BSTH

Belgian Society on Thrombosis and Haemostasis

22ND ANNUAL MEETING 27-28 November 2014 LAMOT Belgium

Final program & Abstracts

Local organiser:

Kristin Jochmans

Department of Haematology
Universitair Ziekenhuis Brussel

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WELCOME TO THE 22ND ANNUAL MEETING OF THE BELGIAN SOCIETY ON THROMBOSIS AND HAEMOSTASIS

Dear Colleagues and friends,

On behalf of the board of the Belgian Society on Thrombosis and Haemostasis(BSTH) it is my privilege to welcome you to Mechelen, the beautiful city of the 'Maneblussers' (Moon Extinguishers'). Mechelen has a rich history, playing an important political and economic role in the Burgundian period. Many historical buildings such as the Sint-Rumbold's Cathedral with the famous 'Rombouts'-tower, the palace of Margaret of Austria, the Town Hall and several stylish houses around the marketplace and along the river Dijle bear witness of this.

For this 22nd Annual Meeting of the BSTH we have drawn up a scientific program with a haemostatic mixture of topics, from science to clinical practice. Different eminent speakers from our country and abroad kindly accepted to share with you their knowledge and expertise during the coming two days. Traditionally, young investigators have the opportunity to present their work as a poster or by oral communication and to have interaction with other participants. Moreover, in an agreeable cooperation with our commercial partners, we succeeded to organise three promising Satellite Symposia.

One must live completely disconnected to ignore the actual financial situation in any sector of the society. I would like to thank explicitly all the companies that have contributed to the support of the BSTH and the organisation of this Annual Meeting. Without their sponsorship, neither the support of young scientists/ physicians by the BSTH, nor the annual gathering of the 'Belgian haemostasis adepts' could take place. On Thursday evening you will have the opportunity to meet each other informally and to enjoy the walking dinner in a relaxing atmosphere.

My only wish is to see you leaving Mechelen after the meeting with good feelings about the content of the program, the quality of the presentations, the overall organisation, the venue, the scientific and social contacts, etc. Just promise to give us feedback in the days and weeks afterwards, in order to stimulate us for an ongoing optimisation of the meeting. All suggestions for future BSTH meetings or initiatives are more than welcome.
Enjoy!

Kristin Jochmans - Universitair Ziekenhuis Brussel
On behalf of the BSTH board and the BSTH Congress Management

ORGANISATION

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BSTH
Belgian Society on Thrombosis and Haemostasis

Congress management

Con-txt

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Fax + 31 (0)38 4650602
Email bsth@con-txt.nl
Web www.con-txt.nl

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 OVERVIEW

THURSDAY NOVEMBER 27TH, 2014	
8.30	Registration desk opening
9.25	Welcome
9.30	Bayer Satellite Symposium Auditorium
10.30	Coffee Break
11.00	Oral Communications: Clinical & Laboratory Auditorium
12.00	Educational I Auditorium
12.45	Lunch Poster presentations
13.15	BSTH General Assembly Dijlezaal
14.15	State of the art I Thrombosis in women Auditorium
16.15	Coffee Break
16.45	LEO Pharma Satellite Symposium Auditorium
19.00	Welcome Reception Scala
19.30	Walking Dinner Scala

FRIDAY NOVEMBER 28TH, 2014	
8.55	Welcome
9.00	BMS-Pfizer Satellite Symposium Auditorium
10.00	Educational II Auditorium
10.45	Coffee Break
11.15	Oral Communications: Basic Research Auditorium
12.15	BSTH news Auditorium
12.45	Lunch Poster presentations
14.00	State of the art II Angiogenesis, haemostasis and cancer Auditorium
16.00	Closure & Reception

PROGRAM **THURSDAY 27 NOVEMBER 2014**

08h30	Registration	
09h25	Welcome	
09h30	**Bayer Satellite Symposium**	
	Chair: C. Hermans - K. Jochmans	
	Substitute or target the coagulation cascade: from a rare bleeding disease to common thrombotic disorders	C. Hermans (Brussel)
	Future therapies in hemophilia A	M.E. Mancuso (Milan)
	Direct oral anticoagulants in daily care: what do we know today and what are the remaining issues?	S. Motte (Brussel)
10h30	Break	
11h00	**Oral Communications: Clinical & Laboratory**	
	Chair: A. Gadisseur - W. Wijns	
	Belgian multicenter study into von Willebrand Disease (B-Will Study): First results	I. Vangenechten (Antwerp)
	Dose finding of rivaroxaban in hemodialysis patients	A. De Vriese (Brugge)
	Rivaroxaban for the treatment of consumptive coagulopathy associated with vascular malformations	C. Vandenbriele (Leuven)
	Evaluation of a rapid lateral flow immunoassay (STic Expert® HIT) in Heparin-Induced Thrombocytopenia (HIT)	I. De Cooman (Ghent)
	Influence of platelet count in platelet rich plasma for adenosine triphosphate release assay	S. Mulliez (Ghent)
	Paul Capel Prize Clinical & Laboratory	
12h00	**Educational I:**	
	Chair: K. Vanhoorelbeke	
	Von Willebrand factor and ADAMTS13: new players in ischemia/reperfusion injury	S. De Meyer (Kortrijk)
12h45	Lunch **Poster presentations**	
13h15	**BSTH General Assembly**	

14h15 **STATE OF THE ART I: Thrombosis in women**

Chair: A. Demulder - P. Hainaut

Pregnancy complications, thrombophilia, and anticoagulants	S. Middeldorp (Amsterdam)
Thrombosis during assisted reproduction	S. Nelson (Glasgow)
Obstetric antiphospholipid syndrome: facts and controversies	J.C. Gris (Nîmes)

16h15 Break

16h45 **LEO Pharma Satellite Symposium**

Chair: P. Hainaut

LMWH and Cancer: Present, Future and Beyond	I. Elalamy (Paris)
Management of Cancer Associated Thrombosis when the Evidence is Lacking, a Real World Experience	S. Noble (Cardiff)

17h45 Closure of day program

RECEPTION & WALKING DINNER **THURSDAY NIGHT**

All registered participants are invited to join the evening program on Thursday night at Lamot: this will be a special occasion to meet the speakers, organisers and board with walking dinner to enjoy in a relaxing atmosphere.

Reception and walking dinner on Thursday night at 19h00

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

19h00 Welcome reception

19h30 Walking dinner

PROGRAM **FRIDAY 28 NOVEMBER 2014**

08h55 Welcome

09h00 **BMS/PFIZER Satellite Symposium**

Chair: M. Sprynger - P. Verhamme

Translating clinical data to real-world use: the importance of medication adherence	B. Vrijens (Luik)
Well-controlled on VKA: to switch or not to switch?	H. Heidbuchel (Hasselt)

10h00 **Educational II**

Chair: K. Devreese

Numbers, Patterns and Timing: a systematic approach to a low platelet count in intensive care patients	A. Greinacher (Greifswald)

10h45 Break

11h15 **Oral Communications: Basic Research**

Chair: F. Mullier - H. Deckmyn

PEAR1: a novel link between IgE-mediated allergy and cardiovascular disease	Y. Sun (Leuven)
'Sleeping Beauty'-mediated gene transfer of ADAMTS13 prevents the onset of TTP-like symptoms in ADAMTS13-deficient mice	S. Verhenne (Kortrijk)
Staphylococcus aureus von Willebrand factor-binding protein exhibits a dual role in bacterial adhesion to the vessel wall	J. Claes (Leuven)
Causes of 'Gray platelets': from genetic studies of inherited thrombocytopenia to functional studies	A. Wijgaerts (Leuven)
Recombinant ADAMTS13 as an effective therapy for acquired thrombotic thrombocytopenic purpura in rats	C. Tersteeg (Kortrijk)

Paul Capel Prize Basic Research

12h15 BSTH news

12h45 Lunch
Poster presentations

14h00 **STATE OF THE ART II: Angiogenesis, haemostasis and cancer**
Chair: M. Hoylaerts - S. Motte

Alternative strategies to target angiogenesis	P. Carmeliet (Leuven)
Risk assessment of venous thromboembolism in cancer	C. Ay (Vienna)
Cancer-related coagulopathies	M. Levi (Amsterdam)

16h00 Closure

16h05 Reception

REGISTRATION

Annual Meeting	€ 115,-
Pre booking rate at registration up to October 15	€ 105,-
Registration on site	€ 140,-
Students*	
Pre booking rate at registration up to October 15	€ 25,-
Registration fee after October 15	€ 35,-
Registration on site	€ 50,-

* status to be attested by letter or mail from mentor.

Registration fee includes:
1. Participation to BSTH scientific meetings
2. Participation to Educational sessions
3. Participation to the Satellite Symposia
4. Breaks, lunches and reception

Registration can be done online at www.bsth2014.com.

Payment must be made online (secured payment page). Local and international payment methods are accepted: Visa, MasterCard, iDEAL, Mister Cash or Bank Transfer.

Registration will be closed on November 24.
After this date only registration on site will be possible.

Evening program:
Congress participants (doctors, nurses, students etc):

Early bird rate until October 15	€ 25,-
After 15 October	€ 35,-
On site	€ 50,-

Delegates of companies/sponsors: only through goodwill sponsoring.

BSTH BOARD 2014

The present members of the BSTH board 2014 are:

PRESIDENT	Alain Gadisseur Universitair Ziekenhuis Antwerpen UZA, Edegem Alain.Gadisseur@uza.be
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	Kristin Jochmans UZ Brussel Kristin.Jochmans@uzbrussel.be
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	Muriel Sprynger Centre Hospitalier Universitaire de Liège Domaine de Sart-Tilman msprynger@gmail.com
	Kristel Vandenbosch (non-voting) Service Thrombose-Hémostase Centre Hospitalier Universitaire de Liège kvandenbosch@chu.ulg.ac.be
	Karen Vanhoorelbeke (non-voting) Laboratory for Thrombosis Research IRF-Ls KU Leuven Kulak karen.vanhoorelbeke@kuleuven-kulak.be

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 26, 2014 19h00 - 24h00
Thursday November 27, 2014 07h00 - 08h30

Exhibition opening hours
Thursday November 27, 2014 08h30 - 19h30
Friday November 28, 2014 08h30 - 16h30

Dismounting
Friday November 28, 2014 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

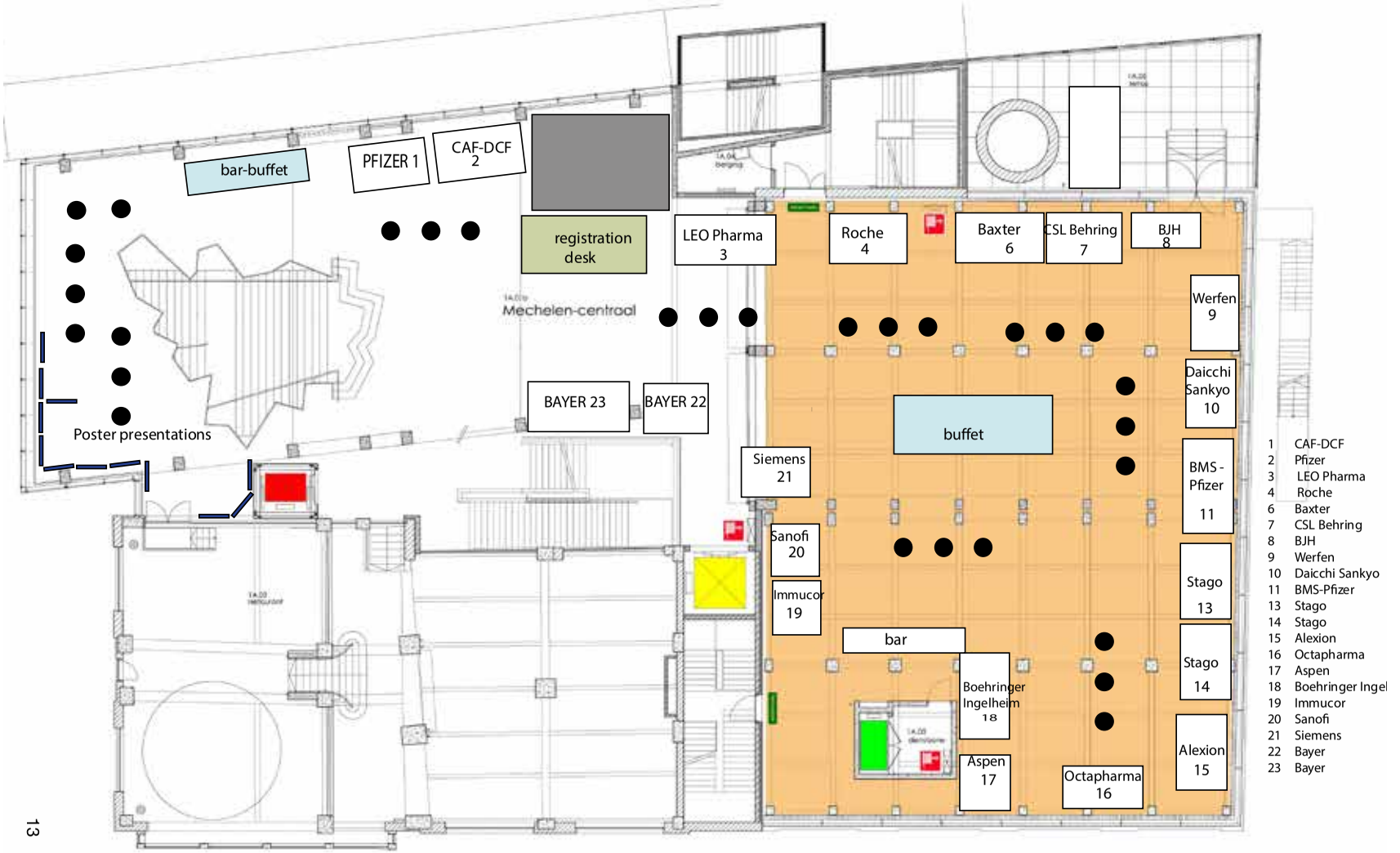
Exhibitors may not use audible electronic, mechanical apparatus, or open audio systems that may be heard outside the exhibitor assigned space. Con-txt, on behalf of BSTH and its organizers, reserves the right to require any exhibitor to discontinue any activity that may cause annoyance or interference 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 27 2014, will forfeit their booth space without refund. The space may be resold or used by the BSTH.


bar-buffet
PFIZER 1
CAF-DCF 2
registration desk
LEO Pharma 3
Roche 4
Baxter 6
CSL Behring 7
BJH 8
Werfen 9
Daicchi Sankyo 10
BMS - Pfizer 11
Stago 13
Stago 14
Alexion 15
Octapharma 16
Aspen 17
Boehringer Ingelheim 18
Immucor 19
Sanofi 20
Siemens 21
BAYER 22
BAYER 23
buffet
bar
Poster presentations
Mechelen-centrool
1 CAF-DCF
2 Pfizer
3 LEO Pharma
4 Roche
6 Baxter
7 CSL Behring
8 BJH
9 Werfen
10 Daicchi Sankyo
11 BMS-Pfizer
13 Stago
14 Stago
15 Alexion
16 Octapharma
17 Aspen
18 Boehringer Ingel
19 Immucor
20 Sanofi
21 Siemens
22 Bayer
23 Bayer

STATE OF THE ART SPEAKERS

S. Middeldorp
Amsterdam

Saskia Middeldorp is Professor of Medicine, Department of Vascular Medicine, Academic Medical Center, Amsterdam, The Netherlands.

Saskia Middeldorp, MD, qualified as a medical doctor at the University of Amsterdam in 1992. She received her training in internal medicine at the Academic Medical Center in Amsterdam and obtained her board licence in 1999 (subspecialty Vascular Medicine, 2002). Since December 2010, she is professor and co-chair of the Department of Vascular Medicine of the Academic Medical Center. She leads the residency program of vascular medicine and the clinical thrombosis and haemostasis research lines of the Department. Her present research focuses on several aspects of hereditary and acquired thrombophilia, women's issues in thrombosis and haemostasis, and the clinical evaluation of new anticoagulants.

S. Nelson
Glasgow

Scott Nelson was appointed directly to the Muirhead Chair in Obstetrics and Gynaecology at the University of Glasgow in August 2008 on completion of his specialist training. He had previously undertaken his medical training in Glasgow with a PhD in fetal therapy from the University of Dundee. Further clinical academic training was undertaken in Glasgow as a SHEFC Clinician Scientist in maternal medicine and assisted conception prior to gaining his CCT. Professors Nelson's clinical interest is the interface of assisted conception and perinatal medicine, with his research focusing on reproductive health across the lifecourse.

