Which FVIII levels are important? In PK-curves, area under the curve (AUC), peak and trough levels are important. The use of the graphs provided by a PK online medical device as an educational tool has contributed to increase adherence in some patients of our Unit.

Conclusion: Personalization of care involves many aspects including tailored prophylaxis, joint health, treatment goals, patient’s preferences, lifestyle and activity adjustment, psychosocial support and treatment adherence.

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ABSTRACTS SATELLITE SYMPOSIUM SHIRE

EXTENDED HALF-LIFE PRODUCTS IN HEMOPHILIA A THE PRESENT AND THE FUTURE OF A NEW TREATMENT ERA

P. TURECEK (VIENNA)

The objectives of this presentation is to overview the new information on personalized care with extended half-life products for haemophilia A. Key clinical data and real world experience with BAX 855 will be communicated.
Definition of extended half-life products for haemophilia A and communication on the benefits of the new class of extended half-life products will be reviewed.

rpFVIII, A NEW APPROACH FOR THE TREATMENT OF PATIENTS WITH ACQUIRED HEMOPHILIA

P. KNÖBL (VIENNA)

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Acquired hemophilia A (AHA) is an autoimmune disease, caused by antibodies blocking the function of clotting factor VIII. The incidence is 1.5/million/year. In half of the patients an underlying disorder (malignancy, pregnancy, drugs, other autoimmune diseases) is associated with AHA. New onset of bleeding (skin hematomas, deep muscle or retroperitoneal hematomas, mucosal bleeding) often leads to further investigations and the diagnosis of AHA. A prolongation of the APTT, without correction in mixing studies, and low FVIII levels confirm the diagnosis. Bleeding is severe in most of the patients and hemostatic therapy is necessary. As factor VIII replacement is insufficient at high inhibitor titers, bypassing agents (recombinant factor VIIa, activated prothrombin complex concentrates) are necessary to stop bleeding. Extracorporeal immunoadsorption may help to lower the inhibitor titer below that threshold. Although spontaneous remissions can occur, immunosuppression (steroids, cyclophosphamides, rituximab) is necessary to eradicate the inhibitor and improve survival, but may cause severe side effects.

Obizur® is a recombinant, porcine-sequence B-domain deleted FVIII, approved as hemostatic therapy of adult patients with acquired hemophilia. As it's antigen structure is different from human FVIII it may escape inhibition by the autoantibodies, although some cross-reactivity may occur. Dosing can be monitored with conventional one-stage FVIII clotting assays. The available clinical data suggest that in 100% of patients with anti-porcine FVIII titers <20 BU/ml responded to repetitive high doses of Obizur® with high FVIII levels, and bleeding was controlled in 24 of 28 patients.

In conclusion, Obizur® represents a valuable new method to control bleeding in patients with acquired hemophilia A. In contrast to bypassing agents, it can be dosed with conventional FVIII assays.

CSL Behring

LEO

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ORAL PRESENTATIONS: CLINICAL & LABORATORY

1. Inhibiting ADAMTS13 activity prevents the loss of high molecular weight VWF multimers in an in vitro left ventricular assist device

2. Increased Acetyl-CoA carboxylase phosphorylation state in platelets identify high risk patients with coronary artery disease: ACCTHEROMA trial

3. The interaction of factor V and tissue factor pathway inhibitor in a myeloma patient with acquired factor V deficiency

4. Reversal of a Dabigatran overload with Idarucizumab: 5 mg may be a very insufficient dose

5. Cryopreservation impairs platelet function but generates procoagulant platelets

A-130 | **INHIBITING ADAMTS13 ACTIVITY PREVENTS THE LOSS OF HIGH MOLECULAR WEIGHT VWF MULTIMERS IN AN IN VITRO LEFT VENTRICULAR ASSIST DEVICE**

S. DECONINCK*[1], A. RAUCH[2,3,4,5], E. ROOSE[1], N. VANDEPUTTE[1], C. TERSTEEG[1], H. DECKMYN[1], S.F. DE MEYER[1], S. SUSEN[4,5], K. VANHOORELBEKE[1]

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ORAL PRESENTATIONS: CLINICAL & LABORATORY

[1]KU Leuven Campus Kulak Kortrijk, Laboratory for Thrombosis Research, IRF Life Sciences, Kortrijk, Belgium,
[2]INSERM Unit 770, Le Kremlin-Bicêtre, France,
[3]UMR_S 770, Univ Paris-Sud, Le Kremlin-Bicêtre, France,
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BACKGROUND: Gastrointestinal (GI) bleedings are one of the leading adverse events of left ventricular assist device (LVAD) therapy and have been associated with acquired von Willebrand syndrome (aVWS). aVWS is characterized by a loss of high molecular weight (HMW) von Willebrand factor (VWF) multimers and hence loss of their haemostatic function. Remarkably, within days after LVAD removal, the loss of HMW VWF multimers is restored and bleeding episodes disappear. The loss of HMW VWF multimers might be explained by an increased shear-induced proteolysis of VWF by ADAMTS13 (A Disintegrin And Metalloprotease with ThromboSpondin type 1 repeats, member 13) due to the high pathological shear stress occurring in the LVAD. Consequently, blocking ADAMTS13 would be an attractive way to rescue the loss of HMW VWF multimers in LVAD patients.

AIM: To investigate whether blocking ADAMTS13 prevents the loss of HMW VWF multimers, thereby preserving VWF size and activity, in an in vitro LVAD circuit.

METHODS: Using an in vitro HeartMate II™ circuit with citrate-anticoagulated human blood, ADAMTS13 mediated VWF proteolysis was studied in the presence of the inhibitory anti-ADAMTS13 monoclonal antibody 3H9 at three different concentrations (20, 4 and 1 µg/mL). A non-functional anti-ADAMTS13 antibody was used as a control. Blood samples were taken 5 minutes before and 5, 30 and 180 minutes after the onset of perfusion in the LVAD system. The plasma samples were analysed for VWF multimers using SDS agarose gel electrophoresis and VWF collagen binding activity (VWF:CB) and VWF ristocetin cofactor activity (VWF:GPIbR) were determined using ELISA.

RESULTS: In the in vitro HeartMate IITM circuit, a significant decrease in HMW VWF multimers was observed in the presence of the control antibody (20 µg/mL, not inhibiting ADAMTS13 function). HMW VWF multimers decreased (12.7 ± 5.7%, 180 minutes after the start of perfusion compared to 41.9 ± 1.7% HMW VWF multimers before perfusion, p=0.01) and this was reflected by a significantly decreased VWF:CB/VWF:Ag ratio (0.6 ± 0.1%, p=0.03) but not by a decrease in the VWF:GPIbR/VWF:Ag ratio (0.9 ± 0.1%). As expected, The loss of HMW VWF multimers was also observed when lower concentrations (4 and 1 µg/mL) of the control antibody were used. Interestingly, blocking ADAMTS13 activity using the anti-ADAMTS13 antibody 3H9 at 20 µg/mL prevented the loss of HMW VWF multimers: HMW VWF multimers were 31.0 ± 6.5%, 180 minutes after the start of perfusion compared to 36.3 ± 1.8% HMW VWF multimers before perfusion. The preservation of HMW VWF multimers was also reflected by the normal VWF:CB/VWF Ag (0.9 ± 0.2%) and VWF:GPIbR/VWF:Ag ratios (1.0 ± 0.2%), 180 minutes after the start of perfusion. Notably, there was also no significant loss of HMW VWF multimers at 180 minutes after the start of perfusion when using 4 µg/mL of the inhibitory antibody (32.7 ± 1.9% compared to 40.8 ± 3.7% before perfusion), but the decrease could not be prevented when 1 µg/mL of antibody 3H9 was used.

CONCLUSIONS: Using an inhibitory anti-ADAMTS13 antibody, we were able to prevent the loss of HMW VWF multimers in an in vitro HeartMate II™ circuit in a dose-dependent manner. Our data unequivocally show that the loss of HMW VWF multimers is due to shear-induced ADAMTS13 digestion. Hence, blocking ADAMTS13 function might be an effective way to treat aVWS in LVAD patients.

A-135 | **INCREASED ACETYL-COA CARBOXYLASE PHOSPHORYLATION STATE IN PLATELETS IDENTIFY HIGH RISK PATIENTS WITH CORONARY ARTERY DISEASE: ACCTHEROMA TRIAL**

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ORAL PRESENTATIONS: CLINICAL & LABORATORY

INTRODUCTION: Thrombin, and to a lesser extent collagen, is the major platelet agonist leading to Acetyl-CoA Carboxylase (ACC) phosphorylation, a genuine substrate of the AMP-activated protein kinase. In the context of atherothrombotic coronary artery disease (CAD) associated with thrombin generation, platelet P-ACC can be an interesting marker of thrombin-induced platelet activation. The role of ACC, a key enzyme involved in de novo fatty acid biosynthesis pathway, in regulating intraplatelet lipid metabolism in CAD patients, remains unexplored.

AIMS: In the present study, we investigated platelet ACC phosphorylation as a risk marker for patients with CAD and its consequences on intraplatelet lipid metabolism (ACCTHEROMA, NCT03034148)

METHODS: A total of 188 consecutive patients (65±12 years) admitted for coronary angiogram were included from March 2015 to February 2016. Based on clinical presentation and angiographic data, patients were classified into four groups (non-CAD, non-significant CAD, stable CAD and acute coronary syndrome). Blood samples were drawn immediately after sheath insertion at the cath lab. Samples were directly processed for platelets isolation and protein extracts were analyzed by immunoblotting. In order to characterize the impact of ACC phosphorylation on intraplatelet lipid metabolism, we performed a lipidomic study by infusion-tandem mass spectrometry (DI-MS/MS).

RESULTS: In consecutive patients admitted for coronary angiogram, platelet P-ACC was clearly increased in patients with demonstrated CAD [median (IQR): 0.48 (0.29-0.73) vs. 0.22 (0.11-0.45), p <0.001)] and was highly associated with acute coronary syndrome (ACS) (OR: 6.71, 95% CI: 2.06 - 21.91, p = 0.002). The addition of platelet P-ACC to other established markers (HsCRP, D dimer) improved the predictive value for ACS (NRI: 0.49, 95%CI: 0.17-0.80, p = 0.002; IDI: 0.06, 95%CI: 0.02-0.10, p = 0.002). In contrast to our initial hypothesis, we observed a poor correlation between thrombin generation markers and platelet P-ACC. However, we identified oxidized low-density lipoprotein as another potential factor accounting for platelet ACC phosphorylation. The lipidomic study revealed that ACC might be involved in the regulation intraplatelet triglycerides in CAD patients.

CONCLUSIONS: Platelet P-ACC is a potential marker for early identification of high-risk patients with CAD. P-ACC may contribute to the regulation of lipid metabolism in platelets of CAD patients with dyslipidemia.

A-104 | **THE INTERACTION OF FACTOR V AND TISSUE FACTOR PATHWAY INHIBITOR IN A MYELOMA PATIENT WITH ACQUIRED FACTOR V DEFICIENCY**

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BACKGROUND / INTRODUCTION: Case report - A 45-year-old woman with multiple myeloma was admitted to the hematology department due to progression of the disease under treatment with carfilzomib and lenalidomide. A prolongation of the activated partial thromboplastin time (aPTT; patient 50.4 seconds; normal range 28-39 seconds) and – shortly afterwards – of the prothrombin time (PT; patient 15.2 seconds; normal range <13 seconds) was noticed. PT and aPTT mixing studies indicated a coagulation factor deficiency and no inhibitor could be detected with the Bethesda assay. A factor V (FV) deficiency was established with a one-stage clotting assay (0.4%; normal range 70-120%) and confirmed with a prothrombinase-based assay in 1:1000-diluted plasma. In the following days the FV level increased again to 7.5% (day 11), 31% (day 18), 64% (day 24) and 128% (day32) respectively. Since FV acts a carrier of the anticoagulant protein tissue factor pathway inhibitor α (TFPIα) in plasma, we also measured TFPIα levels, which increased in parallel to FV from 25% (day 8) to 133% (day 32). In fact, the FV and TFPIα levels showed an excellent correlation (R^2 = 0.984). At no point the patient showed any signs of bleeding. She received several units of fresh frozen plasma and reached complete remission.

RESULTS: The cause of the transient FV deficiency could not be identified. Thrombin generation was measured using Calibrated Automated Thrombinography in the samples taken on different days to evaluate the effects of the acquired FV deficiency on the overall coagulation function. Coagulation was initiated either with a low tissue factor (TF) concentration (1.5 pM) ± neutralizing antibodies against TFPIα or with a high TF concentration (10 pM) ± activated protein C (APC), to optimally monitor both the pro- and anticoagulant pathways. Thrombin generation in the patient's plasma was not measurable at the moment of complete FV deficiency, but rapidly increased in the following days as the FV level increased, reaching a maximum on day 18. Afterwards, thrombin generation decreased again despite the steady increase in FV level.
These findings can be explained by the co-variation between the patient's FV (procoagulant) and TFPIα (anticoagulant) levels over time. When FV was at its lowest levels (0.4% and 7.5%), the hypocoagulable state was the predominant defect and could not be effectively counteracted by the low TFPIα level, as demonstrated by the absent or markedly delayed thrombin generation. At this point, the patient might have been exposed to a bleeding risk. In contrast, when FV had reached a sufficient level (31%) to guarantee maximal prothrombinase activity, the TFPIα level (40%) was still suboptimal, resulting in a hypercoagulable state and possibly an increased risk of thrombosis. This was demonstrated by the elevated thrombin generation (especially at low TF and at high TF with APC) as compared to the normal pool. The imbalance between pro- and anticoagulant pathways was resolved at day 32, when FV and TFPIα had returned to their normal levels, yielding similar thrombin generation as in the normal pool under all experimental conditions.

SUMMARY / CONCLUSIONS: We illustrated the interplay of FV and TFPI in a patient with acquired FV deficiency and showed that the thrombin generation assay, performed under different conditions, can be useful to clarify the complicated mechanisms behind acquired factor deficiencies and their effects on the haemostatic balance.

