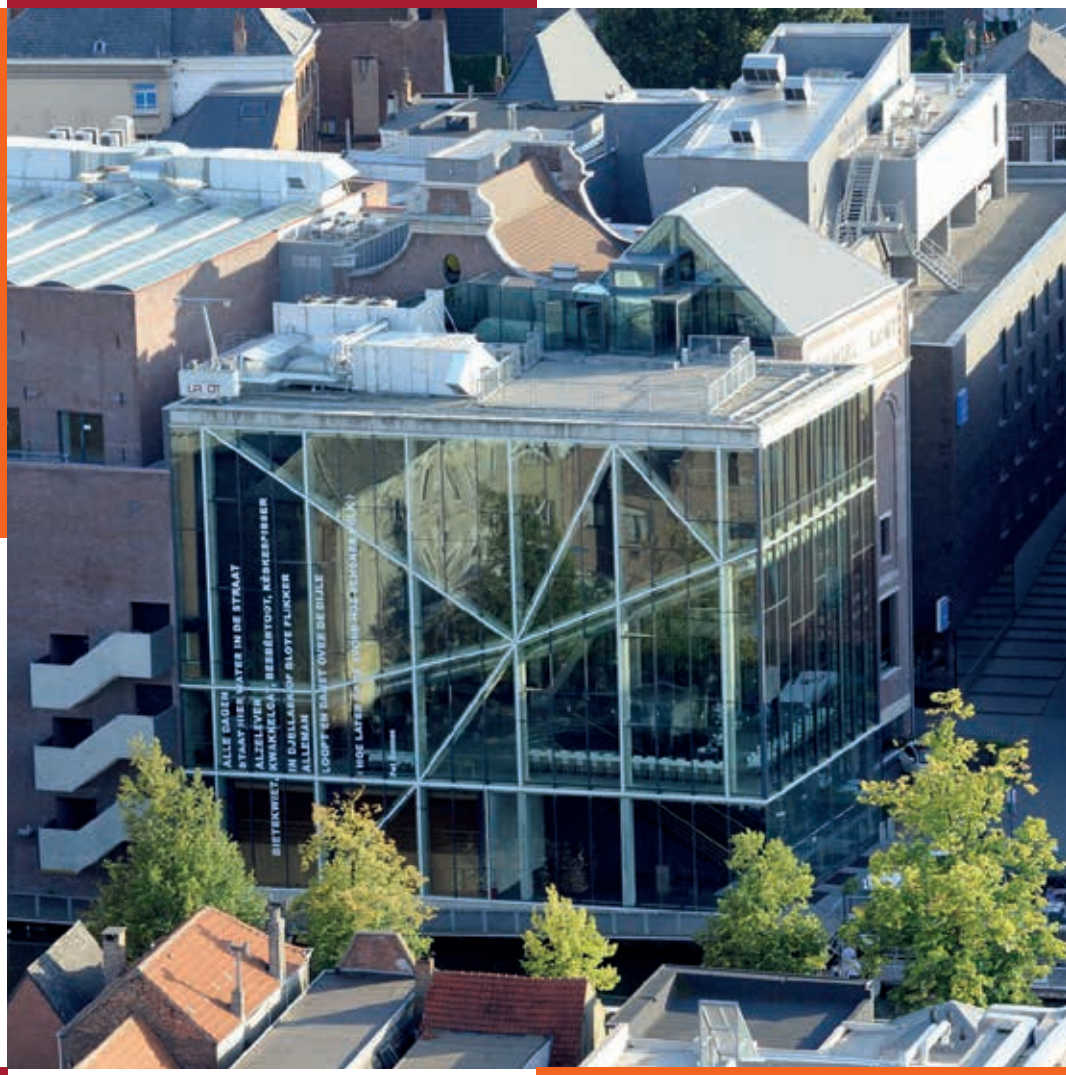
BSTH

Belgian Society on Thrombosis and Haemostasis

26TH ANNUAL MEETING

22-23 NOVEMBER 2018

LAMOT BELGIUM



LOCAL ORGANISER

ANNE DEMULDER

CHU BRUGMANN

MEETING THEME:

NEW INSIGHTS IN THROMBOSIS AND HEMOSTASIS

PROGRAM & **ABSTRACTS**

WWW.BSTH2018.ORG

Roche

REimagine Haemophilia

INVITATION

Nov 22nd 2018

10:15 - 11:15

Roche Satellite Symposium

Exploring new paths for Haemophilia A management

SmPC HEMLIBRA® available upon request
The Roche Symposium is intended for healthcare professionals only.

R.E. Dr Christine Lenaerts - BE/HAEM/1018/0068 - 23/10/2018

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GENERAL INFO

ORGANISATION

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WWW.BSTH2018.ORG

CONGRESS MANAGEMENT

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

DATE

22 - 23 NOVEMBER 2018

VENUE

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

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WELCOME TO THE 26TH ANNUAL MEETING

Dear participants,

I would like to welcome you to the 26th Annual Meeting of the Belgian Society on Thrombosis and Haemostasis (BSTH), once again in the Lamot Congress Center in Mechelen. Mechelen has been our host city for the last few years now, and all our meetings there have been of a high quality, something we wish to repeat this year.

Behind the scenes there have been some changes with our regular logistic congress organizer, Klaske Dam of Con-txt, changing roles and now concentrating on the BSTH society management and a new logistic organizer in the form of Congress Care. These changes were not foreseen last year and have caused many a headache for the board of the BSTH. Some things will be handled differently or will look differently but I hope this will not detract from another high quality BSTH Annual Meeting.

This year the scientific organization has been handled by Prof. Dr. Anne Demulder of the LHUB-ULB, and she has done a marvelous job in putting together another stimulating program. Amidst all the logistic changes she has kept a cool head and concentrated hard on the job in hand undeterred by the chaos, and for this we are very grateful. This year the focus of the state of the art lectures is on thrombosis, and especially on thrombosis and cancer, thrombosis at unusual sites, and the link between microorganisms and thrombosis. The two educational sessions focus on anticoagulation and some of their problems.

We are very thankful for the continued support from the industry, both as sponsors of the BSTH as an organization and as sponsors of the BSTH Annual Meeting. This year the industry organized satellite symposia are all focused on bleeding disorders, which provides a nice balance with the thrombosis topics in the main program.

It is good to see that the thrombosis and haemostasis community in Belgium is thriving and expanding with more and more Belgian researchers and clinicians giving presentations at international scientific meetings or playing prominent roles in international scientific organizations. I hope the BSTH has played a beneficial role in these developments. The oral presentation sessions and the poster walk are a small part of this. Another part is the annual CSL Behring Encouragement Award, judged by the BSTH Board, now in its third year. I am happy to say that interest in this prize has been growing steadily and this year saw 8 submissions for this award, and the winner will be announced on Friday.

I hope you will all enjoy this 26th Annual Meeting of the Belgian Society on Thrombosis and Haemostasis!

Alain Gadisseur
President of the BSTH

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PROGRAM THURSDAY 22 NOVEMBER

08:30		REGISTRATION & COFFEE
09:25		WELCOME
		EDUCATIONAL I *Chairs: T. Vanassche and A. Demulder*
09:30		Should we measure DOACS? *J. Douxfils, Namur*
		SPONSORED SATELLITE SYMPOSIUM - ROCHE **"EXPLORING NEW PATHS FOR HAEMOPHILIA A MANAGEMENT"** *Chair: C. Hermans, Belgium Louvain / Brussels*
10:15		A new treatment option for Haemophilia A patients with inhibitors: addressing the unmet needs *C. Hermans, Louvain / Brussels*
10:45		Laboratory monitoring in a new landscape; What is changing with Emicizumab (Hemlibra®) *M. Jacquemin, Leuven*
		Questions and answers
11:15		BREAK
		ORAL PRESENTATIONS: CLINICAL & LABORATORY *Chairs: C. Orlando and K. Vandenbosch*
11:45	O01	Inherited antithrombin deficiency and thrombosis in childhood: data from a large multicentric cohort *C. Orlando, Brussels*
11:57	O02	Platelet-rich but not RBC-rich areas in stroke thrombi consist of dense fibrin structures, VWF and extracellular DNA *S. Staessens, Kortrijk*
12:09	O03	Lupus anticoagulant hypoprothrombinemia syndrome: a paediatric case report *S. Vandamme, Wilrijk*
12:21	O04	The presence of anti-ADAMTS13 autoantibodies does not change ADAMTS13 antigen levels measured by an in house developed ADAMTS13 antigen ELISA *C. Dekimpe, Kortrijk*
12:33	O05	The concentration of GP1ba magnetic particles is a critical parameter occasionally responsible for poor reproducibility of the VWF:RCo with a chemiluminescent immunoassay. *B. Calcoen, Leuven*

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PROGRAM THURSDAY 22 NOVEMBER

12:45 **PAUL CAPEL PRIZE CLINICAL & LABORATORY**

13:00 LUNCH

14:00 **BSTH GENERAL ASSEMBLY (access for BSTH members only)**

STATE OF THE ART I: THROMBOSIS
Chairs: K. Jochmans and K. Devreese

14:30 Mechanisms and risk factors of thrombosis in cancer
A. Falanga, Bergamo, Italy

15:00 Cancer associated thrombosis: how to prevent, how to treat?
C. Ay, Vienna, Austria

15:30 Approach to thrombosis at unusual sites: Splanchnic and cerebral vein thrombosis
W. Ageno, Varese, Italy

16:00 BREAK

SPONSORED SATELLITE SYMPOSIUM - BAYER
"EMPOWERING PATIENTS TO GO ONE STEP FURTHER"
Chairs: C. Hermans and Le Phu Quoc

16:30 Welcome & Introduction

16:35 Clinical experiences with BAY94-9027, a novel, PEGylated, extended half-life VIII
P. Holme, Oslo University Hospital, Norway

16:55 PEGylation within hemophilia care. What about bio-distribution and metabolism?
A. Baumann, Director principal Scientific Expert Biologics, Bayer Pharmaceutical

17:10 Pharmacoeconomics with a focus on hemophilia
K. Steen Carlsson, Health economics expert, Lund University, Sweden

PROFESSOR GASTON BAELE MEMORIAL LECTURE

17:30 Accomplishments and prospects in Haemostasis, a personal view
M. Hoylaerts, Leuven

18:00 CLOSURE OF DAY PROGRAMME

EVENING PROGRAM

WELCOME RECEPTION

CSL Behring and Novo Nordisk invite all participants on Thursday night at 18:00 hrs. to join the welcome reception.

DINNER

From 19:00 hrs. you're invited to join our dinner at LAMOT at night, with a magnificent view on the River Dijle.

Preregistration is required.
Each participant including delegates of companies can register to join for a fixed price per person (55 EUR).

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PROGRAM FRIDAY 23 NOVEMBER

08:30		REGISTRATION & COFFEE
08:55		WELCOME
		EDUCATIONAL II *Chairs: K. Vanhoorelbeke and J. Emmerechts*
09:00		Auto-immune heparin-induced thrombocytopenia *A. Greinacher, Greifswald, Germany*
		SPONSORED SATELLITE SYMPOSIUM - SHIRE *Chairs: A. Gadisseur and K. Peerlinck*
09:45		Targeting ideal trough and peak levels in hemophilia A patients *Professor Víctor Jiménez Yuste, Chief of Hematology Department; University Hospital La Paz, Madrid, Spain*
10:15		Progressing patient care in Von Willebrand Disease *Professor Sophie Susen, Head of the department of Hemostasis and Transfusion in Lille University Hospital, France*
10:45		BREAK
		ORAL PRESENTATIONS: BASIC RESEARCH *Chairs: H. Deckmyn and C. Oury*
11:15	O06	Open ADAMTS13 conformation in immune-mediated thrombotic thrombocytopenic purpura is induced by anti-ADAMTS13 autoantibodies *E. Roose, Kortrijk*
11:27	O07	The VWF-GPIb interaction mediates thrombo-inflammation in experimental stroke via recruitment of monocytes, neutrophils and T-cells to the brain *F. Denorme, Kortrijk*
11:39	O08	The differential role of platelets and fibrin in adhesion on inflamed and damaged valves in a new mouse model of early staphylococcus aureus endocarditis *S. Meyers, Leuven*
11:51	O09	Visualizing dense granule formation in megakaryocytes using advanced microscopy and its application for Hermansky-Pudlak syndrome *J. Heremans, Leuven*
12:03	O10	P2X1 deficiency causes massive intestinal bleeding along with enhanced neutrophil-dependent thrombosis in colitis *O. Wéra, Liège*

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PROGRAM FRIDAY 23 NOVEMBER

12:15	**PAUL CAPEL PRIZE BASIC RESEARCH**
12:25	**BSTH NEWS**
12:45	LUNCH
13:00	**POSTER WALK**
	STATE OF THE ART II: MICROORGANISMS AS ENVIRONMENTAL FACTOR *Chairs: S. De Meyer and M. Sprynger*
14:00	Staphylococcus aureus: master manipulator of the human hemostatic system *T. Vanassche, Leuven*
14:30	Cardiac device infection: Incidence, diagnosis and treatment *L. Musumeci, Liege*
15:00	Microbiota and thrombosis *C. Reinhardt, Mainz, Germany*
15:30	**POSTER PRESENTATION AWARDS**
15:35	CLOSURE
15:45	RECEPTION

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BSTH BOARD 2018

THE PRESENT MEMBERS OF THE BSTH BOARD 2018 ARE:

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EXHIBITION RULES & REGISTRATION FEES

EXHIBITION RULES

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

FOOD AND BEVERAGE

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

MANNING OF STANDS

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

NOISE

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

SECURITY AND INSURANCE

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

EXHIBITION OPENING HOURS

THU 22 NOV 2018	08:30-19:00
FRI 23 NOV 2018	08:15-16:30

REGISTRATION FEES

	EARLY-BIRD (UNTIL 1 OCT.)	REGULAR (UNTIL 9 NOV.)	LATE & ON-SITE (AS OF 10 NOV.)
MEMBER BSTH			
Regular (MD specialist, MSc specialist, PhD scientist)	100 EUR	110 EUR	140 EUR
MD trainee / PhD student	55 EUR	55 EUR	70 EUR
Nurse, paramedic, technician, data manager, student	25 EUR	30 EUR	40 EUR
NON MEMBER BSTH			
Regular (MD specialist, MSc specialist, PhD scientist)	165 EUR	195 EUR	225 EUR
MD trainee / PhD student	110 EUR	125 EUR	140 EUR
Nurse, paramedic, technician, data manager, student	70 EUR	70 EUR	85 EUR

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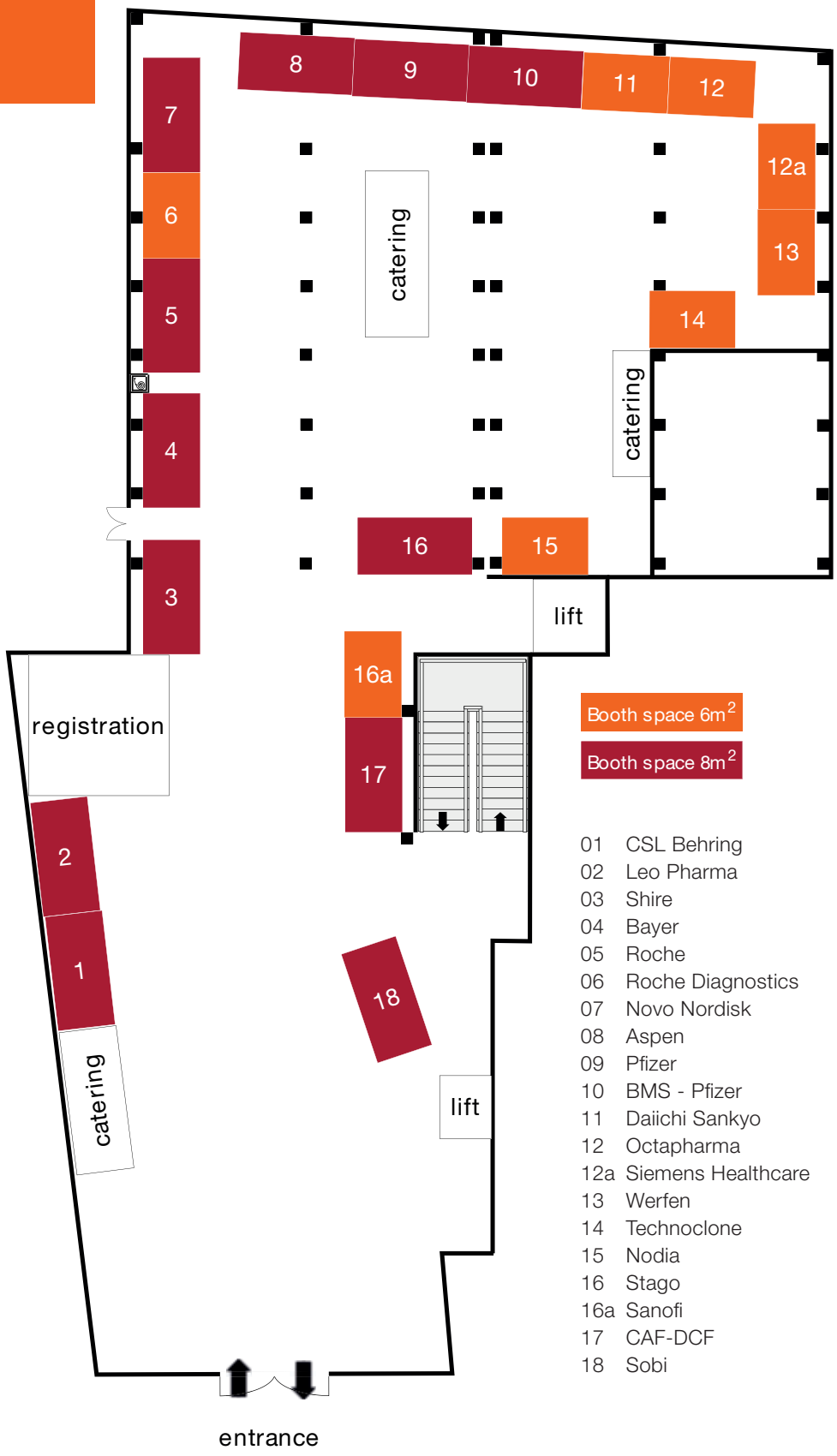
FLOORPLAN



01 CSL Behring
02 Leo Pharma
03 Shire
04 Bayer
05 Roche
06 Roche Diagnostics
07 Novo Nordisk
08 Aspen
09 Pfizer
10 BMS - Pfizer
11 Daiichi Sankyo
12 Octapharma
12a Siemens Healthcare
13 Werfen
14 Technoclone
15 Nodia
16 Stago
16a Sanofi
17 CAF-DCF
18 Sobi

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EDUCATIONAL I

Should we measure DOACS?

J. Douxfils
University of Namur, NAMUR, Belgium

One of the key benefits of the direct oral anticoagulants (DOACs) is that they do not require routine laboratory monitoring. Nevertheless, assessment of DOAC exposure and anticoagulant effects may become useful in various clinical scenarios. The five approved DOACs (apixaban, betrixaban, dabigatran etexilate, edoxaban and rivaroxaban) have different characteristics impacting assay selection and the interpretation of results.
This presetnation provides an updated overview on (i) which test to use (and their advantages and limitations), (ii) when to assay DOAC levels, (iii) how to interpret the results relating to bleeding risk, emergency situations and perioperative management, and (iv) what is the impact of DOACs on routine and specialized coagulation assays.
Assays for anti-Xa or anti-IIa activity are the preferred methods when quantitative information is useful, although the situations in which to test for DOAC levels are still debated. Different reagent sensitivities and variabilities in laboratory calibrations impact assay results.

International calibration standards for all specific tests for each DOAC are needed to reduce the inter-laboratory variability and allow inter-study comparisons. The impact of the DOACs on hemostasis testing may cause false-positive or false-negative results; however, these can be minimized by using specific assays and collecting blood samples at trough concentrations.

Finally, prospective clinical trials are needed to validate the safety and efficacy of proposed laboratory thresholds in relation to clinical decisions.

After being graduated in pharmaceutical sciences in 2011, Dr. Jonathan Douxfils obtained his PhD in biomedical and pharmaceutical sciences in 2015. In 2018, he gained an academic position at the University. The researches directed by Dr. Douxfils play a leading role in the establishment of guidelines for the laboratory measurement of DOACs in the routine setting. These recommendations have been used so far by several Expert Societies involved in the field of thrombosis and hemostasis. He also exercises his expertise as a pharmacovigilance expert at the European Medicine Agency, is co-chairmen of the SSC Control of Anticoagulation at the International Society of Thrombosis and Haemostasis (ISTH) and is also the co-founder and the CEO of QUALIblood, a Contract Research Organization (CRO) aiming to provide the industries, hospitals and universities with all the analytical services for blood investigations and hemocompatibility testing.

Passionate about clinical and laboratory research, he is involved in several projects aiming to solve pharmacological and/or epidemiological problems especially in thrombosis and hemostasis. Thanks to his translational view, i.e. from basic research to post-marketing pharmacovigilance, of the pharmaceutical and biomedical market, his collaborations with key opinion leaders but also with field practitioners, Dr. Jonathan Douxfils puts its expertise and know-how at the services of projects aiming to improve the safety and effectiveness of therapeutic agents in order to promote public health.

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ROCHE SATELLITE SYMPOSIUM

C. Hermans (Louvain / Brussels)

Cedric Hermans currently heads the Hemostasis and Thrombosis Unit and the Hemophilia Center of the Saint-Luc University Hospital in Brussels, Belgium. He was appointed Associate Professor at the Medical School of the Catholic University of Louvain in 2003, Full Professor in 2012 and Vice-Dean in 2015.
Dr. Hermans has (co)-authored more than 200 original articles in international journals and is a member of several scientific societies and international advisory boards. He is past-president of EAHAD and was recently appointed as member of the Board of Directors of the World Federation of Haemophilia. He is the Editor-in-Chief of the Haemophilia Journal.
His main research interests lie in the area of haemostasis and thrombosis, especially clinical studies on the treatment of haemophilia, new anticoagulants, and the management of thrombosis

M. Jacquemin (Leuven)

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

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STATE OF THE ART I

Mechanisms and risk factors of thrombosis in cancer

A. Falanga
Hospital Papa Giovanni XXIII, BERGAMO, Italy

The close relationship between cancer and thrombosis is known since more than a century. Cancer patients present with many types of hemostatic abnormalities and have an increased risk of both thrombotic and hemorrhagic complications. Cancer is associated with a four- to seven-fold increase in the risk of venous thromboembolism (VTE), peaking in the first 3 months following cancer diagnosis. However, even without thrombosis, the majority of cancer patients present with clotting alterations detectable by laboratory tests, which characterize the hypercoagulable state of these subjects. Preventing these complications is clinically relevant because thrombosis is a major cause of morbidity and mortality in cancer patients. The pathogenesis of blood coagulation activation in oncological patients is complex. Multiple clinical factors together with biological procoagulant mechanisms expressed by cancer tissues concur to the activation of blood coagulation and importantly contribute to the overall thrombotic risk of these patients. Clinical factors include general risk factors, as well as disease-specific factors, as the cancer site and stage and anticancer therapies. Besides general clinical risk factors for VTE, also disease-specific clinical factors, i.e. type and stage of the tumor, and anti-cancer therapies increase the thrombotic risk in these patients. Altogether, these factors favor the shift of the hemostatic balance towards a prothrombotic condition, as shown by the appearance of subclinical coagulation changes in almost all of cancer patients, who constantly present with high levels of circulating biomarkers of hypercoagulability. Finally, biological mechanisms are definitly involved in the pathogenesis of cancer-associated thrombosis (CAT). Indeed, tumor cells gain the capacity to activate the host hemostatic system in several ways, and this phenomenon is often driven by the same oncogenes responsible for the cellular neoplastic transformation. The principal procoagulant mechanisms expressed by cancer cells include: i) the activation by cancer cells of the clotting system through the expression of procoagulant properties (such as TF, C], and heparanase), TF-MP, coagulation factors, and fibrinolysis proteins; ii) the activation by cancer cells of the procoagulant potential of host blood vascular cells, i.e. platelets, leukocytes, and endothelial cells. The latter mechanism can occur either by cell-cell direct contact, mediated by specific surface adhesion receptors, and/or by the release of inflammatory cytokines as well as pro-angiogenic and growth stimulating factors (i.e. VEGF, bFG], and G-CSF) by both cancer and host blood cells. The activation of platelets, endothelial cells, and leukocytes produces, among other procoagulant features, the release of procoagulant microparticles and neutrophil extracellular traps. All these phenomena contribute to clotting activation in cancer. Of interest, several genes responsible for the cellular neoplastic transformation drive the programs of hemostatic properties expressed by cancer tissues. A better understanding of such mechanisms can help the development of novel strategies to prevent and treat CAT and to identify new approaches to the cure of cancer.

