BSTH

Belgian Society on Thrombosis and Haemostasis

Final Program & Abstracts

23RD ANNUAL MEETING 19-20 November 2015 LAMOT Belgium

Local organisers:

Marc Hoylaerts
Center for Molecular and Vascular Biology
University of Leuven, Belgium
&
Hans Deckmyn
Laboratory for Thrombosis Research
KU Leuven Kulak, Belgium

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WELCOME

TO THE 23RD ANNUAL MEETING

Dear Colleagues and friends,

We have the pleasure of inviting you to our annual meeting in Mechelen, on behalf of the entire Board of the Belgian Society on Thrombosis and Haemostasis. Those of you who participated in last year's meeting will surely have kept a good memory of this meeting in a city, centrally located in our country and fairly easily accessible from different parts of the country. Also, the omnipresent history in this ancient city and the beautiful location in a former brewery have contributed to the success of last year's meeting. The availability of the right-sized auditorium and several practical facilities needed for the deployment of all our initiatives made it an easy decision to return to Mechelen! For the first time in 23 years, the BSTH annual meeting will go back to its previous choice…. in anticipation of making also this year's meeting a success!

Also for the 23rd meeting, we have put together a program, composed of basic science and clinical topics, relevant to our field. The emphasis this year will be on the link between infection and haemostasis, on the importance of neutrophil extracellular traps in thrombosis, myelofibrosis and innate immunity. As usual, a number of young investigators will have the opportunity to present their work, as a poster or as an oral presentation, after selection by all BSTH board members. Also this year, the best basic and best clinical presentation will be awarded the Paul Capel price. As our society has been transformed into a more modern and open society, with several new board members, the general assembly will be open to all registered members and you will be able to participate in the society's future more actively than ever before! Our pharmaceutical partners in this organization have assembled three symposia, a formula well known to our regular participants. A novelty, introduced as of this year is the networking reception, organized at the end of the first day's program. We strongly recommended all our participants to participate in this 1 hour long event, where new contacts can be made and old friendships will be rejuvenated. We conclude the day with a walking diner throughout the city for those who will join us.

This meeting could not have been organized without the generous support of our Corporate members and the sponsorship of other pharmaceutical and diagnostic partners. Without their support, it would be impossible to maintain the present quality standards of our meeting. Our gratitude is genuine, because we all realize that our economical climate is not yet "back to normal".

It is our hope that you will enjoy the venue for the 23rd meeting and that you will actively participate with the same enthusiasm as last year and the years before. Since this is essentially a meeting by us all for all of us, your input in ongoing initiatives of the BSTH will be strongly appreciated and your ideas are welcome!

Looking forward to meet you in Mechelen,

Marc Hoylaerts & Hans Deckmyn
University of Leuven & KU Leuven Kulak, Belgium
On behalf of the BSTH board

Passionate

about creating
a world where people
with haemophilia
lead fulfilling,
self-determined lives

Sobi is working to develop the next generation of haemophilia treatments.

"Our hope is that in developing these potential treatments we will be able to provide an option with the possibility to further improve protection from bleeding episodes, without increasing the treatment burden for people with haemophilia", said Dr. Roald van der Laan, Medical Science Manager, Belgium – Netherlands – Luxembourg.

Sobi understands that quality and purity are at the heart of haemophilia therapies. Sobi has been manufacturing recombinant factor VIII for well over a decade. And is now working with Biogen to develop the next generation of haemophilia treatments.

The company has a history of innovation in haemophilia treatments that stretches back more than 50 years. Through his work in haemophilia research at Sobi, Principal Scientist Peter Lind has contributed to one of the major transformational stages in haemophilia treatment: bringing recombinant treatments to people with haemophilia. In the 1980s he established the principles for the design of B-domain deleted factor VIII products. Sobi has been a producer of these products since the 1990s.

Sobi's mission is to improve the lives of people with haemophilia and other rare diseases. This commitment drives the company towards innovations in the treatment of haemophilia with the simple goal of providing people with haemophilia choices that help them to live the lives they wish for.

Sobi wants to help close the treatment access gap

Sobi believes that providing access to safe, effective and reliable treatment is a shared societal responsibility. Globally, around 75% of people with haemophilia don't receive regular treatment. Sobi and Biogen have pledged to donate one billion International Units (IU) of clotting factor to humanitarian aid programmes in developing countries over the next 10 years. For the first five years, 500 million IU will support the World Federation of Haemophilia (WFH) Humanitarian Aid Program. This will enable thousands of people in developing countries to access modern treatment over the coming years.

Sobi is co-creating the future

"By working with all members of the community, we aim to support the best possible life-long outcomes for people with haemophilia", says Geoffrey McDonough, MD, President and CEO of Sobi.

Sobi partners with and supports many haemophilia organisations around the world. By listening to the needs of societies, and seeking to help them achieve their objectives, Sobi aims to become a trusted partner for the long term.

Sobi is an international specialty healthcare company dedicated to rare diseases. There are approximately 7,000 recognised rare diseases, the majority of which are without treatment. Sobi's mission is to develop and deliver innovative therapies and services to improve the lives of these patients.

sobi

Pioneer in Rare Diseases

NP-0363

ORGANISATION

BSTH
Belgian Society on Thrombosis and Haemostasis

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Congress management

Con-txt

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GENERAL INFORMATION

Language:
The official language of the BSTH Annual Meeting is English. There will be no simultaneous translation.

Technical Equipment:
Only Power Point projection will be available. All slides will be presented from a central server.

Presenters are being requested to bring their presentation on a memory stick, ZIP drive, CD rom or DVD.

Posters:
Posters should be put on display on Thursday morning upon arrival and have to be removed on Friday in the afternoon after closure.

Accreditation:
Participants will receive a certificate of attendance and accreditation.

Liabilitiy:
Neither the organizers nor the BSTH accept liability for damages and/or losses and any kind which may be incurred by meeting participants.

MEETING VENUE

The Annual Meeting will be organised at Lamot
Van Beethovenstraat 8/10,
B-2800 Mechelen
www.lamot.be

PROGRAM

THURSDAY 19 NOVEMBER 2015

08h30 Registration

09h25 Welcome

09h30 **CSL Behring Satellite Symposium**

TURNING SCIENCE INTO PRACTICE

Chair: K. Peerlinck and C. Hermans

Pharmacokinetic data - translations into clinical practice — J. Rischewski (Hamburg)

Innovative technologies in hemophilia therapeutic proteins — E.M. Mancuso (Milan)

10h30 Break

11h00 **Educational I:**

Chair: M. Sprynger and M. Hoylaerts

Preventing Infective endocarditis via antiplatelet strategies — P. Moreillon (Lausanne)

11h45 **BSTH General Assembly**

12h30 Lunch

13h45 **STATE OF THE ART I: Infection and Hemostasis**

Chair: K. Jochmans and C. Motte

Sepsis, a disorder of platelets — D. Cox (Dublin)

Sepsis and Hemostasis — C. Van't Veer (Amsterdam)

15h15 **Oral Communications: Clinical & Laboratory**

Chair: C. Oury and S. De Meyer

Addition of idarucizumab to plasma samples containing dabigatran allows to use routine coagulation assays to diagnose hemostasis disorders — M. Peetermans (Leuven)

A novel FGA mutation underlying a case of congenital dysfibrinogenemia with unusual clinical presentation — M. Tajdar (Brussel)

ADAMTS13 containing the naturally occurring mutation p.Arg1177Gln adopts an open conformation — E. Roose (Kortrijk)

Elevated basal levels of platelet-bound fibrinogen predict the occurrence of sepsis in ICU: a prospective study — C. Delierneux (Liège)

	The role of ADAMTS13 and VWF in Patients with Acute Ischemic Stroke: a Case Control Study	F. Denorme (Kortijk)
	Paul Capel Prize Clinical & Laboratory	
16h30	Break	
16h45	Baxalta **Satellite Symposium**	
	HAEMOPHILIA, THE JOURNEY CONTINUES	
	Chair: C. Hermans	
	Outcome tools in patients with haemophilia: clinical illustrations	C. Hermans (Brussels)
	Personalized treatment in haemophilia A: what it means today and how to pursue it tomorrow	A. Gringeri (Vienna)
	Gene therapy for hemophilia A and B: the journey continues	T. VandenDriessche (Leuven)
17h45	Closure of day program	

EVENING PROGRAM

THURSDAY 19 NOVEMBER 2015

At Thursday night 18h00 all registered participants are invited to join the welcome reception.

At 19h00 you are invited to join a culinary walk trough Mechelen. As part of a group accompanied by a specialist guide, you will walk from dish to dish, stopping at several nice restaurants and a number of unusual places.

For the latest news and updates on the program please check on www.bsth.be.

Welcome reception & dinner

Venue:
Lamot
Van Beethovenstraat 8/10
B-2800 Mechelen

18h00:
Networking reception

19h00:
Start dinner walk through Mechelen (for participation please register)

PROGRAM

FRIDAY 20 NOVEMBER 2015

08h55 Welcome

09h00 **Satellite Symposium**

RECOGNIZING THE NEEDS OF PEOPLE WITH HAEMOPHILIA A:
INTRODUCING NOVOEIGHT®

Chair: C. Hermans

The development, design and purification process of uroctocog alpha - NovoEight® - Practical consequences	K. Vad (Copenhagen)
One year Real Life Experience With NovoEight®	R.E.G. Schutgens (Utrecht)

10h00 **Educational II:**

Chair: A. Demulder and A. Gadisseur

New insights in the mechanisms of thrombosis	A. Brill (Birmingham)

10h45 Break

11h15 **Oral Communications: Basic Research**

Chair: K. Vanhoorelbeke and C. Orlando

High and long-term expression of von Willebrand factor after Sleeping Beauty transposon-mediated gene therapy in mice	I. Portier (Kortijk)
Platelet Endothelial Aggregation Receptor 1: a novel modifier of neo-angiogenesis	C. Vandenbriele (Leuven)
ADAMTS13-mediated thrombolysis of t-PA resistant occlusions in ischemic stroke	F. Denorme (Kortrijk)
Endothelial cells do not express the vasopressin receptor 2 that is required for the release of FVIII and VWF following DDAVP treatment	L. Feyen (Leuven)

	Inhibition of TAFI and PAI-1 protects mice from cerebral ischemia/reperfusion injury	F. Denorme (Kortrijk)
	Paul Capel Prize Basic Research	
12h15	BSTH news	
12h45	Lunch	
13h15	Poster walk (dessert and coffee will be served in Scala)	
14h00	**STATE OF THE ART II: Platelets / megakaryocytes beyond hemostasis**	
	Chair: K. Devreese and H. Deckmyn	
	Megakaryocytes and myelofibrosis	H. Raslova (Paris)
	Cross-talk between thrombosis and innate immunity	S. Massberg (Munich)
15h30	Closure	
15h30	Reception	

BOARD'15

THE PRESENT MEMBERS OF THE BSTH BOARD 2015 ARE:

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Cécile Oury (non-voting)
GIGA-Cardiovascular Sciences, University of Liège
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MEMBERSHIP OF THE BSTH

1. Membership benefits

The BSTH council has defined the membership benefits for the different categories of membership starting from January 1st 2015. At registration you are enabled to enter your membership for 2015 and pay online.
Further information on membership options and benefits you will find on www.bsth.be

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

2. Annual Meeting Fees

Members	Early registration	Registration	On site registration
MD specialists, MSc specialists, PhD scientists	90 €	100 €	125 €
MD trainees, PhD students	50 €	50 €	65 €
Nurses, paramedics, technicians, data managers, students	20 €	25 €	35 €
Corporate	According to sponsorship/Exhibition booth package		

Non-members			
MD specialists, MSc specialists, PhD scientists	150 €	175 €	200 €
MD trainees, PhD students	100 €	115 €	125 €
Nurses, paramedics, technicians, data managers, students	60 €	65 €	75 €

* excluding separate fee for evening programme

3. Membership Fees (annually)

MD specialists, MSc specialists, PhD scientists	50 €
MD trainees, PhD students	35 €
Nurses, paramedics, technicians, data managers	25 €
Corporate members	5000 € (excl VAT)

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.

EXHIBITION

Exhibition Schedule

Set up

Wednesday	November 18, 2015	19h00 - 22h00
Thursday	November 19, 2015	07h00 - 08h30

Exhibition opening hours

Thursday	November 19, 2015	08h30 - 19h30
Friday	November 20, 2015	08h30 - 16h30

Dismounting

Friday	November 20, 2015	16h30 - 18h00

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

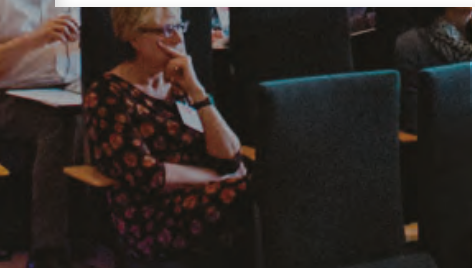
Exhibitors may not use audible electronic, mechanical apparatus, or open audio systems that may be heard outside the exhibitor assigned space. Con-txt, on behalf of BSTH and its organizers, reserves the right to require any exhibitor to discontinue any activity that may cause annoyance or interference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

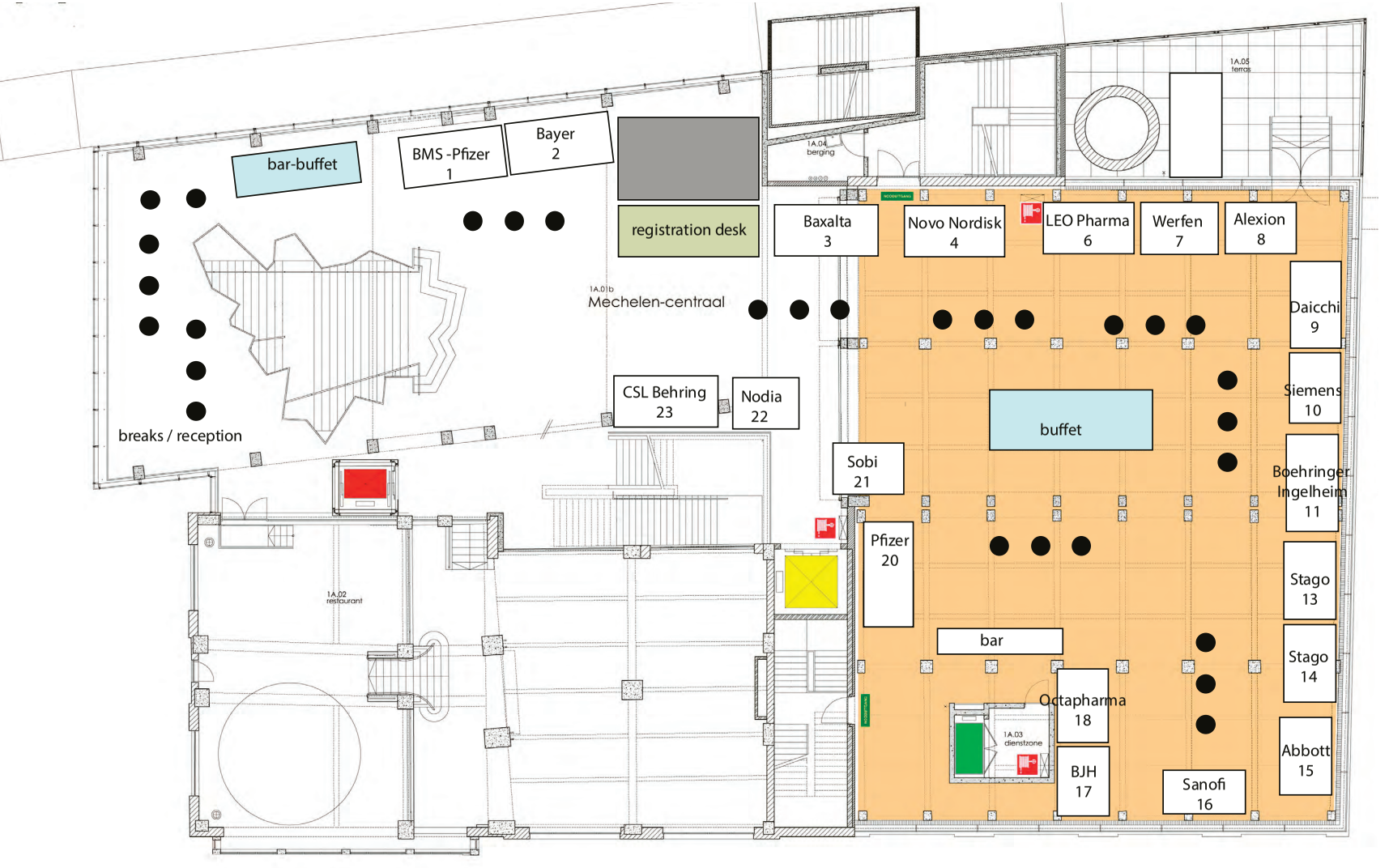
Company / Product Profile

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 19, 2015, will forfeit their booth space without refund. The space may be resold or used by the BSTH.




bar-buffet
BMS -Pfizer
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Bayer
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registration desk
Baxalta
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Novo Nordisk
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LEO Pharma
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Werfen
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Alexion
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1A.05
terras
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berging
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STATE OF THE ART SPEAKERS

D. Cox
Dublin

I graduated with a BSc in Pharmacology (University College Dublin, 1983) and a PhD in Immunology (Dublin City University, 1989). I then led an anti-thrombotic drug discovery group in Fujisawa Pharmaceutical Company, Osaka, Japan for 6-years. I subsequently joined Royal College of Surgeons in Ireland where I am currently a senior lecturer in pharmacology. I am also a part-time lecturer in Pharmaceutical Medicine for Hibernia College.
My research interests are in the development of novel anti-platelet agents and on the role of platelets in infectious diseases. I am an academic editor for PLos One and I am on the advisory board of Journal of Thrombosis and Haemostasis.