A-127 | **REVERSAL OF A DABIGATRAN OVERLOAD WITH IDARUCIZUMAB: 5 MG MAY BE A VERY INSUFFICIENT DOSE**

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We describe the story of a patient admitted to the emergency department in July 2016 with abdominal pain, bradycardia (54 beats/min) and hypotension (108/41 mm Hg). The patient was receiving Dabigatran Etexilate (150 mg b.i.d.) for atrial fibrillation.

The abdominal CT scan showed an occlusion of the celiac trunk. Arteriography confirmed the occlusion with a chronic aspect but a preserved gut vascularization by the other vessels (superior mesenteric artery).

Coagulation parameters were consistent with a Dabigatran overload (Dabigatran dilute Thrombin Time: 657 ng/ml, Quick PT 17%, APTT 2.6 ratio, Thrombin Time > 200 sec). Laboratory tests also showed a renal insufficiency (Cockcroft-Gault 23 mL/min). Very surprisingly, injection of 5 mg Idarucizumab (out of a total hospital allocation of 7.5 mg) did not improve coagulation parameters, which became even worse (table).

The diagnosis of Dabigatran overload was challenged but the presence of a powerful anti-IIa activity was quickly confirmed by an Ecarin Chromogenic Assay (STA®-ECA II, kit kindly provided by Stago), which Dabigatran level agreed with those of dTT test. Other tests ruled out paraneoplastic interferences.

The patient was treated by fluids, antibiotics (tazobactam and amikacin) and norepinephrine due to the circulatory shock. Despite this treatment, and given the unfavorable course of circulatory shock and persistent abdominal pain, a decision for surgical intervention was taken.

Forty hours after admission, and without improvement of coagulation parameters, a left colectomy was performed after injection of 3000 U PCC (35 U/kg, GIHP propositions) without major bleeding. Renal failure and coagulation parameters gradually improved a few days after surgery, as well as the clinical condition of the patient. She left ICU on the 13th day after admission.

Understanding was given by measurement of total Dabigatran by LC - MS/MS that indicated a massive apparition of Idarucizumab-bounded Dabigatran. An in vitro test suggested that a second dose of Idarucizumab would have been sufficient to reverse all the Dabigatran present in patient's blood. Blood coagulation parameters worsening may be due to extravascular Dabigatran re-entering the circulation.

The necessity of a second 5 mg dose has been described in the full cohort analysis of the REVERSE AD study (Pollack 2017), but it seems that it is the first description of a case with an apparent total ineffectiveness of the first dose. This also demonstrates that a strict monitoring of hemostasis parameters is mandatory in the management of a patient with Dabigatran overload and surgical indication or bleeding. In this case, it is necessary to prepare for a possible immediate second dose.

	Quick %	APTT sec	Thrombin time sec	Dabigatran dTT ng/ml	Dabigatran LC-MS/MS ng/ml
Before Idarucizumab	17	87	>200	1070	1514
After 2.5 mg	6	>200	>200	1169	5804
After 5.0 mg	10	141	>200	1024	7837
Before surgery	15	155	>200	1194	1946
48 h after surgery	59	69	184	186	251

A-132 | **CRYOPRESERVATION IMPAIRS PLATELET FUNCTION BUT GENERATES PROCOAGULANT PLATELETS**

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BACKGROUND: Increasing demand and limited shelf-life of platelet concentrates (PC) puts pressure on stock to meet transfusion needs. Cryopreservation of PC could solve this issue especially in a logistically difficult context like rural regions, major disaster or on the battlefield. In addition, cryopreservation of autologous PC may become crucial for rare blood types or severely immunocompromised patients.

AIM: Cryopreserved PC from three independent blood services (USA, Australia (AUS) & Belgium (BEL)) were directly compared to assess variability. Furthermore, a paired comparison of cryopreserved vs control non-cryopreserved platelets was performed to detect quality differences. All experiments were conducted in vitro.

METHODS: Cryoprotection was by addition of 27% (v/v) dimethylsulfoxide (DMSO) in saline to PC until a final concentration of 6% DMSO was reached. Platelets were hyperconcentrated through centrifugation, frozen at -80 °C and thawed at 37 °C. Resuspension after thawing differed by blood service; AUS and BEL used ABO-matched plasma, USA used 0.9% NaCl. Functional investigation of the platelets included flow cytometry, light transmittance aggregometry and microfluidic flow chambers with anticoagulated reconstituted blood onto collagen (Cg). Procoagulation of the platelets was investigated by thrombin generation assay (TGA), rotational thromboelastography (ROTEM) and microfluidic flow chambers with recalcified reconstituted blood onto Cg or onto Cg with tissue factor (TF) in the presence of corn trypsin inhibitor.

RESULTS: In all cases, cryopreservation affected platelet function but to different degrees depending on the production site. Activation of integrin $\alpha_{IIb}\beta 3$ by collagen-related peptide was 15 to 22-fold decreased following cryopreservation, depending on the production site. Similar decreases were found for aggregation in response to a mix of ADP, epinephrine and thrombin with amplitudes reduced from 85% for control non-cryopreserved PC to 1%, 16% and 22% for cryopreserved PC from AUS, USA and BEL, respectively. Thrombus growth rate of anticoagulated reconstituted whole blood was decreased in Cg-coated flow chambers from 0.2 s-1 for control versus 0.005 s-1, 0.06 s-1 and 0.06 s-1 for cryopreserved samples from AUS, USA and BEL, respectively. Similar results were found with recalcified reconstituted blood in both Cg and Cg plus TF-coated flow chambers, yielding slower platelet thrombus as well as fibrin formation. Despite this damage, onset of coagulation was not affected. In ROTEM or TGA onset of coagulation was even faster by 1.5 to 2 fold indicating a procoagulant role of cryopreserved platelets. This may be caused by a 15% to 25% increase in the number of microparticles and/or the increased expression of phosphatidylserine from 3% to 70% in control versus cryopreserved PC, respectively.

CONCLUSION: The impact of cryopreservation on platelet function differs between production sites suggesting that procedural standardization between facilities is required. Cryopreservation impairs platelet function in classical platelet tests while thrombin generation assays and thromboelastography showed accelerated clot initiation. The flow chamber studies did not indicate excessive platelet aggregation or clot formation after cryopreservation.

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ORAL PRESENTATIONS: BASIC RESEARCH

1. An open conformation of ADAMTS13 is a hallmark of acute acquired thrombotic thrombocytopenic purpura

2. P2X1 ion channel regulates neutrophil NETosis in colitis

3. Unraveling anti-spacer immunoprofiles of acquired TTP patients using anti-idiotypic antibodies

4. Plasma of healthy donors contains specific anti-idiotypic antibodies that recognize two cloned human anti-ADAMTS13 autoantibodies

5. Phosphorylation of acetyl-CoA carboxylase by AMPK in platelets controls thromboxane generation, dense granules secretion and thrombus formation.

A-140 | **AN OPEN CONFORMATION OF ADAMTS13 IS A HALLMARK OF ACUTE ACQUIRED THROMBOTIC THROMBOCYTOPENIC PURPURA**

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BACKGROUND: Autoantibodies targeting ADAMTS13 cause acquired thrombotic thrombocytopenic purpura (TTP). The conformation of ADAMTS13 is folded via interaction between its spacer and CUB domains. Disruption of this spacer-CUB interaction by addition of von Willebrand factor or an activating anti-CUB1 antibody 17G2 changes the structure of ADAMTS13 to an open conformation, resulting in exposure of previously cryptic epitopes.

AIM: Determine if the conformation of ADAMTS13 is altered and cryptic epitopes are exposed in acquired TTP patients compared to healthy donors.

METHODS: Murine monoclonal antibodies recognizing cryptic epitopes in the spacer domain of ADAMTS13 (hence recognizing ADAMTS13 with an open conformation) were selected via ELISA. The conformation of ADAMTS13 in healthy donors (n=40), acquired TTP patients (acute (n=63) and remission (n=36)), sepsis (n=63) and hemolytic uremic syndrome (HUS) patients (n=12) was determined via ELISA, in which plasma was added to the antibody 1C4 that recognizes a cryptic epitope in ADAMTS13. As a control, plasma was also incubated with the activating anti-CUB antibody 17G2 which opens the conformation of ADAMTS13 and allows binding to the antibody 1C4 recognizing a cryptic epitope.

RESULTS: We generated and characterized a murine monoclonal anti-spacer domain antibody (1C4) as an antibody that specifically recognizes a cryptic epitope in ADAMTS13. While antibody 1C4 could readily capture MDTCS (the N-terminal part of ADAMTS13), 1C4 only captured full length ADAMTS13 when its conformation was changed by addition of the activating anti-CUB antibody 17G2. Interestingly, ADAMTS13 in healthy donors adopts a folded conformation where the spacer and CUB domains interact and the epitope of 1C4 is inaccessible. However, changing the conformation of ADAMTS13 in healthy donors by addition of activating anti-CUB antibody 17G2 did expose the cryptic epitope of 1C4. As expected, similar results were obtained for sepsis and HUS patients. Intriguingly, the conformation of ADAMTS13 is altered in acute acquired TTP patients as the cryptic epitope of 1C4 was readily available. Furthermore, in 78% of the acquired TTP patients in remission, ADAMTS13 did no longer bind to the antibody 1C4, showing that this open conformation is specific for the acute phase.

CONCLUSION: The conformation of ADAMTS13 in acute acquired TTP patients is open compared to the one in healthy donors, acquired TTP patients in remission, HUS and sepsis patients. Exposure of cryptic epitopes in the spacer domain might induce immune responses and explain autoantibody development in acquired TTP. What changes the ADAMTS13 conformation during an acute TTP episode is now currently under investigation.

A-126 | **P2X1 ION CHANNEL REGULATES NEUTROPHIL NETOSIS IN COLITIS**

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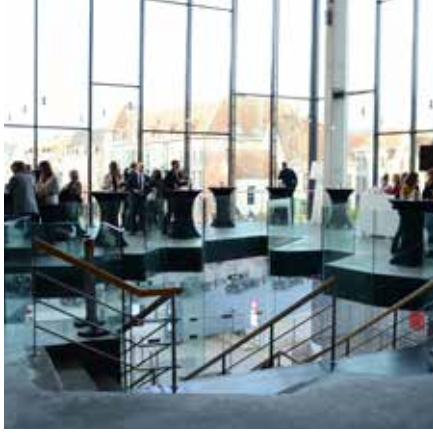

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BACKGROUND/INTRODUCTION: Thrombosis is an extra-intestinal complication that can significantly contribute to morbidity and mortality of patients with inflammatory bowel diseases (IBD). Because pathogenesis of thrombosis in IBD patients is not fully elucidated, and these patients are also at high risk of major gastrointestinal bleeding, thromboprophylaxis management of these conditions remains a challenge.
Neutrophil extracellular traps (NETs) are detected in intestinal biopsies of IBD patients, and it has been reported that neutrophils from these patients release more NETs than neutrophils from healthy individuals. NETs might play an important role both in colitis-associated bleeding and in thrombosis. NETs indeed promote coagulation and thrombus formation. They may also contribute to small vessel vasculitis that can result in tissue damage and subsequent hemorrhages. Our previous studies indicated that, in addition to its role in platelet activation, P2X1 ion channel also contributes to the regulation of neutrophil function.

AIMS: To investigate the mechanisms governing neutrophil function in intestinal inflammation with the ultimate goal to identify new therapeutic targets or strategies to prevent thrombosis and/or bleeding in IBD patients.

METHODS/MATERIALS: We used a mouse model of IBD induced by oral administration of dextran sodium sulfate (DSS) for 7 days. The effects of platelet- or neutrophil-depleting antibodies, and of P2X1 deficiency were compared on the following parameters: disease activity index (diarrhea, bleeding, weight loss), differential blood cell counts, and hematocrit. Histopathology and immunohistochemistry analyses were performed. Plasma and colonic levels of cytokines were analysed by multiplex assays. NET formation by peripheral blood neutrophils was evaluated ex vivo, and in vivo, by measuring plasma levels of pentraxin-3 (PTX-3). Extra-intestinal thrombosis was assessed using a laser-induced injury model of thrombosis in cremaster muscle arterioles. Accumulation of platelets and neutrophils were recorded in real-time.

RESULTS: P2X1-deficient mice showed more intestinal bleeding than wild-type mice, which resulted in anemia after 7 days of DSS treatment. Although platelet counts remained unchanged, P2X1-deficient mice displayed a significant increase of mean platelet volume, typical of younger and more reactive platelets. The drop in hematocrit was inversely correlated with levels of neutrophil infiltration in colon. Consistent with a role for neutrophils in bleeding, neutrophil depletion limited bleeding in wild-type and P2X1-deficient mice.
Interestingly, G-CSF and PTX-3 plasma levels were more elevated in P2X1-deficient mice than in wild-type mice, while levels of IL-6, IL-1-β and TNF-α increased similarly in the two mouse groups. Plasma PTX-3 levels augmented with colitis severity, suggesting a role of NETs in the pathogenesis of colitis. Strikingly, under colitis conditions, P2X1 deficiency promoted thrombosis in laser-injured arterioles, and neutrophils isolated from these mice showed enhanced spontaneous NETosis. Finally, injection of G-CSF (filgrastim, 10µg/day) in DSS-treated wild-type mice stimulated neutrophil production, and exacerbated colitis-associated bleeding.

SUMMARY/CONCLUSION: In colitis, P2X1 deficiency increases G-CSF plasma levels, which may cause mobilization of neutrophils predisposed to NETosis, thereby enhancing intestinal bleeding and extra-intestinal thrombosis.

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A-129 | UNRAVELING ANTI-SPACER IMMUNOPROFILES OF ACQUIRED TTP PATIENTS USING ANTI-IDIOTYPIC ANTIBODIES

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BACKGROUND: Patients suffering from acquired thrombotic thrombocytopenic purpura (aTTP) present with a polyclonal anti-ADAMTS13 autoantibody response with clear immunodominant epitopes in the ADAMTS13 spacer domain. A detailed analysis of immunoprofiles in aTTP patients is not available yet. However, insight into immunoprofiles of patients with other autoimmune disorders such as myasthenia gravis, provided crucial diagnostic and prognostic information.

AIM: In the current study, we investigated whether murine anti-idiotypic antibodies can be used to identify immunoprofiles in aTTP patients.

