2023
PhD Course
&
Lustrum Symposium

Nederlandse Vereniging voor Trombose en Hemostase
The Dutch Society on Thrombosis and Haemostasis

Monday March 27 – Friday March 31

Strandhotel Westduin, Koudekerke, NL

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A word from the NVTH President

Dear NVTH-ers, dear PhD Course & Symposium attendees,

Welcome to the annual NVTH PhD Course & Symposium, which is not only dedicated to sharing science with and learning from colleagues, but in which we will also celebrate the 35th anniversary of the NVTH!

As the NVTH was founded in March of 1988, this year the VIIth Lustrum is held. The Lustrum Committee has worked hard towards a special program that will kick-off Thursday afternoon and will culminate in a *vibrant party* on location in Vlissingen. We are happy to welcome both junior and seasoned NVTH-ers in our midst to celebrate this milestone with us.

But first, the PhD students will have been immersed in all basic and clinical aspects of arterial thrombosis, with ample of time dedicated to get to know each other through group activities (both science- and survival-based…).

Immediately after the PhD Course the NVTH community will gather to enjoy some outstanding thrombosis and hemostasis research shared to you by four excellent keynote speakers, twenty oral abstract presentations, and 39 (!) poster pitches. A program jam-packed with novel science: we are proud to provide a platform for all junior researchers to share and discuss their work.

And of course, your coagulation and NVTH knowledge will be quizzed, and the traditional beach BBQ at Kon-Tiki will allow for plenty of networking.

We wish you a highly informative, fun, and festive NVTH PhD Course and Lustrum Symposium!

On behalf of the NVTH board,

Mettine Bos

NVTH president


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NVTH PhD Course Arterial Thrombosis – 27-29, March 2023

Monday 27th March, 2023

12.00 – 13.00	Registration and lunch
13.00 – 13.05	*Introduction* Marieke Kruip
13.05 – 13.45 (B)	*Atherosclerosis, inflammation and coagulation* Henri Spronk
13.45 – 14.25 (C)	*The clinical presentations of arterial thrombosis* Hugo ten Cate
14.25 – 15.00 (C)	*Peripheral Arterial Disease (PAD)* Jenneke Leentjes
15.00 – 15.30	Coffee break
15.30 – 16.10 (B)	*Platelets in the pathophysiology of arterial thrombosis* Judith Cosemans
16.10 – 16.50 (B/C)	*Cardiovascular diseases in different vascular beds* Heleen Van Beusekom
16.50 – 17.30 (B/C)	*Diagnosis and treatment of acute ischemic stroke* Paula Janssen
18.00 – 19.30	Dinner
19.30 – 21.00	*Next Generation Researchers – Laptop session* PhD students present (max 3 slides) themselves, their project, and their favourite song in small groups

NVTH PhD Course Arterial Thrombosis – 27-29, March 2023

Tuesday 28th March, 2023

07.30 – 08.30	Breakfast
08.30 – 09.10 (B)	*Plaque rupture or plaque erosion; (animal) models for arterial thrombosis* Marijke Kuijpers
09.10 – 09.50 (B)	*Lipoproteins as modulators of atherotrombosis* Miranda van Eck
09.50 – 10.30 (C)	*Women's health in cardiovascular disease* Jeanine Roeters van Lennep
10.30 – 11.00	Coffee break
11.00 – 11.40 (C)	*Antithrombotic treatment and reversal; what are the options?* Renske Olie
11.40 – 12.20 (C)	*primary prevention and diagnosis and treatment of cardiovascular disease* Jur ten Berg
12.20 – 13.00 (B)	*Calcification and treatment with vitamin K antagonists* Leon Schurgers
13.00 – 14.00	Lunch
14.00 – 16.00 (B/C)	*assignment*
16.00 – 17.00 (B/C)	*Presentations*
17.00 -	Dinner
18.30 -	Workshop

NVTH PhD Course Arterial Thrombosis – 27-29 March, 2023

Wednesday 29th March, 2023

07.30 – 08.30	Breakfast
08.30 - 09.10 (C)	*Role of von Willebrand factor and ADAMTS13 in arterial thrombosis* Frank Leebeek
09.10 – 09.35 (B)	*Antiphospholipid syndrome and arterial thrombosis; basic science* Rolf Urbanus
09.35 – 10:00 (C)	*Antiphospholipid syndrome from a clinical point of view* Gerard Jansen
10.00 - 10.30 (C)	*Patient demonstration (antiphospholipid syndrome)* Gerard Jansen
10.30 – 11.00	Coffee break
11.00 – 12.00	*Panel discussion: artificial intelligence* Bjorn van der Ster, William van Doorn
12.00 Lunch	

(B) indicates lectures with Basic science focus
(C) indicates lectures with Clinical focus


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NVTH Symposium – March 29-31,2023

Wednesday March 29th, 2023

12.00 – 13.00 Registration and Lunch

13.00 – 13.30 **Keynote Lecture I – Marjon Cnossen (Erasmus MC) – What the SYMPHONY consortium teaches us on hemophilia** – moderator Heleen van Ommen (Erasmus MC)

13.30 – 14.30 **Session I – Coagulation biochemistry** – moderators Leon Schurgers (CARIM) & Yvonne Jongejan (LUMC)

Todaro, Alic	In vitro and ex vivo rescue of a nonsense mutation (F5 p.Arg1161Ter) responsible for severe coagulation factor V deficiency
Bar Barroeta, Awital	Thrombin activation of the factor XI dimer is a multi-staged process for each subunit
Noordermeer, Tessa	Anti-β2-glycoprotein I antibodies cause activated protein C resistance by interfering with Factor V cleavage at Arginine 506
Frunt, Rowan	Two positively charged patches in the EGF-1 domain of Factor XII synergistically contribute to surface binding and activation

14.30 – 14.45 **Poster Pitches I. Clinical research** – Moderator Ton Lisman (UMCG)

Poolen, Geke	A Neural Network Approach to Predict Recurrent VTE Based on Coagulation Parameters
Poolen, Geke	A decreased endogenous thrombin potential is associated with recurrent venous thromboembolism
Spiegelenberg, Janneke	High fibrinogen and low antithrombin is associated with an increased risk of recurrent cardiovascular event in young ischemic stroke patients
Chen, Qingui	Epidemiology of antithrombotic therapy during pregnancy in The Netherlands (2013-2019)
Chen, Qingui	Anticoagulant use and prognosis in patients with atrial fibrillation and cancer: a nationwide population-based study
Iding, Aaron	Chronic inflammatory diseases increase the risk of post-thrombotic syndrome: a prospective cohort study a prospective cohort study
Ko, Amica	Effect of pegylated asparaginase on coagulation parameters and thrombin generation in adults with acute lymphoblastic leukemia
Barakzie, Aarazo	Effect of intravenous tPA treatment on hemostatic factors in relation to outcome in acute ischemic stroke patients undergoing thrombectomy
Burggraaf, Louise	Trends in anticoagulant treatment duration and outcomes after pulmonary embolism between 2014 and 2019: a nationwide cohort study
Lanting, Vincent	Prediction of on treatment recurrent venous thromboembolism in patients with cancer: an individual patient data meta-analysis of randomized controlled trials
Han, Jihee	The association between microvascular health and coagulation parameters: the Netherlands Epidemiology of Obesity Study

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Kruijt, Mirjam	Next-generation antithrombin diagnostics by mass spectrometry
Camilleri, Eleonora	Bridging therapy and risk of major bleeding and thrombosis in continuous-flow left ventricular assist device patients: a quasi-experimental study
Willems, Ruth	Patients with pancreatic cancer have a disturbed whole blood thrombin generation profile
Monard, Amaury	Evaluation of innovative laboratory tests to predict a thrombotic phenotype in a family with dysfibrinogenemia and a novel FGG mutation

14.45 – 15.15 Coffee/Tea break

15.15 – 15.45 **Keynote Lecture II – Kathleen Freson (KU Leuven) – Culturing genetically modified platelets** – Moderator Ton Lisman (UMCG)

15.45 – 16.45 **Session II – Clinical thrombosis** – Moderators Mandy Lauw (Erasmus MC) & Bente van den Boom (UMCG)

Strijdhorst, Anniek	Risk of on-treatment recurrent venous thromboembolism in patients with active vs cured cancer patients: a post-hoc analysis of Hokusai VTE cancer
Iding, Aaron	Fibrin clot properties identify patients that benefit from catheter-directed thrombolysis: A post hoc analysis of the CAVA trial A post hoc analysis of the CAVA trial
Smeets, Mark	Age- and sex specific risks of major cardiovascular complications and death following elective hip and knee arthroplasty in the Netherlands: a Dutch Hospital Data Registry study
Visser, Chantal	The impact of pulmonary embolism on the long-term health outcomes of COVID-19

16.45 – 17:15 **Poster Pitches II. Basic research** – Moderator Mettine Bos (LUMC)

Bär, Isabel	Phenotypic characterization of Von Willebrand disease type 3 patient-derived ECFCs with a homozygous p.M771V mutation
Schönichen, Claudia	Inflammatory endothelial cells regulate platelet reactivity: using multi-omics to decipher endothelial (dys)regulation of platelets in inflammatory vessel-on-a-chip-models
Smit, Eva	Plasma proteomics in the diagnosis and risk prediction of thrombotic events
Vliet, Sabien van	Characterization of activation intermediates of a factor IX variant with cofactor-independent activity
Zivkovic, Minka	Functional characterization of a nanobody based GPVI specific platelet agonist
Bröker, Vanessa	Tissue factor pathway inhibitor attenuates calcium-induced vascular smooth muscle calcification
Dijk, Willian van	Alterations in thrombin generation after SARS-CoV-2 vaccination and the relation with inflammation

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Dijk, Willian van	Thrombin generation potential and the risk of severe COVID-19 infection
Duijl, Tirsa van	Multiplex quantitation of coagulation and fibrinolysis markers: an upcoming approach to unravel bleeding and thrombotic disorders
Fan, Dongyue	Unveiling mechanisms involved in venous thromboembolism in colorectal cancer patients
Gehlen, Rachel	Epitope Specificity of Anti-prothrombin Antibodies That Express Lupus Anticoagulant Activity
Groten, Stijn	Integrative phosphoproteomics of EC-hemostatic interactions
Kreft, Iris	Quantification of von Willebrand factor proteoforms by mass spectrometry-based proteomics
Arisz, Ryanne	Thrombodynamics: a Novel Assay for Diagnostic Evaluation of Haemophilia A Patients
Baaten, Constance	Platelet activating and fibrin generating properties of distinct vascular smooth muscle cell phenotypes
Diest, Rianne van	Development of in silico models to evaluate direct oral anticoagulant binding to factor Xa variants
Leoni, Michela	Liver sinusoidal endothelial cells as a novel target for tolerance induction to FVIII
Mourik, Dagmar van	Role of contact activation pathway in antiphospholipid syndrome: a case-control study
Veizaj, Dejvid	Using AlphaFold2 and Molecular Dynamics simulations to model factor Xa – substrate binding in silico
Agten, Stijn van	Differential effects of vitamin-K dependent proteins on vascular calcification: chemical protein synthesis and bulk RNA sequencing to unravel cellular pathways
Hordijk, Sophie	Proximity biotinylation proteomics to identify novel regulators of Weibel-Palade body morphology and von Willebrand factor secretion
Steeghs, Danique	Novel luminescent-based ADAMTS13 activity assay
Zheng, K.L.	Platelet reactivity in patients with Atrial Fibrillation and Coronary Artery Disease under Factor IIa inhibitors and Factor Xa inhibitors
Laan, Bas	Automated segmentation and quantitative analysis of Weibel-Palade body morphology, localization and content using CellProfiler

17.10 – 17.30 **Presentation NVTH Awards**

17.30 – 18.45 **Poster Session** + Drinks, Snacks

19.00 **Dinner at Kontiki (beach)**

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NVTH Symposium – March 29-31,2023

Thursday March 30th, 2023

07.30 – 09.00	Breakfast
09.00 – 09.30	**Keynote Lecture III – Roger Schutgens (UMC Utrecht) – Care for the rare** – Moderator Marieke Kruip (Erasmus MC)
09.30 – 10.30	**Session III - VWF and platelets** – Moderators Rolf Urbanus (UMCU) & Magdi Nagy (CARIM)
Jongejan, Yvonne	Effect of allele-selective silencing of von Willebrand factor in mice on experimental bleeding and thrombosis models
Linthorst, Noa	Allele-selective inhibition of mutant von Willebrand factor with small interfering RNAs to ameliorate a von Willebrand disease type 2B phenotype in vivo in mice
Postmus, Tim	N-glycan-mediated shielding of ADAMTS13 effectively prevents binding of pathogenic autoantibodies in high-titer patients with immune-mediated TTP
Zivkovic, Minka	HMB-001 – a novel bispecific antibody accumulating and targeting endogenous FVIIa to activated platelets supports enhanced haemostatic responses in models of Glanzmann thrombasthenia
10.30 – 11.00	Coffee / Tea Break
11.00 – 12.00	**Session IV - Clinical hemostasis** – Moderators Karina Meijer (UMCG) & Iris van Moort (Erasmus MC)
Vaan, Anne de	Pregnancy and Inherited bleeding DisordErs Study: interim safety analysis after revision of the Dutch guidelines.
Zwet, Konrad van der	Emicizumab plasma concentrations are not related to bleeding rates using standard dosing, a call for dose adaptation
Han, Jihee	The association between venous thrombosis-associated genetic variants and coagulation factor levels and thrombin generation potential
Elling, Tessa	Frequency of INR monitoring and their impact on INR control during the COVID-19 pandemic
12.00 – 13.00	Lunch
13.00 – 13.30	**NVTH Annual Meeting**

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13:30 – 14:00	**Keynote Lecture IV – Waander van Heerde (Radboud University Medical Center & Enzyre BV – Past, present and future of Enzyre** – Moderator Coen Maas (UMCU)
14:00 – 15:00	**Session V - Cells & omics –** Moderators Bart van Vlijmen (LUMC) & Tirsa van Duijl (Sanquin)

Bouwens, Bryan	Detection of TFPI citrullination in blood after neutrophil activation by PMA
Del Castillo, Jessica	Peptidomics reveals expected and unexpected proteolytic events in plasma upon activation of the coagulation cascade
Groten, Stijn	The in vitro proteomic landscape across inflamed vascular beds
Ouazzani Chahdi, Naoual	RBC-derived transglutaminase-2 affects thrombus formation

15:00 – 15:30	Coffee / Tea Break
15:30 – 16:00	**NVTH LUSTRUM QUIZ**
16.00 – 16.20	**The 35th birthday of NVTH – Tilman Hackeng (Maastricht University)**
16.20 – 17.15	**Presentation Jeanne Stibbe bokaal** **Quiz results** **Uitreiking Erepenningen**
17.30	**Travel to Vlissingen for lustrum party by bus**
18.00	**Start lustrum party!**
24.00	**End of lustrum party, travel to hotel by bus**

Friday March 31st, 2023

07.00 – 10.00	Breakfast + check-out hotel

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Abstracts Oral Sessions

Wednesday March 29 - Session I. Coagulation biochemistry

In Vitro And Ex Vivo Rescue Of A Nonsense Mutation (F5 P.Arg1161ter) Responsible For Severe Coagulation Factor V Deficiency

A.M. Todaro1, C.M. Radu2, M. Ciccone3, S. Toffanin2, M.L. Serino3, E. Campello2, C. Bulato2, B. Lunghi4, D. Gemmati3, A. Cuneo3, T.M. Hackeng1, P. Simioni2, F. Bernardi4, E. Castoldi1 1Department of Biochemistry, CARIM, Maastricht University, Maastricht (The Netherlands) 2Department of Medicine, Thrombotic and Haemorrhagic Diseases Unit, University of Padua Medical School, Padua (Italy) 3Department of Medical Sciences, Section of Haematology, Sant'Anna Hospital, Ferrara University, Ferrara (Italy) 4Department of Life Sciences and Biotechnology, Section of Biochemistry and Molecular Biology, Ferrara University, Ferrara (Italy)

Introduction Coagulation factor V (FV) deficiency is a rare autosomal recessive bleeding disorder that is usually managed with fresh-frozen plasma. The F5 c.3481C>T nonsense mutation, introducing a premature stop codon in exon 13 (p.Arg1161Ter), is recurrent among FV-deficient patients. Detailed DNA, mRNA and functional analyses in a symptomatic homozygous patient suggested that this mutation might respond to ribosome readthrough agents.
Aim To assess the correction potential and cytotoxicity of five ribosome readthrough agents with different mechanisms of action (G418, ELX-02, PTC-124, 2,6-diaminopurine and amlexanox) in in vitro and ex vivo models of the F5 p.Arg1161Ter mutation.
Methods In vitro model: COS-1 cells transfected with wild-type or mutant FV cDNA were treated with readthrough agents (0-500 μM) for 48 hours. FV activity in conditioned media was determined using a prothrombinase-based assay and thrombin generation in reconstituted FV-depleted plasma. Cell viability was evaluated with an XTT-based assay. Ex vivo model: Following written informed consent, ex vivo differentiated megakaryocytes from a homozygous patient were treated with each readthrough agent for 7 days and FV expression was probed by immunofluorescence with anti-FV antibodies.
Results COS-1 cells transfected with the FV p.Arg1161Ter cDNA secreted hardly any functional FV (FV activity: 0.8±0.3% of wild-type). Treatment with G418, ELX-02 and 2,6-diaminopurine increased FV activity in a dose-dependent manner (up to 4.7-fold, 2.8-fold and 13.6-fold, respectively), while PTC-124 and amlexanox were ineffective. All readthrough agents except ELX-02 showed some degree of cytotoxicity. Untreated patient's megakaryocytes were negative at FV immunostaining, but turned positive upon treatment with all five readthrough agents. Notably, the patient's megakaryocytes were also able to internalise the FV produced by readthrough-mediated rescue of the p.Arg1161Ter mutation, a necessary requirement for maintaining the platelet FV pool in vivo.
Conclusions This study provides in vitro and ex vivo proof-of-principle for the efficacy of ribosome readthrough agents in rescuing the F5 p.Arg1161Ter mutation.

Thrombin Activation Of The Factor XI Dimer Is A Multi-Staged Process For Each Subunit

Awital Bar Barroeta1 , Pascal Albanese2,3, J. Arnoud Marquart1 , Richard A. Scheltema2,3, Joost C.M. Meijers1,4 1 Department of Molecular Hematology, Sanquin, Amsterdam, the Netherlands 2 Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Utrecht, The Netherlands 3 Netherlands Proteomics Centre, Utrecht, The Netherlands 4 Department of Experimental Vascular Medicine, Amsterdam UMC, University of Amsterdam, Amsterdam, the Netherlands

Background: Factor XI (FXI), a protein in the intrinsic coagulation pathway, can be activated by two enzymes. In hemostasis, FXI is activated by thrombin, while FXIIa-mediated activation of FXI is thought to be prothrombotic. The interactions of these enzymes with FXI are transient in nature and therefore difficult to study in a structural context.
Aims: To identify the binding interface between thrombin and FXI and understand the dynamics underlying FXI activation.
Methods: Crosslinking mass spectrometry (XLMS) was used to construct the FXI homodimer and to localize the binding interface of thrombin on FXI. Molecular dynamics simulations were then applied to investigate conformational changes after binding. Additionally, the binding site of nanobody 1C10 – previously shown to inhibit thrombin-mediated activation of FXI – was investigated with hydrogen-deuterium exchange mass spectrometry (HDX MS).
Results: We identified a binding interface of thrombin located on the light chain of FXI. After this initial interaction, FXI undergoes conformational changes driven by binding of thrombin to the apple 1 domain in a secondary step to allow for migration towards the FXI cleavage site. The 1C10 binding site to the apple 1 domain supports this proposed trajectory of thrombin.
Conclusions: Our investigations show that the activation of FXI is a multi-staged procedure. Thrombin first binds to Pro520 in FXI, after which it migrates towards the activation site by first engaging the apple 1 domain and, finally, Arg378. We further validated the results with known mutation sites on FXI and additionally found that Pro520 is conserved in PK. Through this site, thrombin can bind PK even though it cannot activate PK. This detailed analysis of the interaction between thrombin and FXI points a way for future interventions for bleeding or thrombosis.