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C. Van 't Veer
Amsterdam

Cornelis van 't Veer, PhD, associate professor, Center for Experimental and Molecular Medicine, Academic Medical Center, Amsterdam, the Netherlands, has conducted extensive research on how proteins work together and studies the role of the different toll-like receptor (TLR) regulators and blood coagulation factors in bacterial infections.
Dr van 't Veer was born in Zeeland, the Netherlands, and studied biology with a specialisation in medical biology from 1983 to 1989 at the University of Utrecht, Utrecht. After an internship to investigate the interaction of complement factor 4 with C4b-binding protein at the Department of Haematology, Academic Hospital of Utrecht, the department offered him a job as a graduate student in 1989 in the lab of Professor Bonno Bouma, PhD. In 1997, Dr van 't Veer returned to the Netherlands and joined Professor Wim Buurman, PhD, and his group in the Department of Surgery, University of Maastricht, Maastricht, to study inflammation and coagulation.
In 2003, Dr van 't Veer was offered a post with the sepsis group of Professor Tom van der Poll, MD, PhD, Department of Experimental Internal Medicine, Academic Medical Centre, where he now studies the role of different TLR regu-lators and blood coagulation factors in bacterial infections in mice and man.

H. Raslova

Paris

Hana Raslova is Research Director at INSERM, in the Department of Normal and Pathological Hematopoiesis, UMR 1170, at the Gustave Roussy Institute, Villejuif, France

Hana Raslova graduated as Engineer in Chemistry at the technical University in Slovakia in 1996. She received her training in Institute of Virology in Slovakia and in Cancer Research Center in France and qualified as a Docteur ès Sciences at the University Paris 6 in France and as a PhD at Comenius University in Slovakia in 2000 with a specialization in microbiology. She thereafter joined the “Normal and Pathological Hematopoiesis” team at the Gustave Roussy Institute and obtained a permanent position four years later. Since 2015 she leading the research team « From hematopoietic stem cell to megakaryocyte » at INSERM, UMR 1170, in Villejuif.
The research in her team focuses on hematopoietic stem cells and megakaryopoiesis in both hereditary thrombocytopenias predisposing

S. Massberg

Munich

Steffen Massberg is Professor of Cardiology and Director of the Department of Cardiology at the University Clinic Munich, Ludwig-Maximilians University in Germany.

His basic-science research topics include stem-cell biology, platelet biology, mechanisms of arterial and venous thrombosis, immune-cell migration, immune-cell/coagulation crosstalk and bio-imaging (including 2-photon microscopy). He has written over 135 articles published in national and international peer-reviewed journals, including Blood, Nature Reviews Immunology, The Journal of Experimental Medicine and Circulation.

Professor Massberg is the co-ordinator and lead scientist of the European FP7 project PRESTIGE, the co-speaker and principal investigator of the DFG Collaborative Research Unit (SFB) 914 and the principal investigator of the DFG-Research Unit (FOR) 923. He has received many prizes for his work, the most recent being the 2011 Martin Villar Haemostasis Award (together with Dr Bernd Engelmann) and the 2012 Basic Science Award from the German Society of Cardiology (DGK).

EDUCATIONAL SPEAKERS

P. Moreillon

Lausanne

After a Doctorate in Medicine at the University of Lausanne in 1987, Philippe Moreillon pursued post-doctoral studies at Rockfeller University, New York, where he got a PhD in microbiology in 1993. He then worked at the Lausanne University Hospital as Chief of the Antimicrobial Agents Unit and then in the Infectious Diseases Department. Philippe Moreillon became full Professor and Director of the UNIL Institute of Fundamental Microbiology in 2002, with a part-time affiliation at the University Hospital.

His research interests encompass pathogenesis and ecology of Gram positive bacteria, microbial-induced inflammation, and antibiotic resistance. He has more than one hundred scientific contributions in these fields. Vice-Dean of the Faculty of Biology and Medicine between 2002 and 2006, Philippe Moreillon was elected as Vice Rector of the University of Lausanne in 2006. He was re-elected and started his second term in September 2011, in charge of Research and International Relations.

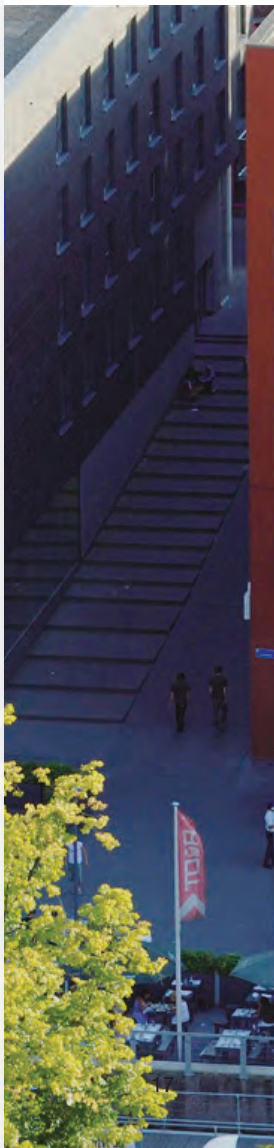
A. Brill

Birmingham

Alex is a Birmingham Fellow in the Institute of Cardiovascular Sciences. Alex has a strong background in different models of thrombosis, inflammation and microscopy. His main research interests are mechanisms of cardiovascular diseases, such as deep vein thrombosis and stroke, and identifying new targets to prevent and treat these disorders.

Alex received his undergraduate and graduate degrees (MD and PhD) from Saratov State University, Russia. He did his first postdoctoral training with Professor Ofer Lider at the Weizmann Institute of Science, (Israel) studying mechanisms of T cell adhesion and migration. In 2007, he moved to Boston (USA) and did another postdoc at Harvard University in the group of Professor Denisa Wagner. Alex joined the Birmingham Platelet Group in March 2013.

Alex has published more than 30 papers in peer-reviewed scientific journals. He was a co-author of a chapter in the “Platelets” textbook. Alex presented results of his research at numerous international conferences.



INFECTION AND HEMOSTASIS

D. Cox (Dublin)

Sepsis, a disorder of platelets

Septicemia occurs when bacteria grow within the circulatory system and is associated with very high mortality. A characteristic feature of septicaemia is the occurrence of thrombocytopenia. This is not an incidental finding as it is due to platelet activation and consumption. These activated platelets aggregate and cause a the formation of thrombi in the microvasculature. This leads to ischemia and ultimately organ failure. All of the major species of bacteria that are associated with sepsis such as Staphylococci and Escherichia coli can also directly activate platelets. All have been shown to induce aggregation in an IgG-FcgRIIa-dependent manner. This direct activation of platelets by bacteria is likely to play a critical role in the pathogenesis of sepsis. Thus, FcgRIIa is a potential novel drug target in sepsis.

C. Van 't Veer
(Amsterdam)

Sepsis and Hemostasis

Platelet function and coagulation are important factors in host defense to several types of bacteria. For infections with Borrelia burgdorferi, group A streptococci, Yersinia pestis and Burkholderia Pseudomallei it is well established that the generation of a fibrin network can be of pivotal importance for adequate host defense. Furthermore we recently showed that platelets are necessary for adequate host resistance to pneumosepsis caused by Streptococcus pneumoniae and Klebsiella pneumoniae. In mice activation of the thrombin receptor PAR4 aids in the host defense to Streptococcus pneumoniae by amplification of initial cytokine production in whole blood. In contrast, reduction of thrombin formation by APC or TFPI is beneficial in baboon studies with E. coli infusion, and accordingly mice with FVIII or TF deficiency display increased host defense to peritoneal induced E. coli sepsis.
From the above it appears that our molecular understanding of the processes instigated by systemic bacteria and the effect on these of the coagulation system in the case of sepsis is still far from complete. This prompted us to investigate how the host response to bacteria in whole blood is actually influenced by coagulation. Whole blood activation assays with LPS or bacteria are typically performed with heparinized blood and overnight incubations while clotting, fibrinolysis, cytokine formation and bacterial killing/clearance are processes that occur in minutes up to 2 hours. We therefore set out to develop a model in which we could determine the short term effect of whole blood coagulation on the host response to bacteria. In this model we observed that in human whole blood exposed to living E. coli TNF expression is inhibited by thrombin formation through increased expression of the inhibitor MKP-1 that dephosphorylates phospho-p38 which is crucial for Hur dependent TNF translation. Down regulation of TNF translation by this thrombin/MKP-1pathway is consistent with the observed increased host resistance to E. coli when thrombin generation is inhibited/prevented since TNF is pivotal for killing of E. coli by macrophages. In contrast, we found that thrombin and thrombin receptor activation in whole blood profoundly potentiates TNF transcription induced by living Streptococcus pneumoniae and as a consequence thrombin greatly boosts TNF expression in reaction to these streptococci. Thus thrombin can be a key regulator in translation or transcription of TNF depending on the bacterial trigger. Differential effects of thrombin on TNF production may have to be taken into account in the course of bacterial infection and sepsis.

PLATELETS / MEGAKARYOCYTES BEYOND HEMOSTASIS

H. Raslova (Paris)

Megakaryocytes and myelofibrosis

The megakaryocyte differentiation (MK-poiesis) is responsible for platelet production and involves two almost unique biological mechanisms in a cell differentiation process: the polyploidisation through a process called endomitosis and the production of mature cells (platelets) through a dynamic cytoplasm fragmentation of megakaryocytes. Proteins necessary for platelet formation and functions are synthetized during megakaryopoiesis and are either components of the cytoplasmic membranes or packaged into granules including dense granules, lysosomes, and α-granules. α-granules, which are the most abundant, contain the extracellular matrix proteins, growth factors, coagulation factors, protease inhibitors and proteoglycans. Recent evidence has shown that megakaryocytes are not only involved in platelet production, but also in the regulation and establishment of the bone marrow environment including the extracellular matrix and the bone. In addition they are also important components of the hematopoietic stem cell niche. Indeed, megakaryocytes produce extracellular matrix and synthesize a lot of growth factors such as TGFβ1 and PDGF, which are usually packaged in α-granules, but can also be secreted. Abnormal secretion of these factors, in particularly TGFβ1, by megakaryocytes in the bone marrow will result in stromal reaction leading to myelofibrosis, osteosclerosis and neoangiogenesis. This abnormal secretion and activation of TGFβ1 can be observed in hereditary or malignant diseases affecting megakaryopoiesis such as Gray platelet syndrome (GPS), a disorder associated with a defect in α-granules or classical non BRC-ABL myeloproliferative neoplasms (MPN). Megakaryopoiesis and platelet production are mainly regulated by the TPO/MPL/JAK2 pathway. The classical non BCR-ABL MPN (Polycythemia vera, PV; essential thrombocythemia, ET; and primary myelofibrosis, PMF) are the consequences of a constitutive activation of this signaling pathway through gain of function mutations of JAK2 and MPL. More recently mutations of calreticulin (CALR) have been identified. These mutations also activate the JAK/STAT pathway by a mechanism, which remains to be understood. The boundaries between these three disorders are not always clear to determine because PV and ET can progress to myelofibrosis. Thus myelofibrosis is an important hallmark of these classical MPNs associated with a poor prognosis. The myelofibrosis observed in MPNs is associated with a defect in megakaryocyte differentiation, which is frequently accompanied by the occurrence of new mutations on genes involved in epigenetic and splicing regulation. A defect in GATA1 translation due to a defect in ribosome biogenesis could be a major determinant of the development of myelofibrosis. GATA1 is one of the key transcription factors regulating MK-poiesis and GATA1 mutations in X-linked syndrome also result in dysmegakaryopoiesis and myelofibrosis.
Whether the mechanisms leading to myelofibrosis in GPS, PMF and myelofibrosis post-PV and ET are common remains to be elucidated. Is the abnormal amplification of MK in bone marrow accompanied by a proportional increase in TGFβ1 secretion and activation sufficient for myelofibrosis development? Or is an enhanced MK activation leading to the premature release of TGFβ1 from α-granules necessary? And finally the important question that should be addressed: how is TGFβ1 locally activated in bone marrow?

S. Massberg (Munich)

Cross-talk between thrombosis and innate immunity

Arterial thrombosis triggers myocardial infarction or stroke, while venous thrombosis may lead to pulmonary embolism, together the most frequent causes of mortality worldwide. Platelet plug formation is a major step of thrombosis; however, recent evidence suggests that in addition to platelets, innate immune effector cells also critically contribute to thrombosis. In vivo imaging approaches have helped to dissect the pathways that control the trafficking of innate immune cells to sites of vascular damage and were essential to define the mechanisms leukocytes operate to foster thrombotic pathways. Over the past years, a large number of thrombosis-related host molecules produced or activated by monocytes and neutrophils have been identified. E.g. activated monocytes and the microparticles (or microvesicles) that they release express intravascular tissue factor (also known as blood cell tissue factor or blood-borne tissue factor) and can promote blood coagulation during thrombus development inside blood vessels. Neutrophils have also evolved cell-specific mechanisms to potentiate thrombosis. When activated, they release neutrophil extracellular traps (NETs), which are comprised of a matrix of DNA and histones that is catapulted out of neutrophils. These NETs are decorated by several components of the antibacterial machinery of neutrophils. However, NETs not only serve antibacterial functions, but also induce a strong procoagulant response. NETs can bind to and activate platelets in the setting of thrombosis. In addition, the extracellular nucleosomes within NETs form a catalytic platform that stimulates the proteolytic activity of neutrophil elastase, which in turn promotes coagulation. While all of these pathways have the potential to trigger pathological thrombosis, we recently showed that they also play a major physiological role in immune defense in a process that we termed immunothrombosis. Indeed, immune cell migration to the vasculature triggering immunothrombosis leads to formation of thrombi, generating an intravascular scaffold that facilitates the recognition, containment and destruction of pathogens, thereby protecting host integrity without inducing major collateral damage to the host. During my talk, I will summarize our recent findings on the trafficking of innate immune effectors to sites of vessel injury and review the mechanisms used by these cells to trigger physiological and pathological thrombosis.

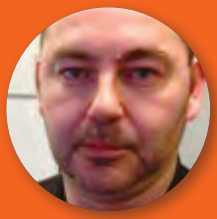
P. Moreillon (Lausanne)

Preventing Infective endocarditis via antiplatelet strategies

Infective endocarditis (IE) is a universally fatal disease if not aggressively treated with bactericidal antibiotics often associated with cardiac surgery. IE pathogenesis relies on multiple factors including predispositions of the host, such as preexisting valve lesions, and features of the pathogens, including surface determinants that promote colonization and invasion of injured endothelia. Since host predisposing factors are largely known, preventing IE has relied on punctual antibiotic administration to at-risk patients prior to medico-surgical interventions promoting bacteremia, such as dental procedures. Yet, epidemiological studies have shown that the risk of IE did not correlate with such procedures, but was rather a lifelong constant threat due to spontaneous low-grade bacteremia occurring during normal activities, such as chewing and tooth brushing. Therefore, antibiotic prophylaxis of EI has been recently mostly abandoned, leaving at-risk patients with no protective measure at all.