Anna Falanga, MD, is Chief of the Department of Immunohematology and Transfusion Medicine and the Thrombosis and Hemostasis Center, at the Hospital Papa Giovanni XXIII, in Bergamo, Italy. After having received her medical degree and board certification in internal medicine at the University of Naples, Dr. Falanga obtained the board certification in Hematology at the University of Verona, Italy. Subsequently, she spent three years working as a postdoctoral fellow at the Mario Negri Institute in Milan, Italy, and a further two years at the University of Colorado School of Medicine, Denver, CO, USA. She has long been involved in the field of research on “Cancer & Thrombosis”. For her experience in the field, she is a member of the panel of experts and a co-chair for the preparation of the Guidelines of prophylaxis and treatment of venous thromboembolism in patients with cancer, for both the Italian Society of Medical Oncology, the American Society of Clinical Oncology, and the European Society of Medical Oncology.

Dr Anna Falanga has authored more than 280 publications. She has been guest editor of 21 books or journal supplements. She is appointed as professor of 'Coagulation disorder' at the School of Hematology, Faculty of Medicine, University Bicocca Milan, Italy, and as a professor of 'Clinical pathology' at the University of Bergamo. She has an active role in several professional societies. She has been Chair of the Scientific and Standardization Committee of the International Society of Thrombosis and Haemostasis (ISTH) (term 2010-2012), co-chair (term 2007-2010) and chair (2011) of the Scientific Committee on Hemostasis of the American Society of Hematology (ASH) and President of the Italian Society on Thrombosis and Hemostasis (SISET) (term 2014-2016). Further, she has been Councilor on Board of the European Hematology Association (EHA) (term 2009-2013), and of the Italian Federation of the Thrombotic Centres (terms 2009-2012 and 2012- 2015). She is currently Councilor on Board of the ISTH (term 2014-2020). In 2009 she received the BACH Investigator Award of the ISTH, and in 2015 the "Harold R. Roberts Medal 2015" of the SSC of ISTH. Other activities of Dr. Falanga include the organization of numerous scientific conferences, including nine biennial International Conferences on "Thrombosis and Hemostasis Issues in Cancer" (ICTHIC), in Bergamo, Italy.

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STATE OF THE ART I

Cancer-associated thrombosis: how to prevent, how to treat?

C. Ay
Medical University of Vienna, VIENNA, Austria

One out of 5 venous thromboembolic events occur in association with cancer. On the other hand up to 20% of patients with cancer develop venous thromboembolism (VTE) during the course of their disease. Importantly there is a wide variation with VTE risk depending on specific risk factors or clinical settings.
Primary thrombophropyhaxis for prevention of cancer-associated VTE (CAT) is recommended after cancer surgery and in hospitalised cancer patients, who are immoblised or in those with acute medical illness. In contrast, routine thromboprophylaxis in the ambulatory setting is still controversially discussed and not uniformly recommened, except for selected patient populations. Improvement in risk assessment is needed to identfiy those patients at highest risk for CAT who might benefit from primary thromboprophylaxis. A recently developed and externally validated clinical prediction model used the cancer type and D-dimer levels to personalise risk assessment for CAT (Pabinger I et al. Lancet Haemtol. 2018 Jul;5(7):e289-e298). Randomized-controlled trials (RCT) with DOAC investigating efficacy and safety of primary thromboprophylaxis in patients at high risk of VTE defined by the Khorana-Score are underway. Results of these trials (CASSINI and AVERT) are awaited to be published soon.

Treatment of CAT is challenging, as both the risk of recurrence and bleeding during anti-coagulation are increased. Low-molecular-weight-heparin (LMWH) has been the standard of care for treatment and secondary prevention of CAT for up to 6 months. Recent RCTs have shown that DOAC (edoxaban or rivaroxaban) are effective in treating CAT, but exhibit an increased risk of major bleeding due to higher rates of gastro-intestinal bleeding with DOAC, however, without increase in intra-cranial or fatal bleeding. A post-hoc analysis of the HOKUSAI VTE Cancer study (edoxaban vs dalteparin) has provided new insights into the types, severity and outcomes of bleeding associated with anticoagulants in cancer patients. Based on the recent evidence DOAC have emerged as an alternative treatment for CAT in patients with cancer, especially in those withouth high risk of bleeding.

Cihan Ay is currently an associate professor in medicine/haemostaseology at the Medical University of Vienna, Austria, and has been a visiting research professor at the University of North Carolina at Chapel Hill, NC, USA (2016-2017).
He serves as co-director of the program for acquired thrombophilia and inherited bleeding disorders at the Clinical Division of Haematology and Haemostaseology, Department of Medicine I, Medical University of Vienna – Vienna General Hospital, Austria.

His research and clinical work focuses on VTE, anticoagulation and bleeding disorders. He has authored over 130 peer-reviewed publications in the field of thrombosis and haemostasis and has been particular successful in contributing to a better understanding of the etiology of VTE in cancer by identifying biomarkers and clinical risk factors for cancer-associated VTE.

He serves as Co-Chair of the Scientific and Standardization Committee (SSC) Subcommittee of the International Society on Thrombosis and Haemostasis (ISTH) on Haemostasis and Malignancy, on the Editorial Board of Journal of Thrombosis and Haemostasis (JTH) and Thrombosis Research, and as an associate editor of Research and Practice in Thrombosis and Haemostasis (RPTH), the new open access journal of ISTH.

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STATE OF THE ART I

Approach to thrombosis in unusual sites: SVT and CVT

W. Ageno
University of Insubria, VARESE, Italy

Venous thrombosis occurring outside of the lower limbs and the pulmonary arteries is generally defined as thrombosis in unusual sites. Although relatively rare, venous thrombosis in unusual sites is often challenging. Given the large variety of clinical conditions, this presentation focused on two of the most relevant sites of venous thromboembolism: the splanchnic and the cerebral veins. Solid abdominal cancer and liver cirrhosis are responsible of up to 50% of all splanchnic vein thrombosis (SVT), the remaining are associated with JAK2 V617F mutation with or without overt myeloproliferative neoplasms, inflammatory diseases or infections, abdominal surgery or trauma. Other very rare predisposing factors include paroxysmal nocturnal haemoglobinuria or Behcet disease, to name a few. Establishing the optimal treatment for SVT patients is challenging, due to severity of presentation in a non-negligible proportion of cases (25% present with gastrointestinal bleeding, others with acute abdomen requiring surgery), the difficult balance between the high risk of recurrent thrombosis and the short and long-term risk of hemorrhagic complications. Observational studies suggest that up to one third of patients with portal vein thrombosis are left untreated in routine clinical practice, whereas low molecular weight heparin (LMWH) is the most commonly prescribed treatment. The long-term risk of VTE recurrence is highest in patients with cirrhosis, followed by patients with cancer and myeloproliferative neoplasms. Major bleeding complications are also highest in patients with cirrhosis. LMWH is recommended as a standalone treatment during the first weeks for patients with cirrhosis, cancer, or thrombocytopenia. For all other patients LMWH and vitamin K antagonists (VKA) represent the treatment of choice. No studies have assessed the efficacy and safety of the direct oral anticoagulants in this setting, but a number of case series have been recently published. .
Cerebral vein thrombosis (CVT) most commonly affects young women with gender related risk factors. Other risk factors include brain tumors, local infections (otitis, mastoiditis, meningitis, in particular in children), head injury, or surgery. Anticoagulant treatment has a crucial role with the aim to avoid thrombus extension and to favor local resolution. There are only two small randomized controlled trials that evaluated the efficacy and safety of heparin, either unfractionated heparin or LMWH for the acute treatment of CVT. Taken together, these studies show a trend towards reduced risk of death or dependence as compared with placebo. A challenging aspect of CVT is related to the concomitant presence of intracranial bleeding at the time of diagnosis in as many as 25-39% of patients.

Based on observational evidence, guidelines are concordant that this should not be an absolute contraindication to anticoagulant treatment. Thrombolytic therapy should be considered only for patients with clinical deterioration despite adequate anticoagulant treatment and without any alternative cause of deterioration. The risk of recurrent thrombosis after CVT was reported to be low, in particular in young women with gender specific risk factors. Risk factors for thrombosis recurrence include male sex, severe thrombophilia, and previous VTE. Studies assessing the role of the direct oral anticoagulants in this setting are ongoing.

Walter Ageno is Professor of Medicine at the University of Insubria and Director of the Short-Stay Medical Unit and Thrombosis Center at the Ospedale di Circolo of Varese, Italy.
Dr. Ageno has authored and co-authored more than 450 articles indexed in MEDLINE, as well as several abstracts presented at national and international meetings and more than 15 book chapters in the fields of venous and arterial thrombosis. He is coordinating international trials on the treatment of unusual site thrombosis and has also been a member of the steering committee or the safety monitoring board in a number of phase III and IV studies on the prevention and treatment of venous thromboembolism. Dr. Ageno is chairman of the Italian Society of Hemostasis and Thrombosis and immediate past-chairman of the Scientific Subcommittee board of the International Society on Thrombosis and Haemostasis. He is deputy editor for Thrombosis and Haemostasis, associate editor for Internal and Emergency Medicine, Blood Transfusion, and Monaldi Archive for Chest Disease and member of the Advisory Board of Vascular Medicine and Journal of Thrombosis and Haemostasis. He has contributed to a number of national and international guidelines on venous and arterial thrombosis.

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BAYER SATELLITE SYMPOSIUM

Clinical experiences with BAY94-9027, a novel, PEGylated, extended half-life factor VIII

P.A. Holme
Oslo University Hospital, OSLO, Norway

Hemophilia A is a rare genetic bleeding disorder caused by the impairment of factor VIII. Prophylactic intravenous injections of factor VIII are currently the standard of care therapy to manage patients with severe hemophilia A. BAY 94-9027 is a novel, PEGylated, recombinant factor VIII for the treatment of hemophilia A. The safety and efficacy of this molecule have been assessed in the PROTECT VIII clinical trial program with an observation period of 5.2 years in total. Professor Pål André Holme will present these results from the PROTECT VIII and PROTECT VIII extension trials. As an active investigator in these trials, professor Holme will also share his personal clinical experiences within these clinical studies.

Pål André Holme is professor of hematology in the Department of Haematology and the Institute of Clinical Medicine at the University of Oslo, and a senior hematologist at Oslo University Hospital, Rikshospitalet in Norway. He graduated from the medical school of the University of Oslo in 1993, and was a research fellow at the Research Institute for Internal Medicine of Oslo University Hospital, where he earned a PhD for studies on platelet activation and platelet-derived microparticle formation in 1996.

Professor Holme has been head of the treatment of adult patients with hemophilia and bleeding-related disorders in Norway since 2006. He participates in several clinical research groups, has been an active investigator in studies on bleeding-related disorders and platelets, and has authored many papers and textbook chapters on these subjects.

Professor Holme participates as an investigator to the PROTECT VIII trial of Bayer.

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BAYER SATELLITE SYMPOSIUM

PEGylation within hemophilia care. What about bio-distribution and metabolism?

A. Baumann
Bayer AG, BERLIN, Germany

Polyethylene glycol (PEG) molecules are synthetic, highly water soluble, inert polymers consisting of repetitive units of ethylene oxide. PEGylation is a strategy used to improve the pharmacokinetic properties of molecules across different therapeutic areas. Over the last few years this strategy has also been applied on factor VIII and IX for the treatment of hemophilia A and B. In BAY 94-9027, a 60 kDa PEG has been fused to a B-domain deleted factor VIII. The effects of this PEG molecule on the bio-distribution and metabolism of factor VIII are assessed in several pre-clinical studies. Professor Andreas Baumann will highlight some important data from these bio-distribution, elimination and toxicology studies and open the floor for questions from the audience.

Professor Baumann is Director and Principal Scientific Expert for Biologics within Translational Sciences at Bayer AG in Berlin, Germany, where his responsibilities cover the global preclinical pharmacokinetic development of biotechnology derived pharmaceuticals. He has 25 years' experience in drug development in the pharmaceutical industry including small molecules and for the last 15 years focused on non-clinical development of Biologics.

Prior his present obligations he was heading the Research Pharmacokinetic Department at Schering AG, Berlin. In parallel, he is Professor of Pharmacology & Toxicology, and has been lecturing for more than 15 years at Universities in Germany. He has published 50 peer-reviewed scientific articles and scientific text book articles and has been presenting regularly at international scientific conferences. Prof. Baumann is member of the BioSafe Leadership Team of the Biotechnology Industry Organization (BIO).

BAYER SATELLITE SYMPOSIUM

Pharmacoeconomics with a focus on hemophilia

K. Steen-Carlsson
Swedish Institute for Health Economics, LUND, Sweden

Pharmacoeconomics is the study of the economic aspects of pharmaceuticals and uses cost-benefit, cost-effectiveness, cost-minimization, cost-of-illness and cost-utility analyses to compare pharmaceutical products and treatment strategies. In this lecture, professor Katarina Steen-Carlsson will guide us through the basic concepts of pharmacoeconomics and focus these concepts on the hemophilia treatment field. This includes a pharmacoeconomic view on extended half-life products and novel therapies for the treatment of hemophilia.

Associate professor Katarina Steen Carlsson, PhD, is research director at the Swedish institute for Health Economics and researcher in health economics at the Lund University. In 1991 she completed her bachelor in Business and Economics at the Lund University in Sweden. After her doctoral research on 'equality of access in health care' she obtained her PhD degree in 1999. From 2002-2009 she occupied postdoctoral positions at the Vårdal Institute, the Swedish Institute for Health Sciences. In 2017 she became associate professor health economics at Lund University. She has been appointed as Scientific Advisor in health economics of Social Services at the Swedish National Board of Health and Welfare and is a member of the Scientific Advisory Committee of the SBU, Swedish Agency for Health Technology Assessment and Assessment of Social Services.

Prof. Steen Carlsson has published over 75 original peer-reviewed articles, review articles, reports and book chapters in various therapeutic areas including hemophilia. She holds positions as a member of the Editorial Board of Haemophilia and the Nordic Journal of Health Economics and as a reviewer for various other scientific journals.

Cédric Hermans (Brussels)

Professor Cédric Hermans currently leads the Hemostasis and Thrombosis Unit and the Hemophilia Center of the Saint-Luc University Hospital in Brussels, Belgium. He was appointed Associate Professor at the Medical School of the Catholic University of Louvain in 2003, Full Professor in 2012 and Vice-Dean in 2015.

Professor Hermans has published over 196 original articles in international journals and is a member of several scientific societies and international advisory boards. He was president of EAHAD and was recently appointed as a member of the Board of Directors of the World Federation of Hemophilia. In July 2018 prof. Hermans became the new Editor-in-chief of the international scientific journal Haemophilia.

His main research interests lie in the area of hemostasis and thrombosis, especially clinical studies on the treatment of hemophilia, new anticoagulants, and the management of thrombosis.

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BAYER SATELLITE SYMPOSIUM

Phu Quoc Lê (Brussels)

Dr. Phu Quoc Lê is head of the General Pediatrics department in the Etterbeek-Ixelles Hospital Centre and half-time resident Pediatric Immuno-Hemato-Rheumato-Oncology at the University Children's Hospital Queen Fabiola (HUDERF) in Brussels. He is also the pediatric lead of the HemoWab group, one of the 5 hemophilia reference centers in Belgium. Dr. Lê completed his specialty training in Pediatrics in 1999 at the Free University of Brussels (ULB) and obtained his PhD in 2018 with a thesis on improving the multidisciplinary care of patients with sickle cell disease. Since 2009 he has a teaching assignment in Pediatric Physiotherapist Specialization at the Scientific Institute for Locomotion of the ULB.

Dr. Lê is a member of several national and international associations within hematology, rheumatology and oncology, publishes in renowned scientific journals, and regularly presents data on international congresses.

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PROFESSOR GASTON BAELE MEMORIAL LECTURE

Accomplishments and prospects in Haemostasis, a personal view

M.F. Hoylaerts
Center for Molecular and Vascular Biology, KU Leuven, LEUVEN, Belgium

The microscopic discovery of platelets by the Italian pathologist Giulio Bizzozero in 1882 implicitly triggered a new research field on the function of this microscopic “dust”. The enormous work done by the all-round German physician Rudolf Virchow, as of 1845 streamlined our present thinking on the relations between vascular pathology, thrombosis and coagulation activation. The French, equally all-round physician Armand Trousseau in 1865 was the first to establish a relation between neoplastic disease and thrombo-embolic disorders, presently known as Trousseau’s syndrome.

Aspirin was one of the first anti-platelet agents in use. Although salicylic acid had been used medicinally since antiquity, the modern aspirin story begins with its synthesis and manufacture in 1899. But it took until 1971, when John Vane, University of London, identified aspirin’s mechanism of action as a dose-dependent inhibition of prostaglan-din synthesis, work he was rewarded a Nobel prize for in 1982, together with Bengt Samuelsson and Sune Bergström. The first (statistically inconclusive!) indications that aspirin might be useful in the secondary prevention of death from heart attack and in the reduction in total mortality of 12% at 6 months and 25% at 12 months, came in 1974 from the first randomised controlled trial of aspirin, work followed by a multitude of subsequent clinical studies, unequivocally having established its benefit.

Yet, it’s efficacy is moderate and the search for antiplatelet drugs continues today, with various degrees of success. Platelets express over 5,000 proteins, their functional regulation subject to polymorphisms and expression variation, often affecting platelet activity and hence the efficacy of anti-platelet treatment. With the identification of specific platelet receptors and the existence of selective signaling cascades in platelets, present research searches to translate these findings in new anti-platelet drugs for the prevention of thromboembolism. Thus, various anti-platelet P2Y12 antagonists and P2Y12-reactive drugs are well-characterized, but the variable degree of the individual platelet response remains a factor of concern, today addressed via the development of anti-platelet drug combinations, the so-called dual anti-platelet therapy. Additional dual anti-platelet therapy may allow more efficacious treatment of poor responders to current combinations of aspirin and ADP-receptor antagonists. However, some of the receptors implicated (the VWF receptor GPIba, the collagen receptors GPVI and a2b1, the thrombin receptors PAR1 and PAR4 and secondary receptors, modulating platelet function) show variable expression or show surprisingly little bleeding when congenitally absent, necessitating well-designed clinical studies.

Although already in 1978, Alfred Copley reviewed the role of platelets in physiological defense mechanisms, today more and more interest is attributed to the platelet’s inflammatory properties, immune regulation and secretion potential in biological repair mechanisms, including their role in thrombo-inflammation, a hot topic, potentially initiating a new class of anti-thrombo-inflammatory drugs.

Early efforts to pharmacologically interfere with thrombotic complications resulted in the discovery of heparin, by Jay McLean and William Henry Howell in 1916. Still, heparin only entered clinical trials in 1935 and it took until the early 1980’s before the mechanism of its anticoagulant action was fully understood. The “sweet clover disease” in the 1920’s, causing a hemorrhagic disease in cattle being fed a mouldy hay, promoted the development of warfarin, which was marketed as a rodenticide in 1948. The transition of warfarin to clinical application was made under the name ‘Coumadin’, which was water soluble, orally bioavailable and its effect was reversed by vitamin. In 1955, Warfarin was given to President Dwight Eisenhower following a myocardial infarction As Duxbury and Poller pointed out; ‘What was good for a war hero and the President of the United States must be good for all, despite being a rat poison!‘. These developments are surprising since the coagulation cascade and the anticoagulant system are only understood correctly since the 1990’s, many different factors having been identified, the importance of which was uncertain for a very long time. Even today, the role of Factor XII vs that of FXI, after careful re-evaluation appears to be correctly understood since recently only. Nevertheless, new structure-assisted design and large molecular screening have identified a wealth of specific anti-thrombin and anti-factor Xa inhibitors, having changed the field of antithrombotic therapy tremendously in just a few years. This has been achieved at a time, where in most fields of medicine, the search for new drugs was hampered for economic reasons, a real achievement in the field of Thrombosis & Haemostasis! Today we search for the holy grail, i.e. prevention of primary thrombosis and VTE, without raining bleeding risks. Several new approaches are at our disposal to achieve these goals. Some of those possibilities and potential of new approaches will be briefly discussed during the presentation.

Marc Hoylaerts studied Biochemistry at the University of Leuven, where he graduated in 1978. He was active for 3 years as a postdoctoral scientist in the field of fibrinolysis, during which time he worked with Prof Désiré Collen in Leuven on tissue-type plasminogen activator. Upon his return to Leuven in 1993, he joined the research team of Prof Jos Vermylen, whose interest was on platelet physiology in health and disease. During the next 25 years, until his retirement end 2017, his work in the Center for Molecular and Vascular Biology of the Department for Cardiovascular Sciences of the KU Leuven focused on the characterization of various platelet receptors and ligands, their activation pathways and the interaction of platelets with inflamed or injured vasculature. In these studies, activation of platelets and of the coagulation cascade were central in the context of human pathologies, such as thrombosis, atherosclerosis, hemostasis dysfunction during air pollution and infective endocarditis. This work has been performed in vitro and in various mouse models, and has been carried out in collaboration with numerous (inter)national postdoctoral scientists, PhD students and clinical scientists.

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EDUCATIONAL II

Anti-PF4/Polyanion antibody induced autoimmune HIT: A new mechanism of autoimmunity

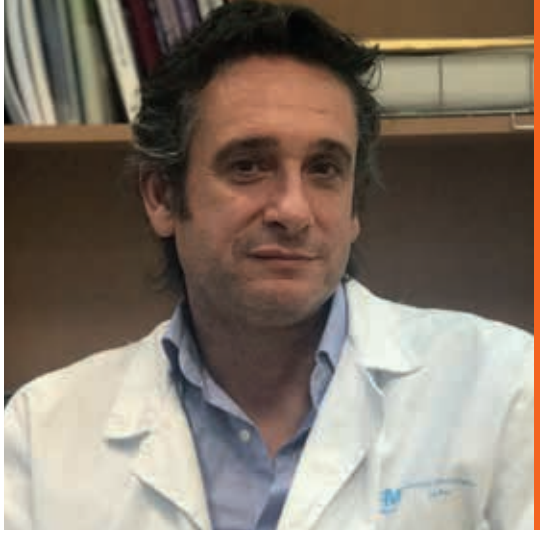
A. Greinacher
Universitätsmedizin, GREIFSWALD, Germany

Background/Introduction:Heparin-induced thrombocytopenia (HIT) is a well known adverse drug effect. While clinical presentation and management of HIT have been worked out during the past decades, the immunological mechanisms are still not fully understood. Platelets contribute to innate immunity. We recently introduced the concept that hereby PF4 plays an important role. Antibodies recognizing a neoepitope on PF4/polyanion complexes opsonize bacteria via binding to PF4 bound to bacterial surface polyanions. When this bacterial host defense mechanism is misdirected to PF4/heparin complexes on platelets and monocytes, the prothrombotic adverse drug reaction HIT occurs. Aims: The lecture will introduce the concept that high negative charges are a danger signal indicating bacterial invasion and that the endogenous protein platelet factor 4 (PF4) “translates” these negative charges into a conformational change recognized by the adapted immune system. Based on the molecular pathogenesis of HIT and autoimmune HIT a new mechanism of antibody mediated autoimmunity will be presented.
Methods/Materials: We assessed binding characteristics of different anti-PF4/polyanion antibodies by single molecule force spectroscopy, CD-spectroscopy and isothermal titration calorimetry and combined this with functional assays.

Results: Anti-PF4/polyanion-antibodies activating platelets only in the presence of polyanions show binding forces to PF4/P-complexes <60pN; antibodies activating platelets in the absence of polyanions (autoimmune-HIT antibodies) show much higher binding forces ~100pN. Most interestingly, these anti-PF4-autoantibodies substitute for heparin. They bind to PF4 alone and thereby induce the same change in the conformation of PF4 as heparin does. This induces exposure of the HIT neo-epitope, and recruits physiologic, polyanion-dependent anti-PF4/P-antibodies into the autoimmune-HIT process, resulting in massive, heparin-independent platelet activation.