J.C. Gris
Nîmes

MM.D., Montpellier University, 1985. Ph.D., doctoral studies: Biology and Health, Montpellier University, 1989. Full professor of Haematology, Montpellier University, 2001. Head of the research team EA 2992, Montpellier University. Jean-Christophe Gris is Head of the Laboratory of Haematology and of the Outpatient Department of Haematology Department of Laboratory Medicine, University Hospital, in Nîmes, France. He is also head of the council for clinical research, Nimes University Hospital. Main fields of research: Haemostasis thrombosis, vascular biology and pregnancy outcome; Haematology-related risk factors for pregnancy loss; Haemostasis-modulating treatments and pregnancy outcome; Haemostasis and venous thrombo-embolic risk; Haemostasis, sepsis and prognosis. Currently: 175 related citations in the PubMed database.

He is a member of the French Society of Haematology, of the European Society of Haematology, of the International Society on Thrombosis and Haemostasis, Associated member of the French Society of Vascular Medicine and member of the Editorial Board of the Journal of Thrombosis and Haemostasis.

P. Carmeliet
Leuven

Laboratory of Angiogenesis and Neurovascular Link, Vesalius Research Center, K.U. and VIB, 3000 Leuven, Belgium.

Peter Carmeliet is Director of the VIB - Vesalius Research Center, at the University of Leuven in Belgium. He graduated as Doctor in Medicine in 1984, and completed his PhD in Medicine in 1989. In 1992, Carmeliet started his own research group with a focus on how blood vessels grow (angiogenesis) in health and disease.

The Carmeliet lab is currently studying how endothelial cells change their metabolism during vascular branching and is exploring the therapeutic potential of targeting endothelial metabolism for anti-angiogenic strategies. The role of several key metabolic enzymes in endothelial cell biology and angiogenesis in vivo are under investigation.

C. Ay
Vienna

MD and scientist at the Vienna General Hospital of the Medical University of Vienna, Austria.

Medical studies at the Medical University of Vienna, Austria and the Ruprecht-Karls-Universität Heidelberg, Germany.
Training in Internal Medicine at the Medical University of Vienna.

Area of speciality: haematology, blood coagulation, thrombosis and bleeding disorders
Research interests: venous thromboembolism, haemostasis, cancer and thrombosis, anticoagulation

M. Levi
Amsterdam

Marcel Levi (1964) is professor of Medicine, Dean of the Faculty of Medicine of the University of Amsterdam, and Chairman of the Executive Board of the Academic Medical Center in Amsterdam, the Netherlands.

After his medical training and specialization in Internal Medicine he obtained his PhD (1991) and was appointed as a Fellow by the Royal Netherlands Academy of Science. He followed a MSc program at the University of Oxford in Evidence-based Health Care and worked at the University of Perugia, Italy and the Center for Transgene Technology and Genetherapy of the University of Leuven, Belgium. He has published more than 700 articles in international scientific journals, has been awarded several international research awards, and serves as an associate editor for many international scientific journals. He is currently vice-chairman of the Netherlands Organization for Medical Research (ZON-MW). He was elected as a honorary fellow of the Royal College of Physicians in the UK and as a member of the Royal Netherlands Academy of Science (KNAW) and is a member of the European Medical Research Council.

EDUCATIONAL SPEAKERS

A. Greinacher
Greifswald

Andreas Greinacher is an M.D. with a specialization in transfusion medicine and hemostasis. His scientific career is focused on platelet disorders, bridging immuno-hematology and hemostasis. He is working at the University Hospital Greifswald, Germany, where he is head of the Institute of Immunology and Transfusion Medicine, the clinical thrombosis and hemostasis service, the hemostasis out-patient clinic, the transfusion and stem cell service and the immuno-hematology laboratory.

Aside from heparin-induced thrombocytopenia and drug dependent thrombocytopenia, he has a major interest in hereditary and acquired platelet disorders. His recent work is focusing on adopting nanotechnology and biophysical methods to investigate platelets and protein changes. He is section editor of several journals in the field of thrombosis and hemostasis and the current chairman of the education committee of the ISTH.

S. De Meyer
Kortrijk

Biomedical Sciences Kulak IRF Life Sciences – Laboratory for Thrombosis KU Leuven Kulak. Current Academic appointment: as Associate Professor, Department of Cardiovascular Sciences, KU Leuven/ Group Biomedical Sciences Kulak.
Simon has won more than 15 travel grants/awards, a.o. the Galenus Prize for Fundamental Pharmacology, Brussels,Belgium, 2012 .
Simon is Co-promotor of 7 PhD students and Promotor of 1 PhD student. Simon is active as a reviewer activities: Regular reviewer for Blood (IF 10.558), Circulation research (IF 9.504), Arteriosclerosis, Thrombosis and Vascular Biology (IF 7.215), Stroke (IF 5.756), Journal of Thrombosis and Haemostasis (IF 5.439), occasional reviewer for Thrombosis and Haemostasis (IF 4.701), Journal of Cerebral Blood Flow Metabolism (IF 5.008), Expert Review of Cardiovascular Therapy, Experimental and Translational Stroke Medicine.
Editorial activities: Member of the editorial board of 'Experimental and Translational Stroke Medicine'.
Professional societies: Member of the Belgian Society of Thrombosis and Haemostasis, Member of the International Society of Thrombosis and Haemostasis, Member of the American Society of Gene Therapy, Member of the European Society for Gene and Cell Therapy. Publications: 38 Internationally peer reviewed papers.

THROMBOSIS IN WOMEN

Pregnancy complications, thrombophilia, and anticoagulants

S. Middeldorp (Amsterdam)

AMC Amsterdam

Women with acquired and inherited thrombophilia are thought to be at increased risk for pregnancy complications, including recurrent pregnancy loss and, depending on the type of thrombophilia, severe preeclampsia. I will review the associations between the types of thrombophilia and types of complications, as well as the currently available clinical trial evidence regarding the use of aspirin and heparin to prevent these pregnancy complications.
In women with antiphospholipid syndrome (APS), guidelines recommend to prescribe aspirin and heparin to women with recurrent miscarriage. The same regimen is suggested for late pregnancy complications by some but not all experts. Aspirin or LMWH to improve pregnancy outcome in women with unexplained recurrent miscarriage has no benefit and should not be prescribed. Whether anticoagulant therapy prevents recurrent miscarriage in women with inherited thrombophilia or in women with severe pregnancy complications, remains controversial because of inconsistent results from trials. Aspirin modestly decreases the risk of severe preeclampsia in women at high risk.

Thrombosis during assisted reproduction

S. Nelson (Glasgow)

Muirhead Professor of Obstetrics & Gynaecology, University of Glasgow

Since the first successful IVF cycle in 1978 there has been a rapid expansion of the number of women receiving assisted conception such that in 2014 almost every primary school class in Europe will have a child born from IVF within it. During this period of therapeutic development there have been multiple case reports of venous and arterial thrombosis occurring in women receiving assisted reproductive technologies even in the absence of pregnancy. These initial observations have now been confirmed, with population prevalence studies demonstrating an overall estimate of 2.66 per 1000 cycles as compared to 0.97 for natural pregnancy. A range of mild to moderate risk factors have now been identified including age, obesity and multiple gestations, however, ovarian hyperstimulation syndrome (OHSS) which affects 5% of IVF cycles incurs a 100-fold increase in risk of venous thrombosis over natural conceptions. Identification of women at risk of OHSS can now be accurately achieved using antral follicle count (AFC) and anti-Müllerian hormone (AMH). For those women with a high AFC or AMH combining a GnRH antagonist with a conventional hCG trigger will reduce the risk of OHSS and still allow a fresh transfer to occur. Elimination of OHSS is however now a reality by avoiding exposure to exogenous hCG. This can be achieved by segmentation of the IVF cycle using a GnRH agonist for final oocyte maturation and then freezing all oocytes or embryos with subsequent replacement of a single embryo in the context of a frozen embryo transfer. This novel approach will ensure a VTE risk equivalent to natural conception and can be combined with conventional thromboprophylaxis strategies.

Obstetric antiphospholipid syndrome: facts and controversies

J.C. Gris (Nîmes)

Department of Haematology, University Hospital, Nîmes and University of Montpellier, France.

Antiphospholipid syndrome (APS) is defined as an autoimmune disease characterised by clinical symptoms associated with a panel of autoantibodies belonging to the so-called antiphospholipid antibody (aPL; i.e. lupus anticoagulant: LA, anticardiolipin antibodies: aCL, anti-β2-glycoprotein I antibodies: aβ2GP1) family. The clinical features are thrombotic manifestations and pregnancy complications (failure, placenta-mediated late complications). The aPL are not only diagnostic of APS but are also believed to play a pathogenic role. Although APS is traditionally considered as a single entity, most of women presenting with pregnancy failure and aPL do not become thrombotic and neither thrombosis nor infarction are common in APS placentae in large-scale histological studies. The obstetrical impact of aPL is thought to occur mainly through the modulation of the trophoblastic cell physiology, molecular mechanisms being progressively depicted. The associations linking obstetrical morbidities and aPL subtypes have variable specificities and sensitivities. There is increasing body of evidence that isolated aCL-associated early recurrent pregnancy loss is largely inconsistent, this clinical manifestation of APS being different form late loss or early delivery with placental insufficiency, over-diagnosis being probably related to the absence of clinically-relevant definitions of a positive aCL depending on the ach precise clinical symptom. LA and aβ2GP1 may be the aPL associated with late adverse obstetric outcomes: the role of aCL and of the IgM isotype is clearly questioned. Some other non-traditional members of the wide aPL family may also be pathogenic.

Prophylactic treatments are based on antithrombotics but there is no clear evidence that LMWH, due to their shortened polysaccharide chains, may be as efficient as UFH used to be, and late pregnancy complications due to placental insufficiency remain abnormally frequent. Babies born to mothers with APS seem to be more prone to neurodevelopmental abnormalities despite treatments given to their pregnant mother. New therapeutic concepts, designed for counteracting aβ2GP1, are progressively emerging. These uncertainties indicate the possible paths for future research as we move toward precision medicine in thrombotic diseases but still have to impose it in obstetrics.

ABSTRACTS STATE OF THE ART LECTURES II

ANGIOGENESIS, HAEMOSTASIS AND CANCER

Alternative strategies to target angiogenesis

P. Carmeliet (Leuven)

Vesalius Research Center, Vlaams Instituut voor Biotechnologie (VIB), University of Leuven (KULeuven)

Angiogenesis, the growth of new blood vessels, plays a crucial role in numerous diseases, including cancer. Anti-angiogenesis therapies have been developed to deprive the tumor of nutrients. Clinically approved anti-angiogenic drugs offered prolonged survival to numerous cancer patients, but the success of anti- angiogenic VEGF-targeted therapy is limited by intrinsic

refractoriness and acquired resistance. New strategies are needed to block tumor angiogenesis via alternative mechanisms. We recently reported that PFKFB3-driven glycolysis importantly regulates the endothelial tip cell function during vessel sprouting, even overruling the potent pro-stalk activity of Notch, and that its loss in endothelial cells causes vascular hypobranching defects. Moreover, partial and transient reduction of glycolysis by blocking PFKFB3 sufficed to reduce pathological angiogenesis. Ongoing studies explore the role of lipid and amino acid metabolism in vessel sprouting, and assess the therapeutic potential of targeting these metabolic pathways for anti-angiogenic therapy.

Risk assessment of venous thromboembolism in cancer

C. Ay (Vienna)

Medical University of Vienna, Department of Medicine I, Clinical Division of Haematology and Haemostaseology

Cancer is a strong and independent risk factor for venous thromboembolism (VTE). The risk of VTE was shown to be increased four- to 7-fold in patients with cancer. However, the risk of VTE is not equal in all cancer patients and the incidence among different groups of cancer patients varies considerably depending on cancer-, treatment- and patient-related factors. According to a recent systematic review and meta-analysis, the annual incidence rate of VTE ranges between 0.5% and 20%. As thromboembolic complications are a significant cause of morbidity and mortality in patients with cancer, their prevention is of utmost clinical importance.
Risk assessment may help identify patients at high risk of developing VTE who may benefit from primary thromboprophylaxis. Therefore, several studies have focused on the investigation of risk factors for occurrence of VTE in patients with cancer, and found that the most important risk factors are the tumor entity, tumor stage and certain anti-cancer treatments. The histological grade of a tumor has also been linked to an increased risk of VTE. Other studies have reported that the probability of VTE is higher in patients with venous diseases (i.e. history of previous VTE and presence of varicose veins).

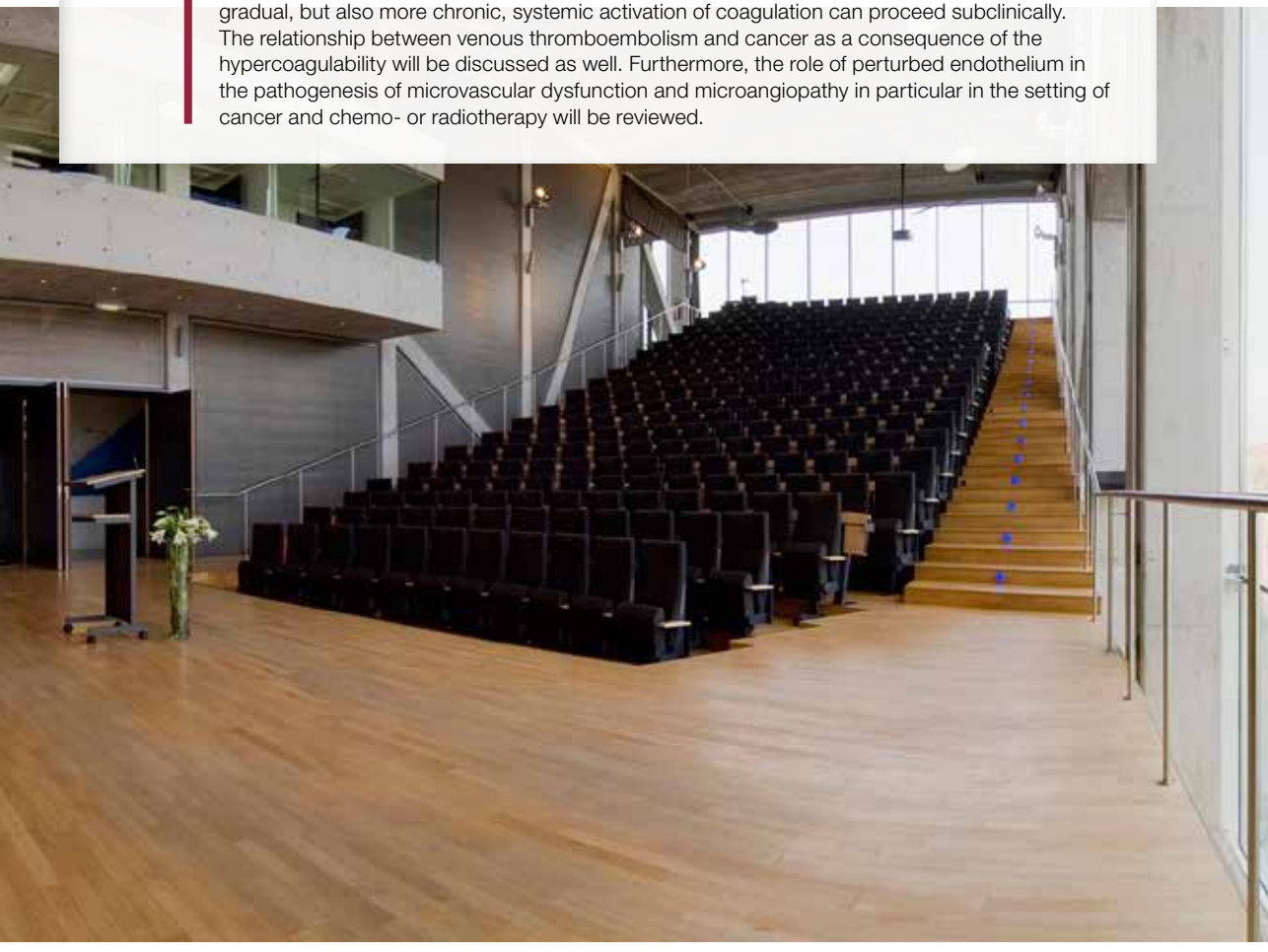
Also laboratory tests and biomarkers have been investigated for their usefulness to predict risk of VTE. Elevated platelet and leukocyte counts and decreased hemoglobin have turned out to be associated with occurrence of VTE. In the Vienna Cancer and Thrombosis Study (CATS), a prospective and observational cohort study, D-dimer, prothrombin fragment 1+2, soluble P-selectin, clotting factor VIII and the thrombin generation potential were identified to be candidate biomarkers for predicting risk of VTE. The role of circulating microparticles in cancer-associated VTE is still not clarified and has to be further investigated.
Consequently, new risk stratification approaches have been adopted to identify patients at high-risk of VTE. The most promising risk assessment models incorporate both clinical parameters and biomarkers. In 2008 Khorana et al. developed such a risk scoring model for prediction of VTE in ambulatory cancer patients scheduled for chemotherapy. Parameters, determined prior to initiation of chemotherapy, that are included in this risk model are: primary site of cancer, pre-chemotherapy platelet count of 350 x 109/l or more, haemoglobin level less than 10 g/dl and/or use of erythropoiesis stimulating agents, pre-chemotherapy leukocyte count of more than 11 x 109/l, and a body mass index of 35 kg/m2 or more. Numerical values (0-2) are assigned to each variable, and patients are stratified into 3 discrete categories according to the total score. This score was validated in the Vienna CATS and other studies. In the Vienna CATS this risk model was expanded by adding two biomarkers (D-dimer and soluble P-selectin), and the prediction of VTE was demonstrated to improve considerably. In the high-risk categories, patients with a VTE rate up to a 20% or higher within 6 months could be identified. However, theses risk stratification tools need to be validated in interventional and randomized controlled trials selecting ambulatory cancer patients for primary thromboprophylaxis, until they can be introduced into clinical practice for routine use.
In summary, the risk of cancer-associated VTE is multifactorial and risk stratification for VTE is feasible in cancer patients.