Revisiting IE prevention as a yearlong measure includes pathogen-specific and host-specific strategies. Pathogen-specific interventions include vaccines, which are complicated because they should simultaneously cover several different bacteria. In contrast, host predisposing factors are more amenable because they are quasi-universal for all IE pathogens. These include the last steps of the coagulation cascade and platelet aggregation, with which Streptococcus spp. Enterococcus spp and Staphylococcus spp. interact in various ways. Using this approach it was recently shown that modulation of platelet aggregation via acetylsalicylic acid and ticlopidine effectively prevented experimental IE due to streptococci and enterococci, as well Staphylococcus aureus to a lesser extend. In contrast, anticoagulation with acenocoumarol did not prevent IE from any organisms. Moreover, the thrombin inhibitor dabigatran specifically prevented experimental IE due to S. aureus, but not other IE pathogens. This appeared to result from the ability of dabigatran to inhibit S. aureus-induced staphylothrombin, which results from direct activation of prothrombin by S. aureus-secreted coagulase and von Willebrand-binding protein.

Taken together these observations suggest that anti-aggregation strategies, which are commonly used to prevent cardiovascular disease recurrences, could be useful as long-term prevention of IE in at-risk patients. Moreover, dabigatran could have a particular niche against S. aureus IE in patients with prosthetic valves, which is associated with a mortality rate of up to 50%. Case-control studies might provide an answer to these questions.



A. Brill (Birmingham)

New insights into the mechanisms of thrombosis

Thrombosis represents an acute clinical problem. In particular, deep vein thrombosis (DVT) develops in about 1 million people in the US and 25,000 in the UK annually. Despite this fact, mechanisms of thrombus development remain incompletely understood.
Glycoprotein VI (GPVI) and CLEC-2 constitute a family of Immunoreceptor tyrosine-based activation motif (ITAM)-containing receptors with similar downstream signaling pathways. Whereas the role of GPVI in arterial thrombosis has been demonstrated, the role of CLEC-2 is still elusive. We addressed the impact of these receptors in thrombosis using an animal model of DVT (stenosis of the inferior vena cava (IVC)) and laser injury in cremaster muscle.
Mice genetically lacking GPVI as well as mice with pharmacologically depleted receptor had a trend to decreased DVT prevalence. After laser injury, thrombi in GPVI-deficient mice started to grow but were unstable, rapidly detached and thrombus size did not reach the wild-type level. This is in agreement with previously reported role of GPVI in thrombus propagation and its ability to bind fibrin. In contrast, CLEC-2 deficiency did not affect thrombosis in the laser injury model. Mice lacking CLEC-2 on platelets were completely protected against DVT. In an effort to delineate mechanisms of this observation, IVC vessel wall was stained for the CLEC-2 ligand podoplanin. We were unable to detect podoplanin expression after 48 h stenosis of the IVC when the thrombosis was checked, although the possibility that podoplanin may become expressed at earlier time points cannot be ruled out. Intravital microscopy of the IVC 6 h after initiation of stenosis demonstrated reduced platelet recruitment, which may suggest down-regulated endothelial activation crucial for thrombus development in this model. The anti-thrombotic phenotype of CLEC-2-deficient mice was not accompanied by prolonged tail bleeding time.
In conclusion, we have demonstrated differential roles of ITAM receptors in thrombosis with GPVI being involved in thrombus growth after laser injury and CLEC-2 in DVT.

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SATELLITE SYMPOSIUM SPEAKERS CSL-BEHRING

J. Rischewski
Luzern

Dr. Johannes Rischewski is Head of the Pediatric Hemophilia Center and Consultant Pediatric Hemato-Oncologist at the Children's Hospital in Luzern, Switzerland, and lecturer in Pediatric Hemato-Oncology at the University of Basel, Switzerland. Dr. Rischewski went to medical school in Freiburg, Germany and in London, UK. He then undertook specialist medical training in pediatrics and pediatric oncology and hematology in Stavanger, Norway, in Kiel and Hamburg, Germany, as well as in Basel, Switzerland. His main areas of expertise include autoimmune disorders such as idiopathic thrombocytopenic purpura (ITP), and other cytopenias, bleeding disorders, childhood cancer and hematology. Dr. Rischewski has a strong interest in the development of new clotting factors, and is actively involved in ongoing clinical trials.

M.E. Mancuso
Milan

Maria Elisa Mancuso (MD, PhD) is an Hematologist and works as a Clinical Assistant at the Angelo Bianchi Bonomi Hemophilia and Thrombosis Center in Milan (Italy). She obtained a post-degree in Clinical and Experimental Hematology and a PhD in Clinical Methodology. She is involved in clinical research and has published several original articles in peer-reviewed journals as Blood, Journal of Thrombosis and Haemostasis, Haematologica, Thrombosis and Haemostasis, British Journal of Haematology and Haemophilia. She is a member of several scientific societies (ISTH, WFH, ASH, EAHAD). She has been involved as co-investigator in several clinical trials, and she takes care of both children and adults with hemophilia with a specific scientific interest in inhibitors and chronic hepatitis C.

J.. Rischewski
(Luzern)

M.E. Mancuso
(Milan)

SATELLITE SYMPOSIUM SPEAKERS BAXALTA

C. Hermans
Brussels

Cedric Hermans currently heads the Division of Haematology, the Haemostasis and Thrombosis Unit and the Haemophilia Centre of the Saint-Luc University Hospital in Brussels, Belgium. He was appointed Associate Professor at the Medical School of the Catholic University of Louvain in 2003, and Full Professor in 2012.

Professor Hermans completed his medical training and specialisation in general internal medicine at the Medical School and the Saint-Luc University Hospital of the Catholic University of Louvain, Belgium. He obtained his PhD in Biomedical Sciences, specialising in Toxicology, in 1999. In 2000 he took up a one year fellowship in the Haemophilia Centre and Haemostasis Unit of the Royal Free Hospital, London. Professor Hermans has published over 140 original articles in international journals and is a member of several scientific societies and advisory boards. He is treasurer and vice-president of EAHAD. Presently, his main research interests lie in the area of haemostasis and thrombosis, especially clinical studies on the treatment of haemophilia, new anticoagulants, and the management of thrombosis.

A. Gringeri
Vienna

Dr Alessandro Gringeri received his medical degree from Milan University School of Medicine in Milan, Italy. He specialized in Clinical and Laboratory Haematology at the Specialty School of Haematology at the University of Milan. He received his professorship in Internal Medicine from University “La Sapienza” School of Medicine in Rome, a Master’s degree in Pharmacoeconomics from Milan University School of Pharmacy in Milan, and a Master’s degree in Health Organization Management from the Luigi Cattaneo University School of Economics in Castellanza, Italy. Dr Gringeri had served for 30 years as Consultant in Haematology at the Angelo Bianchi Bonomi Haemophilia and Thrombosis Centre, in Milan. Since November 2012, Dr Gringeri is Medical Director for the Medical Affairs Hematology department of the international pharmaceutical company Baxalta (formerly Baxter), for which he is based in Vienna, Austria.

He served as medical member of Executive Committee of the World Federation of Haemophilia, member of the Medical Advisory Group of the European Haemophilia Consortium and of the Italian Federation of Haemophilia Associations (FEDEMO).

T. VandenDriessche
Leuven

Dr. VandenDriessche obtained his PhD at the Free University of Brussels (Belgium) in 1992 and was a visiting fellow at the Weizmann Institute for Science (Israel). He continued his research as a post-doctoral fellow at the National Institutes of Health (USA) in the lab of gene therapy pioneers Dr. R. Michael Blaese, Dr. Rick Morgan and Dr. W. French Anderson, where he started his research on gene therapy for hemophilia. He was subsequently recruited to the University of Leuven (Belgium) and the Flanders Institute of Biotechnology (VIB) at the Center for Transgene Technology & Gene Therapy directed by Dr. Désiré Collen. He had joined the lab of Dr. Rob Kotin & Dr. Marshal Nirenberg at the NIH as a sabbatical fellow. He subsequently worked at the Vesalius Research Center headed by Dr. Peter Carmeliet. In 2010, he became Full Professor at the University of Leuven and at the Free University of Brussels, where he is now tenured Director of the Department of Gene Therapy & Regenerative Medicine. He served as President of the European Society of Gene & Cell Therapy.

C. Hermans
(Brussels)

Outcome Tools in the Life of a Patient

Outcome measures are essential to allow a healthcare team provide a proper management plan for people with haemophilia (PWH) that encompasses all aspects of the patient – their ability to participate fully in everyday life, pursue employment and engage in physical and sporting activities. This presentation will consider adult and elderly patients with haemophilia who may or may not have been following a prophylactic regimen of factor treatment for many years. The degree of joint involvement will be considered, as well as the ways in which this translates into issues that compromise simple tasks of everyday living. We will consider how outcome measures can inform decisions about the need and appropriateness of surgical intervention for arthropathy on an individual level. There will also be a consideration of how these instruments can be relevant to shaping treatment plans by helping patients and physicians to share perspectives and align treatment expectations

A. Gringeri
(Vienna)

Personalized treatment in haemophilia A: what it means today and how to pursue it tomorrow

Prophylaxis is the treatment of choice in people with hemophilia. It has been shown to be effective in reducing bleeding frequency in the great majority of patients, about half of whom experience no more than one bleeding event a year. Unfortunately, the remaining half continue to suffer from multiple bleeds that, when occurring in joints, can cause irreversible damage and a consequential impairment of their health-related quality of life and a greater burden for their caregivers, despite of the use of the same product and the same dosing regimen.
In fact, one size does not fit all, and each individual has a different pharmacokinetic (PK) response to clotting factor concentrate. It is important to know this in order to choose the right prophylaxis regimen that better fits with the clinical status of the patient and the patient's desires and needs. MyPKFiT is a tool that can predict the individual PK response with a few blood samples and help the clinician to adjust the dosing to the patient instead of adjusting the patient to the dosing. The information about the PK characteristics of each product and the individual response to them has become increasingly important especially now, with the availability of extended half-life products. In fact these products on one hand allow a more convenient infusion schedule, on the other hand they might provide insufficient FVIII levels to prevent bleeding, particularly when more intensive physical work is made and intervals between infusions are more prolonged.
In conclusion, in the journey towards a bleed-free world, each prevented bleed matters. To achieve this ambitious goal, "one-size-fits-all" prophylaxis regimens are insufficient, while personalized prophylaxis that provides the right product, at the right dose, at the right time, every time, to the right patient, might aspire to accomplish it.

Gene therapy for hemophilia A and B: the journey continues

T. Vandensriessche
(Leuven)

Thierry VandenDriessche1, Nisha Nair1, Hanneke Evens1, Mario Di Matteo1, Federico Mingozzi2, Caroline Le Guiner3, Pieter De Bleser4, Alessio Cantore5, Nathalie Ward6, Simon Waddington6, John Mc Vey6, Philippe Moullier3, Luigi Naldini5 & Marinee K.L. Chuah1.

1Free University of Brussels & University of Leuven, Belgium; VIB, Ghent, Belgium; INSERM U649, 3University of Nantes, France; 2Généthon, France; 5San Rafaelle Telethon Institute for Gene Therapy, Milano, Italy; 6Institute of Child Health, University College London, London, UK.

The robustness and safety of liver-directed gene therapy for hemophilia could be substantially improved by enhancing factor VIII (FVIII) and factor (FIX) expression in the liver. To achieve this, we developed and validated a new computational approach of rational in silico vector design. This approach relies on a genome-wide bio-informatics strategy to identify cis-acting regulatory modules (CRMs) containing evolutionary conserved clusters of transcription factor binding site motifs that determine high tliver-specific gene expression. Incorporation of these CRMs into adeno-associated viral (AAV) and non-viral plasmid and transposon vectors enhanced gene expression in mice liver 10 to 100-fold, depending on the promoter used. In particular, these CRMs resulted in robust and sustained liver-specific FIX expression and induction of FIX-specific immune tolerance in mice. FIX peak levels of 20-35% could be attained in cynomolgus macaques after AAV-based liver-directed gene therapy. Given its small size (72 bp), the most potent CRM is particularly well suited to boost FVIII expression using AAV vectors, allowing the production of physiologic FVIII at low, clinically relevant vector doses. This obviated the need to boost FVIII expression through modification of its coding sequence by incorporating artificial glycosylation sites. Using a sensitive hepatocellular carcinoma-prone mouse model, we found no evidence of increased genotoxic risk of these CRM elements when integrating vectors are employed. By combining codon usage optimization with the hyperactivating FIX-R338L Padua mutation we obtained a 15-fold gain in potency which was validated using different vector platforms including AAV, integration-competent and –defective lentiviral vectors and transposons, allowing the use of lower and thus potentially safer vector doses. The use of computational vector design, synthetic (hyperactive), FVIII and FIX transgenes are promising avenues to further improve the efficacy, feasibility, and safety of hemophilia gene therapy.

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SATELLITE SYMPOSIUM SPEAKERS NOVO NORDISK

K. Vad

Copenhagen

Novo Nordisk A/S

Knud Vad's educational background is a PhD in Molecular Biology which he has complemented with business training. He has worked for Novo Nordisk for twenty-three years starting his career in the Research Department developing micro-organisms for the expression of biopharmaceuticals. He subsequently transferred to Project Management where he has run projects within diabetes, haemostasis and currently in growth disorders as Project Vice President. His responsibility has included the coordination of all activities in the NovoEight® development programmes

R.E.G. Schutgens

Utrecht

University Medical Center Utrecht, The Netherlands

Roger Schutgens is Head of the Van Creveldkliniek, center for benign hematology, thrombosis and hemostasis and co-founder of the UMC Utrecht Red Blood Cell Center at the University Medical Center Utrecht, The Netherlands. As a consultant hematologist and epidemiologist, his main responsibilities include care, research and education in the area of benign hematology, thrombosis and hemostasis.

Dr Schutgens completed his medical degree at the Catholic University of Leuven in Belgium before completing his PhD at Utrecht University in The Netherlands. His areas of focus include patients predisposed to bleeding, hypercoagulable conditions and congenital abnormalities of platelets. He is

The development, design and purification process of turoctocog alpha - NovoEight® - Practical consequences1,2

K. Vad
(Copenhagen)

Turoctocog alfa is a recombinant factor VIII (rFVIII) with a truncated B-domain made from the sequence coding for 10 amino acids from the N-terminus and 11 amino acids from the C-terminus of the naturally occurring B-domain. Turoctocog alfa is produced in Chinese ham-ster ovary cells without addition of any human- or animal-derived materials. The purification process includes an affinity chromatography step ensuring intact rFVIII in the final product. During secretion some rFVIII molecules are cleaved at the C-terminal of the heavy chain (HC) at amino acid 720, and a monoclonal antibody binding C-terminal to this position is used in the purification process allowing isolation of the intact rFVIII. Viral inactivation is ensured by a detergent inactivation step as well as a 20 nm nanofiltration step. Characterisa-tion of the purified protein demonstrated that turoctocog alfa was fully sulphated at Tyr346 and Tyr1664, which is required for optimal proteolytic activation by thrombin. Kinetic as-sessments confirmed that turoctocog alfa was activated by thrombin at a similar rate as seen for other rFVIII products fully sulphated at these positions. Tyr1680 was also fully sulphated in turoctocog alfa resulting in strong affinity for binding to von Willebrand factor (VWF). The non-clinical data thus confirms the haemostatic effect of turoctocog alfa and, together with the comprehensive clinical evaluation, supports the use as FVIII replacement therapy in patients with haemophilia A.

1. NovoEight® Summary of Product Characteristics Jun-2015
2. Lentz SR et al., Expert Opin Orphan Drugs 2014 ; 2(4) : 419-431

R.E.G. Schutgens
(Utrecht)

One year real-life experience with NovoEight® consequences1,2

NovoEight® (turoctocog alfa), a novel third-generation B-domain truncated recombinant factor FVIII (rFVIII), has been developed for the prevention and treatment of bleeds in patients with haemophilia A. In pivotal phase 3 trials, guardian™1 and guardian™3, NovoEight® provided safe and effective prophylaxis in adults/adolescents (≥12 years) and children (<12 years), respectively.1,2 Here we report on clinical experience with NovoEight® at the Van Creveldkliniek, University Medical Center, in Utrecht over the past year. The Van Creveldkliniek provides care for 827 patients with haemophilia, and more than 600 patients with rare coagulation disorders. In 2014, the Van Creveldkliniek was designated a centre of national expertise in haemophilia.

