Nederlandse Vereniging voor Trombose en Hemostase

Nieuwsbrief

Juli 2014

Nieuwsbrief

Inhoud

Nieuwsbrief

Contents

Inleiding

Hierbij de NVTH nieuwsbrief van juli 2014 waarmee we u informeren over ontwikkelingen binnen het gebied van de Trombose en Hemostase en activiteiten van onze vereniging.

De eerste helft van 2014 zit er weer op. We hebben afscheid genomen van aantal prominente bestuursleden. Gelukkig hebben we nieuwe en ambitieuze bestuursleden teruggekregen.

De afgelopen maanden zijn er weer een aantal onderzoekers gepromoveerd. De samenvattingen van het promotieonderzoek vindt u verderop in de nieuwsbrief.

De jaarlijkse NVTH PhD cursus heeft in 2015 het thema Veneuze Trombose en zal gehouden worden van maandag 30 maart - woensdag 1 april, wederom in Golden Tulip Strandhotel Westduin te Koudekerke. Aansluitend zal het NVTH symposium plaatsvinden op woensdag 1 april en donderdag 2 april.

Speciaal voor het vijfde lustrum van de NVTH hebben we een boek samengesteld over de geschiedenis van Trombose en Haemostase Onderzoek in Nederland. Een exemplaar van het Lustrumboek kan aangevraagd worden bij Mark Roest: mroest@umcutrecht.nl.

Namens het NVTH bestuur,

Vriendelijke groeten,

Mark Roest

Introduction

We are proud to present the NVTH newsletter of January 2014th to inform you about developments within the field of Thrombosis and Haemostasis and about activities of our society.

The first half of 2014 is over. We said goodbye to prominent NVTH members and we welcomed new and ambitious board members.

In recent months, there have been several investigators who received their PhD. The summaries of their thesis can be found in this newsletter .

The theme of the annual NVTH PhD course 2015 is: “Venous Thrombosis” and will be held from Monday, March 30 to Wednesday, April 1st, at Golden Tulip Hotel Westduin, Koudekerke. Subsequently, the NVTH symposium will be held from Wednesday, April 1st until Thursday April 2nd.

To celebrate the twenty-fifth anniversary of the NVTH we have made a book on the history of Thrombosis and Haemostasis Research in the Netherlands. Copies can be requested by sending an email to Mark Roest: mroest@umcutrecht.nl.

On behalf of the NVTH Board,

Sincerely,

Mark Roest

Bestuurszaken

In verband met het aflopen van de zittingstermijn is Prof. dr Tilman Hackeng (MUMC) teruggetreden als voorzitter van de NVTH. Het voorzitterschap is overgedragen aan Prof. dr Saskia Middeldorp (AMC).

In verband met het aflopen van zijn zittingstermijn is Prof dr Frank Leebeek (ErasmusMC), teruggetreden als secretaris van de NVTH. Dr. Roger Schutgens (UMCU) is hem opgevolgd als secretaris van de NVTH.

Tevens is Prof. dr Pieter Willem Kamphuisen (UMCG) aangetreden als nieuw bestuurslid.

De benoemingen zijn goedgekeurd door de Algemene ledenvergadering van 10 april 2014.

Administration

Prof. Dr Tilman Hackeng (MUMC) and Prof. Dr. Frank Leebeek (Erasmus MC) have completed their maximum term of board membership and have retired at April 10th.

Prof. Dr. Saskia Middeldorp (AMC), now a board member of the NVTH has replaced prof.dr. Tilman Hackeng to be the new chairman of the NVTH board.

Dr. Roger Schutgens (UMC Utrecht) is the new secretary of the NVTH.

Furthermore, Prof. Dr. Pieter Willem Kamphuisen (UMCG) is a new board member.

New board members were installed at the general meeting of April 10, 2014.

AIO cursus en symposium 2015

De jaarlijkse NVTH PhD cursus heeft in 2015 het thema Veneuze Trombose en zal gehouden worden van maandag 30 maart - woensdag 1 april, wederom in Golden Tulip Standhotel Westduin te Koudekerke. Aansluitend zal het NVTH symposium plaatsvinden op woensdag 1 april en donderdag 2 april.

