BSHT Annual Scientific Meeting 2018

Thursday 8 – Friday 9 November 2018

The Slate, University of Warwick, Coventry, UK

PROGRAMME + ABSTRACT BOOK

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With grateful thanks to this year's meeting sponsors, who make this meeting possible:

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WELCOME

8 November 2018

Dear Colleagues,

It is a great pleasure for me to welcome you to the 2018 Annual Scientific Meeting of the BSHT in the well-equipped facilities of The Slate at the University of Warwick. We return to Warwick this year due to its central location, the intimate feel of the venue and the inclusive accommodation, which I believe enhances interactions and collaborative links within the UK Haemostasis and Thrombosis community.

We have an exciting two days of new and emerging science from the Thrombosis and Haemostasis community. For the first time, we have included a 'clinician-in-training' parallel session, which the committee felt may represent an exciting and useful addition to our normal programme. For those of you with a clinical background, I urge you to attend this session and to provide feedback on this. Our main programme has invited world-leading experts from both overseas and the UK. You will notice that I chose to draw on the expertise of the BSHT committee this year for several of our invited talks, as I felt this was an 'untapped resource'. As always, we also have many excellent abstract presentations that will cover recent findings. The scientist in training and emerging fellows sessions are, in my opinion, highlights of our programme.

Next year, the UK will also play host to the European Congress on Thrombosis and Haemostasis (ECTH) 2019 meeting, which will be held in Glasgow (Oct 2nd-4th). As the host society, this will replace our annual scientific meeting in 2019. As many of you will also already be aware, in 2022, the ISTH will come to London with Beverley Hunt and Robert Ariens as President/Vice-President, respectively. Both the ECTH 2019 and ISTH 2022 represent excellent opportunities for the UK to showcase our strength in haemostasis research on an international stage.

In addition to international meetings, I believe that there is a very important place for national meetings, which are easier and cheaper to attend. These provide excellent opportunities for emerging researchers to present their data and gain recognition. Importantly, these meetings bring together clinicians and scientists from the UK with diverse backgrounds to discuss haemostasis-related issues and to forge collaborative links. I also believe that there is scope in the future to hold joint meetings with other national societies with allied interests. These can be mutually beneficial, and diversify the flavour of our meetings. I hope that you will enjoy the programme over the next couple of days, find the time to make new acquaintances and connections, and that you will find inspiration in the new data and findings presented at this meeting. Finally, I would also like to thank all of our sponsors that help us keep registration costs low. Please do take time to visit their stands and to discuss things with their representatives.

On behalf of the BSHT Committee,

Dr Jim Crawley
President of the BSHT

USEFUL INFORMATION

CERTIFICATES OF ATTENDANCE:

Attendance certificates are available from the registration desk and can be collected from lunchtime on Friday.
Please remember to sign the register on each day of attendance.

EXHIBITION:

The following companies are represented in the exhibition area. Please take time to visit the stands.

- Alexion Pharma UK Limited
- Bayer Public Limited Company
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- LFB Biopharmaceuticals Limited
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- Sebia (UK) Limited
- Sanofi Genzyme
- Stago UK Limited
- Sysmex UK Limited

MEETING DETAILS + ACCOMMODATION:

Meeting address:	The Slate, University of Warwick (*Lakeside Village*), Coventry CV4 7AL
Clinical Training session:	Radcliffe House – Space 1
Registration | Meeting:	The Slate
Refreshments | Exhibition:	The Slate
Posters | Drinks Reception:	The Slate
Dinner (*Thursday*):	Radcliffe House Dining Room *(for ticket holders only)*
Breakfast:	Radcliffe House Dining Room *(residential guests only)*
Accommodation:	Radcliffe House *(adjacent The Slate)* | Check in: from 15:00 hrs | Check out: from 10:00 hrs
Luggage Store:	Luggage can be stored either at Radcliffe House or cloakroom area within The Slate

POSTER SESSION:

The poster session will be held in The Slate during the pre dinner drinks reception on Thursday between 17:55 – 19:15 hrs

WIFI:

Enabled throughout Radcliffe and The Slate FOC.
The University Wi-Fi service provides conference delegates/exhibitors the freedom to use mobile (laptop or handheld) devices to connect to the University network, without needing a fixed wire or network.
Conference park delegates can access the "Warwick Guest" Wi-fi network around campus and within their accommodation. Ask at Conference Reception or any member of the team for assistance if required.
The Warwick Guest network can be used on up to 3 devices at any one time. If delegates have more than 3 devices they will need to register with a different email address to one they used in the first instance.

PROGRAMME – Thursday 8 November

07:00 – 08:00	Breakfast *(Radcliffe House residents only)* *Radcliffe Dining Room*
09:30 – 10:20	Registration, Refreshments + networking *The Slate*
10:20 – 10:30	**Welcome + Introduction** ***Jim CRAWLEY***
10:20 – 12:20	**SESSION ONE: Plenary 1 and Oral Communications 1**
	Co-chairs: Josefin AHNSTROM & Yotis SENIS
10:30 – 11:00	***Profibrinolytic properties of platelets*** ***Nikki MUTCH*** (Aberdeen)
11:00 – 11:05	Discussion
	Oral Communications 1
11:05 – 11:15	1 CRYSTAL STRUCTURES OF THE RECOMBINANT β-FACTOR XIIa PROTEASE WITH BOUND PRO-ARG AND THR-ARG SUBSTRATE MIMETICS *Monika PATHAK, Rosa Manna and Jonas Emsley* Centre for Biomolecular Sciences, School of Pharmacy, University of Nottingham, UK
11:15 – 11:20	Discussion
11:20 – 11:30	2 CHARACTERISATION OF A NOVEL GPIBα KNOCKIN MOUSE THAT LACKS THE LAST 24 AMINO ACIDS OF ITS INTRACELLULAR DOMAIN *Isabelle I. SALLES-CRAWLEY, Adela Constantinescu-Bercu, Kevin J Woollard & James TB Crawley* Centre for Haematology, Hammersmith Hospital Campus, Imperial College London, London, UK
11:30 – 11:35	Discussion
11:35 – 11:45	3 EFFICAY AND SAFETY OF PROTHROMBIN COMPLEX CONCENTRATE IN PATIENTS TREATED WITH RIVAROXABN OR APIXABAN COMAPARED TO WARFARIN PRESENTING WITH MAJOR BLEEDING *Deepa RJ ARACHCHILLAGE [1.2], Sharon Alavian[1], Jess Griffin[1], Kamala Gurung[1], Richard Szydlo[1], Nilanthi Karawitage[1], Mike Laffan[1.2]* [1] Department of Haematology, Imperial College Healthcare NHS Trust, London, UK [2] Department of Haematology, Imperial College London, London, UK
11:45 – 11:50	Discussion
11:50 – 12:00	4 WHAT PHYSIOLOGICAL SURFACE IS RESPONSIBLE FOR THE THROMBIN FEEDBACK LOOP ON FACTOR XI? *Beth. A. WEBB[1], O. M. Posada[1], C. Wilson[1], J. Ahnström[2], J. H. Morrissey[3] and H. Philippou[1]* [1]LICAMM, University of Leeds, Leeds, UK [2]Faculty of Medicine, Imperial College London, London, UK [3]Department of Biological Chemistry, Medical School, University of Michigan, Michigan, USA
12:00 – 12:05	Discussion
12:05 – 12:15	5
12:15 – 12:20	Discussion
12:20 – 13:20	Lunch, networking + exhibition