Anti-B2-Glycoprotein I Antibodies Cause Activated Protein C Resistance By Interfering With Factor V Cleavage At Arginine 506

Tessa Noordermeer1, Soumaya Chemlal1, Jessica E. Molhoek2, Roger E.G. Schutgens1, Maarten Limper3, Philip G. de Groot4, Joost C.M. Meijers5,6, Rolf T. Urbanus1 1. Center for Benign Haematology, Thrombosis and Haemostasis, Van Creveldkliniek, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands 2. Central Diagnostic Laboratory, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands 3. Department of Rheumatology and Clinical Immunology, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands 4. Synapse B.V., Maastricht, the Netherlands 5. Department of Molecular Hematology, Sanquin Research, Amsterdam, the Netherlands 6. Department of Experimental Vascular Medicine, Amsterdam UMC, University of Amsterdam, the Netherlands

Background: The acquired thrombotic risk factor known as lupus anticoagulant (LA) is detected as a phospholipid-dependent prolongation of the clotting time and can be caused by autoantibodies against β2-glycoprotein I (β2GPI). LA is associated with activated protein C (APC) resistance, which might contribute to thrombotic risk in patients with LA. How anti-β2GPI antibodies cause APC resistance is currently unclear. We have previously shown that antiβ2GPI antibodies cause LA by attenuating factor (F)V activation by FXa through a direct interaction with FV. As FV is central to the anticoagulant properties of APC, we hypothesized that the interaction between β2GPI-antibody complexes and FV also causes APC resistance.
Aim: To investigate how anti-β2GPI antibodies induce APC resistance.
Methods: The effects of anti-β2GPI antibodies on APC resistance were studied in LA-positive patient plasma and with monoclonal antibodies.
Results: APC resistance was observed in LA-positive patients with anti-β2GPI antibodies and in normal plasma supplemented with monoclonal anti-β2GPI antibodies using LA-sensitive clotting assays with the snake venom protein C activator protac. Anti-β2GPI antibodies only interfered with APC-mediated cleavage of FV, not FVa: When FV was activated with Russell's viper venom FV activator, the inhibitory effect of anti-β2GPI antibodies on APC activity in plasma was lost. Anti-β2GPI antibodies had no effect on APC-mediated FVa inactivation in a purified system either. Analysis of FV cleavage patterns after incubation with APC indicated that anti-β2GPI antibodies attenuated APC-mediated cleavage of R506 and R306 in FV. APC-mediated cleavage at R506 is required for FV cofactor activity during inactivation of FVIIIa. Assays with purified coagulation factors confirmed that anti-β2GPI antibodies interfered with the cofactor function of FV during inactivation of FVIIIa.
Conclusion: Anti-β2GPI antibodies with LA activity contribute to a procoagulant state by causing APC resistance via interference with the cofactor function of FV during FVIIIa inactivation.

Two Positively Charged Patches In The EGF-1 Domain Of Factor XII Synergistically Contribute To Surface Binding And Activation

Rowan Frunt1 , Hinde El Otmani1 , Simone Smits1 , Chantal Clark2 , Coen Maas1 1CDL Research, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands 2Center for Benign Hematology, Thrombosis and Hemostasis – Van Creveldkliniek, Utrecht, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands

Background. Factor XII (FXII) contributes to medical device-associated thrombosis by binding to foreign surfaces. We and others identified its EGF-1 domain as a surface binding module. Aim. To explore the contribution of two positively charged patches in the FXII EGF-1 domain to surface binding and contact activation.
Methods. We studied surface binding and activation of recombinant FXII variants in buffer or FXIIdeficient plasma in pull-down assays, chromogenic substrate conversion assays and clotting assays.
Results. FXII EGF-1 contains 11 positively charged residues. These can be divided into two distinct patches. The first patch (upstream; K73, K74, K76, H78, K81 and H82) is FXII-specific. The second patch (downstream) contains three histidines (H99, H105 and H110) which are conserved in HGFA and two FXII-specific lysines (K87 and K113). We generated glutamine-substitution FXII variants, neutralizing FXII-specific positively charged residues in the upstream patch (Δupstream), in the downstream patch (Δdownstream) or in both (Δcombined). Compared to wild-type (WT) FXII, kaolin binding was decreased by 55%, 3% and 97% for Δupstream, Δdownstream and Δcombined. Kaolin-triggered prekallikrein activation in buffer was decreased by 71%, 43% and 86%, respectively. All variants were equally susceptible to activation by plasma kallikrein in solution. Reconstitution of WT FXII in plasma results in an aPTT clotting time of 36 (±2) seconds. By comparison, reconstitution with Δupstream, Δdownstream or Δcombined leads to clotting times of 99 (±8), 48 (±2) and 223 (±13) seconds, respectively. Correspondingly, kaolin-triggered kallikrein generation decreased by 69%, 62% and 89%. Finally, we raised VHHs against FXII EGF-1. These prolong aPTT clotting times (lead candidate 113 (±2) seconds vs control VHH 38 (±1)) and inhibit kaolin-driven kallikrein generation in plasma (lead candidate: 74% vs control VHH 8%).
Conclusion. We here identify crucial residues for surface binding of FXII, which may serve as a druggable target.


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Risk Of On-Treatment Recurrent Venous Thromboembolism In Patients With Active Vs Cured Cancer Patients: A Post-Hoc Analysis Of Hokusai VTE Cancer

Anniek Strijdhorst, MD, Department of vascular medicine, Amsterdam University Medical Center, Amsterdam, The Netherlands; Harry R. Büller, MD, Department of vascular medicine, AmsterdamUMC, Amsterdam, The Netherlands; Gary E. Raskob, MD, University of Oklahoma Health Sciences Center, College of Public Health, University of Oklahoma, Oklahoma City, Oklahoma, United States; Michael Grosso, MD, Daiichi Sankyo Pharma Development, Basking Ridge, New Jersey, United States Annelise Segers, MD, ITREAS, Academic Research Organization, Amsterdam, The Netherlands; Marcello Di Nisio, MD, Department of Medicine and Ageing Sciences, University G. D'Annunzio, Chieti, Italy; Nick van Es, MD, Department of Vascular Medicine, Amsterdam University Medical Center, Amsterdam, The Netherlands

Background Trials evaluating anticoagulation for cancer associated thrombosis have used a uniform, broad definition of active cancer, which encompasses patients with local or metastatic cancer, but also those who can be considered cured after surgical resection or other curative treatment. We hypothesized that the incidence of recurrent venous thromboembolism (VTE) would differ between these groups, which might support a different management and more narrow definition of active cancer.
Aim To evaluate the incidence of on-treatment recurrent VTE in patients with acute VTE stratified by components of the active cancer definition.
Methods This was a post-hoc analysis of Hokusai VTE Cancer, an open-label randomized controlled trial comparing edoxaban with dalteparin for the treatment of acute cancer-associated VTE. We classified patients with solid cancer into three groups at time of VTE index event: active cancer with distant metastasis active cancer without metastasis, and cured cancer after surgery or other treatment, without measurable disease. The primary outcome was recurrent VTE during 6-month follow-up.
Results Of the 919 patients with solid cancer in the modified intention-to-treat analysis, 546 (59%) had metastatic disease distant metastasis. 151 (n=16%) had solid cancer without metastasis, and 222 (n=24%) had received treatment with curative intent. Recurrent VTE occurred in 53 patients (10%) with metastatic disease, 8 (5%) in patients with cancer without metastasis, and 13 (6%) in patients considered cured. Compared to patients with metastatic disease, the risk of recurrent VTE was significantly lower in those considered cured (HR, 0.51; 95%-CI, 0.28-0.94).
Conclusion Patients with acute VTE and solid cancer after treatment with curative intent were at significantly lower risk of recurrent VTE than those with distant metastasis. However, the risk of recurrence in the former group was still substantial, which probably does not justify shorter or less intensive anticoagulant treatment.

Fibrin Clot Properties Identify Patients That Benefit From Catheter-Directed Thrombolysis: A Post Hoc Analysis Of The CAVA Trial

Iding AFJ 1,2 , Alkarithi G 3 , ten Cate H 1,2, Ariëns RAS 3 , ten Cate-Hoek AJ 1,2 1. Thrombosis Expertise Center, Heart & Vascular Center, Maastricht University Medical Center, Maastricht, the Netherlands 2. Department of Biochemistry, Cardiovascular Research Institute Maastricht, Maastricht University, Maastricht, the Netherlands 3. Discovery and Translational Science Department, Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, Leeds, UK

Introduction: Catheter-directed thrombolysis (CDT) to improve patency after deep vein thrombosis (DVT) has not conclusively been shown to prevent the post-thrombotic syndrome in randomizedcontrolled trials. However, additional CDT might benefit selected patients that are unlikely to attain patency with standard treatment.
Aims: We assessed whether fibrin clot properties are associated with long-term patency in patients with iliofemoral DVT.
Methods: Patients included in the CAVA trial had blood samples taken at inclusion and at 12 months. Properties of fibrin clot formation and lysis in plasma were determined using turbidity assays, permeation, and confocal microscopy. Patency was defined as >90% iliofemoral vein compressibility on ultrasound assessment at 12 months. The medical ethics committee approved this study, and all patients gave written informed consent.
Results: Of 152 patients, 67 remained after excluding patients without blood samples (n=65), without ultrasound assessment (n=8) or with unmeasurable samples at inclusion (n=12), likely due to low-molecular-weight heparin treatment. Patients' clinical characteristics did not differ between treatment groups. Patency was attained in 50.0% (14/28) of patients with CDT and 25.6% (10/39) of those with standard treatment (p=0.040). In patients on standard treatment not reaching patency, clot properties at inclusion showed shorter lag time, higher maximal clotting rate and higher maximal turbidity, while clot properties were not associated with patency in patients with CDT. No differences were observed at 12 months. Maximal turbidity most accurately predicted patency (c-statistic=0.78). In patients with high maximal turbidity (≥0.70 optical density), none (0/15) attainted patency with standard treatment while 44.4% (4/9) achieved patency with CDT (p=0.012). In contrast, patency did not differ between treatment groups (41.7% vs. 52.6%) in patients with low maximal turbidity.
Conclusion: Fibrin clot properties, especially maximal turbidity, could identify patients unlikely to attain patency with standard treatment. This predictor might be used to select patients that would benefit from CDT in a point-of-care setting.

Age- And Sex Specific Risks Of Major Cardiovascular Complications And Death Following Elective Hip And Knee Arthroplasty In The Netherlands: A Dutch Hospital Data Registry Study

M.J.R. Smeets1 , M.G.J. Gademan1,2, R.G.H.H. Nelissen2 , S.C. Cannegieter1,3, B. Nemeth1,2 1. Department of Clinical Epidemiology, Leiden University Medical Center, The Netherlands 2. Department of Orthopaedics, Leiden University Medical Center, The Netherlands 3. Department of Internal Medicine, section Thrombosis and Hemostasis, Leiden University Medical Center, The Netherlands

Background Total Hip Arthroplasty (THA) and Total Knee Arthroplasty (TKA) are associated with serious (cardiovascular) complications, including venous thromboembolism (VTE), arterial thromboembolism (ATE), major bleeding and death. Although essential to adequately inform patients, stratified absolute risk estimates for these complications are lacking. In the Netherlands, 4-6 weeks thromboprophylaxis is given post THA and TKA.
Aim We aimed to 1) estimate overall risks, 2) assess possible time trends and 3) estimate age- and sex specific risks in the Netherlands over recent years.
Method Data on procedures and diagnoses were obtained from the Dutch Hospital Data registry. All patients with a first primary THA or TKA for osteo-arthritis, conducted in all Dutch hospitals between 2015 and 2020, were included. Outcomes of interest were VTE, ATE, major bleeding and death at 30- and 90-days following surgery. All analysis were stratified for procedure type. For aim 1, the overall risks over 2015 until 2020 were estimated. For aim 2, the risks over the inclusion years were plotted separately. Lastly, risks were estimated after stratification for sex and age. Death was taken into account as competing risk for all analyses.
Results 110,470 patients with a THA and 117,526 patients with TKA were included. The mean age was 69.9 years and 64% was female. Aim 1: overall risks of VTE, ATE, bleeding and death were all below 1%. Aim 2: the risks for any of the adverse outcomes remained stable over the years. Aim 3: across outcomes and for all cardiovascular complications combined, the risks were higher in men and increased with age. Only for THA, a higher risk of VTE was observed in women.
Conclusion Absolute risks of cardiovascular complications and death following THA/TKA have been stable over recent years. Stratification for subgroups will enable orthopaedic surgeons to better inform their patients.

The Impact Of Pulmonary Embolism On The Long-Term Health Outcomes Of COVID-19

Chantal Visser1a, Julia C. Berentschot2a, Cindy M.M. de Jong3 , M. Louisa Antoni4 , Rita J.G. van den Berg-Emons5 , Bram van den Borst6 , Hugo ten Cate7,8,9, Arina ten CateHoek9,10, Dionne Braeken10, J.J. Miranda Geelhoed11, Majanka H. Heijenbrok-Kal5 , Lucia J.M. Kroft12, Jenneke Leentjens13, Anna H.E. Roukens14, Suzanne C. Cannegieter3,15, Frederik A. Klok3 , Merel E. Hellemons2b, Marieke J. H. A. Kruip1b on behalf of the Dutch Covid & Thrombosis coalition. a joint first authors b joint senior authors. 1. Department of Hematology, Erasmus MC, Erasmus University Medical Center Rotterdam, Rotterdam, the Netherlands 2. Department of Respiratory Medicine, Erasmus MC, Erasmus University Medical Center Rotterdam, Rotterdam, the Netherlands 3. Department of Medicine - Thrombosis and Hemostasis, Leiden University Medical Center, Leiden, the Netherlands 4. Department of Cardiology, Heart Lung Centre, Leiden University Medical Center, Leiden, the Netherlands 5. Department of Rehabilitation Medicine, Erasmus MC, Erasmus University Medical Center Rotterdam, Rotterdam, the Netherlands 6. Department of Pulmonary Diseases, Radboud University Medical Center, Radboud University, Nijmegen, the Netherlands 7. Department of Internal Medicine, Division of Vascular Medicine, Maastricht University Medical Center (MUMC+), Maastricht, the Netherlands. 8. Cardiovascular Research Institute Maastricht (CARIM), Maastricht, the Netherlands 9. Department of Biochemistry, Laboratory for Clinical Thrombosis and Hemostasis, Maastricht University, Maastricht, the Netherlands 10. Thrombosis Expertise Center, Maastricht University Medical Center (MUMC+), Maastricht, the Netherlands. 11. Department of Pulmonary Diseases, Leiden University Medical Centre, Leiden, The Netherlands 12. Department of Radiology, Leiden University Medical Center, Leiden, the Netherlands 13. Department of Internal Medicine, Radboud University Medical Center, Radboud University, Nijmegen, Netherlands 14. Department of Infectious Disease, Leiden University Medical Center, Leiden, the Netherlands 15. Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands

Background The incidence of pulmonary embolism (PE) in patients with COVID-19 has been reported to be remarkably high. However, its impact on the long-term health outcomes of COVID-19 patients is unknown.
Aims To compare pulmonary function and patient reported outcome measures (PROMs) between COVID-19 survivors with and without PE 3 months after hospital discharge.
Methods In this multicentre observational study, we aggregated data from existing databases on the follow-up of COVID-19 patients in 4 hospitals in the Netherlands. Health outcomes were evaluated at 3 months after hospitalization for COVID-19 by pulmonary function testing and PROMs. We evaluated the impact of PE on diffusing capacity of the lungs for carbon monoxide (DLCO) (% of predicted), anxiety and depression (HADS, GAD-7, and PHQ-9), cognitive status (CFQ), and health-related quality of life (HRQoL) (EQ-5D-5L) using logistic and linear multivariate models, adjusted for the predefined confounders of age at admission, sex, presence of comorbidities, and stay at intensive care unit. Approval was given by the Erasmus University Medical Centre's ethics committee.
Results We included 465 COVID-19 patients, of whom 102 (21.9%) had developed PE during admission. At 3 months follow-up, patients with PE had significantly more impairment in pulmonary function and lower HRQoL than patients without PE. After adjusting for confounders, the association between PE and lower HRQoL remained (β=-0.069, 95% CI (- 0.12 to -0.017), p=0.009). However, no association was observed between PE and DLCO values after adjustment (β=-2.0, 95% CI (-6.5 to 2.4), p=0.367). Similarly, no association between the psychological outcomes and PE was observed after adjustment (data not shown).
Conclusion We found evidence for a possible association between PE and lower HRQoL 3 months after hospitalization, scoring below the Dutch reference values. No evidence for an effect of PE on impaired diffusion capacity and psychological outcomes was observed.


VarmX

Thursday March 30 - Session III. VWF and platelets

Effect of allele-selective silencing of von Willebrand factor in mice on experimental bleeding and thrombosis models

Yvonne K. Jongejan[1], Noa A. Linthorst[1], Elisa Schrader[2], Sebastiaan N.J. Laan[1], Richard J. Dirven[1], James E. Dahlman[2], Bart J.M. van Vlijmen[1], Cécile V. Denis[3], Jeroen C.J. Eikenboom[1] [1]Department of Internal Medicine, Division of Thrombosis and Hemostasis, Einthoven Laboratory for Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, The Netherlands.
[2.] Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University School of Medicine, Atlanta, Georgia, United States.
[3]Laboratory for Hemostasis, Inflammation and Thrombosis, Unité Mixed de Recherche S1176, Institut National de la Santé et de la Recherche Médicale, Université Paris-Saclay, Le Kremlin-Bicêtre, France.

Background Von Willebrand factor (VWF) plays an important role in the initiation of platelet adhesion. Defective VWF causes bleeding and high VWF plasma levels are associated with thrombosis. We have shown inhibition of endothelial murine Vwf using allele-selective small interfering RNAs (siRNAs) targeting either C57BL/6J (B6) or 129S1/SvImJ (129S) mice.
Aim To investigate the effect of allele-selective Vwf-silencing in F1 crosses of B6 and 129S mice on bleeding and thrombosis.
Methods B6.129S F1 hybrid mice were intravenously injected with nanoparticle-encapsulated siRNAs. 96 hours post-injection, citrated blood and lungs were collected for measurement of plasma VWF:Ag and lung Vwf mRNA levels. Allele-selectivity was tested for siRNAs targeting either Vwf expressed from B6- (siVwf.B6) or 129S-alleles (siVwf.129S), using a non-selective siVwf and scrambled siControl as controls. siVwf.B6-injected B6 and B6.129S mice were subjected to a ferric chloride ($FeCl_3$) experimental thrombosis model (mesenteric vessels) and a tail-clip bleeding assay.
Results Both siRNAs were allele-selective, with siVwf.B6 inhibiting lung Vwf with 41% (B6-allele 72%, 129S-allele 12%) and plasma VWF with 46%, and siVwf.129S inhibiting lung Vwf with 45% (B6-allele 9%, 129S-allele 58%) and plasma VWF with 43%. siVwf.B6-mediated alleleselective Vwf-silencing in B6.129S mice lead to a minor increase (+24 seconds) in bleeding time compared to siControl.B6-treated B6.129S mice, whereas siVwf.B6-treated B6 mice showed a moderate to severe increase (+120 seconds) in bleeding time. $FeCl_3$-treatment only rarely lead to thrombotic occlusions in B6.129S mice. However, in siVwf.B6-treated B6 mice only 50% of the mice developed an occlusion versus 89% of the siControl.B6-treated mice.
Conclusion Allele-selective siRNAs effectively and selectively inhibit Vwf at mRNA and plasma level. 50% VWF reduction had no relevant impact on the bleeding time. The effect of 50% VWF reduction on the $FeCl_3$-thrombosis model was not conclusive and is being analyzed. Funded by the Dutch Thrombosis Foundation (grant #2018-01).

Allele-Selective Inhibition Of Mutant Von Willebrand Factor With Small Interfering Rnas To Ameliorate A Von Willebrand Disease Type 2B Phenotype In Vivo In Mice

N.A. Linthorst1 , Y.K. Jongejan1 , R. Bierings2 , J.E. Dahlman3 , C.V. Denis4 , F.W.G. Leebeek2 , E. Schrader3 , J. Voorberg5 , B.J.M. van Vlijmen1 , J. Eikenboom1 1Department of Internal Medicine, Division of Thrombosis and Hemostasis, Einthoven Laboratory for Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, Zuid-Holland, The Netherlands, 2Department of Hematology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Zuid-Holland, The Netherlands, 3Wallace H. Coulter Department of Biomedical Engineering, Georgia Institute of Technology and Emory University School of Medicine, Atlanta, Georgia, United States, 4Laboratory for Hemostasis, Inflammation and Thrombosis, Unité Mixed de Recherche S1176, Institut National de la Santé et de la Recherche Médicale, Université Paris-Saclay, Le Kremlin-Bicêtre, France, 5Department of Molecular Hematology, Sanquin Research and Landsteiner Laboratory, Amsterdam, Noord-Holland, The Netherlands

Background: Von Willebrand disease (VWD) is the most common inherited bleeding disorder caused by defects in von Willebrand factor (VWF). Current therapies do not inhibit mutant VWF expression. Inhibition of mutant VWF through allele-selective silencing using small interfering RNAs (siRNAs) could potentially improve VWF function and alleviate VWD phenotype.
Aims: Investigate the feasibility of allele-selective inhibition of mutant VWF with siRNAs and its effect on disease phenotype in a VWD-type 2B mouse model.
Methods: Three siRNAs were used: 1) siVwf.B6, a strong selective inhibitor of mouse Vwf expressed from the C57BL/6J (B6) allele, and not from the 129S1/SvImJ (129S) allele, 2) a non-selective siVwf and 3) a scrambled control (siControl.B6). siRNAs were encapsulated in nanoparticles for endothelial targeting in vivo. Homozygous 2B-B6 mice were crossed with 129S mice to create 2B-B6.129S F1 offspring. 96 hours after siRNA injection, citrated blood and lung tissue were collected for determining VWF plasma protein and lung mRNA. In parallel, 2B-B6.129S and WT-B6.129S F1 mice were subjected to a tail-clip bleeding assay.
Results: Treatment of 2B-B6.129S mice with siVwf resulted in a strong median reduction of 86% of plasma VWF levels, while upon treatment with siVwf.B6 a reduction limited to 59% was observed. These findings were comparable to that observed for siRNA-treated WT-B6.129S mice. Upon tail-clipping, 2B-B6.129S mice showed prolonged bleeding compared to WTB6.129S mice (siControl.B6-treated). While treatment with siVwf coincided with prolonged bleeding in both WT-B6.129S and 2B-B6.129S mice, siVwf.B6 coincided with normal bleeding times for all WT-B6.129S mice as well as 4 out of 6 2B-B6.129S mice.
Conclusions: First results regarding allele-selective inhibition of mutant VWF in a VWD-type 2B mouse model are promising. Evaluation of platelet phenotype, mRNA expression, VWF activity and multimer distribution is currently ongoing.
Funding was received from NWO-AES (#18712), Dutch Thrombosis Foundation (#2018-1) and EHA Research Mobility Grant.