Data were collected from patients registered at the Van Creveldkliniek, who had haemophilia A without inhibitors, and had been switched from Refacto AF® to treatment with NovoEight®. Patients were treated with recommended doses of NovoEight® for prophylaxis and the treatment of bleeds. Treatment was switched in line with recommendations.3 One patient underwent three surgical procedures (carpal tunnel surgery, and two total knee prostheses) using NovoEight® to maintain haemostasis. The annualised bleeding rate (ABR) was calculated after prophylaxis with NovoEight®. Endpoints were recorded in line with recommendations from Teitel et al.3

In total, 40 patients with haemophilia A (severe [n=21], moderate [n=4], and mild [n=15]) were switched to receive NovoEight®, and of these, 18 patients (86%) with severe haemophilia A were treated prophylactically. Efficacy among patients receiving prophylaxis with NovoEight® (mean ABR 1.44, range 0-3) was comparable to published data for patients on intermediate-dose Dutch prophylaxis regimen (median 1.3 joint bleeds/year).4 To date, no inhibitors have been detected in any of the 28 treated patients (exposure to NovoEight® 1–332 days), and no adverse events have been reported. Incremental recovery of NovoEight® was well above 2 ([IU/dL]/[IU/kg]), and hence better than expected. Three surgeries were successfully performed in one patient receiving NovoEight®, although one procedure was complicated by infected haematoma, however unrelated to NovoEight®. Patients reported satisfaction with NovoEight® and the injection device.

Overall, clinical experience with NovoEight® in the Netherlands over the past year has been positive; safety and efficacy have been demonstrated in 40 previously treated patients with haemophilia A, including one patient undergoing three surgeries.

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Addition of idarucizumab to plasma samples containing dabigatran allows to use routine coagulation assays to diagnose hemostasis disorders

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Background / Introduction: The presence of dabigatran in patients' samples impacts most coagulation assays and can therefore interfere with the confirmation of a lupus anticoagulant or with the diagnosis of hemostasis disorders developing after the initiation of the anticoagulant treatment, such as vitamin K deficiency or acquired haemophilia. Idarucizumab is a humanized antibody fragment that binds and neutralizes the anticoagulant effect of dabigatran; however it is not known if addition of idarucizumab in vitro to human plasma containing dabigatran can reverse the effects of dabigatran on these assays.

Aims: The objective of this study was to determine whether idarucizumab added in vitro to plasma samples spiked with dabigatran fully neutralises the interference of dabigatran in these diagnostic coagulation assays.
Methods / Materials: In preliminary experiments, we identified the coagulation assays sensitive to dabigatran. These assays were then carried out with normal and abnormal plasma samples spiked with 500 ng/ml dabigatran, with or without a 3-fold molar excess of idarucizumab.

Results: The intrinsic and extrinsic factors levels were decreased by 70% and 30%, respectively, in the presence of dabigatran. These measurements were fully normalised after the addition of idarucizumab. The screen and confirm tests used for the detection of lupus anticoagulant were prolonged by dabigatran, to such an extent that the results obtained with some spiked samples falsely suggested the presence a lupus anticoagulant. Conversely, the addition of dabigatran misleadingly corrected an abnormal APC-resistance ratio. The addition of Idarucizumab also allowed the correct identification of normal and abnormal samples with both these assays.

Summary / Conclusions: The in vitro addition of idarucizumab to plasma samples containing dabigatran authorizes the use of routine coagulation assays by fully neutralizing dabigatran, thereby allowing the diagnosis of hemostasis disorders that are concurrently present in patients taking dabigatran.

A novel FGA mutation underlying a case of congenital dysfibrinogenemia with unusual clinical presentation

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Background / Introduction: Case report - A two weeks old boy was referred to the ICU of the University Hospital Brussels because of an inappropriate increase in head circumference and full fontanel. After an uncomplicated prenatal course, he was born prematurely at a gestational age of 34 weeks and 4 days with a birth weight of 2290 g and a head circumference of 34 cm (90 – 97th percentile,) rising to 36 cm after 2 weeks. A cranial ultrasound documented a marked hydrocephalus with remnants of a subependymal hemorrhage in the right lateral ventricle. A MRI and MR angiography confirmed these findings and demonstrated hemosiderin deposits along the ventricular system causing the hydrocephalus. Thrombosis of the right internal cerebral vein was suspected as it could not be visualized on the venous angiogram. Initial coagulation studies showed a normal PT and aPTT, a slightly reduced fibrinogen activity (165 mg/dL) and a prolonged thrombin time. More profound haemostatic work-up was performed in order to rule out the most common causes of coagulopathy (both bleeding and thrombosis) but no abnormalities were found. The reduced fibrinogen activity was confirmed on a second sample. Detailed family history revealed bleeding symptoms such as menorrhagia and epistaxis on the mothers' side. The baby's mother also showed a decreased fibrinogen activity (146 mg/dl), suggesting the possibility of an inherited fibrinogen disorder. Fibrinogen antigen was determined in the mother and the child and was found to be normal. The low fibrinogen activity versus antigen ratio is compatible with dysfibrinogenemia. Genetic analysis of the fibrinogen genes revealed a novel mutation: both the patient and the mother are heterozygous for a previously unreported frameshift mutation in exon 5 of FGA:

c.1415_1416 ins C, with a termination codon immediately after the insertion (CCT GAT > CCC TGA). This patient underwent daily lumbar punctures to release the excessive amount of cerebrospinal fluid, followed by the placement of a definitive ventriculoperitoneal drain.

Summary / Conclusions: Discussion - Congenital dysfibrinogenemia is a rare qualitative fibrinogen disorder characterized by normal antigen levels of dysfunctional fibrinogen, mainly with autosomal dominant inheritance. Since the first patient was identified in 1958, only 500 cases have been reported. The clinical presentation of inherited dysfibrinogenemia is highly heterogeneous: some patients suffer from bleeding, other from thromboembolic complications or both. In this case, imaging showed thrombosis as well as hemorrhage. In the mother's family mild bleeding symptoms were present, specifically menorrhagia and epistaxis. To this day, only 2 cases of intracranial hemorrhage were described in patients with this disorder. About 100 mutations have been reported in congenital dysfibrinogenemia, mostly missense mutations. In this case, an insertion of one nucleotide was discovered in FGA, leading to a premature termination codon.
Conclusion - We described a case of congenital dysfibrinogenemia in a two weeks old premature boy who presented with thrombosis of the right internal cerebral vein and ventricular dilatation as a result of a grade IV intraventricular hemorrhage. Genetic analysis revealed a previously unreported mutation in exon 5 of FGA. These findings stress the importance of including fibrinogen in the work-up of both thrombotic disorders and bleeding complications.

ADAMTS13 containing the naturally occurring mutation p.Arg1177Gln adopts an open conformation

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Background / Introduction: Thrombotic thrombocytopenic purpura (TTP) is caused by a deficiency in the metalloprotease ADAMTS13, due to mutations in the ADAMTS13 gene (congenital TTP) or presence of anti-ADAMTS13 autoantibodies (acquired TTP). Mutations found in the ADAMTS13 gene of TTP patients are valuable to gain more insight into the working mechanism of the enzyme. Recently, we and others demonstrated that ADAMTS13 adopts a closed conformation through interaction between its distal and proximal domains. Cryptic epitopes in the proximal ADAMTS13 domains become exposed after addition of activating anti-distal domain antibodies or after binding of its substrate von Willebrand factor. The presence of three linker regions in the distal domains of ADAMTS13 seem to account for the flexibility and conformational changes of the enzyme. Our in house developed antibody 6A6, which recognizes a cryptic epitope in the proximal metalloprotease (M) domain of ADAMTS13, is a unique tool to distinguish between an open or closed ADAMTS13 conformation. Whether conformational activation of ADAMTS13 protects the enzyme from proteolysis by other coagulation proteases remains to be investigated.

Aims: In this study we aimed at fully characterizing ADAMTS13 in a TTP patient. In addition, we investigated whether the novel p.Arg1177Gln patient mutation induced a conformational change in the enzyme.

Methods / Materials: Patient plasma samples were used to determine ADAMTS13 antigen levels and the presence of anti-ADAMTS13 antibodies. The exons of the ADAMTS13 gene were sequenced and site-directed mutagenesis was used to introduce ADAMTS13 substitutions in the ADAMTS13-pcDNA6.1 expression vector. Wild type (WT) and mutant proteins were produced through stable HEK293 cell lines. ADAMTS13 activity and conformation were analyzed using the FRETS VWF-73 assay and ELISA.

Results: We performed a detailed laboratory analysis of a patient clinically diagnosed with TTP. An undetectable ADAMTS13 activity, a decreased ADAMTS13 antigen level (0.27 ± 0.06 µg/ml, n = 3) and the presence of inhibitory anti-ADAMTS13 antibodies led to the diagnosis of acquired TTP. In addition, we identified the p.Ala900Val polymorphism and a novel p.Arg1177Gln mutation in the patients' ADAMTS13 gene. Since both substitutions were heterozygous, their effect on TTP pathophysiology was expected to be minimal. Interestingly however, the novel p.Arg1177Gln mutation is situated in the third linker region of ADAMTS13 between its distal T8 and CUB1 domains. Indeed, we recently demonstrated that removal of this linker region resulted in a more active, open ADAMTS13. We therefore investigated the effect of this naturally occurring mutation in the third linker region of ADAMTS13 on the activity and conformation of ADAMTS13. Using the FRETS VWF-73 assay we demonstrated that the p.Arg1177Gln mutant was 2.15 times more active than WT ADAMTS13. In line with this, the more active p.Arg1177Gln mutant had a more open conformation. Indeed, the cryptic epitope in the M domain of ADAMTS13 of our anti-ADAMTS13 monoclonal antibody 6A6 was exposed in the p.Arg1177Gln mutant but not in WT ADAMTS13.

Summary / Conclusions: We here show for the first time that a naturally occurring mutation in the third linker region of ADAMTS13 induces a more open conformation and hence increases the proteolytic activity of ADAMTS13. Whether this open

conformation makes ADAMTS13 more susceptible to proteolysis, thereby contributing to the TTP phenotype remains to be determined.

Elevated basal levels of platelet-bound fibrinogen predict the occurrence of sepsis in ICU: a prospective study.

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Background / Introduction: Platelets, active players in thrombosis, also assist and modulate inflammation and immunity. They may act as circulating sentinel cells that first encounter blood borne infectious products for activation of innate immune responses.

Aims: We evaluated platelet activation markers as potential predictive markers of sepsis among 4 common populations of patients admitted to intensive care unit (ICU).

Methods / Materials: 99 non-infected ICU patients (64 ± 15 year-old) were prospectively screened at day 1-2 (T1) and day 3-4 (T2) of admission after elective cardiac surgery, trauma, acute neurologic dysfunction or prolonged ventilation (>48h). A third sample was drawn when infection was diagnosed (Tx). We evaluated platelet activation by measuring the expression of P-selectin (CD62P) and fibrinogen binding on cell surface before and after stimulation with major platelet agonists (ADP, collagen, and TRAP) in flow cytometric assays. We also measured platelet counts, and plasma levels of IL-2, TNF-a, IL-10, sCD40L, IL-17A, IL-6, IL-7, IFN-g, CRP, fibrinogen, and D-dimers. Clinical scores were recorded at admission.

Results: Patients who developed sepsis (n=19) presented with significantly higher basal levels of platelet-bound fibrinogen (MFI: 2318 [1755-3580] versus 1401 [1058-2128], p=0.00074) at T1 compared with patients who did not get infected (n=80). These patients also showed higher plasma levels of CRP (mg/l: 5.1 [2.5-17.4] versus 2.5 [1.1-9.1], p=0.046), D-dimers (µg/l : 1997 [1015-6375] versus 495 [320-1445], p=0.0008), and lower plasma levels of IL-2 (pg/ml: 1.7 [0-5.4] versus 8.9 [0.84-12], p=0.002) and of sCD40L (pg/ml: 36.3 [29-55.3] versus 73.7 [39.3-110.5], p=0.037). Neurological patients were more susceptible to develop sepsis (Chi-square test). In multivariate logistic regression analysis, the SOFA clinical score, the levels of platelet-bound fibrinogen and the neurological patient group remained predictive of sepsis.

The sensibility and specificity of our predictive model were 61.1% and 94.7%, respectively (PPV=73.3%, NPV=91%).

Summary / Conclusions: Predisposition to sepsis in selected critically ill medico-surgical adults can be identified on day 1-2 of admission based on basal levels of platelet-bound fibrinogen. This platelet activation marker may add incremental prognostic information to clinical scoring.

Table 1. Multivariate logistic regression model of sepsis prediction in intensive care units (n=93)

	ICU-acquired sepsis	
Predictive variables	*Coefficient ± SE*	*p-value*
Age	-0.037 ± 0.031	0.23
Sex	0.81 ± 0.94	0.39
Acute neurologic dysfunction	2.2 ± 1.1	0.050*
Aspirin before admission	1.3 ± 0.99	0.18
Diabetes	-1.1 ± 1.3	0.41
SOFA at admission	0.52 ± 0.16	0.0011**
CRP [1]	-0.35 ± 0.66	0.59
sCD40L [1]	0.28 ± 0.42	0.51
D-dimers [1]	-0.032 ± 0.98	0.97
Platelet-bound fibrinogen[1,2]	5.3 ± 2.1	0.013*

[1] *Logarithmic transformation*
[2] *Basal levels of platelet-bound fibrinogen*

The role of ADAMTS13 and VWF in Patients with Acute Ischemic Stroke: a Case Control Study

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Background / Introduction: Von Willebrand Factor (VWF) plays a major role in platelet thrombus formation. Its activity and thus thrombogenicity is controlled by the metalloprotease ADAMTS13. From animal models it has become clear that absence of VWF is protective in ischemic stroke whereas absence of ADAMTS13 worsens disease outcome. Increasing evidence shows a clear association between VWF levels and acute ischemic stroke in humans but this association is less clear for ADAMTS13.

Aims: To compare plasma levels of ADAMTS13 and the ratio of VWF:ADAMTS13 levels between ischemic stroke patients and healthy controls.

Methods / Materials: In total 105 patients with acute ischemic stoke (AIS) or transitory ischemic attack (TIA) and 97 healthy volunteers (HV) were included. Blood was taken at days 0, 1 and 3 in patients with AIS or TIA and once in HV. ADAMTS13 levels were measured by ELISA and correlated with demographic and clinical parameters by multivariate linear regression and Kruskal-Wallis analysis. VWF:ADAMTS13 ratio's were determined using VWF levels that were measured in a previous study.

Results: Patients with AIS or TIA had significantly lower ADAMTS13 levels on day 0, 1 and 3 after stroke (82.6% ± 21.0%, 85.3% ± 21.6% and 80,2% ± 20.7%; respectively) compared with HV (110.6% ± 26.9%; $p < 0.0001$). Likewise, the VWF:ADAMTS13 ratio was significantly higher in stroke patients on all time points analyzed (2.7 ± 1.9, 2.7 ± 2.3 and 3.1 ± 2.3; respectively) compared with HV (1.1 ± 0.5; $p < 0.0001$). After adjustment for age and sex, these results remained significant ($p < 0.01$).
ADAMTS13 levels were associated with sex ($p = 0.018$). No association was found with age ($p = 0.101$), disease modality (TIA vs. AIS; $p = 0.780$), stroke etiology (TOAST criteria; $p = 0.668$), anti-platelet therapy ($p = 0.198$) or thrombolysis ($p = 0.255$). Lowest ADAMTS13 levels were found in patients with the most severe stroke as assessed with the National Institutes of Health Stroke Scale (NIHSS), the modified Rankin Scale (mRS) and Barthel Index (BI) although this difference did not reach statistical significance ($p > 0.05$). Interestingly, the VWF:ADAMTS13 ratio was significantly associated with stroke severity (NIHSS: $p = 0.048$; mRS: $p = 0.015$ and BI: $p = 0.004$) and stroke modality (AIS or TIA; $p = 0.023$).

Summary / Conclusions: In this study we found significant lower ADAMTS13 levels in ischemic stroke patients compared with healthy controls. This difference was even bigger for the ratio of VWF:ADAMTS13 levels. These results confirm the importance of the VWF/ADAMTS13 axis in ischemic stroke and put forward the ratio VWF:ADAMTS13 as a potential biomarker for stroke risk and severity.

BASIC RESEARCH

High and long-term expression of von Willebrand factor after Sleeping Beauty transposon-mediated gene therapy in mice

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Background / Introduction: Type 3 von Willebrand disease (VWD), the most severe form of VWD, is characterized by the complete absence of the von Willebrand factor (VWF) protein. Current treatment options are limited to exogenic VWF/FVIII

plasma concentrates, which only provide a short-term solution given the relatively short half-life of VWF (8-12h). Since there is a great demand for long-term treatment options, gene therapy could become a promising alternative for the treatment of VWD.