SUMMARY/CONCLUSIONS: Antibody-mediated changes in endogenous proteins triggering binding of otherwise non-pathogenic antibodies may also be important for other antibody-mediated autoimmune disorders in hematology.

Andreas Greinacher, specialized for transfusion medicine, immunohematology and clinical immunology, is full professor and head of the department of transfusion medicine at the Universitätsmedizin Greifswald. His research interests are hereditary and immune mediated thrombocytopenias, especially heparin-induced thrombocytopenia and application of biophysics to understand molecular mechanisms of antigenicity of endogenous proteins. He has published more than 400 papers on these topics.



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SHIRE SATELLITE SYMPOSIUM

Targeting ideal trough and peak levels in hemophilia A patients

V. Jimenez Yuste (Madrid, Spain)

Professor Víctor Jiménez Yuste, will review personalised care in the treatment of hemophila A to recognise the important advances in this area and to consider where improvements may be made in a sustainable way to optimise outcomes.
Using his clinical experience, he will provide input and guidance on the challenges and benefits of tailoring treatment to the individual patient.

Professor Víctor Jiménez Yuste is Head of Hematology Department at Hospital Universitario La Paz, Madrid, Spain and Associate Professor in the Hematology Department at the Autónoma University.
After his medical degree at Valladolid University, Dr. Jiménez Yuste specialised in hematology at La Paz University Hospital. He was awarded his PhD by the Autónoma University of Madrid where he completed his thesis studying hemophilia and HIV infection.
Professor Jiménez Yuste is a member of EAHAD's executive committee and a member of the executive committee of the Spanish Society of Thrombosis and Hemostasis.
His research interests include the management of inherited bleeding disorders, ITP and acquired hemophilia.
He is/ was Principal Investigator in more than 20 studies related to Haematology and and he has authored and co-authored more than 100 papers in various journals about these issues.

SHIRE SATELLITE SYMPOSIUM

Progressing patient care in Von Willebrand Disease

S. Susen (Lille, France)

Professor Sophie Susen, will review the current landscape of von Willebrand Disease (vWD), highlighting the importance of patient-centric care in vWD management and overcoming challenges to improve patient outcomes. She will discuss the importance of innovation and present strategies to elevate standards of care in vWD to engage key stakeholders in the vWD patient journey.

Professor Sophie Susen is Professor of Hematology at Lille Medical school (France) and head of the department of Hemostasis and Transfusion in Lille University Hospital. She has been a member of the expert-group for blood products (2008-2012) at the French regulatory agency (ANSM).

Her PhD and postdoctoral fellowship was based on the role of von Willebrand factor in the context of vascular injury.
Her main research activity focuses on the role of VWF and blood flow via different approaches.

She is the coordinator of the French National network on von Willebrand disease.
Professor Susen is also a board-member of the French Group in Thrombosis and Hemostasis and the Group on Perioperative Hemostasis.
She has been investigator in numerous clinical studies and author or co-author of more than 100 publications in this domain contributing to clinical investigation of new products.

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STATE OF
THE ART II

Staphylococcus aureus: master manipulator of the human hemostatic system

T. Vanassche
University Hospitals Leuven, LEUVEN, Belgium

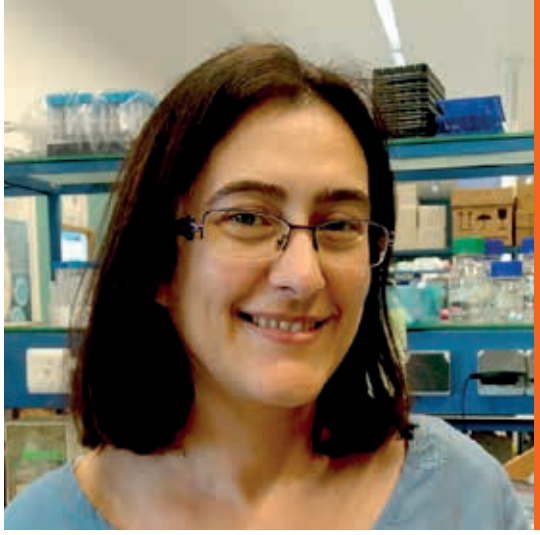
Staphylococcus aureus (S.aureus) is a frequent cause of severe infections. Although S.aureus colonizes up to 50% of the healthy population, it can cause devastating infections when it becomes invasive. As one of the most important sources of bacteremia and infective endocarditis, S.aureus is currently the most deadly infectious agent in the developed world.
Millions of years of co-evolution between S.aureus and the mammalian immune system have led to a delicate equilibrium, where the slightest imbalance can lead to either clearance of bacterial colonization or destruction of the host. It is still not clear how our immune system is able to control S.aureus, but it seems to rely more on innate than acquired immunity, as illustrated by the failed attempts to develop a protective vaccine.

In recent years, it has become clear that the coagulation system is a vital part of innate immunity. Invading pathogens or tissue damage trigger an inflammatory response and, in parallel, activate the coagulation cascade. Innate immune cells release several procoagulant molecules such as tissue factor and degrade natural anticoagulants. Activated neutrophils form neutrophil extracellular traps (NETs) by extruding their DNA in response to bacterial pathogens. Together with polyphosphates released by bacteria these NETs are potent activators of the contact activation pathway. Activation of the coagulation cascade will eventually lead to the generation of thrombin, which converts fibrinogen into insoluble fibrin strands and activates platelets, thereby forming a blood clot. This coagulum can seal off an infection, prevent further bacterial spreading, and serves as a scaffold for an inflammatory response to eliminate the intruder.

However, S.aureus is not only well-equiped to escape many of these immune defense mechanisms; but also uses the entanglement between immunity and vascular biology to its own advantage. Indeed, many virulence factors of S.aureus target the hemostatic system. S.aureus is able to initiate coagulation, to break down fibrin clots, to bind and activate platelets, and to interact with the endothelium and subendothelium.

In this talk, we will explain some of these intriguing mechanisms, and try to learn how we can use a better understanding of the deregulation of coagulation by S.aureus to design novel therapeutic strategies.

Dr. Vanassche earned a medical degree from the University of Leuven in Belgium, and trained in internal medicine and cardiology at the University Hospital in Leuven.
During his clinical cardiology fellowship, he completed a cardiovascular research fellowship at the Center for Molecular and Vascular Biology, University of Leuven, focusing on the interactions between infection, inflammation and coagulation. As part of his doctoral research, he studied how S. aureus dysregulates the host's coagulation to promote its virulence, and evaluated the potential of targeting coagulases as an adjunctive antibacterial strategy in in vitro models, in vivo models, and in a clinical trial.
In parallel, he was also involved in clinical trials in the field of thrombosis and vascular medicine.
After completing his doctoral research, he worked as a clinical research fellow at the Population Health Research Institute, McMaster University, in Hamilton, Canada under the supervision of Dr. J. Eikelboom and Dr. S.
Connolly.
After obtaining his cardiology license, Dr. Vanassche joined the department of Cardiovascular Sciences at University Hospitals Leuven, where he is involved in the intensive cardiac care unit, thrombosis and vascular medicine, and hypertension and cardiovascular prevention.



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STATE OF
THE ART II

Cardiac Device Infection: Incidence, diagnosis and treatment

L. Musumeci, C. Oury, P. Lancellotti
GIGA -ULG, LIEGE, Belgium

Cardiac device-related infections have increased in parallel to the increase in number of pacemakers, cardioverter defibrillators as well as prosthetic valves implantation. Infections can arise just after surgery, primary infections (within a week to one month post-surgery), or appear long after surgery, as secondary infections (after 6 months). Although incidence for pacemakers is rather low, 1 to 10 in 1000 device-year, for prosthetic valves it can reach 1 to 6 cases per 100 patients.
Pacemaker lead and/or prosthetic valve infection can cause cardiac device-related infective endocarditis (CDRIE) or prosthetic valve endocarditis (PVE). Pathophysiology of endocarditis may differ depending on the initial cause, e.g.arising from a primary or a secondary infection. In the case of early infection, bacteria start to grow at the site of the device, colonizing it, while in the case of late infection, the pathophysiology is similar to the native valve endocarditis (NVE), i.e.bacteria access the bloodstream through the mouth (dental procedure), skin (venous catheters or surgical procedure), urinary or gastrointestinal tracts. Once in bloodstream, bacteria may adhere to damaged endothelia and finally start the colonization process. At the site of bacteria colonization we can have thrombus formation, monocyte recruitment and inflammation, leading to what is called mature vegetation.
The type of bacteria colonizing the device depends on the timing of the infection, as well as on the procedure used for the implantation (open-heart surgery or the transcatheter procedure). For PVE, the most common colonizing bacteria is Staphylococcus aureus(50% incidence), and for the other half approximately 10% is due to Coagulase-negative staphylococci (CoNS), 20% to oral streptococci, 10% to other streptococci and 10% to enterococci; other microorganism accounting for <5%.
Since bacteria colonization of cardiac devices is difficult to fight, because of biofilm formation (bacteria in the biofilm are more resistant to the usual antibiotic doses), it is important to prevent infections by lowering the adherence of bacteria to the devices (special anti-fouling coatings) and by reducing bacteremia, for example by antibiotic prophylaxis for dental procedures.
Diagnosis of infective endocarditis is challenging, since the symptoms are not always well defined, ranging from acute sepsis to mild fever. Nevertheless, a definitive diagnosis can be reached using cardiac imaging together with blood culture results (Modified Duke Criteria). Whenever possible, the complete removal of the infected device and surrounding tissue is indicated, since antibiotic treatment is associated with increased risk of recurrence due to bacterial tolerance and antibiotic resistance.
In conclusion, preventive treatment pre and post device implantation has been suggested as a way to lower the risks of CDRIE. There are currently high expectations towards novel biomaterial that would lower not only the risk of bacteria adherence, but also the risk of thrombosis, since microthrombi on surfaces can attract more bacteria.

Lucia Musumeci has graduated in Chemistry and obtained a Master in Biochemistry in 2000 at the University of Naples Federico II, Italy. In 2005 she completed her PhD in Molecular Medicine at the University of Rome La Sapienza. During her PhD at the Sanford Burnham Prebys Institute in San Diego, CA she worked on projects focused on human and bacterial protein phosphatases. She has contributed to the field of immunology by discovering an important human genetic mutation, linked to autoimmune diseases, in the PTPN22 gene encoding for the protein tyrosine phosphatase Lyp1. During her postdoctoral studies at the University of Sydney she gained experience in human genetics discovering bogus SNPs in the dbSNP database. As postdoc at the University of Liege, under the supervision of Dr Cecile Oury, she has uncovered the role of DUSP3/VHR in platelets as an important regulator of collagen and CLEC-2 induced human platelet activation. Now she is Senior Scientist in the Cardiovascular Unit of the GIGA Research Institute in Belgium, collaborating with Cecile Oury and Patrizio Lancellotti on a project focused on the development of an antifouling coating for medical devices.

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STATE OF
THE ART II

Microbiota and Thrombosis

C. Reinhardt
Universitätsmedizin Mainz, MAINZ, Germany

The symbiotic gut microbiota plays pivotal roles in host physiology and the development of cardiovascular diseases, but microbiota-triggered pattern recognition signaling mechanisms impacting thrombosis are poorly defined. We found that germ-free and Toll-like receptor (Tlr) 2-deficient mice have reduced thrombus growth following carotid artery injury relative to conventionally raised controls. Platelet deposition to the carotid artery injury site in germ-free Tlr2-/- and germ-free wild-type (WT) mice was indistinguishable, but colonization with microbiota restored a significant difference in thrombus growth. We identified reduced plasma levels of von Willebrand Factor (VWF) and reduced VWF synthesis specifically in hepatic endothelial cells as a critical factor that is regulated by gut microbiota and determines reduced thrombus growth in Tlr2-/- mice. Static platelet aggregate formation on extracellular matrix was similarly reduced in germ-free WT, Tlr2-/-, and heterozygous Vwf+/- mice that are all characterized by a modest reduction in plasma VWF level. Moreover, administration of VWF rescued defective thrombus growth in Tlr2-/- mice in vivo. Thus, our experiments delineated an unexpected pathway, in which microbiota-triggered TLR2 signaling alters the synthesis of pro-adhesive VWF by the liver endothelium and favors platelet integrin-dependent thrombus growth.

Christoph Reinhardt (born 25.12.1978 in Reutlingen / Germany) studied Biochemistry at the University of Zurich / Switzerland (1998 - 2003). He earned his Ph.D. in Human Biology on “Analyses on Molecular Mechanisms of Activation of intravascular Tissue Factor” in the laboratory of Prof. Bernd Engelmann at the Ludwig-Maximilians-University Munich / Germany (2003 – 2007). From March 2007 to June 2010 Christoph Reinhardt received postdoctoral training in the laboratory of Prof. Fredrik Bäckhed at the Wallenberg Laboratory for Cardiovascular Research at the University of Gothenburg / Sweden, where he worked as an EU Marie Curie Fellow and was trained in germ-free mouse technology. Following his 3 years postdoctoral training, he joined he joined the Center for Thrombosis and Hemostasis in Mainz as a Junior Group Leader within the Professorship “Translational Research in Thrombosis and Hemostasis”. In December 2016 Dr. Reinhardt was appointed an assistant professorship on “Experimental Hemostasis and Gnotobiotics”. His recent work explores the role of commensal microbiota in arterial thrombosis and atherothrombosis.

ORAL PRESENTATIONS: CLINICAL & LABORATORY

Inherited antithrombin deficiency and thrombosis in childhood: data from a large multicentric cohort

C. Orlando[1], B. De la Morena-Barrio[2], M.E. De la Morena-Barrio[2], V. Vicente[2], J. Corral[2], K. Jochmans[1]

[1]Department of Haematology, Vrije Universiteit Brussel (VUB), Universitair Ziekenhuis Brussel, BRUSSELS, Belgium

[2]Servicio de Hematología y Oncología Médica, Centro Regional de Hemodonación, Universidad de Murcia, IMIB-Arrixaca, MURCIA, Spain

O01

CLINICAL & LABORATORY

BACKGROUND: Inherited antithrombin (AT) deficiency is a rare autosomal dominant disorder characterized by a decreased AT activity in plasma and a predisposition to recurrent venous thromboembolism. In contrast to adults, little is known about the presentation of the disorder in the pediatric population as available data are restricted to case reports or small patient cohorts.

AIM: The objective of our study was to investigate the thrombotic presentation of inherited AT deficiency in children (age ≤18), combining data from two patient cohorts from Belgium and Spain, resulting in the largest pediatric dataset on AT deficiency reported to date.

METHODS: This observational, retrospective study was performed on 968 patients of any age from 441 unrelated families with genetically confirmed AT deficiency. In patients where the first thrombotic event occurred before the age of 19, disease history was evaluated, collecting data concerning the site of (recurrent) venous and/or arterial thrombosis, possible risk factors (oral contraceptive use, pregnancy, complicated delivery, obesity, immobilization, infection, surgery and trauma) or other documented thrombophilia.

RESULTS: Seventy-three subjects (7.5%) developed thrombosis before 19 years of age. We observed two periods with a high prevalence of thrombosis: the neonatal period (<30 days, n=15) and adolescence (12-18y, n=49). In newborns, thrombosis often presented at unusual sites such as upper extremities, renal veins and cerebral veins. Half of these were idiopathic while in the other half transient provoking factors as traumatic delivery, infection, trauma or surgery were reported. During adolescence, thrombus localization (deep vein thrombosis and pulmonary embolism) and risk factors (oral contraceptives, surgery and pregnancy) were common to those seen in adults.
We noted a high frequency (n=13, 17.8%) of cerebral sinovenous thrombosis, mainly in the neonatal period (8/13).
Five patients suffered from arterial thrombosis of whom four in the neonatal period, while in two patients a venous thrombosis was present simultaneously.
There was a strikingly severe outcome with fatality in 6 cases (3 neonates) and severe disability in 3 cases, all neonates (psychomotor retardation, limb amputation and tetraplegia). One or more recurrent thrombotic events were observed in 24.6% of the patients.
From a molecular point of view, the subjects in this study predominantly showed type I AT deficiency (76.7%). From the 14 patients with type II AT deficiency, 6 carried the same mutation, p.Leu131Phe (Budapest III), affecting the Heparin Binding Site. Four of them were homozygous while the two heterozygous cases were also carrier of the Factor V Leiden mutation, either heterozygous or homozygous.
Considering the complete cohort of AT deficient subjects of any age, pediatric thrombosis was rare in type II HBS deficiency (8/223, 3.6%), in contrast to type I deficiency (56/604, 9.3%).

SUMMARY/CONCLUSIONS: The results of this observational, retrospective study show severe thrombotic events and a high recurrence rate in pediatric AT deficient patients. We suggest testing for AT deficiency in children from affected families, particularly in case of type I deficiency. Given the high prevalence of cerebral sinovenous thrombosis in the neonatal period, we recommend avoiding invasive procedures, like forceps or vacuum extraction, during delivery in women with known AT deficiency.

O02

CLINICAL & LABORATORY

Platelet-rich but not RBC-rich areas in stroke thrombi consist of dense fibrin structures, VWF and extracellular DNA

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

[1]Laboratory for Thrombosis Research, KU Leuven Campus Kulak, KORTRIJK, Belgium

[2]Department of Medical Imaging, AZ Groeninge, KORTRIJK, Belgium

[3]Department of Neurology, AZ Groeninge/ Antwerp University Hospital, KORTRIJK/ ANTWERP, Belgium

[4]Department of Medical Imaging / Department of Clinical Neuroscience, AZ Groeninge/ Karolinska Institutet, Stockholm, Sweden, KORTRIJK, Belgium

BACKGROUND: Acute ischemic stroke is one of the leading causes of death and disability worldwide. Despite the huge clinical, economical and social burden associated with ischemic stroke, only two strategies are currently available to remove the occluding thrombus and restore blood flow to the brain: (i) pharmacological thrombolysis using tissue plasminogen activator (t-PA) and (ii) mechanical removal of the thrombus via endovascular thrombectomy. However, more than half of the patients receiving t-PA do not respond to the therapy due to so called 't-PA resistance'. In addition, the thrombectomy procedure is not successful in 10-20 % of patients. The factors contributing to such therapy resistance are not known but are most likely linked to specific characteristics of the thrombus that is occluding the blood vessel in stroke patients.

AIMS: The aim of this study was to analyze ischemic stroke thrombi retrieved from patients treated with thrombectomy to better understand thrombus characteristics and structure.

METHODS: A total of 176 thrombi were collected from endovascular treated ischemic stroke patients at Groeninge Hospital (Kortrijk, Belgium). Fresh thrombi were fixed in 4 % paraformaldehyde, embedded in paraffin and cut into 5 µm sections. Histological analysis was performed using hematoxylin and eosin, martius scarlet blue staining, Feulgen staining, and both immunohistochemical and immunofluorescence analysis of von Willebrand factor (VWF), platelets (GPIb), fibrin, DNA and white blood cells (CD45). Multicolor immunofluorescent analysis was performed on a randomly selected subset of thrombi (n = 8).

RESULTS: Stroke thrombi are heterogenous. Nevertheless, histological analysis reveals common structural features. In general, stroke thrombi are composed of red blood cell (RBC)-rich areas, interspersed with platelet-rich areas. On a microstructural level, RBC-rich areas consist of red blood cells that are trapped within a relatively loose fibrin network in a honeycomb structure in which platelets and VWF are absent. In contrast to RBC-rich areas, platelet-rich zones consist of much denser fibrin structures that in addition are lined with VWF. These fibrin-VWF organizations are filled with platelets. White blood cells tend to accumulate on the boundaries between RBC-rich and platelet-rich areas. DNA networks are seen throughout the thrombus in some but not all thrombi and are particularly present in the platelet-rich areas and in the boundary zones.

CONCLUSION: We describe the complex microstructure of patient stroke thrombi. Fibrin and VWF align in dense platelet-rich structures, together with DNA networks that span throughout the thrombus. These observations corroborate our previous findings that VWF and DNA can contribute to t-PA resistance. Whether the dense platelet-rich zones that are interspersed within loosely arranged RBC areas influence efficiency of mechanical thrombus retrieval during thrombectomy is currently being investigated. The identification of these defined thrombus characteristics is crucial for further research to improve acute ischemic stroke therapy.

O03

CLINICAL & LABORATORY

Lupus anticoagulant hypoprothrombinemia syndrome: a paediatric case report

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INTRODUCTION: In a child with bleeding tendency, several etiologies are possible of which congenital disorders e.g. Von Willebrand disease and haemophilia, are the most frequent. An efficient work-up consisting of a thorough personal and familial history, physical examination and stepwise approach to laboratory testing is quintessential in making the right diagnosis. We present a case concerning a child with bleeding symptoms and focus on the diagnostic path leading to a rare but not to be missed disorder.

AIMS: To highlight the importance of lupus anticoagulant hypoprothrombinemia syndrome (LAHPS) in the work-up of bleeding disorders in children and to illustrate its diagnostic algorithm by a case-report.

METHODS/MATERIALS: Routine coagulation studies and lupus anticoagulant (LA) were performed by lupus sensitive Werfen reagents and analyser. Factor assays were performed with a different lupus sensitive reagent (Stago, method 1) and were repeated with LA insensitive reagent (Siemens, method 2). All coagulation tests were performed on 3.2% citrated plasma.

RESULTS: A 21-month old girl of Tibetan origin was admitted to the hospital due to atraumatic bruising. Clinical examination showed multiple ecchymosis spread across the whole body. History was unremarkable except for recent gastroenteritis. The initial laboratory work-up revealed a normal complete blood count but markedly prolonged APTT of 80.8s (reference value: 24.0-39.2s) and low PT of 47% (reference value: 76.0-103.8%). Analysis for LA was positive with no normalization during LA confirmation studies. Evaluation of the clotting factors is visually represented in Table 1. Mixing studies showed normalization of FII.
Based on the history and laboratory work-up, a diagnosis of acquired bleeding disorder LAHPS was made. Immunosuppressive therapy was started; quick and adequate clinical and laboratory response followed.
LAHPS is a rare bleeding disorder, characterized not only by the presence of LA but also by non-neutralizing antibodies to prothrombin leading to rapid clearance of the resulting antigen-antibody complexes. Ultimately this causes hypoprothrombinemia, explaining the bleeding tendency in affected patients. To date, two forms of LAHPS are described according to the presumed trigger: autoimmune disease (LAHPS-AI) and infection (LAHPS-VI).

CONCLUSIONS: We recommend to consider LAHPS in the differential diagnosis of bleeding as this presents a challenge for diagnostic and therapeutic management. The diagnostic difficulty in LAHPS lies within the presence of two antibodies: LA which interferes with certain coagulation tests, primarily when a lupus sensitive reagent is used, and non-neutralizing antibodies against prothrombin leading to in vivo effects. In the work-up of a child with bleeding symptoms accompanied by a prolonged APTT and PT, it is important to think of LA testing as well as factor assays, especially FII. When the diagnosis of LAHPS is made, directed search for a trigger i.e. infection or autoimmune disease should be performed. Immunosuppressants are a valid therapeutic option and their effects must be monitored through FII levels.

Clotting factor	Method 1 % normal activity	Method 2 % normal activity	Reference range (%)
PT-based factors			
FII	13	29	49.4 – 130.0
FV	110	86	73.2 – 188.1
FVII	120	120	47.8 – 124.2
FX	60	-	59.7 – 152.8
APTT-based factors	**LA sensitive reagent**	**LA insensitive reagent**	
FVIII	26	77	59.0 – 167.0
FIX	20	80	52.6 – 128.9
FXI	31	-	58.0 – 154.0
FXII	24	-	50.0 – 200.0

Table 1: Evaluation of clotting factors

The presence of anti-ADAMTS13 autoantibodies does not change ADAMTS13 antigen levels measured by an in house developed ADAMTS13 antigen ELISA

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O04

CLINICAL & LABORATORY

BACKGROUND: Immune-mediated thrombotic thrombocytopenic purpura (iTTP) is characterized by the presence of autoantibodies (autoAbs) against ADAMTS13. ADAMTS13 is a metalloprotease which consists of multiple domains: metalloprotease (M), disintegrin-like (D), cysteine-rich (C), spacer (S), 8 thrombospondin type 1 repeats (T1-8) and 2 CUB domains. ADAMTS13 antigen (Ag) levels are moderately to severely reduced in most iTTP patients, indicating that antibody-mediated clearance is the major pathogenic mechanism. In addition, a severe reduction in ADAMTS13 Ag has recently been shown to be associated with a higher mortality rate.