PROGRAMME – Thursday 8 November

13:20 – 15:10	**SESSION TWO: Plenary 2 and Scientists-in-Training Session**
	Co-chairs: Robert ARIENS & Neil MORGAN
13:20 – 13:50	***Tyrosine phosphatases: critical regulators of platelet production and function*** ***Yotis SENIS*** (Birmingham)
13:50 – 13:55	Discussion
	Scientists-in-Training Session
13:55 – 14:05	Scientist in Training 1 IDENTIFICATION OF FUNCTIONALLY IMPORTANT RESIDUES IN PROTEIN S FOR ITS INTERACTION WITH TFPI *Adrienn TERAZ-OROSZ, Salvatore Santamaria, James TB Crawley, David A Lane and Josefin Ahnström* Centre for Haematology, Faculty of Medicine, Imperial College London, London, UK
14:05 – 14:10	Discussion
14:10 – 14:20	Scientist in Training 2 ANTI-CUB AND ANTI-SPACER ANTIBODIES THAT CONFORMATIONALLY ACTIVATE ADAMTS13 WORK THROUGH ENHANCEMENT OF THE METALLOPROTEASE DOMAIN FUNCTION *Anastasis PETRI[1], An-Sofie Schelpe[2], Nele Vandeputte[2], Hans Deckmyn[2], Simon F De Meyer[2], Karen Vanhoorelbeke[2], James TB Crawley[1]* [1]Centre for Haematology, Imperial College London, London, United Kingdom, [2]Laboratory for Thrombosis Research, KU Leuven, Kortrijk, BELGIUM
14:20 – 14:25	Discussion
14:25 – 14:35	Scientist in Training 3 NOVEL PLATELET-NEUTROPHIL INTERACTION VIA ACTIVATED $\alpha_{IIb}\beta_3$ MEDIATES NETOSIS UNDER FLOW *Adela CONSTANTINESCU-BERCU, Isabelle I. Salles-Crawley, Kevin J Woollard & James T.B. Crawley* Imperial College London, Hammersmith Hospital Campus, Du Cane Road, London, UK
14:35 – 14:40	Discussion
14:40 – 14:50	Scientist in Training 4 CANCER CELLS RELEASE ACTIVE TF-fVIIa COMPLEX WHICH CAN BE DIRECTLY INHIBITED BY APIXABAN *Yahya MADKHALI1, Sophie Featherby1, Anthony Maraveyas2, John Greenman1, Camille Ettelaie1* 1Biomedical Section, School of Life Sciences, University of Hull, Cottingham Road, Hull, HU6 7RX, UK., 2Division of Cancer-Hull York Medical School, University of Hull, Cottingham Road, Hull, HU6 7RX, UK
14:50 – 14:55	Discussion
14:55 – 15:05	Scientist in Training 5 CROSS-TALK BETWEEN THE ALTERNATIVE PATHWAY OF COMPLEMENT AND THE INTRINSIC PATHWAY OF COAGULATION *Samantha L HEAL, Lewis J Hardy, Beth A Webb, Clare L Wilson, Helen Philippou* Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, Leeds, UK
15:05 – 15:10	Discussion
15:10 – 15:40	Refreshments, networking + exhibition
15:40 – 17:45	**SESSION THREE: Plenary 3 and Emerging Fellows**
	Co-chairs: Henry WATSON & Rhona MACLEAN
15:40 – 16:10	***New therapeutic options in the treatment of TTP*** ***Marie SCULLY*** (London)
16:10 – 16:15	Discussion

PROGRAMME – Thursday 8 November

	Emerging Fellows
16:15 – 16:35	***Procoagulant lipids. A future treatment for abdominal aortic aneurysms?*** ***Keith ALLEN-REDPATH*** (Cardiff)
16:35 – 16:40	Discussion
16:40 – 17:00	***Learning how to restrain the world's most prolific killers (platelets)*** ***Mark THOMAS*** (Birmingham)
17:00 – 17:05	Discussion
17:05 – 17:25	***Personalised prophylaxis in Haemophilia A: the population pharmacokinetic approach*** ***Saket BADLE*** (London)
17:25 – 17:30	Discussion
17:30 – 17:50	***Regulation of clot stability by the serine/threonine phosphatase PP1*** ***Zoltan NAGY*** (Birmingham)
17:50 – 17:55	Discussion
17:55 – 19:15	**Poster session + Drinks Reception**
19:30	**Conference Dinner** *Radcliffe House Dining Room (purchased ticket holders only)*