Aims: Achieve long-term expression of VWF using the non-viral Sleeping Beauty (SB)-transposon technology in a mouse model of severe VWD.

Methods / Materials: The SB-transposon technology is a non-viral gene therapy strategy that has promising long-term therapeutic potential because of its integrative features. We constructed three different transposon plasmids containing full length murine (m)VWF cDNA. The first two transposon plasmids contained the mVWF cDNA under control of either the ubiquitous CAG-promoter or the liver-specific α1-antitrypsin promoter. The third plasmid consisted of the mVWF cDNA under control of the liver-specific promoter but in combination with a sandwich SB-transposon design, specifically created to transpose larger transgenes. Each transposon plasmid, together with a second plasmid encoding the SB100X transposase, was targeted to the liver of VWF-deficient mice via hydrodynamic gene delivery. VWF antigen levels were measured at regular time intervals using ELISA and correction of the bleeding diathesis was investigated using a tail-clip bleeding assay and a saphenous vein bleeding model.

Results: Use of the SB-transposon in combination with the CAG-promoter resulted in mVWF levels of 134.8±20.3% 3 days after gene transfer (n=24). These levels declined to 1.3±0.4% (n=21) 116 days after gene transfer, but could still be detected in plasma more than 1 year after gene transfer (< 1% of normal levels, n=5). However, when using the more potent liver-specific promoter, significantly higher VWF-plasma levels were observed (22.1±3.7% 6 months after gene transfer, n=22). Most interestingly, use of the sandwich SB-transposon resulted in very high mVWF levels (>2000%) that stabilized around 42 days (315.1±79.1%, n=13) and remained supraphysiological 1 year after gene transfer (245.0±92.4%; n=9). In these mice, transposition of the transposon into the mouse liver genome was confirmed using splinkerette PCR. Importantly, twelve weeks after gene transfer tail-clip bleeding was (partially) corrected in 3 out of 14 mice and blood loss was significantly reduced in 6 out of 14 mice. Using a saphenous vein bleeding model 36 weeks after gene transfer, a slight (but statistically not significant) reduction in bleeding time was observed in treated mice (139.0±19.4 s; n=9) compared to untreated VWF-deficient mice (220.9±20.5 s; n=17). The proportion of high molecular weight multimers in long-term liver-expressed VWF was lower than in normal plasma VWF, which could explain why some mice were not phenotypically corrected despite high VWF levels.

Summary / Conclusions: The powerful SB-transposon system efficiently transposes the large mVWF cDNA into the host genome, resulting in long-term and sustained VWF-expression in a liver-based gene therapy platform. The sandwich SB-transposon resulted in robust and supraphysiological VWF-expression that was maintained >1 year after gene transfer. Long term partial correction was observed in some mice expressing transgene mVWF.

Platelet Endothelial Aggregation Receptor 1: a novel modifier of neo-angiogenesis

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Background / Introduction: Platelet Endothelial Aggregation Receptor 1 (PEAR1) is a cell membrane protein, expressed on platelets and endothelial cells (ECs). We previously reported that PEAR1 sustains platelet aggregation via αIIbβ3-activation and that knockdown of PEAR1 in megakaryocyte progenitor cells resulted in increased platelet production, revealing a role for PEAR1 in attenuating megakaryopoiesis. Although PEAR1 expression is highly correlated with the expression of Endoglin (ENG), ALK1 and ANG2 (3 genes that are very important in the pathogenesis of Pulmonary Arterial Hypertension and Hereditary Haemorrhagic Telangiectasia), its effective role in EC biology remained to be elucidated.

Aims: The aim of this study was to determine the expression of PEAR1 in human endothelium of various tissues and to investigate its role in ECs in vitro and in (neo)-angiogenesis, using Pear1-/- mice.
Methods / Materials: We found PEAR1 on the cell membrane and on filo- and lamellipodia of human cultured ECs and showed that its expression coincides with CD31 in various tissues (immunofluorescence (IF) and immunohistochemistry (IHC)). PEAR1 expression (qPCR) is variable in ECs of different origin with low expression in ECs with a rapidly proliferating phenotype (e.g. Blood Outgrowth Endothelial Cells; BOECs) and high expression in mature, non-proliferating ECs (e.g. microvascular ECs of liver and heart). Lentiviral knockdown of PEAR1 in cultured ECs doubled EC proliferation (FACS analysis for Edu) and significantly stimulated EC migration (scratch assays), in turn enhancing in vitro tube formation on matrigel through the Akt/PTEN-dependent p21/CDC2-pathway (western blot). Even when physiological blood vessel formation

was unaffected in Pear1-/- mice (IHC), neo-angiogenesis in these mice was significantly increased in a hind limb ischemia ligation model (4.7-fold increase in capillary density in the ligated limb of Pear1-/- mice compared to ligated limbs in WT-mice; increased limb perfusion and capillary density via microCT imaging and Laser Doppler Imaging – Figure 1). PEAR1 knockdown in mice resulted in a 2-fold faster wound closure due to enhanced neo-angiogenesis.

Results: See methods section

Summary / Conclusions:
We established an inverse correlation between endothelial PEAR1 expression and vascular assembly both in vitro and in vivo. These results identified, for the first time, a critical role for PEAR1 in neo-angiogenesis and make this gene of interest for further research in the field of tumour angiogenesis or pulmonary hypertension.

ADAMTS13-mediated thrombolysis of t-PA resistant occlusions in ischemic stroke

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Background / Introduction: Currently, tissue plasminogen activator (t-PA) is the only approved thrombolytic drug for ischemic stroke. However, t-PA does not always result in efficient thrombus dissolution and subsequent blood vessel recanalization, suggesting other factors may be involved in thrombus stabilization. Hence, there is a pressing demand for alternative and/or supporting strategies for acute stroke thrombolysis. ADAMTS13 is a metalloprotease that cleaves von Willebrand factor (VWF), a crucial factor in platelet thrombus formation.

Aims: In this study we assessed the thrombolytic potential of ADAMTS13 in the setting of acute ischemic stroke.

Methods / Materials: Thrombi were retrieved via thrombectomy from patients with ischemic stroke. Thrombus composition was histologically assessed via classical H&E staining, MSB staining and anti – VWF antibodies. The thrombolytic potential of ADAMTS13 was assessed in a murine model of thrombotic stroke in which a VWF-rich thrombus occludes the middle cerebral artery (MCA).

Results: To better understand thrombus composition and its effect on t-PA resistance, we analyzed thrombi retrieved from 31 ischemic stroke patients and found a distinct presence of VWF in various samples. Almost half of these thrombi (48%, 15 out of 31) were particularly rich in VWF, containing over 20% VWF. We hypothesized that ADAMTS13 can exert a thrombolytic effect on these VWF-containing thrombi by cleaving VWF that is holding platelets together. As a proof of concept we showed that recombinant human ADAMTS13 (rhADAMTS13) was able to completely degrade ristocetin-induced platelet-VWF agglutinations. Next, we set up an in vivo murine stroke model in which stroke is caused by a VWF-rich thrombus formed in the right MCA after topical FeCl3 application. Remarkably, administration of t-PA (10 mg/kg) did not lead to MCA recanalization, showing this thrombus was resistant to t-PA induced fibrinolysis. Intriguingly, however, infusion of rhADAMTS13 (400-3500 U/kg) 5 min after occlusion dose-dependently dissolved these t-PA resistant thrombi resulting in fast restoration of MCA blood flow. This ADAMTS13-induced dissolution of MCA thrombi was associated with reduced brain injury 24 h after stroke ($p < 0.005$). Finally, we also assessed the thrombolytic potential of rhADAMTS13 in a more clinically realistic time window. Delayed rhADAMTS13 administration 60 min after occlusion was still capable of destabilizing the thrombus, thereby partially restoring MCA blood flow and reducing ischemic stroke injury ($p < 0.05$).

Summary / Conclusions: These data show for the first time a potent thrombolytic activity of ADAMTS13 on VWF-rich thrombi in the setting of stroke. Since histological analysis of human stroke thrombi showed various amounts of VWF, with some particularly rich in VWF, ADAMTS13 might become useful in future treatment of acute ischemic stroke.

Endothelial cells do not express the vasopressin receptor 2 that is required for the release of FVIII and VWF following DDAVP treatment

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Background / Introduction: Most patients with mild/moderate haemophilia A or type 1 von Willebrand disease can be treated by the administration of the selective V2 vasopressin receptor agonist 1-desamino-8-D-arginine vasopressin (DDAVP), which has been commercialised under the name of Minirin® or Octim® sprays. Administration of this drug causes a 2-3-fold increase in FVIII and VWF concentration in plasma within 30 minutes. These changes are not observed in patients with congenital nephrogenic diabetes insipidus who have a mutation in AVPR2, encoding the vasopressin 2 receptor (V2R). This suggests that this receptor plays a role in the effects of DDAVP on hemostasis. However it is not known whether endothelial cells express V2R for direct activation by DDAVP or whether DDAVP activates these cells to release FVIII and VWF through an indirect mechanism.

Aims: To determine whether DDAVP can activate endothelial cells directly through V2R, we determined whether AVPR2 is expressed in endothelial cells by quantitative RT-PCR analysis.
Methods / Materials: RNA was extracted from intestinal and lung microvascular endothelial cells and from umbilical artery and vein endothelial cells and converted to cDNA. cDNAs were also prepared from commercially available total RNA from human liver and human renal epithelial cells and from CHO cells transfected with an AVPR2 expression vector. For each cDNA sample, FVIII, VWF and AVPR2 expression levels spanning exons 19 to 20, 27 to 28 and 1 to 2, respectively, were evaluated by Taqman qPCR. In each cell line, AVPR2 cDNA levels were normalised against glyceraldehyde 3-phosphate dehydrogenase (GAPDH) cDNA levels and the relative fold increase in expression compared to human renal epithelial cells was calculated using the delta-delta CT method. AVPR2 qPCR specificity was confirmed by analysis of the PCR products by gel electrophoresis.

Results: As expected, AVPR2 cDNA was detected in CHO cells overexpressing V2R and in kidney epithelial cells. In contrast, AVPR2 cDNA levels were below the detectable limit in microvascular intestinal cells, lung endothelial cells, umbilical artery cells and vein endothelial cells. AVPR2 cDNA was also undetectable in liver extract, indicating that liver sinusoidal endothelial cells, which are responsible for the production of most FVIII, do not express the receptor either. VWF cDNA was detected in all endothelial cells tested and in liver extract, albeit at a 30 to 450-fold lower level than that of endothelial cells. FVIII cDNA was detected only in liver extract and, at a 6–fold lower level, in microvascular intestinal cells.

Summary / Conclusions: Although V2R is necessary for the release of FVIII and VWF following treatment with DDAVP, it was not detected in any of the endothelial cells tested here. In addition, expression of this receptor was also not detected in cells from the liver, the organ that produces most of the circulating FVIII. The release of FVIII and VWF following DDAVP administration is therefore likely to be mediated by an indirect mechanism involving either hemodynamic change or the release of mediator(s) that secondarily activate endothelial cells.

Inhibition of TAFI and PAI-1 protects mice from cerebral ischemia/reperfusion injury

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Background / Introduction:
Despite recent advances made in stroke prevention and therapy, ischemic stroke is still one of the leading causes of death and disability in the world. Strikingly, progressive stroke is a feature also seen in patients in which rapid revascularization was achieved. Cerebral ischemia/reperfusion is associated with activation of the coagulation cascade and fibrin deposition in cerebral microvessels contributing to the no-reflow phenomenon. Both thrombin activatable fibrinolysis inhibitor (TAFI) and plasminogen activator inhibitor 1 (PAI-1) attenuate fibrinolysis. Inhibition of TAFI and/or PAI-1 might thus have beneficial effects in cerebral ischemia/reperfusion injury.

Aims: To evaluate the therapeutic potential of inhibiting TAFI and PAI-1 in the setting of cerebral ischemia/reperfusion injury.
Methods / Materials: TAFI and PAI-1 were inhibited by monoclonal antibodies (MA) one hour after transient middle cerebral artery occlusion in mice (MA-TCK26D6 and MA-33H1F7, respectively). Twenty-three hours after reperfusion mice were neurologically scored via the Bederson score and the grip test, thrombotic burden was assessed in the brain by fibrin(ogen) western blotting and cerebral infarct sizes were calculated via TTC-staining.

Results: Both anti-TAFI (25 mg/kg) or anti-PAI-1 (6 mg/kg) MAs significantly decreased cerebral fibrin(ogen) deposition

24 h after stroke compared with an IgG control MA ($p < 0.01$). This decrease in fibrin(ogen) deposition reduced cerebral infarct sizes by 50%: 37.0 ± 5.6 mm3 (n = 14) for anti-TAFI MA and 36.0 ± 6.0 mm3 (n = 18) for anti-PAI-1 MA compared with 80.6 ± 11.1 mm3 (n = 9) for IgG control MA ($p < 0.01$ and $p < 0.05$, respectively). Concurrently, both neurological and motor performance was significantly improved after treatment ($p < 0.01$ and $p < 0.05$ for anti-TAFI MA and anti-PAI-1 MA, respectively). Interestingly, targeting of PAI-1 or TAFI with a lower dose of inhibiting antibody (1 mg/kg, n = 10/group) lost its protective effect, but combined targeting of TAFI and PAI-1 at the same low dose reduced both cerebral fibrin(ogen) deposition and infarct sizes by 50% (42.5 ± 8.0 mm3; n = 11; $p < 0.05$). Finally, no cerebral hemorrhages were observed in any of the conditions tested.

Summary / Conclusions: We demonstrated that inhibition of TAFI or PAI-1 is protective in a mouse model of cerebral ischemia/reperfusion. Importantly, combined inhibition showed a synergistic protective effect. These results put forward combined TAFI and PAI-1 inhibition as a promising safe target for the treatment of cerebral ischemia/reperfusion injury.

CLINICAL & LABORATORY

Assessment of sensitivity for coagulation factor deficiency in routine coagulation tests

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Background / Introduction: Factor sensitivity of activated partial thromboplastin time (APTT) and prothrombin time (PT) depend on reagent composition and laboratory equipment. Clinical and Laboratory Standards Institute (CSLI) guidelines recommend a prolongation of the APTT/PT when factor levels are below 30% (1). Recommendations state that every laboratory should assess their own factor sensitivities. According to literature, assessment according to the CLSI with factor deficient plasmas can give inconsistent results. The use of well-defined patient samples is advised but difficult to obtain (2).

Aims: To establish and compare factor sensitivities by the use of factor deficient plasmas and patient samples.

Methods / Materials: APTT and PT were performed with the STA-PTTA and Neoplastine CI plus reagent on the STA-R Evolution (Diagnostica Stago, Asnières, France). Intrinsic and extrinsic factor levels were analyzed with STA-C.K.Prest and Neoplastine CI plus reagent, respectively. In-house made normal pooled plasma (NPP), lyophylized STA-immunodeficient and STA-deficient plasmas were used to make dilution series (all by Stago). A series of 10 spiked samples with increasing factor concentrations by mixing increasing volumes of NPP with factor deficient plasma and a series of 10 single factor deficient patient plasmas were evaluated for each coagulation factor. APTT or PT and respectively intrinsic or extrinsic factors were measured. Factor sensitivity was deduced graphically. The factor sensitivity is defined as the level of factor from where on a prolongation of APTT or PT was observed.

Results: The table represents factor sensitivities for APTT and PT determined by the spiking experiment and by the use of factor deficient patient samples. Except for FIX, FXI and FXII, levels are comparable.

	Factor sensitivity - spiking experiment	Factor sensitivity - patient samples
FII	37%	ND
FV	35%	42%
FVII	48%	44%
FX	41%	53%
FVIII	50%	55%
FIX	52%	69%
FXI	65%	81%
FXII	46%	78%

Factor sensitivities for APTT (FVIII, FIX, FXI and FXII) and PT (FII, FV, FVII and FX).

Summary / Conclusions: Factor sensitivity of STA-PTTA and Neoplastine CI Plus is in accordance with CLSI (<30%). Very mild deficiencies of FXI that may lead to an important bleeding risk will be detected also. Results of this study should be carefully interpreted as factor sensitivity is depending on the whole laboratory setting. Discrepancy between the sensitivity level obtained through spiking experiments and patient sample analysis were observed only in the APTT.