AIMS: We aimed at validating our in-house ADAMTS13 Ag ELISA in terms of sensitivity, reproducibility and background and investigated whether ADAMTS13 Ag determination is influenced by the presence of anti-ADAMTS13 autoAbs.

METHODS: In our in-house ADAMTS13 Ag ELISA, ADAMTS13 is captured by the anti-ADAMTS13 monoclonal antibody (mAb) 3H9 (anti-M) and detected using a mixture of biotinylated anti-ADAMTS13 mAbs 17G2 (anti-CUB1) and 19H4 (anti-T8) and HRP-labeled streptavidin. Detection limit (DL, 3xSD above mean of blank) and quantification limit (QL, minimum measurable concentration with coefficient of variation (CV) < 20%) were determined by performing six replicates of serial dilutions of normal human plasma (NHP), in six assays. The influence of iTTP autoAbs on ADAMTS13 Ag determination was tested using 20 purified iTTP IgG samples. First, NHP was pre-incubated with the purified iTTP IgGs and ADAMTS13 Ag levels in this mixture were determined. Next, the presence of overlapping epitopes between the assay mAbs (3H9, 17G2 or 19H4) and iTTP IgGs were studied in a competition ELISA on coated recombinant human (rh)ADAMTS13.

RESULTS: The DL of our in-house ADAMTS13 Ag ELISA is 2.52 ng/mL (1/396.9 NHP dilution) and the QL is 7.00 ng/mL (1/142.9 NHP dilution). The CV for each NHP dilution (1/6.7; 1/11.1; 1/18.5; 1/31.0; 1/51.4; 1/85.7; 1/142.9) was 3.0%, 3.5%, 3.1%, 3.0%, 2.6%, 2.3% and 3.6%, respectively. Additionally, the ADAMTS13 Ag measurements in NHP were similar with or without pre-incubation of purified iTTP IgGs, indicating that iTTP autoAbs do not interfere with the ADAMTS13 Ag determination. Moreover, a competition ELISA in which the binding of the assay mAbs (3H9, 17G2 and 19H4) to rhADAMTS13 in the presence of purified iTTP IgGs was tested, showed that none of the purified iTTP IgGs competed with 3H9 or 17G2 for rhADAMTS13 binding, and only one iTTP IgG sample competed with 19H4 for rhADAMTS13 binding.

SUMMARY/CONCLUSION: Our in-house ADAMTS13 Ag ELISA has been validated in terms of sensitivity, reproducibility and background. Additionally, we showed that the presence of anti-ADAMTS13 autoantibodies does not change ADAMTS13 antigen levels. The high affinity of our murine anti-ADAMTS13 mAbs for plasma ADAMTS13 possibly explains why the presence of low(er) affinity patient anti-ADAMTS13 autoAbs do not interfere in the ELISA.

O05

CLINICAL & LABORATORY

The concentration of GP1ba magnetic particles is a critical parameter occasionally responsible for poor reproducibility of the VWF:RCo with a chemiluminescent immunoassay

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BACKGROUND/INTRODUCTION: A sensitive chemiluminescent immunoassay using magnetic particles coated with GP1bα has been validated on an automated analyser, the AcuStar® (Werfen), to measure von Willebrand factor ristocetin cofactor activity (VWF:RCo). Occasional unexplained variability in the controls of this assay has prompted a further evaluation of the kits.

AIM: To determine whether variations in the amount of GP1bα particles in the cartridges impair the reproducibility of the assay.

METHODS/MATERIALS: VWF:RCo levels were measured using an AcuStar® according to the manufacturer's instructions. The GP1bα particle suspension in the cartridge was controlled by spectrophotometry. The impact of a reduced concentration of GP1bα particles on VWF:RCo measurement was determined by replacing the particles in the cartridge with buffer. For this purpose, an aliquot of 200 µl was removed from the cartridge and its absorbance was measured at 450 nm. After centrifugation, the supernatant of the aliquot was reinserted into the cartridge while the pellet with GP1bα particles was discarded. This manipulation was repeated 6 times for each cartridge.

RESULTS: Optical density of various kits within a given batch.
A variation of up to 29% in the optical density of GP1bα particles was noticed between the cartridges .
Impact of a reduced GP1bα particle concentration on the measurement of VWF:RCo levels.
A linear correlation was observed between the concentration of GP1bα particles and the levels of VWF:RCo measured in the control pool plasma (Fig. 1A). A 10% reduction in GP1bα particle concentration resulted in a 7.3% decrease in VWF:RCo levels. Accordingly, the reduced levels of GP1bα particles in some kits may have biased the assays.
Our data did not allow us to determine whether the variability in GP1bα particle concentration was due to an insufficient filling of the kits or to an incomplete resuspension of the GP1bα particles. However, anecdotal observations suggest that the latter occurs. Indeed, in one kit we recently observed an almost colourless GP1bα particle suspension. Unexpectedly, a ring of GP1bα beads was found attached to the cover of the vial (Fig. 1B). Following another similar observation, the kit was turned upside down and subsequently thoroughly shaken. This additional mixing successfully brought the GP1bα particles into suspension.

CONCLUSIONS: Our observations indicate that the resuspension of the GP1bα particles is a critical step in the preparation of the cartridges used to measure VWF:RCo levels with an AcuStar®. The control of GP1bα particle concentration by spectrophotometry should help laboratories to identify the cause of an unexpected assay variability.

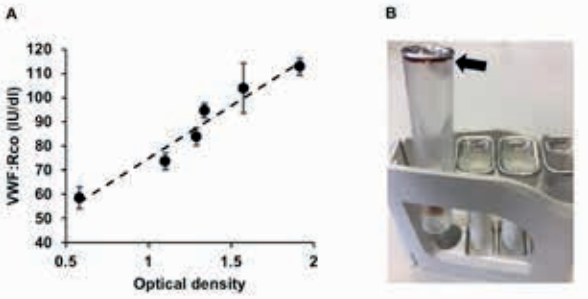

Figure 1. GP1bα magnetic particles and the VWF:RCo assay
A. Measurement of VWF:RCo as a function of the concentration of GP1bα magnetic particles. B. A ring of GP1bα particles (see arrow) remains attached to the cover of the vial after the preparation of the cartridge.

ORAL PRESENTATIONS: BASIC RESEARCH

Open ADAMTS13 conformation in immune-mediated thrombotic thrombocytopenic purpura is induced by anti-ADAMTS13 autoantibodies

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O06

BASIC RESEARCH

BACKGROUND: Deficient ADAMTS13 activity (TS13:act <10%) induced by anti-ADAMTS13 autoantibodies (autoAbs) causes immune-mediated thrombotic thrombocytopenic purpura (iTTP). Recently we showed that an open ADAMTS13 conformation is characteristic for acute iTTP patients, while folded ADAMTS13 was found in 78% of iTTP patients in remission with an TS13:act >50%. However, also iTTP patients in remission with a persistent (<10%) or moderately restored (10-50%) TS13:act have been described, but their ADAMTS13 conformation is unknown. Intriguingly, the factor responsible for inducing open ADAMTS13 in iTTP patients remains elusive. Identifying the cause of open ADAMTS13 in iTTP will help better understand the pathophysiology of iTTP and could help appreciate the prognosis and better manage the prevention of subsequent relapses.

AIM: Determine ADAMTS13 conformation in plasma of iTTP patients during acute TTP and remission when TS13:act is <10%, moderately restored (10-50%) or >50% and investigate if anti-ADAMTS13 autoAbs induce conformational changes in ADAMTS13.

METHODS: TS13:act was determined in 120 iTTP plasma samples from 4 different centers (Marseille, Milan, Budapest, Utrecht). Samples were categorized according to the presence of clinical symptoms (acute versus remission) and their TS13:act in remission (>50%, 10-50%,<10%). Next, ADAMTS13 conformation was determined in all samples using our ADAMTS13 conformation ELISA. Additionally, presence of anti-ADAMTS13 autoAbs was also determined via ELISA. Finally, IgG's from 18 acute iTTP plasma samples were purified and added to folded ADAMTS13 from healthy donor (HD) plasma to test whether iTTP IgGs are able to induce the open HD ADAMTS13 conformation.

RESULTS: Of the 120 iTTP plasma samples, 46 were obtained during the acute (clinical signs present) and 74 during the remission phase (clinical signs absent). Further subdividing remission samples showed that TS13:act was >50% in 41, 10-50% in 14 and <10% in 19 samples. ADAMTS13 was open in 98% (45/46) of the acute samples and folded in 71% (29/41) of the remission samples with TS13:act >50%, confirming our previous results. Interestingly, ADAMTS13 was open in 93% and 89% of remission samples with TS13:act 10-50% and <10%, respectively (chi square, P<0.0001). Since anti-ADAMTS13 autoAbs influence TS13:act in iTTP patients, we next could demonstrate that open ADAMTS13 conformation was linked with presence of anti-ADAMTS13 autoAbs (chi square, P<0.0001) suggesting that anti-ADAMTS13 autoAbs could be a factor able to induce an open ADAMTS13 conformation in iTTP patients. To further test this hypothesis, we purified IgGs from 18 acute iTTP plasmas with open ADAMTS13 and added them to HD plasma containing closed ADAMTS13, where 14 of the 18 patient IgG pools (78%) did induce the open conformation in HD ADAMTS13, indicating that patient anti-ADAMTS13 autoAbs indeed can induce conformational changes in ADAMTS13.

CONCLUSION: We show that ADAMTS13 is in the open conformation not only in iTTP patient plasma during the acute phase but also in remission when TS13:act is <10% or 10-50%. Hence, the presence of open ADAMTS13 in those remission patients indicates that the underlying pathophysiology is still ongoing, emphasizing the need for a close monitoring of those patients. In addition, anti-ADAMTS13 autoAbs were identified as a factor responsible for the change in conformation in ADAMTS13 in iTTP.

O07

BASIC RESEARCH

The VWF-GPIb interaction mediates thrombo-inflammation in experimental stroke via recruitment of monocytes, neutrophils and T-cells to the brain

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INTRODUCTION: Von Willebrand factor (VWF) is crucial for hemostasis by capturing platelets at sites of vascular damage. Recently, also an inflammatory role for VWF has emerged. Previously, we and others have shown that mice deficient in VWF are protected from ischemic stroke. Intriguingly, mainly VWF-mediated platelet adhesion and not VWF-mediated platelet aggregation was found to be detrimental in the acute phase of ischemic stroke. Hence, both platelets and VWF contribute to stroke progression in a way that is not strictly related to thrombus formation, but most likely also involves an acute inflammatory component. However, how VWF mediates thrombo-inflammatory ischemic stroke brain damage is currently unclear.

AIM: To investigate the potential inflammatory role of the VWF A1-platelet GPIb interaction in the ischemic stroke brain.

METHODS: VWF knockout (KO) and wildtype (WT) mice were subjected to 60min of cerebral ischemia, followed by 23h of reperfusion. To study the role of the VWF - platelet GPIb interaction, mice were treated with a nanobody specifically blocking the GPIb-binding site in the VWF A1 domain (KB-VWF-006bi) or a control nanobody (KB-VWF-004bv), 1h after stroke onset. Twenty-four hours after stroke, mice were neurologically scored, cerebral infarct sizes were measured, and flow cytometric and immunohistological analysis of immune cell recruitment to the brain was performed.

RESULTS: In a first set of experiments, the acute cerebral immune response after stroke was compared between VWF WT and KO mice by flow cytometric analysis of single cell suspensions prepared from brain tissue. Twenty-four hours after stroke, the amount of recruited white blood cells in the ipsilesional hemisphere of VWF KO mice was 2-times less as compared to VWF WT mice (p=0.02). Upon further analysis, we found two-fold less proinflammatory monocytes (p=0.03), five-fold less neutrophils (p=0.01) and four-fold less T-cells (p=0.002) in the ischemic brain of VWF KO mice compared to VWF WT mice. Interestingly, immunohistological analysis revealed that most of the recruited neutrophils and T-cells were located within the microcirculation of the ipsilesional hemisphere.
Next, we investigated whether pharmacological inhibition of VWF-mediated platelet adhesion could reduce immune cell recruitment to the brain and thereby protect mice from ischemic stroke brain damage. To test this, we utilized a nanobody specifically inhibiting the interaction between the VWF A1 domain and the platelet receptor GPIb. Interestingly, inhibition of the VWF-A1–platelet GPIb interaction 1h after stroke onset led to a reduced recruitment of white blood cells to the ischemic brain compared to control treated mice. More specifically, recruitment of proinflammatory monocytes was two-fold less (p=0.02), neutrophil recruitment was five-fold less (p=0.0003) and T-cell recruitment was two-fold less (p=0.02) in VWF A1 nanobody treated mice. Importantly, this reduced recruitment of white blood cells to the ipsilesional hemisphere was accompanied by an improved neurological outcome (p=0.02) and reduced cerebral infarct sizes (p=0.001).

CONCLUSIONS: Inhibition of the interaction between VWF and platelet GPIb reduces the pro-inflammatory effects of VWF in experimental stroke, revealing an inflammatory component of the VWF-GPIb interaction in the ischemic brain. This puts forward inhibition of the VWF-GPIb interaction as a promising strategy to reduce thrombo-inflammation in ischemic stroke.

The differential role of platelets and fibrin in adhesion on inflamed and damaged valves in a new mouse model of early staphylococcus aureus endocarditis

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O08

BASIC RESEARCH

BACKGROUND/INTRODUCTION: In no other disease the interplay between bacteria and coagulation is so crucial as in infective endocarditis (IE). Although *Staphylococcus aureus(S. aureus)* possess a variety of factors that enables adherence to the endothelium, how S. aureusadheres to the cardiac valves remains undetermined. Furthermore, there is an unmet need in preventing IE, which is mainly due to the fact that previous animal models did not allow sufficiently detailed insights in the early steps of this disease.

AIMS: Therefore, this study aims to determine the role of the coagulation system in adherence of *S. aureus* to the cardiac valves in a clinically relevant mouse model.
Methods/Materials: By using a new mouse model, we could look at bacterial adhesion to the aortic valve. To this end, we intravenously injected Texas red labeledS. aureusin C57BL/6 mice after which a catheter was introduced in the carotid artery and advanced beyond the aortic valve. Subsequently, this catheter was used to locally stimulate or damage the endothelium. Afterwards, the catheter was removed and mice were immediately sacrificed. Adherence was determined by analyzing cryosections with confocal microscopy and quantifying adhesion by Imaris. To determine which coagulation specific factors are involved in adherence to damaged and inflamed valves, we conducted electron and fluorescent (A-546 fibrinogen, A-649 anti-GPIb antibody for platelets, isolectine B4 A-594 for endothelium) microscopy. The role of platelets in S. aureusadherence to damaged and inflamed valves was addressed by injecting mice with a platelet depletion antibody (anti-GPIb) or a control (isotope antibody) one hour before surgery. Finally, we injected a Srta mutant of S. aureusto investigate if cell-wall anchored proteins allow *S. aureus* to adhere to the valve.

RESULTS: Electron microscopy images of inflamed aortic valves revealed the presence of platelets and fibrin in an early vegetation. Additionally, fluorescent images showed a layer of fibrin on damaged valves, whereas on inflamed valves platelets were more abundant. Furthermore, the role of platelets in the inflammatory model was confirmed by platelet depletion experiments. In platelet depleted mice, *S. aureus* adhered significantly (p=0.0003) less on inflamed valves (platelet depletion: log(1.514±0.5834), control: log(2.857±0.7357)), whereas on damaged valves (log(2.672±0.4406)) adhesion was not significantly reduced (p=0.547) in comparison with the control (log(2.861±0.6748)). Injection of the Srta mutant revealed that adhesion on damaged valves is mediated by cell-wall anchored proteins (log(2.221±0.4785), control: log(2.792±0.1842), p=0.0422)), in contrast to inflamed valves (log(3.277±0.7867), control: log(3.365±0.3363), p=0.7890)).

SUMMARY/CONCLUSIONS: Adhesion on inflamed valves is mediated by platelets, in contrast to damaged valves where fibrin most likely plays a role. In the inflamed model, adhesion to platelets and thereby to the endothelium is not mediated by proteins on the cell wall of *S. aureus*. The exact factors that allows *S. aureus* to adhere to platelets on inflamed valves will be further addressed. In conclusion, these results reveal that adhesion occurs differently on inflamed and damaged valves, suggesting that anti-adherence therapy will be distinct for these two populations.

O09

BASIC RESEARCH

Visualizing dense granule formation in megakaryocytes using advanced microscopy and its application for Hermansky-Pudlak syndrome

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BACKGROUND: Hermansky-Pudlak syndrome (HPS) is a rare inherited platelet disorder characterized by oculocutaneous albinism and bleeding diathesis due to a dense storage pool defect (SPD). Autosomal recessive variants causing HPS have been identified in ten genes (*HPS1-10*), all affecting components of the endo/lysosomal lineage. Diagnosis of such SPDs has been mainly limited to platelet electron microscopy and ATP secretion assays. With the emergence of super resolution microscopy, labeling and imaging small platelet granules (150-500 nm) has become feasible. We will exploit the possibilities of this technology by also assaying megakaryocytes (MKs), thus modelling the complete process of granule biogenesis during platelet formation. HPS patients will be included in the analysis to reach a better understanding of important players involved in granulopoiesis.

AIMS: In depth characterization of platelet and MK granules by setting up advanced microscopy techniques for healthy controls and applying these to study the granule defect in HPS.

METHODS: The high-throughput sequencing gene panel 'ThromboGenomics' was used to diagnose HPS patients. Different platelet assays were performed to characterize their SPD including electron microscopy, flow cytometry for alpha and dense granule markers after platelet activation with different agonists, mepacrine assays, ATP secretion tests and structured illumination microscopy (SIM) labelling alpha and dense granules. Also, CD34+ hematopoietic stem cells were isolated from peripheral blood and differentiated into MKs for healthy controls and an HPS patient. Different compartments of the endosomal system were visualized using 3D confocal microscopy and SIM.

RESULTS: SIM and other advanced microcopy techniques were optimized to study alpha and dense granule formation during megakaryopoiesis by testing different granule-specific markers. Next, the technology was applied for an HPS patient with a homozygous start loss variant in *HPS6*. The patient's platelets showed abnormal dense granule cores, reduced surface levels of CD63 (but not CD62P) after platelet activation, lower levels of mepacrine uptake and release and an absent ATP secretion. SIM demonstrated a decreased number of CD63+ vesicles, together showing that the patient has a dense SPD with reduced counts and defective release of both membrane protein and cargo. Further, to model the vesicular compartments in MKs via imaging, cell size was considered, as well as the number of nuclei, by dividing the cells into ploidy groups (N=1, N=2, N=4-8). For healthy MKs, a linear increase in vesicle number was observed with ploidy and the transport of dense granule (and lysosome) membrane proteins via the endosomal system was low, indicating that granule maturation is a highly specific process. Immunostaining of MKs of the HPS6 patient revealed a decrease in dense granule (and lysosome) precursor vesicles, as well as early endosomes. A slight reduction in late endosomes was also found, while transport vesicles were normal. These preliminary findings indicate that dense granule formation is aberrant in HPS patients and that the early endosomes might be an unexpected player in the support of dense granule formation.

SUMMARY: Advanced microscopy has provided novel insights on physiological granule biogenesis and the defects in HPS, demonstrating the feasibility and power of using such state-of-art imaging platforms to study granule formation during megakaryopoiesis.

P2X1 deficiency causes massive intestinal bleeding along with enhanced neutrophil-dependent thrombosis in colitis

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O10

BASIC RESEARCH

BACKGROUN/INTRODUCTION: Hemostatic imbalance often accompanies acute and chronic inflammatory disease. Beyond complex interactions between platelets, leukocytes and endothelia, it has recently been reported that red blood cells (RBCs) also contribute to thrombus formation. In mice, a high hematocrit was associated with increased thrombosis while anemia led to prolonged tail bleeding time. However, whether this phenomenon would also apply to the inflammatory setting remains unknown. Yet, this can have important clinical implications since in pathologies such as inflammatory bowel diseases (IBD), anemic patients have a high risk of thrombosis.
We previously showed that P2X1 ion channel expressed on both platelets and neutrophils is instrumental in preserving vascular integrity in intestinal inflammation. Indeed, in a model of acute colitis, P2X1-/- mice develop anemia due to massive bleeding.

AIMS: To determine if and how anemia that develops under intestinal inflammatory conditions can lead to a prothrombotic tendency.

METHODS/MATERIALS: We used P2X1$^{-/-}$ mice in a model of acute colitis induced by oral administration of dextran sodium sulfate (DSS). Intestinal bleeding score was determined and anemia was characterized. Blood neutrophil count and plasma levels of G-CSF were analyzed. Extra-intestinal thrombosis was assessed using a laser-induced injury model of thrombosis in cremaster muscle arterioles. Accumulation of platelets, neutrophils and fibrin generation were recorded in real-time.

RESULTS: P2X1$^{-/-}$ mice showed more intestinal bleeding than wild-type (WT) mice, which resulted in anemia characterized by lower RBC count, hematocrit and hemoglobin concentration. Mean corpuscular volume (MCV) and red cell distribution width (RDW) were increased in P2X1$^{-/-}$ mice compared to wild-type (WT) mice. Spleens of P2X1$^{-/-}$ mice were enlarged and contained higher fraction of immature erythroblasts, indicative of regenerative anemia. Strikingly, P2X1$^{-/-}$ mice exhibited higher neutrophil count that was due to increased G-CSF levels. In our extra-intestinal thrombosis model, DSS-treated P2X1$^{-/-}$ mice showed a strong increase of neutrophil accumulation at the site of arteriolar wall injury. Fibrin generation was reinforced at the site of injury despite impaired platelet recruitment in the growing thrombi.

SUMMARY/CONCLUSIONS: In intestinal inflammation, anemia can be associated with a prothrombotic tendency. In contrast to classical non-inflammatory thrombosis, extra-intestinal thrombi are enriched in neutrophils and contain less platelets and RBCs. This process may be driven by G-CSF-dependent neutrophil mobilization.

ABSTRACTS POSTERS

P01

ABSTRACTS POSTERS

Evaluation of the cobas t 711 coagulation analyzer: a single center experience

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BACKGROUND: The cobas t 711 is a novel, fully automated coagulation analyzer from Roche Diagnostics. The first parameter release includes routine coagulation tests (APTT, PT, Fibrinogen and D-Dimer) and measurement of antithrombin (AT) activity.

AIMS: We evaluated the performance characteristics of the routine coagulation parameters (+AT) and we performed a method comparison study with the ACL TOP700/500 instruments (Werfen). We assessed FVIII- and lupus-sensitivity of the different APTT reagents ('APTT', 'APTT Screen' and 'APTT Lupus') and FVII-sensitivity of the PT reagent.

METHODS: Between-run variation was assessed by running internal quality controls twice daily during 10 days. Within-run variation was determined on 10 repeat measurements on normal and abnormal pooled plasma. Method comparison with ACL TOP700/500 was performed on left-over samples from daily routine and, for antithrombin, supplemented by samples from genetically confirmed AT deficient patients. Reagents used on ACL TOP700/500 were APTT-SP, PT Recombiplastin 2G, Q.F.A. Thrombin and D-Dimer HS500 (Werfen) and Innovance Antithrombin (Siemens). Factor sensitivity was assessed with dilution series of normal pooled plasma in factor deficient plasma of the factor of interest. For FVIII-sensitivity, APTT was measured with the different APTT reagents on cobas t 711 and with APTT-SP on ACL TOP700. For FVII-sensitivity, we measured the PT on both cobas t 711 and ACL TOP700 with their respective PT reagent. Lupus sensitivity was assessed by measuring APTT on samples from 10 patients positive for lupus anticoagulant.

RESULTS: Between-run variations fell within the specifications provided by the manufacturer, except for fibrinogen and antithrombin (normal control). However, all were <7%.
Within-run precision was excellent for all parameters (<2%), except for D-Dimer. This higher CV (6.1%) can be explained by the high concentration of D-Dimer in the plasma pool (i.e. ~4200 ng/mL FEU).
Method comparison showed good agreement between 'APTT Screen' on cobas t 711 with APTT-SP, while 'APTT' and 'APTT Lupus' did not. A possible explanation could be the use of silica as activator in both 'APTT Screen' and APTT-SP. PT results (% and INR) on cobas t 711 showed good correlation with PT Recombiplastin 2G. Fibrinogen values correlated well in the normal range but tended to be lower in the higher range when compared to fibrinogen values on TOP700/500. For D-Dimer, 8 samples with positive result on ACL TOP700/500 had a normal result (<500 ng/mL FEU) on cobas t 711. None of these patients suffered from venous thromboembolism.
There was an overall good agreement between antithrombin activity on both systems, except for AT deficient samples. Some samples from type II AT deficient patients yielded normal results, a known shortcoming of anti-IIa based assays, as the one on cobas t 711.
Off the three different APTT reagents, 'APTT Screen' was the most sensitive to FVIII while only 'APTT Lupus' was sensitive to Lupus Anticoagulant. Factor FVII-sensitivity of PT on cobas t 711 was identical to PT Recombiplastin 2G.