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07:00 – 08:00	Breakfast *(Radcliffe House residents only)* *Radcliffe Dining Room*
08:00 – 08:30	Registration, Refreshments + networking *The Slate*
08:30 – 10:10	**SESSION FOUR: Plenary 4 and Oral Communications 3**
	Co-chairs: Catherine BAGOT & Chris GARDINER
08:30 – 09:00	***Cancer and thrombosis*** ***Henry WATSON*** (Aberdeen)
09:00 – 09:05	Discussion
09:05 – 09:15	Transfer time to parallel session
	CLINICAL TRAINING SESSION (Parallel Session) *Radcliffe Space 1* *Chair: Keith GOMEZ* 09:15 – 09:40 ***Mike LAFFAN*** (London) ***Global haemostatic assays in clinical practice*** 09:40 – 10:10 ***Pratima CHOWDARY*** (London) ***Novel therapies for haemophilia***
	Oral Communications 2
09:10 – 09:20	6 DISSIMILAR EFFECTS OF DIRECT ORAL ANTICOAGULANTS ON THE RELEASE OF TF-POSITIVE MICROVESICLES AND CANCER CELL PROLIFERATION *Sophie FEATHERBY[1], Yahya Madkhali[1], Anthony Maraveyas[2], Camille Ettelaie[1]* [1]Biomedical Section, School of Life Sciences, University of Hull, Cottingham Road, Hull, HU6 7RX, UK., [2]Division of Cancer-Hull York Medical School, University of Hull, Cottingham Road, Hull, HU6 7RX, UK
09:20 – 09:25	Discussion
09:25 – 09:35	7 MICRORNA-223 IN PLATELET-DERIVED EXTRACELLULAR VESICLES DOWNREGULATES TISSUE FACTOR EXPRESSION IN THP-1 MONOCYTIC CELLS *Mary E W COLLIER, Ashley R Ambrose, Alison H Goodall* Department of Cardiovascular Sciences, University of Leicester and NIHR Cardiovascular Biomedical Research Unit, Glenfield Hospital, Leicester LE3 9QP
09:35 – 09:40	Discussion
09:40 – 09:50	8 PREVALENCE OF ANTI-PROTEIN C ANTIBODIES AND ACQUIRED ACTIVATED PROTEIN C RESISTANCE IN SYSTEMIC LUPUS ERYTHEMATOSUS *Giuseppe A. Ramirez[1, 2, 3], Hannah Cohen[4, 5], Ian Mackie[4], David Isenberg[1, 2], Maria EFTHYMIOU[4]* [1]Centre for Rheumatology, Division of Medicine, University College London, London, UK [2]Department of Rheumatology, University College London Hospitals NHS Foundation Trust, London, UK[3]Università Vita-Salute San Raffaele, Milan, Italy[4]Haemostasis Research Unit, Department of Haematology, University College London, London, UK[5]Department of Haematology, University College London Hospitals NHS Foundation Trust, London, UK
09:50 – 09:55	Discussion
09:55 – 10:05	9 PATIENTS WITH RECURRENT VENOUS THROMBOEMBOLISM HAVE LESS ELASTIC CLOTS THAN THOSE WITH NON-RECURRENT DISEASE *Stephen R. BAKER[1], Michal Zabczyk[2], Anetta Undas[2], Robert A. S. Ariëns[1]* [1] Theme Thrombosis, Department of Discovery and Translational Science, Leeds Institute of Cardiovascular and Metabolic Medicine, University of Leeds, UK. ~ [2] Institute of Cardiology, Jagiellonian University Medical College, Krakow, Poland and John Paul II Hospital, Krakow, POLAND
10:05 – 10:10	Discussion

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10:10 – 10:40	Refreshments, Refreshments + networking *The Slate*
	CLINICAL TRAINING SESSION (Parallel Session) *Radcliffe Space 1* *Chair: Keith GOMEZ* 10:40 – 11:10 ***Rhona MACLEAN*** (Sheffield) ***When & how to thrombolyse in venous thrombosis*** 11:10 – 11:35 ***Keith GOMEZ*** (London) ***Genomics in the haemostasis clinic***
10:40 – 12:30	**SESSION FIVE: Plenary 5 & Oral Communications 3**
	Co-chairs: Nikki MUTCH & Isabelle SALLES-CRAWLEY
10:40 – 10:50	10 ERP44, A NEW THIOL ISOMERASE ENZYME THAT IS PRESENT IN PLATELETS *Shuruq ALSUFYANI, Lisa-Marie Holbrook, Jonathan M Gibbins* Institute for Cardiovascular and Metabolic Research, School of Biological Sciences, University of Reading, Harborne Building, Whiteknights, Reading, Berkshire, RG6 6AS, UK
10:50 – 10:55	Discussion
10:55 – 11:05	11 CHARACTERISATION OF A GENETICALLY MODIFIED FXIII-A L34V MODEL AND POTENTIAL FOR VASCULAR STUDIES *Adomas BARANAUSKAS, Robert A.S. Ariëns, Cédric Duval* Discovery and Translational Science Department, Leeds Institute of Cardiovascular And Metabolic Medicine, The LIGHT Laboratories, University of Leeds, Clarendon Way, LS2 9NL, UK
11:05 – 11:10	Discussion
11:10 – 11:20	12 BTK INHIBITION INHIBITS CLEC-2 MEDIATED PLATELET ACTIVATION *Joshua HINDS[1], Stephanie K Watson[1], Guy Pratt[2], Phillip LR Nicolson[1]* [1]Institute of Cardiovascular Sciences, College of Medical and Dental Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK [2]Department of Haematology, Queen Elizabeth Hospital, Birmingham, B15 2TH, UK
11:20 – 11:25	Discussion
11:25 – 11:35	13 INCIDENCE OF THROMBOCYTOPENIA AND HEPARIN INDUCED THROMBOCYTOPENIA IN PATIENTS RECEIVING EXTRACORPOREAL MEMBRANE OXYGENATION (ECMO) COMPARED TO CARDIOPULMONARY BYPASS (CPB) AND THE LIMITED SENSITIVITY OF PRE-TEST PROBABLITY SCORE *Deepa RJ ARACHCHILLAGE [1,2,3], Sanjay Khanna[1], Christophe Vandenbriele[1], Jonathan Dutton[1], Alex Rosenberg[1], TV Aw[1], Winston Banya[1], Mike Laffan [2,3], Stephane Ledot[1], Brijesh Patel[1,3]* [1] Royal Brompton & Harefield NHS Foundation Trust, London, UK [2] Imperial College Healthcare NHS Trust, London, UK [3] Imperial College London, London, UK
11:35 – 11:40	Discussion
	Summer Student Presentations
11:40 – 11:45	Summer Student 1 THE DEVELOPMENT OF IMPROVED BIO-ENGINEERED EXPRESSION CASSETTES FOR GENE THERAPY OF HAEMOPHILIA A *Ellie HOLDEN*
11:45 – 11:50	Summer Student 2 NEUTRALISATION OF EXTRACELLULAR HISTONES TO PROMOTE FIBRINOLYSIS AND REDUCE HISTONE CYTOTOXICITY *Evgenia TSAOUSI*