Registratie 2015:

Inschrijving open:	20 oktober 2014
Deadline Inschrijven PhD cursus:	16 januari 2015
Deadline Abstracts:	16 januari 2015
Deadline Pre-registratie symposium:	16 januari 2015
Deadline Registratie symposium:	20 februari 2015

PhD course and Symposium 2014

The theme of the annual NVTH PhD course 2015 is: “Venous Thrombosis” and will be held from Monday, March 30 to Wednesday, April 1st, at Golden Tulip Hotel Westduin, Koudekerke. Subsequently, the NVTH symposium will be held from Wednesday, April 1st until Thursday April 2nd.

Registration 2015:

Registration open :	20 oktober 2014
Deadline registration PhD cursus:	16 januari 2015
Deadline Abstracts:	16 januari 2015
Deadline Pre-registration symposium:	16 januari 2015
Deadline Registration symposium:	20 februari 2015

PhD graduation

Name:	Liesbeth M. Kager
Titel:	Coagulation and fibrinolysis in tuberculosis, melioidosis and beyond.
Department:	Center for Experimental and Molecular Medicine, AMC , Amsterdam
Defense:	24 januari 2014
Promotores:	Prof. dr. T van der Poll
Co-promotores:	Dr. Kees van ’t veer, Dr. Joost Wiersinga

TB is een chronische bacteriële infectieziekte waarmee 1/3 van de wereld bevolking geïnfecteerd is. Deze ziekte is moeilijk te behandelen, therapieën duren maanden en er is veel resistentie tegen de huidige antibiotica. Melioidose is een acute, snel verlopende ernstige infectieziekte, veel voorkomend in Zuidoost Azië en Noord-Australië. Uiteindelijk overlijdt 30% van de patiënten, meestal door bloedvergiftiging, ondanks dat zij geschikte antibiotica krijgen. Door de hoge mortaliteit, makkelijke toegankelijkheid en makkelijke manier van verspreiden staat melioidose op de lijst van mogelijke bioterroristische wapens. Het (bloed)stollingssysteem speelt een belangrijke rol in de anti-bacteriële afweer. Er was echter weinig bekend over de rol van (bloed)stolling tijdens tuberculose en melioidose. De kennis, voortkomend uit dit proefschrift, draagt bij aan verder inzicht in de afweermechanismen van de gastheer tijdens tuberculose en melioidose, wat uiteindelijk kan leiden tot het ontdekken van nieuwe aangrijpingspunten voor het ontwikkelen van nieuwe behandelingen voor tuberculose en melioidose. Wij hebben onderzocht hoe het stollingssysteem geactiveerd raakt bij patiënten met longtuberculose, als onderdeel van de anti-bacteriële afweer en gekeken of deze effecten hetzelfde zijn in de bloedbaan als in de long zelf. Verder hebben we onderzocht wat de effecten zijn van de verschillende eiwitten die betrokken zijn bij de bloedstolling en fibrinolyse op de antibacteriële afweer tijdens tuberculose en melioidose. De belangrijkste conclusies van dit onderzoek zijn: 1) Tijdens tuberculose raakt het stollingssysteem geactiveerd, waarbij muizenstudies echter geen duidelijke effecten op de afweer lieten zien wanneer specifieke eiwitten betrokken bij de bloedstolling -in het bijzonder het proteïne C systeem en de fibrinolyse- uitgeschakeld worden. 2) Tijdens melioidose daarentegen, dragen eiwitten betrokken bij de bloedstolling en met name het proteïne C systeem en fibrinolyse duidelijk bij aan de afweerrespons van de gastheer.

Verder weten we dat geactiveerd proteïne C soms beschermend werkt tijdens ernstige sepsis. Intraveneuze toediening geeft echter een verhoogde kans op (intracraniele) bloedingen. Wij hebben onderzocht wat het effect is van intrabronchiaal toegediend recombinant geactiveerd proteïne C tijdens endotoxine-

geïnduceerde longinflammatie. Intrabronchiale toediening van APC bleek niet beschermend en ontstekingsremmend te werken maar, integendeel, stimuleerde het de activatie van stollingsfactoren en afgifte van cytokines. Geconcludeerd werd daarom dat lokale toediening van recombinant geactiveerd proteïne C geen plaats heeft in de behandeling van (ernstige) longontsteking.