METHODS: We selected three cloned human anti-spacer autoantibodies to generate anti-idiotypic antibodies in mice. The three cloned anti-spacer autoantibodies (autoantibody 1, 2 and 3) each had different characteristics with either a strong, weak or no inhibitory effect on ADAMTS13 function and they had no or only partially overlapping epitopes. Mice were separately injected with each cloned anti-spacer autoantibody to generate monoclonal antibodies. Next, anti-idiotypic antibodies were selected based on their ability to specifically block the binding of the anti-spacer autoantibodies 1, 2 or 3 to ADAMTS13. Finally, anti-spacer immunoprofiles of 99 acute idiopathic aTTP patients were determined by detecting which of the three anti-idiotypic antibodies could capture their respective anti-spacer autoantibodies from patient plasma.

RESULTS: Murine antibodies against all 3 cloned human anti-spacer autoantibodies could be produced. We generated 2, 16 and 2 hybridoma clones producing antibodies against anti-spacer autoantibody 1, 2 and 3 respectively. Using the competition assay, we identified anti-idiotypic antibodies for each of the anti-spacer antibodies. Screening patient plasma on the 3 anti-idiotypic antibodies revealed that anti-spacer autoantibody 1 was present in 41%, anti-spacer autoantibody 2 in 51% and anti-spacer autoantibody 3 in 27% of the patients. Next, specific anti-spacer immunoprofiles were established based on the presence of different combinations of anti-spacer autoantibody 1, 2 or 3 in the plasma of the patients. Anti-spacer immunoprofile 1 was present in 6%, profile 2 in 15%, profile 3 in 3%, profile 4 in 16%, profile 5 in 5%, profile 6 in 5%, profile 7 in 14% and profile 8 in 35% of the patients (Table 1). Immunoprofile 2 (anti-spacer autoantibody 2), 4 (anti-spacer autoantibody 1 and 2), 7 (anti-spacer autoantibody 1, 2 and 3) and 8 (none of the anti-spacer autoantibodies 1, 2 or 3) were represented the most.

anti-spacer immunoprofile	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
anti-spacer antibody	1	2	3	1 & 2	1 & 3	2 & 3	1 & 2 & 3	/
% patients	6	15	3	16	5	5	14	35

Table 1: Distribution of the different anti-spacer immunoprofiles in aTTP patients

CONCLUSION: We show that anti-idiotypic antibodies can be used to capture anti-spacer autoantibodies from patients' plasma. Additionally, we have determined specific anti-spacer immunoprofiles in aTTP patients. We will now determine whether these specific immunoprofiles allow the identification of prognostic factors to predict death and disease recurrence in aTTP patients.

A-145 | PLASMA OF HEALTHY DONORS CONTAINS SPECIFIC ANTI-IDIOTYPIC ANTIBODIES THAT RECOGNIZE TWO CLONED HUMAN ANTI-ADAMTS13 AUTOANTIBODIES

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INTRODUCTION: Acquired thrombotic thrombocytopenic purpura (aTTP) is an autoimmune disorder characterized by the presence of autoantibodies against the VWF cleaving protease ADAMTS13. Intriguingly, anti-ADAMTS13 autoantibodies are also present in 5% of the healthy population, and epitopes of anti-ADAMTS13 autoantibodies in healthy individuals and aTTP patients partially overlap. It is currently unknown why healthy individuals with anti-ADAMTS13 autoantibodies do not develop aTTP. Interestingly, it has been shown that healthy individuals can have e.g. anti-factor VIII antibodies, and that specific anti-idiotypic antibodies against anti-factor VIII autoantibodies spontaneously develop in these individuals.

AIMS: To investigate whether healthy individuals with anti-ADAMTS13 autoantibodies have specific anti-idiotypic antibodies against these autoantibodies. The presence of such anti-idiotypic antibodies might explain why these healthy donors do not develop aTTP.

METHODS/MATERIALS: Two human anti-ADAMTS13 autoantibodies (h2G9 and h5D3) were cloned from B-cells from a healthy individual using single cell sorting, expressed and their binding to ADAMTS13 was analyzed using ELISA. The VH and VL sequences from these antibodies were grafted on the constant region of a murine antibody to generate m2G9 and m5D3. Binding of m2G9 and m5D3 to ADAMTS13 was again tested using ELISA. Finally, a 96-well plate was coated with m2G9 and m5D3, blocked and plasma of healthy donors was added. Anti-idiotypic antibodies bound to m2G9 and m5D3 were detected with antibodies recognizing the constant region of human antibodies.

RESULTS: We have cloned and expressed two human anti-ADAMTS13 autoantibodies (h2G9 and h5D3) from a healthy donor and showed that they bound to ADAMTS13 using ELISA. Grafting the VH and VL sequences of h2G9 and h5D3 on a murine backbone generated m2G9 and m5D3 which bound to ADAMTS13 with a similar affinity as h2G9 and h5D3. We next set up an ELISA where m2G9 and m5D3 were coated and plasma of healthy donors was added to check for the presence of anti-idiotypic antibodies that specifically recognize m2G9 and m5D3.
We used plasmas of eight healthy donors, 6 of which were positive for anti-ADAMTS13 autoantibodies. Interestingly, in four of these six donor plasmas, anti-idiotypic antibodies that recognize m2G9 and m5D3 could be detected. Anti-idiotypic antibodies that recognize m2G9 and m5D3 were also detected in one sample found negative for anti-ADAMTS13 autoantibodies.

CONCLUSIONS: Our study approach shows the presence of anti-idiotypic antibodies in plasma of healthy donors. At present, we can only screen for 2 types of anti-idiotypic antibodies (the ones recognizing 2G9 and 5D3). Therefore, we are currently cloning more anti-ADAMTS13 autoantibodies from healthy donors which should allow us to screen for more different types of anti-idiotypic antibodies to find out a possible effect this interaction might have. This will lead to the investigation of the presence or absence of anti-idiotypic antibodies in aTTP patients which might shed some light on the pathophysiology of aTTP.

A-137 | **PHOSPHORYLATION OF ACETYL-COA CARBOXYLASE BY AMPK IN PLATELETS CONTROLS THROMBOXANE GENERATION, DENSE GRANULES SECRETION AND THROMBUS FORMATION**

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INTRODUCTION: AMPKα1 is activated in thrombin- or collagen-stimulated platelets. Acetyl-CoA carboxylase (ACC), the bona-fide substrate of AMPK, is phosphorylated/inhibited upon platelet stimulation but its role in platelets has never been investigated. ACC is a central regulator of lipid metabolism.

HYPOTHESIS: Given the roles of lipids in platelets, namely structural, energy storage and signaling, we hypothesized that ACC phosphorylation could affect platelet functions and thrombus formation.

METHODS: Platelets were isolated from a knock-in mouse model (KI) expressing a genetically modified ACC that can no longer be phosphorylated/inhibited by AMPK. In vitro, platelet adhesion and thrombus formation were measured using a flow chamber-based assay. Hemostasis was assessed via the measurement of bleeding time. Thrombosis was studied upon ferric chloride-induced carotid artery injury.

RESULTS: Platelets express the ACC1 isoform. Thrombin or collagen stimulation led to a rapid increase in ACC phosphorylation in WT platelets. However, baseline and thrombin- or collagen induced ACC phosphorylation remained undetectable in KI platelets. In vitro, thrombus formation on collagen-coated surface was augmented under flow, in the absence of ACC phosphorylation.
In vivo, KI mice had a shorter bleeding time and exhibited an increased thrombus growth reflected by a higher increment in thrombus surface area over a 10-minute time interval, compared to WT mice (KI, 7242 $\mu m^2 \pm 1265$; WT, $2520 \mu m^2 \pm 1219$, $P<0,05$). Mechanistically, platelets from KI mice showed an increased collagen-induced thromboxane release and dense granules secretion (ATP release: KI=0.49 nmoles±0.03; WT=0.34 nmoles±0.05, $P<0,05$). Interestingly, lipidomic analysis revealed that KI platelets had increased levels of arachidonic-acid containing phosphatidylethanolamine plasmalogen, which are major contributors of arachidonic acid and thromboxane generation following platelet stimulation.

CONCLUSIONS: Platelet ACC phosphorylation by AMPK regulates phospholipids content which in turn, downregulates dense granules and thromboxane secretion, and thrombus formation. ACC phosphorylation is a counterregulatory mechanism limiting thrombus formation.

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POSTER PRESENTATIONS

1. Platelets are a major source of pro-tumoral serum amyloid A proteins during inflammation-induced colorectal carcinogenesis
2. Is aPTT a good predictor of reliable INR in chronic intermittent hemodialysis patients?
3. Impact of a rapid centrifugation method on the accuracy of direct oral anticoagulants level and turnaround time.
4. The effect of a pneumatic tube transportation on coagulation tests
5. Differences in heparin and enoxaparin sensitivity of three commercially available PT reagents.
6. Development of appropriate and culturally adapted educational tools on haemophilia for the patients from Ivory Coast and their families.
7. Influence of enoxaparin (Clexane®) on lupus anticoagulant testing with HemosIL dRVVT (IL) and HemosIL SCT (IL) on ACL TOP
8. Performances evaluation of a new PT reagent STA® - NeoPTimal for PT/INR measurement and exogenous coagulation factor assays
9. Performances evaluation of a new coagulation analyser, STA R Max® 2
10. A patient with a type 2 CB von Willebrand disease: laboratory diagnosis and response to desmopressin
11. Major autoantibody reactivity serology and minor symptoms in a SLE patient : a case report.
12. Pre-analytical Stability of Coagulation Parameters in Plasma Stored at Room Temperature
13. Role of von Willebrand factor in abdominal aortic aneurysms
14. A patient with acquired factor X deficiency and metastatic transitional cell carcinoma of the bladder : is there a link between metastasis and factor deficiency in solid tumours?
15. Systemic Thrombolysis and Endovascular Thrombectomy in Severe Acute Ischemic Stroke after Dabigatran Reversal with Idarucizumab
16. Successful and cost-effective use of Fc-Fused FIX during major orthopaedic surgery
17. ADAMTS13 in experimental malaria-associated acute respiratory distress syndrome
18. Monitoring issues of Direct anti-Xa Oral Anticoagulants and Low Molecular Weight Heparins, during bridging with LMWH therapy.
19. Dabigatran etexilate in the treatment of localized intravascular coagulopathy associated with venous malformations
20. Effect of ABCB1 genetic polymorphisms on the transport of rivaroxaban in HEK293 recombinant cell lines
21. Diagnostic performance evaluation of Quantia D-dimer ® reagent for pulmonary embolism in clinical practice

A-123 | **PLATELETS ARE A MAJOR SOURCE OF PRO-TUMORAL SERUM AMYLOID A PROTEINS DURING INFLAMMATION-INDUCED COLORECTAL CARCINOGENESIS**

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BACKGROUND: Clinical and experimental evidence support a role for inflammation in the development of colorectal cancer, though the mechanisms are not fully understood. Beyond thrombosis and hemostasis, platelets are key actors of inflammation; they also have been involved in cancer. However, whether platelets participate in the link between inflammation and cancer is unknown.

AIM: To investigate the contribution of platelets and platelet-derived proteins to inflammation-elicited colorectal tumor development.

METHODS: We used a clinically relevant mouse model of colitis-associated cancer. Platelet secretion and their reactivity to thrombin were assessed at different stages of carcinogenesis. We conducted an unbiased proteomic analysis of releasates of platelets isolated at pre-tumoral stage to identify soluble factors that might act on tumor development. Plasma levels of the identified proteins were measured during the course of carcinogenesis. We then treated the mice with clopidogrel to assess its efficacy to inhibit platelet release reaction and tumor development.

RESULTS: At pre-tumoral stage, hyperactive platelets were a major source of circulating pro-tumoral serum amyloid A (SAA) proteins. Clopidogrel prevented the early elevation of plasma SAA, decreased colitis severity, and delayed the formation of dysplastic lesions and adenocarcinoma. Platelet inhibition hindered the expansion and function of immunosuppressive myeloid cells as well as their infiltration in tumors, while tissue CD8 T cells were augmented. Platelets or releasates of platelets from cancer mice both were able to polarize myeloid cells toward an immunosuppressive phenotype.

CONCLUSIONS: Thus, platelets promote initiation of colitis-associated cancer by releasing pro-tumoral SAA proteins. Platelet releasates enhance myeloid cell immunosuppressive function. Antiplatelet agents may help prevent inflammation-elicited carcinogenesis by restoring antitumor immunity.

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A-122 | **IS APTT A GOOD PREDICTOR OF RELIABLE INR IN CHRONIC INTERMITTENT HEMODIALYSIS PATIENTS?**

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INTRODUCTION: INR samples for monitoring vitamin K antagonist(VKA) therapy in patients on chronic intermittent hemodialysis(CIHD) often contain unfractionated heparin(UFH) depending on the sampling timeframe and access port. UFH originates from contamination by the catheter lock solution or from administration after starting hemodialysis(HD). Prothrombin time(PT) reagents for INR analysis contain heparin-neutralizing agents to guarantee reliable INR in patients with VKA and concomitant heparin anticoagulation. This neutralization is only complete if UFH is below a certain threshold. Above this threshold, INR is falsely prolonged. However, in daily routine, measuring UFH concentrations on these samples would be too expensive and time-consuming.

AIM: Most activated partial thromboplastin time(aPTT) reagents are sensitive to heparin and could be a cheaper and faster alternative than measuring UFH in INR samples. We investigated which aPTT level corresponds with the UFH threshold at which UFH neutralization is complete. This threshold could be a good alternative to predict a reliable INR in CIHD patients instead of measuring UFH concentrations.

METHODS: Fourteen CIHD patients on VKA were included after informed consent (median age (min-max): 81 (70-88), male:female ratio 9:5). All patients had a central venous catheter(CVC) access locked with a solution containing UFH. INR was determined at 3 time points: at the beginning (time point 0; t0), 30(t30) and 60 minutes(t60) after starting HD. Citrated plasma samples (0.109M, BD Vacutainer) were obtained from the CVC at t0 and from the arterial access of the HD circuit at t30 and t60. Twelve patients participated at 3 different HD sessions and 2 patients only at once session, resulting in 113 samples (exclusion 1 outlier).
PT (INR) (ReadiPlasTin), aPTT (SynthasII) and UFH (Liquid Anti-Xa) were simultaneously measured on ACL TOP 500 (Werfen). UFH is neutralized by polybrene in the INR reagent up to UFH of 1 IU/mL. By ROC analysis, a aPTT cut-off at which UFH is below 1 IU/mL and a UFH cut-off at which aPTT is significantly prolonged, were determined. A significantly prolonged aPTT was defined as being above the upper limit of the reference range (24.8-34.4s) +10%. All statistics were performed by MedCalc for Windows (MedCalc Software).