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The order of draw: much ado about nothing?

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Background / Introduction: The pre-analytical phase is crucial for good quality laboratory results. The 'order of draw' has been advocated since 1982 to reduce the risk of cross-contaminating blood tubes with additives from a previously filled tube. The current 'correct order of draw' is: blood cultures, coagulation (citrate), non-additive, clotactivator, NaHeparin, Liheparin, EDTA, acid citrate dextrose and fluoride/oxalte tubes. Furthermore: a discard tube is te be used if the citrate tube is the first te be drawn.

Aims: We wanted to evaluate the effect of the 'order of draw' on the prothrombin time/international normalized ratio (PT/ INR) and the activated partial thromboplastin time (APTT) when the citrate tube is drawn as the first tube, second tube or after a heparin, EDTA or serum tube with clot activator.

Methods / Materials: The study was conducted in two phases. In each phase, approxately 100 consecutive outpatients referred to the oral anticoagulant clinic of the Onze-Lieve-Vrouw hospital in Aalst, Belgium, for the routine monitoring of their oral antivitamin K medication. According to the experimental design, multiple blood tubes were collected using the Sarstedt Safety Monovette System (Sarstedt, N€umbrecht, Germany). In phase 1, the following tubes were collected in this order, respectively: citrate – citrate (reference) – lithium heparin – citrate, and in phase 2: serum with clot activator – citrate – citrate (reference) – K3-EDTA –citrate.

Table 2. Summary of the results

	PT (INR)		APTT (s)	
	Median (IQR)	Mean bias (95% CI) 95% significance	Median (IQR)	Mean bias (95% CI) 95% significance
Phase 1				
Reference	2.6 (2.1–3.0) (n = 95)		33.9 (31.6–37.0) (n = 95)	
First tube	2.6 (2.1–3.0) (n = 95)	0.001579 (−0.02008 to 0.02323) P = 0.6205	34.5 (31.8–37.0) (n = 95)	0.1379 (−0.04729 to 0.3231) P = 0.0227
After heparin	2.6 (2.1–3.0) (n = 94)	0.002021 (−0.01107 to 0.01511) P = 0.4915	33.7 (31.4–36.7) (n = 93)	−0.1742 (−0.4069 to 0.05855) P = 0.2668
Phase 2				
Reference	2.7 (2.2–3.3) (n = 91)		34.0 (31.4–37.0) (n = 93)	
After EDTA	2.7 (2.2–3.3) (n = 91)	−0.01264 (−0.04161 to 0.01633) P = 0.7603	34.0 (31.2–36.8) (n = 93)	−0.2249 (−0.4012 to −0.04870) P = 0.0016
After serum	2.7 (2.2–3.3) (n = 91)	0.007033 (−0.01554 to 0.02961) P = 0.3388	34.4 (32.0–37.4) (n = 93)	0.2180 (0.032860 to 0.4032) P = 0.0215

PT, prothrombin time; INR, international normalized ratio (reference range: <1.2); APTT, activated partial thromboplastin time (reference range: 24–31 s); CI, confidence interval; IQR, interquartile range.
P-values derived from Wilcoxon signed rank test.

The collected tubes were transported to the laboratory within 15 min after collection and centrifuged at 1500 g for 10 min at room temperature. Following separation, the PT/INR and APTT were measured on all citrate tubes using a Siemens BCS XP coagulation analyser, using Innovin as the PT reagent and Actin FSL as the aPTT reagent (Siemens Healthcare, Brussels, Belgium). The results of the analysis were expressed as median and interquartile range. The size and significance of the bias between the results of the different tubes and the reference tube in both experiments were determined with Wilcoxon signed rank tests and Bland–Altman plots using MEDCALC for WINDOWS version 11.6 (MedCalc Software, Ostend, Belgium). A P-value <0.05 was considered statistically significant.

Results:
We studied 193 patients receiving oral anticoagulation. A summary of the results is available in the table below. There was no statistically significant influence on the PT/ INR. The same applies for the APTT measured on a citrate tube drawn after a heparin tube. There was a small, but statistically significant bias on the APTT when the citrate tube was drawn as the first tube, after an EDTA tube or after a serum tube with clot activator. We consider this bias (max. 0.2 s) as not clinically significant.

Summary / Conclusions: The order of draw has no significant influence on the PT/INR and APTT when measured on a Sarstedt citrate tube filled without the use of a discard tube or after a heparin, EDTA or serum tube with clot activator. These results are in line with other studies that evaluated the necessety of the discard tube for coagulation testing and studies that evaluated the influence of potential cross-contamination of blood tube additives on selected biochemistry tests. Our results add to the growing evidence that nowadays, using modern vacuum blood collection systems, the order of draw is indeed much ado about nothing.

Comparison of HIL interferences on three hemostasis analyzers and contribution of a preanalytical module for routine coagulation assays

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Background / Introduction: Preanalytical errors are an important part of routine coagulation laboratory errors, with consequences on laboratory efficacy and on patient safety. The leading preanalytical issues in hemostasis include insufficient filled tubes, clotted samples and hemolyzed, lipemic or icteric samples (HIL). Automation in preanalytical problems detection has recently been implemented on specific hemostasis analyzers for the detection of HIL, filling volume and undue clotting.

Aims: 1° To assess the added value of a preanalytical module integrated to ACL TOP 550 (Werfen) on coagulation results by comparison with visual inspection of the samples.
2° To evaluate the interference of cell-free hemoglobin (hemolysis), bilirubin (icterus) and turbidity (lipemia) on a mechanical analyzer (STA-R Evolution, Stago) and on two optical analyzers with an integrated preanalytical module (CS-5100, Siemens and ACL TOP 550).

Methods / Materials: A total of 3058 samples of the routine coagulation laboratory were randomly collected and routine coagulation tests, including prothrombin time (PT), activated partial thromboplastin time (aPTT), D-dimer and fibrinogen, were performed on STA-R Evolution and ACL TOP 550. Preanalytical problem detection was performed by visual check of the samples and by the new preanalytical module integrated on ACL TOP 550. Additional tests were performed by adding increasing concentrations of exogenous solutions of hemoglobin (Hb), bilirubin or triglycerides (TG) to different pools of plasma. The pools were tested for PT, aPTT, fibrinogen and D-dimer on STA-R Evolution, CS-5100 and ACL TOP 550.

Results: Visual inspection detected statistically less samples than the preanalytical module integrated on ACL TOP 550 (1.9 % vs 6.6 %) ($p < 0.001$). The majority of the samples were rejected for poor filling (1.6 % with the visual inspection and 6.1% by ACL TOP 550). ACL TOP 550 flagged 27 samples (0.9%) on basis of the HIL index, but didn't detect clots although present in 12 samples (0.4 %) as detected manually.
The impact of HIL on hemostasis testing was automate- and reagent- dependent. Test results deviated from Ricos bias criteria (Ricos et al., 2014) at a level equal or greater than the hemolysis and lipemia visual threshold. Unlike on optical analyzers, D-dimer results on the mechanical analyzer were strongly influenced by hemolysis. A significant bias was observed on all analyzers for PT, aPTT and fibrinogen with icteric pools but not on D-dimer levels. Icteric samples were mainly detected by the preanalytical module.

Summary / Conclusions: HIL interferences appear on assays when the concentration of Hb, bilirubin or TG exceeds a certain threshold that is analyzer- and reagent- dependent. Automatic and standardized check of routine coagulation samples by ACL TOP 550 increases the accuracy and consistency in detection of preanalytical issues as compared to visual

inspection only. To our opinion, the main advantages are the standardization and automation of the detection of insufficient filled tubes and icteric samples that are not detect visually. The technology had no advantages for the detection of undue clotting. Automatic threshold for hemolysis and lipemia detection could be useful if more accurate than the visual detection to determinate the level influencing test results. From this study, we learn that whatever the automate used (mechanical or optical), HIL influenced the results of routine coagulation tests, and it should be assessed if the interference is clinically significant or not before delivering the result to the clinician.

Phenotypic characterization of a patient with Glanzmann Thrombasthenia caused by a novel homozygous mutation in the ITGA2B gene

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Background / Introduction: Glanzmann Thrombasthenia (GT) is a rare congenital bleeding disorder caused by a defect in platelet receptor αIIbα3. It is characterized by impaired platelet aggregation that classically causes mild to severe mucocutaneous bleedings. We present here the case of a 52-year old woman who was diagnosed with GT at birth due to umbilical cord bleeding. In 2009, the patient presented with hemorrhage in the upper gastro-intestinal tract, which led to hemorrhagic shock. Platelet transfusion turned out to be inefficient, as the patient had developed anti-platelet antibodies (anti-HLA class II, anti-GPIbIX and anti-αIIbα3). Hemostasis was restored using recombinant FVIIa. Since then, the patient exhibited recurrent but moderate epistaxis.

Aims: To better characterize the patient hemostatic dysfunction and identify the underlying αIIbα3 mutation.

Methods / Materials: Initial laboratory work-up consisted in standard routine tests including whole blood cell count and coagulation screens (prothrombin time, PT; activated partial thromboplastin time, aPTT; thrombin time, TT and fibrinogen levels). Primary hemostasis was evaluated by measuring closure time using PFA-100® and by light-transmission aggregometry in platelet-rich plasma (PRP). Platelet surface αIIbα3 levels were assessed in whole blood by flow cytometry analysis using PerCP-coupled anti-CD61 antibody. Clot retraction was evaluated in whole blood drawn on a tube containing a clot activator (silica particles) and incubated for 24h at 37°C. In addition, thrombus retraction was assessed in PRP in which erythrocytes (2%, V/V) were added to visualize the platelet clot. Either calcium (20 mM) or thrombin (1 U/ml) was added to the PRP and tests tubes were incubated at 37°C. Thrombus formation and retraction were evaluated every 30 minutes up to 4h. ITGA2B and ITGB3 genes were analyzed using high resolution melting and direct exon sequencing.

Results: Laboratory investigations displayed the classical phenotypic presentation of GT. Blood cell count and in particular platelet count was within normal range (150-350 x103/mm3). Routine coagulation tests (PT, aPTT, TT) showed no abnormality. PFA-100 closure time was markedly prolonged using both collagen-epinephrine (>300 sec) and collagen-ADP cartridges (>292 sec). As expected, PRP aggregation assay showed absence of platelet response to ADP (5 µM), collagen (2 µg/ml), epinephrine (5 µM) and arachidonic acid (1 mM), while ristocetin-induced agglutination remained normal. The expression of αIIbα3 integrin was reduced to 14% of normal as compared to an age-matched control population. Clot retraction was severely impaired in both recalcified PRP and whole blood. Thrombus retraction was virtually absent 20 min and up to 4 hours upon recalcification and remained strongly inhibited even 4 hours after addition of 1 U/ml thrombin, as compared to PRP from a healthy donor. Genetic analysis revealed a previously unidentified mutation in exon 18 of the ITGA2B gene. The missense mutation (c.1722A>C) led to the substitution of the Asp591 to an Ala residue of the αIIb subunit. Intriguingly, our patient was homozygous for the mutation although no notion of consanguinity appeared in her family history.

Summary / Conclusions: We identified a novel mutation in the αIIb subunit resulting in Glanzmann Thrombasthenia.

Pneumatic tube transport is feasible for Rotem coagulation tests in a Central laboratory

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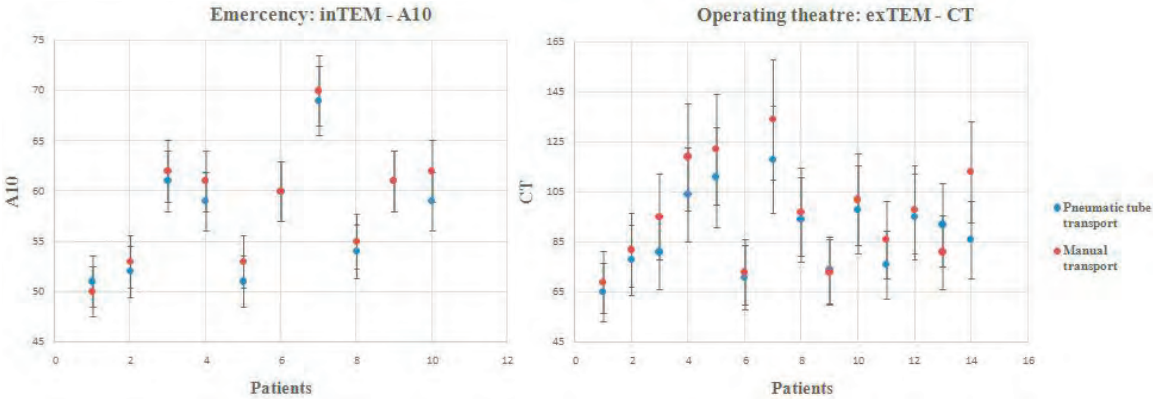
Background / Introduction: Rotational Thromboelastometry (ROTEM®, Pentapharm, Allemagne) measures the viscoelastic properties during clot formation and lysis in whole blood and permits to detect hemostatic changes quickly by 5 tests as

inTEM, exTEM, hepTEM, fibTEM and apTEM which were considered as a Point-Of-Care-Test (POCT).
Aims: ROTEM analysis could be done near the patient and the physician to benefit an immediate therapeutic decision, owever these important tests done by non professional technicians could decrease the quality and reproducibility of the results. Performing these analyses in a central laboratory with a real-time transmission of graphic results and a rapid transport of blood sample could be an alternative. The aim of our study was to evaluate the influence of our hospital pneumatic tube transport system on the result of ROTEM tests in comparing with the results of manual transport.

Methods / Materials: Four hospital units of our hospital in which ROTEM analysis was needed were investigated, including Operating Theatre (N=14); Intensive Care Unit (N=11); Emergency (N=10) and Delivery Unit (N=10). Among these 45 patients, there were 17 Women and 28 Men with a median age of 44 year-old [18 – 45]. Only 1 patient suffered of clinical bleeding. For all patients, two citrated whole blood samples were collected simultaneously and sent separately to the laboratory, one by the pneumatic tube transport system and the other by manual transport. The difference of 7 parameters {CT (clotting time), A5, A10, A30 (clot firmness (mm), 5, 10 and 30 minutes respectively after CT, Li30 (lysis index after 30 minutes), MCF (maximum clot firmness) and alpha angle} for all 5 ROTEM tests between these two transport systems were analysed by a t-Student test or a Mann-Whitney U test (Graphpad Prism 5) and the correlation coefficient of these results were also calculated. The inter-run reproductibilitie (CV%) of the 35 parameters were established.

Results: No statistically significant difference ($p > 0.05$) between the results obtained by two different transport methods was found for all 5 ROTEM tests when we grouped all four units ($n = 45$). However, we found a statistically significant ifference for A10 of inTEM ($p = 0.022$, correlation coefficient $r = 0.9810$, $y = 0.019 + 1.017$ x, Inter-run CV% = 5%) and for CT of exTEM ($p = 0.011$, correlation coefficient $r = 0.8994$, $y=-6.838 + 1.158$ x, Inter-run CV% = 18%) in Emergency and Operationg Theatre respectively when we analysed the results in individual Units. If we took into account the inherent error in these analysis, we found that its differences were inferior to the inter-run CV which were within an acceptable error.

Summary / Conclusions: In conclusion, we can consider that there is no statistically significant difference for all 5 ROTEM tests between the pneumatic tube transport and the manual transport. In our hospital, it is feasible to implement a ROTEM in a central laboratory using a pneumatic tube transport with a real-time transmission of graphic results.



Clotting time or Rosner index for mixing test interpretation makes a difference in the overall lupus anticoagulant test result.

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Background / Introduction: Lupus anticoagulant (LAC) testing includes a screening, mixing and confirmation step. Mixing the patient plasma (PP) with pooled normal plasma (PNP) discriminates between an inhibitor and coagulation factor deficiencies. Guidelines on LAC testing by the ISTH-SSC (2009) and by the CLSI (2014) are a useful step towards standardization by providing precise guidelines on who and how to test as well as on mixing test interpretation. However, a lack of consensus remains whether to express mixing tests in terms of clotting time (CT) or index of circulating coagulant (ICA = [(CT of mixture PP:PNP- CT PNP)/CT PP]x100). In daily practice, we noticed that both interpretation methods might differ and lead to an opposing LAC conclusion. Additionally, the influence of anticoagulant therapy on both methods of interpretation has not yet been investigated.

Aims: To contribute to a simplification and standardization of the LAC three-step interpretation on the level of the mixing test.