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11:50 – 11:55	Summer Student 3 STRUCTURAL BASIS OF THE LEUKOCYTE INTEGRIN MAC-1 I-DOMAIN INTERACTION WITH THE PLATELET GP1Bα N-TERMINAL DOMAIN *Ruiqi NG*
	Plenary 5
11:55 – 12:25	***Mechanisms of thrombus formation in mice*** ***Christophe DUBOIS*** (France)
12:25 – 12:30	Discussion
12:30 – 13:30	**BSHT AGM** & Lunch, networking + exhibition
13:30 – 15:20	**SESSION SIX: Plenary 6 & Oral Communications 4**
	Co-Chairs: Jim CRAWLEY & Keith GOMEZ
13:30 – 14:00	***Modifying serpin specificity to therapeutic effect: The creation of SerpinPC, a pan-haemophilia agent*** ***Jim HUNTINGTON*** (Cambridge)
14:00 – 14:05	Discussion
	Oral Communications 4
14:05 – 14:15	14 A PRACTICAL METHOD FOR REDUCING INTERFERENCE BY LIPAEMIA FOR COAGULATION TESTS *Chris GARDINER, Philip Lane, Ian J Mackie* Haemostasis Research Unit, 1st Floor, 51 Chenies Mews, University College London, London WC1E 6HX
14:15 – 14:20	Discussion
14:20 – 14:30	15 PARTIAL RESCUE OF NATURALLY OCCURING ACTIVE SITE FACTOR X VARIANTS THROUGH DECREASED INHIBITION BY TFPI AND ANTITHROMBIN *Josefin AHNSTRÖM, Joseph Temenu, Michael A. Laffan and David A. Lane* Centre for Haematology, Faculty of Medicine, Imperial College London, London, UK
14:30 – 14:35	Discussion
14:35 – 14:45	16 ASSESSING THE IMPACT OF NICE GUIDELINE NG89 ON HOSPITALISED MEDICAL PATIENTS AT RISK OF VENOUS THROMBOEMBOLISM *Will LESTER[1], Keval Dabhi[2] and Deepak Chandra[3]* Centre for Clinical Haematology, University Hospitals Birmingham, Edgbaston, Birmingham, B15 2TH Health Informatics department, Yardley Court, Edgbaston, Birmingham, B15 1JD Department of Clinical Haematology, Royal Stoke University Hospital, University Hospitals of North Midlands NHS Trust, ST4 6QG
14:45 – 14:50	Discussion
14:50 – 15:00	17 THE CRITICAL ROLE OF TISSUE FACTOR PATHWAY INHIBTOR (TFPI) UNDER FLOW AND *IN VIVO* *Isabelle I. SALLES-CRAWLEY*, Josefin Ahnström* & James TB Crawley** Centre for Haematology, Hammersmith Hospital Campus, Imperial College London, London, UK
15:00 – 15:05	Discussion
15:05 – 15:15	18 ANTIBODY-MEDIATED CLEARANCE OF ADAMTS13 IN ACQUIRED THROMBOTIC THROMBOCYTOPENIC PURPURA: A ROLE FOR MONOCYTES? *Mary I UNDERWOOD 1, Mari R Thomas 2, Marie A Scully 2, James TB Crawley 1* 1 Centre for Haematology, Imperial College London, Hammersmith Hospital Campus, Du Cane Road, London W12 0NN, UK 2 Department of Haematology University College Hospital, London NW1 2BU, UK
15:15 – 15:20	Discussion
	Meeting Close

SPEAKER SUMMARIES

Profibrinolytic properties of platelets

Dr Nicola J MUTCH

PhD
Reader in Thrombosis & Haemostasis
University of Aberdeen, School of Medicine, Medical Sciences and Nutrition, Institute of Medical Sciences, Aberdeen, UK

Platelets are integral to thrombus formation yet harbour numerous fibrinolytic proteins. Platelet aggregates anchor the fibrin network via the integrin αIIbβ3, stationing them at a prime location to deposit fibrinolytic proteins at their site of action. My group has demonstrated profibrinolytic functions of platelets during growth of the aggregate and has shown that they limit thrombus growth and stability. We have shown direct binding of plasminogen to the surface of activated platelets and the generation of functional plasmin activity. This plasmin activity is protected from inhibition by $\alpha_2$antiplasmin and can drive local fibrinolysis. We are the first to identify the presence of the novel plasminogen receptor, $PlgR_{KT,}$ on human and mouse platelets. $PlgR_{KT}$ is a novel differentiation-induced integral membrane plasminogen receptor that exposes a C-terminal lysine on the cell surface. In addition we recently documented a role for the coagulation protein factor XIIa (FXIIa) in plasminogen activation, a process that is dramatically augmented by platelet-derived polyphosphate. Accumulation of fibrinolytic proteins on the platelet surface is potentially key in driving endogenous lysis of thrombi within the core of thrombi where solute transport of plasma –derived proteins is low. These mechanisms are potentially an instrumental process by which nature limits thrombus growth which could be exploited to promote thrombus resolution in disease.

Tyrosine phosphatases: critical regulators of platelet production and function

Professor Yotis SENIS

PhD
University of Birmingham, Institute of Cardiovascular Sciences, Institute of Biomedical Research, Birmingham, UK

Platelets play a vital role in thrombosis and haemostasis, yet it remains ambiguous how the number and reactivity of platelets is regulated. Recent findings from our group have established the receptor tyrosine phosphatase CD148 and the immunoreceptor tyrosine-based inhibitory motif (ITIM)-containing receptor G6b-B as critical regulators of these processes. CD148 works in conjunction with the tyrosine kinase Csk to regulate Src family kinase (SFK) activity in platelets and megakaryocytes (MKs), setting their threshold of activation. Deletion of CD148 and/or Csk in the MK lineage in mice disrupts the SFK equilibrium, resulting in aberrant platelet count and function, and reduced thrombosis and haemostasis. Mice lacking both CD148 and Csk exhibit paradoxical bleeding despite having significantly elevated SFK activity. This is due to upregulation of negative feedback mechanisms, including G6b-B, which forms an inhibitory complex with the tyrosine phosphatases Shp1 and Shp2, reducing platelet reactivity to vascular injury. Intriguingly, congenital null and loss-of-function mutations in G6b-B results in severe macrothrombocytopenia, MK clusters and myelofibrosis in the bone marrow in mice and humans, demonstrating the critical role of this receptor in inhibiting platelet and MK activation. From this work, CD148 and G6b-B have emerged as potential therapeutic drug targets in the prevention and treatment of thrombosis, macrothrombocytopenia and myelofibrosis.

SPEAKER SUMMARIES

New therapeutic options in the treatment of TTP

Professor Marie SCULLY

BSc, PRCPath, MRCP, MD
Consultant haematologist
UCLH, Haematology, London, UK

TTP is an acute life threatening condition, untreated has a mortality of 90%. Despite the advances in regular plasma exchange therapy, replenishing the missing enzyme ADAMTS 13 and immunosuppressive therapy, the mortality was still at least 20%. The use of monoclonal anti CD 20 therapy has improved outcomes when used early in disease presentation, reducing time to remission and preventing relapses. Furthermore, rituximab therapy can be used to prevent further TTP episodes. However, even with intense therapy, there remains a mortality risk until rituximab is effective. There are also those cases with significant refractory disease or who have an exacerbation despite initially prompt response to treatment. The completion of a phase III international, randomized control trial and subsequent license of Caplacizumab provides an adjunct to therapy and protection while immunosuppressive therapy takes effect. It is a novel nanobody therapy, which binds to the A1 region of VWF, preventing further platelet binding and reduces microvascular thrombi formation. In conjunction, the role of recombinant ADAMTS 13, confirmed in phase I therapy, should, in the future, simplify treatment algorithms. The current studies are in congenital TTP cases, where the optimal treatment paths are not documented. Further investigation into congenital TTP may help with insight into the longer term effects of ADAMTS 13 levels on patient morbidity.