Cancer-related coagulopathies

M. Levi (Amsterdam)

Department of Medicine, Academic Medical Center, University of Amsterdam, the Netherlands

The association between cancer and thrombosis is known for years. Besides the well-recognized connection between venous thromboembolism and malignancies, there are, however, also other manifestations of cancer-related activation of coagulation and (micro)vascular dysfunction. In fact, coagulation derangements and vascular disturbances in patients with cancer cover a wide spectrum of diseases and various clinical manifestations. We will highlight the mechanisms that play a role in the systemic activation of coagulation in cancer patients, in its most severe form manifested as disseminated intravascular coagulation. Clinically, DIC in cancer has in general a less fulminant presentation than the types of DIC complicating sepsis and trauma. A more gradual, but also more chronic, systemic activation of coagulation can proceed subclinically. The relationship between venous thromboembolism and cancer as a consequence of the hypercoagulability will be discussed as well. Furthermore, the role of perturbed endothelium in the pathogenesis of microvascular dysfunction and microangiopathy in particular in the setting of cancer and chemo- or radiotherapy will be reviewed.



ABSTRACTS **EDUCATIONAL I**

Von Willebrand factor and ADAMTS13: new players in ischemia/reperfusion injury

S. De Meyer (Kortrijk)

Laboratory for Thrombosis Research KULeuven Kulak

Thrombus formation is of paramount importance in the pathophysiology of acute ischemic stroke. Current antithrombotics used to treat or prevent cerebral ischemia are only moderately effective and bear an increased risk of severe bleeding. von Willebrand factor (VWF) has long been known to be a key player in thrombus formation at sites of vascular damage. While the association between VWF and coronary heart disease has been well studied, knowledge about the role of VWF in stroke is much more limited. However, in recent years, an increasing amount of clinical and preclinical evidence has revealed the critical involvement of VWF in stroke development. This overview summarizes the latest insights into the pathophysiologic role of VWF-related processes in ischemic brain injury under experimental conditions and in humans. Potential clinical merits of novel inhibitors of VWF-mediated platelet adhesion and activation as powerful and safe tools to combat thromboembolic disorders including ischemic stroke are discussed.

ABSTRACTS **EDUCATIONAL II**

Numbers, Patterns and Timing: a systematic approach to a low platelet count in intensive care patients

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Institut für Immunologie und Transfusionsmedizin, Universitätsmedizin Greifswald, Germany

The most frequent cause of severe thrombocytopenia in hospitalized patients is chemotherapy-induced hypoproliferative thrombocytopenia. In the critically ill, non-chemotherapy patient thrombocytopenia is also frequent but more challenging from a diagnostic point of view because of the multifactorial pathogenesis of this disorder. Five major mechanisms can result in thrombocytopenia, which are listed in Table 1. Thrombocytopenia is both a pathologic entity and a sign of severe illness. Severely sick patients often lack the capacity of normal platelet production due to toxic effects on the bone marrow and in addition may present with several causes for increased platelet consumption such as bleeding or infection. Treatment should target the underlying disease. Platelet transfusions are indicated in bleeding patients, while there is no strong evidence supporting prophylactic transfusions in critically ill patients. In some patients thrombocytopenia may even indicate an increased risk for macrovascular (e.g. in heparin-induced thrombocytopenia [HIT]) or microvascular (e.g. in thrombotic thrombocytopenic purpura [TTP]) thrombosis. These prothrombotic causes of thrombocytopenia require different management strategies than other causes of thrombocytopenia. Therefore it is most important for treatment decisions to differentiate between a prohemorrhagic and a prothrombotic disorder. Beside the absolute platelet count, interpretation of the platelet count course is helpful to distinguish between different etiologies. Whereas a moderate decrease in platelet counts within the first three days

after major surgery or severe infection is rather typical, an absent or blunted platelet count increase after five days indicates continuing critical illness and a worse outcome.

Post-surgical patients: The platelet count typically declines after major surgery reaching a nadir between day 1 and day 4. The magnitude of the platelet count decrease during the first 3-4 days after surgery reflects the extent of the tissue trauma or blood loss and is primarily caused by platelet consumption. Thereafter, platelet counts increase constantly reaching the pre-surgery level between day 5 and day 7 and peak at around day 14 after surgery. A platelet count decrease in the second week of ICU-treatment should prompt further diagnostic workup, even if the absolute platelet count is still within a range not associated with a substantial increase in the bleeding risk, as this may be an indicator for immune mediated complications e.g. heparin-induced thrombocytopenia.

Medical patients: The platelet course in medical ICU-patients depends on the underlying disease and is usually not influenced by an accidental or iatrogenic tissue trauma. Conditions predisposing for thrombocytopenia are sepsis, use of extracorporeal circuits, intravascular devices, disseminated intravascular coagulation, multi organ failure, and recent cardiopulmonary resuscitation. Medical ICU-patients in whom the underlying disease is successfully treated, typically show a moderate platelet count decrease after admission with a recovery after about five days.

In critically ill patients thrombocytopenia is often associated with platelet function defects. By far the most common cause of an impaired platelet function is the intake of anti-platelet drugs. Other drugs can also inhibit platelet function e.g. the serotonin reuptake inhibitors (antidepressants), or antiepileptic drugs. There are many other causes leading to acquired platelet disorders: infectious diseases (malaria, Dengue hemorrhagic fever) cause thrombocytopenia; uremia, is associated with multiple platelet function defects; in chronic liver disease platelets are altered by increased thrombin generation, which activates platelets, and by increased levels of fibrin split products which bind to platelets; myeloproliferative disorders (essential thrombocytemia, polycythemia vera) are associated with very heterogeneous platelet function defects and in paraproteinemia the pathologic proteins may block platelet receptors or impair the interaction of platelets and von Willebrand factor; chronic platelet activation, e.g. due to extracorporeal circuits, or cardiac assist devices leads to acquired storage pool deficiency, as well as shedding/degradation of platelet membrane proteins and receptors; immune thrombocytopenias and rarely anti-platelet antibodies which impair platelet function but do not cause thrombocytopenia.

Table 1: Different mechanisms of thrombocytopenia

Onset of thrombocytopenia		
At admission	**Absent platelet recovery or slow decrease after initial recovery**	**Rapid decrease after initial recovery**
infusion of plasma expanders and fluids		
Hemodilution		
infusion of plasma expanders and fluids transfusion of red blood cells and plasma		
increased platelet consumption		
major blood loss massive tissue trauma DIC SIRS/ sepsis severe pulmonary embolism diabetic ketoacidosis HELLP-syndrome promyelocytic leukemia malaria microangiopathic desease	persistent bleeding repeated major surgery sepsis/nosocomial infection multiorgan failiure dialysis extracorporeal circulation DIC	SIRS / sepsis DIC pulmonary embolism bleeding
increased sequestration		
Hepatosplenomegaly Hypothermia	circulatory shock resuscitation	
decreased production		
intoxication (drugs, toxins, herbals) acute leukemia myelodysplasia metastatic bone marrow infiltration chronic liver disease viral infections (EBV,CMV,HCV,HIV) irradiation	intoxication viral infections metastatic bone marrow infiltration viral infections acute and chronic liver failure iron depletion folate-deficiency	
graft failiure after HSCT hereditary macrothrombocytopenia hemophagocytosis	vitamin-B12 deficiency chemotherapy	
platelet destruction		
immune thrombocytopenia thrombotic thrombocytopenic purpura early onset HIT antiphospholipid syndrome viral infections (HIV,HCV) drug induced immune thrombocytopenia (out patients) autoimmune disease (lupus)	post surgery TTP	HIT drug induced immune thrombo-cytopenia GPIIb/IIIa -inhibitor induced thrombocytopenia posttransfusion purpura passive transfusion of platelet alloantibodies

SATELLITE SYMPOSIUM SPEAKERS

Cedric Hermans MD, PhD, FRCP (Lond, Edin) (Brussels)

Haemostasis and Thrombosis Unit, Division of Haematology, Catholic University of Louvain, Brussels
Cedric HERMANS is currently heading the Division of Haematology, the Haemostasis and Thrombosis Unit and the Haemophilia Centre of the Saint-Luc University Hospital, Brussels, Belgium where he was appointed Full Professor in 2012. Dr. Hermans has published over 125 original articles in international journals and is a member of several scientific societies and international 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.

Maria Elisa Mancuso (MD, PhD), (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 is involved in the care of both children and adults with hemophilia with a particular scientific interest in inhibitors and chronic hepatitis C.

Serge Motte MD, PhD (Brussels)

Dr Motte is Professor of Health Economics, Health Economics Research Centre, Management of Institutions of care and nursing research, School of Public Health, Université Libre de Bruxelles and Head of the Thrombosis and Antithrombotic Treatment Clinic, Erasme University hospital, Université Libre de Bruxelles, Brussels. He qualified as a medical doctor in 1982, went on to complete his post-graduate training in Internal Medicine in 1987, and was awarded his PhD in 1997, all at the Université Libre de Bruxelles. He has an executive master's degree in management in health care institutions (2009) from the Université Libre de Bruxelles.
His major research interests include the epidemiology, prevention, treatment, economic burden of venous thromboembolism and the management of healthcare institutions. Dr Motte has authored and co-authored over 40 publications on a variety of subjects.

Substitute or target the coagulation cascade: from a rare bleeding disease to common thrombotic disorders

Professor Cedric HERMANS, MD, FRCP (Lon, Edin), PhD,
Full Professor, Catholic University of Louvain

SUBSTITUTE OR TARGET THE COAGULATION CASCADE: FROM A RARE COAGULATION DISEASE TO COMMON THROMBOTIC DISORDERS. SUCCESS STORY OF KOGENATE® AND XARELTO®

Parallel advances in contrasting diseases such as haemophilia and common thrombotic diseases, both arterial and venous, have recently clearly demonstrated the major successes achieved by either replacing or inhibiting specifically one of the clotting factors of the coagulation cascade.

Two randomized, controlled, parallel-group pioneering trials studies using Kogenate®, a recombinant F8 concentrate, have indeed validated the concept that intravenous prolonged substitutive therapy of F8 deficiency provides major physical and well-being benefits not only in children with severe haemophilia A when initiated early but also in adults when started later in life. The large experience accumulated with Kogenate® also shows that F8 concentrate produced by biotechnology represents a convenient and safe alternative, devoid of potential infectious risk, to plasma-derived F8 concentrate for patients with F8 deficiency. Currents efforts to improve the unfavorable kinetics of F8 by modifying its structure and prolonging its persistence in blood should render replacement therapy with Kogenate® not only easier and but also more efficient by allowing a more prolonged correction.

Parallel to the successes of replacement therapy for the rare patients with haemophilia, direct and specific inhibition of activated factor X (FXa) with an oral agent such as Rivaroxaban (Xarelto®) has recently been found to provide a convenient and more efficient alternative to classical oral or parenteral anticoagulants (VKA and LMWH) for the numerous patients requiring a curative or preventive treatment of both venous and arterial thrombosis, mainly patients with (non-valvular) atrial fibrillation at risk of embolic events. With the exception of patients with severely reduced renal dysfunction at risk of drug accumulation (CrCL < 15 ml/min), Xarelto® is becoming a privileged antithrombotic agent for the many patients requiring anticoagulant therapy. Complementary to Xarelto®, Cardio-aspirin® remains the antiplatelet of choice used alone or in conjunction with other antiplatelet agents for the many patients at risk or with established arterial disease.

With Kogenate®, Xarelto® and Cardioaspirin®, Bayer has provided the medical community and the rare patients with haemophilia (1.000 in Belgium), the numerous candidates for prolonged oral anticoagulation (> 100.000) and the even larger group requiring antiplatelet therapy (1.000.000) with an armamentarium of innovative, safe and efficient haemostatic and antithrombotic agents. These agents are constantly being further validated and improved to correct efficiently and safely the defective clotting system typical of rare or common diseases.

References: 1. Manco-Johnson MJ, J Thromb Haemost. 2014;12: 119–122]. J Thromb Haemost. 2013;11(6):1119-1127. 2. Manco-Johnson MJ, N Engl J Med. 2007;357(6):535-544. 3. Coyle TE, J Thromb Haemost. 2014 Apr;12(4):488-96.

LB11.2014.2003

Future therapies in hemophilia A

Maria Elisa Mancuso, (MD, PhD)
Angelo Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy

Replacement therapy is the cornerstone of hemophilia A treatment and it can be delivered either on demand in case of bleeding episodes or as prophylactic treatment in order to prevent bleeds. Despite its proven safety and efficacy, replacement therapy still has some drawbacks that render is accomplishment quite challenging, especially in some patient groups. In fact, such therapy can be administered only by intravenous injections and, owing to the limited half-life of Factor VIII (FVIII), it should be administered quite frequently in order to maintain a measurable trough level.
The progress of hemophilia therapy was very fast in the last 3 decades during which plasma-derived products became safer, and the recombinant technology offered the opportunity of safe and effective new generation products manufactured without the use of human or animal proteins. Nowadays, the available portfolio of FVIII concentrates is quite large however up to now the vast majority of hemophilic patients worldwide have no access to therapy due both to cost and availability issues.

The next future will witness a relevant change in the therapeutic horizon of patients with hemophilia A since new standard recombinant products will be licensed so increasing product choice and availability and FVIII molecules with extended half-life will facilitate treatment feasibility. Two new standard recombinant FVIII products will be soon available, a B domain truncated rFVIII named turoctocog alfa manufactured by Novo Nordisk, and a full-length FVIII molecule that is the evolution of sucrose-formulated rFVIII manufactured by Bayer. Both molecules were safely and effectively used both for treating and preventing bleeding episodes in previously treated patients (PTPs) of any age. Studies on previously untreated patients (PUPs) are ongoing for both molecules. The increased availability of FVIII concentrates should favour cost decrease due to competition on the market thus rendering replacement therapy more accessible in less wealthy countries and making prophylaxis possible for many more patients.

On the other hand, some new modified FVIII molecules have been studies in the recent past and some of them will enter the market very soon. The main modification was aimed at extending FVIII half-life in order to attain and maintain measurable (and so protective) trough levels with less frequent injections. The extension of FVIII half-life is a hard task because of the binding with von Willebrand Factor and indeed up to now only a 1.5-fold prolongation has been obtained. Technologies used to extend FVIII half-life were fusion with Fc fragment of immunoglobulins and PEGylation. Moreover, PEGylation can be random or site-specific and may imply the use of PEG moiety of different molar sizes. One Fc-fusion and 3 PEGylated rFVIII molecules have been developed and have been employed in the frame of Phase 2/3 studies to explore their safety and efficacy to treat and prevent bleeding episodes in severe hemophilia A PTPs. PUPs studies are also ongoing.

LB11.2014.2003

Direct oral anticoagulants in daily care: what do we know today and what are the remaining issues?

S. Motte

SATELLITE SYMPOSIUM SPEAKERS

Professor Ismaïl Elalamy (MD, PhD), (Paris)

Professor Ismaïl Elalamy is a Professor of Haematology and Head of the Haematology Department at Tenon University Hospital, Paris, and current President of the French Society of Angiology. Prof Elalamy has been involved in the field of thrombosis and antithrombotic therapies since 1991, and his research interests include improving therapeutic strategies, identifying new biomarkers and the application of novel diagnostic techniques to the clinic. He was a Post-Doc fellow at the Pasteur Institute, Paris, from 1998-2003, Associate Professor of Haematology at the APHP, Paris, from 1998-2008, and has also been Head of "Thrombosis and Cancer Research Group" since 2005 now attached to INSERM U938 "Cancer Biology and Therapeutics". In his current position, Prof Elalamy has established an expert centre for HIT diagnosis and management in Paris, and a research centre for cancer and thrombosis, with specialized clinical and laboratory activities in platelet studies.
Prof Elalamy holds positions as Councillor of GFTC and the MLTD, as well as General Secretary of GITA. Additionally, he is involved in a French educational programme in Morocco and Tunisia, on behalf of ISTH, focusing on haemostasis and thrombosis.
Since 1998, Prof Elalamy has given over 200 symposia and plenary lectures at international congresses, on a diverse range of issues including; platelet activation, heparin-induced thrombocytopenia, acquired and hereditary thrombophilia, antithrombotic strategies and resistance to anti-platelet agents. He has written over 180 peer reviewed articles and collaborated on several books on haemostasis and thrombosis, as well as editing the first French language book on Heparin-induced Thrombocytopenia in 2006.