Tot slot ontwikkelden wij een muismodel voor bot-tuberculose, waarin we aantoonde dat er na een incubatietijd van 10 maanden sprake is van mycobacteriële invasie in de botten. Ook werden er bij MRI aanwijzingen voor beenmerginfiltratie aangetoond. Met een nieuw muismodel voor bot-tuberculose kan de etiologie hiervan bestudeerd worden, evenals nieuwe behandelingsmodaliteiten.

Name: Joke Konings
Titel: The role of coagulation factor XII in fibrin clot formation and fibrinolysis
Department: Biochemistry, Maastricht University
Defense: January 23th, 2014
Promotores: Prof. Dr. H. ten Cate
Co-promotores: Dr. J.W.P Govers-Riemslag

The structure of fibrin clots and their susceptibility to fibrinolysis are important determinants of the thrombotic risk. Clots characterized by a dense fibrin network and resistance to fibrinolysis are associated with an increased risk of thrombosis. The contribution of coagulation factor XII (FXII) to arterial thrombosis is less straightforward. Results from clinical studies are ambiguous, low levels of FXII and high levels of activated FXII (FXIIa) are associated with arterial thrombosis, however not in all clinical studies. FXIIa is able to initiate the intrinsic pathway of coagulation and thereby contributes to thrombin formation. Furthermore, FXIIa can convert plasminogen into plasmin and thereby stimulate fibrinolysis.

The aim of the work described in this thesis, was to determine how FXII influences the fibrin clot structure and the susceptibility to fibrinolysis.

We determined the influence of FXIIa on fibrin clot formation, structure and fibrinolysis. We observed an increase in fibrin fiber density and clot stiffness in the presence of α-FXIIa. In plasma, this increase was dependent on two mechanisms: 1) formation of thrombin via activation of the intrinsic pathway of coagulation, and 2) direct interaction of FXIIa with fibrin(ogen). In binding experiments we showed that purified FXII and α-FXIIa, but not β-FXIIa, bound to purified fibrinogen and fibrin with nanomolar affinity. Immunostaining of human carotid artery thrombi showed that FXII(a) co-localized with areas of dense fibrin(ogen) deposition. From these results, we concluded that FXIIa modulates the fibrin structure via activation of the intrinsic pathway of coagulation and via direct interaction with fibrin(ogen).

Next, we studied the contribution of α-FXIIa to clot stability and fibrinolysis in the presence of low levels of tissue plasminogen activator (tPA). α-FXIIa directly converted plasminogen into plasmin and reduced clot lysis time in the presence of tPA. This reduction in clot lysis time was caused by an earlier onset of plasmin formation.

We determined the influence of fibrin clot formation, fibrinolysis and / or contact activation in three patient groups. First, we measured the activation of the contact activation system in patients with a first acute myocardial infarction (AMI) during the acute event and 3 and 6 months after the AMI. The degree of contact activation was determined in plasma as the levels of activated factor XI (FXIa), FXIIa and kallikrein in complex with C1 esterase inhibitor (C1INH) and the levels of FXIa in complex with α1-antitrypsin (AT). The levels of FXIa-C1INH were elevated during the

acute event compared to the steady-phase 3 and 6 months after the AMI. The levels of FXIa-AT, FXIIa-C1INH and kallikrein-C1INH did not change over time. Since the levels of FXIIa-C1INH were not elevated, activation of FXI during the acute phase did probably not result from contact activation.

Next, we determined if in patients that develop stent-thrombosis (ST) after a percutaneous coronary intervention (PCI), the fibrin clot structure and fibrinolysis are altered compared to control PCI patients (patients that received a stent but did not develop ST). In this study, there were no significant differences in fibrin clot formation and fibrinolysis between ST patients and control PCI patients.

Patients with hereditary angioedema due to a deficiency in C1INH (HAE-C1INH) have impaired inhibition of the contact activation system. This leads to activation of the contact system and release of bradykinin (which mediates the symptoms observed in HAE-C1INH patients). However, these patients do not have an increased risk of thrombosis. Our results showed consumption of both prekallikrein and FXI in these patients, therefore the absence of increased thrombosis in these patients is probably due to the control of the coagulation system by other inhibitors, such as AT, and increased fibrinolysis.