N-Glycan-Mediated Shielding Of ADAMTS13 Effectively Prevents Binding Of Pathogenic Autoantibodies In High-Titer Patients With Immune-Mediated TTP

Tim Postmus1 , Nuno AG Graca1 , Bogac Ercig2 , Brenda Luken3 , Berangere S Joly4,5,, Karen Vanhoorelbeke6 , Agnes Veyradier4,5, Paul Coppo4,5 and Jan Voorberg1,7 1Department of Molecular Hematology, Sanquin-Academic Medical Center Landsteiner Laboratory, Amsterdam, The Netherlands. 2Division of Biochemistry and Oncode Institute, The Netherlands Cancer Institute, Amsterdam, Netherlands 3Sanquin Innovation, Amsterdam, The Netherlands 4Centre National de Référence des Microangiopathies Thrombotiques, hôpital SaintAntoine, AP-HP. Sorbonne Université, Paris, France. 5Service d'hématologie biologique, hôpital Lariboisière et EA3518 Institut de Recherche Saint-Louis, AP-HP. Nord, Université Paris Cité, Paris, France. 6 Laboratory for Thrombosis Research, IRF Life Sciences, KU Leuven Campus Kulak Kortrijk, Kortrijk, Belgium. 7Department of Experimental Vascular Medicine, Amsterdam UMC, Amsterdam, The Netherlands.

Thrombotic Thrombocytopenic Purpura (TTP) is a rare thrombotic disease, affecting the lives of between 1.5 and 6 individuals per million per year. Patients affected by TTP show a great deficiency (<10%) in ADAMTS13 activity. ADAMTS13 is responsible for cleavage of VWF multimer and subsequently the accumulation of platelets at the sites of vascular perturbation. During episodes of immune TTP (iTTP) patients develop antibodies against various domains of ADAMTS13, but predominantly to the spacer domain. Binding of these autoantibodies to ADAMTS13 is the underlying cause for the reduced cleavage capability of ADAMTS13 and thereby the formation of platelet rich microthrombi. In a previous study we have shown that introduction of an N-glycan (NGLY-3) around the main autoantibody epitope on the spacer domain can reduce autoantibody binding to ADAMTS13. Here, we build upon this by generating two new ADAMTS13 N-glycan variants (NGLY-7 and NGLY-8), and combination mutants with the previously studied N-glycan (NGLY-3+7 and NGLY-3+8). 50 plasma samples of iTTP patients were screened against these new NGLY variants for reduced binding as well as increased FRETS-VWF73 activity when compared to wtADAMTS13. These newly tested patient samples further reinforced our previous findings that N-glycans can shield ADAMTS13 from pathogenic antibody binding. N-glycan modified variants of ADAMTS13 were able to reduce antibody binding down to 27% of wtADAMTS13 binding. Additionally, ADAMTS13 activity in the presence of iTTP plasma was restored from 37% for wtADAMTS13 up to 81% for N-glycan modified ADAMTS13. Collectively our findings show that N-glycan modified variants of ADAMTS13 can effectively reduce pathogenic antibody binding to ADAMTS13 as well as restore ADAMTS13 activity, especially in samples with high titer inhibitors.

HMB-001 – A Novel Bispecific Antibody Accumulating And Targeting Endogenous Fviia To Activated Platelets Supports Enhanced Haemostatic Responses In Models Of Glanzmann Thrombasthenia

Minka Zivkovic1, Prafull S. Gandhi2, Henrik Østergaard2, Amalie C. Bonde2, Catherine J. Rea2, Benny Sørensen2, Johan H. Faber2, Roger E. G. Schutgens1, Søren E. Bjørn2, Rolf T. Urbanus1 1 Center for Benign Haematology, Thrombosis and Haemostasis, van Creveldkliniek, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX, Utrecht, the Netherlands. 2 Hemab Therapeutics, Ole Maaløes Vej 3, 2200 Copenhagen, Denmark

Background: Glanzmann thrombasthenia (GT) is a severe platelet disorder caused by fibrinogen receptor αIIbβ3 deficiency. Acute bleeds in GT patients can be managed with recombinant factor VIIa (rFVIIa). HMB-001 is a bispecific antibody that binds to endogenous FVIIa with one arm and targets it to TLT-1 receptors on activated platelets with its second arm. Multiple-dose subcutaneous administration of HMB-001 in cynomolgus monkeys showed accumulation of endogenous FVIIa.
Aims: Evaluate the potentiation of FVIIa activity by HMB-001 in GT models.
Methods: Platelet TLT-1 expression and plasma FVIIa levels were evaluated in blood samples from GT patients and healthy controls. Targeting of rFVIIa to activated platelets by HMB-001 was evaluated with FACS. Effects of HMB-001 were evaluated with light transmission aggregometry and αIIbβ3-inhibited (GT-like) platelets or with a microfluidic flow chamber and GT-like blood. Results were confirmed in blood of GT patients.
Results: TLT-1 expression on activated GT platelets was normal and plasma FVIIa levels in GT patients and healthy controls were similar. Flow cytometry showed improved binding of rFVIIa to activated platelets with HMB-001. Aggregation of activated GT-like platelets was absent but occurred when fibrin formation was initiated with rFVIIa. Aggregation onset shortened with increasing rFVIIa concentrations. HMB-001 potentiated the effect of rFVIIa 10-fold but had no effect in absence of rFVIIa. Similar results were obtained with GT platelets. HMB-001 activity was TLT-1 dependent, as excess of soluble TLT-1 attenuated the HMB-001 activity. Perfusion of recalcified GT-like whole blood over a collagen surface resulted in platelet adhesion, but not fibrin formation. rFVIIa caused a dose-dependent increase in fibrin formation. HMB-001 strongly potentiated FVIIa-dependent fibrin formation on adhered platelets, which was confirmed in GT patient blood.
Summary/conclusions: HMB-001 potentiates FVIIa-mediated, fibrin-dependent platelet aggregation and enhances haemostatic reponses in models of GT. HMB-001 therefore has potential to treat and prevent bleeds in GT.

NODIA

Pregnancy And Inherited Bleeding Disorders Study: Interim Safety Analysis After Revision Of The Dutch Guideline

A de Vaan1 , MJHA Kruip2 , J Eikenboom3 , MC Punt1 , SEM Schols4 , FCJI Heubel-Moenen5 , M Coppens6 , L Nieuwenhuizen7 , ABU Makelburg8 , M Peters9 , JJ Duvekot10, JM Middeldorp11 , KWM Bloemenkamp12, REG Schutgens1 , AT Lely12 , KPM van Galen1 Affiliations 1 Center for Benign Haematology, Thrombosis and Haemostasis, van Creveldkliniek, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands 2 Department of Hematology, Erasmus MC, Erasmus University Medical Center Rotterdam Rotterdam, The Netherlands. 3 Department of Internal Medicine, Division of Thrombosis and Hemostasis, Leiden University Medical Center, Leiden, The Netherlands. 4 Department of Hematology, Radboud University Medical Center and Hemophilia Treatment Center Nijmegen-Eindhoven-Maastricht, Nijmegen, The Netherlands. 5 Department of Haematology-Internal Medicine, MUMC+, Maastricht, the Netherlands 6 Department of Vascular Medicine, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; 7 Department of Haematology, Maxima Medisch Centrum, Eindhoven, the Netherlands. 8 Department of Hematology, University Medical Center Groningen, Groningen, the Netherlands. 9 Department of Pediatric Hematology, Emma Children's Hospital, University Medical Centers, location AMC, Amsterdam, the Netherlands 10 Department of Gynaecology and Obstetrics, Erasmus MC, Erasmus University Medical Center, Rotterdam, the Netherlands 11 Department of Obstetrics, Leiden University Medical Center, Leiden, the Netherlands 12 Department of Obstetrics, WKZ Birth Centre, Division Woman and Baby, University Medical Center Utrecht, Utrecht, the Netherlands

Background: Due to high incidence of postpartum hemorrhage (PPH, ≥500 ml) and severe PPH (≥1000 ml) in women with von Willebrand disease (VWD) or hemophilia carriers (HC) (34% and 10%, respectively) the Dutch guideline concerning pregnant women with inherited bleeding disorders has been revised. Since 2018, pregnant women with VWD and HC receive prophylactic clotting factors (CF) if third trimester CF levels are <80 IU/dL instead of <50 IU/dL. Target peak CF levels at delivery were raised from 100 IU/dL to 150 IU/dL. The primary aim of the PRIDES is to assess the incidence of severe PPH after guideline revision.
Aim: Interim analysis on included patients. Safety analysis of the first 24 inclusions in the ≤50 IU/dL group. If ≥9 cases of severe PPH occur in this group, premature guideline re-revision will be mandatory.
Methods: Ongoing multicenter cohort study. Pregnant women with VWD or HC were treated by the revised guideline with care coordinated by hemophilia treatment centers in the Netherlands.
Outcomes: Since 2018, 137 pregnancies (70 VWD, 77 HCs) were included. Mean age was 33.8 years (SD 4.1) History of PPH was reported in 22% (n=30/137). In the <80 IU/dL group, 90% (n=43/48) received prophylactic CF. In the ≥80 IU/dL group, 7% (n=6/89) received on demand CF treatment. Severe PPH occurred in 25% (n=12/48) of the group <80 IU/dL. The incidence of severe PPH in the women ≤50 IU/dL was 25% (n=6/24).
Conclusion: This interim analysis shows a persistent high incidence of severe PPH of 25% in the group with third trimester CF levels ≤50 IU/dL despite augmented prophylactic CF treatment. Safety analysis does not dictate premature guideline revision. Final results of the PRIDES are expected within 2-3 years.

Emicizumab Plasma Concentrations Are Not Related To Bleeding Rates Using Standard Dosing, A Call For Dose Adaptation

Konrad van der Zwet1 Lize F.D. van Vulpen1 Paul R. van der Valk1 Idske C.L Kremer Hovinga1 , Karin van Galen1 Roger E.G. Schutgens 1, Kathelijn Fischer 1 1 Center for Benign Haematology, Thrombosis and Haemostasis, Van Creveldkliniek, University Medical Center Utrecht, Utrecht University, The Netherlands

Background: Emicizumab prophylaxis result in highly effective bleeding prevention in persons with hemophilia A (PWHA), but its high cost limits widespread access. HAVEN-trials and several case series suggest adequate bleeding control at low emicizumab concentrations and/or dosing, below the standard dose (6 mg/kg/4weeks).
Aim: To correlate emicizumab concentrations with bleeding rates in PWHA using standard dosing.
Methods: Monocenter observational cohort, consecutive PWHA receiving emicizumab ≥4 weeks were included. Emicizumab was dosed according to label (± 6mg/kg/4weeks, rounded to vials) at 1-4 weekly intervals. Treated bleeding data and emicizumab concentrations during maintenance were extracted from patient files between January 2021-December 2022. Emicizumab concentrations were measured at least annually, using a validated mass spectrometry-method. Emicizumab concentrations groups were categorized in quartiles. Mean annualized (joint) bleeding rates (A(J)BR) were estimated and compared across quartiles using negative binomial regression.
Results: 127 patients (93% with severe hemophilia A) were included.8% had an active inhibitor. Median age at start of emicizumab was 23.3 years (range: 0.5–78.3) and duration of therapy was 1.8 years (IQR 1.2–2.2).
Median emicizumab concentration during maintenance was 61.2 µg/ml (IQR 45.4–75.0). Overall mean ABR and AJBR were 0.61 (95CI 0.46–0.80) and 0.29 (95CI 0.21–0.41). We observed the following mean A(J)BR across the emicizumab quartiles:

- Q1 (<45 µg/ml): mean ABR: 0.61 (95CI 0.35-1.08) and mean AJBR: 0.25 (95CI 0.11-0.55).
- Q2 (45-59.9 µg/ml) mean ABR: 0.30 (95CI 0.15-0.60) and mean AJBR: 0.29 (95CI 0.07-0.39).
- Q3 (60 – 74.9) mean ABR 0.73 (95CI 0.44-1.19) and mean AJBR: 0.25 (95CI 0.12 – 0.52)
- Q4 (≥75 µg/ml) mean ABR of 0.77 (95CI 0.47-1.27) and mean AJBR: 0.48 (95CI 0.27-0.86).

Higher emicizumab concentrations did not decrease A(J)BR.
Conclusions: This study confirmed low A(J)BR on emicizumab prophylaxis. Bleeding rates did not correlate with emicizumab concentrations, suggesting no added value of higher emicizumab concentrations and/or dosing.

The Association Between Venous Thrombosis-Associated Genetic Variants And Coagulation Factor Levels And Thrombin Generation Potential

Jihee Han1 , Ruifang Li-Gao1 , Renée de Mutsert1 , Frits R. Rosendaal1 , Astrid van Hylckama Vlieg1 1Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands

Background Recent three large meta-analyses of genome-wide association studies for venous thromboembolism (VTE) identified over 130 genetic variants associated with VTE. However, the mechanisms by which recently described and therefore less explored VTE-associated genetic variants increase the risk of VTE remain unclear.
Aims We investigated the association between 61 recently described VTE-associated genetic variants, and the levels of coagulation factor (F) VIII, FIX, FXI, and fibrinogen as well as thrombin generation parameters (lag time, peak, endogenous thrombin potential, time-to-peak, and velocity), which were considered intermediate phenotypes for VTE.
Methods This study was conducted on 5341 participants with European ancestry of the Netherlands Epidemiology of Obesity study. The associations between VTE-associated genetic variants and the levels of coagulation factors and thrombin generation parameters were examined using linear regression analyses, adjusting for age, sex, oral contraceptive use, hormone replacement therapy, and menopausal status. The Medical Ethical Committee of the Leiden University Medical Center, Leiden, The Netherlands approved the study. All participants gave their written informed consent.
Results The mean (SD) age of participants was 56 (6) years and 56% were women. Of the 61 genetic variants, 19 were negatively associated with one or more of coagulation factor levels and thrombin generation parameters, of which MAP1A rs55707100 was most strongly associated with each thrombin generation parameter as well as FXI levels (-5.43 % per allele, 95% CI: -8.61, -2.26). Among the 14 genetic variants associated with increased coagulation, ST3GAL4 rs35257264 was the most strongly associated with each thrombin generation parameter as well as FVIII levels (8.30 % per allele, 95% CI: 2.29, 14.31).
Conclusions These results suggest that the genetic variants likely increase the risk of VTE by affecting the coagulation pathway. Our findings contribute to a better understanding of biological mechanisms by which genetic variants increase the risk of VTE.

Frequency Of INR Monitoring And Their Impact On INR Control During The COVID-19 Pandemic

Tessa Elling1 , Nic J. G. M. Veeger2 , Derk Hardeman3 , Adrian Kruit4 , Jos G.J. Pouwels5 , Vladimir Tichelaar6 , Karina Meijer1 1 Department of Hematology, University Medical Centre Groningen, University of Groningen, Groningen, the Netherlands 2 Department of Epidemiology, University Medical Centre Groningen, University of Groningen, Groningen, the Netherlands 3 Thrombosis Service Zuid-West Friesland, Sneek, the Netherlands 4 Trombosedienst v.h. Med. Diagn. Centrum, Drachten, the Netherlands 5 Trombosedienst Treant Zorggroep, Emmen , the Netherlands 6 Certe Thrombosis Service, Groningen, the Netherlands.

Background: International normalized ratio (INR) monitoring is needed to maintain optimal anticoagulation intensity in patients treated with a vitamin K antagonist (VKA). The COVID-19 pandemic may have reduced the INR testing frequency. Whether the COVID-19 pandemic had an impact on INR monitoring as well as on INR control is however not clear.
Aims: To evaluate whether there is a difference in INR testing frequency and INR control between the COVID-19 pandemic and the pre-pandemic period.
Methods: All patients managed by one of the four Thrombosis services located in the northern region of the Netherlands were included using a dynamic cohort design. The study period (i.e. December 23, 2018 to December 23, 2020) was divided into eight quartiles, and patient inclusion was evaluated at the start of each quartile. The INR return interval and the time in therapeutic range (TTR) were calculated during each quartile and compared between the pandemic and pre-pandemic period. Comparisons were made to the same period of the previous year.
Results: In each quartile ~20.000 patients were included; 55% were male and mean age was 80 years (table 1). In each quartile, the majority of the patients had a TTR above 70%. INR return interval increased during the COVID-19 pandemic, especially at the start of the pandemic (28.0 [IQR 19.3-38.5] vs. 25.1 [IQR 16.3-32.7] days, p-value < 0.01). There was no decrease in TTR, even a small numerical increase in TTR was seen (72.8 [IQR 51.1-100.0] vs. 75.0 [IQR 54.4-100.0], p-value <0.01).
Conclusion: Compared to the pre-pandemic period, the frequency of INR monitoring decreased during the COVID-19 pandemic. However, the increase in INR return interval did not affect TTR. Therefore, we conclude that VKA therapy is not immediately compromised when circumstance mandate a reduced frequency of INR monitoring.


Roche

Detection Of TFPI Citrullination In Blood After Neutrophil Activation By PMA

Bryan Bouwens, MSc. Maastricht university – department of Biochemistry – CARIM S. Thomassen. Maastricht university – department of Biochemistry – CARIM E. Magdeleyns. Maastricht university – department of Biochemistry – CARIM Prof. Dr. R.R. Koenen. Maastricht university – department of Biochemistry – CARIM Prof. Dr. T.M. Hackeng. Maastricht university – department of Biochemistry – CARIM

Background: Neutrophils are known to undergo NETosis, the externalization of their DNA and other intracellular contents to entrap and kill invading pathogens. NETosis has been shown to enhance blood coagulation during inflammation. Previously, it was shown that TPFI, an important anticoagulant, also binds to neutrophil extracellular traps (NETs), and is inactivated through cleavage by elastase. PAD4 is a crucial enzyme during the NETosis process, which is localized intracellularly but expelled from the cell upon neutrophil activation. In this research, we investigate the effect of PAD4 on TFPI and probe the changes to TFPI activity in full blood and plasma.
Aim: The aim of this work is to elucidate effects of neutrophil activation and subsequent consequences for TFPI activity induced by PAD4 measured via thrombin generation .
Methods: Neutrophils in whole blood were either stimulated to undergo NETosis using phorbol 12- myristate 13-acetate (PMA) or suppressed using metoprolol. Plasma derived from this blood was subsequently analyzed for presence of citrullinated TFPI by western-blotting. Effects on coagulation were measured by thrombin generation.
Results: Activation of neutrophils in whole blood resulted in a substantial increase in both peakheight and endogenous thrombin potential. Western blotting enabled the detection of the presence of citrullinated TFPI while suppression of neutrophil activation by metoprolol showed no detectable levels of citrullinated TFPI. Solid-phase competitive interaction studies revealed that the C-terminus of full-length TFPI dose-dependently binds to PAD4, thereby facilitating citrullination of crucial arginine-residues.
Conclusion: The findings indicate that TFPI is citrullinated after neutrophil activation in whole blood. Since FXa is the main target of TFPI, it is speculated that this explains the substantial increase in thrombin generation. This prompts for further investigation of inflammation-related diseases for citrullination of TFPI and possibly other coagulation factors. It can be hypothesized that citrullination TFPI contributes to the risk thrombosis in patients with thrombophilia or inflammation.