Dr. Simon Noble (Cardiff)

Dr Simon Noble is a Clinical Reader in Palliative Medicine at Cardiff University and Honorary Consultant at the Royal Gwent Hospital in Newport. His research interests include the management of venous thromboembolism (VTE) in cancer, quality of life effects of VTE and their therapies, clinical decision making in VTE management and the patient journey.

He is on the faculty for the 1000 Lives Plus Campaign in Wales acting as medical lead for the thromboprophylaxis intervention. He also chairs the All Wales Thromboprophylaxis Group and the All Wales Thrombosis Campaign for Lifeblood, in his capacity as Lifeblood Medical Director for Wales. He was co author on the International Practice Guidelines for the Management of cancer associated thrombosis and is currently Co-chair of the ISTH Scientific subcommittee for haemostasis and malignancy.

He manages the South East Wales Cancer Associated Thrombosis (CAT) Service which looks after 340 new cases of CAT each year. He holds several grants regarding management of CAT in advanced disease. He is currently chief investigator in the international study PELICAN which looks at quality of life and patient experience of CAT in various countries and health settings. He has published 100 original papers and abstracts.

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LMWH and Cancer: Present, Future and Beyond

I. Elalamy
Hôpital TENON UPMC PARIS and INSERM U938

Cancer is a naturally acquired hypercoagulable state. Venous thromboembolism (VTE) is associated with higher risks of recurrence and bleeding and a higher mortality rate in patients with cancer. The specific profile of cancer patients combining frequent co-morbidities, co-medications using various anti-tumoral therapies and the cancer progression itself, represent major therapeutic challenges for the choice of a long-term anticoagulant treatment.

Low-molecular-weight heparins (LMWH) are at the present the preferred option for the treatment and the secondary prevention of VTE according to current guidelines, since their clinical benefit is significantly superior to vitamin K antagonists (VKA). Target-specific oral anticoagulants (TSOA), orally active are a potential attractive alternative to LMWH in the future but this feature is not already proven. Several lines of evidence demonstrated that LMWH are not only potent antithrombotic drugs but these complex polysaccharides lead to many other biological effects with a potential interest in cancer context.
During this presentation, we'll discuss all these aspects trying to understand why LMWH is the optimal choice of a long term anticoagulation in patients with cancer.

Management of Cancer Associated Thrombosis when the Evidence is Lacking, a Real World Experience

Dr. S. Noble
Cardiff University

432BE13PR07668/141086 – November 2014

ABSTRACTS
SATELLITE SYMPOSIUM

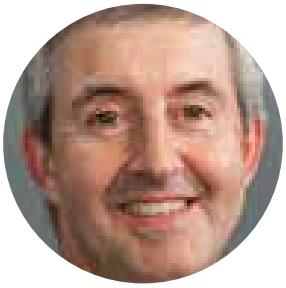
Translating clinical data to real-world use: the importance of medication adherence
B. Vrijens

Well-controlled on VKA: to switch or not to switch?
H. Heidbuchel

SATELLITE SYMPOSIUM SPEAKERS

Dr. Bernard Vrijens
Chief Science Officer, MWV Healthcare, Assosicate Professor of Biostatistics University of Liege, Belgium

Bernard Vrijens is Chief Science Officer at MWV Healthcare. He was General Manager of the AARDEX Group, prior to AARDEX becoming part of MWV Healthcare in 2012. He is also Associate Professor of Biostatistics at the University of Liège, Belgium. Dr. Vrijens holds a PhD from the Department of Applied Mathematics and Informatics at the University of Ghent, Belgium. Dr Vrijen's currently leads a research programme investigating (a) the most common errors in dosing using a simple but robust taxonomy, (b) particular dosing errors that can jeopardise the efficacy of a drug, and (c) the optimal measurement-guided medication management programme that can enhance adherence to medications and maintain long-term persistence. Dr. Vrijens is a co-author of *two* book chapters, over 60 peer-reviewed scientific papers, and named as inventor on two patents. He is a founding member and managing director of the European Society for Patient Adherence, Compliance, and Persistence, and an active member of several EU- and US-funded collaborative projects around the theme of adherence to medications.



Professor Hein Heidbuchel, MD, PhD FESC, Hasselt University and Heart Center Hasselt, Belgium

Professor Heidbuchel (°1961) graduated as MD from the University of Leuven, Belgium, in 1986. He went on to undertake a PhD in Physiology (1992), gained specialist degrees in Internal Medicine (1993) and Cardiology (1995), and a special accreditation in Hospital Administration and Management (2005). He trained as a Resarch Fellow of the NIH (Fogarty International Center) at the University of Oklahoma (Prof. Dr. W. Jackman) in 1993 and 1994, supported by a Fulbright-Hays grant-in-aid, and was a Fundamental Clinical Investigator of the Fund for Scientific Research Flanders from 1996 to 2006. Professor Heidbuchel is a Fellow of the European Society of Cardiology (FESC). He held the position of Chair of the Educational Committee of the European Heart Rhythm Association (EHRA) from 2009 till and was Chair of the Section on Sports Cardiology of the European Society of Cardiology from 2010 till 2012, and was part of the EHRA and EACPR Board during that time. He is a Member of the EHRA Research Network since 2008. He was multiple times a Scientific Committee Member of the EuroPRevent Congress Program Committee and a Member of the Europace Congress Program Committee (EHRA). He is the European Co-ordinating Clinical Investigator of the EuroEco trial and Belgian coordinator of many international clinical trials, DSMB member of international trials and active clinical investigator of more than 25 trials. Professor Heidbuchel served 6 years as Associate Editor for the European Heart Journal,is currently an International Editorial Board Member of Europace, Acta Cardiologica, and Arrhythmia and EP Review, and an Editorial Advisory Board Member for Advances in Venous Arterial Thrombosis. He is reviewer for multiple international journals.


Daiichi-Sankyo

ABSTRACTS SELECTED FOR ORAL PRESENTATION
CLINICAL & LABORATORY

Belgian multicenter study into von Willebrand Disease (B-Will Study): First results

I. Vangenechten[1], K. Jochmans[2], P. Peters[3], K. Devreese[4], C. Hermans[5], S. Motte[6], A. Gadisseur[1]
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Background/ Introduction
Von Willebrand Disease (VWD) is an autosomally inherited bleeding disorder caused by a quantitative or qualitative defect of von Willebrand factor (VWF). It is classified in type 1 (quantitative defect), type 2 (qualitative defect), and type 3 (absence of VWF), and further subclassifications. The current ISTH classification is based on measurement of VWF:Ag, VWF:RCo, Ristocetin Induced Platelet Aggregation (RIPA) and VWF multimers.

Aims
In a multicenter study, organized by the Antwerp University Hospital, a family-based analysis of VWD in Belgium was initiated in 2011 under auspices of the BSTH, with patient accrual starting in 2012.Blood samples were collected from patients with suspected or known VWD with one of the following characteristics: VWF:Ag<35%, VWF:RCo<35%, VWF:RCo/VWF:Ag<0.7, VWF:CB/VWF:Ag<0.7, FVIII:c/VWF:A <0.5, or positive low concentration RIPA, and grouped in families. From each family the proband was included in the study together with at least one affected sibling or parent.

Methods/Materials
At this moment, blood has been collected from 123 representing 77 families with suspected VWD. FVIII:c, VWF:Ag, VWF:RCo, VWF:CB, VWFpp, VWF-FVIII binding (if indicated), VWF multimers and molecular analysis were performed at Antwerp University Hospital. Platelet Function Analyzer and RIPA testing were done locally.

Results
Based on laboratory tests FVIII:c, VWF:Ag, VWF:RCo, VWF:CB, VWFpp and VWF multimers the distribution of different subtypes of VWD is as follows: VWD type 1 in 29/123 patients, and type 2 in 21/123 patients (type 2A in 12/125, type 2M in 8/123 and type 2N 1/123), with 10/123 still unconfirmed/unclassified. All cases of type 3 VWD (5/123 patients) were based on the presence of (often asymptomatic) type 1 VWD in both parents.

Summary/Conclusions
Molecular analysis is still ongoing with currently 56/123 patients fully analyzed and the remainder partially. So far, 27 mutations in the VWF gene have been found in 55/123 patients, of which 7 are new to the ISTH VWD database (http://www.vwf.group.shef.ac.uk/), and awaiting gene expression studies. In 2/123 patients heterozygous type 2N mutations were detected.

Dose finding of rivaroxaban in hemodialysis patients

A.S. De Vriese[1], R. Caluwé[2], E. Bailleul[3], D. De Bacquer[4], D. Borrey[5], S. Ameye[5], E. Hysselinckx[3], B. Van Vlem[2], S.J. Vandecasteele[1], J. Emmerechts[5]
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Background/ Introduction
The use of vitamin K antagonists for the prevention of stroke and systemic embolism in dialysis patients with non-valvular atrial fibrillation is controversial. However, no good alternatives are presently available. The anti-FXa antagonist rivaroxaban is contraindicated in patients with a CreaCl < 15 ml/min, for lack of pharmacokinetic, pharmacodynamic

Aims
IThe aim of the present study was to characterize the pharmacokinetics and pharmacodynamics of rivaroxaban in chronic hemodialysis patients without residual renal function. and clinical data.

Methods/Materials
Study Design: A two-centre non-blinded cohort study.
Setting & Participants: Eighteen chronic hemodialysis patients without residual renal function.
Interventions: Three evaluations were performed: 1) A single dose of 10 mg rivaroxaban was administered at the end of three subsequent dialysis sessions and the area under the curve (AUC) and effect on coagulation parameters was measured during 44 hours. 2) A single dose of 10 mg rivaroxaban was given 6 to 8 hours before a dialysis session and the effect of dialysis on rivaroxaban levels was evaluated. 3) To assess potential accumulation, rivaroxaban 10 mg was given once daily and the AUC was measured during 24 hours on day 1 and day 7. Rivaroxaban concentrations were measured both by a chromogenic anti-FXa assay and by LC-MSMS. Prothrombin time (PT) was assessed using two different recombinant PT reagents and expressed as ratios.

Results
The mean AUC0-44 of rivaroxaban plasma levels after a single dose of 10 mg was 2072 µg/L/hour, the mean Cmax was 172.6 µg/L and the mean terminal elimination half-life time was 8.6 hours. The highest PT ratio measured was 1.56 and 1.70 at 4 hours for Innovin® and Recombiplastin® respectively. Dialysis had no appreciable effect on the plasma levels of rivaroxaban nor on PT values. Mean Ctrough after multiple daily doses of 10 mg was 32.9 µg/L.

Summary/Conclusions
This is the first study investigating the pharmacokinetics and pharmacodynamics of rivaroxaban in hemodialysis patients without residual renal function. A dose of 10 mg rivaroxaban results in a similar drug exposure as published for 20 mg in healthy volunteers. Rivaroxaban is not eliminated by dialysis. There was no accumulation after multiple daily dosing. Rivaroxaban 10 mg daily may be used as an alternative for vitamin K antagonists in hemodialysis patients.

Rivaroxaban for the treatment of consumptive coagulopathy associated with vascular malformations

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Background/ Introduction
The localized activation of coagulation in vascular malformations can lead to a consumptive coagulopathy characterized by elevated D-dimers and a consumption of fibrinogen and platelets, eventually giving rise to a bleeding tendency. By reducing coagulation activation, anticoagulant treatment with heparin is able to halt this haemostatic dysregulation and the associated bleeding diathesis. As there is no recommended optimal dose, treatment is individually titrated according to the effect on the haemostatic profile and the achieved anti-Xa activity.

Aims
We included our Aims in the Results section

Methods/Materials
This is a clincal case reports, no specific Methods/Materials

Results
Here, we present a case of a consumptive coagulopathy due to a vast vascular malformation of the trunk with a sustained correction of the fibrinogen depletion and associated bleeding tendency both with subcutaneous enoxaparin and with the oral factor Xa inhibitor rivaroxaban. Heparin potentiates the effect of antithrombin, which reduces the thrombin-induced conversion of fibrinogen by inhibiting factor Xa and IIa. Although it may seem counterintuitive to treat a bleeding tendency with an anticoagulant agent, a reduction of the localized coagulation reverses the pathophysiological process underlying the consumption of clotting factors (1) (Figure 1). Despite the biochemical and clinical effect of LMWHs, the need for long-term parenteral treatment may defer both physicians and patients from treatment. Therefore we opted to switch to the oral factor Xa inhibitor rivaroxaban with positive biochemical (Figure 1) and clinical results. Rivaroxaban is a diral oral factox Xa inhibitor approved for the prevention of thromboembolic complications in atrial fibrillation (2), for the prevention of VTE after major orthopaedic surgery, and for the treatment of acute VTE as well as the secondary prevention of recurrent VTE (3, 4).

Summary/Conclusions
We illustrate a successful transition from the LMWH enoxaparin to rivaroxaban for this patient with pronounced consumptive coagulopathy and we demonstrate that the oral factor Xa inhibitor rivaroxaban can be an effective and more convenient alternative for LMWH to reverse consumptive coagulopathy in patients with large vascular malformations. The dose of the Xa inhibitor needs to be tailored in function of the effect on the bleeding tendency and the laboratory coagulation parameters.

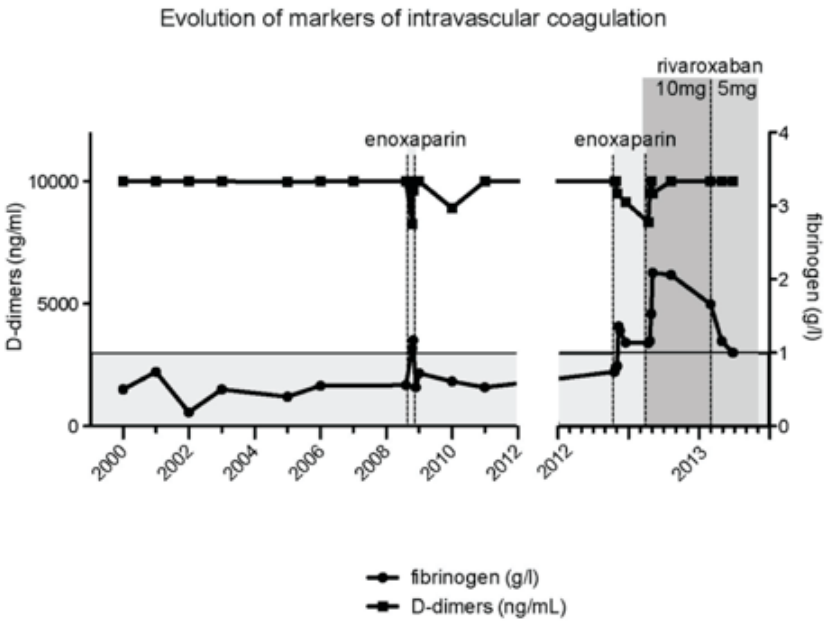

Figure 1. Evolution of markers of intravascular coagulation in relation to treatment with enoxaparin or rivaroxaban

References
1. Verstraete M, Amery A, Vermylen C, Robyn G. Heparin treatment of bleeding. Lancet. 1963;1(7278):446.
2. Patel MR, Mahaffey KW, Garg J, Pan G, Singer DE, Hacke W, et al. Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. The New England journal of medicine. 2011;365(10):883-91.
3. EINSTEIN Investigators, Bauersachs R, Berkowitz SD, Brenner B, Buller HR, Decousus H, et al. Oral rivaroxaban for symptomatic venous thromboembolism. The New England journal of medicine. 2010;363(26):2499-510.
4. EINSTEIN-PE Investigators, Buller HR, Prins MH, Lensin AW, Decousus H, Jacobson BF, et al. Oral rivaroxaban for the treatment of symptomatic pulmonary embolism. The New England journal of medicine. 2012;366(14):1287-97.

Evaluation of a rapid lateral flow immunoassay (STic Expert® HIT) in Heparin-Induced Thrombocytopenia (HIT)

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Background/ Introduction
Recently, a rapid lateral flow immunoassay (STic Expert® HIT, Stago Diagnostica) became available for the diagnosis of Heparin-Induced Thrombocytopenia (HIT), a heparin-related complication caused by antibodies against heparin-platelet factor 4 (H-PF4). Patients with HIT suffer from a very high risk of thrombosis and death, which makes a prompt diagnosis essential.