SUMMARY/CONCLUSION: All evaluated assays on the cobas t 711 showed good analytical and clinical performance. The differences in APTT and antithrombin observed in the method comparison can be attributed to reagent-specific characteristics like activator (for APTT) and analysis method (for AT).

P02

A challenging case of acquired hemophilia manifesting as hematuria

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CASE PRESENTATION: A 69 year-old female with a history of stroke and hypertension presented to the emergency department with acute abdominal pain and macroscopic hematuria. The patient consulted her general practitioner two days prior admission for the same motives and was prescribed antibiotics (ciprofloxacine) to treat a urologic infection. She was routinely taking aspirin for stroke prevention.

INVESTIGATION: Upon admission, the physical examination was unremarkable, aside from the abdominal pain.Initial laboratory workup showed signs of inflammatory syndrome. Leucocytes counts (predominantly neutrophils) were elevated. Fibrinogen levels were high and CRP was elevated as well. Iron levels were low but no anemia was found. aPTT was moderately prolonged (37 sec; local reference interval: 20 – 35 sec).

Urology examination revealed urethritis and pyelonephritis. The patient was discharged a few days later with a urinary catheter and antibiotic treatment.
Persistent hematuria led the patient to come back to the hospital 3 weeks later. This time, she presented with large bruises and anemia. A significantly prolonged aPTT (43.2 sec) with a normal PT (10,7 sec) were found leading to thorough hemostasis laboratory investigations. Coagulation factors FVIII and FIX, and von Willebrand Factor were measured: only FVIII:c levels were dramatically reduced to 2%. A mixing test was performed: the Rosner index was 18.8%, evocative of the presence of an inhibitor. The specific FVIII inhibitor assay, the Bethesda assay, using the Nijmegen modification, reported 10.8 Bethesda Unit, confirming the diagnosis of acquired hemophilia.
Aspirin treatment was stopped, while immunomodulatory therapy with methylpredinosolone (1 mg per kg once daily) and cyclophosphamide (1.5 mg per kg) was initiated. aPTT and FVIII:c steadily normalized in the following weeks. FVIII:c increased to 30% by week 4 and normal levels were achieved by week 6 of treatment.

DISCUSSION: Acquired hemophilia is a rare bleeding disorder, caused by autoantibodies against specific coagulation factor, generally FVIII. These inhibitors may develop with malignancy or autoimmune disease. Acquired hemophilia occurs more frequently in the elderly.
In this context of urinary infection, hematuria was primarily considered as a consequence of the infection and the possibility of acquired hemophilia was overlooked despite the abnormal aPTT. This led to delayed diagnosis and appropriate treatment, and thus increased the risk of severe bleeding manifestation.
In our patient, the development of the anti-FVIII inhibitors most likely resulted from a tumoral process, as a pulmonary adenocarcinoma was discovered later on during the follow up. A lung biopsy was performed using recombinant activated Factor VII (Novoseven®) to maintain hemostasis during this invasive procedure, without hemorrhagic complication.

CONCLUSIONS: Even in the absence of “typical” bleeding signs, an isolated prolongation of aPTT should always be further investigated to exclude the risk of acquired hemophilia. In this case, at her first admission, our patient did not present anemia nor bleeding signs (no bruising), other than the hematuria that could have resulted solely from her urinary infection.
This case should raise awareness on atypical, mild, presentation of acquired hemophilia.

P03

Prevalence of heterozygous type 2N Von Willebrand disease mutations in a Belgian survey

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INTRODUCTION: Von Willebrand Disease (VWD) is an inherited bleeding disorder caused by reduction (type 1), dysfunction (type 2) or absence (type 3) of Von Willebrand Factor (VWF). VWD type 2N is a qualitative variant in which binding of VWF to Factor VIII is decreased leading to a mild haemophilia phenotype. This recessive condition is caused by a homozygous or compound heterozygous mutation located in the D’/D3 domain (including exon 18-28) of VWF. Most frequent well-documented causal mutations of VWD type 2N are reported within exon 19, 20 and 21. The prevalence of type 2N VWD is very low in the general population (%).

AIMS: The prevalence of the most common heterozygous type 2N VWD mutations within the Belgian population.

METHODS: 851 blood samples were collected anonymously by three university hospitals throughout Belgium; UZ Antwerp, UZ Brussel and CHU Liège from patients not referred for bleeding-related problems. Sanger sequencing was performed on exon 19, 20 and 21, including intron/exon boundaries.

RESULTS: A heterozygous variant of p.R816W (exon19) and p.R854Q (exon 20) was found in respectively 1/851 (0.12%) and 8/851 (0.94%) of all analyzed individuals. Variant p.R924Q (exon 21) variously described as type 1, 2N and as polymorphism was found in 27/851 (3.17%) individuals.

CONCLUSIONS: These results show a prevalence of heterozygous mutations of type 2N VWD around 1% of the Belgian population (p.R816W, p.R854Q). The prevalence of the p.R854Q variant with ~1% is in accordance with previous estimations made by Casonato et al. in Italy and by Eikenboom et al. in the Netherlands.
p.R924Q mutation variously described as type 1, 2N or just a polymorphism has a higher prevalence ~3% but clinical significance is unclear. With the prevalence of 1% for the type 2N allele type 2N VWD has to be kept in mind whenever the diagnosis of haemophilia A is suspected.

P04

Differences in FVIII measurements of plasma spiked with AFSTYLA® generated with one-stage clotting assay using different commercial APTT reagents compared with chromogenic method

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BACKGROUND: The APTT based One-Stage Clotting assay (OSA) is the more routinely used assay for measuring clotting factors. Recently the Chromogenic Substrate assay (ChS) has been proposed as the most accurate potency assignment for the novel recombinant FVIII, single chain AFSTYLA® (CSL Behring).

AIM: To identify the most appropriate assay for monitoring of patients treated with AFSTYLA® by comparing results of FVIII:C using ChS and OSA with different commercial activators.

METHODS: Measurements of FVIII:C were done on depleted plasma samples spiked with different concentrations of AFSTYLA® (0.09-0.8U/ml) provided by the Blood Center of Wisconsin. For the potency assignment the Chromogenic assay was used. These were analyzed using ChS: Biophen FVIII:C (Hyphen Biomed) and OSA: Silica based STA-PTTA (Stago), TriniCLOT aPTT (Tcoag); Kaolin based STA-CK-Prest (Stago), Pathromtin SL (Siemens); and Ellagic acid based Actin FS (Siemens), Synthafax (IL). All assays used Factor VIII deficient plasma (Siemens) and were performed on STA-C analyzer (Stago).

RESULTS: Discrepancies were observed between OSA and ChS. Across all concentrations, the accuracy (observed concentration vs theoretical spiked concentration) ranged from 40-122% for the OSA and 100-123% for the ChS. Ratios for OSA/ChS of 0.33-1.0 were observed across different OSA activators and concentrations. Divergence of results increased by increased concentration while the most comparable results were seen at 0.09U/ml AFSTYLA®.

CONCLUSION: Results of OSA and ChS were comparable within low range (≤0.15U/ml). ChS had acceptable results for all concentrations with a tendency to overestimate, especially at higher concentration. The ChS is the most correct assay, which was used as potency assignment for the spiked samples, and is recommended to use for monitoring if patients treated with AFSTYLA®. No one single conversion factor, as prescribed by the manufacturer, can be applied because of varying ratio for OSA/ChS. Among OSA no suitable assay can be identified.

P05

Differences in activity of plasma spiked with IDELVION® generated with one-stage clotting assay using different commercial APTT reagents compared with chromogenic method

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BACKGROUND: APTT based One-Stage Clotting assay (OSA) is routinely used for measuring clotting factors. Recently the Chromogenic Substrate assay (ChS) has been proposed as the most accurate assay for novel extended half live (EHL) clotting factor products, especially in FVIII and now also FIX. The EHL recombinant FIX preparation, IDELVION® (CSL Behring), an albumin fusion protein, has important laboratory issues.

AIM: To identify the most appropriate FIX assay to monitor patients under treatment with IDELVION® by comparing results of ChS and OSA with different commercial activators.

METHODS: Measurement of FIX was done on depleted plasma samples spiked with different concentrations IDELVION® (0.05-0.8U/ml) provided by the Blood Center of Wisconsin. For the potency assignment the one stage assay with Pathromtin SL was used. The analysis made use of ChS: Biophen FIX (Hyphen Biomed) and OSA: Silica based STA-PTTA (Stago), TriniCLOT aPTT (Tcoag); Kaolin based STA-CK-Prest (Stago), Pathromtin SL (Siemens); and Ellagic acid based Actin FS (Siemens), Synthafax (IL). All assays used Deficient Factor IX plasma (Stago) and were performed on STA-C analyzer (Stago).

RESULTS: Different FIX results were observed among OSA and also between OSA and ChS. Across all levels accuracy (observed vs targeted spiking concentration) ranged from 40-225% for the OSA and 110-152% for the ChS. Varying ratios of 0.33-2.0 for OAS/ChS were seen across different OSA activators and concentrations. At each level, ChS and OSA using Synthafax highly

overestimated, while CK-Prest underestimated. Pathromtin SL was more in line with theoretical spiking levels. Results from both methodologies were more divergent for levels >0.2U/ml IDELVION® but similar at 0.05U/ml.

Conclusion: OSA using Pathromtin SL is the most recommended assay for monitoring FIX levels in patients treated with IDELVION®. OSA using different activators obtained varying results with severe overestimation by Synthafax, and underestimation by CK-Prest. The proposed ChS constantly overscored levels, especially >0.2U/ml IDELVION®.

P06

Evaluation of von Willebrand factor multimer distribution using a new semi-automated von Willebrand multimeric assay, HYDRAGEL VW multimer (Sebia)

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BACKGROUND: Laboratory diagnosis and (sub)classification of von Willebrand disease (VWD) is based on a multistep process requiring the evaluation of von Willebrand Factor (VWF) multimeric distributions. VWF multimer electrophoresis is labor-intensive, and not standardized technique, only performed in specialized laboratories. Recently, the first commercial semi-automated assay, HYDRAGEL VW multimer assay (Sebia), was developed.

AIM: Evaluation of performance characteristics of the HYDRAGEL assay. Results were compared with the current in-house multimeric assay in order to evaluate whether the HYDRAGEL can improve VWD diagnosis in routine laboratories and may replace the current method.

METHODS: Assessment of intra- and inter- run accuracy, analytic sensitivity, establishment of reference intervals using 40 normal donors, and method comparison with in-house method (181 VWD patients: 42 type 1, 78 type 2A, 19 type 2B, 23 type 2M, 7 type 2N and 12 type 3 VWD) according to manufacturer's instructions using the HYDRAGEL VWF multimer Visualization kit (Sebia) on a HYDRASYS 2 scan (Sebia). The current in-house method was developed by Budde et al. (DIN58988) using low resolution (<1.5%) and high resolution (>1.5%) SDS-agarose gel electrophoresis, colored with specific anti-human VWF and conjugated anti-IgG antibody directed against the first antibody followed by chemiluminescene visualization.

RESULTS: For the intra-assay variability, assessed with a normal and type 2A VWD plasma on one single gel, the coefficient of variation (CV) was respectively 8.2% and 0.9% for LHWM, 6.2% and 6.6% for IMWM and 6.6% and absent for HMWM. Inter-assay variability using normal control plasma data from 20 gels, the CV was 9% for LHWM, 4% IMWM and 4% HMWM. Normal reference ranges were established [LMWM 10.2-24.6 %AUC, IMWM 24.0 – 37.2 %AUC and HMWM 40.8 – 63.2 %AUC]. No significant difference between blood group O and non-O (Δ = 0.58%; 95%CI, -83.2 - -80.9) was seen. In the method comparison, concordant results were obtained in 162/181 (89.5%) cases. Discordant results were observed in 10% of all cases. The HYDRAGEL observed a reduction of HMWM within patients carrying mutations linked to type 2M (Leu1288Arg (n=4), Leu1382Gln (n=1), Val1414Gln (n=2) and Arg1315Cys (n=5)) and type 2B (Pro1266Leu (n=1), Arg1341Trp (n=5), unknown mutation (n=1)) where the in-house method observed a normal distribution.

CONCLUSION: The HYDRAGEL VW multimer assay is a within one-day semi-automated assay demonstrating overall comparable results with the current in-house method. The estimated normal ranges were in line with those found by Bowyer et al. in Sheffield, UK. The rapid ready to use kit can be used to reduce workload by rapidly screening on abnormal multimer patterns. Using one single gel concentration the HYDRAGEL was not able to visualize the multimer triplet structure. It could be a useful as an additional screen of VWF multimers for VWD diagnosis in routine laboratories.

P07

Evaluation of an automated von Willebrand factor collagen binding assay, HemosIL® AcuStar® VWF:CB

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INTRODUCTION: Von Willebrand Disease is bleeding disorder caused by a reduction (type 1), dysfunction (type 2) or absence (type 3) of Von Willebrand Factor (VWF). After diagnosis of VWD, the classification of type 2 VWD requires an exhaustive test panel including VWF:collagen binding capacity (VWF:CB). The VWF:CB assay, which is restricted to specialized laboratories, is sensitive to the loss of the high molecular weight multimers (HMWM) of the VWF and therefore a preferential measure of the presence of HMWM.

AIM: Evaluation of the performance characteristics of an automated VWF:CB assay and comparing it with an ELISA based method, which is currently in use.

METHODS: The automated HemosIL® VWF:CB assay (Instrumentation Laboratory, Bedford, USA) was performed on an AcuStar® (Instrumentation Laboratory) and the ELISA based assay, Zymutest® VWF:CB, (Hyphen Biomed, Neuville sur Oise, France) was determined on an EZ Read 4000 Microplate Reader (Biochrom Ltd, Cambourne, UK). Inter-assay and intra-assay variation were evaluated using normal and abnormal control material included in the HemosIL VWF:CB kit. Citrated (9:1) platelet poor plasma of forty five healthy donors (21 blood group O and 23 non-O) were analyzed for the establishment of normal reference ranges. Twenty seven samples from patients suspected for VWD and seventeen previously diagnosed type 2A VWD patients were used to correlate both methods.

RESULTS: Inter-assay coefficient of variation (CV), calculated using the data of a normal and abnormal sample from twenty runs, was respectively 5,4% and 4,9%. Intra-run variation was assessed with the normal and abnormal control, tested ten times in one run. CV was 6.8% for the normal and 5.0% for the abnormal control. Results of forty five healthy volunteers were cross referred to the reference ranges provided by the manufacturer. In accordance with the CLSI EP28 guidelines these reference ranges, taking into account the effect of blood group with significant difference between blood group O and non-O ($p<0.05$), could be used. This results in: [47.0 – 170.7/dl] for blood group O, [61.1 – 193.3U/dl] for non-O and [50.5 – 181.2U/dl] for all. Comparing VWF:CB results of twenty seven diagnostic samples and seventeen type 2A VWD patients obtained with both methods, HemosIL® VWF:CB and Zymutest® VWF:CB, no significant difference was found ($p>0.05$).

CONCLUSIONS: The HemosIL® VWF:CB on AcuStar® showed concordant results with the current ELISA based method, Zymutest® VWF:CB for both analytic and clinical decisions. This new automated assay is time-saving, stable, more cost effective, less exposed to variables (i.e. temperature, working skills,…) compared to the current ELISA based method, and therefore it would be an useful addition to VWD diagnosis in routine laboratories if an AcuStar® analyzer is available.

P08

Resolving DOAC interference on aPTT, PT and lupus anticoagulant testing by the use of activated carbon

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INTRODUCTION: Direct oral anticoagulants (DOACs) affect laboratory coagulations tests and hamper their interpretation. DOAC-STOP tablets (Haematex) can remove clinically relevant DOAC concentrations in vitro without significantly altering coagulation tests (Exner et al. Thromb Res. 2018). The adsorption of dabigatran by DOAC-STOP resembles experiments with activated carbon (AC) (Van Ryn et al. Thromb Haemost. 2010). Also in vivo administration of activated carbon after apixaban ingestion resulted in neutralizing the effect.(Wang et al. Am J Cardiovasc Drugs. 2014).

AIMS: We evaluated if AC could be used to resolve DOAC interference on PT, aPTT and lupus anticoagulant (LA) assays.

METHODS: AC (Norit Carbomix) was added in the desired concentration to citrate plasma samples, gently mixed for 5 min, and centrifuged (2 cycles of 2000g, 5 min). Clear supernatants were analyzed using Werfen reagents on an ACL-TOP. AC interference on PT (ReadiPlasTin), aPTT (SynthASil), anti-FXa (Liquid Anti-Xa), DTI, and LA screening panel (SCT and dRVVT screen/mix/confirm) was determined on citrate plasma from 5 healthy adult controls spiked with AC: 0, 20, 40, 80, 120, and 160 mg/mL. DOAC concentrations, PT and aPTT results were compared before and after addition of 20 mg/mL AC to citrate plasma samples from 29 patients receiving DOAC therapy: dabigatran (n=4), rivaroxaban (n=16), apixaban (n=7), edoxaban (n=2). To evaluate whether AC selectively removes DOACs, citrate plasma samples from patients receiving LMWH (n=10) or coumarin (n=10) therapy were evaluated. To determine whether AC resolves DOAC interference on LA screening, 20 mg/mL AC was added to platelet-free citrate plasma from patients with positive LA (n=9), negative LA (n=19), false-positive LA due to DOAC therapy (n=15), and false-positive LA due to coumarin therapy (n=3). LA results were interpreted according to ISTH guidelines.

RESULTS: An AC concentration of 20 mg/mL led to the lowest interference on PT, aPTT, and LA screening tests. It was thus selected for further experiments. After addition of 20 mg/mL AC to citrate plasma from 29 treated patients, all DOAC concentrations were below the LoQ of the anti-Xa and DTI assays, except for 2 apixaban samples which were only slightly above the LoQ: 16,4 and 15,5 ng/mL vs. LoQ 15 ng/mL. In these 29 patients, AC also removed DOAC interference on PT and aPTT. In patients receiving coumarin and LMWH therapy, clinically unimportant differences were found between PT and aPTT results before and after addition of 20 mg/mL AC. Of the 15 LA samples with interference due to DOAC therapy, 14 samples became negative and 1 had a positive result after addition of AC (patient known with APS syndrome). Interference due to coumarin therapy was not resolved by AC addition. All 19 LA negative samples remained negative. However, of the 9 positive LA samples 3 became negative after AC addition. These were however positive results around the cut-off. This phenomenon could be explained by the fact that the negative pooled plasma that was used to calculate the cut-off ratios was not treated with AC. This will be addressed in future experiments.

CONCLUSIONS: Our preliminary data are encouraging and suggest that AC selectively removes DOAC interference on PT, aPTT, and LA assays.

P09

Comparison of the effect of manual sample transport and two different pneumatic transportation systems on coagulation assays

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INTRODUCTION: Pneumatic transportation systems (PTS) are widely used in hospitals as a cost-effective means to lower the total turn-around time and to reduce the workload associated with transporting blood samples from wards to the clinical laboratory. However, as samples are exposed to various influences during this type of transport (e.g. changing air pressure, velocity, vibration), PTS may affect sample quality and test results. Studies investigating the effect of PTS on laboratory tests yielded conflicting results, especially for coagulation assays.

AIMS: The aim of this study was to evaluate the effect of manual sample transport and two different PTS on platelet activation and coagulation assays.

METHODS: Citrated blood samples were collected from 20 healthy volunteers without known history of coagulation disorders, of which 3 took NSAIDs prior to blood sampling. Immediately after blood collection, samples (n = 4 per transport condition) were sent to the core laboratory by i) manual transport by courier, ii) the regular, bidirectional, multi-line PTS (Aerocom® AC 3000 MultiLine, Aerocom), iii) a newly installed, ultrarapid, unidirectional, monotube PTS (Tempus600 Vita®, Timedico). Upon arrival in the laboratory, the following analyses were performed: platelet activation via flow cytometric detection of p-selectin (CD62p) expression on platelets (FACSCanto™ II, BD), platelet function tests: INNOVANCE® PFA-200 with collagen/EPI and collagen/ADP cartridges (Siemens) and light transmission aggregometry (TA-8V, Stago) following platelet activation by ADP 2.5 and 5.0 µM, epinephrine 10 µM, arachidonic acid 1.0 µM (Kordia) and U46619 1.0 µM (Sigma-Aldrich), and thromboelastrometry (ROTEM delta, Instrumentation Laboratory).

RESULTS: Transport of blood samples via the Aerocom PTS resulted in significantly increased platelet activation (35.4 ± 2.9% CD62p expression), compared to manual transport (28.9 ± 2.6%; $p = 0.040$) and transport via the Tempus600 PTS (25.6 ± 1.9%; $p = 0.008$). This effect was not reflected in the results of platelet function tests, as no significant differences were observed for PFA-200 and light transmission aggregometry assays for all three transport conditions. ROTEM analysis showed significantly shorter clotting times (CT) in samples transported via the Aerocom PTS for EXTEM assays ($p < 0.001$) and via the Tempus600 PTS for EXTEM ($p = 0.002$), INTEM ($p = 0.005$) and APTEM ($p = 0.005$) assays, compared to manual transport. However, as all results stayed within normal ranges, these differences did not lead to clinically relevant effects.

CONCLUSIONS AND FUTURE PERSPECTIVES: In our population of healthy volunteers, sample transport via a conventional multi-line PTS and a new ultrarapid monotube PTS mainly influenced clotting times in ROTEM analyses, without causing clinically relevant effects . Platelet function tests were unaffected. To further investigate the effects of PTS, additional coagulation assays (PT, aPTT, thrombin generation, lupus anticoagulant testing) will be performed in the near future on stored citrated plasma samples of the included volunteers.

Method validation of the new Stago PT reagent STA-NeoPTimal for PT/INR measurement

P10

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BACKGROUND & AIM: The protrombin time (PT) is a routine clotting assay, used to assess the extrinsic coagulation pathway and for follow-up of Vitamin K antagonist (VKA) therapy. Our aim was to evaluate a new PT reagent, STA-NeoPTimal (Stago), prepared from rabbit tissue factor with an International Sensitivity Index (ISI) approaching 1.

MATERIALS AND METHODS: Evaluation of STA-NeoPTimal was performed on the Stago STA-R Evolution and STA R Max 2 analyzers. Within-run imprecision (n=7), between-run imprecision (n=10) and accuracy were assessed with Lyphocheck Coagulation Control Level 1 and 2 (Biorad). Method comparison for PT was performed with STA-Neoplastin CI Plus (Stago) including 333 routine samples (healthy volunteers, patients with liver pathology, on VKA therapy, with extrinsic factor or fibrinogen deficiency) using Passing-Bablok regression, Bland-Altman analysis and qualitative comparison by contingency tables Evaluation of results was performed in measured seconds, percentage and INR. Similarly, method comparison was performed for extrinsic factor one-stage clotting assays FII (n=23), FV (n=70), FVII (n=75) and FX (n=69). Additionally, effect of direct oral anticoagulant (DOAC) therapy (apixaban, dabigatran, edoxaban and rivaroxaban) was evaluated for both reagents, using a serial dilution of 6 samples with ascending concentrations for every DOAC. Interference of hemolysis was assessed by spiking 4 samples from healthy volunteers with hemolysate.