In conclusion, we have found that FXII has a dual role in coagulation and fibrinolysis. During clot formation, activation of FXII leads to a stronger fibrin clot, whereas during fibrinolysis, activation of FXII leads to the formation of plasmin and a faster onset of fibrinolysis. We postulate that during clot formation, FXIIa stabilizes the thrombus by activating the intrinsic pathway of coagulation and by interaction with the fibrin network. This action prevents embolization. During fibrinolysis, FXIIa helps to initiate plasmin formation at low levels of tPA and thereby prevents vessel occlusion.

Naam:	Marcel Schouten
Titel:	The protein C in severe infection.
Afdeling:	Center for Experimental and Molecular Medicine, AMC , Amsterdam
Verdediging:	14 maart 2014
Promotores:	prof. dr. T. van der Poll en prof. dr. M.M. Levi
Co-promotores:	dr. C van 't Veer

Pneumonia is a major cause of sepsis, with the pneumococcus, *S. pneumoniae*, being the most frequently isolated micro-organism. Influenza is another major cause of mortality from pulmonary infectious disease. The second most common cause of sepsis is peritonitis, with *Escherichia (E.) coli* being an important pathogen involved. In severe infection and sepsis systemic inflammation leads to activation of the coagulation system and inhibition of anticoagulant systems and fibrinolysis. Activation of coagulation with resulting fibrin deposition are essential parts of the host defense in an attempt to contain the invading microorganisms and inflammatory response. However, an exaggerated response can lead to aggravation of disease. It is becoming increasingly clear, that components of the coagulation system are vice versa able to modulate inflammation. One of the important anticoagulant systems, the protein (PC) system, is downregulated in sepsis. In this thesis we further eludicate the crosstalk between inflammation and coagulation in severe infection, with a focus on the endogenous PC system. Different components of the PC system were studied in mouse models of severe infectious disease.

<u>Part I: Endogenous protein C in severe infection</u>

In chapter 2 a state of the art overview was presented of what was known thus far on the crosstalk between inflammation and coagulation in severe infection and sepsis, with a special focus on the endothelium.

In chapter 3 we used tmonoclonal antibodies that specifically inhibit the anticoagulant effects of endogenous APC (mAb 1591) or both the anticoagulant and cytoprotective effects of APC (mAb 1609). We show that in pneumococcal pneumonia and sepsis endogenous PC inhibits coagulation and that both the cytoprotective and anticoagulant properties of endogenous APC contribute to its anticoagulant effects.

In chapter 4 the role of PC in lethal H1N1 influenza A pneumonia was studied. Endogenous PC decreased pulmonary coagulation activation as evidenced by lower levels of thrombin-antithrombin complexes (TATc) and fibrin degradation products (FDP) and the absence of intravascular thrombus in control antibody as compared to anti-PC treated mice. PC limited lung histopathology, which is in line with anti-inflammatory properties ascribed to APC. Remarkably, endogenous PC increased neutrophil influx into the alveoli, which is in contrast with previous studies suggesting that APC inhibits neutrophil recruitment. In line, anti-PC treated mice had lower protein levels in BALF, suggesting that the lung barrier function is compromised by PC. Finally, anti-PC treated animals had a survival advantage of 18 hours. To our knowledge, this is the first study in which a beneficial effect of PC inhibition on outcome is shown.

In chapter 5, we show that endogenous PC decreases local and systemic activation of coagulation and enhances systemic fibrinolytic activity in murine *E. coli* peritonitis. Moreover, we demonstrate that endogenous PC transiently lowers bacterial outgrowth, possibly by preventing bacteria to use fibrin clots to escape from killing. These data reveal endogenous PC as an important regulator of the antibacterial response during abdominal sepsis.