Aims
Although the diagnosis is based on clinical criteria according to Warkentin's 4T scoring system1, the diagnosis based on clinical symptoms only is not straightforward. This implies the necessity of a rapid and reliable laboratory assay.

Methods/Materials
Citrated plasmas (stored at -80°C) from 158 patients with clinical suspicion of HIT were analysed with a rapid lateral flow immunoassay (STic Expert® HIT) and ELISA (Asserachrom® HPIA IgG, Stago Diagnostica). Definite HIT was diagnosed in 20 patients with positive result with a flowcytometric CD62p functional assay2,3,4. The Warkentin 4T score was determined. Results for STic Expert® HIT were read by three readers. For ELISA a cut-off was calculated for each run as described by the manufacturer.
Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for both assays were determined using Bayesian analysis (MedCalc). Interreader agreement for STic Expert® HIT was defined on the basis of kappa agreement (IBM SPSS Statistics 22 and R-project for Statistical Computing).

Results
Asserachrom® HPIA IgG had a sensitivity of 100.00% and specificity of 81.16%. PPV and NPV were 43.48% and 100.00%, respectively. Sensitivity for STic Expert® HIT was 95.00% for all readers and specificity ranged between 84.06% and 84.96% for the three readers. PPV and NPV varied between readers from 46.34% to 48.72%, and 99.12% to 99.15%, respectively. Interreader variability (kappa agreement) between two readers varied from 0.812 to 1.000 and Fleiss' Kappa agreement for the three readers was 0.875. One sample with a 4T score of 3 was false negative with STic Expert® HIT for the three readers. The false negative result was confirmed by a different test performer and three other, blinded readers. Asserachrom® HPIA IgG resulted in an OD of 0.725 for this sample.

Summary/Conclusions
Asserachrom® HPIA IgG showed a 100.00% sensitivity compared to 95 % for STic Expert® HIT. Specificity was slightly lower for Asserachrom® HPIA IgG when compared to STic Expert® HIT. Interreader variability for STic Expert® HIT was very good. STic Expert® HIT provides a time saving, easy and ready to use method for detection of HIT antibodies. However, one patient with a 4T score of 3 was false negative with STic Expert® HIT.

References
1. Greinacher A. and Warkentin T.E. Recognition, treatment, and prevention of heparin-induced thrombocytopenia: review and update. Thrombosis Research. 2006: 118, 165-176.
2. Denys B et al. A clinical-laboratory approach contributing to a rapid and reliable diagnosis of heparin-induced thrombocytopenia. Thrombosis Research. 2008: 123, 137-145.
3. Tomer A. A sensitive and specific functional flow cytometric assay for the diagnosis of heparin-induced thrombocytopenia. British Journal of Haematology. 1997: 98, 648-656.
4. Tomer A. et al. Determination of heparin-induced thrombocytopenia: a rapid flow cytometric assay for direct demonstration of antibody-mediated platelet activation. American Journal of Hematology. 1999: 61, 53-61.

Influence of platelet count in platelet rich plasma for adenosine triphosphate release assay

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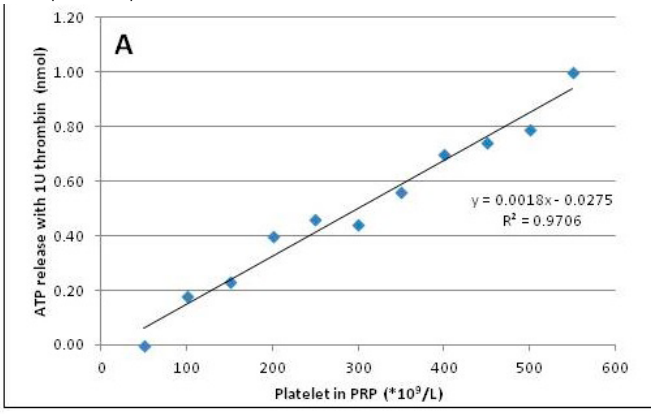
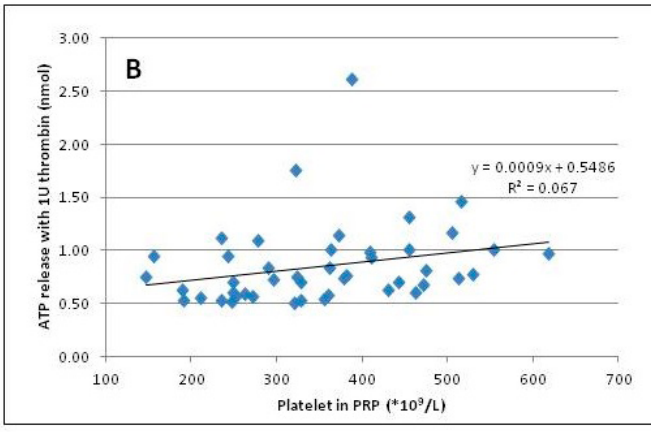
Background/ Introduction
Platelet dense granule release assays are recommended for diagnosing platelet function disorders and are commonly performed by bioluminescent assays that measure stored adenosine triphosphate (ATP) release. The bioluminescent assays are typically performed using a lumi-aggregometer and platelet rich plasma (PRP). PRP should be prepared by centrifuging blood samples at 200 x g for 10 min (Cattaneo et al. 2013).
For the light transmission platelet aggregation (LTA) guidelines are available on the platelet count in PRP. LTA results could be inaccurate when the platelet count in PRP is lower than 150*10^9/L (Cattaneo et al. 2013). When the platelet count in PRP is >600*10^9/L, the platelet count should be adjusted using platelet poor plasma (PPP) (Harrison et al. 2011). The ATP release assay using PRP may also be influenced by the platelet count in PRP. However, there are no recommendations about the platelet count in PRP to perform the ATP release assay.

Aims
We evaluated the influence of platelet count in PRP on the ATP release assay, performed by lumi-aggregometry (Chrono-Log, Havertown PA, USA).

Methods/Materials
In the initial investigation of bleeding disorders we perform LTA and ATP release. We retrospectively evaluated samples of patients with a suspected bleeding diathesis, but with normal LTA with several agonists (adenosine diphosphate, collagen, ristocetin, arachidonic acid, and thromboxane analogue U46619) and normal ATP release (> or = 0.5 nmoles) with 1U thrombin (n=45). We correlated the ATP release with the platelet count in PRP. We also performed dilution series of one patient sample with platelet count in PRP between 50-550*10^9/L and correlated with ATP release.

Results
In serial dilution samples a linear correlation (r^2=0.9706) between ATP secretion and the platelet count was observed (see Figure 1A). For a platelet count below 300*10^9/µL ATP release was under the reference limit (<0.5 nmol). The mean ATP secretion of the patient samples (n=45) was 0.85 nmol (range, 0.50-2.61 nmol) and the mean platelet count in PRP was 352*10^9/µL (range, 147-618*10^9/µL). The correlation in patient samples was weaker (r^2=0.067) (see Figure 1B), due to patient variability. Patients with a platelet count between 147 and 300*10^9/µL (n=17) showed a normal ATP secretion.





Summary/Conclusions
We observed a linear correlation between ATP release and platelet count in PRP. Our dilution series showed that the platelet count should be >300*10^9/µL. However, there may be an inter-individual variability. Because the ATP release is depended on the platelet count in PRP, guideline development might be helpful to improve practices for performing ATP release assays.

PEAR1: a novel link between IgE-mediated allergy and cardiovascular disease

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Background/ Introduction

Cardiovascular disease is a leading cause of mortality worldwide which occurs when platelets inappropriately aggregate and occlude the vasculature serving organs. Genome-wide association studies have identified several loci associated with cardiovascular disease susceptibility and platelet function, with independent studies highlighting polymorphisms linked to Platelet and Endothelial Aggregation Receptor-1 (PEAR1) as being responsible for natural variation in response to platelet agonists. In activated platelets, PEAR1 is redistributed from cytoplasmic α-granules to the membrane and is phosphorylated and thereby stabilizes aggregates. An important unanswered question in understanding the mechanism of PEAR1 function is the identification of its activating ligand during platelet aggregation.

Aims

To investigate how PEAR1 signaling is initiated, we sought to identify its extracellular ligand by creating a protein microarray representing the secretome and receptor repertoire of the entire human platelet.

Methods/Materials

A list of secreted and membrane proteins expressed by human platelets was compiled and a total of 178 plasmids encoding 173 proteins and their complexes were synthesized, and expressed in mammalian cells.

Results

Using a soluble recombinant PEAR1 protein and a systematic screening assay designed to detect extracellular interactions, we identified the high-affinity immunoglobulin E (IgE)-binding subunit Fc ε R1 α , as a physiological PEAR1 ligand. The Fc ε R1 α -PEAR1 interaction was potently inhibited by pre-complexing Fc ε R1 α with IgE, suggesting that IgE is an intrinsic plasma-borne restrictive regulator of platelet aggregation and that IgE could potently inhibit the Fc ε R1 α -PEAR1 interaction, suggesting links between platelet function and allergy. Consistent with this, Fc ε R1 α -mediated PEAR1 signaling and stable platelet aggregate formation were inhibited by exogenous IgE. Finally, omalizumab, an anti-IgE therapeutic, relieved IgE-mediated regulation of the Fc ε R1 α -PEAR1 interaction and platelet aggregate stability in perfusion assays (Figure 1), consistent with recent clinical data on omalizumab use.

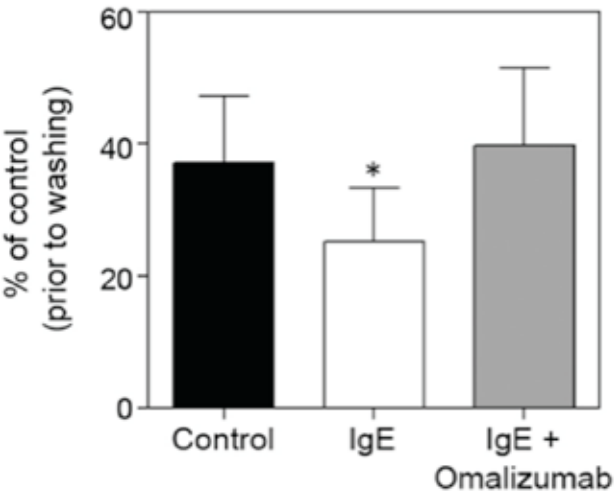

Figure 1 - IgE reduces the stability of collagen-induced aggregates, an effect relieved by omalizumab. The reduction in the size of aggregates was measured after a 15 minute wash out phase under flow in a perfusion chamber. The data are averaged from three individuals, bars indicate mean ± SEM; n = 3, * P < 0. calculated using a repeated measures ANOVA with Tukey's multiple comparison test.

Summary/Conclusions
The identification of Fc ε R1 α as an activating ligand for PEAR1 and the finding that IgE can inhibit this interaction suggests a mechanistic link between circulating IgE levels and platelet function. Healthy individuals have low circulating IgE levels (~0.5 nM) which match the affinity of IgE for Fc ε R1 α, resulting in sub-saturating Fc ε R1 αoccupation. In atopic patients where IgE levels can increase ten-fold, saturation of platelet Fc ε R1 α by IgE with the consequent loss of PEAR1 signaling may mechanistically explain a lack of responsiveness to multiple agonists reported in platelets isolated from asthmatic patients. The controlled in vivo reduction of circulating IgE by clinical intervention can be achieved by a therapeutic anti-IgE monoclonal antibody, omalizumab, which prevents IgE binding to Fc ε R1 α . We have shown that omalizumab can relieve IgE-mediated inhibition of the Fc ε R1 α-PEAR1 interaction and possibly provides an explanation for the increased risk of cardiovascular disease/stroke associated with omalizumab use. In summary, we believe that the platelet receptor protein microarray will be a valuable tool in cardiovascular disease research, and the detailed investigation of the Fc ε R1 α -PEAR1 interaction and its regulation by endogenous IgE provides a mechanism to explain previously under-appreciated interactions between allergy and cardiovascular disease.

‘Sleeping Beauty’-mediated gene transfer of ADAMTS13 prevents the onset of TTP-like symptoms in ADAMTS13-deficient mice

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Background/ Introduction
In humans, congenital deficiency of A Disintegrin And Metalloprotease with ThromboSpondin type 1 motif (ADAMTS13) may cause the life-threatening disease thrombotic thrombocytopenic purpura (TTP). The current treatment of choice is plasma infusion. However, further research is required because plasma infusion exposes patients to the risk of allergies, infections, and fluid volume overload.

Aims
We tested the use of the non-viral ‘Sleeping Beauty’ (SB) transposon system to integrate the murine ADAMTS13 (muADAMTS13) gene and to prevent the onset of TTP-like symptoms in Adamts13-/- mice.

Methods/Materials
The SB transposon system (muADAMTS13-transposon and transposase expressing plasmid) was delivered by hydrodynamic tail vein injection in Adamts13-/- mice. Transgene muADAMTS13 present in plasma was measured using a muADAMTS13:Ag ELISA, its proteolytic activity was assessed using a FRETS-VWF73 assay and VWF multimers were analyzed using SDS agarose gel electrophoresis. Mice expressing transgene muADAMTS13 were challenged with recombinant human VWF (rhuVWF) at different time points to induce TTP-like symptoms. The TTP-like phenotype was evaluated by the occurrence of severe thrombocytopenia and organ damage. Adamts13-/- mice hydrodynamically injected with 0.9% NaCl were used as controls (non-treated mice).

Results
The co-injection of the transposon plasmid with the transposase-expressing plasmid resulted in high levels of active transgene muADAMTS13 (299±46%, 221±33%, and 156±26% at day 7, 28 and 98 days after injection respectively). Moreover, transgene muADAMTS13 was active as it digested FRETS-VWF73 ex vivo and ultra-large VWF multimers in vivo. Interestingly, the presence of transgene muADAMTS13 protected Adamts13-/- mice from TTP-like symptoms after challenge with rhuVWF at 7, 28, or 70 days as no severe thrombocytopenia and severe organ damage were observed in SB-treated mice compared with non-treated mice.

Summary/Conclusions
We successfully used the non-viral SB transposon system to realize long-term expression of transgene muADAMTS13 in Adamts13-/- mice. This transgene muADAMST13 was active, capable of digesting UL-VWF multimers in vivo, and able to prevent the onset of TTP-like symptoms in Adamts13-/- mice.

Staphylococcus aureus von Willebrand factor-binding protein exhibits a dual role in bacterial adhesion to the vessel wall

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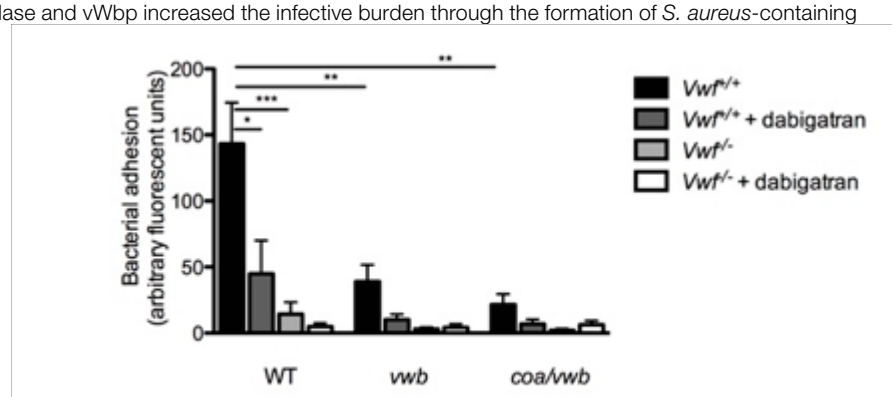
Background/ Introduction
Staphylococcus aureus (S. aureus) is the most frequent cause of life-threatening endovascular infections and infective endocarditis. Adhesion of *S. aureus* to blood vessels under shear stress requires von Willebrand factor (VWF), however, the mechanisms are incompletely understood. Several bacterial factors have been proposed to interact with VWF, including von Willebrand factor-binding protein (vWbp), a secreted coagulase that contributes to *S. aureus* pathophysiology by activating the host's prothrombin. Staphylothrombin, the resulting complex of a bacterial coagulase and prothrombin, converts fibrinogen into insoluble fibrin.

Aims
We aim to investigate the role of coagulase activity and VWF binding in the initiation and pathogenesis of endovascular infections, and the contribution of vWbp in these two functions in particular.