Cancer and thrombosis

Dr Henry WATSON

MD
Consultant Haematologist and Honorary Professor of Medicine
Aberdeen Royal Infirmary and University of Aberdeen, Aberdeen Royal Infirmary, Aberdeen, UK

Venous thromboembolism is a common complication of malignant disease. The factors that contribute to this risk are numerous and include the need for surgical intervention and biopsy, treatment with chemo-radiotherapy and the use of indwelling central lines. However there are many aspects of malignancy that drive the development of thrombosis and there is evidence that many of the features of tumour biology that contribute to this prothrombotic nature also add to the tumours capacity to invade tissue and to form metastases. The current standard of care for patients with cancer associated VTE is with low molecular weight heparin – but this is changing as data on DOACs in this situation emerge.

SPEAKER SUMMARIES

Mechanisms of thrombus formation in mice

Professor Christophe Dubois

PhD
Lab Leader
Aix Marseille University, C2VN, INSERM UMR-S1263, Faculty of Pharmacy, Marseille, FRANCE

The mechanisms involved in thrombus formation following a laser-induced injury will be discussed here. We will focus on the roles played by neutrophils, platelets, endothelial cells and microparticles on thrombus formation in living mice.

Modifying serpin specificity to therapeutic effect: The creation of SerpinPC, a pan-haemophilia agent

Professor James HUNTINGTON

PhD
University of Cambridge, Department of Haematology, Cambridge, UK

Hemophilia is caused by defect or deficiency in either factor (f) VIII (hemophilia A, HA) or fIX (hemophilia B, HB), and is usually treated by on-demand or prophylactic intravenous infusions. In about 30% of HA patients and 2% of HB patients the replacement factor is recognized as a foreign protein and inhibitory antibodies are generated. These 'inhibitor patients' can only be treated on-demand with 'bypassing' agents such as NovoSeven and several marketed clotting factor concentrates, such as FEIBA. All current treatments restore hemostasis by augmenting the concentrations of procoagulant factors. However, it is also possible to rebalance the hemostatic system by inhibiting natural anticoagulant pathways. Of particular interest is activated protein C (APC) which proteolyticaly inactivates the complex that produces thrombin (prothrombinase, composed of fVa and fXa). Coinheritence of partial APC resistance (fVLeiden) has been found to ameliorate bleeding frequency and severity in hemophilia patients, presumably by allowing prothrombinase more time to produce thrombin. We engineered a serpin (serine protease inhibitor) specific for APC over other coagulation proteases by making three mutations in α_1-antitrypsin. It was tested for efficacy and safety in mouse models. The variant, *SerpinPC*, rescued thrombin generation in vitro and fully restored hemostasis in HA and HB mice. The safety of *SerpinPC* was tested by treating wild-type mice with daily intravenous doses of 100mg/kg for 7 days. *SerpinPC* was well tolerated, with no overt signs of toxicity, no increase in inflammatory markers or evidence of thrombosis. In addition, *SerpinPC* did not contribute to lethality in an LPS model. *SerpinPC* is a safe and effective hemostatic agent that could potentially treat all forms of hemophilia, regardless of inhibitor status.

Global haemostatic assays in clinical practice

Professor Mike LAFFAN
DM FRCP FRCPath
Professor of Haemostasis and Thrombosis, Imperial College London, Haematology, Hammersmith Hospital, London, UK

Global tests of haemostasis are attractive because do not require knowledge of a specific diagnosis and seem well suited to complex acquired disorders of haemostasis that are seen in hospital practice. They may also reflect anticoagulant therapy and thrombotic risk as well. Although they have been available for many years, problems with standardization and reproducibility as well as establishing clinical correlation have limited their introduction into standard practice. Their global nature may also not provide a specific therapeutic approach. These factors have led to only limited approval by NICE. For haematologists, a major problem is that they are generally performed as point of care tests rather than in the coagulation laboratory which limits their ability to become familiar with their use. Nonetheless, their use is becoming more widespread and referrals commonly ask for help with their interpretation. In response to this the BSH has recently published guidelines on viscoelastic test of haemostasis.

Novel therapies for haemophilia

Dr Pratima CHOWDARY
MBBS, FRCPath
Consultant Haematologist
Royal Free Hospital, KD Haemophilia and Thrombosis Centre, London, UK

Replacement therapy with missing factor VIII or IX in severe haemophilia A and B patients respectively for bleed management and preventative treatment or prophylaxis is standard of care with good outcomes. Current management of bleeding in patients with inhibitors include treatment either with recombinant activated factor VIIa (rVIIa) or activated prothrombin complex concentrate (aPCC). They are called as bypassing agents as mechanisms underpinning their haemostatic efficacy are based on the restoration of thrombin generation through pathways that potentially contribute minimal amount of thrombin under normal conditions. In addition to on-demand therapy they are also used for prophylaxis, but the effectiveness is much less compared to prophylaxis in non-inhibitor patients.

Restoration of thrombin generation through novel mechanisms has become the focus of innovation to overcome limitations imposed by protein replacement therapy. Two broad approaches that restore thrombin generation through novel mechanisms are currently in clinical trials. The first includes the development of factor VIII mimetic, a bi-specific antibody that bridges FIXa and FX resulting in the generation of FXa, and this process is regulated by substrate availability rather than inhibition of co-factor activity.This bi-specific antibody called emicizumab in phase 3 studies resulted in 87% reduction in bleeds in severe haemophilia A patients with inhibitors. The second approach consists of diminishing the activity of natural inhibitors, including attenuation of antithrombin (AT) activity, the serine protease inhibitor responsible for inhibiting both common, initiation and amplification pathways. This has been achieved by fitusiran, a novel RNA interference (RNAi) therapeutic, i.e. gene silencing that targets AT synthesis in the liver. The other mechanism of rebalancing haemostasis through diminished inhibition is related to decreased activity of tissue factor pathway inhibitor (TFPI) that inhibits the initiation pathway. This is achieved through monoclonal antibodies that target the kunitz domains of TFPI.

When & how to thrombolyse in venous thrombosis

Dr Rhona MACLEAN
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Consultant Haematologist,
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Whilst anticoagulation is the optimal treatment for many patients with acute venous thromboembolic disease, there is evidence that thrombolysis may the treatment of choice in certain clinical situations (both in patients with pulmonary embolism, and those with deep vein thrombosis). In this case-based session, with reference to the literature, decisions as to when, and how, to thrombolyse will be discussed.

Genomics in the haemostasis clinic

Dr Keith GOMEZ
MBBS PhD
Consultant Haematologist
Royal Free London NHS Foundation Trust, Haemophilia Centre and Thrombosis Unit, London, UK

Over the last two decades we have seen revolutionary advances in genetic analysis. In this area the UK is a global leader in translating new techniques for patient benefit. While there has been a massive expansion in our genetic testing repertoire, there have also had to be changes in how these tests are used and incorporated into the patient pathway. This has led to the discovery of new genetic causes of disease and a better understanding of the phenotypic consequences of genetic variation. It has also brought new challenges for healthcare professionals and providers and a host of new practice guidelines. Through case-based discussion, this presentation will go through the development of next generation sequencing and its use in clinical practice.