<u>Part II: EPCR, PAR-1 and the lectin-like domain of thrombomodulin in pneumococcal disease.</u>

In this part we investigated three receptors of the endogenous PC system in pneumococcal disease. The endothelial protein C receptor (EPCR) is a major player in the PC system, both for activation of PC and the

cytoprotective effects of APC. In chapter 6 we studied the role of EPCR in pneumococcal disease. In contrast to what was known on the role of EPCR in systemic challenge models, we found a detrimental role for EPCR in pneumococcal pneumonia and sepsis as reflected by less bacterial outgrowth and an attenuated pro-inflammatory response in EPCR knock out (KO) mice, and, conversely, higher bacterial loads and an enhanced pro-inflammatory response in mice overexpressing EPCR on their endothelium. It is not probable that enhanced activation of PC alone is responsible for this, since our studies on endogenous PC, described in chapter 3, did not identify a detrimental role in pneumococcal infection.
Data accumulate on coagulation-induced enhancement of inflammation, notably by activation of protease-activated receptors (PARs). To further establish the role of PAR-1 in infection we studied this receptor in murine pneumococcal pneumonia in chapter 7. We show that PAR-1 impairs host defense, as reflected by a reduced lethality, lower bacterial loads, histopathology scores and neutrophil influx in PAR-1 KO mice. Understanding the role of PAR-1 signaling in infection is difficult due to multiple and in part opposite effects ascribed to this receptor. Therefore, PAR-1 at this moment does not represent a straightforward therapeutic target.
In chapter 8 we studied the lectin-like domain of thrombomodulin (TM), which, in contrast to its EGF-like repeat, is not involved in thrombin-induced PC activation. In contrast to sterile inflammation models in which loss of the lectin-like domain of TM renders mice more sensitive to lung injury and endotoxemia we showed that deletion of the domain in pneumococcal pneumonia results in an improved host defense as evidenced by lower bacterial loads in blood and liver at 48 hours after infection, less pulmonary inflammation and a better survival. The clinical relevance of this finding is important to recognize, since soluble TM is being studied for use in the clinic.

Part III: Factor V Leiden in pneumococcal pneumonia and influenza
The factor V Leiden (FVL) mutation - a missense mutation in the FV gene that replaces arginine at position 506 with glutamine, resulting in resistance of activated FV (FVa) to inactivation by APC - is a major risk factor for venous thrombo-embolism. Since some data suggest a survival benefit for carriers of the FVL mutation in severe infection, this mutation was studied more extensively. In chapter 9 a review of data on the FVL mutation in infection was presented. Experimental and clinical studies show inconsistent results as to a difference in survival from sepsis in carriers of the mutation. Additional analyses in larger cohorts of septic patients or long-term prospective studies in patients with a FVL mutation will be required to clarify the issue whether a heterozygous FVL mutation is protective during severe infection and sepsis.
In chapter 10 we studied the effect of the FVL mutation in pneumococcal pneumonia, both in an untreated and in a clinically more relevant antibiotic treated setting. The FVL mutation had no consistent effect on activation of coagulation in the presence or absence of antibiotics. Moreover, it had no effect on pulmonary inflammation and bacterial outgrowth. Remarkably, homozygous FVL mice were strongly protected against death when treated with ceftriaxone. This bears resemblance with findings in patients with severe sepsis. Since FXIII, the main crosslinker of fibrin, has been linked to organ failure in septic shock we performed FXIII blots. Indeed, FXIII was depleted in homozygous FVL mice as compared to WT and heterozygous FVL mice. More research is needed to investigate how the FVL mutation impacts survival in murine and human pneumonia in the context of antibiotic therapy.
Chapter 11 describes a study on the effect of the FVL mutation in lethal H1N1 influenza A pneumonia. We show extensive local and systemic activation of coagulation during lethal influenza as reflected by increased lung and plasma levels of TATc and FDP and fibrin deposition. Like in pneumococcal pneumonia, the FVL mutation did not impact the procoagulant response, histopathology or survival. Taking together the data from both FVL studies we found no clear indications for a beneficial role for heterozygosity for the FVL mutation in infectious disease.

Part IV: Recombinant murine activated protein C in pneumococcal pneumonia and influenza.