Methods/Materials
We measured adhesion of S. aureus Newman to VWF, collagen and activated endothelial cells (ECs) in a micro parallel flow chamber. By using either pharmacological coagulase inhibition, targeting both coagulases (staphylocoagulase and vWbp), or by using mutant deficient in either vWbp (*vwb)*, staphylocoagulase (coa) or both, we studied the contribution of *VWF* binding, coagulase activity and platelet interactions in shear-dependent *S. aureus* adhesion. *In vivo* adhesion of *S. aureus* was evaluated in the mesenteric circulation of wildtype (WT) and *Vwf*-deficient mice by using real-time fluorescence video-microscopy.

Results
We found a shear-dependent increased adhesion of *S. aureus* to the (sub)endothelium, dependent on interactions between vWbp and the A1-domain of VWF. Adhesion was enhanced by coagulase-mediated fibrin formation that clustered bacteria and recruited platelets into microthrombi. Coagulase inhibition and inhibition of platelet αIIb β3 reduced *S. aureus* adhesion. *In vivo*, deficiency of vWbp or VWF, and inhibition of coagulase activity reduced S. aureus adhesion to the vessel wall.

Summary/Conclusions
We conclude that vWbp is a key protein involved in early steps of vascular infections by *S. aureus* through a unique synergism between its shear-dependent interaction with VWF and its coagulase activity. vWbp mediates bacterial binding to collagen and ECs under shear via vWbp-VWF interactions. Bacterial adhesion is enhanced by S. aureus mediated fibrin formation and by *S. aureus*-fibrin-platelet interactions. S. aureus adhesion to activated mesenteric endothelium required both VWF and vWbp, whereas the coagulase activity of staphylocoagulase and vWbp increased the infective burden through the formation of *S. aureus*-containing microthrombi.



"The initial bacterial adhesion to activated endothelium in vivo is VWF en vWbp mediated"

Causes of 'Gray platelets': from genetic studies of inherited thrombocytopenia to functional studies

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Background/ Introduction
The BRIDGE-BPD consortium (www.bridgestudy.org) performed exome sequencing to discover novel regulators of megakaryopoiesis and platelet function in patients with bleeding and platelet disorders (BPDs). A heterozygous insertion (c.261-262insC) was detected in the GFI1B gene of a Belgian macrothrombocytopenia patient (Chen, Science, 2014). Electron microscopy of her enlarged platelets closely resemble the defects that we previously described for X-linked macrothrombocytopenia due to GATA1 D218 mutations (Freson, Blood, 2001). Both present with enlarged platelets with membrane inclusion and paucity of alpha granules. Others have even described GATA1 and GFI1B defects in literature as 'Gray platelet syndromes (GPS)'. However, the genetic cause of the real GPS is due to recessive mutations in NBEAL2 that is expected to be involved in alpha granule formation. We also studied platelets from a patient homozygous for a nonsense mutation in NBEAL2 (W2480X).

Aims
Based the striking phenotypic resemblance of gray platelets in these 3 different genetic diseases, we hypothesize that GATA1 regulates NBEAL2 expression via interaction with GFI1B. Both transcription factors have zinc fingers that can potentially interact with each other or indirectly via DNA. Our aim is to unravel the interplay between GFI1B, GATA1 and NBEAL2 in megakaryopoiesis with a focus on alpha granule formation and trafficking.

Methods/Materials
Platelet extracts and CD34+ hematopoietic stem cells (HSC) from GATA1-D218Y and NBEAL2-W2480X patients were used for immunoblots and in vitro megakaryopoiesis experiments, respectively. GATA1 binds the sequence (A/T)GATA(A/G) in regulatory elements of many lineage-restricted genes. ChIP-sequencing experiments revealed 3 GATA binding sites in a regulatory region upstream of the NBEAL2 gene. By cloning this fragment with binding sites BS1, BS2 and BS3 in a luciferase reporter construct and modifying these sites by mutagenesis, we investigated the importance of GATA1/GATA2 transcription regulation of the NBEAL2 gene. Using a GST pull-down assays we studied the binding between GST-coupled GATA1-WT, GATA1-D218Y and GATA2 with GFI1B zinc fingers.

Results
Platelet extracts from GATA1 defective platelets revealed low NBEAL2 levels. In vitro megakaryopoiesis experiments using GATA1-D218Y and NBEAL2-W2480X HSC showed abnormal vWF staining in alpha granules. Preliminary results of the luciferase assays showed that by blocking GATA BS1, a higher promoter activity and by blocking BS2, a lower promotor activity was obtained. The mutation in BS3 didn't give a clear difference in the promotor activity in comparison to the wild type sequence. Interestingly, overexpression of GATA2 in combination with the NBEAL2 promotor with a mutation in BS1 resulted in a 9-fold increase of the activity while the combination with the NBEAL2 promotor with a mutation in BS2 resulted in a significant decrease of activity. This confirms that the GATA binding sites are important for the regulation of the promoter of NBEAL2 and its expression is influenced by GATA2. Results of the GST pull-down assay further showed that GFI1B binds with a stronger affinity to GATA1-D218Y in comparison to the GATA1-WT.

Summary/Conclusions
Additional binding experiments are necessary to elucidate the exact mechanism by which GFI1B and GATA1 stimulate NBEAL2 expression but we here provide a first evidence for their interplay via direct interactions.

Recombinant ADAMTS13 as an effective therapy for acquired thrombotic thrombocytopenic purpura in rats

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Background/ Introduction
The size of von Willebrand factor (VWF) multimers is regulated via proteolysis by a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13). A deficiency in ADAMTS13 activity is associated with the pathogenesis of thrombotic thrombocytopenic purpura (TTP), a life-threatening condition in which episodes of thrombotic microangiopathy damage kidneys, heart and brain. Most TTP patients suffer from acquired TTP, where circulating anti-ADAMTS13 autoantibodies are causative for the decreased ADAMTS13 activity. Current treatment consists of daily plasma exchange, but this is time consuming, expensive and not without risk. Therefore, improved therapies are highly warranted.

Aims
We aim at developing a new rat model for acquired TTP, in which circulating inhibitory antibodies against ADAMTS13 are present. With this model we next want to investigate the therapeutic efficacy of recombinant ADAMTS13.

Methods/Materials
Rats (Sprague-Dawley) were injected with 650 U/kg polyclonal goat anti-ADAMTS13 IgG to inhibit endogenous rat ADAMTS13 activity. Next, human recombinant ADAMTS13 (rhADAMTS13) was injected in different doses and ADAMTS13 activity and immune complex formation was determined over time to measure antibody binding to endogenous and rhADAMTS13. A rat model for acquired TTP was next developed by injecting rats with the goat anti-ADAMTS13 antibodies and challenging them with 2000 U/kg recombinant human VWF (rhVWF) to trigger TTP symptoms. The efficacy of rhADAMTS13 to treat acquired TTP in these rats was studied by subsequent injection of different doses of rhADAMTS13 (400, 800 and 1600 U/kg). Blood and organs were collected to determine the clinical pathology.

Results
The polyclonal antibody against ADAMTS13 completely blocked endogenous rat ADAMTS13 activity, as no activity of the enzyme is detected within 24 hours after injection. Upon subsequent administration of rhADAMTS13 to the rats, circulating inhibitory antibodies immediately complexed a large proportion of rhADAMTS13 as measured by the presence of circulating immune complexes. Despite the formation of immune complexes, a dose-dependent increase in rhADAMTS13 activity was observed indicating that administration of rhADAMTS13 was able to override circulating anti-ADAMTS13 inhibitory antibodies. When rats with inhibited anti-ADAMTS13 activity were challenged with rhVWF, they displayed severe TTP symptoms such as thrombocytopenia, hemolytic anemia, increased lactate dehydrogenase activity and the presence of VWF-rich thrombi in kidneys and brain. Treatment with increasing doses of rADAMTS13 resulted in restoration of these TTP symptoms, as appreciated by a normalization of the platelet count, increased proteolysis of high molecular weight VWF multimers, decreased lactate dehydrogenase activity and the absence of VWF-rich microthrombi in kidney and brain.

Summary/Conclusions
We have established a small laboratory animal model for acquired TTP. Using this model, we have demonstrated that rhADAMTS13 is able to override circulating anti-ADAMTS13 inhibitory antibodies, and is able to restore TTP symptoms in rats. Therefore, rhADAMTS13 is an effective therapy for acquired TTP in rats and holds promising value for future clinical use for the treatment of this severe and life-threatening disease.

ABSTRACTS SELECTED FOR POSTER PRESENTATION
CLINICAL & LABORATORY

Evaluation of the chromogenic dosage of FXII and its application to angioedema type III

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Background/ Introduction
Hereditary angioedema (HAE) is a disease characterized by recurrent episodes of angioedema, which most often affect the skin or mucosal tissues of the upper respiratory and gastrointestinal tracts. It is the consequence of an excessive production of bradykinin. Three types of HAE have been described. Type I is due to a deficiency of C1 inhibitor (C1-INH) which plays a role in regulating bradykinin production, type II are due to the presence of dysfunctional C1-INH, whereas type III is characterized by normal C1-INH levels, and is called HAE with normal C1 inhibitor. A minority of these patients present with mutations in factor XII resulting in a gain of function. Published studies indicate that Chromogenic dosage of FXII is more adapted than the usual one-stage method to detect high level of FXII in this population.

Aims
We evaluated a chromogenic method to measure FXII levels in patients suspected of angioedema with normal C1 -INH and compared these results to the usual one stage method with special attention to high level of FXII.

Methods/Materials
The chromogenic FXII kit (Coachrom FXII Test Kit) was tested on STA-Evo® automate (kinetic measure) and in microplate (end point measure). Normal citrated plasmas were used to evaluate reference range, linearity, quantification and detection limits, repeatability, reproducibility, accuracy, interference with lipemia, icteria and hemolysis. One stage method was performed with the CRYOcheck®, FXII deficient plasma, using Cephascreen ® (Stago) reagent on STA-Evo®. Thirthy-one plasmas from patients suspected of HAE with normal C1-INH were evaluated using the 3 methods.

Results
Chromogenic method adapted on microplate showed the lowest bias, variability and highest linearity limit. Normal reference range of FXII for the Chromogenic method in microplate was narrower compared to both the kinetic method and the one stage method (78-127% versus 55-163% and 61-240% respectively). 13/31 plasmas of patients suspected of angioedema with normal C1 -INH showed FXII levels above the reference range with the method adapted in microplate, versus 8 and 9 only with the method on STA and chronometric method respectively.

Summary/Conclusions
These results confirm that chromogenic dosage of FXII could be more adapted to diagnose abnormal FXII levels in this population. This method can be reliably adapted on a routine automate. However, these results should be interpreted together with clinical and genetic data.

Adenosine triphosphate release assay with different concentrations of thrombin for the assessment of platelet function disorders and correlation with platelet count in storage pool disease

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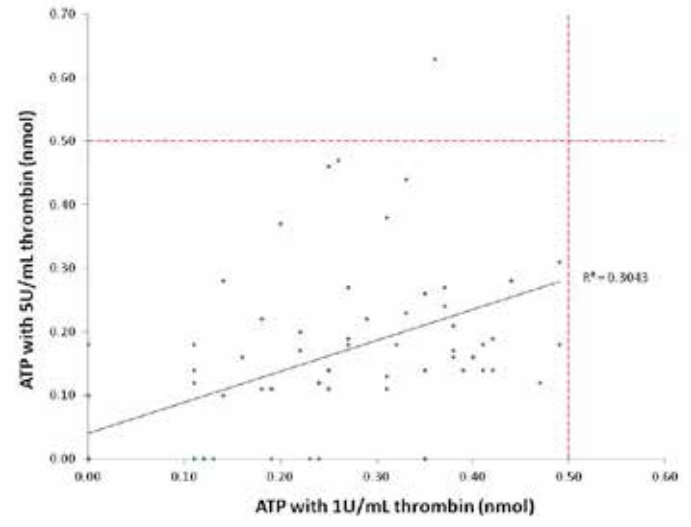
Background/ Introduction
Inherited platelet disorders constitute a heterogeneous group of bleeding. A subgroup of these disorders are characterized by deficiency in the number of granules, granule content or their release mechanisms. The measurement of platelet dense granule content (adenosine triphosphate (ATP)) by lumi-aggregometry is useful in the diagnosis of platelet disorders. Dense granules are rich in adenosine triphosphate (ATP), after activation the content is secreted, further enhancing both platelet adhesion and activation. Secretion of reduced amount of ATP is indicative of either a release defect or a storage pool disease (SPD), by increasing the concentration of the agonist, a distinction could be made. Whether the platelet count of platelet rich plasma (PRP) may influence the amount of ATP secretion is unknown.

Aims
We evaluated the usefulness of activation with different concentrations of thrombin (1 and 5 U/mL) for the detection of platelet dense granule disorders and evaluated the influence of the platelet count in PRP on the ATP secretion in patient with SPD.

Methods/Materials
We retrospectively compared patient samples with reduced ATP secretion (<0.5 nmoles) activated with 1U/mL and 5U/mL thrombin (n=73). We correlated these data with results of platelet function analyzer (PFA), light transmission platelet aggregation (LTA) with several agonists and electron microscopy of the thrombocytes. The influence of the platelet count (between 100-600*10^9/L) in PRP was evaluated in patient samples with SPD (n=24).

Results
There is a linear correlation between the ATP secretion with 1U/mL versus 5U/mL thrombin (r^2=0.304) (Figure 1). In only 18% of the patients the ATP secretion increased with 5U/mL thrombin. 16 patients (25 samples) were confirmed with the diagnosis of SPD.

All patients had a reduced ATP secretion with 1U/mL thrombin. The median of the ATP release was 0.13 and 0.00 nmoles, respectively activated with 1 and 5U/mL thrombin. The most common impairment in LTA was observed with epinephrine (54%). The PFA and LTA with other agonist (adenosine diphosphate, collagen, ristocetin, arachidonic acid, and thromboxane analogue U46619) was impaired in 21% of the samples. There was no correlation between the ATP secretion and platelet count in PRP (range, 121-595*10^9/L)



Heparin and low molecular weight heparin: influence of anti-Xa/anti-IIa ratio on activated partial thromboplastin time

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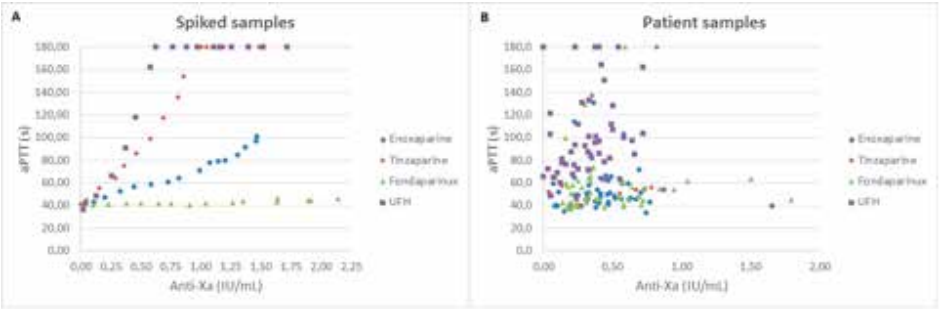
Background/ Introduction
Unfractionated Heparin (UFH) and low molecular weight heparines (LMWH) are anticoagulants widely used in prevention and treatment of thrombo-embolic disease. They bind to antithrombin and increase its inhibition of coagulation factors Xa and IIa. The length of the LMWH determines the ratio of anti-Xa/anti-IIa effect. The longer, the more anti-IIa activity. LMWH treatment does not need monitoring except in specific populations such as pregnant women, children and patients with impaired renal function. The recommended method for monitoring is a colorimetric anti Xa assay. Nevertheless, activated partial thromboplastin time (aPTT), influenced by both FXa and FIIa activity, is often determined in these patients as part of a coagulation screening panel and therefore the sensitivity of aPTT reagent towards LMWH can be informative.

Aims
We evaluated the effect of UFH, (Heparin, LEO Pharma), two LMWHs (Enoxaprin (Clexane®, Sanofi-Aventis), Tinzaparin (Innohep®, LEO Pharma)) and Fondaparinux (Arixtra®, GlaxoSmithKline) on our routine aPTT reagent. Based on literature, anti-Xa/anti-IIa ratios of these products are 1, 3.3 and 1.8, respectively. Fondaparinux selectively inhibits FXa. In addition, we investigated the influence of anti-Xa/anti-IIa ratios on aPTT.

Methods/Materials
Samples of patients treated with UFH, Enoxaparin and Fondaparinux (each n=50) and Tinzaparin (n=10) were collected. Furthermore, normal pool plasma was spiked with different types of heparin (0 to 1,5 IU anti-Xa/mL). aPTT (PTT A, Diagnostica Stago), anti-Xa (Biophen Heparin LRT, Hyphen BioMed) and anti-IIa (Biophen Heparin anti-IIa, Hyphen BioMed) were measured.