RESULTS: Regression analysis between aPTT and UFH resulted in R^2=0.916. The aPTT cut-off at which UFH is below 1 IU/mL was 254s. The UFH cut-off at which aPTT is significantly prolonged was 0.056 IU/mL. At t0, 76% had levels above the detection limit of 0.040 IU/mL and 14% were above 1 IU/mL, even after taking a discard tube and extensive flushing of the CVC. At t30 and t60, only 1 sample had UFH levels > 1 IU/mL because of a wrong sampling access, i.e. at the venous instead of the arterial access port of the HD circuit.

	t0 (n=37)	t30 (n=38)	t60 (n=38)
Median UFH (IU/mL)	0.127	0.424	0.350
Median aPTT (s)	49.4	78.9	69.6
UFH >0.040 IU/mL (n) (%)	28 (76)	34 (89)	36 (95)
UFH > 1 IU/mL (n) (%)	5 (14)	1 (3)	1 (3)

CONCLUSION: aPTT measured by SynthasII correlates well with UFH. Median UFH were far under the threshold of UFH neutralization of 1 IU/mL by the INR reagent. aPTT is significantly prolonged at a UFH ≥ 0.056 IU/mL. In our hands, aPTT can predict a reliable INR in CIHD patients if ≤254s. Samples taken at de CVC site on t0 were often contaminated with UFH. It is therefore most reliable and practical to measure INR on samples taken at the arterial access port of the HD circuit 30 or 60 minutes after starting HD.

A-111 | **IMPACT OF A RAPID CENTRIFUGATION METHOD ON THE ACCURACY OF DIRECT ORAL ANTI-COAGULANTS LEVEL AND TURNAROUND TIME**

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BACKGROUND: There is an urgent need for readily and rapidly available tests to measure direct oral anticoagulants (DOACs) in different clinical settings such as assessing anticoagulant activity in patients with bleeding, determining the suitability for thrombolytic therapy for acute ischemic stroke or guiding the physician in the administration of reversal agents.
One way to reduce the turnaround time (TAT) (i.e time from registration of the blood sample in the laboratory to first result published) is to reduce the duration of centrifugation.

AIMS:
1. To compare the impact of 2 centrifugation protocols (standard protocol: A versus short centrifugation protocol: B) on the accuracy of screening and specific coagulation assays.
2. To measure the TAT in a real-life setting.

METHODS/MATERIALS: Sixty-four plasma samples from patients treated with apixaban and 28 plasma samples from patients treated with rivaroxaban were included in the study. Study was approved by local ethic committee and all patients gave their written informed consent. Blood was taken by venipuncture in the antecubital or femoral vein and collected into 0.109 M sodium citrate (9:1 v/v) tubes. Platelet-poor plasma (PPP) was obtained from the supernatant fraction after centrifugation for 15 minutes (min) at 1500g at room temperature (protocol A) or 3 min at 4400 g at room temperature (protocol B). For each patient, the plasmas collected by protocol A and B were analysed simultaneously. All the analysis were performed on fresh samples on a STA-R MAX coagulometer (Diagnostica Stago®). Activated partial thromboplastin time (aPTT, CKPrest), Prothrombin time (PT, Recombiplastin), Thrombin Time (TT) and Fibrinogen (Clauss assay) were performed on all samples. Rivaroxaban and apixaban plasma concentrations were quantified using the STA-Liquid Anti-Xa assay (STA LAX) and liquid chromatography coupled with mass spectrometry (LC-MS/MS). The TAT was calculated for the standard centrifugation protocol.
Comparison of the 2 centrifugation protocols was performed using the t-test (normal distribution) or the Wilcoxon test (non-normal distribution).

RESULTS: For apixaban, there is no difference between protocol A and protocol B for aPTT (mean A: 30,4 sec, mean B: 30,4 Sec, p: 0.68) , TT (mean A: 17,5 sec, mean B: 17,4 Sec, p: 0.29), Fib (mean A: 344,5mg/dl , mean B: 343,1 mg/dl, p: 0.10) and STA LAX (mean A: 68.4ng/ml, mean B: 67.5ng/ml , p: 0.052). There is a statistically significant difference for PT (mean A: 80.6%, mean B: 79.7%, p: 0.026), but this is not clinically relevant.
The median TAT was 61.0 min (min: 30.0 min, max: 208.8 min).
For rivaroxaban, there is no difference between protocol A and protocol B for TT (mean A: 17, 9 sec, mean B: 17, 8 Sec, p> 0.2), and STA LAX (mean A: 77.3 ng/ml, mean B: 77.3 ng/ml, p: 0.9604). There is a statistically significant difference for aPTT (mean A: 32, 3 sec, mean B: 32,6 Sec, p: 0.0132), PT (mean A: 73.8%, mean B: 72.6 %, p: 0.0197), and Fib (mean A: 377,0 mg/dl , mean B: 369,3 mg/dl, p: 0.0209) , but these are not clinically relevant.
The median TAT was 67.6 min (min: 43.3 min, max: 177.4 min).

CONCLUSIONS: A rapid centrifugation protocol is useful to reduce the TAT of DOACs concentration without any impact on the accuracy of the results.
Measuring DOAC levels with a short TAT (<45min) is feasible but needs continuing training of all the staff members and reducing the delay between result delivery (result available in the lab) and technical validation (result available for the physicians).

A-146 | THE EFFECT OF A PNEUMATIC TUBE TRANSPORTATION ON COAGULATION TESTS

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BACKGROUND / INTRODUCTION: Pneumatic tube transportation is routinely used to send biological samples from hospital wards to the laboratory. This transportation presents several advantages in term of cost and efficiency. Indeed, it reduces the turnaround time (TAT), a robust quality indicator.
For hemostasis testing, except for platelet function testing, this system is generally acceptable as long as the pneumatic system does not induce excessive vibration and shock that may denature proteins, activate platelets and may thus affect lab results.

AIMS: The aim of our study was to validate our pneumatic tube transport of coagulation samples by evaluating its influence on routine coagulation tests results in comparison to gentle transportation Methods

MATERIALS: A total of 15 healthy volunteers (7 women and 8 men-medium age 44 (29-60) were collected at the outpatient clinic. Two citrated tubes were simultaneously taken by subject and sent separately to the laboratory, by the pneumatic tube transport system or by gentle transportation.
18 coagulation parameters were determined on each sample: prothrombin time PT, activated partial prothrombin time aPTT, thrombin time TT, fibrinogen level, coagulation factor level (II, V, VII, X, VIIIc, IX, XI, XII), FvW:Rag and RCF, D-dimer , antithrombin AT. Differences of the results according these two transport systems were analyzed by a Wilcoxon matched pairs test- (Graphpad Prism 5) and the correlation coefficient of these results were also calculated.

Coagulation test	Pneumatic transport	Gentle transport	Wilcoxon matched pairs test (p)
PTT (%)	100.5	101.4	0.216 NS
INR (ratio)	0.99	0.98	0.305 NS
APTT (s)	28.7	28.5	0.498 NS
APTT (ratio)	0.95	0.95	0.313 NS
TT (s)	16.2	16.1	0.199 NS
Fibrinogen (mg/dl)	305.6	308.9	0.493 NS
AT (%)	105.3	105.4	0.897 NS
DDIM (ng/ml)	180.0	219.3	0.575 NS
FII (%)	99.8	103.9	0.080 NS
FV (%)	110.7	110.4	0.883 NS
FVII (%)	92.3	93.1	0.615 NS
FX (%)	110.9	112.3	0.432 NS
FVIII (%)	164.3	151.9	0.125 NS
FIX (%)	168.7	159.1	0.096 NS
FXI (%)	151.7	139.1	0.069 NS
FXII (%)	146.1	139.0	0.477 NS
Rcf (%)	118.3	117.1	0.816 NS
Rag (%)	114.6	111.1	0.185 NS

Statistical analysis for all studied parameters

SUMMARY / CONCLUSIONS: In conclusion, as no differences in the results were obtained for each parameter we could validate the pneumatic tube transportation of our samples.

A-107 | **DIFFERENCES IN HEPARIN AND ENOXAPARIN SENSITIVITY OF THREE COMMERCIALLY AVAILABLE PT REAGENTS.**

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AIM: Comparison of the response in PT obtained with each of the reagents (Dade Innovin (Siemens) ; RecombiplasTin 2G (IL) ; and ReadiPlastin (IL)) when increasing concentrations of heparin or enoxaparin were added to a sample of normal plasma. The degree to which PT results are prolonged depends on the reagent formulation.

According to the package inserts, Dade Innovin reagent is insensitive to unfractionated heparin (UFH) up to ± 2 IU/mL, and RecombiPlasTin and ReadiPlastin up to ± 1 IU/mL on the ACL TOP family for PT. The ReadiPlastin reagent is formulated to be insensitive up to 1,4 IU/mL LMWH for PT.

MATERIAL & METHODS: The sensitivity study was conducted using normal pooled plasma spiked with lithium heparin plasma or sodium enoxaparin (Clexane®) plasma. On each spiked sample, the anti-Fx activity was measured. Furthermore, we checked the sensitivity of APTT Synthasil (IL).

Reagents :
- PT Dade Innovin (Siemens)
- PT RecombiplasTin 2G (IL)
- PT ReadiPlastin (IL)
- APTT SynthasIL (IL)
- Liquid anti-Xa reagent (IL)

Instrument : ACL TOP 550

RESULTS: cfr. Figure 1. The degree to which PT results are prolonged depends on the reagent formulation: PT ReadiPlastin > RecombiPlastin > Dade Innovin.

N°	Theoretical concentration Heparine (IU/ml)	Anti-F Xa activiteit	PT Innovin		PT RecombiPlastin		PT ReadiPlastin		aPTT Synthasil	
		X_m (U/mL)	X_m (sec)	Ratio	X_m (sec)	Ratio	X_m (sec)	Ratio	X_m (sec)	Ratio
1	**0**	**0,04**	9,0	1,0	11,3	1,0	11,5	1,0	29,0	1,0
2	**0,5**	**0,59**	9,8	*1,1*	12,1	*1,1*	12,2	*1,1*	111,5	*3,9*
3	**0,7**	**0,78**	9,9	*1,1*	12,4	*1,1*	12,6	*1,1*	154,4	*5,3*
4	**1**	**1,05**	10,2	*1,1*	12,5	*1,1*	12,9	*1,1*	250,4	*8,6*
5	**1,5**	**1,48**	10,6	*1,2*	12,9	*1,1*	14,4	*1,2*	-	-
6	**2**	**2,06 ***	11,1	*1,2*	15,1	*1,3*	20,0	*1,7*	-	-
7	**2,5**	**2,45 ***	11,8	*1,3*	19,6	*1,7*	31,2	*2,7*	-	-
8	**3**	**3,01 ***	12,8	*1,4*	36,0	*3,2*	62,9	*5,5*	-	-

N°	Theoretical concentration Enoxaparin (IU/ml)	Anti-F Xa activiteit	PT Innovin		PT RecombiPlastin		PT ReadiPlastin		aPTT Synthasil	
		X_m (U/mL)	X_m (sec)	Ratio	X_m (sec)	Ratio	X_m (sec)	Ratio	X_m (sec)	Ratio
1	**0**	**0,05**	9,3	1,0	11,1	1,0	11,4	1,0	29,3	1,0
2	**0,4**	**0,55**	9,4	*1,0*	11,5	*1,0*	11,7	*1,0*	43,3	*1,5*
3	**0,8**	**1,00**	9,7	*1,0*	11,8	*1,1*	12,1	*1,1*	58,8	*2,0*
4	**1**	**1,19**	9,7	*1,0*	11,8	*1,1*	12,4	*1,1*	66,0	*2,3*
5	**1,5**	**1,76**	10,0	*1,1*	12,3	*1,1*	13,1	*1,1*	86,2	*2,9*
6	**2**	**2,25 ***	10,2	*1,1*	12,7	*1,1*	13,8	*1,2*	114,0	*3,9*
7	**2,5**	**2,79 ***	10,1	*1,1*	12,7	*1,1*	13,6	*1,2*	128,7	*4,4*

X_m : samples were tested in duplicate, and each pair of results was averaged.
Ratio: Each APTT / PT result (sec) was normalized against APTT / PT (sec) without heparin addition.
* outside linear range of test. - no result.

CONCLUSION: Overall, PT Dade Innovin reagent showed the least influence by increasing heparin / enoxaparin concentrations, wherease PT ReadiPlastin reagent was more sensitive for this interference. The interference cut-offs according to the package inserts were confirmed in this sensitivity study.

A-138 I **DEVELOPMENT OF APPROPRIATE AND CULTURALLY ADAPTED EDUCATIONAL TOOLS ON HAEMOPHILIA FOR THE PATIENTS FROM IVORY COAST AND THEIR FAMILIES.**

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The Haemophilia Treatment Center of the Cliniques universitaires Saint-Luc in Brussels is the partner of the haemophilia center of Yopougon in Abidjan, Ivory Coast, since 2014 in the frame of the twinning program of the World Federation of Haemophilia.
One of the goals of the twinning is to provide education to the patients with haemophilia (PWH), the haemophilia carriers and their family. A good understanding of haemophilia, it's clinical features, treatment options and mode of transmission is indeed critical to get a better awareness of the disease, prevent the complications, raise the number of diagnosed cases and improve the quality of care.

Numerous educational tools (booklets, movies, slide kits...) about haemophilia have been developed over the last decades and are available in several languages. However, their content, the topics adressed (eg pre-implantatory diagnosis), the specificities of the language used (words/idiomatic expressions) and the iconography (eg pictures of caucasian patients without any signs of arthropathy) used in the available materials are rarely appropriate and therefore are less efficient to provide a pertinent and useful source of information to the PWH, the carriers and their family.