Peptidomics Reveals Expected And Unexpected Proteolytic Events In Plasma Upon Activation Of The Coagulation Cascade

Jessica Del Castillo1 , Floris P.J. van Alphen2, Carmen van der Zwaan2, Marleen Zuurveld1, Kamran Bakhtiari1, Arie J. Hoogendijk1, Herm-Jan Brinkman1,3, Joost C.M. Meijers1,3, Alexander B. Meijer1 1Department of Molecular Hematology, Sanquin Research, Amsterdam, The Netherlands. 2Department Research Facilities, Sanquin Research, Amsterdam, The Netherlands 3Department of Experimental Vascular Medicine, Amsterdam UMC, Amsterdam, The Netherlands

Background: Hemostasis is driven by several proteolytic events resulting in the formation and dissolution of a fibrin clot. The proteolytic processing of procoagulant and anticoagulant plasma proteins has been well characterized. However the impact on other plasma proteins is not fully understood. Insight therein may increase our understanding in the putative link between coagulation and other biological processes in plasma.
Aims: To assess proteolytic events in plasma that are associated with hemostasis.
Methods: Citrated plasma from healthy individuals was recalcified and the extrinsic pathway of coagulation was initiated with tissue factor in the absence or presence of platelets. Resulting endogenous peptides were extracted and analyzed by LC-MS/MS. Spectra were interpreted with PEAKSX De Novo algorithm. Identified peptides were mapped in silico against the Human UniProt database. This is a novel approach that allows making snapshots at distinct time points of the proteolytic events in and around the coagulation cascade.
Results: Upon activation of coagulation by tissue factor, peptides were generated including (truncated) variants of activation peptides of amongst others fibrinogen and factor XIII. In addition, we identified fibrinogen-derived peptides that could be mapped to cleavages by thrombin and plasmin. Also, protease inhibitors were processed such as alpha1-antitrypsin, antithrombin and alpha-2-macroglobulin. Surprisingly, In the presence of platelets, an increased number of proteolytic peptides were observed of prothrombin, vitronectin and proteins of the complement system including C4a, C4b, and C7.
Conclusions: Our data reveal the general impact of coagulation on plasma proteins. We found expected and novel proteolytic events upon activation of coagulation. Our results demonstrate that peptidomics can be leveraged to obtain functional insights of proteolysis during coagulation and fibrinolysis, and may be a useful approach to identify novel modulators of hemostasis

The In Vitro Proteomic Landscape Across Inflamed Vascular Beds

Stijn Groten1 , Eva Smit1 , Maartje van den Biggelaar1,2* , Arjan Hoogendijk1* 1 Department of Molecular Hematology, Sanquin Research, Amsterdam 1066 CX, the Netherlands. 2 Department of Biomolecular Mass Spectrometry and Proteomics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Utrecht, The Netherlands *equal contribution

Endothelial cells (ECs) mediate critical steps in the delicate balance of haemostasis and inflammation within tissues. Although it is becoming increasingly clear that ECs are heterogenous between vascular beds, how this organotypic diversity influences the sitespecific dynamics of inflammation and haemostasis is unknown. Moreover, how inflammatory triggers are mediated on a molecular level between vascular beds is mostly based on transcriptomic approaches and remains incompletely characterized.
To study the proteomic diversity of inflammation between vascular beds we obtained 6 primary human EC types, 3 donors each, and included widely used in vitro EC models; human umbilical cord ECs (HUVECs) and blood outgrowth ECs (BOECs), to characterize the proteomic landscape of in vitro cultured primary ECs both in steady state and after IFNγ inflammation.
We observed majorly overlapping proteomic signatures between all ECs in steady state, and detected limited EC markers specific to a single organ. Abundances of coagulation and complement mediators were similar between organs and showed only minor organ-specific regulation of, for example, PLAU (low in HUVECs), SERPING1 (high in BOECs) and TFPI2 (low in brain ECs). Differentially regulated proteins showed high donor-to-donor variation, but overall grouping of BOECs and liver sinusoidal/cardiac ECs versus HUVECs and brain/kidney glomerular ECs. Simulation with IFNγ yielded an identical inflammatory signature in all cells, independent of cell heterogeneity. Upregulated processes included hallmark IFNγ responses such as antigen-presentation (HLAs, TAPs, CD74), anti-viral proteins (GBPs, IFITs) and chemokines (CXCL9, CXCL10, CXCL11) after IFNγ stimulation. This proteomic landscape across vascular beds characterizes in vitro cultured primary ECs, puts these in the context of established in vitro models and indicates a highly conserved IFNγ response across ECs regardless of heterogeneity. This study provides important fundaments to support the determination of future in vitro studies on the molecular dissection of site-specific inflammatory-haemostatic interactions.

RBC-Derived Transglutaminase-2 Affects Thrombus Formation.

Naoual Ouazzani Chahdi1 , Judith J. de Vries2 , Hande Eyisoylu2,3, Hanke Matlung1 , Chi Hau4 , Rienk Nieuwland4 , Gijsje H. Koenderink3 , Moniek P.M. de Maat2 , Joost C.M. Meijers1,5, Robin van Bruggen1 1 Department of Molecular Hematology, Sanquin Research, Amsterdam, The Netherlands 2 Department of Hematology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, Netherlands 3 Department of Bionanoscience, Kavli Institute of Nanoscience, Delft University of Technology, Delft, the Netherlands 4 Department of Experimental Clinical Chemistry, Amsterdam UMC, The Netherlands 5 Department of Experimental Vascular Medicine, Amsterdam UMC, The Netherlands

Background: A venous clot mainly consists of red blood cells (RBCs), which contribute largely to the size and stability of a venous clot. However, it remains unclear if and how RBCs affect clotting. FXIIIa, an enzyme that crosslinks fibrin, strongly affects the mass and appearance of a clot. Additionally, transglutaminase-2 (TG2), a protein that has a function highly homologous to FXIIIa and is also expressed by RBCs, may play a role in clot formation.
Aim: To investigate whether TG2, expressed by RBCs, affects clot formation.
Method: Clots were formed by incubating human recalcified citrated blood with tissue factor in the absence or presence of TG2 inhibitors. Moreover, TG2 knock-out (KO) RBCs were generated using CRISPR/Cas9 in hematopoietic stem cells, and used to form clots. Contracting clots were visualized by scanning electron microscopy (SEM) as well as fluorescence microscopy and fibrin formation was studied using time-lapse imaging. Lastly, microparticle flow cytometry was used to investigate RBC-derived vesicles.
Results: Inhibition of TG2 activity resulted in clots that had fewer and thinner fibrin fibers at the exterior of the clot. When using TG2 KO RBCs a less dense fibrin network was formed in the clots, which supported the findings using the TG2 inhibitor. Moreover, our data revealed that most of the fibrin was localized in the inside of the clot, when TG2 was inhibited or absent. Interestingly, when both the TG2 inhibitor and phospholipids, containing phosphatidylserine (PS), were added, the fibrin network seemed to be unaffected.
Conclusion: TG2 expressed by RBCs has an effect on the fibrin structure during clot formation.


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Abstracts of Poster Pitches

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A Neural Network Approach To Predict Recurrent VTE Based On Coagulation Parameters

Geke Poolen1,2, Rolf Urbanus1 , Geert-Jan Geersing4 , Veronica Schmalschlager3 , Harmen Middelveld3 , Roger Schutgens1 , Bas de Laat2,3, Romy de Laat-Kremers2 . 1 Center for Benign Haematology, Thrombosis and Haemostasis, Van Creveldkliniek, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands 2 Department of Data Analysis and Artificial Intelligence, Synapse Research Institute, Maastricht, the Netherlands 3 Department of Functional Coagulation, Synapse Research Institute, Maastricht, the Netherlands 4 Department General Practice, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands.

Background: After a first venous thromboembolism (VTE), the recurrence rate is high, regardless of treatment, and increases if anticoagulation is discontinued. Accurate prediction of a patient's individual recurrence risk can aid in medication decisions after six months of standard anticoagulation. Nevertheless, it remains difficult to predict which patients will suffer from recurrent VTE with existing clinical prediction models.
Aim: Using neural networks to predict recurrent VTE based on coagulation parameters.
Methods: We developed two neural networks for the prediction of recurrent VTE with samples from the randomized controlled Vista trial which included 869 patients with a history of VTE of whom 96 patients had recurrence within two years. Both neural networks use thrombin generation, thrombin dynamics and coagulation factors (prothrombin, antithrombin, fibrinogen, FVIII, Von Willebrand Factor and active Von Willebrand Factor), in addition to age, sex and body mass index. The first and second neural network were developed with coagulation parameters obtained respectively during and after anticoagulation.
Results: With logistic regression only increased D-dimer levels and lower endogenous thrombin potential (ETP) were associated with an increased risk of recurrent VTE. Therefore, we applied neural networking methodology to integrate all acquired data into a prediction model for recurrent VTE. The first neural network, based on coagulation parameters measured during anticoagulation predicts recurrent VTE with a sensitivity of 82%, and a specificity of 44%. The second neural network, based on coagulation parameters measured after stopping anticoagulation, is more accurate. The sensitivity of this neural network is 99%, and the specificity 45%. Additionally, the positive and negative predictive values of the latter neural network were 67% and 97%, respectively.
Conclusions: Neural networks methodology seems suitable for the prediction of complex thrombotic events such as VTE recurrence. However, our findings need to be validated clinically, and further fine-tuning of the neural network could improve the specificity.

A Decreased Endogenous Thrombin Potential Is Associated With Recurrent Venous Thromboembolism

Geke C. Poolen1 , Romy M.W. de Laat-Kremers2 , Veronica Schmalschlager3 , Harmen Middelveld3 , Geert-Jan Geersing4 , Rolf T. Urbanus1 , Bas de Laat2,3, Roger E.G. Schutgens1 . 1 Center for Benign Haematology, Thrombosis and Haemostasis, Van Creveldkliniek, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands 2 Department of Data Analysis and Artificial Intelligence, Synapse Research Institute, Maastricht, the Netherlands 3 Department of Functional Coagulation, Synapse Research Institute, Maastricht, the Netherlands Synapse Research Institute, Maastricht, The Netherlands. 4 Department General Practice, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands

Background: Recurrence of venous thromboembolism (VTE) is difficult to predict with clinical parameters only. Thrombin generation (TG) parameters might be useful in the prediction of recurrent VTE. However, the association between recurrence and TG is unclear and sometimes contradictory. Thrombin dynamics (TD) can be used to further unravel TG data by discriminating prothrombin conversion and thrombin inactivation.
Aims: To investigate the association of TG and TD parameters with VTE recurrence.
Methods: We used stored samples from the Vista trial, a randomized controlled trial evaluating treatment duration of VTE based on the Vienna Prediction Model. 869 patients with unprovoked VTE were recruited at nine local thrombosis services in the Netherlands. Two years of follow-up was obtained and primary outcome was recurrence. Plasma samples were collected during (T=0; N=600) and one month after stopping anticoagulation (T=28; N=514). TG was measured by calibrated automated thrombinography and TD parameters were calculated. Associations between parameters and recurrence were analyzed with logistic regression after correction for age, sex, d-dimer, index event and hormone use.
Results: 11% of patients had recurrent VTE. None of the TG parameters were associated with recurrence when measured during anticoagulation (T=0). In a multivariate analysis, a 100-point increase of ETP at T=28 was associated with a reduced risk of recurrence measured with PPP reagent low (OR 0.91, 95%CI 0.84-0.99) and PPP reagent (OR 0.89, 95%CI 0.81-0.99). Other TG parameters (at T=28) were not associated with recurrence. TD parameters of prothrombin conversion and thrombin inactivation were not associated with recurrent VTE either. Furthermore, D-dimer levels were associated with increased recurrence risk (OR 1.04 per 100ug/ml increase, 95%CI 1.02-1.07).
Conclusion: We observed increased D-dimer levels and a decreased ETP in our patient population with recurrent VTE. This could indicate an increased turn-over of coagulation factors in patients with an increased risk of recurrent VTE.

High Fibrinogen And Low Antithrombin Is Associated With An Increased Risk Of Recurrent Cardiovascular Event In Young Ischemic Stroke Patients.

Janneke P. Spiegelenberg1,2, Mark Roest1 , Bas de Laat1 , Marleen M.H.J. van Gelder2 , Saskia Middeldorp3 , Anil M. Tuladhar4 , Jenneke Leentjens3 , Frank-Erik de Leeuw4 . 1 Synapse Research Institute, Maastricht, the Netherlands. 2 Department of Internal Medicine, Radboud university medical center, Nijmegen, the Netherlands. 3 Department for Health Evidence, Radboud university medical center, Nijmegen, the Netherlands. 4 Department of Neurology, Center for Neuroscience, Radboudumc, Donders Institute for Brain, Cognition and Behaviour, Nijmegen, the Netherlands.

Background: Patients with ischemic stroke at young age (18-50 years) are at increased risk of recurrent cardiovascular events, despite antithrombotic treatment. Enhanced coagulation parameters may predict recurrent arterial thrombosis. The aim of the study is to determine associations between coagulation parameters and risk of recurrent ischemic arterial events after a first ischemic stroke or transient ischemic attack (TIA) in young patients.
Method: We included patients with first-ever TIA or ischemic stroke that occurred between 1980 and 2010 from the FUTURE cohort. Blood collection was performed in 2010 and follow-up on recurrent ischemic events started in 2010 until 2023. The following coagulation parameters were measured: factor VIII, d-dimer, fibrinogen, antithrombin, and prothrombin. In addition, thrombin generation was determined using 1pM and 5pM tissue factor, and thrombomodulin in plateletpoor plasma to assess the functional capacity of plasma to form thrombin. Primary outcome was a composite of recurrent ischemic stroke, TIA, acute coronary syndrome or peripheral artery disease. We applied Cox proportional hazard models to estimate hazard ratios (HR) with 95% confidence intervals (CI).
Results: Among 327 included patients (median age 42.1 years, 56% women), 69 patients experienced a recurrence during a mean follow-up of 6.4 years. In univariate analyses, a 10% increase of antithrombin activity (HR 0.86, 95% CI=0.77-0.96) and a 1 µg/ml increase of fibrinogen (HR 1.45, 95% CI=1.04-2.02) were associated with recurrence. Other coagulation parameters were not associated with recurrent events. After correction for potential confounding, the association with antithrombin remained (HR 0.80, 95% CI=0.70-0.91), whereas the HR for fibrinogen slightly diminished (1.40, 95% CI=0.95-2.06).
Conclusion: In this study, most coagulation parameters were not associated with recurrent cardiovascular events after stroke or TIA at young age. Antithrombin activity, however, was associated with a reduced risk of recurrent ischemic arterial events, whereas fibrinogen seemed to be associated with an increased risk.

Epidemiology Of Antithrombotic Therapy During Pregnancy In The Netherlands (2013-2019)

Qingui Chen, Nienke van Rein, Suzanne Cannegieter, Luuk Scheres Department of Clinical Epidemiology, Leiden University Medical Center

Aims: To update epidemiology of antithrombotic therapy during pregnancy, including prescription patterns, maternal outcomes, and their time trends.
Methods: Dutch prenatal registry (including data on all pregnancies with a gestational age ≥22 weeks) was linked to nationwide registries from Statistics Netherlands (containing outpatient medication prescriptions, hospital diagnoses, and mortality). Antithrombotic therapy before and during pregnancies, and maternal outcomes during pregnancies and postpartum periods were determined and compared by calendar years.
Results: 1,122,711 pregnancies (of 822,115 women) between 2013 and 2019 were included (30.5±4.7 years old; 78.9% Caucasian). 0.4% (n=3,983) had ≥1 anticoagulant prescription within six months before pregnancies, where the majority was low-molecular-weight heparin (LMWH, ≈80%). This increased to 1.1% (n=12,519) during pregnancies, and LMWH remained the majority (>97%), while only 97 and 446 pregnancies had ≥1 prescription of direct oral anticoagulant or vitamin K antagonist, respectively. For antiplatelet, 0.2% (n=2,547) received it before pregnancies, which increased to 2.3% (n=25,715) during pregnancies. Noticeably, both LMWH and antiplatelet had been increasingly prescribed since the 5th-8th week, where DOAC and VKA use decreased, especially for prior users. Between calendar years, the pregnancies showed similar baseline characteristics and antithrombotic agent use both before and during pregnancies, except antiplatelet use during pregnancies increased from 0.8% in 2013 to 5.7% in 2019. For maternal outcomes, those who received antithrombotic therapy during pregnancies showed significantly higher incidence of both bleeding and thromboembolism than those who did not, but no notable time trends in incidence of these outcomes were observed. An extra analysis found the incidence of preeclampsia/eclampsia during pregnancies decreased to 2.3% in 2019 after reaching the peak (2.5%) in 2015.
Conclusion(s): The Dutch pregnant population in recent years saw broadly constant anticoagulant use and maternal outcomes (i.e., bleeding and thromboembolism), but an increase in antiplatelet use.

Anticoagulant Use And Prognosis In Patients With Atrial Fibrillation And Cancer: A Nationwide Population-Based Study

Qingui Chen1 , Nienke van Rein1 , Tom van der Hulle2 , Serge A Trines3 , Henri H Versteeg4 , Frederikus A. Klok4 , Suzanne C. Cannegieter1,4 1 Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, The Netherlands 2 Department of Medical Oncology, Leiden University Medical Center, Leiden, The Netherlands 3 Department of Cardiology, Heart Lung Center, Leiden University Medical Center, Leiden, The Netherlands 4 Department of Medicine, section of Thrombosis and Hemostasis, Leiden University Medical Center, Leiden, The Netherlands

Background: Anticoagulant use and prognosis of patients with both atrial fibrillation (AF) and cancer have rarely been investigated with recent and large-scale data. Aims: To describe the anticoagulation patterns and prognosis of this specific patient population in The Netherlands (2015-2019).
Methods: Using nationwide registries from Statistics Netherlands, all Dutch inhabitants with incident AF and prior cancer were identified as the Cancer-AF cohort (n=29,782), and all individuals with incident cancer and prior AF were identified as the AF-cancer cohort (n=25,024). Within each cohort, anticoagulant use in the six months before and after having both conditions was examined using data on outpatient medication prescriptions. After stratification by calendar years of the incident AF or cancer diagnosis, anticoagulant use and prognosis (i.e., ischemic stroke, major bleeding, venous thromboembolism [VTE], and allcause mortality) within the 1-year follow-up after having both conditions were compared between years.
Results: After having both AF and cancer, the (6-month) proportion of receiving ≥1 anticoagulant prescription increased from 43.7% to 68.5% in the Cancer-AF cohort, while it decreased from 82.1% to 70.5% in the AF-cancer cohort. From 2015 to 2019, the proportion of days covered by anticoagulants within the one year after having both conditions slightly increased from ≈60% to ≈70% in both cohorts, driven by an increase in use of direct oral anticoagulants (DOACs), while use of vitamin K antagonists and heparin group decreased. Meanwhile, the incidence of adverse events and survival remained broadly stable over time, although the incidence of VTE in the AF-cancer cohort seemed to decrease. The absolute 1- year risk of major bleeding remained high (>3%).
Conclusion(s): Patients with both AF and cancer in The Netherlands were increasingly anticoagulated in the DOAC era, without a measurable change in prognosis.


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Chronic Inflammatory Diseases Increase The Risk Of Post-Thrombotic Syndrome: A Prospective Cohort Study

Iding AFJ 1,2 , Limpens TMP 2 , ten Cate H 1,2, ten Cate-Hoek AJ 1,2 1. Thrombosis Expertise Center, Heart & Vascular Center, Maastricht University Medical Center, Maastricht, the Netherlands 2. Department of Biochemistry, Cardiovascular Research Institute Maastricht, Maastricht University, Maastricht, the Netherlands

Introduction: Post-thrombotic syndrome (PTS) is a common complication of deep vein thrombosis (DVT). Inflammation is known to play a major pathogenic role in PTS, possibly by promoting residual venous obstruction (RVO). Nevertheless, the potential impact of chronic inflammatory diseases (CIDs) and their anti-inflammatory treatment on the prevalence of PTS has not been studied thus far.
Aims: We assessed the risk of PTS in patients with CIDs, stratifying for treatment.
Methods: Consecutive patients with proximal DVT were followed prospectively for two years between 2003 and 2020. CIDs included inflammatory bowel disease, rheumatic diseases, and gout. Patients with active malignancy were excluded. RVO was assessed by compressive ultrasound after 3-6 months. PTS was diagnosed using the Villalta score after 6-24 months. Odds ratios (ORadj) were calculated by logistic regression adjusted for relevant patient characteristics. The medical ethics committee approved this study.
Results: In total 82 of 801 patients had CIDs (10.2%). Patients with CIDs were older (median 65 vs. 58 years), but similar for other patient characteristics. RVO was more strongly associated with PTS in patients with CIDs (ORadj 4.22 [1.25-14.30]) compared to those without CIDs (ORadj 2.08 [1.37-3.15]). Overall, patients with CIDs had a similar prevalence of RVO (36.8 vs. 41.4%), but more often developed PTS (35.4% vs. 18.9%, $p < 0.001$) than those without CIDs (ORadj 2.37 [1.39-4.06]). In patients with CIDs that received anti-inflammatory treatment (n=46, 56%), the prevalence of RVO was much lower than in those with untreated CIDs (26.1% vs. 53.3%, p=0.027). Accordingly, the risk of PTS was particularly high in patients with untreated CIDs (ORadj 3.56 [1.70-7.43]) compared to those without CIDs.
Conclusion: Patients with CIDs were at an increased risk to develop PTS. Notably, the risk of PTS was lower in patients with treated CIDs, which might be due to a favorable effect of anti-inflammatory treatment on RVO-related venous pathology.

Effect Of Pegylated Asparaginase On Coagulation Parameters And Thrombin Generation In Adults With Acute Lymphoblastic Leukemia

A.K. Ko1 , M.B. Tijssen1 , M.P.M. de Maat1 , J.C.M. Meijers2,3, S. Middeldorp2,4, F.W.G. Leebeek1 , B.J. Biemond5 , A.W. Rijneveld1 , M.N. Lauw1,5 1 Department of Hematology, Erasmus University Medical Center, Rotterdam; The Netherlands 2 Department of Vascular Medicine, Amsterdam University Medical Center, Amsterdam; The Netherlands 3 Department of Molecular Hematology, Sanquin Research, Amsterdam; The Netherlands 4 Department of Internal Medicine, Radboud university medical center, Nijmegen; The Netherlands 5 Department of Hematology, Amsterdam University Medical Center, Amsterdam; The Netherlands

Background. Intensification of asparaginase treatment in pediatric-inspired chemotherapy regimens for adults with acute lymphoblastic leukemia (ALL) has improved survival, but also increased venous thromboembolism (VTE) risk with incidences reported up to 30%. The pathogenesis of VTE in ALL remains poorly understood, especially in adults due to scarce data.
Aims. To compare coagulation parameters and thrombin generation over time in adults treated for ALL with and without pegylated asparaginase (PEGAsp) during the first remission-induction cycle (RI1), and to evaluate potential associations with VTE.
Methods. 340 adults with newly diagnosed ALL were included in the HOVON-100 trial (Rijneveld et al, 2022). Patients ≤40 years received PEGAsp on day 8 and 21 in RI1, whereas patients >40 years received no PEGAsp. We collected consecutive plasma samples at baseline and every 7 days during RI1. Antithrombin, fibrinogen, D-dimer and FVIII were assessed using Sysmex CS-5100. Thrombin generation was measured by calibrated automated thrombography. Median and IQR values were calculated per parameter for each age group.
Results. Symptomatic VTE occurred in 113/340 (33%) HOVON-100 patients. We collected samples of 99 patients from 12 Dutch centers; 40 of 99 patients (40%; 25/40 ≤40 years, 15/40 >40 years) experienced VTE during ALL treatment, including 13 in RI1. Antithrombin was clearly reduced in patients ≤40 years treated with PEGAsp compared to those >40 years without PEGAsp. Fibrinogen, D-dimer and FVIII levels did not differ between groups. In all patients, thrombin generation (i.e. endogenous thrombin potential (ETP)) was higher at baseline compared to normal pooled plasma. ETP further increased in patients with PEGAsp, particularly during antithrombin levels nadir. No association was observed between antithrombin, thrombin generation and VTE, possibly because of insufficient power.
Conclusion. Antithrombin reduction and increased thrombin generation were observed in adults with ALL during RI1, particularly in patients treated with PEGAsp. No association with VTE was found.