Results
Anti-Xa/anti-IIa ratios of all heparins were close to the ratios found in literature.
Spiked samples showed a good correlation between anti-Xa activity and aPTT: R^2 0,98-0,99. For Enoxaparin the upper limit of the therapeutic range (1,0 IU/mL) correlated with an aPTT of < 80s. Fondapariux had almost no effect on the aPTT, illustrating that aPTT is mostly dependent on the anti IIa activity. UFH samples with anti-Xa activity > 0,6 IU/mL resulted in an aPTT > 180s, hence, not covering the therapeutic range for UFH (0,3-0,7 IU anti-Xa/mL). Patient samples resulted in lower aPTT values illustrating that the anti-IIa effect in spiked samples is stronger compared to physiological conditions. After exclusion of 3 outliers (outside 95% prediction limits (PL)) a simple linear regression analysis with 95% PL between aPTT and anti-Xa was done for Enoxaparin. The upper limit of the therapeutic range (1,0 IU/mL) correlated with an aPTT of 50,3s (95% PL: 57s). Overall, there was a poor correlation between anti-Xa activity (any heparin) and aPTT. Linear regression illustrated some outliers; three samples were confirmed lupus anticoagulant positive. Other in vivo effects (f.i. high FVIII, low or high fibrinogen, low antithrombin, factor deficiencies or simultaneous therapy with other anticoagulants) could not be further explored due to insufficient sample volume.

Summary/Conclusions
These results confirm that chromogenic dosage of FXII could be more adapted to diagnose abnormal FXII levels in this population. This method can be reliably adapted on a routine automate. However, these results should be interpreted together with clinical and genetic data.



Thrombin generation in the plasma of sickle cell trait adults shows prothrombotic profile as compared to normal adults.

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Background/ Introduction
Sickle cell trait (AS) affects over 100 million individuals worldwide. Even considered as a benign carrier state, these people may have rare complications particularly thromboembolic (TE) diseases.

Aims
We aimed to characterize thrombin generation (TG) in AS adults using parameters assessed by Calibrated Automated Thrombography, a global coagulation assay.

Methods/Materials
27 AS adults were evaluated and compared to 31 healthy adults (AA) using the CAT method (Calibrated Automated Thrombogram®). Platelet free plasma (PFP) was obtained from 0,109M citrated blood after double centrifugation, and frozen at -80°C. TG was triggered using 1pM tissue factor (TF) and 4µM phospholipids (PL), in two different conditions, namely with and without addition of thrombomodulin (TM). CAT parameters were analyzed (Lagtime (mn), Peak (nM), TtPeak (min), Velocity index (nM/min), ETP (nM). Individual parameters were also measured : complete blood count, protein C activity, free protein S, factor VIII, vW factor (Ag and Ac), D-dimer, antithrombin, fibrinogen, prothrombin time (PT) and activated partial thromboplastin time (aPTT). Mann Whitney test was used to compare AS patients to healthy adults for each parameter. This study was approved by the local Ethics Committee and informed consent was obtained from each participant.

Results
Without addition of TM, AS group showed significantly higher ETP (p=0.015), higher peak (p=0.019) and higher velocity index (p=0.04). Lagtime was similar in the two groups. With addition of TM, AS highlighted a significantly higher ETP (p=0.036). D-dimer levels (p=0.011) as well as neutrophil (p=0.008) and monocyte (p=0.033) counts were significantly higher in the AS group as compared to the AA group.

Summary/Conclusions
This study suggests a hypercoagulable state in AS adults as compared to AA adults. These results could explain the higher risk of TE described in AS adults. A larger AS cohort should be investigated to confirm these observations. Moreover the underlined mechanisms and their clinical significance are to be explored.

The Use of Temporary Central Venous Catheters for Optimizing Continuous Infusion in Hemophilia Patients Undergoing Major Surgical Procedures

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Background/ Introduction
Continuous infusion (CI) of clotting factor concentrate has facilitated surgical procedures and intensive replacement therapy in hemophilia patients. The advantage of CI over bolus infusions is ability to maintain steady-state levels of coagulation factors and moreover, to reduce the total amount of factor concentrate spent. CI is commonly delivered through a peripheral vein. However, a significant number of hemophilia patients have distorted peripheral veins which can compromise continuous flow of factor concentrate needed for successful treatment. Use of central venous catheter can ease the application of CI. By searching the literature, we found only a few case reports describing the use of temporary non-tunneled central venous catheters (CVC) for administrating CI in patients with hemophilia.

Aims
The aim of this study was to evaluate the efficacy and safety of short-term used non-tunneled CVC for CI during surgical procedures in hemophilia patients.

Methods/Materials
In this study we have retrospectively studied patients with hemophilia that had temporarily used non-tunneled CVC for CI of factor concentrate during and after major surgery in the Saint-Luc University Hospital in Brussels between August 2000 and April 2014. The indication for CVC usage was a major surgery with anticipated need for CI of factor concentrate longer than 5 days.

CVC was inserted by an experienced anesthesiologist in the operating room after the induction of general anesthesia and normalization of APTT. Before the CVC insertion, the patient would have already received bolus of clotting factor concentrate and have the CI started through the peripheral vein. Upon placement, the CI was switched to the CVC. The CVC was kept in place until leaving hospital or cessation of the need for continuous infusion.

Results
During the study period, 40 male patients with hemophilia A or B (37 and 3 patients, respectively) underwent 67 major surgical procedures covered by CI of factor concentrate delivered through CVC. Patients, age 21 -81, had severe, mild or moderate disease (33, 5 and 2 patients, respectively). Patients had altogether 65 CVC for 67 surgical procedures. Patients underwent orthopedic, gastrointestinal and cardiovascular surgery. The CVC were placed in the right jugular vein (58%), the left jugular vein (18%), the left subclavian vein (8%) and right subclavian vein (3%), while the data were missing in 6 patients. Median duration of catheter was 12 days (range 5 to 107 days). No complications related to the CVC were noted. We searched for bleeding at the site of puncture of the catheter, signs of local infection, pneumothorax following placement of CVC, catheter thrombosis, malfunction of the catheter and surgical site infection.

Summary/Conclusions
Based on results of this study, we can conclude that the use of short-term non-tunneled CVC should be considered in patients with hemophilia undergoing major surgery with the need for prolonged CI of factor concentrates. By placing CVC we can ensure undisturbed flow of factor concentrate during CI and preserve peripheral veins for the future concentrate administration.

Endogenous thrombin generation potential: an added value parameter to individualize prophylaxis treatment in pediatric haemophiliac patients?

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Background/ Introduction
Haemophilia is a genetic disorder caused by a deficient factor VIII (haemophilia A) or factor IX (haemophilia B). It's a rare X-linked recessive disease. The clinical features overall depend on the levels of residual factors.

Nevertheless, significant differences have been noted in patients having the same level of coagulation factors, which is therefore not reflecting the patient clinical phenotype.

Aims
Thrombin generation assay (TGA), a method of global evaluation of the coagulation process, has been used in this study to find a relationship between clinical phenotype and TGA parameters. Moreover it could be a very important tool in both the follow-up and treatment adaptation of haemophiliac patients procedures in hemophilia patients.

Methods/Materials
We analysed the computerized medical charts (starting from the diagnosis to January 2014) and some plasma samples (taken between July 2008 and January 2014) of 35 haemophiliac A and B patients. Following exclusions criteria only 18 patients were retained. 31 citrated blood samples of these 18 patients were analysed with TGA using Calibrated Automated Thrombinography ® (CAT) as described by Hemker et al (Thrombinoscope®, Maastricht, The Netherlands). We evaluated Endogenous thrombin potential (ETP) as it represents the whole quantity of thrombin generated during the all coagulation process. Individual analysis of each patient including the computation of a clinical score defined as the number of bleeding episodes/ duration of treatment was confronted to ETP.

Results
A threshold ETP value was determined below which the bleeding frequency is increased. Moreover a statistically significant relationship between ETP and clinical phenotype was revealed.

Summary/Conclusions
To conclude, a relationship between ETP and the clinical features of haemophiliac patients was demonstrated. We suggested that TGA may be of added value in the follow-up and individualized prophylactic treatment of haemophiliac patients.

Value and implications of the anti-Xa activity monitoring in pregnant women receiving prophylactic LMWH: a retrospective study

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Background/ Introduction
Continuous infusion (CI) of clotting factor concentrate has facilitated surgical procedures and intensive replacement tLow molecular weight heparins (LMWH) have stable pharmacokinetics. However, physiologic changes during pregnancy can alter pharmacokinetics of LMWH and make the biodisponibility of the drug less predictable. When starting prophylactic treatment with LMWH in pregnant woman, most physicians calculate the initial LMWH dose based on the body weight and adapt the dose according to the weight gain. An alternative method is to monitor the anti-Xa activity and adjust the LMWH dose accordingly. LMWH are for the moment the only anticoagulants available for prophylaxis of thrombo-embolism in pregnant women and prevention of severe pregnancy complications.

Aims
Therefore, we conducted a study addressing the rationality of performing an anti-Xa activity monitoring regularly during pregnancy.

Methods/Materials
We retrospectively analyzed data from pregnant women undergoing prophylaxis with LMWH followed at the Hemostasis and Thrombosis Unit of the Saint-Luc University Hospital Unit. All the patients that had anti-Xa activity done at least three times were included in the study. The measurements of anti-Xa were done regularly every 6 weeks and the dose of LMWH was increased in case of anti-Xa activity lower than 0.3 U/ml. Initial LMWH dose was adjusted to the patient body weight and its thrombotic risk. The first measurement of anti-Xa was done 10 days afterwards.

Results
Out of 173 women evaluated in the study, 79% needed LMWH dose adjustment.97 patients (56%) had one dose adjustment, 35 patients (20%) two and 5 patients (3%) three. The first augmentation of LMWH dose was needed before the patient gainedbody weight in 36,5% of cases. The average increase of the LMWH dose was 49%, 100% and 116% for the one, two and three dose adjustments, respectively. Under-dosing of LMWH, defined as anti-Xa activity <0,2 U/mL, was noted in 12% of patients on the first monitoring versus 3,5% on the last monitoring.No severe side effects occurred during the treatment. Five patients (3%) had unfavorable outcome of the pregnancy.

Summary/Conclusions
This study demonstrates that regular monitoring of anti-Xaactivity can help in maintaining the prophylactic levels of LMWH throughout the pregnancy. We also showed that the drop of anti-Xa activity does not always parallel with the weight gain in pregnant women. Therefore we recommend that LMWH dose adjustment should not be made based solely on the weight gain of the patient during pregnancy.

Validation of a ready to use Clauss fibrinogen

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Background/ Introduction
The STA®-Liquid fib kit is a new reagent ready to use for the quantitative determination of fibrinogen levels in plasma by Clauss method.

Aims
This evaluation includes an assay of repeatability, reproducibility, stability and a correlation with the STA®-Fibrinogen 5 kit. This one is realized on STAR-Evolution® (Stago) within the CHU de Charleroi.

Methods/Materials
Repeatability and reproducibility are evaluated on three levels, the first by 21 intra-run determinations of citrate plasma samples and the second by two determinations during 15 days of two controls and a pool high. This pool is aliquoted and frozen at -80°C.
In a purpose of comparison, the stability study is realized on both STA®-Liquid fib and STA®-Fibrinogen 5® kit. This one consists of a measure of two controls and a pool high by both kits, once a day, until appearance of a drift of the method. Our targets values and our confidence intervals are defined by the study of reproducibility. The correlation between the two kits is realized on 30 citrate plasma samples covering the entire pathophysiological range of the fibrinogen

Results
The CV (%) obtained for the 3 levels of the repeatability are lower than those reported by the firm. Concerning the reproducibility, CV (%) of the STA®-Liquid fib kit are conform to those of GEHT (Table 1). The firm claims, for the STA®-Liquid fib, an onboard stability of 10 days and a stability of 5 days for the STA®-Fibrinogen 5 kit. The results show no drift over an evaluation period of 35 days for STA®-Liquid fib kit against a clear drift after 15 days for Fibrinogen 5® kit.
The equation of the regression line (y=0,9802x + 0,0799) demonstrates a lack of proportional and constant bias throughout the pathophysiological range studied.

	Repeatability				Reproducibility		
	Sample 1	Sample 2	Sample 3		Control Low	Control N	Pool high
n	21	21	21	n	30	30	30
Mean (g/L)	2,17	3,3	5,39	Mean (g/L)	1,23	3,16	5,93
SD (g/L)	0,07	0,06	0,09	SD (g/L)	0,06	0,21	0,28
CV (%)	3,24	1,75	1,6	CV (%)	4,93	6,78	4,72
CV_{firm}* (%)	4,9	2,1	2,1	CV_{GEHT}** (%)	7,6	7,6	7,6
*CV_{firm}: Indicative manufacturer's CV				**CV_{GEHT}: standards of acceptability of GEHT 2014			

Summary/Conclusions
The performances of the STA®-Liquid fib kit meet the expectations beforehand fixed. Its stability on board is remarkable. The ease of use of the kit (liquid format) requires no longer critical manual reconstitution steps of the reagent and contributes to improve good practice of the technologist.

ADAMTS13 destabilizes thrombi in a mouse model of thrombotic focal cerebral ischemia

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Background/ Introduction
Ischemic stroke occurs when blood thrombi occlude one major or multiple smaller intracerebral arteries. Currently, rapid thrombolysis using tissue-plasminogen activator (t-PA) is the only approved therapeutic treatment for ischemic stroke, but it has many serious limitations. ADAMTS13 is a metalloprotease that cleaves von Willebrand factor (VWF), a crucial factor in thrombus formation. By cleaving ultra-large and hyper-reactive VWF multimers into smaller, less thrombogenic ones, ADAMTS13 has been shown to have both antithrombotic and anti-inflammatory properties.

Aims
In this study, we analyzed whether ADAMTS13 can destabilize blood thrombi in the setting of ischemic stroke by cleaving VWF that is linking platelets together.

Methods/Materials
We used a mouse model of focal cerebral ischemia in which a thrombus is generated in the right middle cerebral artery (MCA) by topical application of FeCl3. Two different types of injury were used: a severe injury in which permanent occlusion of the MCA is always achieved and a threshold injury after which the endogenous thrombolytic system is capable of inducing spontaneous recanalization.

Results
A substantial difference between ADAMTS13 knock-out (KO) and wild type (WT) mice was observed after a threshold thrombotic injury to the MCA. In ADAMTS13 KO mice an occlusive thrombus was formed faster compared to WT animals. Moreover, ADAMTS13 KO mice developed significantly larger cerebral infarctions 24h later. The origin of this discrepancy was found in the early stages after thrombus formation. In ADAMTS13 KO mice, a stable thrombus was able to form which occluded blood flow in the MCA. WT mice on the other hand showed more spontaneous recanalization, rescuing the brain from cerebral ischemia. We were able to demonstrate that this effect was at least partially due to ADAMTS13, since injection of recombinant ADAMTS13 was able to restore MCA blood flow and rescue the brain from larger cerebral infarctions, even when it was administered after thrombus formation.
We next examined the prothrombolitic potential of recombinant ADAMTS13 in a model of occlusive thrombotic MCA injury. In this model all mice formed a stable thrombus and no spontaneous recanalizations were observed. Injection of rADAMTS13, 5 min or 60 min after occlusion, restored MCA blood flow and significantly reduced infarct size, demonstrating the thrombolytic potential of ADAMTS13

Summary/Conclusions
These results point towards a destabilizing role for ADAMTS13 in cerebral thrombotic occlusions. Hence, ADAMTS13 may have pro-thrombolytic potential that could be exploited in the management of acute ischemic stroke. In the future ADAMTS13 could serve as an adjuvant for tPA assisted thrombolysis in ischemic stroke.

PEAR1 upregulation during megakaryopoiesis is dependent on changes in DNA methylation

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Background/ Introduction
Platelet Endothelial Aggregation Receptor 1 (PEAR1) is a cell-cell contact receptor predominantly expressed in platelets and endothelial cells. A number of Single Nucleotide Polymorphisms (SNPs) in the PEAR1 gene were identified to be associated with variable platelet response to activation and cardiovascular outcomes, highlighting the cardiovascular importance of this protein. PEAR1 sustains activation of the platelet integrin alphaIIbBeta3, but also regulates megakaryopoiesis. It is progressively more actively transcribed during differentiation of CD34+ stem cells towards the later stages of megakaryocyte (MK) specification, following a similar pattern as that observed for GATA1, a transcription factor playing a key role in megakaryopoiesis and erythropoiesis (Kauskot et al. Blood 2013). Knocking-down PEAR1 in CD34+ cells increased the proliferation of immature MKs, whereas terminal MK maturation (proplatelet formation) was not affected (Kauskot et al. Blood 2013). Interestingly, expression profiling has revealed massive PEAR1 expression in normal human bone marrow derived MKs and platelets, while only transient PEAR1 positivity was noted in myeloid precursors (Kauskot et al. Blood 2013). Epigenetic events, such as DNA methylation, have been described to regulate a number of biological processes, including cell differentiation. PEAR1 is encoded by 22 exons located on chromosome 1q23.1 (Figure 1A). Two CpG islands are located (1) at the promoter/5'Un-Translated Region (5'UTR) and (2) spanning exons 7-9 (CpG28 and CpG70, respectively, Figure 1A). PEAR1 CpG28 represents a potential DNA methylation dependent regulatory region because of its location in the PEAR1 gene

Aims
The aim of the study was to investigate DNA methylation dependent mechanisms of PEAR1 expression regulation throughout megakaryopoiesis

Methods/Materials
We have used the Sequenom EpiTYPER to study DNA methylation at the CpG28 PEAR1 region (Figure 1A) in CD34+ cells differentiating into MKs. A total of 32 CpG sites across this region were analyzed at 4 different time points of differentiation. Validation was performed by means of bisulfite sequencing and DNA methylation specific enzymatic digestion.