We developed several educational tools (booklets, an educational game and a slide kit presentation) in collaboration with the ivorian medical and paramedical teams and the local patient association. The content and the topics addressed have been adapted with a specific focus on to the local issues encountered in haemophilia care (eg prevention of bleeds due to circumcision). A special attention was also paid to linguistic and cultural aspects to improve the level of understanding and raise the impact of the information. We tried to use as much as possible pictures and smart draws to overcome potential literacy difficulties. In order to be the best in line with the ethnicity of the target population of the tool, the illustrations were essentially based on photographies taken on site during clinics and cartoons representing african subjects. The short and long-term impact of these original tools is currently being evaluated in a prospective study.

A-120 | **INFLUENCE OF ENOXAPARIN (CLEXANE®) ON LUPUS ANTICOAGULANT TESTING WITH HEMOSIL DRVVT (IL) AND HEMOSIL SCT (IL) ON ACL TOP**

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AIM: dRVVT (screen/confirm) and SCT (screen/confirm) assays are performed to diagnose the presence of lupus anticoagulant (LA). At least two screening assays, based on different properties, should be performed before the possibility of LA is excluded.

The aim of this evaluation was to investigate the influence of increasing concentrations enoxaparin, added to a sample of normal pooled plasma, on reagents commercially used to detect LA. The degree to which LA results are influenced by the presence of LMWH depends on the reagent formulation.

According to the package inserts, LMW/UH heparin interference up to 1 IU/mL is neutralized by polybrene in dRVVT Screen/Confirm reagent (IL), whereas in SCT Screen/confirm reagent (IL). LMW/UH heparin may exhibit falsely prolonged clotting times which could lead to incorrect results (IL). To check for heparin contamination, we routinely perform a thrombin time (TT) on each sample tested for LA.

MATERIAL & METHODS : The sensitivity study was conducted using normal pooled platelet-free plasma spiked with sodium enoxaparin (Clexane®) platelet-free plasma.

Reagents :
- HemosIL Trombin Time (IL)
- HemosIL Silica Clotting Time (Screen/Confirm/CaCl2) (IL)
- HemosIL dRVVT Screen (IL)
- HemosIL dRVVT Confirm (IL)
- SynthASil APTT (IL)

Instrument : ACL TOP 550

RESULTS: Figure 1
- Clearly noticeable influence of enoxaparin in APTT and TT results.
- No difference in interpretation of SCT / dRVVT screen results; screen ratio is maintained < 1,2 (cut off), despite added concentrations of enoxaparin up to 2,5 IU/mL
- SCT confirm reagent is more sensitive than dRVVT confirm reagent
- Overall interference with enoxaparin doesn't mimic a LA patern.

N°	Added concentration Enoxaparin (IU/ml)	aPTT	TT	Lupus anticoagulant assays												
				SCT screen		SCT mixture		SCT confirm		SCT Screen/ confirm TR	dRVVT screen		dRVVT mixture		dRVVT confirm		dRVVT Screen/ confirm TR
		sec	sec	sec	ratio	sec	ratio	sec	ratio	ratio	sec	ratio	sec	ratio	sec	ratio	ratio
1	0	29,3	16,8	30,9	0,8	33,2	0,86	30,6	0,79	1,01	25	0,72	28,2	0,81	24,3	0,73	0,99
2	0,4	39	24,5	34,3	0,89	36,1	0,94	40,7	1,05	0,87	23,5	0,67	26,5	0,76	23,8	0,71	0,95
3	0,8	49,1	39,3	35	0,9	37,5	0,98	45,1	1,16	0,78	24,1	0,69	26,6	0,76	24,9	0,74	0,93
4	1	54,4	50,6	35,4	0,91	37,5	0,98	48	1,24	0,74	24,9	0,71	26,5	0,76	25,6	0,76	0,93
5	1,5	70,6	139	37	0,96	37,2	0,97	52,9	1,36	0,70	27,8	0,8	26,9	0,77	28,2	0,84	0,95
6	2	85,3	failed	40,3	1,04	36,9	0,96	59,9	1,54	0,67	31,2	0,89	27,7	0,79	32,1	0,96	0,93
7	2,5	100,7	failed	43,9	1,13	37,4	0,97	66,1	1,7	0,67	36,4	1,04	28,3	0,81	37,5	1,12	0,93

CONCLUSION: Overall, no clinical significant influence is shown by increasing enoxaparin concentrations up to 2,5 IU/ml for LA testing. Performing of TT is useful to detect contamination of LMW/UH heparin . LA results need to be interpreted with caution and with awareness of the many variables that may affect the results.

A-121 | **PERFORMANCES EVALUATION OF A NEW PT REAGENT STA® - NEOPTIMAL FOR PT/ INR MEASUREMENT AND EXOGENOUS COAGULATION FACTOR ASSAYS**

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BACKGROUND/INTRODUCTION: A new prothrombin time reagent, STA® - NeoPTimal (Stago, Asnières sur Seine, France) prepared from rabbit tissue factor with ISI close to 1 was tested and compared to two well-known used reagent, STA® - Neoplastine® R (recombinant human thromboplastin) and STA® Neoplastine® CI PLUS (rabbit tissue factor) both manufactured by Stago. PT and exogenous factors assays were performed on a STA R Max® 2, a new coagulation analyser manufactured by Stago.

AIMS: The aim of this study was to evaluate the analytical and clinical performances of STA® - NeoPTimal, the new PT reagent on STA R Max® 2.

METHODS/MATERIALS: In the CHU de Charleroi (Belgium), we compared STA® - NeoPTimal with STA® - Neoplastine® R and STA® Neoplastine® CI PLUS for PT/INR (all with manufacturer's pre-calibration) and STA® - NeoPTimal with STA® - Neoplastine® R for factor assays. We selected a wide range of normal and abnormal fresh plasmas (230 samples): healthy patients, patients with hepatic failure and patients on vitamin K antagonist (VKA) therapy.

Method comparison for factor assays was performed on STA® - Neoplastine® R and STA® - NeoPTimal on a minimum of 30 samples (healthy patients, patients with hepatic failure and patients on VKA therapy). Analytical performances were evaluated with intra-run precisions and inter-run precisions on quality controls. Analytical performances were assessed by calculating mean, standard deviation and coefficient of variation for intra-run and inter-run precisions.

RESULTS: Analytical performances are compliant with GFTH ("Groupe Français d'études sur l'Hémostase et la Thrombose") and GRAAL (GRoupe d'Aide à l'Accreditation des Laboratoires). Clinical performances show good correlations. Results are presented in the table below:

Linear Regression	**Normal + VKA (INR)**	**Liver failure (%)**	**FII (%)**	**FV (%)**	**FVII (%)**	**FX (%)**
n	Normal = 60 VKA = 112	58	31	33	32	30
NeoPT/ NEoR (y=)	0.72x+0.37	1.00x+0.64	0.79x+5.14	0.97x+3.31	0,93x+7.29	0,97x+1.99
NeoPT/ NeoCI+ (y=)	1,13x-0.15	0,98x-0.88	NT	NT	NT	NT

S=slope I=Intercept NT = not tested

SUMMARY/CONCLUSIONS: The evaluation of the new thromboplastin, STA® - NeoPTimal, shows good results. The analytical performances are compliant with the expected specifications. Method comparisons show consistency between STA® - NeoPTimal versus STA® - Neoplastine® R and STA® - NeoPTimal versus STA® Neoplastine® CI PLUS for PT/INR. The factor assays correlations show good results between the two thromboplastins tested. The new thromboplastin STA® - NeoPTimal demonstrates good analytical and clinical performances for PT/INR measurements and exogenous factor assays.

A-118 | **PERFORMANCES EVALUATION OF A NEW COAGULATION ANALYSER, STA R MAX® 2**

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BACKGROUND/INTRODUCTION: STA R Max® 2 is a new high throughput coagulation analyzer developped by Diagnostica Stago (Asnières sur Seine, France), able to perform clotting, chromogenic and immunoturbidimetric tests simultaneously. It is also equipped with a preanalytical module including check volume function.

AIMS: The aim of this study was to evaluate the performances of the STA R Max® 2 before integration in the routine lab using Prothrombin Time (PT), Activated Partial Thromboplastin Time (APTT) and Fibrinogen (Fib) for clotting method (viscoelastic detection), Antithrombin (AT) for chromogenic method and D-Dimer (DDi) for immunoturbidimetric method.

METHODS/MATERIALS: Reagents STA® - Neoplastine® R for PT, STA® - PTTA for APTT, STA® - Liquid Fib for fibrinogen, STA® - Stachrom® AT III for AT and STA® - Liatest® D-DI PLUS for DDi, all from Stago were used for this study. For PT, Fib and DDi, manufacturer's pre-calibrations were used. Analytical performances of the STA R Max® 2 were evaluated with intra-run precisions on quality controls and pool of plasmas and inter-run precisions on quality controls. Analytical performances were assessed by calculating mean, standard deviation and coefficient of variation for intra-run and inter-run precisions. Method comparisons STA R Max® 2 versus STA-R Evolution® were performed using more than 30 patient's plasmas per test and analyzed using linear regressions.

RESULTS: CV for intra-run and inter-run precisions were all compliant with the GFHT ("Groupe Français d'études sur l'Hémostase et la Thrombose") and GRAAL ("GRoupe d'Aide à l'Accreditation des Laboratoires") guidelines. The linear regressions for all the tests evaluated show a good coefficient of correlation, slope and intercept and are presented in the table below.

STA R Max® 2 versus STA-R Evolution®	r (regression coefficient)	Slope	Intercept
PT (%)	1,00	0,980	0,339
APTT (sec.)	0,98	0,930	2,231
FIB (g/L)	0,99	0,983	0,139
AT (%)	0,99	1,073	-6,618
DDi (µg/mL)	0,99	0,984	-0,0527

SUMMARY/CONCLUSIONS: The performance of the new analyzer (STA R Max® 2) was highly equivalent to the analyzer actually used at the lab both in analytical performances and patients results. This will allow us to switch from the STAR Evolution to the STA R Max® 2 in a transparent manner for clinicians.

A-119 | A PATIENT WITH A TYPE 2 CB VON WILLEBRAND DISEASE: LABORATORY DIAGNOSIS AND RESPONSE TO DESMOPRESSIN

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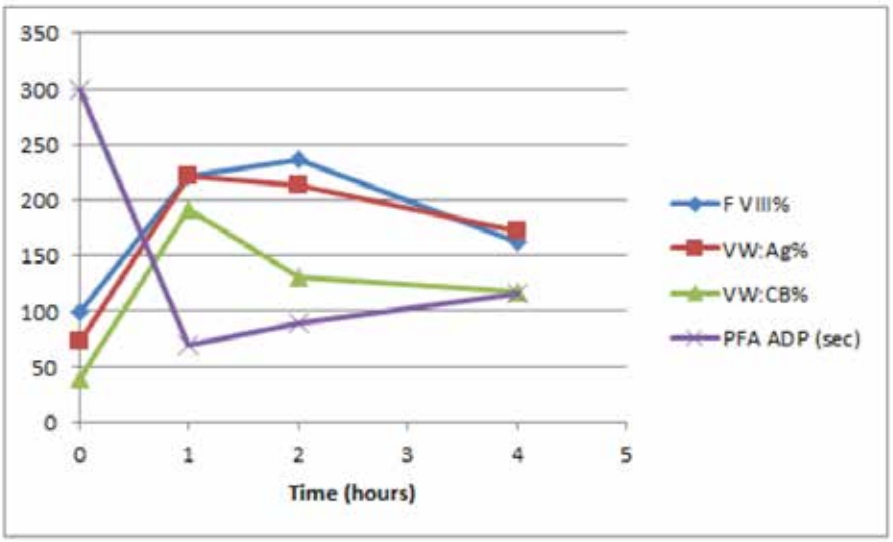
BACKGROUND/INTRODUCTION: Von Willebrand disease (VWD) is a rare inherited hemorrhagic disorder. Four hereditary types are described: type 1, 2, 3 and platelet-type. We described here a patient with a rare type 2 VWD called “Collagen Binding” (CB) where collagen binding assay and electrophoresis of von Willebrand Factor (vWF) multimers were key elements for diagnosis. A desmopressin test was performed to evaluate its efficiency.

CASE DESCRIPTION: A 45-year-old female was investigated for a history of menorrhagia, per-operative hemorrhage and ecchymosis. Her Tosetto bleeding score was 6. Excepting PFA coll/ADP which was increased at 300 sec, all subsequent tests were in a normal range: aPTT and PT, factor VIII, von Willebrand factor antigen (STA® - Liatest® VWF:Ag, Stago France) levels of 72%, Willebrand factor RCo (BC von Willebrand Reagent, Siemens Germany) was 56% with a normal VWF:RCo/VWF:Ag ratio at 0.78. Von Willebrand factor CB assay (Asserachrom® VWF:CB assay, Stago France) was reduced at 39% with a pathologic VWF:CBA/VWF:Ag ratio at 0.54. She has two children who have a similar profile of vWF. Desmopressin test was performed with a dose of 24 µg adjusted to body weight, results are illustrated on figure. A vWF multimers electrophoresis (Hydragel 5 von Willebrand multimer, Sebia) was also performed and demonstrated a decrease in the high molecular weight fraction (HMW): 26.6% with a healthy plasma control at 51.3%. Patient is included in the B-Will study and a genotypic test will be performed.



Coagulation assays (F VIII%, VW:Ag%, VW:CB%, PFA ADP sec) performed in the course of a Desmopressin test at time 0, 1, 2 and 4 hours.

DISCUSSION: These results allow us to conclude to a type 2 CB VWD associated with an alteration of the electrophoretic pattern (HMW loss of 50%). Some studies demonstrated that HMW fraction, in this pathology, may be either normal or present a “smear” (1). We highlighted a very good response to desmopressin with a complete normalization of PFA occlusion time at 1 and 2 hours. PFA test was a clear milestone in the diagnostic process but to date, it doesn’t seem that we have enough information about its sensitivity to recommend it as a screening tool for this rare form of VWD. Thus, despite their variable sensitivity, VWF:CB assay are essential in the evaluation of hemorrhagic diatheses of unknown origin as described previously (2).