Effect Of Intravenous Tpa Treatment On Hemostatic Factors In Relation To Outcome In Acute Ischemic Stroke Patients Undergoing Thrombectomy

Aarazo Barakzie 1 , A.J. Gerard Jansen1 , Fabiano Cavalcante2 , Magdolna Nagy6,7, Diederik W.J. Dippel3,4, Aad van der Lugt5 , Yvo B. W. E. M. Roos2 , Charles B. L. M. Majoie2 , Hugo ten Cate6,7, Moniek P. M. de Maat1 , on behalf of the CONTRAST consortium. 1Department of Hematology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands. 2Department of Radiology and Nuclear Medicine, Amsterdam UMC, University of Amsterdam, Amsterdam Neuroscience, Amsterdam, The Netherlands. 3Department of Neurology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands. 4Department of Public Health, Erasmus MC, University Medical Center, Rotterdam, The Netherlands. 5Department of Radiology and Nuclear Medicine, Erasmus MC, University Medical Center, Rotterdam, The Netherlands. 6Department of Biochemistry, CARIM School for Cardiovascular Diseases, Maastricht University Medical Centre, The Netherlands. 7Thrombosis Expertise Center and Department of Internal Medicine, Maastricht University Medical Centre, The Netherlands.

Background Patients with acute ischemic stroke (AIS) are treated with intravenous tPA (IVT), followed by thrombectomy (EVT). Recently, the MR CLEAN NOIV trial showed no beneficial effect of IVT before EVT compared to EVT alone, indicating a need for biomarkers that predict clinical outcome. We postulate that biomarkers of hemostasis and fibrinolysis indicate the efficacy of thrombolysis.
Aim To study the impact of intravenous tPA on hemostatic biomarkers in relation to outcome in AIS patients undergoing EVT.
Methods We studied 214 of the 539 AIS patients included in MR CLEAN-NOIV trial who underwent IVT followed by EVT (N=108/266) or EVT alone (N=106/273). Blood samples were collected on admission and 24h after reperfusion. Selected hemostatic markers, including von Willebrand factor (VWF), ADAMTS13 (A disintegrin and metalloprotease with thrombospondin 1 repeats 13 (VWFcleaving protease)), ß-thromboglobulin (ß-TG), soluble glycoprotein VI (sGPVI), Factor VIII (FVIII), Fibrinogen (FBG), endogenous thrombin potential (ETP), tissue plasminogen activator (tPA), ddimer (DD), and clot lysis time (CLT) were measured in all blood samples and correlated with the 90 days outcome measured with the post-event modified Rankin Scale (mRS).
Results In patients with EVT alone, ADAMTS13, ß-TG, FVIII, FBG, ETP, and CLT values were significantly increased at 24h compared to on admission values. In patients with IVT plus EVT, ß-TG, FVIII, and DD values were significantly increased and sGPVI significantly decreased at 24h. VWF levels at 24h after IVT followed by EVT were significantly increased (2.41 [1.94-3.05] vs 2.24 [1.32-2.49], p= 0.03) and ADAMTS13 activity was significantly decreased (64.88 [51.90-77.70] vs 76.05 [52.35-89.40], p=0.01) in patients with unfavorable outcome compared to patients with favorable outcome.
Conclusion Treatment with IVT prior to EVT led to increased VWF and decreased ADAMTS13 values which were associated with poor clinical outcome, implicating a more prominent role of endothelial and platelet activation.

Trends In Anticoagulant Treatment Duration And Outcomes After Pulmonary Embolism Between 2014 And 2019: A Nationwide Cohort Study.

J.L.I. Burggraaf1 , Q. Chen1 , N. van Rein1,2, S.C. Cannegieter1,3 1Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands 2Department of Clinical Pharmacy and Toxicology, Leiden University Medical Center, Leiden, the Netherlands 3Department of Thrombosis and Hemostasis, Leiden University Medical Center, Leiden, the Netherlands

Background: Pulmonary embolism (PE) is treated with anticoagulants for at least three months. Thereafter, based on risk of recurrence and bleeding, some patients may benefit from indefinite anticoagulation. In the last decade guidelines have changed recommendations regarding indefinite treatment, which nowadays should be considered for patients with a first unprovoked PE. However, it is unclear whether prescription patterns and thereby outcomes have in fact changed during this period in line with these recommendations.
Objective: Describing time trends in the duration of anticoagulant treatment and outcomes after a first PE in the Netherlands between 2014 and 2019.
Methods: Patients with a first PE diagnosis were included using data from Statistics Netherlands. Patients who died or experienced recurrent PE within three months were excluded. We assessed prescriptions of anticoagulants within and beyond three months. Incidence rates (IRs) of major bleeding and recurrent VTE within 3-9 months after the PE were calculated.
Results: 49.904 patients (mean age 64.2 years, 49.6% male) had a first PE, of whom 22.3% had a malignancy. From 2014 to 2019 the use of vitamin K antagonists (VKA) within the first three months decreased from 77.4% to 9.1% whereas the use of DOACs increased from 1.2% to 76.1% of patients. The proportion of patients with ≥2 anticoagulant prescriptions between 3-9 months, indicating prolonged anticoagulation, increased from 61.4% (2014) to 67.9% (2019), which proportions were 59.8% to 66.9% for patients without malignancy, respectively. IRs of major bleeding within 3-9 months remained stable (2.18 to 2.17/100 person-years), whereas IRs of recurrent VTE decreased from 3.60 to 1.41/100 person-years (adjusted hazard ratio 0.38; 95% confidence interval 0.28–0.52).
Conclusion: Between 2014 and 2019 the use of prolonged anticoagulation after a first PE increased. Meanwhile, the incidence of major bleeding remained stable, whereas the incidence of recurrent VTE within 3-9 months decreased.

Prediction of on treatment recurrent venous thromboembolism in patients with cancer: an individual patient data meta-analysis of randomized controlled trials

Vincent R. Lanting[1,2,3], Floris T.M. Bosch[1,2,3], Andrea Marshall[4], Annie M. Young[4], Agnes Y.Y. Lee[5,6], Marcello Di Nisio[7], Gary E. Raskob, Pieter W. Kamphuisen[1,2,3], Harry R. Büller[1,2], Nick van Es[1,2]

1. Amsterdam UMC location University of Amsterdam, Vascular Medicine, Meibergdreef 9, Amsterdam, The Netherlands.
2. Amsterdam Cardiovascular Sciences, Pulmonary Hypertension and Thrombosis, Amsterdam, The Netherlands.
3. Tergooi hospitals, Internal Medicine, Van Riebeeckweg 212, Hilvserum, The Netherlands.
4. Warwick Clinical Trials Unit, Warwick Medical School, University of Warwick, Coventry, UK
5. Department of Medicine, University of British Columbia, Vancouver, BC, Canada
6. British Columbia Cancer Agency, Vancouver, BC, Canada
7. G. d Annunzio University, Chieti, Italy
8. Hudson College of Public Health University of Oklahoma Health Sciences Center Oklahoma City OK

Background About 5-8% of patients with cancer-associated venous thromboembolism (VTE) develop recurrence during anticoagulant treatment. Identification of high-risk patients may guide decisions about treatment intensity and duration.
Aim To evaluate clinical predictors for on-treatment recurrent cancer-associated VTE.
Methods Data were used from three randomized controlled trials evaluating efficacy and safety of various anticoagulants for cancer-associated VTE: Hokusai VTE Cancer[1], SELECT-D[2] , and CATCH[3]. Adjudicated on-treatment recurrent VTE during 6-month follow-up was the primary outcome. Eleven candidate predictors were selected based on potential association with recurrent VTE from literature and availability in the three databases. Associations were assessed by calculating odds ratios (OR) with 95% CI in a two-stage meta-analysis using the Knapp-Hartung method, with heterogeneity assessed by I^2.
Results The combined dataset comprised 2,366 cancer patients with acute VTE. The median age was 63 years (IQR 56-70), 53% of patients were female, and 56% had metastatic disease (Table 1). Patients received dalteparin (31%), tinzaparin (19%), edoxaban (22%), rivaroxaban (9%), or warfarin (19%). Recurrent VTE during anticoagulant treatment in the 6-month follow-up occurred in 159 patients (6.7%): 81 (3.4%) with pulmonary embolism, 76 (3.2%) with DVT, and 2 (0.1%) with another localization. The median time between initial event and recurrence was 44 days (IQR 20-77). Odds ratios for the candidate predictors ranged from 0.60 to 1.65, with the strongest association observed for metastatic disease (OR 1.63; 95%-CI, 1.52-1.74).
Conclusion This individual patient data meta-analysis of three large trials identified metastatic disease as a strong clinical predictor for recurrent VTE in patients with cancer during anticoagulant treatment. This preliminary analysis is a first step in the development of a prediction model for recurrent VTE in cancer patients.

1. Raskob, G.E., et al., *Edoxaban for the Treatment of Cancer-Associated Venous Thromboembolism.* N Engl J Med, 2018. **378**(7): p. 615-624.
2. Young, A.M., et al., *Comparison of an Oral Factor Xa Inhibitor With Low Molecular Weight Heparin in Patients With Cancer With Venous Thromboembolism: Results of a Randomized Trial (SELECT-D).* J Clin Oncol, 2018. **36**(20): p. 2017-2023.
3. Lee, A.Y.Y., *CATCH: a randomised clinical trial comparing long-term tinzaparin versus warfarin for treatment of acute venous thromboembolism in cancer patients.* BMC Cancer, 2013(13): p. 284.

Prediction Of On Treatment Recurrent Venous Thromboembolism In Patients With Cancer: An Individual Patient Data Meta-Analysis Of Randomized Controlled Trials

Vincent R. Lanting1,2,3, Floris T.M. Bosch1,2,3 , Andrea Marshall4 , Annie M. Young4 , Agnes Y.Y. Lee5,6 , Marcello Di Nisio7 , Gary E. Raskob, Pieter W. Kamphuisen1,2,3, Harry R. Büller1,2, Nick van Es1,2 1. Amsterdam UMC location University of Amsterdam, Vascular Medicine, Meibergdreef 9, Amsterdam, The Netherlands. 2. Amsterdam Cardiovascular Sciences, Pulmonary Hypertension and Thrombosis, Amsterdam, The Netherlands. 3. Tergooi hospitals, Internal Medicine, Van Riebeeckweg 212, Hilvserum, The Netherlands. 4. Warwick Clinical Trials Unit, Warwick Medical School, University of Warwick, Coventry, UK 5. Department of Medicine, University of British Columbia, Vancouver, BC, Canada 6. British Columbia Cancer Agency, Vancouver, BC, Canada 7. G. d Annunzio University, Chieti, Italy 8. Hudson College of Public Health University of Oklahoma Health Sciences Center Oklahoma City OK

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The Association Between Microvascular Health And Coagulation Parameters: The Netherlands Epidemiology Of Obesity Study

Lushun Yuan1 , Jihee Han2 , Anouk I.M. van der Velden1 , Hans Vink3, 4, Renée de Mutsert2 , Frits R. Rosendaal2 , Astrid van Hylckama Vlieg2 , Ruifang Li-Gao2,5, T.J Rabelink1 , Bernard M. van den Berg1 1Einthoven Laboratory for Vascular and Regenerative Medicine, Department of Internal Medicine, Nephrology, Leiden University Medical Center, Leiden, the Netherlands. 2Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands. 3Department of Physiology, Cardiovascular Research Institute Maastricht, Maastricht, the Netherlands. 4MicroVascular Health Solutions LLC, Alpine, Utah, USA. 5Metabolon Inc., Morrisville, North Carolina, USA

Background Dysregulation of the coagulation system is involved in coronary heart disease (CHD) pathogenesis and can be induced by endothelial glycocalyx (EG) perturbation. However, little is known about the link between EG function and coagulation parameters, and whether this relation differs between men and women.
Aims We sought to examine the association between EG function and coagulation parameters in a middle-aged Dutch population and to assess potential sex differences in these associations.
Methods Using baseline measurements of 771 participants from the Netherlands Epidemiology of Obesity study, associations between glycocalyx-related perfused boundary region (PBR) derived from sidestream dark-field imaging and coagulation parameters (factor (F) VIII, FIX, FXI, thrombin generation parameters, and fibrinogen) were investigated using linear regression analyses, adjusting for age, sex, BMI, smoking, menopausal, hormone therapy, C-reactive protein, glycoprotein acetyls, and leptin, followed by sex-stratified analyses.
Results The median age was 56 (51-61) years and 53% were women. In the total population, only PBR feed vessel, indicating poorer glycocalyx status, was associated with fibrinogen levels. There was a sex difference in the associations between PBR and coagulation parameters. No associations were observed in men, while in women one SD PBR (both total and feed vessel) was associated with elevated FIX activity (1.8%, 95% CI: 0.3-3.3, and 2.0%, 95% CI: 0.5-3.4) and plasma fibrinogen levels (5.1 mg/dL, 95% CI: 0.4-9.9, and 5.8 mg/dL, 95% CI: 1.1-10.6) after adjusting for confounders. Furthermore, one SD PBR capillary was associated with elevated FVIII activity (3.5%, 95% CI: 0.4- 6.5) and plasma fibrinogen levels (5.3 mg/dL, 95% CI: 0.6-10.0) after adjusting for confounders. No associations with other coagulation parameters were observed.
Conclusion We showed a sex-specific association between microcirculatory health and procoagulant factors, which suggests microvascular health may be considered in the early development of CHD in women.

Next-Generation Antithrombin Diagnostics By Mass Spectrometry

M. Kruijt, M.M. Treep, C.M. Cobbaert, L.R. Ruhaak Department of Clinical Chemistry and Laboratory Medicine, Leiden University Medical Center, Leiden, The Netherlands

Introduction Hereditary deficiency of antithrombin (AT), causes thrombophilia with high risk for venous thromboembolism. Activity tests are recommended for diagnosing AT deficiency but confer partial information and may lead to diagnostic uncertainty. AT exists in various molecular forms, caused by mutations and post-translational modifications (e.g. α- and β-AT), which vary in heparin affinity and activity (Peterson et al., 1985). Mass spectrometry (MS) allows molecular characterization and can refine the diagnosis of AT deficiency.
Objective To enable precision diagnostics for AT deficiency, an MS-based test for characterization of AT proteoforms was developed and analytically validated (Kruijt et al., manuscript submitted, 2022). The test provides molecular information on (dys)functional AT proteoforms to support medical decision-making.
Method AT was captured from 0.25 µL citrate plasma in a total volume of 50 µL (200x dilution in ABC) 96- well plates coupled to an α-AT VHH-antibody. Following denaturation, alkylation, and digestion, proteotypic peptides were quantified relative to internal standard peptides using MS. External calibration was based on native citrate plasmas. Automated liquid handling enables sample preparation in under 4 hours. Analytical validation was performed in agreement with CLSI C-62A and included precision, linearity, measuring range intervals, and carry-over.
Results A total of 23 peptides, including 4 glycopeptides, were included to enable identification of most genetic variations. Total imprecision of 5 different samples for the quantifying peptide LVSANR was 4.7-8.2%, with a linear measuring range between 0.08 – 2.58 µmol/L (reference values: 1.07-1.49 µmol/L; based on LVSANR). Analysis of a patient sample, for which activity test results were inconclusive, identified a p.Pro73Leu Basel mutation.
Conclusion Next generation AT diagnostics by MS allows molecular characterization of AT that fulfills analytical requirements of diagnostic tests. Future clinical performance studies of the molecular AT test will unravel the potential of AT molecular forms in relation to personalized diagnostics and therapy.

Bridging Therapy And Risk Of Major Bleeding And Thrombosis In Continuous-Flow Left Ventricular Assist Device Patients: A Quasi-Experimental Study

Eleonora Camilleri MD1 , Robin P.W. Roovers BSc1,2, Eva Janssen MD3 , Meindert Palmen MD PhD4 , J. Wouter Jukema, MD PhD3 , Nienke van Rein PharmD PhD1,2, Laurens F. Tops MD PhD3 1 Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands, 2 Department of Clinical Pharmacy & Toxicology, Leiden University Medical Center, Leiden, The Netherlands, 3 Department of Cardiology, Leiden University Medical Center, Leiden, the Netherlands, 4 Department of Thoracic Surgery, Leiden University Medical Center, Leiden, the Netherlands

Background Bridging with low molecular weight heparin (LMWH) is recommended in continuous-flow left ventricular assist device (CF-LVAD) patients during subtherapeutic anticoagulation. However, the consequence of bridging on the risk of bleeding and thrombosis are unknown.
Aim To assess the risk of major bleeding and thrombosis associated with bridging in CF-LVAD patients, through a regression discontinuity (RD) design.
Methods We included CF-LVAD patients implanted at Leiden University Medical Center between 2010- 2021. Outcomes were major bleeding (Interagency Registry for Mechanically Assisted Circulatory Support[INTERMACS] type 3, 4, 5) and thromboembolic events (pump thrombosis, arterial non-central nervous system and venous thromboembolism). We estimated incidence rates and hazard ratios (HR) with 95% confidence intervals (95%CI) by time-dependent Cox regression. Using a RD design, we compared patients using LMWH due to a subtherapeutic international normalized ratio (INR) to patients with no LMWH and an INR just in target range. As LMWH is initiated based on an INR below target range and INR is subject to random variability, patients just below and in the INR target range are comparable, mimicking a randomized trial. Analyses were performed including INRs±0.1, ±0.2, ±0.3, ±0.4 and ±0.5 around the INR threshold.
Results 77 patients were included, with a median age of 69 years, 60(78%) were male and 35 (45%) had INTERMACS classification 3. We included 35,466 INRs during 91 patient-years of followup. Bridging was associated with increased major bleeding rates in all analyses. In particular, the risk of major bleeding was 6.7-fold increased (95%CI 1.2-38.0) during bridging compared to non-bridging considering INR+0.1, and 3.4-fold (95%CI 1.4-8.2) considering INR±0.5. While thrombotic events were infrequent, bridging showed a trend towards increased risk of thrombosis (HR up to 6.6, 95%CI 0.9-47.3).
Conclusions During bridging with LMWH, CF-LVAD patients had a 6.7-fold increased risk of major bleeding and a potential increased risk of thrombosis.

Patients With Pancreatic Cancer Have A Disturbed Whole Blood Thrombin Generation Profile

Ruth Willemsa,b,c,d, Mark Roestc , Bas de Laatc , Hugo ten Catea,b , Judith de Vos-Geelend,e a Thrombosis Expert Center Maastricht and Department of Internal Medicine, Section Vascular Medicine, Maastricht University Medical Center, Maastricht, the Netherlands b Cardiovascular Research Institute Maastricht (CARIM), School for Cardiovascular Diseases, Maastricht, the Netherlands c Synapse Research Institute, Maastricht, the Netherlands d Department of Internal Medicine, Section Medical Oncology, Maastricht University Medical Center, Maastricht, the Netherlands e GROW, School for Oncology and Developmental Biology, Maastricht, the Netherlands

Background Thromboembolic disease is a major complication in patients with pancreatic ductal adenocarcinoma (PDAC), causing morbidity and mortality. One in five patients with newly diagnosed PDAC develops a venous thromboembolism (VTE) and VTE development is associated with a decreased overall survival time. Patients with PDAC often have altered blood cell counts, which are further affected by systemic therapy. The high thromboembolic risk in patients with PDAC may be caused by pro-coagulant effects of pancreatic cancer on blood cells.
Aims To investigate the impact of pancreatic cancer on blood cell dependent coagulation, using the whole-blood thrombin generation (WB-TG) assay, to further characterize the high thrombosis risk in this population.
Methods The TROCOPAC study is a prospective observational case-control study. Before the initiation of systemic therapy (FOLFIRINOX or nab-paclitaxel-gemcitabine), blood was collected from patients with locally advanced or metastatic PDAC. WB-TG profiles of these patients were compared to healthy individuals. 18 patients with PDAC were included and 13 healthy individuals.
Results In WB-TG, patients with PDAC have a significantly higher endogenous thrombin potential (ETP) than healthy individuals (2741 vs. 1760 nM*minutes, p=0.0041). The time to reach the thrombin generation peak is elongated in patients with PDAC when compared to healthy individuals (16.97 vs. 14.47 minutes, p=0.0197).
Conclusions Patients with PDAC have an increased WB-TG ETP and prolonged WB-TG TTP in comparison to healthy individuals. The increased potential to generate thrombin corresponds with the high thrombosis incidence in PDAC. The elongated TTP on the contrary may explain the higher bleeding risk in those patients. We hypothesize that red blood cells and platelet abnormalities in PDAC disturb the thrombosis and bleeding homeostasis.