To study a possible beneficial effect of exogenous APC in pneumonia and sepsis, as had been demonstrated earlier for recombinant human (rh-)APC in human sepsis, we in Part IV studied the effects of the recombinant murine variant of APC (rm-APC) in different models of pulmonary infection. In the first two chapters of this section we studied the effect of rm-APC in pneumococcal pneumonia. In chapter 12 we administered rm-APC after 24 hours of infection, either or not as an add-on to antibiotic therapy. In this study rm-APC, as expected, inhibited pulmonary activation of coagulation. When added to ceftriaxone, rm-APC improved survival as compared to ceftriaxone alone, which was in line with the earlier mentioned human sepsis study (PROWESS). To our knowledge, this is the first preclinical study in which APC has a positive effect on survival in the setting of severe infection with concurrent antibiotic treatment. Since in this study rm-APC did not impact on the pulmonary levels of 55 inflammatory mediators in the context of antibiotic therapy whereas earlier studies in contrast had shown effects of APC on pulmonary inflammation we in chapter 13 performed a study in which rm-APC was administered as early as 12 hours after infection. Indeed, in this study, rm-APC exerted a broad inhibitory effect on the local production of many proteins implicated in inflammation in pneumonia.
Finally, in chapter 14 we studied the effect of rm-APC in influenza. Lethal H1N1 influenza resulted in systemic and pulmonary activation of coagulation and in inhibition of fibrinolysis due to enhanced release of PAI-1. Rm-APC inhibited coagulation activation in plasma and lungs, and partially reversed inhibition of fibrinolysis. Rm-APC also temporarily reduced pulmonary viral loads, but it did not impact lung inflammation or survival.