Results
In order to link DNA methylation to PEAR1 expression, we analyzed PEAR1 CpG28 methylation via Sequenom EpiTYPER during CD34+ cell differentiation into MKs. CpG specific analysis on each of the 32 CpG sites evaluated, identified 7 CpG sites, the methylation of which increased 3-4 fold from day 0 to day 14 of differentiation (Figure 1B). Further analysis revealed that these CpG sites are co-localized in a region with binding sites for CTCF, a transcription factor involved in several biological processes, the binding of which is affected by DNA methylation.

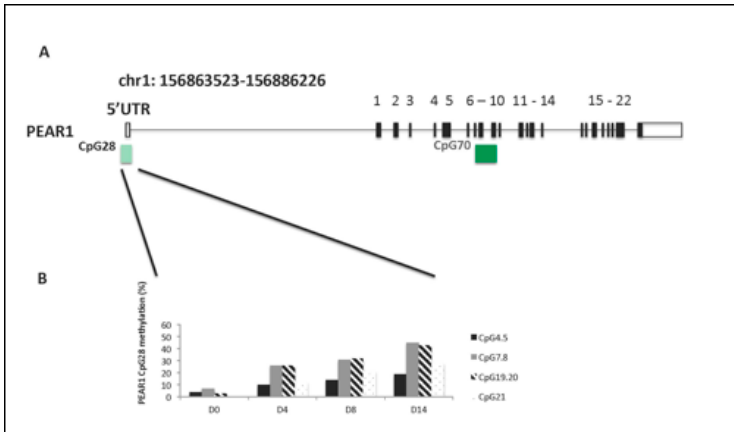

Figure 1. PEAR1 DNA methylation increases during CD34+ cells differentiating into MKs
(A) Genomic representation of PEAR1 (chr1: 156863523-156886226). Exons are depicted as black boxes, untranslated regions as white boxes. Two CpG islands of 28 and 70 CpG sites, respectively, are located in the gene (**CpG28**, 28 CpGs, depicted as light green box: 156863416-156763711; **CpG70**, 70 CpGs, depicted as dark green box: 156877770-156878649).
(B) PEAR1 CpG28 methylation via Sequenom EpiTYPER in CD34+ cells during differentiation into MKs, at day 0 (D0), 4 (D4), 8 (D8) and 12 (D12). Methylation values are displayed for CpGs 4.5, 7.8, 19.20, 21. This region contains binding sites for the methylation sensitive transcription factor CTCF.

Summary/Conclusions
We propose for the first time DNA methylation as a regulatory mechanism of gene expression in megakaryopoiesis. The increase in DNA methylation of the PEAR1 promoter paralleled its expression during megakaryopoiesis. Since DNA methylation has previously been linked to gene silencing, further studies are needed to address how an increase of DNA methylation can enhance gene transcription. PEAR1 regulation might represent a paradigm for the expression of other relevant genes involved in hematopoiesis and in cardiovascular disease.

Platelets contribute to colitis-associated carcinogenesis : evidence from a mouse model

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Background/ Introduction
Chronic inflammatory diseases, like ulcerative colitis and Crohn's disease, are associated with increased risk of colorectal cancer. Nonetheless, the factors linking inflammation to tumor development remain poorly defined. Recent findings indicate that inflammation promotes the accumulation of myeloid derived suppressor cells (MDSC) that down-regulate immune surveillance and antitumor immunity, thereby facilitating tumor development. Beside their role in thrombosis and hemostasis, platelets have been involved in inflammation and in tumorigenesis. Increased platelet number and activity, as well as coagulation abnormalities, often associate with inflammatory conditions as with a wide variety of malignancies. However, the role of platelets in colitis associated carcinogenesis (CAC) has never been investigated..

Aims
The aim of this work was to study the modifications of platelet number and activity accompanying CAC and to investigate the effect of a common antiplatelet therapy on colitis-induced MDSC accumulation and tumor development.

Methods/Materials
We used clopidogrel-treated mice in the well-established Azoxymethane (AOM)/Dextran Sulfate Sodium (DSS) mouse model of colitis-associated cancer. Using flow cytometry and cell sorting, we assessed changes in blood and spleen cell populations and we evaluated colon infiltration by immune cells during the course of carcinogenesis. Platelet count, activation and reactivity were monitored throughout the protocol. A clinical scoring system was applied to evaluate colitis severity (weight loss, stool consistency, and rectal bleeding). The number and size of the tumors were measured at the end of the experiments. Tumor proliferation rate was calculated by immunohistochemical analysis of Ki-67 on colon sections.

Results
The AOM DSS treatment provoked profound changes in platelet and myeloid cell counts during the period preceding the apparition of tumors. The platelet count increased while monocytic and granulocytic MDSC accumulated very rapidly in the blood, in the spleen and in the colon of the mice. The number of activated circulating platelets also significantly increased. Platelets were more reactive to ex vivo stimulation with thrombin. Clopidogrel administration limited diarrhea and did not worsen bleeding. It prevented the AOM DSS triggered platelet activation, without affecting thrombocytosis. The initial MDSC accumulation occurred normally. However, at the time of tumor development, the amounts of granulocytic MDSC were more elevated in the blood of clopidogrel-treated animals as compared to untreated animals. The numbers of mature monocytes were concomitantly lowered. Interestingly, we found a significant decrease of tumor numbers in the clopidogrel group. This decrease correlated with drastically reduced colon and tumor infiltration by myeloid cells (CD11b+).

Summary/Conclusions
Thus, the antiplatelet drug clopidogrel inhibits colitis-induced carcinogenesis, possibly by affecting myeloid cell recruitment at inflammatory sites and in tumors.

The flexible tail of the hemostatic enzyme ADAMTS13 is able to shield the active site

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Background/ Introduction
Enzymes regulating blood coagulation and thrombosis are activated through proteolytic digestion of their zymogen forms. In contrast, the activity of the plasma metalloprotease ADAMTS13 is regulated by a different unique working mechanism. Indeed, its activity is controlled by force-induced conformational changes in its substrate, the von Willebrand factor (VWF).

Aims
Whether ADAMTS13 itself undergoes conformational changes that also regulate its activity, is currently unknown.

Methods/Materials
Anti-ADAMTS13 antibodies were generated by the immunization of Balb/C mice with recombinant human ADAMTS13, and ADAMTS13 variants were used for epitope mapping. The functional effects of these antibodies were tested using the FRETS-VWF73 assay and an in-house developed ADAMTS13-VWF binding assay. Changes in accessibility of antibody epitopes were evaluated using an immuno assay. Electron microscopy studies were performed in the presence and absence of antibodies in order to determine the conformation of ADAMTS13.

Results
Twenty-five of the 31 developed anti-ADAMTS13 antibodies had an epitope in the carboxyterminal tail domains. Interestingly, 12 of these anti-tail antibodies stimulated the proteolytic activity of the MDTCS head domains more than two-fold, thereby proving that manipulation of the tail influences the catalytic activity of the head and indicating that this happens through a shielding effect of the tail. In addition, 15 of the anti-tail antibodies stimulated the binding of ADAMTS13 to VWF up to 373%, revealing that additional VWF binding sites are exposed upon conformational activation of ADAMTS13. Furthermore, a cryptic epitope in the head was exposed upon activation with anti-tail antibodies, supporting the existence of a shielded (tail shields catalytic head) and unshielded conformation of ADAMTS13. Finally, electron microscopy studies in the absence and presence of anti-ADAMTS13 antibodies identified a high number of different conformations of ADAMTS13, indicating a very flexible structure of the full-length enzyme.

Summary/Conclusions
Our data show that due to the flexibility of the full-length enzyme, the tail is able to shield the catalytic head domains, thereby dampening its activity. Hence, next to the VWF-dependent regulation of ADAMTS13 activity, activity is additionally regulated by its own tail domains.

Interplay between coagulation and fibrinolysis in S. aureus infections

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Background/ Introduction
Staphylococcus aureus (S. aureus) is an important cause of skin and soft tissue infections as well as intravascular catheter infections. Interestingly, S. aureus produces not only coagulases that trigger fibrin formation, but also staphylokinase (SAK), the most fibrin-specific plasminogen activator. This marked fibrin-specificity of SAK is due to protection by fibrin of the SAK-plasmin complex against rapid inhibition by alpha-2-antiplasmin (β2AP). Whereas the importance of fibrin generation for both S. aureus abscess formation and adhesion to foreign body surfaces has been shown, the role of SAK remains unclear.

Aims
To elucidate the role of SAK-induced human plasminogen activation in relevant S. aureus disease models.

Methods/Materials
We used an adenoviral vector for human plasminogen expression, to overcome the species-selectivity of SAK in a murine model. First, skin infection was induced by subcutaneous injection of 2x108 CFU of S. aureus Xen36.

The constitutive bioluminescence of this strain allowed for non-invasive follow-up of bacterial spreading and density. The model was tested in α2AP knock-out mice (α2AP -/-) and their littermate controls. The interactions between SAK, human plasminogen, murine plasminogen, α2AP, fibrin and bacteria were studied in vitro using the chromogenic substrate S-2403 to assess plasmin generation. Gelatin zymography was used to study activation of the gelatinase subfamily of matrix metalloproteinases (MMP-2 and MMP-9) in lesional skin tissue sections.
Second, a jugular vein catheter infection model was performed in human plasminogen expressing mice using S. aureus strains with different levels of SAK production. A flow chamber model allowed us to study bacterial adhesion to a plasma-coated surface. Scanning electron microscopy images of S. aureus biofilms of different strains were acquired in diverse media with or without added prothrombin, fibrinogen or plasminogen.

Results
In the murine skin infection model, bioluminescence imaging showed higher bacterial loads and increased spreading of infection in human plasminogen-expressing mice compared to wild-type mice. The absence of α2AP accentuated this phenotype, but close to the initial abscess site the SAK-plasminogen complex can be protected from β2AP action by either the bacterial surface or by fibrin produced by coagulase-positive S.aureus. Lesional skin extracts showed more gelatinase activation in α2AP -/- mice expressing human plasminogen, compared to wild type controls without human plasminogen.
Thick biofilms with fibrin strands were formed if S.aureus was cultured in plasma compared to TSB. Inhibition of thrombin or plasmin activity changed biofilm phenotype, pointing to the role of both coagulation and fibrinolysis in biofilm structure. SAK gene expression was regulated by quorum sensing, with an increase of SAK/staphylocoagulase ratio in dense bacterial communities. High production of SAK interfered with bacterial-induced fibrin formation and adhesion to foreign body surfaces, thus impairing successful biofilm infection.

Summary/Conclusions
Fibrin-specific SAK action and downstream gelatinase activation allows the local spreading of S. aureus from the initial fibrin-enclosed abscess site. In S. aureus foreign body infections, SAK production leads to release of bacteria from the fibrin-containing biofilm structure.

Three photochemical pathogen inactivation methods impair platelet function with different underlying biochemical mechanisms

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Background/ Introduction
Pathogen inactivation of platelet concentrates is used to reduce chances of transfusion transmitted infections. Three photochemical methods are published; two with exogenously added photosensitizer (riboflavin [RF] and amotosalen [AS]) and one without [UV-C].

Aims
Pathogen inactivation may increase the storage lesions observed in platelets, but it is unclear to what extent and why.

Methods/Materials
Platelet concentrates prepared from buffy coats and stored in additive solution were treated with pathogen inactivation and compared to paired untreated controls. Platelet aggregation, flow cytometry, and thrombus formation under flow were measured in function of storage to determine the impact of these novel treatments.

Results
In the murine skin infection model, bioluminescence imaging showed higher bacterial loads and increased spreading of All three methods irreversibly and significantly decreased thrombus formation over immobilized collagen in vitro compared to untreated controls. RF treatment additionally caused premature platelet degranulation and integrin αIIbβ3 activation as shown by P-selectin and PAC1 measurements. Furthermore, the difference between low and high-dose agonist aggregations was larger for RF than controls, indicating reduced signal sensing and/or amplification. Premature activation was not found in AS treatment, but these platelets no longer agglutinated in response to low-dose ristocetin (0.6mg/mL). Platelet rolling on immobilized VWF was however normal only in the presence of tirofiban, a potent integrin αIIbβ3 inhibitor. Moreover, decreased PAC1 binding was seen in response to 30μM SFLLRN peptide and 6ng/mL convulxin. Together these data indicate significantly reduced signal transduction from several receptors to the integrin αIIbβ3. Like RF and AS,

UV-C treatment caused reduced thrombus formation, but this was only significant from day five on. No loss of aggregation or integrin activation in response to several agonists at different concentrations was seen. Yet, increased spontaneous PAC1 binding to UV-C treated platelets was seen.

Summary/Conclusions
The three pathogen inactivation methods significantly impact the in vitro thrombus formation onto collagen under flow by different biochemical mechanisms; UV-C alone induces increased PAC1 binding without affecting aggregations. The RF method prematurely activates platelets, affecting subsequent signal amplification in aggregation while AS platelets translate activatory signals less efficiently to integrin αIIbβ3.

Apheresis platelet concentrates with persistent particles

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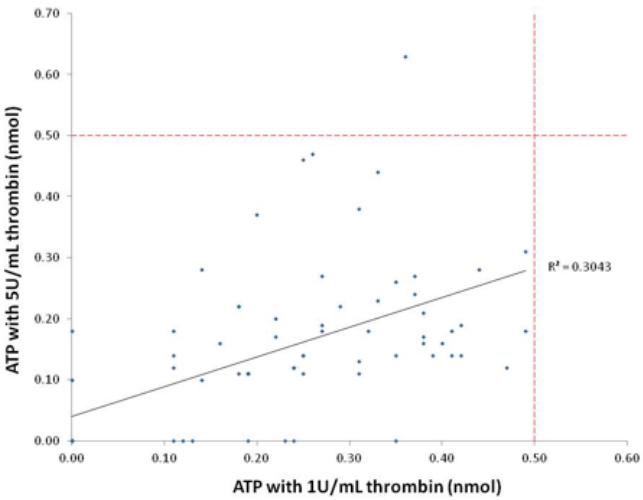
Background/ Introduction
During platelet apheresis, aggregates often appear. These are generally transient but sometimes persist causing wastage.

Aims
In this study, we sought to identify factors that contribute to the formation of persistent aggregates.

Methods/Materials
Several donor and donation characteristics as well as platelet variables were investigated. Donations with persistent aggregates (PA) were compared to unpaired aggregate free donations (AF).

Results
In a six month data monitoring period, 187 donations contained PA (3.6% of all procedures). Most strikingly, the proportion of donors with at least one previous PA donation was twofold higher in the PA group (P<.0001) indicating a donor-related factor. Predonation donor platelet counts were significantly higher (mean±SD: 286±50 vs. 268±49 x106 platelets per µL, P<.001) but no differences were found for gender, body mass index or age. A small but significantly higher hematocrit was noted. Products with PA contained significantly more platelets, a consequence of higher donation volumes. Analyses on day six showed that the pH was lower (7.18±0.16 vs 7.36±0.17, P<.001, n=15), in accordance with higher lactic acid concentrations. No differences were seen for GPIb expression, phosphatidylserine exposure or activated integrin αIIbβ3. However, significantly more P-selectin was detected on PA platelets (P=.02, n=15) pointing to increased alpha-degranulation, which was confirmed by higher cytokine concentrations in the supernatant. Agglutination to low (P=.02) but not high-dose ristocetin was increased in PA products, while no differences were seen for collagen or SFLLRN peptide induced aggregations. Finally, PA were efficiently dislodged by plasmin mediated thrombolysis but not by the integrin αIIbβ3-fibrinogen inhibitor RGDS.



versus 1U/mL thrombin; dotted lines indicate cut-off for reduced ATP secretion"

Summary/Conclusions
There is a higher chance of PA when a donor with at least one previous PA donation presents. However, this parameter by itself insufficiently predicts PA showing that auxiliary factors are involved. Products with PA have acceptable quality but functional studies are warranted.

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