Evaluation Of Innovative Laboratory Tests To Predict A Thrombotic Phenotype In A Family With Dysfibrinogenemia And A Novel FGG Mutation.

Amaury L.L. Monard, Department of Haematology, Maastricht Medical Centre +, Maastricht, The Netherlands Floor C.J.I. Heubel-Moenen, Department of Haematology, Maastricht University Medical Centre +, Maastricht, The Netherlands Elisabetta Castoldi, Department of Biochemistry, Maastricht University, Maastricht, The Netherlands Ilaria De Simone, Department of Biochemistry, Maastricht University, Maastricht, The Netherlands Paola E.J. van der Meijden, Department of Biochemistry, Maastricht University, Maastricht, The Netherlands Kanin Whichapong, Department of Biochemistry, Maastricht University, Maastricht, The Netherlands Willam P.T.M. van Doorn, Department of Central Diagnostic Laboratory, Maastricht University Medical Centre +, Maastricht, The Netherlands Stefano Spada, Department of Biochemistry, Maastricht University, Maastricht, The Netherlands Dave L.S. Hellenbrand, Department of Central Diagnostic Laboratory, Maastricht University Medical Centre +, Maastricht, The Netherlands Alexander D.M. Stork, Department of Internal Medicine, St Anna Hospital, Geldrop, The Netherlands Hugo ten Cate, Department of Biochemistry, Maastricht University, Maastricht, The Netherlands Erik A.M. Beckers, Department of Haematology, Maastricht University Medical centre, Maastricht, The Netherlands Yvonne Henskens, Department of Central Diagnostic Laboratory, Maastricht University Medical Centre +, Maastricht, The Netherlands

Background: Hypodysfibrinogenemia is a rare hereditary fibrinogen disorder characterized by quantitative and qualitative fibrinogen defects. These fibrinogen defects can cause thrombotic and hemorrhagic phenotypes. Unfortunately, predicting the phenotype in a specific patient is often not possible with routine coagulation tests.
Aims: To characterize the phenotype and the genetic profile of a family with hypodysfibrinogenemia and to investigate the ability of innovative tests to predict bleeding and/or thrombotic phenotypes in asymptomatic family members.
Methods: The proband, a 60-year-old woman with both bleeding and thrombotic complications who is currently on DOAC treatment, and two daughters were referred to our Hemophilia Treatment Center (HTC) for phenotypical and genotypical analysis of a congenital fibrinogen disorder (CFD). Extensive laboratory testing was done, as well as DNA-sequence analysis and molecular modelling. Thrombin generation and microfluidic testing were also performed to investigate their ability in phenotype prediction.
Results: Fibrinogen activity and antigen levels led to the diagnosis of dysfibrinogenemia in the proband and hypodysfibrinogenemia in both daughters. In all three cases, the same heterozygous missense mutation in the FGG gene was uncovered. This likely pathogenic mutation leads to the p.(Tyr375Cys) amino acid change. Molecular modeling predicted possible conformational changes or covalent dimerization of the fibrinogen molecule. Thrombin generation was elevated in one daughter. Microfluidic testing showed enhanced fibrin formation in both daughters, regardless of the coagulation trigger.
Conclusion: We described a family with hypodysfibrinogenemia in whom a novel heterozygous missense mutation in the FGG gene was found, possibly leading to conformational changes or covalent dimerization of the fibrinogen molecule. Furthermore we showed that microfluidic testing and thrombin generation can indicate a thrombotic phenotype in these patients, not detected with routine coagulation tests. Informed consent and approval from the medical-ethical committee from Maastricht (METC azM/UM) were obtained.


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Phenotypic Characterization Of Von Willebrand Disease Type 3 Patient-Derived Ecfcs With A Homozygous P.M771V Mutation

Isabel Bär1 , Petra Bürgisser1 , Calvin van Kwawegen1 , Jan Voorberg2 , Jeroen C. J. Eikenboom3 , Frank W.G. Leebeek1 , Ruben Bierings1 1Department of Hematology, Erasmus University Medical Centre, Rotterdam, the Netherlands 2Molecular Hematology, Sanquin Research and Landsteiner laboratory, Amsterdam University Medical Centre, University of Amsterdam, the Netherlands 3Department of Internal Medicine, Division of Thrombosis and Hemostasis, Leiden University Medical Centre, Leiden, the Netherlands

Background: Von Willebrand disease (VWD) is the most common inherited bleeding disorder. Most patients carry missense mutations in the Von Willebrand Factor (VWF) gene. The severity and type of VWD can however vary drastically between patients due to heterogeneity of the disease genotype. A family with p.M771V mutations has been diagnosed with type 2N and type 3 VWD.
Aims: Characterization of patient endothelial colony forming cells (ECFCs) with a p.M771V mutation to gain insight in the molecular mechanism that underpins the severe VWD phenotype.
Methods: ECFCs are isolated from venous blood of two patients selected from the Willebrand in the Netherlands (WiN) cohort that carry the p.M771V mutation. Biosynthesis, storage, and secretion of VWF was studied using biochemical methods (VWF ELISA, immunoblotting, VWF multimer gel electrophoresis), as well as confocal microscopy.
Results: VWF activity <0.1 U/ml were measured in both patients' plasma. Proteolytic processing of proVWF into mature VWF and VWF propeptide (VWFpp) was impaired, judging from high ratio of proVWF:VWF and the low abundance of VWFpp in p.M771V ECFC lysates. Intracellular VWF content and secreted VWF were severely reduced in p.M771V ECFCs compared to ECFCs of healthy control donors. Multimer analysis of intracellular and secreted VWF showed a deficiency of high molecular weight (HMW) VWF multimers. Immunofluorescent staining of VWF revealed the absence of Weibel-Palade bodies in p.M771V ECFCs. The remaining intracellular VWF was found retained in the endoplasmic reticulum (ER).
Conclusion: The severe endothelial disease phenotype observed in p.M771V ECFCs suggests that ER retention of VWF and failure to undergo subsequent processing steps is the basis of the severe bleeding phenotype of VWD3 patients with homozygous mutations at M771.

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Inflammatory Endothelial Cells Regulate Platelet Reactivity: Using Multi-Omics To Decipher Endothelial (Dys)Regulation Of Platelets In Inflammatory Vessel-On-A-Chip-Models

Claudia Schoenichen,1,2 Isabella Provenzale,1,3 Annekathrin Ludt,4 Fiorella Solari,5 Federico Marini,4 Audrey Cleuren,6 Kerstin Jurk,2 Monika Stoll,7 Albert Sickmann,5 Marijke J.E. Kuijpers,1,8 Johan W.M. Heemskerk1,9 . 1Department of Biochemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, The Netherlands; , 2Center for Thrombosis and Hemostasis, University Medical Center of the Johannes Gutenberg-University of Mainz, Germany , 3 Institute for Cardiovascular and Metabolic Research (ICMR), School of Biological Sciences, University of Reading, Reading (UK), 4 Institute of Medical Biostatistics, Epidemiology and Informatics (IMBEI), University Medical Center of the Johannes Gutenberg-University, Mainz, Germany, 5 Leibniz-Institut for analytical sciences – ISAS – e.V. Dortmund, Germany, 6Oklahoma Medical Research Foundation, Oklahoma City, OK, USA, 7Departments of Biochemistry and Genetic Epidemiology and Statistical Genetics, CARIM School for Cardiovascular Diseases, Maastricht University, The Netherlands, 8Thrombosis Expertise Centre, Heart and Vascular Centre, Maastricht University Medical Centre, Maastricht, The Netherlands, 9 Synapse Research Institute Maastricht, The Netherlands

Background: Thrombosis due to endothelial inflammation occurs in a variety of chronic or acute cardiovascular diseases. Despite many treatment options, the risk of recurrent thrombotic events is increased in patients. How endothelial cells regulate platelet reactivity and thrombus formation in these diseases is only partially understood.
Aim: To discover mechanisms of platelet inhibition by endothelial cells in healthy state, understand how inflammation alters endothelial cells and how the endothelial dysregulation affects platelet signaling events and functionality.
Methods: Human umbilical vein endothelial cell (HUVEC) transcriptome was sequenced after control, TNFalpha or LPS treatment. In whole blood, platelet activation, thrombus formation and coagulation on healthy or inflamed HUVEC was assessed in real-time using a high shear vessel-on-a-chip model. Platelet activation (PAC1-binding) after exposure to healthy/inflamed HUVEC followed by agonist stimulation was measured using flow cytometry. Changes in protein phosphorylation levels of platelets exposed to (treated) EC were assessed by a TMT based quantitative phosphoproteomics approach.
Results: The HUVEC transcriptome is significantly altered in inflammatory conditions. This effect was stronger for TNFalpha vs. LPS. In high-shear whole blood perfusion over sub-confluent HUVEC, pre-treatment of EC with TNFalpha increased thrombus surface area, platelet intracellular calcium rises and fibrin formation. Purified platelets showed decreased reactivity to agonist stimulation after exposure to healthy HUVEC. This protective effect was abolished if EC were treated with an inflammatory stimulus. Key changes in the phospho-proteome (3.0k phosphosites) in EC-exposed platelets are currently validated to uncover most relevant signaling alterations due to TNFalpha or LPS treatment of the cells.
Conclusion: The combined use of omics and microfluidic approaches reveals a complex regulation of inflammation-modulated interactions between EC and platelets.

Plasma Proteomics In The Diagnosis And Risk Prediction Of Thrombotic Events

Eva R. Smit1*, Iris C. Kreft1*, Eleonora Camilleri2*, Louise Burggraaf2 , Nienke van Rein2 , Bart J.M. van Vlijmen2,3,4 , Anne-Marije Hulshof5 , Bas van Bussel5 , Frank van Rosmalen5 , Tom van de Berg5 , Yvonne Henskens5 , Hugo ten Cate5 , Jonathan Coutinho6 , Marieke Kruip7 , Jeroen Eikenboom3 , Suzanne C. Cannegieter2,3,4, Maartje van den Biggelaar1 in collaboration with BEAT-COVID group and Dutch COVID & Thrombosis Coalition 1Department of Molecular Hematology, Sanquin Research, Amsterdam, the Netherlands 2Department of Clinical Epidemiology, Leiden University Medical Center, Leiden, the Netherlands 3Department of Internal Medicine, Division of Thrombosis and Hemostasis, Leiden University Medical Center, Leiden, The Netherlands 4Department of Clinical Pharmacy and Toxicology, Leiden University Medical Center, Leiden, the Netherlands. 5Cardiovascular Research Institute Maastricht, Maastricht University Medical Center, Maastricht, the Netherlands 6Department of Neurology, Amsterdam University Medical Center, Amsterdam, the Netherlands 7Department of Hematology, Erasmus MC, Erasmus University Medical Center, Rotterdam; Dutch COVID & Thrombosis Coalition (DCTC) *co-first authors

Venous thromboembolism (VTE), including deep vein thrombosis, pulmonary embolism and cerebral venous sinus thrombosis, is a common, multicausal disease that results in serious short-term and long-term health problems. Despite knowledge on known genetic and life-style risk factors, it is hard to predict which individuals are likely to develop VTE. Therefore, in clinical practice, there is a need for improved biomarkers in the diagnosis, disease management and risk prediction of (recurrent) VTE. Here, we performed crosssectional and longitudinal mass spectrometry-based plasma profiling on subsets of different thrombotic cohorts, including BLEED, MEGA, CVST, BEAT-COVID and MUMCCOVID (n=366 patients). Over 500 proteins were reliably and accurately quantified across 451 plasma samples. Mass spectrometry-based data correlated well with blood cell counts and laboratory parameters. Unbiased clustering analysis demonstrates co-regulated variation in plasma proteins and protein complexes that are associated with coagulation and complement as well as with systemic inflammatory and immune responses. Patients treated with vitamin K antagonists (BLEED cohort) show decreased levels of vitamin Kdependent proteins, including F2, F9, F10, PROS1 and PROZ. In the CVST patients, we observed increased levels of acute phase plasma proteins, including CRP, VWF and SAA1. COVID-19 (n=66 patients) disease severity was reflected on the patients plasma proteome, most prominently by increased plasma levels of CRP, VWF, fibrinogen and calprotectin. However, neither COVID nor non-COVID patient plasma profiles revealed clear differences between patients that did versus patients that did not develop (recurrent) VTE. Plasma proteomes of patients were highly individual-specific, as the majority of plasma proteins varied between patients, but remained stable within individuals. Our study emphasizes the value of longitudinal study design combined with integration of clinical and proteomics data and shows the promise of plasma profiling to inform on individual disease status by highlighting its potential use in thrombosis research.

Characterization Of Activation Intermediates Of A Factor IX Variant With Cofactor-Independent Activity

Sabien D. van Vliet1* , Jafar Vatandoost1,2* , Shuzhen Cheng1 , Ka Lei Cheung1 , Naweed Shifai1 , Marina Moreno Mayorga1 , Viola J.F. Strijbis1 , Mettine H.A. Bos1 1Dept. of Internal Medicine, Div. of Thrombosis and Hemostasis, Einthoven Laboratory for Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, the Netherlands; 2Dept. of Biology, Hakim Sabzevari University, Sabzevar, Iran; * equal contribution.

Background: Conversion of zymogen factor (F)IX to its activated serine protease state FIXaβ proceeds through sequential cleavage at Arg145 and Arg180. Proteolysis at Arg145 results in the activation intermediate FIXα that displays no FX converting activity, while single cleavage at Arg180 generates catalytically active FIXaα. We have previously generated FIX-FIAV comprising specific modifications in the serine protease domain, thereby rendering this variant capable of functioning independently of the cofactor FVIII. Whether these gain-of-function modifications impact the activity of the intermediate FIXα/FIXaα species remains to be determined.
Aims: Generate recombinant FIX variants comprising substitutions at the Arg145 and Arg180 activation sites to establish a framework for detailed characterization of the FIX activation species.
Methods: Recombinant FIX variants FIX-R145Q, FIX-R180Q, and FIX-R145Q-R180Q were stably expressed, generated, purified to homogeneity, and functionally assessed for activation and specific clotting activity.
Results: Under conditions in which wild-type (WT-)FIX was efficiently converted to FIXaβ, FIXR180Q was mostly proteolyzed to FIXα while FIX-R145Q was only partially converted to FIXaα. These findings confirm that cleavage at Arg145 is most efficient, which is required for Arg180 to become available for subsequent proteolysis. Upon introducing R145Q into FIX-FIAV, the FIX-specific clotting activity observed was similar to that of FIX-WT-R145Q, suggesting that the 'FIAV' modifications do not affect Arg180 cleavage. Preliminary analysis of the FVIII-specific clotting activity indicated that FIX-FIAV-R145Q displays FVIII-independent activity comparable to FIXFIAV. As anticipated, no clotting activity was observed for FIX variants comprising both R145Q and R180Q substitutions. Purification of FIX-FIAV-R180Q and analysis of the specific clotting activity is ongoing; these data will provide insight on its putative FVIII-specific gain-of-function state.
Conclusions: These FIX variants allow for analysis of the FIX activation pathways and intermediates, which contributes to knowledge on the mode-of-action of therapeutic FIX variants. We next aim to obtain detailed insight into the contribution of activation intermediates to FVIII-(in)dependent FIX function.

Functional Characterization Of A Nanobody Based GPVI Specific Platelet Agonist

Minka Zivkovic1 , Elisabeth Pols - van Veen1 , Silvie AE Sebastian1,2, Suzanne JA Korporaal2 , Rolf T Urbanus1 1 Center for Benign Haematology, Thrombosis and Haemostasis, Van Creveldkliniek, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands 2 Central Diagnostic Laboratory, University Medical Center Utrecht, Utrecht, the Netherlands

Background: Glycoprotein VI (GPVI) is a platelet specific collagen receptor required for platelet activation on extravascular collagen during hemostasis. Platelet reactivity towards collagen is always assessed during diagnostic workup of platelet disorders. GPVI can be activated by inducing receptor clustering with its ligands collagen, synthetic cross-linked collagen-related peptide (CRPXL), or the snake venom tetramer protein convulxin. However, these agonists are hard to standardize and difficult to produce. Nanobodies are small recombinant proteins consisting of the complementarity determining region of camelid heavy-chain antibodies. They are highly stable, easy to produce and ideal candidates for developing a stable GPVI agonist for diagnostic assays.
Aims: To develop a stable nanobody-based GPVI agonist.
Methods: Lama glama were immunized with recombinant extracellular domains of GPVI and a phage display library was generated. Nanobodies were selected, produced as monomers or dimers in Escheria coli and purified. Tetramers were generated via C-terminal fusion of dimers with click chemistry. Nanobody constructs were functionally characterized with ligh transmission aggregometry (LTA) in platelet rich plasma (PRP) and whole blood FACS analysis.
Results: A nanobody with high affinity for recombinant GPVI was selected, optimized for expression and produced. FACS analysis confirmed the nanobody reacted with human GPVI on platelets. The nanobody fully inhibited CRP-XL induced P-selectin expression and fibrinogen binding in whole blood and attenuated collagen induced platelet aggregation in PRP. Neither monomers or dimers resulted in platelet activation in LTA or flow cytometry settings at concentrations ≥ 1 µM. However, tetramers of the nanobody caused full platelet aggregation in LTA, as well as P-selectin expression and fibrinogen binding in whole blood FACS analysis, with an EC50 of 2.3 ± 0.1 nM.
Conclusion: Nanobody tetramers to GPVI act as strong platelet agonists and may serve as stable GPVI agonists in diagnostic assays.
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Tissue factor pathway inhibitor attenuates calcium-induced vascular smooth muscle calcification

Vanessa Bröker1,2, Stella Thomassen1 , Elisabetta Castoldi1 , Kanin Wichapong1 , Asim Akbulut1 , Philip Wenzel1,2,3,4 , Leon Schurgers1 , Tilman Hackeng1 1Department of Biochemistry, Cardiovascular Research Institute Maastricht, Maastricht University, Maastricht, The Netherlands 2Center for Thrombosis and Hemostasis, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany 3Department of Cardiology, University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany 4German Center for Cardiovascular Research (DZHK), partner site Rhine-Main, University Medical Center of the Johannes Gutenberg University Mainz, Mainz, Germany

Tissue factor pathway inhibitor (TFPI) acts as a constitutive inhibitor of the extrinsic coagulation pathway by inhibition of the tissue factor/FVIIa/FXa complex. Next to that, TFPI also exhibits a protective role in thrombo-inflammation and vascular remodeling. The aim of this project was to identify the role of TFPI in vascular remodeling and vascular calcification.
Here, we used CRISPR-Cas9 technology to generate human induced pluripotent stem cells (iPSC) deficient in TFPI. Next, iPSC were differentiated into vascular smooth muscle cells (iVSMC) and driven into contractile and synthetic phenotypes to analyze expression of VSMC marker, inflammatory, and calcification-related genes. Ultimately, cells were treated with calcification-inducing medium.
CRISPR/Cas9 modification resulted in cells synthesizing non-functional truncated (Δ4 AA in K2 domain) TFPI. In silico decomposition energy studies revealed that the shortened protein lacks binding to FXa. Comparing gene expression in TFPI-deficient- vs. wild-type iVSMC, a significant decrease in the contractile VSMC markers αSMA and CNN1 was observed. While αSMA expression was reduced in both contractile and synthetic cells, a decline of CNN1 was seen in the contractile phenotype only. Furthermore, expression of the inflammatory chemokine MCP-1 was considerably reduced in contractile TFPI-KO iVSMC. There was no differential gene expression seen for the synthetic marker S100A4, the inflammatory cytokine IL-6, and the osteogenic marker BMP4. Increasing calcium concentrations resulted in significantly increased calcification of TFPI-deficient iVSMC compared to wild-type iVSMC. Extracellular addition of recombinant TFPI reversed this effect.
We show that functional TFPI plays a protective role in the development of vascular calcification. Calcium regulation is highly dependent on the VSMC phenotype. Enhanced calcification in knockout iVSMC might therefore derive from a phenotypic switch into a less contractile state, as suggested by the downregulation of contractile markers in TFPI-KO cells. Future studies including RNAseq and protein analysis will elucidate pathways involved in this process.

Alterations In Thrombin Generation After SARS-Cov-2 Vaccination And The Relation With Inflammation

W.J. Van Dijk, MD Phd Student M.L.M. Prins, MD Phd Student L.G. Visser, Professor, MD, Phd A. Van Hylckama Vlieg, Phd F.R. Rosendaal, Professor, MD, Phd Leiden University Medical Center

Background In The First Months In Which SARS-Cov-2 Vaccines Were Administered, Thrombotic Events Following Vaccination Were Reported. This Study Assesses Changes In Coagulation Parameters Following Intramuscular (IM) Or Intradermal (ID) SARS-Cov-2 Primary Immunization Using The Mrna-1273 LPN (Moderna) Vaccine And The Association Between Potential Coagulation Changes And The Inflammatory Response.
Methods This Study Was Embedded In A Randomized Trial Studying The Non-Inferiority Of ID Fractional Dose Administration Of The Moderna Vaccine [Roozen, Medrxiv 2021]. Healthy Unvaccinated Participants, 18-30 Years, With No History Of COVID-19, Were Randomized Between 1/5th Fractional Dose ID And Standard Dose IM. Informed Consent Was Obtained And This Study Protocol Was Approved By The Local Medical Ethical Committee. Blood Was Drawn At Baseline (Prior To Vaccination (Day(D)1)) And One Week After The Second Dose (D36). Changes In Coagulation Parameters (Thrombin Generation (TG); Primary Endpoint Peak Height) And The Inflammatory Response (Hscrp) Were Assessed Between Both Timepoints Using Linear Regression Analysis.
Results The Analyses Included 123 Participant. Peak Height Increased After Vaccination, Especially In The IM Group (Delta Total 21; 95% Confidence Interval (CI) -1.6 – 44.1). Other Parameters Of Thrombin Generation Also Indicated An Increased Coagulability After Vaccination. Hscrp Increased Only After IM Vaccination (Delta IM 1.1; 95% CI 0.1 – 2.1). There Was No Association Between The Change In Peak Height And The Change In Hscrp (Beta 1.6; 95%CI -3.5 – 6.6). However, Change In Several Secondary TG Endpoints Were Associated With Change In Hscrp (E.G., ETP: Beta 28; 95%CI 7.6- 48.3). Igg Binding Antibodies Against SARS-Cov-2 Spike And RBD Proteins Were Similar In ID And IM Vaccinated Participants.
Conclusions These Results Indicate An Increase In Coagulability After SARS-Cov-2 Vaccination, Which Was Associated With The Inflammatory Response. While ID Administration Induced A Similar Efficacy As IM Administration, ID Administration Was Associated With Less Thrombin Activation And Inflammation.