Procoagulant lipids. A future treatment for abdominal aortic aneurysms?

Dr Keith ALLEN-REDPATH
PhD
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Lipoxygenases (LOXs) are lipid peroxidising enzymes known to be involved in atherosclerosis. There are several isoforms of LOXs in mammals, neutrophil 5-LOX, murine monocyte/macrophage 12/15-LOX or human 15-LOX, platelet 12-LOX (p12-LOX) and murine eosinophil 12/15-LOX or human 15-LOX. Recently, genetic deletion of macrophage 12/15-LOX in Apolipoprotein E deficient mice ($ApoE^{-/-}$) significantly reduced atherosclerosis development. However, the role of p12-LOX has never been investigated in atherosclerosis. Furthermore, 12/15-LOX or p12-LOX role in AAA, a more severe atherosclerotic phenotype, has not been studied. More recently LOXs have been observed to generate oxidized phospholipids (OxPL's) that consist of an eicosanoid attached to either phosphatidylcholine (PC) or phosphatidylethanolamine (PE) that have been found to potentiate the coagulation cascade. However, whether they can contribute to atherosclerosis or AAA development is not known. I observed that genetic deletion of p12-LOX in $ApoE^{-/-}$ significantly protects against atherosclerosis *in vivo*. Additionally, $ApoE^{-/-}$ mice genetically deficient in either p12-LOX or 12/15-LOX and treated with 1.1mg/kg/day of Ang II for 2 weeks to induce AAA were also significantly protected against AAA development. With this in mind I investigated whether addition of PE lipids into mice lacking p12-LOX and ApoE would restore an AAA phenotype as seen in $ApoE^{-/-}$ mice. I observed that addition of 12-HETE-PE combined with tissue factor did not restore phenotype however, when these lipids were administered to $ApoE^{-/-}$ mice it significantly inhibited Ang II induced AAA. Mechanistically it is unclear how this protects against AAA development however the addition of procoagulant lipids may interact with coagulation factors preventing it from exerting a damaging effect within the vasculature.

Learning how to restrain the world's most prolific killers (platelets)

Dr Mark THOMAS
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Academic Clinical Lecturer in Cardiovascular Medicine
University of Birmingham, Institute of Cardiovascular Sciences, College of Medical and Dental Sciences, Birmingham, UK

Prof Stan Heptinstall at the University of Nottingham inspired my interest in discovering new ways to inhibit platelets to treat patients with heart attacks, which are also known as acute coronary syndromes (ACS). We explored novel approaches to dual inhibition of platelet $P2Y_1$ and $P2Y_{12}$ ADP receptors and subsequently investigated using platelet P-selectin tests to assess efficacy of platelet $P2Y_{12}$ inhibitors in patients with ACS. During my MRC Clinical Research Training Fellowship, under the mentorship of Prof Robert Storey at the University of Sheffield, we focussed on the effect of platelet $P2Y_{12}$ inhibitors on inflammation and whether this may contribute to their clinical efficacy. We showed that platelet $P2Y_{12}$ inhibitors have a major effect on systemic inflammation induced by bacterial endotoxin in an experimental in-vivo human model. We also demonstrated additional effects of ticagrelor on neutrophil function, mediated by adenosine. In addition, we investigated the influence of diabetes and morphine-use on pharmacodynamic response to platelet $P2Y_{12}$ inhibitors in patients with prior history of ACS in the PEGASUS study and an experimental medicine study respectively. I am now working with Prof Steve Watson at the University of Birmingham to translate exciting new discoveries in platelet biology into novel treatments for patients with cardiovascular disease. I am currently focussing on the platelet GPVI receptor and its relative role in thrombosis and haemostasis respectively.

Personalised prophylaxis in Haemophilia A: the population pharmacokinetic approach

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Clinical Research Fellow / Hematology Specialty Registrar
Queen Mary University of London, Barts and The London School of Medicine and Dentistry, London, UK

Prophylaxis with factor VIII is standard of care in management of severe haemophilia A. However, the current approach has been described as 'one size fits all'. Individual pharmacokinetics (PK) of infused FVIII differ between patients. PK can be used to guide prophylaxis in each individual in a personalised approach to prophylaxis. We present aspects to be considered when using personal pharmacokinetics and findings from the UKPK study: the first UK wide trial to use individual pharmacokinetics in dosing discussions for men with severe haemophilia A.

Regulation of clot stability by the serine/threonine phosphatase PP1

Dr Zoltan NAGY
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Research Fellow
University of Birmingham, Institute of Cardiovascular Sciences, College of Medical and Dental Sciences, Edgbaston, Birmingham, UK

Serine/threonine (Ser/Thr) kinases are essential for platelet activation and inhibition. Classical examples include protein kinase C, which is essential for thrombin and ADP-mediated platelet activation, whereas protein kinase A inhibits platelet activation in response to prostacyclin. However, phosphatases that reverse these phosphorylation events remain incompletely defined. Platelet contractility is a highly dynamic actin-myosin-dependent function, essential for platelet spreading, clot retraction and thrombus stability that is regulated by Ser/Thr phosphorylation. Mutations in the MYH9 gene, which encodes a heavy chain isoform of myosin, result in an autosomal dominant disorder called MYH9-related disease characterized by congenital macrothrombocytopenia and mild bleeding. Platelets from MYH9-deficient mice do not support clot retraction, hence thrombus stability is compromised and tail bleeding is increased. Phosphorylation of key residues in myosin light chain by myosin light chain kinase (MLCK) leads to a conformational change in the associated myosin heavy chain resulting in mechanical force generation, whereas dephosphorylation of these residues by myosin phosphatase inhibits myosin activity, precluding contractility. In platelets, the regulation of myosin activity and the role of myosin phosphatase in regulating adhesion and clot retraction remains unclear. Myosin phosphatase is a multi-subunit complex, consisting of the Ser/Thr phosphatase PP1 catalytic subunit, a substrate targeting subunit and an inhibitory subunit. By exploring published proteomics datasets we found that components of the myosin phosphatase are highly expressed in platelets, including the catalytic subunit PP1delta, the substrate targeting subunit Mypt1, and the inhibitory subunit CPI-17. A multidisciplinary approach is being taken to better understand the function of platelet myosin phosphatase, including the use of knockout mouse models, proteomics and pharmacological inhibitors. Targeting platelet myosin phosphatase may provide a novel strategy for regulating clot stability in patients with thrombotic complications.