RESULTS: Imprecision was acceptable with coefficients of variation (CV) varying between 0%-1.90% for within-run and 1.44-3.27% for between-run imprecision. Bias ranged from 0.29-4.63% for Level 1 and from 3.97-23.27% for Level 2. A higher bias for Level 2 was observed when results were expressed as measured seconds, compared to % or INR. Furthermore, there was no specific target available for the STA-NeoPTimal reagent. Passing-Bablok analysis revealed no systematic or proportional error between both methods with an intercept of 0,64 (95% CI -0,33-1,00) and a slope of 1.01 (95% CI 1,00-1,29). Bland-Altman analysis showed that results with STA-NeoPTimal were on average 2.6% (95% CI -10.3 – 15.6) higher. Table 1 revealed 8 discrepancies in the qualitative comparison of both methods, all due to minor differences in percentages. Comparison of the factor assays was acceptable with only small statistically significant errors, but no clinically relevant differences between both methods: percentage difference between both methods varied from -3.1% (FVII) to 4.1% (FX). The effect of DOAC on PT is similar for both reagents: PT is affected (PT<70%) by the different DOAC's from a concentration of 158 ng/mL (STA-NeoPTimal) or 161 ng/mL (STA-Neoplastin CI Plus) for apixaban, 63 ng/mL or 40 ng/mL for dabigatran, 60 ng/mL or 47 ng/mL for edoxaban and 79 ng/mL or 61 ng/mL for rivaroxaban. Hemolysis, tested up to a concentration of 0.18 g/dL Hb, affected PT measured with STA-Neoplastin CI Plus more compared to STA-NeoPTimal, but for neither of the reagents the effect was significant.

CONCLUSIONS: The analytical performance of the Stago STA-NeoPTimal PT reagent was good. Method comparison for PT and extrinsic factor assays showed good correlation to STA-Neoplastin CI Plus. STA-NeoPTimal is a good alternative for STA-Neoplastin CI Plus.

		STA-NeoPTimal		
		<70%	*>70%*	*total*
STA-Neoplastine CI Plus	*<70%*	134	7	141
	>70%	1	191	192
	total	135	198	333

Table 1: 2x2 contingency table for comparison of PT measured with STA-NeoPTimal and STA-Neoplastine CI Plus reagents (Stago).

P11

Effect of unfractionated heparin and low molecular weight heparin on the measurement of PT with two Stago PT reagents

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BACKGROUND & AIM: The protrombin time (PT) is a routine clotting assay, used for follow-up of Vitamin K Antagonist (VKA) therapy and to screen for extrinsic factor (FII, FV, FVII, FX) deficiencies. In most commercial PT reagents, a heparin neutralizer (polybrene, protamine sulfate) is added to limit the sensitivity of the PT for unfractionated heparin (UFH) or low molecular weight heparin (LMWH), but each manufacturer's thromboplastin has a different sensitivity to the effect of heparin. This study aims to assess the effect of UFH and LMWH on two Stago PT reagents: STA-NeoPTimal and STA-Neoplastine CI Plus, both from rabbit brain origin.

MATERIALS AND METHODS: A sample series was prepared by spiking normal pooled plasma (NPP) with ascending concentrations of unfractionated heparin (UFH) (Heparine Leo®) and low molecular weight heparin (LMWH) (Clexane®), ranging between 0 and 3.0 U/mL anti-Xa. Samples were measured for PT with STA-NeoPTimal and STA-Neoplastine CI Plus on STA-R Evolution (Stago).

RESULTS: For LMWH, no effect on PT(%), measured with STA-Neoptimal, is observed for concentrations tested up to 2.4 IU/mL antiXa. Figure 1 shows that STA-Neoplastine CI Plus is more sensitive to LMWH interference, since a prolongation of PT is observed from concentrations of 2.0 IU/mL on. According to the package insert of both reagents, PT is insensitive to LMWH up to 1.5 IU/mL. For UFH, a limited interference for STA-NeoPTimal is observed starting from concentrations of 3.0 IU/mL. Similarly, interference of UFH occurs at lower concentrations of UFH (2.0 IU/mL) with the STA-Neoplastine CI Plus reagent. The product leaflets state that there is no interference of UFH up to 1.0 IU/mL. Since therapeutic concentrations of heparin and LMWH range from 0.3-0.7 IU/mL and 0.6-1.0 IU/mL respectively, only supra-therapeutic heparin or LMWH concentrations will exert an effect on PT.
Conclusions: Low molecular weight heparin and unfractionated heparin interference in the measurement of PT with STA-NeoPTimal and STA-Neoplastine CI Plus (Stago) occurs but at higher concentrations than stated in the package insert. For both reagents, interference is observed only at supra-therapeutic concentrations of LMWH or UFH, STA-Neoplastine CI Plus being more sensitive to heparin interference compared to STA-NeoPTimal.

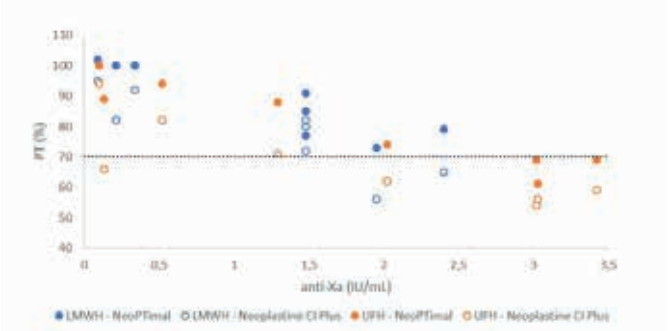

Figure 1: Effect of LMWH and UFH, expressed as antiXa IU/mL, on PT(%), measured with STA-NeoPTimal and STA-Neoplastine CI Plus. Horizontal dotted line

P12

Sensitivity of the new Stago PT STA-NeoPTimal reagent for factor deficiency on Stago STA-R Evolution

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BACKGROUD & AIM: The protrombin time (PT) is a routine clotting assay, used for follow-up of Vitamin K antagonist (VKA) therapy and to screen for extrinsic factor (FII, FV, FVII, FX) deficiencies. According to the CLSI recommendations, sensitivity of the PT reagent for detection of factor deficiencies must be between 30-45%1. In this study, we aimed to assess the sensitivity of the new Stago PT reagent, STA-NeoPTimal (rabbit brain origin) for the different extrinsic factors.

MATERIALS AND METHODS: A dilution series (n=10) with factor concentrations ranging from 0% - 100% was prepared by mixing different ratios of normal pool plasma and factor deficient plasma (Immunodef II, Deficient V, Deficient VII, Deficient X; Stago. For every sample, extrinsic factors were measured and PT was analyzed in duplo with STA-NeoPTimal on STA-R Evolution (Stago). Although not recommended by the manufacturer, one-stage clotting assays for FII were performed using immunodeficient plasma. Results for PT were expressed in in percentage. Additionally, for calculation of limit of detection (LoD=mean + 3.3*standard deviation) of extrinsic factor assays, the 0% factor concentration samples were measured in six-fold.

RESULTS: As shown in Table 1, the PT (expressed in %) measured by STA-NeoPTimal reagent shows different sensitivity for each extrinsic coagulation factor. For FII, sensitivity is insufficient since the CLSI criterium of 30% is not reached. Consequently, for FII, moderate (10-40%) factor deficiencies may be missed2.
LoD was good for FVII and FX (<1%) but too high for FV and FII to classify a deficiency as a severe deficiency2. In contrast to FII, this is of less importance for FV since there is a weak correlation between bleeding risk and factor level. Calculated LoD's were lower than the LoD's mentioned in the package insert of the (immuno)deficient plasmas.
Conclusions: The Stago PT STA-NeoPTimal reagent shows acceptable sensitivity for extrinsic factor deficiency when the CLSI criterium is applied, except for FII, where sensitivity of PT expressed in % is suboptimal (26%). LoD was lower than stated by the manufacturer and below 1% for FVII and FX.

[1]H47-A2 CLSI: One-Stage PT and aPTT test, approved guideline, second edition
[2]Mumford A et al. Guideline for the diagnosis and management of the rare coagulation disorders. Br J Hematol 2014, 167, 304-3026

Extrinsic factor	Sensitivity of PT for factor deficiency*	LoD (%) calculated	LoD (%) package insert
	%		
FII	26	1.29	3
FV	40	1.87	2
FVII	50	0.86	2
FX	36	<0.68	1

Table 1: Sensitivity of the STA-NeoPTimal PT reagent for factor deficiency of FII, FV, FVII or FX and limit of detection (LoD) of the extrinsic factor assays.
**: level of Factor from where on PT is below the reference value (70%)*

The value of laboratory screening vs. clinical judgment in HIT diagnosis: experiences in three Belgian hospitals

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P13

INTRODUCTION: The diagnosis of Heparin-induced thrombocytopenia (HIT) remains challenging as it is based on both clinical judgment and laboratory testing. Clinical scoring systems, of which the Warkentin 4T-score is the most used, have been implemented to guide the clinical decision. Recent literature has suggested that a low probability 4T-score appears sufficiently robust to exclude HIT and that only patients with an intermediate and high probability score require further laboratory evaluation.

AIMS: Our study was initiated to document the role of laboratory results on the definitive clinical HIT diagnosis in 3 Belgian hospitals.

METHODS: All patients who were evaluated for HIT antibodies between March 2018 and July 2018 in the following Belgian hospitals were included: Onze-Lieve-Vrouwziekenhuis Aalst-Asse-Ninove (OLVZ), AZ Sint-Jan Brugge, and the University Hospital Antwerp (UZA). Clinical and laboratory data were gathered and the Warkentin 4T-score was calculated. If unfractionated heparin (UFH) or low molecular weight heparin (LMWH) was stopped or switched, the platelet count was registered in the following days to document a significant increase in platelet numbers: normalization of the platelet count in <10 days="" or="" a="" platelet="" increase=">50x103/µL in ≤2 days. Different laboratory screening methods were used for HIT antibody detection: ID-PaGIA Heparin/PF4 Antibody Test (Bio-Rad, OLVZ Aalst), LIFECODES PF4 IgG (Gen-Probe, AZ Sint-Jan Brugge) and Acl Acustar (Werfen, UZA). Considering all clinical and laboratory data, patients were classified in the "HIT+" or "HIT-" group based on a consultation between the lab and the treating clinician. Statistical testing was performed using MedCalc. Significance testing was done using a Mann-Whitney U test.

RESULTS: We included 35 patients who received UFH or LMWH and had a calculated Warkentin 4T-score and a laboratory testing for HIT.
A Warkentin 4T-score of ≥4 was found in 10/35 patients (29%). A positive laboratory screening was obtained in 6/35 (17%) patients with one weak positive result.
5 patients received a final clinical diagnosis of HIT. The median Warkentin 4T-score for the clinical HIT+ group was 5 (range: 3–6), which was significantly higher in comparison to the HIT- group (median 3, range 0–4, p= 0,0024). Of these 5 HIT patients, 4 tested positive on laboratory screening. Characteristics of those patients with a clinical HIT can be found in table 1. An overview of the sensitivity, specificity, PPV and NPV of Warkentin 4 T-score and laboratory results in comparison to the clinical diagnosis of HIT can also be found in table 1.
When assessed separately, neither a negative 4T-score nor a negative laboratory test could safely rule out the presence of HIT. Only when the 4T-score was assessed in combination with the laboratory test, a sensitivity and a NPV of 100% was reached.
We found in our study a limited PPV and specificity of immunological HIT test and the combination of these tests with a 4-T score. This is supported by literature. Therefore all positive results should be confirmed by functional assays.

CONCLUSIONS: Our preliminary data show that no single approach of 4T-score or laboratory analysis was sufficient for exclusion of HIT. Because the limited amount of samples, further investigation is necessary.

Table 1. Statistical characteristics and overview 5 patients with clinical HIT

Statistical characteristics

	TP	FN	FP	TN	Sensitivity	Specificity	PPV	NPV
4 T score (cut-off ≥ 4) vs clinical diagnosis HIT	4	1	6	24	80%	80%	40%	96%
Laboratory test vs clinical diagnosis HIT	4	1	2	28	80%	93%	67%	97%
Combination 4T score (cut-off ≥ 4) or laboratory test vs clinical diagnosis HIT	5	0	8	22	100%	73%	39%	100%

Characteristics of the 5 patients with a clinical HIT

	Laboratory result	Laboratory test used	4T score	Type heparin	PLT low (x10³/µL)	Decline PLT (%)	Sequelae	Elevation PLT after stop heparin?
1	Negative	*ID-PaGIA Heparin/PF4 Antibody Test (Bio-Rad)*	6	LMWH	38	72	Thrombosis and thrombopenia	Yes
2	Positive	*ID-PaGIA Heparin/PF4 Antibody Test (Bio-Rad)*	5	LMWH	20	79	Thrombosis	Yes
3	Positive	*ID-PaGIA Heparin/PF4 Antibody Test (Bio-Rad)*	5	LMWH	72	70	Thrombopenia	Yes
4	Positive	*ID-PaGIA Heparin/PF4 Antibody Test (Bio-Rad)*	3	UFH	44	80	Thrombopenia	Unknown
5	Positive	AcI Acustar (Werfen)	6	UFH	11	95	Thrombosis	Yes

Table 1. Statistical characteristics and overview 5 patients with clinical HIT

P14

Preliminary evaluation of ADAMTS-13 antibody testing

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INTRODUCTION: Thrombotic thrombocytopenic purpura (TTP) is a disorder that can be fatal if no appropriate treatment is given in time. First line tests, such as complete blood cell count and hemolytic markers, allow a presumptive diagnosis. However, decreased ADAMTS-13 activity (<10%) is considered the only specific marker in TTP diagnosis. In acquired TTP, the presence of antibodies (Ab) requires a longer duration of treatment. Both neutralising Ab and non-neutralising Ab that increase clearance have been reported. Immunological methods target both Ab and are more sensitive. Mixing studies detect neutralising Ab. No recommendation exists regarding conditions for mixing studies, the method or which Ab should be analysed (neutralising and/or non-neutralising Ab).

AIMS: The aim of this study was to optimize mixing studies for detecting ADAMTS-13 neutralising Ab using our current ADAMTS-13 activity assay and to compare it with an ELISA Ab assay that detects all Ab to ADAMTS-13.

METHODS/MATERIALS: The ADAMTS-13 Activity ELISA assay (Technoclone) was used to detect neutralising Ab, expressed in Bethesda units (BU). For optimisation of the mixing study different normal human plasma pool (NPP) (Stago or Cryopep), incubation time (1 hour or 2 hours), incubation temperature (room temperature or 37°C), and diluent (reaction buffer – Technoclone, imidazole buffer 0,1 M, inactivated NPP) were compared. We also verified the inactivation procedure (58°C, 30 min). We compared our results to the BU concentration of the laboratory UZLeuven (dr. M. Jacquemin). referred to as reference method.
The ADAMTS-13 INH ELISA assay (Technoclone) was used to detect both neutralising and non-neutralising Ab according to the instructions of the manufacturer.
We applied both methods to leftover frozen citrated plasma (platelet poor) from TTP cases (adults with ADAMTS-13 activity <10%), 15 healthy persons and 3 patients positive for lupus anticoagulant. 3 samples from the ECAT ADAMTS-13 II program (2017-M2 and M4) were tested.

RESULTS: Optimal conditions for the detection of neutralising Ab (as compared to the reference method) by mixing study were inactivation of patient plasma (58°C, 30 min), dilution in reaction buffer, mixing with Stago NPP, and incubating 1 h at 37 °C.
Most samples from TTP cases were positive for anti-ADAMTS-13 Ab (Table) with both assays, except for case 3 even though a low BU titer had been reported in this sample by the reference lab. This may be due to a repeated freeze-thaw cycles. In case 4 neutralising Ab were found even though the ELISA assay was negative, which may highlight the complementarity of both antibody detection methods.
ECAT samples were all in agreement with the assigned value.
All of the healthy persons and lupus patients had an ADAMTS-13 activity >60% as expected. No neutralising Ab were found with the ADAMTS-13 Activity mixing assay (<0.25BU). We could confirm the cutoff level of 12 U/ml that was suggested by the supplier using ADAMTS-13 INH ELISA assay.

SUMMARY: We optimised a functional anti-ADAMTS-13 Ab detection method and applied it to samples in parallel with an anti-ADAMTS-13 Ab ELISA assay. Both methods seem of complementary value in the diagnosis of TTP, though more samples are needed before conclusions can be made.

Sample	ADAMTS-13 activity (%)	ADAMTS-13 Activity mixing assay Antibody titer BU (reference lab)	ADAMTS-13 INH ELISA assay Antibody concentration U/ml
TTP case 1	0	1,2 (1,9)	18
TTP case 2	0	478 (1236,5)	>1504
TTP case 3	3	<0,25 (2,0)	4 (<cutoff)
TTP case 4	3	2,7 (2,9)	8 (<cutoff)
TTP case 5	0	9,8 (9,5)	82

Table. ADAMTS-13 activity and anti-ADAMTS-13 antibody concentrations in TTP cases using ADAMTS-13 INH ELISA and mixing assay

Optimisation of the calibration curve for the accurate measurement of low FVIII levels with one-stage assays

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P15

BACKGROUND: The monitoring of factor VIII (FVIII) replacement therapy in haemophilia A patients relies on the accurate measurement of FVIII activity over a large concentration range. However, a significant overestimation of FVIII levels lower than 5 IU/dl with one-stage assays has been reported by recent studies with extended half-life recombinant FVIII (EHL rFVIII) (Bowyer et al., Haemophilia 2017;23:e469-e470; Bulla et al. Haemophilia. 2017;23:e335-e339). Former studies have demonstrated that the reagents used to generate calibration curves and to dilute samples may have a serious impact on the accuracy of the measurement of low FVIII levels (Cinotti et al. J Thromb Haemost 2006; 4: 828–33).

AIMS: The objective of this study was to evaluate the effect of using FVIII-deficient plasma or diluent buffer to dilute the FVIII standard on the measurement of different types of FVIII (native FVIII, full length recombinant FVIII, EHL rFVIII) using either a single dilution or multiple dilutions (parallelism method).

METHODS: We generated calibration curves by diluting a standard plasma pool with different FVIII-deficient plasmas or with a commercial diluent buffer. Using these calibration curves, we measured FVIII levels in FVIII-deficient plasma spiked with plasma FVIII, a full length recombinant FVIII (Advate®) and an extended half-life recombinant FVIII (Elocta®). FVIII levels were also analyzed in spiked samples prediluted two- and four-fold, either in diluent buffer or in FVIII-deficient plasma to evaluate parallelism. To limit the use of FVIII-deficient plasma in the parallelism experiments we also developed an automated method diluting the sample in 10% FVIII-deficient plasma on a ACL-TOP 500R.

RESULTS: Coagulation times of the FVIII reference standards diluted with diluent buffer were substantially longer than those diluted with a FVIII-deficient plasma. This resulted in an important overestimation of FVIII levels lower than 25 IU/dl in all spiked samples (plasma FVIII, FL rFVIII, EHL rFVIII) and in the false detection of FVIII activity (≥ 1 IU/dl) in FVIII-deficient plasma.
Similarly, the parallelism experiments carried out with samples prediluted in diluent buffer rather than in FVIII-deficient plasma led to unacceptable discrepancies between the FVIII levels measured at the different sample predilutions (coefficient of variation (CV) of the FVIII levels measured with the different predilutions of the samples > 20%). By contrast, the automated method with predilution of the samples in diluent buffer supplemented with 10% FVIII-deficient plasma provided acceptable results (CV < 20%).

SUMMARY/CONCLUSIONS: Our data confirm that the generation of calibration curves by dilution in FVIII-deficient plasma is crucial for the accurate measurement of low FVIII levels. When serial dilutions of samples are analyzed, predilution in FVIII-deficient plasma is required to respect the parallelism criteria. These methods should be more generally implemented on coagulation instruments.

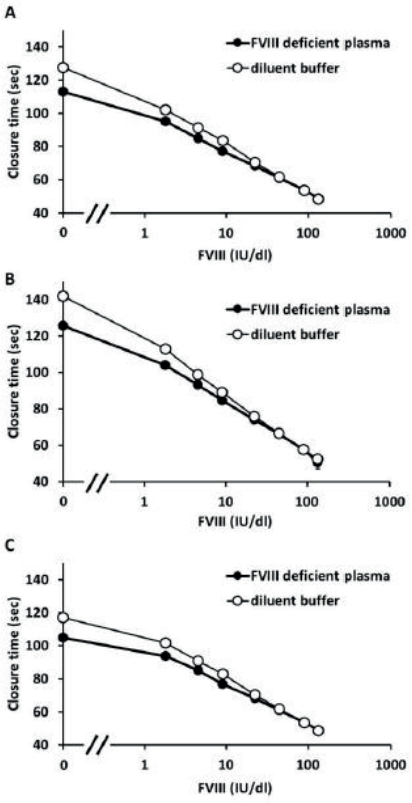

Figure 1. Generation of FVIII calibration curves with diluent buffer or FVIII-deficient plasma.
Eight-point calibration curves were generated by diluting an in-house pooled normal plasma standard with either diluent buffer or FVIII-deficient plasma. A, Siemens; B, Werfen; C, Grifols FVIII-deficient plasma. Data are shown as the mean of triplicates. The mean CV of the triplicates were ≤ 2%.

P16

How to deal with samples with errors on APTT, PT and fibrinogen due to optical clot detection?

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INTRODUCTION/ BACKGROUND: Analysis of routine coagulation parameters PT, APTT and fibrinogen (FG) is performed on the automated coagulation analyzer Sysmex CS5100 (Siemens Healthcare Diagnostics) in our laboratory. A limitation of optical clot detection is the inability to produce correct results in turbid samples. While recollection not always solves this problem, mechanical clot detection could be an alternative method.
In our laboratory, samples with no correct result on the Sysmex CS5100 due to non-interpretable curves, are reanalyzed with the manual benchtop analyzer STart (Stago). On both analyzers, the same reagents (with equal heparin and factor sensitivity) are used. To facilitate the exchangeability and interpretation of results for clinicians, a correlation-equation is used to convert the results from the STart for APTT, PT and FG.

AIMS: Evaluation of the stability and applicability of the correlation-equation, used since the implementation of the CS 5100 5 years ago, by correlating the converted STart results with results from the optical analyzer CS 5100 for the routine coagulation parameters APTT, PT and FG.

METHODS/MATERIALS: Citrated platelet poor plasma of 30 patients, selected to cover the total measurement range, were analyzed on the Sysmex CS 5100 for APTT, PT and FG and afterwards on the Stago STart. On both analyzers, the same reagents (APTT: Actin FS (Siemens), PT: Innovin (Siemens), FG: Dade Thrombin (Siemens)) were used. Both analysis were performed within 4 hours after blood collection. Measurements on the STart were performed in duplo after analyzing a positive and negative control (Control P/N/ Citrol 2 (Siemens)).
Statistical analysis were calculated with MedCalc Statistical Software version 17.5.5 (MedCalc Software bvba, Ostend, Belgium).

RESULTS: For the 30 included samples, an excellent correlation coefficient was found for APTT, PT%, PT INR and FG. Statistical characteristics can be found in Table 1.

SUMMARY/ CONCLUSIONS: The correlation-equation, used since 5 years, is applicable for the determination of the 3 tested coagulation parameters. With excellent correlation coefficients for all tested parameters (APTT, PT and FG), we can conclude that the STart is a reliable alternative for the analysis of routine coagulation parameters for samples with errors due to optical detection. With the use of the same reagents on both analyzers and a correlation-equation to convert the results, we can rapport reliable results to the clinicians even for turbid samples with reaction errors.

Table1: Statistical characteristics of the correlation between STart (Stago) ar 5100 (Sysmex) for the coagulation parameters APTT, PT and FG.

Coagulation parameter	Correlation coefficient	P-value	Measured range
APTT	0,97	P< 0,0001	22,5-72,6 sec
PT %	0,97	P< 0,0001	10-121%
PT INR	0,99	P< 0,0001	0,93-8,75
FB	0,98	P< 0,0001	88-783 mg/dl

Table 1: Statistical characteristics of the correlation between STart (Stago) and CS 5100 (Sysmex) for the coagulation parameters APTT, PT and FG.

P17

Establishment of a thrombin generation threshold in severe haemophiliac patients under prophylaxis to further determine an added value of this test in the personalized treatment: a pilot study

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BACKGROUND: Treatment of severe haemophiliac patients is based on replacement of the deficient factor. Until recently, the theoretical through level admitted to avoid spontaneous bleeding in all patients was >1%. Current recommendation is to personalize the factor dosage to reach a through level that could be higher, depending of individual factors like age, life style, pharmacokinetics… On the other hand, it has been demonstrated that thrombin generation TG could better reflect the phenotype of the patient than the factor level.

AIMS: We aimed to measure TG during pharmacokinetics undergone in every patient included in the study to assess the potential benefit of this test in the personalized follow-up/treatment. Chromogenic (CSA) and chronometric (OSA) FVIII levels were both measured.