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Thrombin Generation Potential And The Risk Of Severe COVID-19 Infection

W.J. van Dijk, MD PhD student 1 Konstantin Föhse, MD 2 Mihai G. Netea, PhD 2 CH van Werkhoven, PhD 3 Marc JM Bonten, MD, PhD 3 Martin P. Grobusch, MD, PhD 4 Maurice A.A.J. van den Bosch, MD, PhD 5 F.R. Rosendaal, PhD 1 A. van Hylckama Vlieg, PhD 1 1. Leiden University Medical Center 2. Radboud University Medical Center Nijmegen 3. University Medical Center Utrecht 4. Amsterdam University Medical Center 5. OLVG Amsterdam

Background: COVID-19 is associated with a wide variety of disease severity. Given coagulation disorders are frequently reported in SARS-CoV-2 infections, a possible marker for severe COVID-19 could be thrombin generation potential (TGP).
Methods: This study was embedded in the BCG-PRIME study, a randomized controlled trail that aimed to assess whether BCG vaccination was associated with a reduced risk of (severe) COVID-19 infection in community-dwelling adults over 60 years of age with chronic somatic comorbidity or recent hospitalization or major surgery. In participants not using anticoagulants, TGP was determined prior to BCG or placebo vaccination. Participants were followed for six months, using app-based self-monitoring, and national databases on test results and healthcare use. Severe COVID-19 was defined as the need for healthcare interventions (antibiotics or steroids; hospital admission, ICU admission) or COVID-19 related death. Peak height of the TGP was analyzed as the primary endpoint. Hazard ratios (HR) were estimated with Cox regression after stratification of the peak height at the median of the distribution, adjusted for age, sex, BMI, frailty, and comorbidities. Informed consent was obtained and this study protocol was approved by the local medical ethical committee.
Results Within the 1148 included participants, 59 had a positive SARS-CoV-2 test during follow-up of whom 19 experienced severe COVID-19. The HR of a COVID-19 infection in participants with a high TGP compared with a low TGP was 0.9 (95% confidence interval (CI) 0.5-1.5). For severe COVID-19 the HR was 1.6 (95% CI 0.6 – 4.1). Other TGP markers were not associated with (severe) COVID-19.
Conclusions Thrombin generation potential was not associated with the risk of COVID-19 infection, but possibly with a severe outcome.

Multiplex Quantitation Of Coagulation And Fibrinolysis Markers: An Upcoming Approach To Unravel Bleeding And Thrombotic Disorders

Tirsa T. van Duijl1 , Eva R. Smit1 , Mariëtte G. Boon-Spijker1 , Carmen van der Zwaan1 , Joost C.M. Meijers1,2, Maartje van den Biggelaar1 1 Department of Molecular Hematology, Sanquin Research, Amsterdam, the Netherlands 2 Department of Experimental Vascular Medicine, Amsterdam UMC, Amsterdam, the Netherlands.

Background: A variety of laboratory tests are needed to define the pathology underlying a bleeding or thrombotic disorder in an individual patient. Despite the diagnostic tools available, the etiology of the disorder remains unexplained in most patients with a mild clinical phenotype. Aims: To determine levels of plasma proteins involved in coagulation and fibrinolysis to obtain protein signatures underlying bleeding phenotypes.
Methods: A coagulation and fibrinolysis protein biomarker panel was composed based on the Tier 1 diagnostic gene list endorsed at the ISTH 2022, supplemented with common inhibitors and regulators of fibrinolysis and coagulation. Targeted mass spectrometry (MS) was performed with a bottom-up proteomics approach to determine the plasma levels of multiple target proteins in a single assay. Factor-deficient plasmas or plasmas derived from non-human species were used in calibration strategies for absolute protein quantitation in conventional (U/mL) or molar units.
Results: Twenty-eight proteins of the coagulation and fibrinolysis panel were quantified from plasma by parallel reaction monitoring (PRM) of 115 peptides in a single 44 minutes MS run. Peptide measurands for quantitation were selected based on theoretical characteristics (e.g. uniqueness and localization in the protein domain) and empirical data from unbiased plasma proteomics studies (e.g. detectability and inter-peptide agreement). VWF, platelet-derived PF4 and PBP, and the coagulation factors fibrinogen (α,β,γ), prothrombin, FV, FVII, FIX, FX, FXI, FXII, FXIIIA, FXIIIB, were quantified in plasma, whereas FVIII was below the limit of detection (±<10 nM) (Figure 1). The plasma levels of antithrombin, protein C, protein S, TAFI and plasminogen as representative inhibitors and regulations of coagulation and fibrinolysis were determined by MS, but quantification of ADAMTS13 and TFPI required upfront enrichment from plasma.
Conclusion(s): Targeted proteomics enables multiplex quantitation of coagulation and fibrinolysis markers and can now be applied to study the clinical value of the all-in one diagnostic screening approach

Unveiling Mechanisms Involved In Venous Thromboembolism In Colorectal Cancer Patients

Dongyue Fan1 , Monika Yanovska1 , Nick van Es2 , Yassene Mohammed3 , Jeroen Buijs1 , Araci M. R. Rondon1 , Henri H. Versteeg1 1 Division of Thrombosis and Hemostasis, Einthoven Laboratory for Experimental Vascular Medicine, Leiden University Medical Center, The Netherlands 2 Department of Vascular Medicine, Amsterdam Cardiovascular Science, Amsterdam University, Medical Centers, University of Amsterdam, Amsterdam, The Netherlands 3 Center for Proteomics and Metabolomics, Leiden University Medical Center, The Netherlands

Introduction: Venous thromboembolism (VTE) is a critical factor that contributes to poor prognosis and it is an important cause of mortality in cancer patients. Colorectal cancer (CRC) patients are at increased risk of VTE due to a hypercoagulable state, and the incidence of VTE is 5-10% in these patients. Therefore, identifying the risk of VTE in individual patients with CRC and prescribing anticoagulant therapy in advance has great potential. Currently, there is still a lack of understanding of which mechanisms are involved in VTE in CRC-associated VTE.
Aim: To study biological mechanisms involved with VTE in CRC patients.
Material and methods: Blood plasma from CRC patients and CRC patients with VTE were analyzed by multiplexed quantitative targeted proteomics. The organ-on-a-chip model (OrganoPlate Graft, Mimetas B.V.) was used to mimic cancer-associated thrombosis. Endothelial permeability was evaluated by adding a high molecular weight protein (TRITCDextran 155 kDa) to the vessel, or with electric cell-substrate impedance sensing experiments (ECIS). The thrombin generation was evaluated by adding pooled blood plasma and FluCa-kit.
Results: The multiplexed proteomics showed that 8 proteins were upregulated in plasma from CRC who suffered from VTE: prothrombin, angiotensinogen, IgM, CD5-like, ficolin-3, IgG, apolipoprotein L1 and apolipoprotein B-100. Our organ-on-a-chip model showed that RKO colorectal cancer cells spheroids reduced the permeability of endothelial cell-based vessels by the 2-fold change and increased the formation of thrombin inside the vessel more than 10 times.
Conclusions: Our data suggest that multiplexed quantitative targeted proteomics is an interesting method to study which proteins play a significant role in CRC-associated VTE. RKO spheroids reduced endothelial permeability and promoted thrombin generation in the organ-ona-chip model.
Perspective: Our next aim is to use inhibitors (e.g., antibodies) for the 8 proteins upregulated in CRC patient plasma to determine the role of these proteins in cancer-associated thrombosis in CRC patients.

Epitope Specificity Of Anti-Prothrombin Antibodies That Express Lupus Anticoagulant Activity

Rachel Gehlen 1 , Tessa Noordermeer 2 , Walid Chayoua 1 , Marisa Ninivaggi 1 , Romy de LaatKremers 3 , Mark Roest 5 , Philip G. de Groot 1 , Rolf T. Urbanus 2 , and Bas de Laat 1,2,4 1 Department of Functional Coagulation, Synapse Research Institute, Maastricht, the Netherlands 2 Van Creveldkliniek, University Medical Center Utrecht, Utrecht University, Utrecht, the Netherlands 3 Department of Data Analysis and Artificial Intelligence, Synapse Research Institute, Maastricht, the Netherlands 4 Department of Protein Engineering, Synapse Research Institute, Maastricht, the Netherlands 5 Department of Platelet pathophysiology, Synapse Research Institute, Maastricht, the Netherlands

Background: The antiphospholipid syndrome (APS) is an autoimmune disorder characterized by the persistent presence of antiphospholipid antibodies. Some of these autoantibodies exert lupus anticoagulant (LAC) activity, a laboratory phenomenon characterized by the prolongation of phospholipiddependent coagulation tests. Recently, our group identified that LAC-positive anti-prothrombin antibodies activate platelets via the platelet receptor, FcγRIIA. However, it is unknown where these antibodies bind on prothrombin.
Aims: To investigate the binding epitope of monoclonal and APS patient anti-prothrombin antibodies with LAC activity.
Method: Fragments of prothrombin were designed and produced in Chinese hamster ovary cells. Prothrombin fragment 1, fragment 2 and fragment 1.2 were coated on ELISA platesto study epitope specificity. LAC-positive monoclonal anti-prothrombin antibodies (3B1 and 28F4), LAC-negative anti-prothrombin monoclonal antibodies (11H2 and 9C6) and plasma from APS patients were studied. A dilute Russell's viper venom time (DRVVT) test was used to evaluate whether recombinant prothrombin constructs could neutralize the effect of LAC-positive anti-prothrombin antibodies. Furthermore, a fragment 1.2 nanobody was produced by immunizing Lama's followed by phage display. The ability of this nanobody to compete with LAC-positive anti-prothrombin antibodies for the prothrombin binding site was tested using the DRVVT test.
Results: The LAC-positive monoclonal anti-prothrombin antibodies recognized prothrombin fragment 2. Interestingly, LAC-negative anti-prothrombin antibodies bound exclusively to fragment 1. LACratios of patient samples correlated significantly with anti-fragment 2 antibody levels ($p< 0.05$, $\rho=0.38$), but not with anti-fragment 1 antibody levels. Furthermore, incubation with prothrombin fragment 2 and the F1.2 nanobody almost completely reversed the prolonged DRVVT clotting time in plasma containing LAC-positive anti-prothrombin antibodies, but not in plasma containing antiβ2GP1 antibodies. In contrast, this neutralising effect was not observed when incubated with prothrombin fragment 1
Conclusion: Our data show that LAC-positive anti-prothrombin antibodies react with fragment 2 of prothrombin. We were able to neutralize a prothrombin-dependent LAC with fragment 2 and with a novel nanobody directed against fragment 1.2

Integrative Phosphoproteomics Of EC-Hemostatic Interactions

Stijn A. Groten1 , Bart L. van den Eshof1 , Floris P.J. van Alphen1 , Alexander B. Meijer1,2 , Maartje van den Biggelaar1,2*, Arie J. Hoogendijk1* 1Department of Molecular Hematology, Sanquin Research, Amsterdam, The Netherlands 2Department of Biomolecular Mass Spectrometry and Proteomics, Utrecht Institute for Pharmaceutical Sciences (UIPS), Utrecht University, Utrecht, The Netherlands *authors contributed equally

Background: The vascular endothelial cell (EC) monolayer plays a crucial part in maintaining hemostasis. An extensive array of receptors allow ECs to dynamically act on disruptions. The impact of individual receptors on EC signal transduction has been the subject of various studies. However insight into discordant and concordant EC signaling remains limited.
Objective: To investigate EC signaling events initiated by hallmark players in hemostatic signaling.
Method: Using unbiased quantitative mass spectrometric based phosphoproteomics we delineated differences and overlaps in EC signaling in response to stimulation with histamine, thrombin, PAR1-4 agonists, APC, Sphingosine-1-phosphate receptor 1 agonist and VEGF. To extend this analysis from single receptors to the signaling invoked by multiple receptors, we furthermore studied the phosphoregulation by factors released by or present within platelets.
Results: We observed that protease activated receptor signaling is highly similar, while distinct from histamine induced signaling networks. Based on this, we identified signaling events that associate with the observed differences in endothelial barrier function disruption due to histamine versus PAR agonists.
Conclusion: This study provides an integrative phosphoproteomic analysis of hallmark EC stimulating factors that highlight the interplay of hemostatic-endothelial signaling.

Quantification Of Von Willebrand Factor Proteoforms By Mass Spectrometrybased Proteomics

Iris C. Kreft1 ,Tirsa T. van Duijl1 , Calvin van Kwawegen2 , Ferdows Atiq2 , Winny Phan1 , Margo B.P. Schuller1 , Carmen van der Zwaan1 , Alexander B. Meijer1 , Arjan J. Hoogendijk1 , Ruben Bierings2 , Frank W.G Leebeek2 , Maartje van den Biggelaar1 , also on behalf of the WiN study group. 1 Department of Molecular Hematology, Sanquin Research, 1066 CX Amsterdam, the Netherlands 2 Department of Hematology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

Von Willebrand Disease (VWD) is the most common inherited bleeding disorder which is characterized by either low (type 1) or absent (type 3) levels of Von Willebrand factor (VWF) or by the occurrence of dysfunctional proteoforms of VWF (type 2). The relationship between genotypic variation, quantitative levels of VWF and its proteoforms in the circulation and bleeding phenotype is complex and largely unknown. Here, we applied both discovery-based data dependent (DDA) as well as targeted workflows to quantify VWF, VWF propeptide and a variety of pathogenic proteoforms in plasma from VWD patients. Citrate plasma samples from 10 healthy individuals and 65 participants of the Willebrand in the Netherlands (WiN) study1 were analyzed by unbiased proteomics to characterize VWF and by targeted proteomics to quantify VWF, VWFpp and five selected pathogenic VWF variants (C1190R, F1293L, R1306W, R1374H & Y1584C). Unbiased plasma proteomics is able to detect alterations in VWF plasma levels which are in high concordance with diagnostic ELISA levels. Overall VWF sequence coverage was >70% and 16 pathogenic VWF proteoforms resulting from missense mutations, frame shifts or exon deletions were characterized. In addition, we were able to discriminate between homozygous and heterozygous mutations. The targeted assay allowed us to quantify VWF and VWFpp in all healthy individuals as well as in all type 1 and type 2 patients, but not in VWD type 3. VWFpp/VWFmature ratios were increased in type 2 patients with a variant in the D'D3A1 domains. Surprisingly, we observed a spread in the variant-to-wild type stoichiometry. Our study highlights the potential importance of detecting and quantifying VWF proteoforms in plasma, which remains to be studied in relation to the VWD bleeding phenotype.

[1] De Wee et al., Determinants of bleeding phenotype in adult patients with moderate or severe von Willebrand disease. Thromb Haemost. 2012 Oct;108(4):683-92.

Thrombodynamics: A Novel Assay For Diagnostic Evaluation Of Haemophilia A Patients

Ryanne A. Arisz1 , Lorenzo G.R. Romano1 , Janneke de Vries1 , Frank W.G. Leebeek1 , Saskia E.M. Schols2 , Jeroen Eikenboom3 , Moniek P. M. de Maat1 , on behalf of the SYMPHONY consortium. 1Department of Hematology, Erasmus MC, Erasmus University Medical Center Rotterdam, Rotterdam, the Netherlands 2Department of Hematology, Radboud University Medical Center, Nijmegen, The Netherlands and Hemophilia Treatment Center Nijmegen-Eindhoven-Maastricht, The Netherlands 3Department of Internal Medicine, Division of Thrombosis and Hemostasis, Leiden University Medical Center, Leiden, The Netherlands

Background: The bleeding tendency in patients with haemophilia A is associated with FVIII activity levels and these are therefore used for classification of disease severity. However, also other determinates may influence bleeding tendency. A potentially valuable new assay to assess the hemostatic potential is thrombodynamics analysis, in which spatiotemporal clot growth and thrombin generation are measured in plasma.
Aim: To correlate thrombodynamics analysis with disease classification in haemophilia A patients.
Methods: We included nineteen patients with a mild (FVIII 0.12 IU/mL; n=5), moderate (0.04 IU/mL; n=7) or severe classification (<0.01 IU/ml; n=7) with blood sampling at least 48h after last prophylaxis treatment. Thrombodynamics (LLC Hemacore, Moscow, Russia) was performed in platelet poor plasma according to the instructions of the manufacturer. One plasma sample of a mild haemophilia A patient could not be measured due to a high lipid concentration and was excluded for analysis.
Results: Clot size (median 1172 µm [326] for mild, 1098 µm [63] for moderate and 804 µm [59] for severe patients) and clotting speed (46 µm/min [6] for mild, 46 µm/min [3] for moderate and 39 µm/min [3] for severe patients) decreased in line with disease severity. Clot density, endogenous thrombin potential (ETP), and maximum thrombin concentration did not correlate with FVIII activity. In these parameters, a large variation between patients with similar severity was observed. Most, but not all patients, showed a trend between the clot density and ETP.
Conclusions: We identified clot density and ETP as parameters that present novel information on clot formation in haemophilia influenced by a other determinants apart from FVIII activity. The correlation between ETP and clot density might suggest differences in the fibrin network properties between patients. Further analysis regarding clinical outcome of bleeding is necessary to establish whether these parameters are associated with bleeding tendency and to evaluate their additional diagnostic value.

Platelet Activating And Fibrin Generating Properties Of Distinct Vascular Smooth Muscle Cell Phenotypes

Constance C. F. M. J. Baaten1,2, Alicia Veninga1 , Armand M. G. Jaminon1 , Femke de Vries1 , Leon J. Schurgers1 , Paola E. J. van der Meijden1,3 1Department of Biochemistry, Cardiovascular Research Institute Maastricht, Maastricht University, Maastricht, The Netherlands 2 Institute for Molecular Cardiovascular Research, University Hospital Aachen, RWTH Aachen University, Aachen, Germany 3Thrombosis Expertise Centre, Heart and Vascular Centre, Maastricht University Medical Centre+, Maastricht, The Netherlands

Background: Upon endothelial injury or dysfunction, vascular smooth muscle cells (VSMC) and extracellular matrix can be exposed to the circulation and thereby activate the haemostatic system. VSMCs actively support platelet plug assembly and fibrin formation. Moreover, pathological conditions can drive VSMCs from a quiescent, contractile phenotype towards a proliferative synthetic phenotype, potentially altering their thrombogenicity.
Aim: To study the thrombogenicity of contractile versus synthetic VSMCs and to characterize thrombi formed on these distinct VSMC phenotypes.
Methods: Primary human VSMCs were isolated from healthy aortic tissue, obtained from Maastricht University Medical Centre+. Collection, storage, and use of tissue and human aortic samples agreed with the Dutch Code for Proper Secondary Use of Human Tissue. Upon written informed consent, blood samples were collected from healthy donors free from antithrombotic drugs. VSMCs were cultured on glass coverslips and maintained as contractile VSMCs or differentiated towards synthetic VSMCs using PDGF-BB. To determine VSMC-mediated platelet adhesion and fibrin formation under flow, recalcified citrated whole blood was perfused over VSMCs at 1000 s-1 for 7 min using the Maastricht flow chamber.
Results: Synthetic VSMCs induced the formation of larger thrombi with more fibrin compared to contractile VSMCs. Interestingly, we observed significantly more erythrocytes present in and around thrombi formed on synthetic VSMCs, suggesting upregulated expression of receptors and/or ligands for erythrocyte adhesion by synthetic VSMCs. To determine whether thrombus formation on synthetic VSMCs is more driven by coagulation, whole blood was pre-treated with dabigatran, inhibiting thrombin. Dabigatran strongly suppressed fibrin formation and reduced platelet adhesion and aggregation for both VSMC phenotypes as well, indicating that thrombus growth on synthetic as well as contractile VSMCs is highly dependent on coagulation activation.
Conclusion: Collectively, our findings point to a higher thrombogenicity of synthetic VSMCs compared to contractile VSMCs.

Development Of In Silico Models To Evaluate Direct Oral Anticoagulant Binding To Factor Xa Variants

Rianne van Diest1 , Dejvid Veizaj2 , David A. Poole III1 , Pieter H. Reitsma2,3, Daniël Verhoef2,3, Daan P. Geerke1 , Mettine H.A. Bos2 1AIMMS Division of Molecular and Computational Toxicology, Department of Chemistry and Pharmaceutical Sciences, Vrije Universiteit, Amsterdam, The Netherlands; 2Department of Internal Medicine, Division of Thrombosis and Hemostasis, Einthoven Laboratory for Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, the Netherlands; 3VarmX B.V., Leiden, The Netherlands.