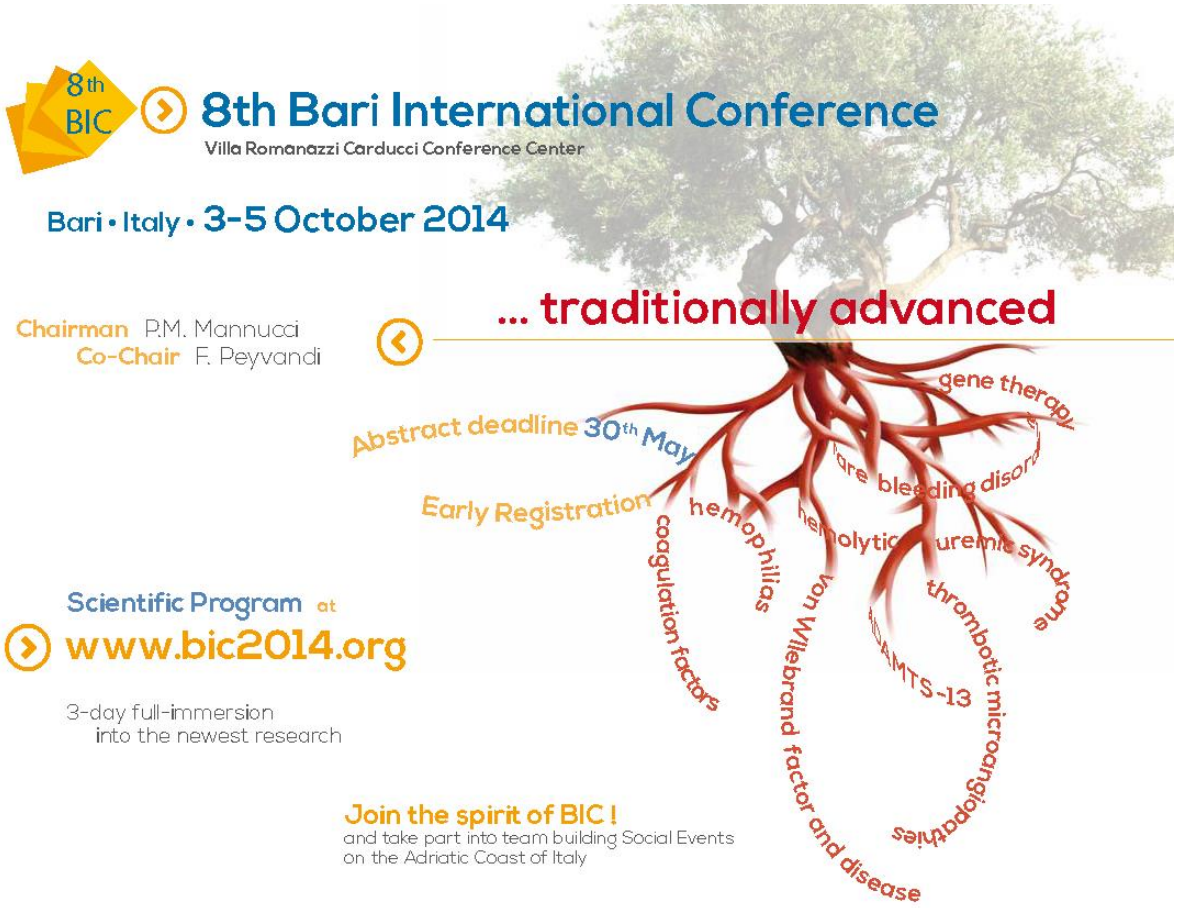
Conclusions

Different studies on the endogenous PC system and on rm-APC and FVL in experimental infection were described. Our studies are consistent in showing that endogenous PC in various models at various time points lowers coagulation activation as it does in the uninfected situation. Moreover, we showed in different models that endogenous PC lowers PAI-1, hereby potentially facilitating fibrinolysis, which had only been shown in vitro. Our studies indicate that different components of the PC system impact on host defense in different ways. As we have seen in our FVL and rm-APC studies, phenotypes can be changed to a large extent when the effect of a protein or receptor is studied in infection with concommittant antibiotic treatment. Our rm-APC studies show the importance of timing of interventions. Our studies should be interpreted with caution upon extrapolation to human sepsis and pneumonia. We studied homogenous animals. In human sepsis, patients differ with respect to the first organ that was affected, the causative micro-organism, the phase of the disease in which they are, their age, race and comorbidity. This heterogeneity perhaps could explain at least partially why so many sepsis trials, for instance the recent PROWESS-shock trial, have negative endpoints.

Naam: Vivian Du
Titel: The Expanding Horizons in Thrombosis and Hemostasis
Afdeling: Laboratory for Clinical Chemistry and Haematology, UMC Utrecht
Verdediging: 16 Juni 2014
Promotores: prof. dr. PhG. De Groot
Co-promotores: dr. B. de Laat
:

The Expanding Horizons in Thrombosis and Hemostasis

In the studies described in this thesis, we investigated the role of erythrocytes in thrombus formation. In contrast to the common notion that erythrocytes are bystanders in thrombus formation, we found that erythrocyte can actively influence thrombus formation by interacting with platelets. Erythrocytes can bind to platelets under flow conditions. The binding of red blood cells to platelets is mediated by ICAM-4 on the red blood cell membrane and its receptor protein $\alpha_{IIb}\beta_3$ on the platelet membrane. Blocking the ICAM-4 mediated erythrocyte-platelet interaction resulted in reduced thrombus formation. Drugs that target the red blood cell-platelet interaction may become one of the new therapeutic strategies to treat or prevent thrombotic conditions, such as heart attack and stroke. In the studies described in this thesis, we also investigated the mechanisms by which the transfused platelets stored at cold conditions are cleared rapidly after transfusion. Platelet products from blood banks are currently stored at room temperature, which leads to a short shelf life (max 7 days) and the risks of bacterial contamination. Cold storage (0-4°C) would be better in preserving the quality of platelet products. However, the cold-stored platelets are known to be cleared rapidly from the circulation after transfusion. Our study showed that low temperature can induce a structural change in GP1b on the platelet surface, which leads to platelet apoptosis. The apoptotic platelets are cleared by macrophages. By interfering with the GP1b structural change, we may be able to prevent platelet apoptosis and render platelet cold storage more feasible.


8th BIC
8th Bari International Conference
Villa Romanazzi Carducci Conference Center
Bari • Italy • 3-5 October 2014
Chairman P.M. Mannucci
Co-Chair F. Peyvandi
... traditionally advanced
Abstract deadline 30th May
Early Registration
gene therapy
hemophilias
coagulation factors
von Willebrand factor and disease
ADAMTS-13
thrombotic microangiopathies
Scientific Program at
www.bic2014.org
3-day full-immersion
into the newest research
Join the spirit of BIC !
and take part into team building Social Events
on the Adriatic Coast of Italy