SPEAKER BIOGRAPHIES

Dr Pratima CHOWDARY
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Consultant Haematologist
Royal Free Hospital, KD Haemophilia and Thrombosis Centre, London, UK

Dr Chowdary is Co-Centre Director for the Haemophilia centre, clinical lead for specialist coagulation lab and serves as a treasurer for the United Kingdom Haemophilia Centre Doctors Organisation. Her major research interests include personalised management of hemophilia, outcome assessments, hemophilic arthropathy and acquired coagulopathy. She chairs the UKHCDO working party on musculoskeletal issues in patients with haemophilia, and member of the UKHCDO lab working party and a panel member for the National Institute for Health Research, Health Technology Assessment Programme.

Professor Christophe DUBOIS
PhD
Lab Leader
Aix Marseille University, C2VN, INSERM UMR-S1263, Faculty of Pharmacy, Marseille, FRANCE

Christophe Dubois obtained his PhD in 2003 from the University of Aix Marseille. He moved to Boston as postdoctoral follow in the lab of Barbara and Bruce Furie. During this time, we published several papers on the mechanisms of thrombus formation in mice by real time intravital microscopy. He became an associate Professor in cell biology in 2006 and full Professor in vascular biology in 2015 at the University of Aix Marseille, France. Since 2006, he leads a research team focused on the consequences of the interactions of platelets and circulating cells on thrombosis by real time intravital microscopy.

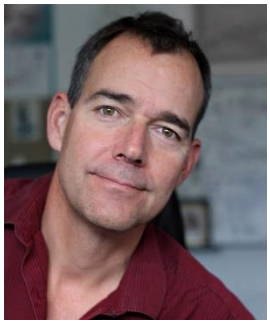
Dr Keith GOMEZ
MBBS PhD
Consultant Haematologist
Royal Free London NHS Foundation Trust, Haemophilia Centre and Thrombosis Unit, London, UK

Dr Keith Gomez trained at St Thomas' Hospital Medical School, University of London, and graduated in 1992. After studying haematology in the North London training programme he joined the Haemostasis Research Group at the Medical Research Council in London. The primary area of research for his doctoral thesis was the development of models of tissue factor-initiated coagulation.

After award of his PhD Dr Gomez took up his current post as Consultant and Honorary Senior Lecturer in Haemostasis in the Haemophilia Centre and Thrombosis Unit at the Royal Free Hospital in London. His main research interests include the genetic basis of inherited disorders of coagulation, the diagnosis and management of platelet disorders and the clinical management of the rarer factor deficiencies.

Dr Gomez chairs the UK ThromboGenomics reporting MDT, the UKHCDO Genetics Working Party and the National Quality Assurance Advisory Panel for Haematology. He is also the secretary of the Haemostasis and Thrombosis Task Force for the British Society of Haematology.

SPEAKER BIOGRAPHIES



Professor James HUNTINGTON
PhD
University of Cambridge, Department of Haematology, Cambridge, UK

Jim graduated in 1989 from the University of Kansas with bachelor's degrees in chemistry and mathematics. He later worked as a chemist at Alza Corp. then obtained a PhD from Vanderbilt. His postdoc was at the University of Cambridge. He was appointed principal investigator at the Cambridge Institute for Medical Research in 1999, University Reader in 2007 and Professor of Molecular Haemostasis in 2011. His current research focuses on determining the structures of the Xase and prothrombinase complexes. He has founded several biotech companies, including: XO1, SuperX and Rebalance in the field of thrombosis; ApcinteX to treat haemophilia; Z Factor to treat alpha-1-antitrypsin deficiency; and a reagents company, Cambridge ProteinWorks

Professor Mike LAFFAN
DM FRCP FRCPath
Professor of Haemostasis and Thrombosis
Imperial College London, Haematology, Hammersmith Hospital, London, UK

Michael Laffan is Professor of Haemostasis and Thrombosis in the Department of Haematology at Imperial College, London, UK, and Director of the Hammersmith Hospital Haemophilia Centre. His principal research interests are the structure-function relationship of von Willebrand factor, in particular the role of glycosylation, and the mechanisms and regulation of thrombin generation. He edited the 12th edition of Practical Haematology, is chair of the UK Working Party on VWD and the BSH Haemostasis and Thrombosis Task Force.

Dr Rhona MACLEAN
MB ChB, FRCP, FRCPath
Consultant Haematologist
Sheffield Teaching Hospitals NHSFT, Haematology, Royal Hallamshire Hospital, Sheffield, UK

Rhona Maclean is Clinical Lead in Haemostasis and Thrombosis at Sheffield Teaching Hospitals NHSFT. She developed the trust thrombosis service, and, with respiratory colleagues in 2011, a joint haematology/respiratory pulmonary embolism service, and with colleagues in vascular radiology, a thrombectomy service.

SPEAKER BIOGRAPHIES

Dr Nicola J MUTCH
PhD
Reader in Thrombosis & Haemostasis
University of Aberdeen, School of Medicine, Medical Sciences and Nutrition
Institute of Medical Sciences, Aberdeen, UK

Nicola carried out her undergraduate and postgraduate studies at the University of Aberdeen where her PhD focussed on cross-talk between coagulation and fibrinolysis. Nicola took up a post-doctoral position at the University of Illinois at Urbana-Champaign. Nicola returned to the UK to take up an independent fellowship in cardiovascular biology at the University of Leeds. Subsequently she obtained a personal fellowship from the British Heart Foundation and returned to Aberdeen where she is currently a Reader. Her research focuses on the cross-talk between haemostatic pathways and how this modulates physiological and disease processes.

Professor Marie SCULLY
BSc, PRCPath, MRCP, MD
Consultant haematologist
UCLH, Haematology, London, UK

Marie Scully is professor of haemostasis and thrombosis at UCL and clinical lead for TTP TMAs and acquired thrombocytopenic conditions at UCLH. She has published within these areas and regularly reviews for international journals. She supervises post graduate trainees and is involved in investigator led and commercial clinical studies, including first in man to international phase III. She is clinical lead for the commissioning of a national TTP service.

Professor Yotis SENIS
PhD
University of Birmingham, Institute of Cardiovascular Sciences, Institute of Biomedical Research, Birmingham, UK

Professor Yotis Senis obtained his BSc(Hons), MSc and PhD in Pathology from Queen's University, Canada. He was awarded a BHF Intermediate Basic Science Research Fellowship in 2009, followed by a BHF Senior Fellowship and Chair in Cellular Haemostasis at the University of Birmingham in 2013. In his research, he takes a multidisciplinary approach to elucidate how tyrosine phosphatases regulate platelet production and function, with the overall objective of identifying novel therapeutic targets for the treatment of thrombosis and bleeding disorders.