CONCLUSION: This case showed that although Willebrand factor RCo was in the normal range, electrophoretic pattern was able to confirm type 2 VWD. Similarly, a specific test for von Willebrand Collagen Binding function remains mandatory in the evaluation of an unexplained hemorrhagic diathesis. It would be necessary to gather cases of type 2 CB VWD in a international registry which could evaluate the sensitivity of each laboratory assays and the efficiency of desmopressin therapy.

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A-142 | **MAJOR AUTOANTIBODY REACTIVITY SEROLOGY AND MINOR SYMPTOMS IN A SLE PATIENT : A CASE REPORT.**

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INTRODUCTION: Systemic lupus erythematosus (SLE) is a multi-organ system autoimmune disease with clinical and serological heterogeneity. The antiphospholipid syndrome (APS) is a systemic autoimmune disorder characterized by a combination of vascular thrombosis and/or pregnancy morbidity, and the presence of antiphospholipid antibodies (aPL), namely, the lupus anticoagulant (LA) and/or anticardiolipin antibodies (aCL) and/or anti-b2 Glycoprotein I (anti-b2GPI)(Revised Sapporo criteria). A great variety of other clinical features, for example thrombocytopenia (< 100 x10^3/µL, APS nephropathy, cardiac valve involvement, livedo reticularis and non-thrombotic central nervous system manifestations has been described, but were not included in the criteria. APS was first recognized in patients with systemic lupus erythematosus (SLE) and was then found at lower frequency in patients with other autoimmune disorders such as rheumatoid arthritis, Sjogren's syndrome, scleroderma, vasculitis, diabetes mellitus and Crohn's disease. The syndrome may also be independent of any underlying disease (i.e., primary APS). 40% of SLE patients are tested positive for aPL.

AIM: We present a case with major positivity of autoantibody reactivity serology and minor SLE and no APS symptoms. Clinicians were alarmed by the laboratory results but the patient had nearly any symptom.

CLINICAL CASE: A 33 years old man presented in February 2017 with multiple skin lesions and mild photosensitivity. This lesions were diagnosed as lichen planus. An investigation for autoimmune disease was made. Because he had insufficient clinical criteria but corresponding non-infectious serology for SLE (low complement, anti-nuclear antibody positive, anti-ds-DNA negative, anti-Sm antibody positive) he was treated with hydroxychloroquin. In September 2017 he presented with mild pain and swelling in the left lower leg. A new clinical and laboratory assessment was made. A splenomegaly was found but no DVT or new clinical findings. Laboratory investigation revealed an autoimmune hemolytic anemia (Hb 9.6 g/dL), mild thrombocytopenia (113 x10^3/µL) and extreme levels of aPL (LAC dRVVT and SCT strongly positive; aCL IgG 137 GPL U/mL (< 40); aCL IgM >1000 MPL U/mL (<40); anti-α2GPI IgG 110778 U/mL (<60); anti-β2GP1 IgM 2701 U/ml (<20). According to the Systemic Lupus International Collaborating Clinics (SLICC) classification criteria, the patient had now the definite diagnosis of SLE. The diagnosis of APS could not be made because of the lack of clinical criteria. aPL testing on a second occasion, after at least 12 weeks, is not yet performed. However, triple aPL positivity is the most frequent scenario among patients with clinical significant aPL. Low dose aspirin, corticosteroids, and supportive stockings were added to the treatment.
According to a multicenter prospective study, patients with a high-risk profile for aPL (triple positivity) and no prior thromboembolism are considered to have a risk for thrombotic complications of 37.1% by ten years. It has been estimated that APS may develop in up to 50-70% of patients with both SLE and aPL after 20 years of follow-up.

CONCLUSION: Despite the presence of thrombocytopenia, autoimmune hemolytic anemia and the triple aPL positivity, this SLE patient is not classified as having definite APS. Although, he has a high risk to develop clinical manifestation of APS in the future.

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A-105 | PRE-ANALYTICAL STABILITY OF COAGULATION PARAMETERS IN PLASMA STORED AT ROOM TEMPERATURE

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INTRODUCTION: Haemostasis testing is influenced by many pre-analytical variables, such as storage time and temperature, that can affect the stability of coagulation factors and influence the results of coagulation assays. The stability of coagulation parameters may also depend on measurement principle and reagent used for analysis.

AIM: The aim of this study was to investigate the stability of haemostasis tests after storage of aliquoted plasma at room temperature (RT) for different time periods and to evaluate whether a longer storage period is acceptable compared to the current Clinical and Laboratory Standard Institute (CLSI) H21 A5 guidelines 2008. By using two different fully automated haemostasis analysers with corresponding reagents, the influence of different measurement principles and reagents on the stability of coagulation parameters was also investigated.

METHODS: Blood samples from 20 healthy volunteers were obtained, immediately processed to platelet poor plasma and aliquoted. Aliquots were stored at RT for 0h, 2h, 4h, 6h, 8h, 12h, 24h and 48h. Routine haemostasis tests (PT, aPTT, fibrinogen and D-dimers) were performed on non-frozen plasma. Coagulation factors (FII, FV, FVII, FX, FVIII, FIX, FXI and FXII), von Willebrand factor antigen (vWF:Ag) and von Willebrand factor activity (vWF:Act) were performed batch-wise on frozen plasma aliquots.
All routine tests and coagulation factors were determined on STA-R Max® (Diagnostica Stago®, S.A.S., France) and ACL-TOP® 350 CTS (Instrumentation Laboratory, Werfen, Bedford USA). Analysis of PT and aPTT were performed by multiple reagents. VWF:Ag and vWF:Act were measured on the AcuStar® (Instrumentation Laboratory, Werfen, Bedford USA).
Statistically significant differences compared to baseline results were defined by a Friedman test and a post hoc Wilcoxon signed rank test with Bonferroni adjustment. A clinically relevant change compared to the initial measurement (analysis at 0h storage at RT) was denoted as a percentage change of > 10 % according to the 99% confidence interval (CI).

RESULTS: For both analysers, a decrease in factor activity of > 10% (according to the 99% CI) was observed for FV after 2h, FVIII after 4h and for FII, FVII and FX after 48h of storage at RT. A clinically significant decline of FVII activity, determined by ACL-TOP®, was observed at 24h storage (99% CI: -13.3% - -0.7%). A statistically significant and clinically relevant increase in FXII activity was seen after 48h storage at RT.
For aPTT, a statistically significant difference was seen after 6h storage and mean percentage changes of > 10% were observed after 48h of storage for all three aPTT reagents used. Statistically significant differences, but no clinically relevant changes were observed after 48h storage for PT, fibrinogen and FIX. D-Dimers, FXI, vWF:Ag and vWF:Act were found stable up to 48h of storage at RT.

Coagulation assay	Acceptable storage time at RT (CLSI H21 A5)	Acceptable storage time at RT (our findings)*
aPTT	4h	12h - 24h
PT	24h	24h - 48h
Fibrinogen	4h	48h
D-Dimers	4h	48h
FVIII	4h	2h
FIX	4h	48h
FXI	4h	48h
FXII	4h	24h
FII	4h	24h
FV	4h	< 2h
FVII	4h	12h - 24h
FX	4h	24h
VWF:Ag and VWF:Act	4h	48h

Table: comparison of the acceptable storage at room temperature according to the CLSI guidelines and according to our study findings.
Clinical and Laboratory Standard institute (CLSI) H21 A5. RT: Room Temperature.
* Percentage change > 10% compared to baseline results according to the 99% confidence interval was noted as a clinically relevant change.

CONCLUSIONS: Overall, compared to the limits given by the current CLSI guidelines, for most coagulation parameters investigated in this study a longer storage period could be accepted. Time intervals for FVIII and FV dosage were shorter than recommended by the CLSI guidelines. For PT determination our findings were consistent with the current CLSI guidelines.

A-143 | **ROLE OF VON WILLEBRAND FACTOR IN ABDOMINAL AORTIC ANEURYSMS**

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BACKGROUND: Abdominal Aortic Aneurysm (AAA) refers to a permanent, localized dilation of the abdominal aorta that exceeds its normal diameter by 50%. When left untreated, AAAs have a high risk of rupture, which is associated with a high mortality rate. Although its pathophysiology is still not completely understood, AAA is typically characterized by progressive inflammation of the aortic wall and the presence of an intramural thrombus.
Von Willebrand factor (VWF) is a large multimeric plasma protein, known for its role in haemostasis, thrombosis and inflammation. Whether the thromboinflammatory activity of VWF is involved in AAA pathogenesis or progression is currently unknown.

AIM: To unravel the role of VWF in AAA pathophysiology using an angiotensin II continuous infusion-induced AAA mouse model.

METHODS: To induce AAA, both wild type (WT) and VWF-deficient mice ($Vwf^{-/-}$) were subcutaneously implanted with osmotic pumps, continuously releasing a dose of 1 µg/kg/min angiotensin II (AngII). Survival in both groups was monitored for the course of the experiment. After 28 days, the surviving mice were sacrificed to harvest the abdominal aortas in order to determine AAA incidence. Aortic cryosections were stained with hematoxylin and eosin to visualize the aneurysms microscopically, after which the maximal aortic diameter and AAA severity in both groups were evaluated.

RESULTS: After implantation of the AngII-infusing osmotic pumps, a trend towards reduced survival in $Vwf^{-/-}$ mice was observed. While all (15/15) WT mice survived, 3/16 $Vwf^{-/-}$ mice had to be euthanized within the first week after implantation due to severe hemorrhage in the aortic region. However, this trend was not statistically significant ($p=0.0827$). After 28 days, AAAs were observed in both groups of mice. AAA incidence in WT mice was 33% (5/15), which was not statistically different from $Vwf^{-/-}$ mice (54%; 7/13). Also the severity of the AAAs and their maximal aortic diameters were comparable in both mouse strains.

CONCLUSION: Overall, no significant differences were observed between WT and $Vwf^{-/-}$ mice after angiotensin II infusion-induced AAA. These data suggest that, at least in this mouse model, the role of VWF in AAA pathophysiology is limited.

A-109 | **A PATIENT WITH ACQUIRED FACTOR X DEFICIENCY AND METASTATIC TRANSITIONAL CELL CARCINOMA OF THE BLADDER : IS THERE A LINK BETWEEN METASTASIS AND FACTOR DEFICIENCY IN SOLID TUMOURS?**

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INTRODUCTION: Acquired deficiency of factor X is commonly found during antivitamin K therapy in prevention of thrombosis or as a consequence of liver disease. Less fequently, it has been observed as an isolated deficiency in a number of disease states. It has been associated with amyloidosis and haematologic malignancies, and few non amyloid related cases have been reported. Association with solid tumors is rare. We would like to present a patient with a transitional cell carcinoma who developed a factor X deficiency.

CASE DESCRIPTION: A 67-year-old man was diagnosed with a localised transitional cell carcinoma of the bladder. He was first admitted to our hospital in january 2014 for a second opinion consultation. Transurethral resection of the bladder took place and neoadjuvant chemotherapy was administered at our hospital, followed by a cystectomy. During follow up suspicious lesions were noticed on medical imaging and a lung biopsy was carried out. Pathological examination reveiled a metastasis of the transitional cell carcinoma. The patient subsequently received stereotactic body radiotherapy to all lung lesions, yet had progressive disease 6 months later. He was then enrolled in a clinical trial (NCT02826564) in which he received pembrolizumab (Keytruda) and stereotactic body radiatiotherapy to one lung lesion according to the study protocol.

During follow up prothrombin time (PT), activated partial thromboplastin time (aPTT) and fibrinogen concentration were measured to assess his blood's clotting status. First measurements were within reference ranges, but after the lung metastastes were noticed and prior to administration of pembrolizumab, a prolongation of the PT (20s; reference ranges : 11.5 – 14.5s) and APTT (51s; reference ranges 28.9 – 38.1s) was detected. Vitamine K was administrated but could not correct the PT or aPTT.

In the laboratory diagnostic workup, mixing patient's plasma with normal pooled plasma showed correction of aPTT (37.6s) and PT (14.2s) (ref ranges 11.5 – 14.5s). Clotting factor activities for the extrinsic factors were determined by a one-stage clotting assay in a 1:10 sample predilution. FII, V and VII levels were within normal ranges with 105%, 71% and 98% activity, but FX activity was 21 % (all factor reference ranges 70-120%). Testing was repeated in higher predilutions of 1:40 and 1:100, without increase of FX activity (20%). These findings were confirmed three weeks later on a control sample.

DISCUSSION: This is, to our knowledge, the first case of an acquired deficiency of factor X associated with an urothelial carcinoma. We suggest that there might be a link with metastasis of solid tumours, as the FX deficiency occurred simultaneously with the presence of metastases, in accordance with previously published articles discussing this deficiency in solid tumours with metastasis. This theory is consistent with the status quo of the lab results and progressive lesions on medical imaging on the other hand.

A-103 | **SYSTEMIC THROMBOLYSIS AND ENDOVASCULAR THROMBECTOMY IN SEVERE ACUTE ISCHEMIC STROKE AFTER DABIGATRAN REVERSAL WITH IDARUCIZUMAB**

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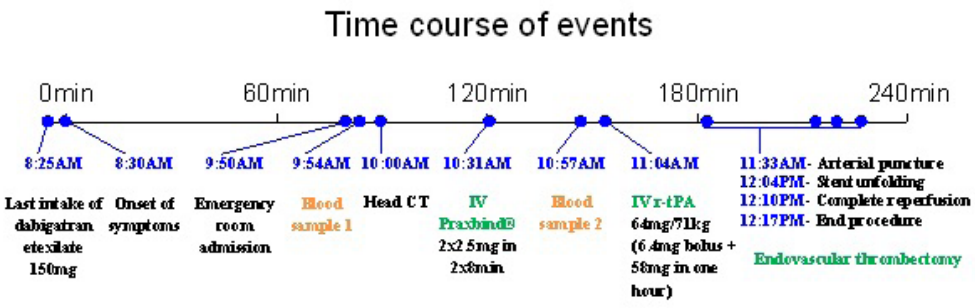
BACKGROUND: Systemic thrombolysis in acute ischemic stroke used to be discouraged in patients under effective anticoagulation by direct oral anticoagulants (DOAC). The recent approval in 2015 of idarucizumab, a humanized monoclonal antibody fragment for immediate and specific reversal of dabigatran, may allow this subgroup of patients to regain eligibility for thrombolysis.