METHODS: 24 severe haemophilia A patients under prophylactic treatment were included after approval of the local Ethical committee. Annual bleeding rate ABR was collected for each patient. Citrated blood samples were obtained at 5 time points: before factor injection T0, 30 minutes, T1, T2 and T24 hours after injection. Poor platelets plasmas were obtained after a double centrifugation 15 minutes at 1900 g and stored at -80°C until processing. Factor half-life (T ½) was estimated thanks to WAPPS-Hemo. FVIII was measured on STA-Evo (Stago) using OSA (Cephascreen® aPTT reagent (Stago) and CRYOcheck® FVIII deficient plasma (Precision Biologic) and CSA (Hyphen Biomed®) assays.
TG using the Calibrated Automated Thrombogram® was triggered with low level of tissue factor (PPP LOW reagent). Endogenous Thrombin Potential (ETP) was monitored.
The ETP at T0 for each patient was expressed in function of the total dose of factor administrated in one year (RETP0). The performance of RETP at time 0 for ABR was determined using a ROC curve.

RESULTS: The following patient's data are expressed in terms of median and range. Age: 13.3 y (2.9 – 60.8), weight 51.3 kg (14 - 97) follow-up 88 months (9 – 217), total dose of factors 2743 UI/kg/year (1171 – 4531), ABR 1 (0 – 3), T ½ 10.5 hours (4.5 -20.2). FVIII levels measured by the two methods were significantly correlated at the 5 time points. Using the ROC curve, a RETP0 <0.100 reach a sensitivity of 73.3% and a specificity of 75% for ABR (Pvalue 0.03321, likehood ratio 2.93). Using this value, we determined an ETP threshold value of 276.9 nM below which the bleeding frequency would increase (sensitivity of 80%, specificity 92.3%, Pvalue 0.001268, likehood ratio 10.4).

SUMMARY/CONCLUSIONS: This pilot study encourages us to continue to use OSA to determine FVIII for classical factor concentrates, which is easier and less expensive cheaper than the CSA. We don't have yet enough data for long acting products. Our study demonstrated that a relationship between ETP at time 0 and the patient phenotype could be established with the determination of an ETP threshold. This threshold could now be prospectively used to adapt prophylactic treatment to minimize bleeding risk, hoping to demonstrate an added value of this test in a larger cohort of patients.

P18

Evaluation of the TAV8® (Stago) aggregometer in the clinical laboratory

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BACKGROUND/INTRODUCTION: Thrombocytes are essential players in normal hemostasis. Throughout the years, the in vitro measurement of the aggregation profile of thrombocytes has remained an important test for the evaluation of primary hemostasis. Such a profile can be obtained by measuring the thrombocytes' capacity to aggregate in the presence of specific activators or agonists. The addition of specific concentrations of these agonists to platelet rich plasma (PRP) leads to activation of the thrombocytes and concurrent characteristic aggregation curves that can be measured by monitoring the light transmission in the PRP using an aggregometer.

AIMS: The aim of this study was to evaluate the use of the TAV8® (Stago) aggregometer for the measurement of aggregation profiles in the clinical laboratory, this in comparison to the CHRONO-LOG®490 (Avant Medical) aggregometer.

METHODS/MATERIALS: Citrated blood was collected from both healthy volunteers (n=14) and patients (n=10) to prepare PRP. Five different agonists were used in this study: ADP (5 µM, 2.5 µM, 0.5 µM) (Chrono-Log®), ristocetin (1.5 mg/ml, 0.5 mg/ml, 0.25 mg/ml) (Chrono-Log®), collagen (5 µg/ml and 2 µg/ml) (Chrono-Log®), arachidonic acid (1 mM) (HART® Biologicals) and epinephrine (10 µM) (HART® Biologicals). First, a method comparison between the TAV8® and the CHRONO-LOG®490 aggregometer was done. In addition, the repeatability, the influence of the number of platelets and several stability studies were performed on both aggregometers. Also, reference values could be set up based on the results of the healthy volunteers.

RESULTS: For the patient samples, a very good correlation (range r-values 0.8811-0.9968) was found between the maximal aggregation percentages on both aggregometers. Also, the clinical evaluation of the profiles showed good agreement. The

repeatability was excellent on both aggregometers, with CV-values ranging from 3.05% to 4.51% on the CHRONO-LOG®490 and from 4.19% to 7.70% on the TAV8®. Evaluation of the influence of the number of platelets in the PRP on the aggregation profile showed that only for arachidonic acid (1mM) and epinephrine (10µM) a significant decrease in aggregation was measured with decreasing number of platelets (101.103 ± 5.103 platelets/µL and 81.103 ± 2.103 /µL versus 336.103 ± 22.103 /µL.

CONCLUSIONS: Evaluation of the TAV8® aggregometer in comparison to the CHRONO-LOG®490 showed that the TAV8® can be used for measuring the aggregation profiles in the clinical laboratory when an anomaly in primary hemostasis is suspected. Since the TAV8® aggregometer has eight channels for simultaneous measurement (versus two on the CHRONO-LOG®490) and has modern software, measurements can be performed faster and data-processing and -storage are more user-friendly.

Evaluation of an Automated light transmission aggregometry

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P19

The purpose of this study is to evaluate platelets function in a normal population and to compare the results between automated LTA on CS2500 (Sysmex from Siemens) and the reference aggregometer PAP- 8 (Biodata)

MATERIAL/METHODS: Samples (n=17) were obtained on healthy volonteers taking no medication interfering with platelet function.
Blood samples were collected in Vacutainers (BD) containing buffered sodium citrate at the concentration 3.2%. Platelet-rich plasma (150-480 10^3 /µl) and Platelet-poor plasma were prepared within 4h after blood collection.
Platelet aggregations were performed on CS2500i using agonists at the recommended concentration by ISTH: ADP (2µmol/l), Arachidonic Acid (1mmol/l), Collagen (2µg/ml), Epinephrine (5µmol/l), Ristocetin (1,2mg/ml) and Ristocetin (0.6mg/ml). For each test, 20 µl of agonist is added at 140µl of sample of PRP.
Reaction cuvettes with stirred bars must be were manually introduced in the CS. The panel composed by the combination of the different agonists was automatically analyzed by the device with no further manipulation required.
Concentrations of agonists used on the LTA PAP-8 analyzer, were as follow: ADP (5µmol/l), Arachidonic Acid (0.5mmol/l), Collagen (2.5µg/ml), Ristocetin (1.2mg/ml and 0.6mg/ml). An optical calibration must be performed before each utilization. For each test, 30µl of agonist is added manually to a volume of at minimum 270µl of PRP and in the same time the counter time must be set on. These manipulations must be repeated for each agonist.

RESULTS: None spontaneous aggregation occurred during this comparison.
All samples showed normal curves of aggregation for each agonist either with PAP-8 or CS2500i. The normal values of % of maximal aggregation of these samples were calculated by percentile 2.5 and percentile 97.5%. All samples performed on the PAP-8 have results in normal values available for this automat.

SUMMARY: The results of this preliminary assessment are in agreement with the PAP-8 aggregometer. The ease of handling when performing this test with an automated LTA on the CS2500 will increase its availability, offer a better standardization and reduce the turnaround time.
However, its specificity and sensitivity to detect platelet disorders needs to be further determined on pathological samples

	ADP %	AA %	COLL %	EPI %	RISTO%	RISTO/2 %
CS2500						
Mean	80.65	87.35	87.76	84.88	84.59	4.21
SD	6.36	5.37	3.73	4.11	3.74	3.96
Reference values %	66,8- 89,4	77,8-93,6	80-92	77,2-91,4	79,4-90,8	0,49-13,08
PAP8						
Mean	88.24	81.59	76.18	NA	88.47	5.24
SD	7.75	7.13	3.89	NA	7.64	4.28
Reference values %	67-100	74-100	75-100	NA	81-100	<10

P20

Evaluation of the new kit STA-vWf RCO (STAGO) measuring Willebrand factor activity and comparison with two SIEMENS kits: BC von Willebrand Reagent and Innovance vW activity

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BACKGROUND / INTRODUCTION: Ristocetin cofactor assay has become one of the key elements in the diagnosis of von Willebrand disease (vWD), the most common hereditary hemostasis disorder, over the years. Its measurement is associated with that of vWf (von Willebrand factor) antigen for the diagnosis of type II vWD, in the measurement of the "vWf RCO / vWf-Ag" ratio.

AIMS: Evaluation of the new STAGO "STA-vWf RCO" kit for ristocetin cofactor assay, and comparison with two kits for vWf assay in the diagnosis of von Willebrand disease.
Methods / Materials: STA-VWF: RCO (Diagnostica Stago) on STA-R® MAX2, BC VWF Reagent (Siemens Healthcare Diagnostics) and Innovance AC (Siemens Healthcare Diagnostics) on BCS-XP®. 81 patient samples for repeatability at different levels and quality controls for reproducibility. 53 patient samples were used for the comparative study, of which 53, 11 were Type II abnormalities.

RESULTS: For reproducibility, control 1 (46% -76%) has a SD of 6.48 and a CV of 10.85%, and control 2 (20% -38%) has an SD of 4.83 and CV of 16.41%. The repeatability was done on three levels, low (21%), medium (59%) and high (108%) with respectively for CV, 9.24%, 8.85% and 7.9%. In the vWD type II screening 11 samples were used, a correlation between the "STA-VWF RCO / vWf-Ag" VS "INNOVANCE AC / vWf-Ag" and "STA-VWF RCO / vWf-Ag" VS "BC VWF Reagent / vWf-Ag" ratios were made, and the results are described below.

Table 1: Comparison between the activities of the three kits and the activity / antigen ratios.
SUMMARY / CONCLUSIONS: This new STA-VWF: RCO kit shows satisfactory results with a repeatability of less than 10% and a reproducibility of less than 17%, in agreement with the "Acceptability Standards in Hemostasis" of the GEHT 2014. The results for the screening of the vWD type II are significantly consistent. This kit is therefore in use as a second-line in large-scale laboratories but can be used routinely in laboratories of smaller scales.

Kits	r	Slope (a)	Intercept (b)
INNOVANCE AC VS STA VWF RCO	0.976	0.7965	5.6712
BC VWF VS STA VWF RCO	0.966	0.8431	4.1961
STA-VWF RCO / vWf-Ag VS INNOVANCE AC / vWf-Ag (activity/antigen ratio)	0.9002	0.1554	0.6812
STA-VWF RCO / vWf-Ag VS BC VWF Reagent / vWf-Ag (activity/antigen ratio)	0.6926	0.5351	0.1971

Table 1: Comparison between the activities of the three kits and the activity / antigen ratios.

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A novel ADAMTS13 conformation ELISA shows conformational activation of rat ADAMTS13 with exposure of cryptic epitopes in vitro

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INTRODUCTION: Thrombotic thrombocytopenic purpura (TTP) is characterized by a severe deficiency in the multidomain metalloprotease ADAMTS13, consisting of a metalloprotease (M), disintegrin-like (D), cysteine-rich (C), spacer (S), 8 thrombospondin type 1 repeats (T1-8) and 2 CUB domains. Human ADAMTS13 can adopt different conformations and several monoclonal anti-T2-CUB2 antibodies (mAbs) are able to induce an increased proteolytic activity (FRETS-VWF73 assay) by opening ADAMTS13, and subsequently revealing cryptic epitopes in human ADAMTS13. Recently, our group has shown that ADAMTS13 adopts an open conformation in acute immune-mediated TTP. Therefore, animal models mimicking conformational changes in ADAMTS13 would be of great importance to further elucidate the pathophysiology of TTP.

AIM: We aimed at inducing conformational changes in rat ADAMTS13 using anti-ADAMTS13 mAbs and to develop an ELISA to discriminate between open and folded rat plasma ADAMTS13.

METHODS: First, cross-reactivity of the activating anti-human ADAMTS13 mAbs 17G2 (anti-CUB1) and 19H4 (anti-T8) with rat ADAMTS13 was tested in ELISA. Next, rat plasma ADAMTS13 activity was determined in the absence/presence of the activating mAb 19H4, using the FRETS-VWF73 assay. Thereafter, a panel of anti-ADAMTS13 mAbs recognizing cryptic epitopes in human ADAMTS13 were screened for cross-reactivity with cryptic epitopes in rat ADAMTS13 (pre-incubated with 19H4) using ELISA. Finally, an ELISA was developed to discriminate between rat plasma ADAMTS13 in the absence (folded) and presence (open) of the activating mAb 19H4.

RESULTS: Of the activating anti-human ADAMTS13 mAbs, only 19H4 bound to rat ADAMTS13 in ELISA. In addition, mAb 19H4 induced a 2.3 fold increase in rat plasma ADAMTS13 activity in the FRETS-VWF73 assay. The cloned patient anti-S Ab I-9, which recognizes a cryptic epitope in human ADAMTS13, cross-reacted with rat ADAMTS13 but only after pre-incubation of rat ADAMTS13 with mAb 19H4. Finally, a novel rat ADAMTS13 conformation ELISA was developed, in which only open rat ADAMTS13 is captured by Ab I-9 e.g. upon pre-incubation of rat ADAMTS13 with mAb19H4, and detected by biotinylated crossreacting polyclonal rabbit-anti-mouse ADAMTS13 Abs.

SUMMARY/CONCLUSION: Activating conformational changes can be induced in rat ADAMTS13 upon addition of the anti-T8 mAb 19H4. The folded vs open rat ADAMTS13 conformation can be discriminated in our novel in-house ELISA by using the anti-S Ab I-9, which recognizes a cryptic epitope in ADAMTS13. This unique tool can now be used to further investigate conformational changes in ADAMTS13 in vivo and to elucidate whether exposure of cryptic epitopes in ADAMTS13 can evoke an anti-ADAMTS13 immune response in the rat.

Anti-CUB1 or anti-spacer antibodies that increase ADAMTS13 activity act by allosterically enhancing metalloprotease domain function

P22

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BACKGROUND: ADAMTS13 circulates in a folded conformation, which is mediated by interactions between the C-terminal CUB domains and its central Spacer domain. Binding of ADAMTS13 to the VWF D4-CK domains disrupts the CUB-Spacer interaction, inducing a structural change that extends ADAMTS13 into an open conformation that enhances catalytic efficiency ~2-fold. This mechanism supports a model in which ADAMTS13 unfolding induces exposure of an exosite in the Spacer domain that interacts with the VWF A2 domain, increasing the affinity between the two molecules, and, therefore, the rate of proteolysis. The D4-CK-mediated conformational activation of ADAMTS13 can be mimicked in vitro with the use of antibodies (Abs) that disrupt the CUB-Spacer interaction, such as the previously published anti-CUB antibody, Ab17G2. We recently generated a novel, activating antibody against the Spacer domain (Ab3E4).

AIM: To characterize the mechanism by which the Ab17G2 and Ab3E4 enhance the catalytic efficiency of ADAMTS13.

METHODS: The effects of the Ab17G2 and Ab3E4 on the activity of ADAMTS13 were studied using FRETS-VWF73. The effects of the Ab17G2 and Ab3E4 on the kinetics of VWF96 (VWF G1573-R1668) proteolysis were characterized using an in-house assay. ELISA was used to investigate conformational changes in ADAMTS13 induced by the Ab17G2 and Ab3E4.

RESULTS: Both Ab17G2 and Ab3E4 enhanced FRETS-VWF73 proteolysis by ~1.7-fold. This result was reproduced using the VWF96 substrate; the Ab17G2 and Ab3E4 enhanced the catalytic efficiency (kcat/Km) of ADAMTS13 by ~1.8- and ~2.0-fold, respectively. The activation was dependent on the conformational extension of ADAMTS13, since the Abs could not enhance the activity of an ADAMTS13 variant that lacks the TSP2-CUB2 domains (MDTCS). Surprisingly, ADAMTS13 activation was not mediated through exposure of the Spacer or Cys-rich domain exosites as previously proposed, as the Ab17G2 and Ab3E4 efficiently enhanced proteolysis of VWF96 variants in which the Spacer/Cys-rich exosite binding sites were disrupted. Kinetic analysis of VWF96 proteolysis showed that the Ab17G2- and Ab3E4-induced activation of ADAMTS13 is primarily manifest through a ~1.5- to ~2-fold increase in enzyme turnover (kcat). Thus, contrary to the current model, this suggests that the conformational extension of ADAMTS13 influences the functionality of the active site, and not substrate binding affinity (Km). Incubating ADAMTS13 with either

Ab17G2 or Ab3E4 exposed a cryptic epitope in the metalloprotease domain that was specifically detected by an Ab binding to this cryptic site in ELISA, further corroborating that the Abs induce a conformational change in ADAMTS13 affecting the M domain.

CONCLUSION: Abs can be used as tools for understanding the structure/function of enzymes. Using activating Abs against the Spacer and CUB1 domains of ADAMTS13, we show for the first time that the activation of ADAMTS13 following its unfolding is not a result of exposure of a functional exosite in Spacer/Cys-rich domain that increases affinity to VWF. Rather, our data are consistent with an allosteric activation mechanism upon the metalloprotease domain. We propose that ADAMTS13 unfolding causes a conformational change in the active site that further activates the enzyme. We are currently investigating whether the D4-CK-induced enhancement of ADAMTS13 proteolytic activity is also mediated by conformational changes in the active site.

P23

Betrixaban: Impact on routine and specific coagulation assays - A practical laboratory guide

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BACKGROUND/INTRODUCTION: Betrixaban is a novel direct oral factor Xa inhibitor approved by the Food and Drug Administration for prophylaxis of venous thromboembolism in patients hospitalized for an acute illness at risk for thromboembolic complications. Assessment of the anticoagulant effect of betrixaban may be useful in some situations. Also, clinicians need to know how coagulation assays are influenced.

AIMS: To determine which coagulation assay(s) should be used to assess the impact of betrixaban on hemostasis and provide laboratory guidance for their interpretation.

METHODS/MATERIALS: Betrixaban was spiked at final concentrations ranging from 0 to 250ng/mL in platelet-poor plasma. These concentrations cover the on-therapy range (from ± 9ng/mL at Ctrough to ± 122ng/mL at Cmax for 40 and 120mg once daily dose). We tested different reagents from several manufacturers and assessed betrixaban impact on routine (PT, aPTT) and more specific coagulation assays (chromogenic anti-Xa, TGA, DRVVT). We also tested several hemostasis diagnostic tests.

RESULTS: A concentration dependent prolongation of aPTT, PT and dRVVT is observed. The sensitivity mainly depends on the reagent. FXa chromogenic assays show high sensitivity and a linear correlation both depending on the reagent and/or the methodology. Several methodologies applicable for other direct factor Xa inhibitors have to be adapted. TGA may be efficient to assess the pharmacodynamics of betrixaban for low concentration but its turnaround time and the lack of standardization are limitations.

SUMMARY/CONCLUSIONS: Our results demonstrate an important lack of sensitivity for routine tests as reported for other DOACs. As it has been observed with other DOACs, chromogenic anti-Xa assays seems to be interesting candidates to assess the presence of low levels of betrixaban, below 120ng/mL, but adapted methodologies should be evaluated to improve sensitivity of these reagents for on-therapy range. However, this study show that adapted-chromogenic anti-Xa assays are the most appropriate assays to measure the anticoagulant effect of betrixaban. Broadly used coagulation assays such as PT or aPTT are not appropriate to efficiently estimate plasma drug concentration due to several limitations and a lack of sensitivity. The dRVV-T could be informative in certain circumstances due to its good sensitivity and its low LOQ. CAT® gives further information on the coagulation process but its use in clinical settings is limited. Finally, betrixaban significantly affects several hemostasis diagnostic tests, such as lupus anticoagulant or other thrombophilia testing, and this needs to be taken into consideration when requesting and interpreting such tests.

Improvement of chromogenic anti-Xa assay to measure betrixaban concentration in plasma

P24

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BACKGROUND/INTRODUCTION: Betrixaban, a novel direct oral factor-Xa inhibitor, has received its market authorization on the 23th of June 2017 in the United States (US) for the prophylaxis of venous thromboembolisms (VTE) in adult patients. Although direct oral anticoagulants (DOACs) do not require routine monitoring, the assessment of their effect on coagulation may be useful in some clinical situations (e.g. detecting drug accumulation in acute renal or hepatic failure; planning urgent invasive procedure; …). Our previous study mentioned that chromogenic anti-Xa assays seem to be useful to estimate the amount of betrixaban in plasma. Nevertheless, the sensitivity of available tests is limited and improvement is needed to encompass the on-therapy range which is from ± 9ng/mL at Ctrough to ± 122ng/mL at Cmax for 40 and 120mg once daily dose which is quite lower, even transformed in molar, than other direct factor Xa inhibitors.

AIMS: To improve the sensitivity of chromogenic anti-Xa assays to betrixaban by adjusting the sample dilution scheme of three frequently used commercial products.

METHODS/MATERIALS: Betrixaban was spiked at final concentrations ranging from 0 to 500ng/mL in normal pooled plasma. Three commercial tests were tested (Biophen® DiXal® (Hyphen BioMed, France), STA®Liquid Anti-Xa (Diagnostica Stago, France), HemosIL® Liquid Anti-Xa (Instrumentation Laboratory, USA)) and adaptation of the sample dilution were performed. In parallel, our newly proposed dilution schemes were tested on plasma spiked with UNF (Heparin Leo®, Leo Pharma, Denmark), with LMWH (tinzaparin sodium, Innohep®, Leo Pharma, Denmark) or with fondaparinux (Arixtra®, GSK, UK) to evaluate the sensitivity of these adapted assays to heparins and derivatives.

RESULTS: Results showed a concentration-dependent decrease in OD/min inversely proportional to the dilution of the samples. For the current (1/50) Biophen®DiXal®showed a ½OD/min of 447ng/mL whereas our adapted method of Biophen®DiXal®with a sample diluted 10-fold showed a ½OD/min of 81ng/mL. The CVs were always below 1.0%. Improvement of the LOD and LOQ were also noticed (from 10 to 31ng/mL for the current method and from 3 to 5ng/mL for the adapted method). Same results are observed with the other reagents. While modifications improve the sensitivity of this test, results also showed an increased sensitivity to heparins and fondaparinux for the Biophen®DiXal®which is, with the normal dilution procedure (i.e. 1/50), insensitive to indirect FXa inhibitors.

SUMMARY/CONCLUSIONS: Our results showed that the improvement of current methodologies makes the chromogenic anti-Xa assays more sensitive to betrixaban. However, this lower initial dilution of the samples makes the specific buffer of the Biophen®DiXal®inefficient to prevent sensitivity towards heparins and derivatives. This may be a problem in case of bridging therapy due to an overestimation of betrixaban concentrations. To avoid the cross-interference between DOACs and heparin and/or LMWH, the conception and the optimization of a new buffer should be explored.

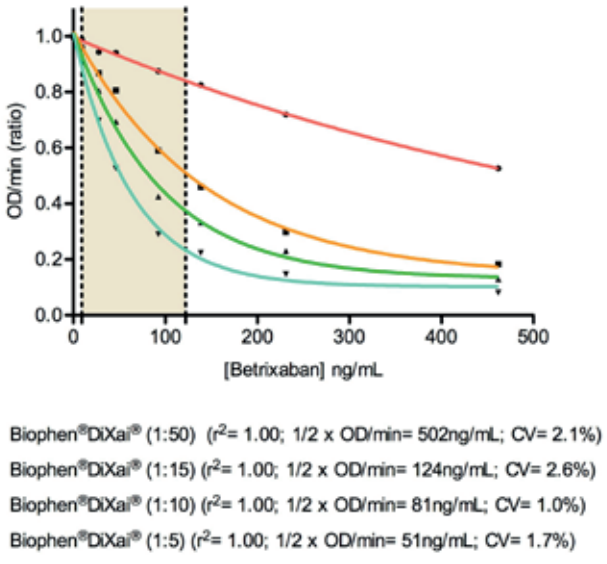

Figure 1: Impact of betrixaban on adapted methodologies with Biophen®DiXal®

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Clot waveform analysis: Determination of optimal wavelength to assess the fibrin coagulation process

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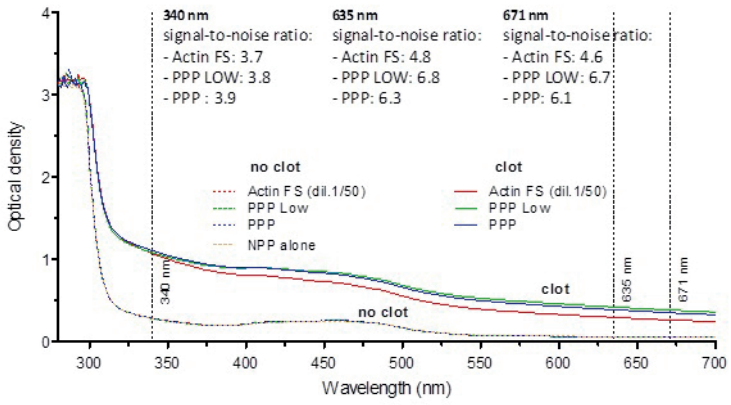
ABSTRACT: The fibrin generation test (FGT) monitors the coagulation process using a TECAN analyzer and provides an analysis of the optical waveform generated during clotting test. This allows the exploration of the coagulation process with more information than global coagulation tests (i.e. prothrombin time, thrombin time or activated partial thromboplastin time). The principle behind this test is also called clot waveform analysis (CWA), which is used to measure and record the time required for a plasma to clot. This technique assesses coagulation endpoint by measuring change in optical density. In the literature, routine tests generally measure fibrin clot formation at different wavelengths (340nm, 635nm or 671nm). The choice of wavelength for detection is crucial to the sensitivity of the assay, but the question is not dealt in the previous SSC report from subcommittee of the working group of the FVIII and IX on CWA

AIMS: This study tries to determine which wavelength is the most sensitive and specific to distinguish non-clotted and clotted plasma samples (the best signal-to-noise ratio)./provides the best signal-to-noise to monitor fibrin clot formation.