Background: The direct oral anticoagulants (DOACs) apixaban, rivaroxaban, and edoxaban inhibit factor (F)Xa (FXa). For prevention and/or treatment of bleeding complications related to DOAC use, specific reversal agents are required. Encouraged by previously engineered F174-substituted FXavariants with decreased sensitivity towards FXa-DOACs, we are exploring in silico approaches for rational design of novel FXa-variants with reduced FXa-DOAC binding while retaining catalytic activity. Whereas crystal structures of FXa in complex with apixaban/rivaroxaban are available as starting point for analyses, a 3D-structure of the FXa-edoxaban complex is lacking.
Aims: Develop in silico models of FXa-DOAC complexes for use in FXa mutation studies with the overall goal of identifying FXa variants with retained substrate and impaired FXa-DOAC binding.
Methods: Using HADDOCK, edoxaban was docked in FXa employing a crystal structure of FXa complexed with an edoxaban derivative as template (PDB ID 2EI8). For the FXa-rivaroxaban complex F174A and F174S variants were studied with Molecular Dynamics (MD) simulations using AMBER. The binding affinity was analyzed by performing free energy computations and evaluating root-mean-square atom-positional deviations.
Results: The docking results provided three different binding poses of edoxaban, from which the second pose displayed an orientation almost identical to the template substrate. The aromatic halogen-containing moiety of edoxaban was positioned in the S1 binding pocket of FXa, similar to the aromatic P1 groups of apixaban and rivaroxaban. FXa-variants F174A and F174S demonstrated distorted interactions with the morpholinone moiety of rixaroxaban and an average lower binding free energy compared to wild-type FXa. However, the data showed significant fluctuations between replicates.
Conclusions: Our docking results are a starting point for MD simulations of the FXa-edoxaban complex. However, fluctuations in the FXa-rivaroxaban data show that our MD protocol needs improvement. Therefore, we will optimize input parameters. Once revised, this model will be used to identify new FXa-variants with reduced DOAC binding and study prothrombin binding.

Liver Sinusoidal Endothelial Cells As A Novel Target For Tolerance Induction To FVIII

Michela Leoni1 , Mariarosaria Miranda1 , Karin Fijnvandraat2 , Sebastien Lacroix-Desmazes3 and Jan Voorberg1 , on behalf of the EDUC8 consortium. 1Department of Molecular Hemostasis, Sanquin Research, Amsterdam, The Netherlands 2Department of Pediatric Hematology, Amsterdam UMC, University of Amsterdam, Emma Children's Hospital, Amsterdam, The Netherlands 3Sebastien Lacroix-Desmazes, Centre de recherche des Cordeliers, INSERM, Sorbonne Université, Université de Paris, Paris, France

Background: A major complication of current treatment of hemophilia A is the development of FVIII inhibitory antibodies. Currently, immune tolerance induction (ITI) is used to eradicate FVIII inhibitors. Since ITI fails in 30% of patients within the EDUC8 consortium we are exploring novel ways to induce tolerance to FVIII.
Aim: Explore the potential of targeting FVIII domains to liver sinusoidal endothelial cells (LSEC) since these cells have been shown to promote tolerance instead of immunity towards the antigens presented on their surface.
Methods: Human hepatic sinusoidal endothelial cells were cultured and characterized. We studied their phenotype by FACS analysis and confocal imaging using specific antibodies against FVIII, stabilin2 and other markers of LSEC and endothelial cells. In a parallel we started to produce new recombinant protein that can target LSEC thanks to the binding of stabilin-2 present on the surface of the cells and contain a FVIII immunogenic domain.
Results: Our results show that primary human hepatic endothelial cells express von Willebrand factor. Primary human liver endothelial cells also express stabilin-2 on the cell membrane as confirmed by our experiments employing confocal microscopy. FACS analysis confirmed these findings and provided evidence for the expression of FVIII, previously shown to be expressed on LSEC. We also started the production of a new recombinant protein containing FVIII and stabilin-2 targeting domains. In parallel we are exploring the functional domains of stabilin-2 involved in the internalisation of FVIII and FVIII/VWF complexes.
Conclusion: We hypothesize that LSECs are a potential target for the induction of tolerance. The primary human hepatic endothelial cells used in this study express several LSEC specific markers. The presence of stabilin-2 on the surface of these cells will allow us to target this receptor employing recombinant proteins containing FVIII immunogenic domains with the ultimate goal of inducing tolerance to FVIII

Role Of Contact Activation Pathway In Antiphospholipid Syndrome: A Case-Control Study

Valérie L.B.I. Jansen* 1,2 , Dagmar J.M. van Mourik* 1,2 , Michiel Coppens1,2 , Saskia3 Middeldorp, Harry R. Büller1 , Henri M.H. Spronk4,5,6 , Magdolna Nagy4,5 , Thijs E. van Mens1,2 *Contributed to this work equally 1. Amsterdam UMC location University of Amsterdam, (Experimental) Vascular Medicine, Meibergdreef 9, Amsterdam, Netherlands 2. Amsterdam Cardiovascular Sciences, Pulmonary Hypertension & Thrombosis, Amsterdam, The Netherlands 3. Department of Internal Medicine, Radboud university medical center, Nijmegen, The Netherlands 4. Department of Biochemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, The Netherlands 5. Thrombosis Expertise Center, Heart and Vascular Center, Maastricht University Medical Center, Maastricht, The Netherlands 6. Department of Internal Medicine, Maastricht Unviversity Medical Center, Maastricht, The Netherlands

Background: Antiphospholipid syndrome (APS) is an acquired autoimmune thrombophilia. APS antibodies have a range of effects on the coagulation system contributing to the prothrombotic state. However, the relative contribution of the contact activation pathway to the pathogenesis has not been elucidated. Given the prominent role of neutrophil extracellular traps (NETs) in APS and the role of NETs in coagulation, contact activation may contribute to the prothrombotic state.
Aim: To assess the contact activation pathway in APS patients compared to healthy controls.
Methods: We included thrombotic and/or obstetric APS diagnosed patients according to the Sydney classification criteria and healthy controls from our APS biobank. Subjects had no recent clinical events. Contact activation and downstream activation were assessed by measuring coagulation enzyme:inhibitor complexes in platelet-poor plasma. Activated coagulation factor XII:antithrombin (FXIIa:AT), FXIIa:C1-inhibitor (FXIIa:C1Inh), FIXa:AT and thrombin (T):AT complexes were measured utilizing novel in-house developed ELISAs. Our primary outcome was total FXII activation as measured by the sum of FXIIa:AT and FXIIa:C1Inh. Concentrations were log-transformed to achieve normal distribution and compared using independent t-test.
Results: We included 73 APS patients and 19 healthy controls. Baseline characteristics show no differences between the groups. Patients had 63.0% lupus anticoagulant positivity, 11.0% triple positive, 72.6% had a thrombotic phenotype and 49.3% an obstetric phenotype. The level of activated FXII did not differ between APS patients and healthy controls (p=0.55). 40 APS patients were using anticoagulants. Anticoagulant use was not associated with activated FXII (p=0.25). Patients on anticoagulants had lower thrombin and FIX activation. The subgroup of patients not using anticoagulants did not have elevated thrombin and FIX activation compared to controls.
Conclusion: This study shows that there is no difference in FXII activation between APS patients and healthy controls. At group level, APS patients without recent thrombosis do not have significantly increased contact activation pathway activity.

Using Alphafold2 And Molecular Dynamics Simulations To Model Factor Xa – Substrate Binding In Silico

Dejvid Veizaj1 , Rianne van Diest2 , David A. Poole III2 , Pieter H. Reitsma1,3, Daniël Verhoef1,3, Daan P. Geerke2 , Mettine H.A. Bos1 1Dept. of Internal Medicine, Div. of Thrombosis and Hemostasis, Einthoven Laboratory for Vascular and Regenerative Medicine, Leiden University Medical Center, Leiden, the Netherlands; 2AIMMS Division of Molecular and Computational Toxicology, Department of Chemistry and Pharmaceutical Sciences, Vrije Universiteit, Amsterdam, The Netherlands; 3VarmX B.V., Leiden, The Netherlands

Background: Factor (F)Xa is the serine protease responsible for proteolytic activation of prothrombin to thrombin. Thisinvolves cleavage at R271 and R320 and proceeds via two pathways, generating intermediates prethrombin-2 and meizothrombin, respectively. Attempts to experimentally establish FXa-prothrombin 3D-structures have led to conflicting results. Developing in silico models to investigate binding of the prothrombin cleavage regions would aid in further characterization of natural FX-variants in addition to assisting in developing therapeutic FX-variants.
Aims: Develop an in silico approach to achieve high-quality 3D-models of FXa/FXa-variants in complex with prothrombin cleavage sequences and characterize the binding.
Methods: Protein sequences were supplied to DeepMind's AlphaFold2 algorithm. Generated 3Dstructures were investigated using distance metrics corresponding to the key interactions for FXaprothrombin binding. Molecular Dynamics (MD) simulations were performed using the AMBER biomolecular simulation package and free energies of binding were computed.
Results: Using AlphaFold2 a series of 3D structures of FXa-prothrombin R271 sequences were generated in which the FXa specificity pocket residue D189 (chymotrypsin numbering) was in close proximity (~2.5 Å) to the side chain of prothrombin residue R271. Similar distances were observed between the catalytic FXa residue S195 and R271 in prothrombin. This suggests that the prothrombin R271 peptides are advantageously positioned for catalysis. Similar 3D-structures were also generated for FXa-prothrombin R320 sequences, albeit that the number of structures meeting the requirements for ~2.5 Å distances between prothrombin R320 and FXa residues D189 and S195 was ~2-times lower. MD simulations were performed starting from 3D-structures varying in prothrombin sequence length to compute binding free energies to facilitate comparisons with in vitro data.
Conclusions: Using AlphaFold2 we generated 3D structures of FXa and FXa-variants in complex with natural substrate sequences as starting point for computational simulations. This in silico workflow could be used to verify FXa-variants for substrate binding and activity.

Differential Effects Of Vitamin-K Dependent Proteins On Vascular Calcification: Chemical Protein Synthesis And Bulk RNA Sequencing To Unravel Cellular Pathways

Stijn M. Agten, Dennis P.L. Suylen, Anouk Gentier, Leon J. Schurgers, Tilman M. Hackeng Department of Biochemistry, Cardiovascular Research Institute Maastricht (CARIM), University Maastricht, Maastricht, Netherlands

Background The small family of vitamin K dependent (VKD) proteins, also termed Gla-proteins, play an essential role in human physiology and pathology. VKD proteins regulate coagulation, mineralization and cell proliferation. Their importance is illustrated by the fact that mouse knockout models or human deficiencies lead to embryonic lethality, accelerated ageing, or premature death. Surprisingly, while coagulation and mineralization have little in common, VKD proteins affect the critical pathological process of vascular calcification. Mechanisms by which they modulate calcification, however, are largely unknown.
Aims To elucidate how Gla-containing VKD proteins regulate vascular calcification.
Methods We have developed a chemical protein synthesis platform that provides access to both uncarboxylated and carboxylated variants of VKD proteins. Proteins were tested for their ability to inhibit in vitro precipitation of calcium phosphate. In addition, cellular calcification was evaluated by adding VKD proteins to human vascular smooth muscle cells (hVSMC) cultured under calcifying conditions. Finally, bulk RNA sequencing was performed of cells treated with different VKD proteins.
Results Chemical access was obtained to carboxylated and uncarboxylated variants of three VKD proteins; Matrix Gla Protein (MGP), osteocalcin, and the Gla-domain (amino acid 1-46) of protein S. Uncarboxylated proteins were unable to inhibit in vitro calcium phosphate precipitation and calcification of hVSMCs. While all carboxylated proteins inhibited in vitro biophysical precipitation, significant differences were observed in biological calcification inhibition of hVSMCs. Both protein S Gla and MGP could fully inhibit calcification, yet RNA sequencing showed distinct differences in gene expression of hVSMCs treated with protein S Gla and MGP.
Conclusion VKD proteins from the mineralization and coagulation subfamily interfere with vascular calcification. Differences in inhibitory activity may point to different modes of action. This is complemented by the differences in gene expression observed. Project is funded by the Dutch Heart Foundation (2019T013) to SMA

Proximity Biotinylation Proteomics To Identify Novel Regulators Of Weibel-Palade Body Morphology And Von Willebrand Factor Secretion

Sophie Hordijk1 , Jeroen Demmers2 , Ruben Bierings1 1Hematology, Erasmus University Medical Centre, Rotterdam, the Netherlands 2Proteomics Center, Erasmus University Medical Centre, Rotterdam, the Netherlands

Defects in Von Willebrand factor (VWF) that impair its synthesis and secretion in endothelial cells (ECs) can cause quantitative von Willebrand Disease (VWD) in patients with bleeding tendencies. When synthesized, VWF is processed through the secretory pathway and stored in secretory organelles called Weibel-Palade bodies (WPBs). The regulatory network that controls trafficking and secretion of VWF involves many dynamic, transient and/or weak interactions that can be identified using novel proteomic strategies. Our aim is to map the composition of the regulatory network of the EC secretory pathway using proximity biotinylation proteomics and determine which of these proteins influence VWF secretion.
Proximity-dependent biotinylation is performed using turbo BirA biotin ligase (TurboID) tagged to the longin SNARE SEC22B, a known regulator of WPB morphology which resides on the interface between the endoplasmic reticulum (ER) and the Golgi. The TurboIDSEC22B fusion is nucleofected into human umbilical vein endothelial cells (HUVECs). After 60 minutes of labeling with biotin, biotinylated proteins within cell lysates are precipitated using streptavidin beads. Label free quantitative DIA mass spectrometry based proteomics will be used for identification and quantitation of target proteins.
Expression of the TurboID-SEC22B fusion constructs in ECs is localized to the ER and Golgi. Proteins in its direct environment are biotinylated through the SEC22B recruited BirA ligase, as visualized using cellular staining with fluorescently-tagged streptavidin. Labeling of intracellular proteins can be increased by prolonging biotin incubation time. The known SEC22B interactor and WPB regulator syntaxin-5 (STX5) is successfully enriched from the HUVEC lysate, indicating that our biotinylation strategy is capable of picking up specific and relevant components of the endothelial secretory pathway. Our preliminary results show that TurboID biotin labeling is a promising tool to identify novel regulators of VWF trafficking in ECs.

Novel Luminescent-Based ADAMTS13 Activity Assay

D. Steeghs1 , J. Merx1,3, M. van Geffen1 , C.J. van de Ridder1 , T.J. Boltje3 , M. van der Bosch1 , C. van 't Veer1 , WL. van Heerde1,2 . 1Enzyre B.V, Novio Tech Campus, Nijmegen, The Netherlands; 2Hemophilia Treatment Centre Nijmegen-Eindhoven-Maastricht, Department of Hematology, Radboudumc, Nijmegen, The Netherlands. 3Radboud University, Department Synthetic Organic Chemistry, Nijmegen, The Netherlands

Background: The activity of Von Willebrand Factor (VWF) is modulated by ADAMTS13, a metalloprotease that cleaves VWF in the A2 domain. Deficiency in ADAMTS13 results in the disorder thrombotic thrombocytopenia purpura (TTP). ADAMTS13 activity analysis for initial diagnosis of TTP is the first-tier assay recommended by the ISTH in 2020. A point-of-care ADAMTS13 test could aid in the therapeutic decision-making process.
Aims: To develop a sensitive luminescent ADAMTS13 activity assay for a point of care platform.
Methods: A chimeric protein construct was expressed in BL21 E. coli containing the VWFA2 domain (with the ADAMTS13 cleavage site Y1605-M1606) in frame with N-terminal NanoLuc® Luciferase, a C-terminal His-tag, and a free cysteine at the C-terminus to enable purification and immobilization, respectively. This construct (N-NLuc-VWFA2-C) was purified and shown to be cleaved by ADAMTS13 by SDS-PAGE in the expected fragments. N-NLuc-VWFA2-C coupled via the free cysteine to commercially available maleimide-coated plates was also cleaved by ADAMTS13 as shown by the released luciferase activity in the supernatant transferred to wells with furimazine substrate. Titration of recombinant ADAMTS13 (TAK-755, Takeda) in ADAMTS13-deficient plasma was performed to verify sensitivity towards ADAMTS13 activity levels in plasma. The luminescent ADAMTS13 activity assay (ADAMTS13lum) was compared to the TECHNOZYM® ADAMTS13 Activity ELISA from Technoclone, using samples from ECAT surveys.
Results: Titration of rADAMTS13 in deficient plasma showed excellent concentration dependent luminescence of the ADAMTS13lum assay over the range of 0-2 IU/ml (R2 0.997). Estimation of ADAMTS13 activity levels of 4 ECAT samples with a range between 0.07 to 0.82 IU/ml with the ADAMTS13lum assay showed high level of agreement (deviance <6%) with the levels estimated by TECHNOZYM® ADAMTS13 Activity ELISA.

Platelet Reactivity In Patients With Atrial Fibrillation And Coronary Artery Disease Under Factor IIa Inhibitors And Factor Xa Inhibitors

KL.Zheng MD(1), WL Bor MD(1) ,A Verburg MD(1), E Aarnink MD(1), JC Kelder MD, PhD(1), CM Hackeng PhD(2), JM ten Berg MD PhD(1,3) Departments of Cardiology(1), Clinical chemistry (2) St. Antonius Hospital Nieuwegein, the Netherlands, (3)University Medical Centre Maastricht, Maastricht University, Maastricht, the Netherlands.

Background Dabigatran is a direct oral anticoagulant(DOAC). Recently, several observational studies point to increase of myocardial infarction(MI) among patients using dabigatran, including the landmark trial RE-LY. Ever since, there have been discussions on whether dabigatran increases the risk of MI.
Objective To investigate the platelet activation in patients with atrial fibrillation(AF) and coronary artery disease(CAD) treated under Factor Xa inhibitorand Factor IIa inhibitor
Methods This prospective, single-centre, observational, crossover study included 40 patients diagnosed with AF and CAD on a steady state level NOAC between July 2021 and December 2022. Patients switched medication immediately after inclusion for 2 weeks. The primary outcome was platelet reactivity measured by P-selectin activation by flow-cytometry analysis. Secondary markers coagulation markers as measured by the TEG6s and T-TAS AR chip. Paired t-test were used to evaluate the differences in platelet activation under Xa and II inhibitors.
Results There was no significant difference in the P-selectin expression under Factor Xa inhibitors(M=19.9, SD=9.5) and Factor IIa inhibitors(M=20.6, SD=9.7); t(38)=-0.32, p=0.75). Area under the flow curve over 30 minutes(AUC30) was significantly higher under Factor Xa inhibitors compared to Factor IIa inhibitors. Occlusion time(OT) was significantly higher under Factor IIa inhibitors(M=1063 seconds, SD=447) compared to Factor Xa inhibitors(M= 824 seconds, SD=353);t(36)=-3.4, p=0.0015)
Conclusion There was no difference in platelet activation under Xa inhibitors and IIa inhibitors in patients with AF and CAD. The AUC30 was significantly higher in Factor Xa inhibitors and OT as measured by T-TAS AR chip was significantly higher under Factor IIa inhibitors.

Automated Segmentation And Quantitative Analysis Of Weibel-Palade Body Morphology, Localization And Content Using Cellprofiler

Sebastiaan N.J. Laan1,2, Richard J. Dirven1 , Jeroen Eikenboom1 and Ruben Bierings2 for the SYMPHONY consortium
1. Department of Internal Medicine, Division of Thrombosis and Hemostasis, Leiden University Medical Center, Leiden, The Netherlands; 2. Department of Hematology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands. Funding: Netherlands Organization for Scientific Research (NWO) in the framework of the NWA-ORC (NWA.1160.18.038) and from the Landsteiner Stichting voor Bloedtransfusie Research (LSBR-1707).

Background & Aim One of the most used and versatile methods to study number, dimensions, content and localization of secretory organelles is confocal microscopy analysis. However, considerable heterogeneity exists in the number, size and shape of secretory organelles that can be present in the cell, as is the case for Weibel-Palade bodies (WPBs), the cigar-shaped secretory organelles of endothelial cells. One thus needs to analyze large numbers of organelles for valid quantification of these parameters. For this purpose we aimed to develop an automated, unbiased method to process and quantitatively analyze organelle morphology and content from microscopy data.
Methods The open source software CellProfiler was used to establish two analysis pipelines, called OrganelleProfiler and OrganelleContentProfiler, aimed at quantification morphometric parameters and content of organelles, respectively. Endothelial colony forming cells (ECFCs) stained for WPB content protein Von Willebrand factor (VWF), cell junctional marker VE-cadherin, endoplasmic reticulum marker PDI and WPB maturation marker Rab27A were imaged by confocal microscopy and analyzed using OrganelleProfiler and OrganelleContentProfiler pipelines. Cell profiler measurements were checked for validity by manual quantification using Fiji image analysis software.
Results We used OrganelleProfiler to quantify cell count and size, and WPB count, size, shape, and relative position to cell nucleus and periphery on a cell-to-cell basis. Characterization of two distinct phenotypic classes of ECFCs using OrganelleProfiler showed significant differences in WPB morphometry and localization. Using OrganelleContentProfiler to quantify secondary signals located in or on the organelle or in the cytoplasm we were able to establish that Rab27A but not PDI are found in or on WPBs.
Conclusion To conclude, the OrganelleProfiler and OrganelleContentProfiler pipelines provide powerful highprocessing quantitative tools for analysis of cell and organelle characteristics.