AIMS: This case report aims, with the clinical context and accurate laboratory tests of dabigatran plasma level during reversal, at a better selection and follow-up of patients who may benefit from idarucizumab. However, no causal links can be drawn from a single case study, and further prospective studies are warranted to evaluate the optimal management strategy.

METHODS: Case report.

RESULTS: We report the case of a 55-year-old woman, treated by dabigatran etexilate 150mg bid for atrial fibrillation with a known thrombus in the left auricle, presenting with mutism and right hemiparesis in the setting of a severe acute ischemic stroke. Neurological status on admission was NIHSS (National Institutes of Health Stroke Scale) 20. Initial additional tests included (1) a blood sample showing disturbances in hemostasis testing with a thrombin time of 80.7 seconds –as expected in a patient treated by dabigatran (therapeutic serum level of 61.4 ng/mL)– and (2) a head computed tomography angiography highlighting an hyperacute left carotid T occlusion. Ten minutes after reversal by idarucizumab, blood tests showed a massive decrease in dabigatran serum levels (0.9 ng/mL) and normalization of the thrombin time (14.2 seconds). Evolution was rapidly favorable after systemic thrombolysis and mechanical thrombectomy.



Time course of events

All three treatments were well-tolerated and, given the high embolic risk, the intake of dabigatran was resumed on the third day after the stroke. The patient was discharged on day 8 with a NIHSS of 5. No hemorrhagic or procoagulatory complications were observed.

CONCLUSIONS: This case report, along with previous reports, suggests that every patient presenting with an acute stroke despite dabigatran therapy (last intake <24h or unknown) should be evaluated for reversal by idarucizumab, making him eligible for safe and effective intravenous thrombolysis. It has been shown to be feasible, well-tolerated and easy to manage in an emergency room or stroke unit.

A-131 | SUCCESSFUL AND COST-EFFECTIVE USE OF FC-FUSED FIX DURING MAJOR ORTHOPAEDIC SURGERY

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Extended half-life Fc-fused FIX (Fc-FIX) (Alprolix®) represents an attractive and validated alternative for prophylactic replacement therapy in patients with severe haemophilia B (HB). There have been few reports of the use of Alprolix® in patients requiring major orthopaedic surgery and the optimal modalities of Fc-FIX in this setting have not yet been formally established. We report the case of a 63-year-old patient with severe HB on secondary prophylaxis with Alprolix® (6000 U every 10 days / body weight: 70 kgs / AJBR: 0) admitted for left-knee total arthroplasty. His Fc-FIX half-life estimated by WAPPS-Hemo was 122 hours (73-170). One hour before surgery, he received a single bolus of 7000 U Alprolix® (100 U/kg) raising FIX from 9 % (trough level measured 10 days after last infusion) to 132 % (one-stage APTT assay). Six hours post-op, FIX was measured at 80 %. During the post-operative period, 3000 U Fc-FIX were given on days 1-5 and on day 7 with a FIX maintained between 51 to 62 % on Days 1-5 and measured at 45 % on Day 7 (last infusion on Day 5). There was no significant blood loss during the peri- and post-operative period. Rehabilitation started on day 1 and the patient was discharged on day 7 post-op. In total, 7 boluses of Fc-FIX were given with a cumulative dose of 25000 U. By comparison, this is less than half the dose of standard FIX given by continuous infusion that would have been required in the same setting (more than 56.000 U considering 6U/kg/h 3 days and 4U/kg/h 4 days). After discharge, prophylaxis with 3000 U of Alprolix® U twice weekly was reinitiated for 2 weeks followed by 6000 U/10 days. There was no adverse event as well as no evidence of clinical or subclinical venous thrombosis as ruled out by bilateral venous doppler US performed on day 7. Thirty days post-op with the use of 43000 units and 12 boluses, the patient had fully recovered and able to practice his daily sportive activities. This clinical case illustrates the haemostatic efficacy, ease of administration, perfect tolerance and cost-effectiveness of Fc-Fused FIX given by bolus infusions as replacement therapy for patients with haemophilia B undergoing major orthopaedic surgery, not only in the peri-operative period but also during the entire rehabilitation phase.

A-139 | ADAMTS13 IN EXPERIMENTAL MALARIA-ASSOCIATED ACUTE RESPIRATORY DISTRESS SYNDROME

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BACKGROUND: Malaria is a global health burden, resulting in 212 million clinical cases and 429,000 deaths in 2015. The pathophysiological mechanisms of severe malaria are complex and involve factors that remain poorly understood. Clinical studies have demonstrated that severe malaria is associated with increased levels of von Willebrand factor (VWF), accumulation of its hyperactive form, and a significant reduction in ADAMTS13 activity. Recent studies have shown that VWF is playing a role in malaria pathogenesis. However, the role of ADAMTS13 in malaria pathology has not been investigated.

AIMS: To study the role of ADAMTS13 in malaria using a murine model of malaria-associated acute respiratory distress syndrome.

MATERIALS AND METHODS: Wild-type (WT) and ADAMTS13 knockout (Adamts13 $^{-/-}$) mice on a mixed background (C57BL/6J,129X1/SvJ and CASA/Rk) were inoculated with 104 Plasmodium berghei (Pb) NK65-infected erythrocytes. Blood samples were taken to assess the levels of VWF antigen and multimers. Giemsa-stained blood smears were made to determine parasitemia. Pulmonary edema was assessed by measuring protein levels in bronchoalveolar lavage fluid.

RESULTS: During the course of infection, plasma VWF levels were significantly increased in both infected WT and Adamts13 $^{-/-}$ mice, especially at 3 days after infection (2-fold increase; $P<0.0001$). Parasitemia levels were comparable between WT and Adamts13 $^{-/-}$ mice following PbNK65 infection. Alveolar leakage in the lungs was seen at 8 days after infection and was not significantly different in infected Adamts13 $^{-/-}$ mice compared to WT. Correspondingly, mortality rates were similar in both genotypes. Both infected WT and Adamts13 $^{-/-}$ mice developed a marked reduction of high molecular weight (HMW) VWF multimers at the end-stage disease, indicating that this loss of HMW VWF is ADAMTS13-independent.

CONCLUSION: Our data suggest that PbNK65-mediated murine malaria infection is associated with early elevated levels of plasma VWF, in accordance with human malaria. The loss of HMW VWF multimers at the end-stage occurred independently of ADAMTS13. In contrast to our previous findings on VWF, ADAMTS13 itself does not influence the development of either parasitemia, lung pathology or survival. These findings emphasize the putative role of VWF in malaria pathogenesis, that is largely ADAMTS13 independent.

A-108 | MONITORING ISSUES OF DIRECT ANTI-XA ORAL ANTICOAGULANTS AND LOW MOLECULAR WEIGHT HEPARINS, DURING BRIDGING WITH LMWH THERAPY.

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INTRODUCTION: Low molecular weight heparins (LMWH) are used in the prevention or treatment of thromboembolism. They require less need for laboratory monitoring because of their simple fixed or weight-based dose regimen and their predictable dose-response relationship. In certain populations monitoring of anti-Xa effect is necessary. Nowadays direct anti-Xa oral anticoagulants (DOAC) have become widely available and are replacing the heparinoids and vitamin K antagonists (VKA), having advantages over those older classes of anticoagulants. Recently published perioperative guidelines are discussing the measurement of residual anticoagulant levels of those drugs during bridging with LMWH. DOAC might interfere with the anti-Xa used for UFH/LMWH or vice versa.

AIM: The purpose of this study was to investigate if there was a dose-related effect of increasing concentrations of Xa-inhibiting DOACs (Rivaroxaban and Apixaban), in samples with LMWH, applying an anti-Xa assay calibrated with UFH/LMWH-specific standards. Subsequently we inversed our study design and estimated the anti-Xa activity of the samples spiked with LMWH on the specific anti-Xa DOAC assays.

MATERIALS & METHODS: Anti-Xa assays were performed on STA R MAX coagulation analyser (Diagnostica Stago, Asnières, France)
with STA-Liquid anti-Xa reagent and calibrators for Rivaroxaban, Apixaban and UFH/LMWH and BIOPHEN Heparin LRT (LMWH) and DiXal reagent (Rivaroxaban) and their calibration materials (Hyphen Biomed, Neuville sur Oise, France).
Samples were spiked with Clexane 10.000 IU/ml (Sanofi, Maisons-Alfort, France) and a calibrator (Stago Rivaroxaban calibrator or Stago Apixaban calibrator).

Pooled normal plasma was used to prepare 20 samples (set A) with double-spiked concentrations of Clexane and Rivaroxaban, and another set of 20 samples (set B) with Clexane and Apixaban. Both sets were analysed using a calibration curve for UFH/LMWH and a curve for Rivaroxaban (set A) or Apixaban (set B).

RESULTS: A different pattern of interference seemed to occur on the two different methods for Rivaroxaban anti-Xa measurement when double-spiked with LMWH and Rivaroxaban.

Interference with Rivaroxaban and interference with Apixaban gave similar results on the LMWH anti-Xa assay, whilst additionally spiked with LMWH. This observation was seen with both methods (Stago and Hyphen).

In general all assays (anti - Xa LMWH and DOAC) were affected by the other anticoagulant class and no linear dose–response effect was observed on any assay if concomitant anti-Xa inhibiting activity of another drug was present.

CONCLUSION: The anti-Xa assays are inaccurate for detection of profylactic or therapeutic levels of DOACs when bridging with LMWH is applied and vice versa. Clinicians should not base their clinical decision making on anti-Xa assay results when administering LMWH and direct antiXa DOAC concomitantly.

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A-106 | DABIGATRAN ETEXILATE IN THE TREATMENT OF LOCALIZED INTRAVASCULAR COAGULOPATHY ASSOCIATED WITH VENOUS MALFORMATIONS

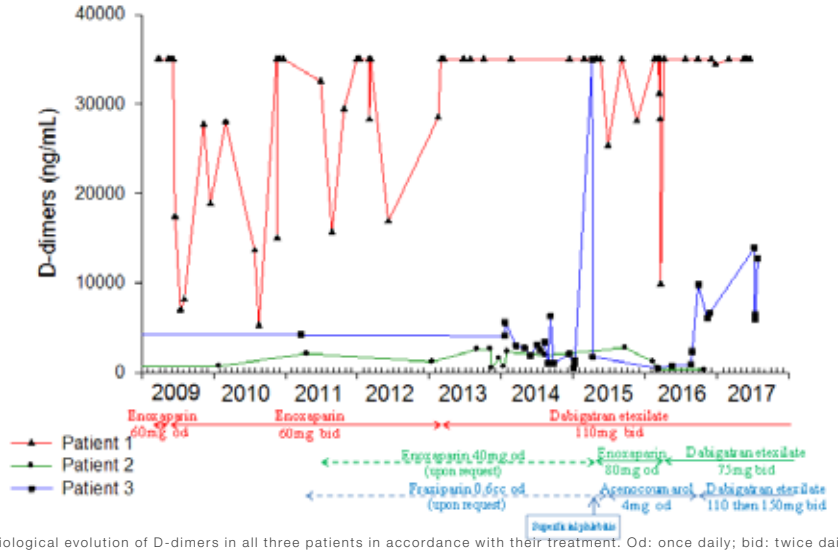
Q. BINET*, C. LAMBERT, C. HERMANS

BACKGROUND: Venous malformations (VM) are congenital slow-flow vascular malformations. A common complication is localized intravascular coagulopathy (LIC), which results from chronic, localized, intraluminal activation and consumption of clotting factors and is characterized by elevated D-dimer and, in severe cases, decreased fibrinogen levels. The medical gold-standard for treatment is anti-coagulation by Low Molecular Weight Heparin (LMWH) which has been reported to improve pain, decrease thrombosis, improve laboratory parameters and reduce hemorrhagic complications. However, the use of subcutaneous injections of LMWH has several limitations and the indications for therapy remain controversial. Conversely, direct oral anticoagulants (DOAC) have demonstrated numerous advantages as they do not require monitoring and have minimal dietary or drug interactions. Because of the availability of a rapid and specific reversing agent such as idarucizumab, dabigatran etexilate given at a validated low dose –tailored in function of the bleeding tendency and laboratory hemostasis parameters– offered in our view the most balanced risk-benefit profile in patients with extensive VM and at high risk of thrombohemorrhagic events.

AIMS: This case series aims at adding to the evidence of a clinical and biological benefit of dabigatran etexilate over LMWH in patients with VM-LIC.

METHODS: We present the very first small case series of 3 patients with VM complicated by chronic LIC in whom a long-term treatment by dabigatran etexilate, a direct thrombin inhibitor, was initiated in the setting of ambulatory hemostasis consultations. The primary outcomes were the absence of thrombohemorrhagic events and the symptomatic benefit (reduced pain and discomfort). Secondary outcomes were the hemostatic biological features (D-dimers, fibrinogen). Data for this study were collected retrospectively, and patients were followed until September 2017.

RESULTS: Clinically, drug tolerance was excellent in all 3 patients. Moderate hemorrhagic complaints occurred in 2 out of 3 patients but there was no thromboembolic event under dabigatran therapy. Whereas dabigatran was as effective as LMWH to control pain in Patients 1 & 3, Patient 2 reported a major and persistent symptomatic relief on pain, swelling and heaviness feeling. Biologically, dabigatran treatment showed various modifications in D-dimers level but the fibrinogen level and platelet count remained unchanged. In Patient 1, D-dimers increased above the upper detection limit of 35000ng/mL. Conversely, D-dimers dropped to indosable levels (<250ng/mL) in patient 2. In patient 3, D-dimers moderately increased under dabigatran, regardless of the dose (110 or 150mg bid), compared to acenocoumarol.



Biological evolution of D-dimers in all three patients in accordance with their treatment. Od: once daily; bid: twice daily.

CONCLUSIONS: Dabigatran etexilate may provide a viable alternative to LMWH in the long term treatment of VM-LIC. Indeed, the oral agent can be an effective and more convenient drug than subcutaneous LMWH in this indication. Moreover, the recent availability of idarucizumab, a monoclonal antibody designed for the reversal of anticoagulant effects of dabigatran, provides a decreased risk of hemorrhagic hazard, particularly in patients at high hemorrhagic risk or with dangerous VM because of their location.