METHODS: The normal pool plasma (NPP) was screened at wavelength from 280 to 700nm, by step of 1nm on a TECAN® analyzer, to provide absorbance spectra of non-clotted and clotted plasma. The coagulation process was triggered by either the intrinsic pathway of the coagulation using Actin FS® (Siemens, Germany) or by the extrinsic pathway using PPP Reagent® or PPP Reagent Low® (Thrombinoscope BV, The Netherland's). Briefly, 20µL of reagent were mixed with 80µL of NPP in a 96-well microtiter plate and incubated for 3 minutes at 37°C. For clotted plasma, the coagulation process was initiated by the addition of 20µL of CaCl2 at 100mM while 20µL of physiological saline was added in non-clotted plasma to harmonize the volume of the reaction. The signal-to-noise between the 2 conditions was then calculated.

RESULTS: At baseline (i.e. before the sample starts to clot for the experiment with the addition of CaCl2), no difference in the absorbance was observed between the 2 conditions, whatever the wavelength used, showing that the addition of CaCl2 (before the coagulation process has started) has no impact on the absorbance of the sample. However, as expected, once the coagulation process has begun, clotted samples showed higher absorbance values than non-clotted samples, whatever the wavelength used. Interestingly, the absorbance of clotted and non-clotted samples was higher at 340nm than at 635 and 671nm ($p<0.05$). However, the signal-to-noise ratio was significantly higher at 635 or 671nm than at 340nm (e.g. 3.8 at 340nm; 6.8 at 635nm and 6.7 at 671nm with the PPP Reagent Low®) due to very low baseline absorbance at 635 and 671nm. Of note that the signal-to-noise ratio is higher with inducers of the extrinsic pathway of the coagulation (i.e. PPP-Reagent® and PPP-Reagent Low®).

CONCLUSION: This study confirms that the choice of the wavelength is crucial for CWA and the subsequent FGT. Wavelengths above 550nm seems preferable to assess fibrin clot formation. At 340nm, the signal-to-noise is lower and the analysis could be affected by individuals' characteristics (e.g. bilirubin) and should therefore be avoided.



Absorbance spectra of non-clotted and clotted plasma

Assessment of inner-filter of the reagents of the coagulation pathway on baseline absorbance in clot waveform analysis

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ABSTRACT: The principle of clot waveform analysis (CWA) is used to record the time required for a plasma to clot. This technique assesses coagulation endpoint by measuring change in optical density. The choice of wavelength for optical detection is crucial to the sensitivity of the assay and the use of colored reagents could interfere with initial absorbance plasma in this optical measurement method.

AIMS: This study aims to determine the inter-individual variability of baseline absorbance value in healthy subjects at the preferred wavelength and the impact of reagents from the intrinsic, extrinsic and common coagulation pathway on baseline absorbance.

METHODS: The fibrin clot formation is initially measured from 80µL of plasma mixed with 20µL of inducer of the intrinsic or extrinsic pathway. The coagulation process is triggered by the addition of 20µL of 100mM CaCl2.
To assess the inter-individual variability on baseline absorbance, 50 individual plasma (IP) were screened. To measure of baseline absorbance, the 20µL of reagent and the 20µL of CaCl2 were replaced by 40µL of phosphate buffer saline (PBS), this prevents the coagulation process.Thus, 80µL of IP were mixed with 40µL of PBS and absorbance was measured at 340, 635 and 671nm.
To investigate the inner-filter effects of reagents on plasma samples, the NPP was mixed with reagents of the intrinsic pathway (aPTT). To avoid triggering coagulation pathway, the 20µL of CaCl2 were replaced by 20µL of PBS.So, 80µl of NPP were mixed with 20µl of reagent and 20µl of PBS and absorbance was measured at 340, 635 and 671nm.
As reagents for prothrombin time (PT), thrombin time (TT) and dilute Russell's Viper venom time (DRVVT) contain calcium ions, the adjunction of these reagents on plasma form a clot within 15-40 seconds, limiting the assessment of the inner-filter effect of reagents on plasma. Thus, plasma samples were replaced by serum samples to avoid activation of the coagulation process. The inter-individual variability was assessed using the serum from 6 healthy individuals. The baseline absorbance of these sera were assessed at the same wavelengths than the IP samples which means that 80µL of individual serum (IS) were mixed with 40µL of PBS to preserve the dilution ratio. To assess the inner-filter effect of reagents on serum, NPS was screened with reagents of the extrinsic and common pathway. Twenty µL of reagents were mixed with 20µL of PBS and 80µL of NPS and screened at 340, 635 and 671nm.

RESULTS: The inter-individual variability on plasma and serum samples, for the 3 wavelengths tested, was ranging from 24% to 26% and from 38% to 40%. Except for STA®-C.K.Prest®, the reagents used in this study had no significant effect on the baseline absorbance(p<0.01). The intra-reagent coefficients of variation (CV) were below 5% for absorbance, except for STA®-C.K.Prest® (<5% at 340 and >10% at 635 and 671nm). PT, TT and DRVVT reagents did not have a significant effect on the baseline absorbance of serum(p>0.05). Compared to the range of measurement (mean ± 1*SD) obtained with the IS, none of the reagents showed a disturbance on the baseline absorbance.The intra-reagent CV were below 3% for absorbance.

CONCLUSION: Despite colorful appearance of reagents, most of commercialized reagents, excepting STA®-C.K.Prest®, can be used to perform CWA, suggesting that reagents containing kaolin should be avoided. The use of colorless reagents is not recommended but can be useful to limit interferences.

	340nm			635nm			671nm		
	Mean ± SD	CV (%)	p-value (<0.05)	Mean ± SD	CV (%)	p-value (<0.05)	Mean ± SD	CV (%)	p-value (<0.05)
Reagents of the intrinsic coagulation pathway									
Actin FS®	0.2977 ± 0.0798	1.20%	0.4798	0.0623 ± 0.0010	1.61%	0.9754	0.0574 ± 0.0007	1.33%	0.9525
SynthAFax®	0.3306 ± 0.0040	1.78%	0.9003	0.0639 ±0.0009	1.41%	0.8443	0.0591 ± 0.0005	0.93%	0.7961
SynthASil®	0.2919 ± 0.0052	1.74%	0.6079	0.0585 ± 0.0015	2.52%	0.7111	0.0540 ± 0.0011	2.04%	0.7232
Cephen®	0.3001 ± 0.0016	0.53%	0.9585	0.0708 ± 0.0026	3.68%	0.3552	0.0648 ± 0.0008	1.32%	0.3409
STA®-PTT-A®	0.3595 ± 0.0008	0.08%	0.1872	0.0755 ± 0.0009	1.15%	0.1581	0.0696 ± 0.0009	1.32%	0.1277
STA®-Cephascreen®	0.3318 ± 0.0036	1.08%	0.4640	0.0633 ± 0.0014	2.19%	0.8943	0.0584 ± 0.0013	2.17%	0.8625
STA®-C.K.Prest®	**0.7788 ± 0.0160**	**4.63%**	**<0.001**	**0.1740 ± 0.0178**	**10.12%**	**<0.001**	**0.1558 ± 0.0187**	**10.88%**	**<0.001**
Reagents of thrombin generation test									
PPP Reagent Low®	0.2670 ± 0.0035	1.30%	0.5091	0.0575 ± 0.0008	1.33%	0.6367	0.0534 ± 0.0007	1.33%	0.6634
PPP Reagent®	0.2690 ± 0.0030	1.10%	0.5365	0.0577 ± 0.0006	1.10%	0.6518	0.0532 ± 0.0009	1.74%	0.6546
PPP Reagent High®	0.2662 ± 0.0018	0.68%	0.4982	0.0570 ± 0.0003	0.54%	0.5948	0.0534 ± 0.0010	1.85%	0.6634
Serum									
	340nm			635nm			671nm		
	Mean ± SD	CV (%)	p-value (<0.05)	Mean ± SD	CV (%)	p-value (<0.05)	Mean ± SD	CV (%)	p-value (<0.05)
Reagents of the extrinsic coagulation pathway									
ReadiPlaT n®	0.4169 ± 0.0069	1.66%	0.7583	0.0735 ± 0.0006	0.75%	0.7804	0.0670 ± 0.0007	1.08%	0.8981
RecombiPlasTin 2G®	0.4218 ± 0.0019	0.44%	0.7188	0.0727 ± 0.0009	1.20%	0.7483	0.0661 ± 0.0010	1.51%	0.8584
Dade Innovin®	0.5853 ± 0.0013	0.34%	0.9724	0.0718 ± 0.0008	1.05%	0.7155	0.0652 ± 0.0006	0.85%	0.8161
PT HTF®	0.4373 ± 0.0048	1.10%	0.5995	0.0912 ± 0.0015	1.62%	0.5404	0.0826 ± 0.0006	0.76%	0.4452
PT Excel®	0.4556 ± 0.0075	1.64%	0.4745	0.0917 ± 0.0004	0.44%	0.5242	0.0830 ± 0.0007	0.87%	0.4313
PT Excel S®	0.4495 ± 0.0016	0.34%	0.5142	0.0907 ± 0.0004	0.42%	0.5554	0.0830 ± 0.0003	0.39%	0.4302
STA Neoplastine®	0.3955 ± 0.0015	0.38%	0.9392	0.0730 ± 0.0013	1.79%	0.7599	0.0667 ± 0.0018	2.67%	0.8829
STA Neoplastin CI®	0.4771 ± 0.0044	0.92%	0.3514	0.1004 ± 0.0008	0.75%	0.2930	0.0912 ± 0.0001	0.06%	0.2252
STA Neoplastin CI Plus®	0.4788 ± 0.0109	2.28%	0.3434	0.1009 ± 0.0010	0.99%	0.2848	0.0915 ± 0.0016	1.74%	0.2199
STA Neoplastin R®	0.4088 ± 0.0016	0.38%	0.8258	0.0795 ± 0.0006	0.69%	0.9790	0.0721 ± 0.0003	0.37%	0.8660
Reagents of the common coagulation pathway									
DRVV Screen®	0.4102 ± 0.0018	0.43%	0.8140	0.0810 ± 0.0006	0.75%	0.9195	0.0703 ± 0.0005	0.70%	0.9474
DRVV Confirm®	0.4498 ± 0.0055	1.23%	0.5121	0.0868 ± 0.0015	1.68%	0.6945	0.0790 ± 0.0013	1.65%	0.5729

Summary of impact of the reagents from the intrinsic, extrinsic and common pathway on the baseline absorbance of plasma and serum.

P27

Von Willebrand factor deficiency does not influence angiotensin II-induced abdominal aortic aneurysm formation in mice

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BACKGROUND: Abdominal aortic aneurysm (AAA) refers to a permanent, localized dilation of the abdominal aorta that exceeds its normal diameter by 50%. When left untreated, AAAs have a high risk of rupture, which is associated with a high mortality rate. AAA pathophysiology is characterized by progressive inflammation, proteolytic degradation, vessel wall destabilization and thrombus formation. Von Willebrand factor (VWF) is a multimeric plasma glycoprotein, known for its role in hemostasis, thrombosis, vascular stability and inflammation. Clinical studies have reported elevated VWF levels in AAA patients and suggested VWF as a potential biomarker for AAA growth. However, the exact role of VWF in AAA pathophysiology is currently not clear.

AIM: To investigate the potential thromboinflammatory involvement of VWF in AAA pathophysiology using an angiotensin II continuous infusion-induced AAA mouse model.

METHODS: AAA formation was induced in both wild type and VWF-deficient mice by subcutaneous implantation of an osmotic pump, which continuously released a dose of 1 µg/kg/min angiotensin II for 28 days. Survival in both groups was monitored for the duration of the experiment. After the infusion period, the surviving mice were sacrificed to harvest the abdominal aortas in order to determine AAA incidence and severity. Cryosections of the suprarenal segments were analyzed with different histological procedures to visualize the aneurysm and the associated intramural thrombus.

RESULTS: After implantation of the angiotensin II-infusing osmotic pumps, 19% (3/16) of VWF-deficient mice did not reach the end of the 28 day-infusion period. In comparison, all wild type mice (n=15) survived; however, this difference was not statistically significant (p=0.08). After 28 days, both AAA incidence and severity were assessed from the isolated suprarenal segments. Overall AAA incidence was not statistically different between VWF-deficient mice (7/13; 54%) and wild type animals (5/15; 33%) (p=0.4). Also, overall severity was not significantly different between the two groups after classification of the mice based on the appearance of the abdominal aorta. Accordingly, the maximal abdominal aortic diameter in wild type animals (1.6 ± 0.7 mm) was comparable to the maximal diameter in VWF-deficient mice (1.9 ± 0.6 mm, p = 0.3). Despite increased VWF plasma levels (169 ± 45%) in the wild type mice after the angiotensin II infusion period, these levels were not correlated with AAA severity. Additionally, detailed histological analyses of important hallmarks of AAA, including elastic degradation, intramural thrombus formation and leukocyte infiltration were assessed. Four wild type mice (27%) developed an aneurysm with an intramural thrombus containing VWF. Interestingly, four VWF-deficient mice (31%) also formed an intramural thrombus in the absence of VWF. Measurement of both fibrin and red blood cell content in these thrombi did not reveal differences in thrombus composition between both groups. In addition, staining of leukocytes in the suprarenal aortic tissue revealed a similar extent of inflammation in both wild type and VWF-deficient mice.

CONCLUSION: Overall, no significant differences were observed between wild type and VWF-deficient mice in angiotensin II infusion-induced AAA. These data suggest that, at least in this mouse model, the role of VWF in AAA pathophysiology is limited.

P28

The mechanism of the loss of HMW VWF multimers after left ventricular assist device implantation is different between humans and sheep

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BACKGROUND: Acquired von Willebrand syndrome has been linked to the bleeding diathesis observed in patients treated with an LVAD as they present with a loss of high molecular weight (HMW) von Willebrand factor (VWF) multimers. We previously (BSTH 2017) showed for the first time that by specifically blocking

ADAMTS13, using the inhibitory anti-ADAMTS13 monoclonal antibody (mAb) 3H9, the loss of HMW VWF multimers was prevented in an in vitro LVAD circuit with human blood. However, it remains to be determined if blocking ADAMTS13 is an effective novel therapy to rescue the loss of HMW VWF multimers in a preclinical animal model. Aim: To investigate if an inhibitory anti-ADAMTS13 mAb can (1) prevent the loss of HMW VWF multimers in an in vitro Impella CP® system with ovine blood and (2) rescue the loss of HMW VWF multimers in an in vivo preclinical ovine Impella CP® model.

METHODS: Since mAb 3H9 does not cross react with sheep ADAMTS13, we used a novel inhibitory anti-ADAMTS13 mAb 17C7 (30 µg/mL), which potently inhibits both human and sheep ADAMTS13 and a non-inhibitory anti-ADAMTS13 mAb 5C11. Ovine blood was circulated through an in vitro Impella CP® system (n=4) and blood was sampled 5 min before and 5, 30 and 60 min after start of the pump. Plasma samples were analysed for VWF multimers.
For the in vivo study, Impella CP® pumps were implanted in sheep (n=8). One dose of 600 µg/kg of the inhibitory mAb 17C7 (n=4) or phosphate buffered saline (PBS) (n=4) was injected 1 day after Impella implantation. Blood was sampled before implantation, 30 minutes and 1 day after pump implantation and 1 and 2 days after injection of mAb 17C7 or PBS. ADAMTS13 inhibition and the loss of HMW VWF multimers were determined.

RESULTS: In vitro, control experiments (using mAb 5C11) lead to a 54±11% reduction of HMW VWF multimers in the Impella CP® circuit, 60 min after pump initiation (p=0.003). In contrast to our previous in vitro data with human blood and the mAb 3H9, blocking ovine ADAMTS13 using the mAb 17C7 did not prevent the loss of HMW VWF multimers in the in vitro Impella CP® circuit as HMW VWF multimers were still 42±10% decreased, 60 min after start of perfusion (p=0.003). The efficacy of mAb 17C7 was also tested in a preclinical ovine Impella CP® model. As expected, HMW VWF multimers significantly decreased one day after Impella implantation (n=8, 55±8.6% decrease, p<0.0001) and HMW multimers remained decreased when PBS was injected (n=4, 53±8.0% decrease). However, in agreement with the in vitro experiments, injection of mAb 17C7 did not rescue the loss of HMW VWF multimers (n=4, 57±12% decrease) although ADAMTS13 was blocked in all sheep (n=4, 8.2±4.7% ADAMTS13 activity, 1 day after injection of 17C7 compared to 81.0±53.6% before injection). To prove that the mechanism of the loss of HMW VWF multimers is different between sheep and humans, we performed an in vitro Impella CP® experiment with human blood and showed that addition of mAb 17C7 (as observed with mAb 3H9) did prevent the loss of HMW VWF multimers (14.6±17% decrease versus 68±7.1% with PBS).

CONCLUSION: Blocking ADAMTS13 in an in vitro Impella CP® circuit with ovine blood, could not prevent the loss of HMW VWF multimers. Although ADAMTS13 could be inhibited in a preclinical ovine Impella CP® model, the shear-induced proteolysis of VWF could not be rescued. Taken together, these data indicate that in sheep other mechanisms than ADAMTS13 are responsible for the loss of HMW VWF multimers.

Why do some grafts, used in RVOT (Right Ventricular Outflow Tract) revalvulation, get infected and others do not?

P29

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BACKGROUND: RVOT reconstruction in congenital heart disease can be surgically done using cryopreserved pulmonary homograft (CH) and alternatively xenografts such as the bovine jugular vein (BJV) valved conduit. Despite this good therapeutic alternative recent clinical studies report an increased risk of infective endocarditis (IE) in BJV. This raises the question of why such valves are more prone to IE than homografts.

OBJECTIVES: We investigate whether different graft tissues promote interactions with plasma components and blood cells and therefore enhance the risk for S. aureus adhesion to valve tissue.

METHODS: Tissues, prepared as for clinical use, were incubated with fluorescently labelled fibrinogen (Fg) as one of abundant plasma proteins. Then, S. aureus adhesion to the same tissues was assessed under flow conditions using a parallel plate flow chamber after tissue preincubation with PBS, human plasma, albumin or serum. Moreover, tissue susceptibility to interaction with platelets was evaluated upon perfusing whole blood using a colorimetric assay. To document a contribution of Fg-mediated pathway to the interplay bacteria-tissue-platelets, bacterial mutants and anti-platelet drugs were employed. Protein binding to tissues was quantified with fluorescence microscopy and bacterial adhesion was evaluated by CFU counting on blood agar. Bacteria and platelets were visualized on the tissues with confocal or electron microscopy.

RESULTS: Bovine pericardium patch presented higher protein binding (P< 0.05) compared to BJV and CH. Although not significant, there is a slight trend to higher Fg interaction with BJV than with CH. After incubation with plasma S. aureus adhesion to BJV increased significantly under flow conditions compared to the respective controls (human serum P< 0.05 and albumin P< 0.01). Both bacterial and platelet adhesions to BJV were greater in relation to CH (P< 0.001 and P< 0.05 respectively). Moreover, deletion of clfA hampered bacterial adhesion to BJV (P=0,07) as well as eptifibatide significantly reduced (P< 0.001) platelet reactivity towards BJV.

CONCLUSIONS: Our results indicate that the plasma glycoprotein fibrinogen modulates differently S. aureus adhesion to the tested tissues, especially BJV. The role of the Fg-mediated pathway is of vital importance for S. aureus recruitment to endovascular tissues. Similarly grafts differ in their susceptibility to bind platelets and this might predispose for bacterial adhesion. The interaction Fg-integrin allbb3 receptor strongly contributes to the binding of platelets to the graft tissue. Moreover, the correlation analysis reveals common pathway(s) that potentially mediates recruitment of both platelets and bacteria.
Future studies will focus on endothelialization of grafted tissues in vivo and how this affects lesion formation and development of valvular infection. Moreover, anti-platelet therapy will be addressed to study its potentialbeneficial effect on IE prevention.

P30

VWF deficiency is associated with reticulocytosis and increased parasite accumulation in experimental malaria-associated acute respiratory distress syndrome

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BACKGROUND: Malaria remains a global health problem, with 216 million documented cases, resulting in 445,000 deaths in 2016. One of the most severe symptoms is malaria-associated acute respiratory distress syndrome (MA-ARDS), characterized by marked pulmonary inflammation. Patient studies have demonstrated that severe malaria is associated with acute endothelial cell activation, accumulation of highly active von Willebrand factor (VWF) multimers, and decreased ADAMTS13 activity.

AIMS: To investigate how VWF affects the pathogenesis of MA-ARDS.

METHODS: 10^4 *Plasmodium berghei (Pb)* NK65 parasites were injected intraperitoneally in wild-type (WT) and VWF knockout (Vwf-/-) mice which are on a C57BL/6 background. Blood samples were taken to assess plasma VWF and ADAMTS13 levels as well as platelet counts. Parasitemia levels were monitored by examination of Giemsa-stained blood smears. Total protein levels in bronchoalveolar lavage fluid were determined as a measure for pulmonary edema. Quantitative RT-PCR was used to determine parasite accumulation in the lungs. Reticulocyte counts were evaluated using flow cytometry, and in vivo biotinylation was performed to monitor red blood cell (RBC) lifespan.

RESULTS: *Pb*NK65-infected mice had increased plasma VWF levels and reduced ADAMTS13 activity levels (2.2-fold increase, $p<0.0001$ and 1.7-fold decrease, $p<0.01$, respectively). Malaria-associated thrombocytopenia was VWF-independent, as thrombocytopenia was observed in both WT and *Vwf*-/- mice. *Vwf*-/- mice died more rapidly compared to WT mice (9 days vs 10 days post-infection, $p<0.001$). Lung edema could not explain this shortened survival time, since alveolar permeability, measured by protein leakage, was 2-fold lower in *Vwf*-/- mice compared to WT animals ($p<0.01$). Parasite accumulation, on the other hand, was significantly increased in *Vwf*-/- mice compared to WT mice both in blood circulation and lung tissue (1.9-fold and 8-fold increase at day 8 post-infection, $p<0.0001$ and $p<0.001$, respectively). Remarkably, reticulocyte counts were elevated approximately 2-fold in *Vwf*-/- mice following infection. This possibly explains the high parasitemia in *Vwf*-/- mice since PbNK65 preferentially infects reticulocytes. Interestingly, biotinylation experiments revealed that the RBC lifespan in non-infected *Vwf*-/- mice was significantly shorter than that observed in WT mice ($p=0.001$), which could account for the increased reticulocyte counts in *Vwf*-/- mice upon malaria infection.

CONCLUSION: In accordance with patient studies, experimental MA-ARDS is associated with early elevated plasma VWF levels and decreased ADAMTS13 activity. Thrombocytopenia is independent of VWF. Parasite accumulation might contribute to shorter survival in Vwf-/- mice, rather than alveolar leakage. VWF deficiency is associated with increased numbers of reticulocytes following malaria infection, which lead to an increase in parasite accumulation. This increase in reticulocyte production could be due to the faster RBC clearance and production in Vwf-/- mice.

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