Methods / Materials: 148 consecutive patient samples with LAC request and a prolonged screening step, and 77 samples from patients non-suspicious for LAC treated with vitamin K antagonists (VKA, n=37) or new oral anticoagulants (NOAC, n=40) were retrospectively evaluated. Both an activated partial thromboplastin time (aPTT) and a dilute Russel viper venom time (dRVVT) were used. We focused on the interpretation differences for mixing tests expressed as CT or ICA and compared the overall LAC conclusion within each distinct group of concordant and discordant mixing test results.

Results: By the interpretation of the screen mix CT, 10 (dRVVT) and 16 (aPTT) more LAC positive patients samples (including 10 and 3 samples from previously diagnosed anti-phospholipid syndrome patients, respectively) were detected, classified as LAC negative considering the ICA in the LAC three-step interpretation. Discordant mixing test results in dRVVT LAC analysis in the VKA samples without LAC request were exclusively due to a prolonged dRVVT screen mix ICA and showed a screen mix CT below the cut-off. In this cohort, screen mix ICA values showed a significant correlation to INR (P=0.0001). Mutual false positive screen mix ICA and CT results were observed for 90% of the tested NOAC samples. We report a higher false positivity rate for LAC testing in NOAC-treated patients (43%) compared to VKA patients (11%).

Summary / Conclusions: Although no golden standard exist to denote LAC positivity, mixing test evaluation in the present study based on the CT showed a higher sensitivity and specificity for both dRVVT and aPTT systems compared to the ICA. Therefore, we advise to use the CT in respect to the 99th percentile cut-off for interpretation of mixing steps in order to reach the highest sensitivity and specificity in LAC detection.

An epidemiological evaluation of the incidence of deep venous thrombosis and pulmonary embolism in patients with hip or knee replacement surgery and of its impact on the average length of stay and hospitalization cost

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Background / Introduction: Various published sources report an incidence of symptomatic deep venous thrombosis (DVT) and pulmonary embolism (PE) in patients undergoing hip or knee replacement ranging between 0.3 and 2%.

Aims: This study aimed at assessing the in-hospital incidence of DVT/PE after major orthopaedic surgery in Belgium and the impact of these complications on the length of stay and hospitalization costs using retrospective data.

Methods / Materials: The incidence of DVT/PE, the average hospitalization cost and the average length of stay (LOS) among patients hospitalized for hip or knee replacement surgery were estimated using the longitudinal IMS Hospital Disease Database (year 2014), including data (diagnoses, procedures, costs) on 25% of Belgian hospital beds. Stays were searched based on ICD-9-CM procedure codes corresponding to hip replacement (81.51-81.52-81.53) and knee replacement (81.54-81.55). Occurrence of DVT/PE was identified with ICD-9 diagnosis codes 451.1-451.2-453.4 (as primary or secondary diagnosis). The impact of a DVT/PE complication on LOS/ cost was assessed through Wilcoxon non-parametrical tests. The use of anti-thrombotics (low-molecular weight heparins [LMWH], unfractionated heparins, novel oral anti-coagulants [NOAC]) was also tracked in the eligible population.

Results: 8,390 stays with hip replacement and 7,260 stays with knee replacement were retrieved in the database. In both subgroups, more than 80% of the patients were on LMWH (mainly nadroparin) and between 13-17% were on NOAC

Type of anti-coagulant	Hip replacement (n=8,390)	Knee replacement (n=7,260)
Low molecular weight heparins (LMWH)	86.7%	83.0%
Enoxaparin	37.5%	34.4%
Tinzaparin	0.1%	0.1%
Nadroparin	49.9%	49.0%
Unfractionated heparin	7.3%	1.2%
Novel oral anti-coagulants (NOACs)	13.6%	16.8%
Apixaban	0.3%	0.2%
Dabigatran	0.6%	0.3%
Rivaroxaban	12.7%	16.3%

Use of anti-thrombotics in the eligible population

The number of stays with a DVT/PE episode was respectively equal to 40 and 47 within the two subgroups, resulting in an incidence of 0.48% in patients with hip replacement and 0.65% in patients with knee replacement. LOS of patients with a DVT/PE episode was about twice as high after both hip (25.9 vs. 13.74 days; p<0.001) and knee (18.9 vs. 9.4 days; p<0.001) replacement. Hospitalization costs were also significantly higher in case of DVT/PE complication (€18,665 vs. €11,642 in hip replacement; €15,150 vs. €10,111 in knee replacement; p<0.001 in both cases). Multivariate regression models controlling for age and gender confirmed these conclusions.

Summary / Conclusions: The incidences of symptomatic DVT and PE reported in clinical trials could be confirmed based on this retrospective search. The occurrence of DVT/PE increases dramatically both LOS and hospitalization costs in patients undergoing hip or knee surgery.

Comparison of point-of-care instruments I-STAT, ACTPlus and Hemochron Signature Plus for measurement of activated clotting time (ACT)

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Background / Introduction: Measurement of activated clotting time (ACT), which is used to monitor anticoagulation therapy with high heparin concentrations, is a point-of-care test frequently used in different clinical settings. The test is based on complete activation of the intrinsic coagulation cascade, with fibrin clot formation as an end-point. Since reference ranges and therapeutic ranges are method dependent, results obtained with various point-of-care instruments cannot be used interchangeably.

Aims: This study aimed to compare the performance characteristics of the I-STAT (Abbott), ACTPlus (Medtronic) and Hemochron Signature Plus (Accriva Diagnostics) point-of care instruments for measurement of ACT. The ACTPlus instrument has two cartridge channels that are measured simultaneously, whereas I-STAT and Hemochron use a single cartridge. For Hemochron, LR-ACT cartridges were used in low range heparin concentrations, HR-ACT cartridges for heparin concentrations from 2IU/ml on.

Methods / Materials: Three ACTPlus, two I-Stat and two Hemochron Signature Plus instruments were validated. Within-run and between-run imprecision was determined using quality controls of normal (low) and abnormal (high) range. For equipment validation, blood samples (n=30) of volunteers whether (n=20) or not (n=10) spiked with heparin were used. Reproducibility was determined by comparing duplicate channel results per instrument for ACTPlus. Results of ACT on each type of instrument were compared by parallel measurement on two instruments. Acceptance criterion for duplicate and parallel measurements was set as <=12% spread error. Linearity in the range of 0 – 5IU/ml heparin was evaluated per instrument type.

Results: The table shows the detailed results. Overall, all instrument types performed equally when using quality control materials with coefficient of variation ranging between 1.3-12.5%. However using blood samples, ACTPlus showed a significant proportion (41%) of duplicate measurements not fulfilling the acceptance criteria of 12%. Concerning parallel measurement, I-STAT showed a 100% acceptance rate for parallel measurements, compared to 90% for Hemochron Signature Plus and <50% for ACTPlus. Linearity was good on all instrument types. Heparin concentrations gave comparable ACT-values on all instrument types in the low range, but values differed when measuring in the high range.

	ACTPlus 1	ACTPlus 2	ACTPlus 3	I-STAT 1	I-STAT 2	Hemochron 1	Hemochron 2
CV% low range (within-run; between-run)	2.2 - 4.0	6.7 - 3.2	2.4 - 8.6	1.9 - 1.8	1.5 - 1.8	4.1 - 8.1	7.2 - 12.5
CV% high range (within-run; between-run)	3.0 - 5.6	4.9 8.3	3.5 - 10.2	2.2 - 3.2	3.0 - 4.9	1.3 - 1.5	2.0 - 3.2
Duplicate measurements 1							
Measurements that fulfill ≤12% criterion	7/17	11/17	12/17	/	/	/	/
Average difference of accepted measurements (%)	1.4	2.4	1.8	/	/	/	/
Parallel measurements between instruments							
Measurements that fulfill ≤12% criterion	12/34 2	10/34 3	16/34 4	30/30		27/30	
Average difference of accepted measurements (%)	5.7	8.0	5.3	2.5		4.3	
Linearity (correlation coefficient)	R2=0.9823			R2=0.9639		R2=0.9409	

1 ACTPlus: resulting from 2 channels; 2 Comparison T1-T2; 3 Comparison T2-T3; 4 Comparison T3-T1

Summary / Conclusions: This pilot study illustrates that evaluation of point-of-care ACT-instruments should not be limited to quality control material, but should also include blood samples. Overall, I-STAT performed better than Hemochron Signature Plus and ACTPlus. When using 12% spread error as criterion, 41% of measurements performed on ACTPlus failed. These results are unacceptable in clinical practice, and require confirmation in real life settings.

ABSTRACTS
POSTER PRESENTATIONS

BASIC RESEARCH

Endogenous plasmin levels control the development of acute episodes of thrombotic thrombocytopenic purpura in mice

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Background / Introduction: A deficiency in ADAMTS13 activity is associated with the life-threatening disorder thrombotic thrombocytopenic purpura (TTP), where patients suffer from episodes of thrombotic microangiopathy. We previously demonstrated that exogenous plasmin is able to rescue Adamts13-/- mice from TTP implying that plasmin may act as a backup mechanism for ADAMTS13 mediated digestion of VWF.

Aims: Elucidation of the role of endogenous plasmin in the control of TTP episodes in mice.
Methods / Materials: ADAMTS13 or PAI-1 activity was blocked using monoclonal antibodies in either uPAR-/-, α2-antiplasmin-/- or wild type (WT) mice. TTP was triggered using recombinant (r)VWF. In vivo cleavage of platelet decorated VWF strings was visualized in FeCl3 injured mesenteric venules, after labeling platelets with Rhodamine 6G.

Results: WT mice with blocked ADAMTS13 activity developed acquired TTP when triggered with 2000 U/kg rVWF, demonstrated by severe thrombocytopenia (158 ± 77 x103 plt/µl; 685 ± 42 x103 plt/µl at baseline, n=15), while 250 U/kg rVWF did not induce TTP in these animals (541 ± 67 x103 plt/µl, n=10). Interestingly however, uPAR-/- mice without ADAMTS13 activity did developed TTP when triggered with 250 U/kg rVWF (209 ± 44 x103 plt/µl, n=10). Furthermore, VWF string cleavage in these mice was strongly delayed to 18.9 ± 2.7 sec compared to 8.8 ± 0.9 sec in WT mice. Hence, blocking both endogenous plasmin generation via uPAR as well as ADAMTS13 activity render mice more susceptible to TTP. In contrast, VWF string cleavage was accelerated to 5.1 ± 0.2 sec in α2-antiplasmin-/- mice with inhibited PAI-1 and ADAMTS13 activity. In addition, the latter mice did not develop TTP upon triggering with 2000 U/kg rVWF (569 ± 267 x103 plt/µl, n=16), demonstrating that unrestrained endogenous plasmin is able to prevent acute TTP episodes.

Summary / Conclusions: Endogenous plasmin activated via uPAR is able to cleave VWF. These endogenous plasmin levels control the development of acute TTP episodes in mice.

Dextran sulphate triggers platelet aggregation via direct activation of PEAR1

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Background / Introduction: Dextran sulphate (DxS; Mr 500kD) induces fibrinogen receptor (αIIbα3)-activation via CLEC-2/Syk-signalling and via a Syk-independent SFK/PI3K/Akt-dependent tyrosine kinase pathway in human and murine platelets. The platelet surface receptor, responsible for the DxS-induced Syk-independent Akt-activation, has hitherto not been identified.

Aims: Since both DxS and PEAR1 induce sustained platelet aggregation via the SFK/PI3K/Akt pathway in platelets, and since activation of PEAR1 in human platelets is independent from Syk, we hypothesized that the Syk-independent DxS-

induced platelet aggregation involves PEAR1. The aim of this study was to investigate whether DxS-induced SFK/PI3K/Akt-signalling occurs via direct phosphorylation of PEAR1 and whether the activation of CLEC-2/Syk and PEAR1 by DxS suffices to explain DxS-induced platelet aggregation.

Methods / Materials: We found that DxS elicited a concentration-dependent aggregation of human platelets resulting from direct PEAR1-activation by DxS. Blocking the PEAR1 receptor, in combination with a selective Syk-inhibitor completely abrogated the DxS-driven platelet aggregation. The DxS-induced Syk-phosphorylation was not affected in Pear1-/- platelets, but Akt-phosphorylation was largely abolished. As a result, the aggregation of Pear1-/- platelets was reduced and reversible, i.e. aggregates were less stable compared to wild-type platelet aggregates. Moreover, DxS-induced Pear1-/- platelet aggregation was fully abrogated by Syk-inhibition (Figure 1), indicating that the remaining platelet aggregation of Pear1-/- platelets was Syk-dependent. Hence, the Pear1/c-Src/PI3K/Akt- and CLEC-2/Syk-signalling pathways are independently and additively activated during platelet aggregation by DxS.

Results: See methods section

Summary / Conclusions: The DxS-induced aggregation of human and murine platelets is the result of simultaneous activation of PI3K/Akt through direct PEAR1-phosphorylation and parallel Syk-signalling through CLEC-2.

ADAMTS13-induced destabilization of the von Willebrand factor A2 domain

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Background / Introduction: Distal (T2-CUB2) domains of ADAMTS13 are crucial for binding to folded VWF. Shear-induced unfolding of VWF exposes the high affinity binding sites for the proximal (MDTCS) domains, thereby enabling proteolysis. The exosites in the distal domains that are involved in VWF binding have however not been elucidated yet.

Aims: Identification of the crucial distal domain(s) in the ADAMTS13-VWF interaction.

Methods / Materials: ADAMTS13 binding to folded VWF was assayed by ELISA. VWF was captured with an anti-A1 domain monoclonal antibody (mAb). Full-length ADAMTS13 (FL-ADAMTS13) or variants were added and binding was detected with specific anti-ADAMTS13 mAbs. Proteolysis of folded VWF was also studied, under static non-denaturing conditions.

Results: The T8 domain comprised the major binding site for the interaction with folded VWF, by using ADAMTS13 variants with individual distal domain deletions. In line with this, partial inhibition of ADAMTS13 binding (79 ± 6%, n=5) was observed with an anti-T8 domain mAb. Unexpectedly, an anti-spacer (S) mAb also inhibited ADAMTS13 binding (≤31 ± 2%, n=3). The observed inhibition was however not due to steric hindrance with the distal domains, as a FL-ADAMTS13 variant with point mutations in the spacer domain also showed reduced binding to folded VWF (≤16 ± 6%, n=3). Hence, ADAMTS13 binding to folded VWF via its distal domains induces a conformational destabilization in the VWF A2 domain that exposes its spacer binding site. This was supported by the reduced binding of FL-ADAMTS13 when a disulfide bridge-locked A2 domain variant was used (35 ± 11% compared to WT-VWF, n=3). Interestingly, when folded VWF was incubated with the MDTCS variant for 5 to 16 h, no proteolysis occurred (due to the folded structure). However, when FL-ADAMTS13 was added, proteolysis of VWF did occur (for 5 and 16 h respectively 2.8 ± 1.2 and 3.1 ± 0.4-fold increase, n ≥ 5), implying ADAMTS13-induced destabilization of the A2 domain.

Summary / Conclusions: Binding of ADAMTS13 to folded VWF via its distal T8 domain leads to destabilization of the VWF A2 domain, making it more susceptible for proteolysis.

A mouse model for acquired thrombotic thrombocytopenic purpura

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Background / Introduction: Acquired thrombotic thrombocytopenic purpura (TTP) is a life-threatening thrombotic microangiopathy, linked to an autoimmune deficiency of the metalloprotease ADAMTS13. In order to develop and evaluate novel therapies for this disease, suitable animal models are necessary. Current animal models of acquired TTP (baboon, rat and mouse) all obtain an acquired deficiency through the administration of anti-human ADAMTS13 (hADAMTS13) antibodies (Abs).

Aims: Development of a well-characterized acquired TTP mouse model, using inhibitory anti-mouse ADAMTS13 (mADAMTS13) monoclonal Abs (mAbs) of mouse origin.

Methods / Materials: Anti-mADAMTS13 mAbs of mouse origin were generated by the immunization of Adamts13-/- mice with recombinant mADAMTS13. Inhibitory mAbs were identified in vitro and subsequently in vivo (in Adamts13+/+ mice) using the FRETS-VWF73 assay. The in vivo characteristics of these mAbs were further analyzed by determination of ADAMTS13 antigen, Ab level and platelet count. Mice were challenged with recombinant human VWF (rhVWF) and analyzed for TTP symptoms by measuring the platelet count and lactate dehydrogenase (LDH) activity.