A-133 | EFFECT OF ABCB1 GENETIC POLYMORPHISMS ON THE TRANSPORT OF RIVAROXABAN IN HEK293 RECOMBINANT CELL LINES

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BACKGROUND: Direct oral anticoagulants (DOAC) are substrates for the ABCB1 transporter (also called P-glycoprotein), an active efflux pump. ABCB1 polymorphisms have been previously reported to influence the pharmacokinetics of several drugs such as immunosuppressants and tyrosine kinase inhibitors. Recently, in vivo studies have suggested that genetic variants might contribute to the inter-individual variability in DOAC plasma concentrations.

AIMS: To evaluate in vitro the effect of the most common coding ABCB1 single nucleotide polymorphisms (SNP), 1236C>T-2677G>T-3435C>T, and the coding ABCB1 1199G>A SNP on the transport activity towards rivaroxaban.

METHODS: HEK293 cells were transfected to overexpress the ABCB1 wild-type (1236C-2677G-3435C, 1199G) or variant proteins (1236C-2677G-3435T, 1236T-2677T-3435T or 1199A). Cell surface ABCB1 expression was characterized by flow cytometry. Recombinant cells were incubated for 120 min with 5 different concentrations of rivaroxaban (from 50 ng/ml to 1000 ng/ml). The intracellular accumulation of rivaroxaban was quantified by LC-MS/MS analysis.

RESULTS: The overexpression of ABCB1 decreased significantly the intracellular accumulation of rivaroxaban, when compared to control cells (Figure 1, $p<0.01$). This confirms the involvement of ABCB1 in the active transport of rivaroxaban. For both the ABCB1 1236C>T-2677G>T-3435C>T and 1199G>A SNPs, the transport activity towards rivaroxaban was similar between cells overexpressing the ABCB1 wild-type and variant proteins (Figure 1, $p>0.05$).

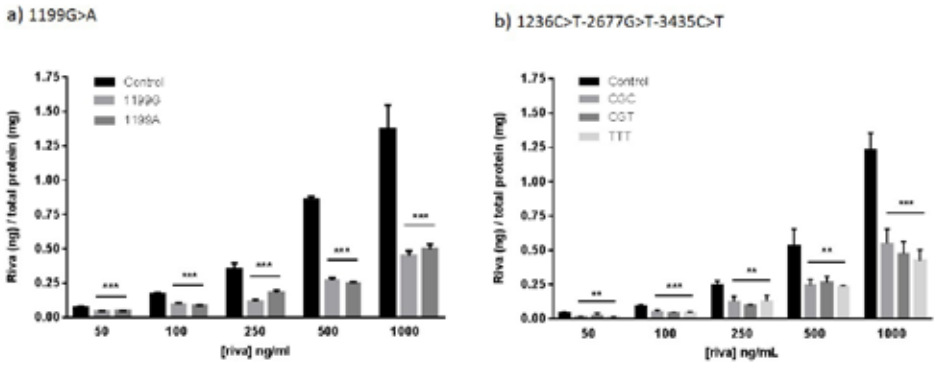

Figure 1: Intracellular accumulation of rivaroxaban after 120min of incubation (N=3) at different concentrations in **(a)** HEK $_{control}$ (i.e. empty vector), HEK $_{1199G>A}$ or **(b)** HEK $_{control}$, HEK $_{1236C>T-2677G>T-3435C>T}$. The absolute amount of rivaroxaban (in ng) was divided by the total amount of proteins in cell extracts (in mg). * Compared to control cells: *p<0.05 **p<0.01 ***p<0.001.

CONCLUSIONS: The intracellular accumulation of rivaroxaban was influenced by the overexpression of ABCB1. The ABCB1 coding SNPs that were evaluated in the present study had no significant effect on the efflux of rivaroxaban in HEK293 cell lines. They are unlikely to contribute to the inter-individual variability in rivaroxaban plasma concentrations.

A-147 | **DIAGNOSTIC PERFORMANCE EVALUATION OF QUANTIA D-DIMER ® REAGENT FOR PULMONARY EMBOLISM IN CLINICAL PRACTICE**

R. HARKATI[1], V. LEJEUNE[1], P. LEFÈVRE[1], J. FREYMANN[2]

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BACKGROUND: Veinous thromboembolic disease (VTED) is an entity of high incidence and high morbi-mortality. Pulmonary embolism (PE) is a frequent complication of VTED. The suspicion of the diagnosis of PE rests on the establishment of a score (Wells and revised Geneva score) based on various clinical criteria aimed at establishing a low, medium or high clinical probability. This score will guide the clinician on the relevance of direct diagnostic tests (angioscan, ventilation and perfusion lung scintigraphy, ultrasound).

D-dimer assay is appropriate for low and moderate clinical probability of PE. Indeed, its high negative predictive value will help to exclude pulmonary embolism with a probability close to 100% without the need of imaging studies.

D-dimers are degradation products of fibrin stabilized by factor XIII. They can be increased in a large number of pathological situations other than VTED and PE such as: disseminated intravascular coagulation (DIC), atrial fibrillation, artery dissection or sepsis.

Many reagents are available on the market. Among these, Werfen's Hemosil® D-dimer HS-500 reagent has a negative predictive value of 100% for the exclusion of VTED and PE when the d-dimers' concentration is less than 500 ng /ml. This makes it a reagent of choice for use in clinical practice.

AIMS: Quantia® D-dimer reagent (Abbott) has a cut-off set at 200 ng / ml. It is a very rarely used reagent with poorly documented literature. At the Princesse Paola hospital's laboratory, we tested and compared the diagnostic performance of the Hemosil® D-dimer HS 500 reagent and the Quantia® D-dimer reagent in order to determine the adequacy of the latter in clinical practice.

METHODS / MATERIALS: D-dimer assay was performed simultaneously with both reagents on a cohort of 123 patients admitted to the emergency department for whom a d-dimer assay was prescribed on the basis of a low to moderate clinical suspicion of PE or VTED. The analysers used were ACL-TOP®750 for Hemosil® D-dimer HS 500 and Architect® c8000 for Quantia® D-dimer. D-dimer assay required a correctly filled sodium citrate tube.

RESULTS: Of the 123 patients studied, 21 were found to have pulmonary embolism or deep venous thrombosis (17% of patients) according to classical imaging tests (pulmonary angioscan, V/P lung scintigraphy, ultrasonography). We did not detect any false negatives for both techniques: the sensitivity was 100% for both reagents. The specificity of Hemosil® D-Dimer HS 500 was higher than that of Quantia® D-dimer: 49% vs 28%. Negative predictive value (NPV) is 100% for both reagents, positive predictive value (PPV) is 11% for Hemosil® D-dimer HS500 and 7% for Quantia® D-dimer.

SUMMARY / CONCLUSION: The Quantia® D-dimer reagent has thus proved to have a very poor specificity with the current reference values. This can potentially generate additional costs by generating unnecessary medical imaging exams for patients with low to moderate clinical probability of PE. This demonstrates the necessity of further optimizing its reference values in order to improve the specificity of this reagent while, at the same time, guaranteeing the NPV of 100% needed for the exclusion of PE.

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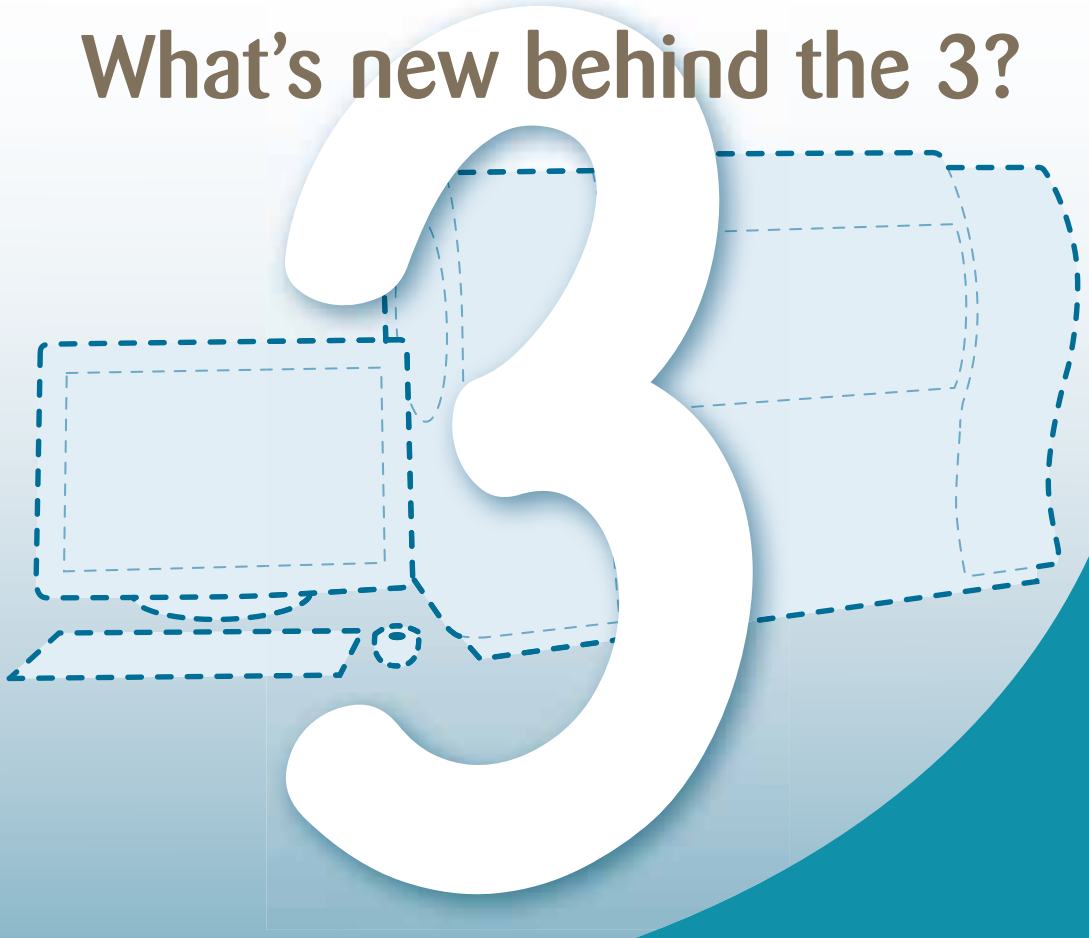

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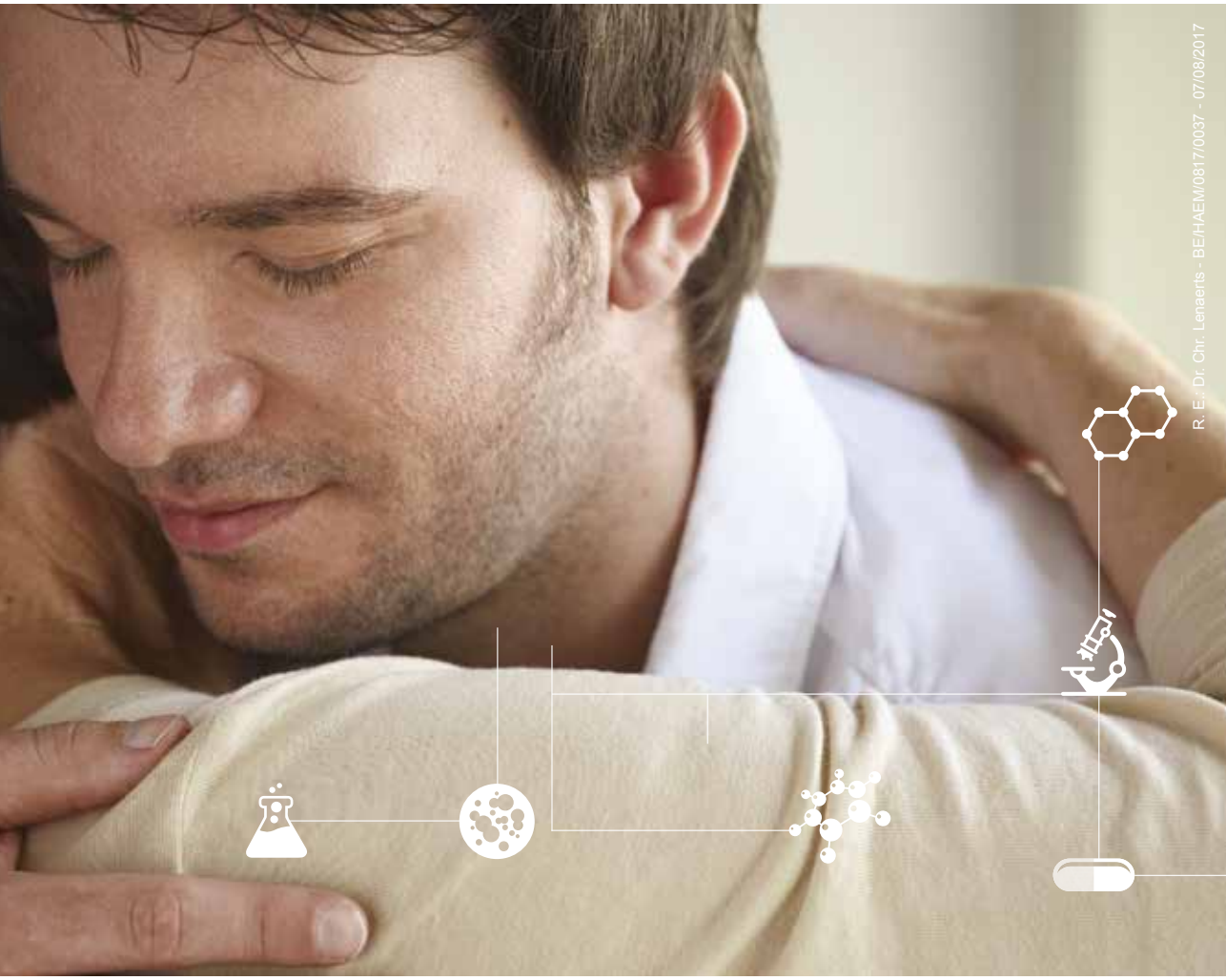

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