Results: A large panel (n = 19) of novel anti-mADAMTS13 mAbs was generated. Four of the anti-mADAMTS13 mAbs partially inhibited mADAMTS13 activity in vitro (α68-90% inhibition). Combining two of these inhibitory mAbs (13B4 and 14H7) resulted in α100% in vitro inhibition. In vivo (in Adamts13+/+ mice), complete inhibition (96 ± 4%, one day post injection) was also achieved by the injection of mAbs 13B4 and 14H7. Subsequent challenge with rhVWF resulted in TTP symptoms like severe thrombocytopenia and increased LDH activity. Interestingly, the inhibitory mAbs were also ideally suited to induce long-term ADAMTS13 deficiency as one bolus injection induced full inhibition for more than 7 days. In addition, the large panel of mAbs additionally allowed us to develop a sensitive assay to detect plasma mADAMTS13.

Summary / Conclusions: A novel acquired TTP mouse model was obtained through the development of inhibitory anti-mADAMTS13 mAbs. Consequently, this model creates new opportunities in the development and evaluation of novel therapies for patients with TTP. The availability of long-term inhibitory anti-mADAMTS13 mAbs further allows investigating the role of ADAMTS13 in other diseases.

Phosphorylation of platelet acetyl-CoA carboxylase is increased in human and mice atherosclerosis and could be an indicator of persistent thrombin generation.

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Background / Introduction: Acetyl-CoA carboxylase (ACC) regulates fatty acids synthesis and oxidation. AMPK phosphorylates ACC on Ser79 and inhibits its activity. We recently showed that thrombin is the only platelet agonist leading to AMPK activation and subsequent ACC phosphorylation in human platelets.

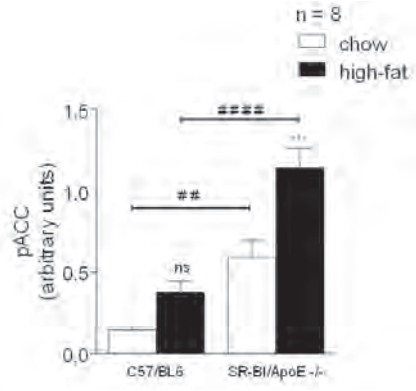
Aims: We hypothesised that there is a relationship between platelet ACC phosphorylation and persistent thrombin generation in vivo. This work aims to assess whether ACC phosphorylation could be correlated to atherosclerosis, a thrombogenic situation.

Methods / Materials: Platelet ACC phosphorylation was analysed in patients referred to diagnostic coronarography and in a murine model of atherosclerosis (SR-BI/ApoE-/-).
In humans, thrombin generation was assessed and the demonstration as well as the severity of the coronary artery disease were based on the coronary angiogram. In mice, the gravity of the disease was scored through histological assessment of lipid deposit and macrophages accumulation in the aortic root. Furthermore, mice platelets activation was evaluated via the detection of PKC substrates phosphorylation.

Results:
Humans with a proven coronary artery disease show a significant increase in platelet ACC phosphorylation as compared to control patients (patients who underwent coronary angiogram, but negative for coronary artery disease). In mice, we confirm that atherosclerosis is associated with an increase in platelet activation and we show a correlation between the level of platelet ACC phosphorylation and the severity of atherosclerosis (Figure 01).

C57BL/6 or SR-BI/ApoE-/- mice were fed a chow- or high-fat diet for 12 weeks. Mice platelets were isolated and ACC phosphorylation analysed by western blot and quantified.

Summary / Conclusions: Platelet ACC phosphorylation is associated to atherosclerosis and, accordingly to our hypothesis, could be an indicator of persistent thrombin generation. This could identify patients at high risk of subsequent thrombotic event and who could benefit for a long-term antithrombin treatment in addition to the classical antiplatelet therapy.



Identification of a novel staphylococcal binding partner for soluble von Willebrand factor-binding protein crucial for adhesion of Staphylococcus aureus to the vessel wall via von Willebrand factor

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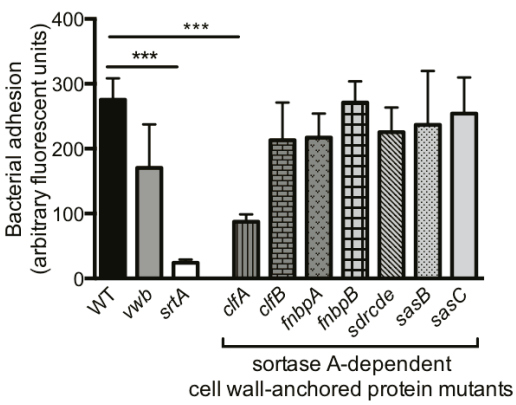
Background / Introduction: Staphylococcus aureus (S. aureus) is the most frequent pathogen causing life-threatening endovascular infections and infective endocarditis. Compared to other pathogens, infective endocarditis caused by S. aureus has a higher mortality and is more frequently associated with severe complications. In order to establish endovascular infections, S. aureus needs to bind to the vessel wall and persist despite being exposed to the strong shear forces of the rapidly flowing blood. Adhesion of S. aureus to blood vessels under shear stress requires von Willebrand factor (VWF). We previously described that staphylococcal von Willebrand factor-binding protein (vWbp) interacts with VWF fibers, enabling flow-controlled bacterial adhesion to endothelial cells and to subendothelial matrix via VWF. However, the mechanism through which the soluble vWbp anchors to the bacterial cell wall remains uncertain. S. aureus expresses a number of bacterial cell wall-anchored proteins that mediate bacterial adhesion to the host and contribute to the pathogenesis of endovascular infections. Most of these proteins are positioned in the bacterial cell wall by a mechanism that involves cleavage of a conserved Leu-Pro-X-Thr-Gly (LPXTG) motif. A mutation in the srtA gene (sortase A) leads to a defect in the anchoring of about 20 S. aureus cell wall-anchored proteins.

Aims:
We hypothesized that soluble vWbp is able to interact with a staphylococcal cell wall-anchored protein, hereby facilitating the adhesion of S. aureus to endothelial cells and to subendothelial matrix via VWF.

Methods / Materials: We measured the adhesion of S. aureus Newman (WT) and mutants deficient in vWbp (vwb), SrtA (srtA) or in SrtA-dependent cell wall-anchored proteins to VWF, vWbp and endothelial cells. Fluorescently labeled bacteria were perfused over a glass surface coated with VWF, collagen, vWbp or endothelial cells in a micro parallel flow chamber. The contribution of exogenously produced vWbp added to the medium was analyzed during shear-dependent S. aureus adhesion.

Results:
We found that exogenously produced vWbp restored vwb binding to VWF under flow. Likewise, compared to binding of the WT strain, the vwb strain was able to adhere to coated vWbp under flow, illustrating that both soluble and coated vWbp are able to interact with a surface receptor on S. aureus. In addition, soluble vWbp mediated S. aureus recruitment to VWF-coated coverslips. In contrast, the srtA strain failed to adhere to vWbp or to VWF in flow. We further investigated the adhesion profile of several SrtA-dependent cell wall-anchored protein deletion mutants to vWbp, VWF and endothelial cells. We identified Clumping factor A (ClfA) as a binding partner for soluble vWbp.



Micro parallel flow chamber perfusion over coated vWbp with fluorescently labeled WT, vwb, srtA, clfA, clfB, fnbpA, fnbpB, sdrcde, sasB and sasC strains, shear rate: 1000 s-1, n ≥ 5. Results are expressed as mean±SEM. *** P < 0,001.

Summary / Conclusions: We conclude that vWbp interacts both with sheared VWF and with a SrtA-dependent staphylococcal cell wall-anchored protein, more specifically with ClfA. The complex formed by VWF-vWbp-ClfA is crucial to mediate the adhesion of S. aureus to VWF.

ADAMTS13 deficiency in mice does not aggravate diet-induced liver steatosis

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Background / Introduction: ADAMTS13 (A Disintegrin And Metalloproteinase with ThromboSpondin type-1 motif, member 13) specifically cleaves ultralarge (UL) multimeric von Willebrand Factor (VWF), thereby preventing accumulation of these multimers and, in turn, platelet clumping and formation of microthrombi. ADAMTS13 is mainly produced by hepatic stellate cells, and numerous studies have suggested a functional role of ADAMTS13 in the pathogenesis of liver diseases.

Aims: To investigate a potential role of ADAMTS13 in development of non-alcoholic steatohepatitis (NASH).

Methods / Materials: Two models of diet-induced steatosis in ADAMTS13 deficient (Adamts13-/-) and wild-type (WT) control mice were used. VWF antigen levels and multimers in plasma were determined by ELISA. Livers of mice were removed and used for isolation of RNA, triglyceride quantitation, H&E and VWF staining to visualize steatosis and microthrombi, respectively. Protein extracts were used for plasmin-α2-antiplasmin ELISA. Expression of different inflammatory, steatosis and fibrinolytic markers was detected by qPCR.

Results: High fat diet feeding, as compared to normal chow, resulted in enhanced liver triglyceride levels (63.6 ± 3.2 versus 12.7 ± 0.76 mg/dl/mg tissue for WT mice, $p < 0.0001$; 65.3 ± 6.8 versus 11.3 ± 1.4 mg/dl/mg tissue for Adamts13-/- mice, $p < 0.0001$) and enhanced steatosis (49.5 ± 0.9 versus 10.9 ± 2.8 % for WT mice, $p < 0.0001$; 62.1 ± 4.3 versus 35.5 ± 5.2 % for Adamts13-/- mice, $p = 0.002$) without, however, differences between the two genotypes. Expression of markers of inflammation, oxidative stress, steatosis and fibrosis was affected by diet but not by genotype. However, platelet-rich microthrombi were more abundant in the liver of obese Adamts13-/- as compared to obese WT mice or to lean Adamts13-/- mice. Hepatic plasmin-α2-antiplasmin complex levels were lower for lean Adamts13-/- than WT mice, whereas no significant difference was found between obese Adamts13-/- and WT mice (1.5 ± 0.11 versus 2.4 ± 0.10 ng/mg protein for mice fed a SFD, $p = 0.02$; 3.2 ± 0.18 versus 4.9 ± 0.15 for obese mice, $p = 0.93$), indicating less activation of the fibrinolytic system in livers of lean Adamts13-/- mice. Also on a steatosis diet, devoid of methionine and choline, no significant effect of ADAMTS13 deficiency on development of liver steatosis was observed.

Summary / Conclusions: Thus, our data in two murine models confirm that obesity promotes NASH, but do not support a detrimental role of ADAMTS13 in its development, nor a compensatory role of plasmin in removal of platelet-rich microthrombi from the liver in the absence of ADAMTS13.

GARP expressed on platelets does not contribute to hemostasis and thrombosis in mice

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Background / Introduction: Platelets play a crucial role in hemostasis and thrombosis. Identifying the function of new platelet receptors can unravel yet unexplored pathways regulating platelet physiology. Glycoprotein-A Repetitions Predominant protein (GARP or LRRC32) is present on among others human platelets and endothelial cells. Evidence for its involvement in thrombus formation was suggested in GARP deficient zebrafish (1).

Aims: The present study investigates the role of GARP on murine platelets in hemostasis and thrombosis using platelet-specific conditional GARP knock out mice (GARP cKO).

Methods / Materials: Cre expression driven by the PF4 promoter was used to generate transgenic mice specifically lacking GARP expression on megakaryocytes and platelets. Hematological parameters (platelets, mean platelet volume, white blood cells, red blood cells, hemoglobin and hematocrit) and platelet surface glycoprotein expression (GPIb, GPVI and αIIb) were determined in GARP cKO and littermate controls. Platelet function was measured ex vivo by flow cytometry (P-selectin

expression, αIIbβ3 activation and fibrinogen binding), spreading analysis and aggregometry using PAR4-activating peptide (PAR4-AP) and CRP as agonists. Additionally, collagen-induced adhesion and aggregation under flow and clot retraction were analysed. In vivo tail bleeding time and occlusion time of the mesenteric and carotid artery after FeCl3-induced thrombosis were also determined.

Results: No GARP could be detected on platelets of GARP cKO mice, in contrast to their controls, where an increased GARP expression was detected upon platelet activation. GARP cKO mice had normal hematological parameters and their platelets had normal surface GPIb, GPVI and αIIb glycoprotein expression. GARP cKO platelets furthermore displayed normal agonist induced activation, spreading on fibrinogen and aggregation responses. Absence of GARP did not influence clot retraction and had no impact on thrombus formation on collagen-coated surfaces under flow. In line with this, platelet GARP deficiency did neither affect the tail bleeding time nor change the occlusion time in the carotid or mesenteric artery after FeCl3-induced thrombosis.

Summary / Conclusions: Although previous zebrafish studies have demonstrated an important role for GARP in thrombus formation (1), the present results provide direct evidence that platelet GARP is not important in hemostasis and thrombosis in mice. Whether GARP on endothelial cells might influence thrombus formation claims the need for further investigation of the GARP receptor.

1. O'Connor et al Blood. 2009;113:4754-62

Binding to von Willebrand factor enables S. lugdunensis to overcome shear stress and cause infective endocarditis

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Background / Introduction: Staphylococcus lugdunensis (S. lugdunensis) is an emerging cause of infective endocarditis and vascular infections. Since heart valves are subjected to high blood flow, S. lugdunensis must have mechanisms to overcome shear stress in order to colonize the heart valves and cause endocarditis

Aims: In this study, we investigate the role of platelets and von Willebrand factor (vWF) in the initial adhesion of S. lugdunensis in vascular infections and endocarditis.
Methods / Materials: S. lugdunensis adhesion to vWF, collagen and endothelial cells was studied in a parallel flow chamber model in the absence and presence of platelets. In vivo adhesion of S. lugdunensis was evaluated in a mouse microvasculature perfusion model and a newly developed infective endocarditis model.

Results: S. lugdunensis, opposed to other coagulase negative staphylococci, is able to bind to vWF under flow. By interacting directly to VWF, S. lugdunensis increases its adhesion to the subendothelial martrix and to activated endothelial cells. Moreover, the release of VWF in activated vessels of the mesenteric microcirculation leads to recruitment of S. lugdunensis to the vessel wall. In order to study the importance of VWF in S. lugdunensis endocarditis, we developed a new mouse model of inflammatory endocarditis. In this model we can elicit infective endocarditis by inducing local inflammation of the valve endothelium through local infusion of histamine, follow by infusion of S. lugdunensis. Hereby early infective vegetations are formed. This model therefore provides a unique glimpse into the initial pathophysiology of endocarditis. When this model is performed in VWF knockout mice the infective lesions are significantly reduced.

Summary / Conclusions: S. lugdunensis binds directly to vWF, which proves to be essential for withstanding shear forces and for successful adhesion of this pathogen to the vessel wall and the cardiac valves. This mechanism explains why S. lugdunensis causes more aggressive infections, including endocarditis, compared to other coagulase negative staphylococci.

P2X1 ion channels limit the formation of extra-intestinal neutrophil-rich thrombi in acute colitis

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Background / Introduction: Acute and chronic inflammatory diseases are associated with hemostasis disorders. This phenomenon depends on complex interactions between platelets, leukocytes and endothelia but the underlying mechanisms remain largely unknown. ATP-gated P2X1 ion channels expressed on platelets and neutrophils could play a role in the crosstalk between inflammation and thrombosis.

Aims: To investigate whether P2X1 ion channels contribute to acute colitis and to extra-intestinal thrombosis associated with this inflammatory condition.

Methods / Materials: We used P2X1-/- mice in a model of acute colitis induced by dextran sodium sulfate (DSS). Disease activity index (DAI) was determined to assess colitis severity (diarrhea, bleeding, weight loss). Differential counts of peripheral leukocytes and platelet counts were measured. Plasma and colonic levels of cytokines were analyzed. Extra-intestinal thrombosis was assessed using a laser-induced injury model of thrombosis in cremaster muscle arterioles. Accumulation of platelets, neutrophils and fibrin generation were recorded in real-time.

Results: P2X1-/- mice displayed a higher DAI score due to higher intestinal blood loss as compared to control animals. These mice did not develop leukocytosis, and the levels of plasma and colonic cytokines, especially RANTES and IFN-g, were significantly reduced. In our extra-intestinal thrombosis model, DSS-treated P2X1-/- mice showed a strong increase of neutrophil accumulation at the site of arteriolar wall injury. Fibrin generation was reinforced at the site of injury despite impaired platelet recruitment in the growing thrombi.

Summary / Conclusions: P2X1 ion channels would play a protective role in acute colitis by maintaining platelet-dependent intestinal vascular integrity and limiting the formation of deleterious neutrophil-rich thrombi.

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