Dr Henry WATSON
MD
Consultant Haematologist and Honorary Professor of Medicine
Aberdeen Royal Infirmary and University of Aberdeen, Aberdeen Royal Infirmary, Aberdeen, UK

Henry Watson is a Consultant Haematologist at Aberdeen Royal Infirmary. He specialises in all aspects of clinical haemostasis and thrombosis.

Scientist in Training – 1

IDENTIFICATION OF FUNCTIONALLY IMPORTANT RESIDUES IN PROTEIN S FOR ITS INTERACTION WITH TFPI

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Centre for Haematology, Faculty of Medicine, Imperial College London, London, UK

Background
The extrinsic pathway of coagulation is regulated by tissue factor pathway inhibitor (TFPI) through direct binding and inhibition of factor (F)Xa and TF/FVIIa/FXa. For full inhibitory activity, TFPI require its cofactor, protein S. Protein S enhances the inhibition of FXa ~4-10 fold, thereby reducing the affinity of TFPI/FXa to below the plasma concentration of TFPI. The cofactor function is dependent upon a TFPI/protein S interaction involving the LG1 subunit of the protein S sex hormone-binding globulin (SHBG)-like domain. However, the detailed interaction site in protein S is not yet known.

Aim
To determine functionally important amino acid residues within protein S LG1, involved in the interaction with TFPI.

Methods
We created recombinant protein S variants where we either inserted an additional N-linked glycan attachment sites or substituted clusters of surface exposed charged residues for alanine in LG1. All variants were screened for TFPI and APC cofactor function in plasma based thrombin generation assays and for C4BP binding affinity. Variants of interest were further investigated in FXa inhibition assays.

Results
Six N-linked glycan variants were tested for TFPI cofactor function in plasma. Two variants, protein S D253T and Q427N/K429T, displayed decreased TFPI cofactor activity (~50-60% compared to wild-type), while showing normal APC cofactor function and C4BP binding. The location of these two inserted N-linked glycans formed the basis for the design of three alanine composite variants where clusters of 4-6 surface exposed charged residues were substituted for alanine. One variant, protein S K255A/E257A/D287A/R410A/K423A/E424A exhibited completely abolished TFPI cofactor function in plasma and FXa inhibition assays while retaining normal APC cofactor function and affinity for C4BP.

Conclusion
Through inserting additional N-linked glycans and substituting surface exposed charged amino acid residues within protein S LG1 we have identified a potential TFPI interaction site, involving protein S residues Lys255, Glu257, Asp287, Arg410, Lys423 and Glu424.

Scientist in Training – 2

ANTI-CUB AND ANTI-SPACER ANTIBODIES THAT CONFORMATIONALLY ACTIVATE ADAMTS13 WORK THROUGH ENHANCEMENT OF THE METALLOPROTEASE DOMAIN FUNCTION

Anastasis Petri[1], An-Sofie Schelpe[2], Nele Vandeputte[2], Hans Deckmyn[2], Simon F De Meyer[2], Karen Vanhoorelbeke[2], James TB Crawley[1]
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Background:
ADAMTS13 circulates in a folded conformation that is stabilised through intra-molecular interactions between the C- and N-terminal domains. Binding of ADAMTS13 to the VWF D4-CK domains reportedly extends ADAMTS13 into an open conformation that enhances its activity by ~2-fold. It was previously proposed that ADAMTS13 unfolding results in exposure of a functional exosite in the Spacer domain that interacts with VWF, increasing substrate-binding affinity. Certain antibodies have been generated that disrupt the folded conformation of ADAMTS13 (such as the Ab17G2 anti-CUB mAb) and so mimic the conformational activation of ADAMTS13 *in vitro*. We recently generated a novel activating mAb (Ab3E4) that recognises the central Spacer domain of ADAMTS13.

Aims:
To characterize the mechanism underlying conformational activation of ADAMTS13.

Methods:
Antibody-induced conformational activation of ADAMTS13 were studied by ELISA. The effects of Ab17G2 and Ab3E4 on ADAMTS13 activity were studied by kinetic analysis of VWF A2 domain fragment proteolysis.

Results:
Ab17G2 and Ab3E4 enhanced FRETS-VWF73 proteolysis by ~1.7-fold. This was reproduced using VWF96 as a substrate. Kinetically, the catalytic efficiency (k_{cat}/K_m) of ADAMTS13 proteolysis of VWF96 was enhanced by ~1.8-to-2.0-fold. Conformational activation was dependent on ADAMTS13 unfolding, since the activity of an ADAMTS13 variant lacking the TSP2-CUB2 domains (MDTCS) was not enhanced. Surprisingly, ADAMTS13 activation was not dependent on exposure of the Spacer/Cys-rich domain exosites, as previously proposed, as proteolysis of VWF96 variants with ablated Spacer/Cys-rich exosite binding sites was still enhanced. Michaelis-Menten analysis of VWF96 proteolysis showed that the antibody-induced ADAMTS13 activation is primarily manifest through ~1.5-to-2.0-fold enhanced substrate turnover (k_{cat}). Contrary to the current model, the conformational extension of ADAMTS13 influences the functionality of the metalloprotease domain active site more than the substrate-binding affinity (K_m). The ability of Ab17G2 and Ab3E4 to conformationally alter the metalloprotease domain of ADAMTS13 was further demonstrated by an ELISA using anti-metalloprotease domain antibodies that recognise conformationally sensitive epitopes, corroborating antibody-induced conformational changes influencing the active site.

Summary/Conclusions:
We show for the first time that antibody-induced conformational activation of ADAMTS13 is not a result of Spacer/Cys-rich domain exosite exposure. Rather, we propose that ADAMTS13 unfolding causes a conformational change in the active site that further activates the enzyme.

Scientist in Training – 3

NOVEL PLATELET-NEUTROPHIL INTERACTION VIA ACTIVATED $\alpha_{IIb}\beta_3$ MEDIATES NETOSIS UNDER FLOW

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Background:
Platelet-leukocyte interactions are important in diverse pathophysiological settings including atherogenesis, infection and DVT. All previously characterised interactions between platelets and leukocytes, require at least one of the cells to be fully activated (e.g. via P-selectin-PSGL-1, CD40L-CD40). However, a recent study showed that platelets bound to von Willebrand factor (VWF) under flow acquire the ability to bind leukocytes. We hypothesized that under flow VWF A1-GpIbα interaction 'primes' platelets, enabling them to form novel interactions with leukocytes.

Methods:
VWF A1 domain was purified and captured onto linear or bifurcated microchannels. Labelled whole or plasma-free blood was perfused at defined shear rates ($50s^{-1}$ to $1000s^{-1}$). Platelet and leukocyte binding was recorded in real-time.

Results:
Binding of GPIbα to VWF A1 under flow 'primed' platelets causing intracellular Ca^{2+}-release and activation of $\alpha_{IIb}\beta_3$. VWF-'primed' platelets captured neutrophils and T-cells (but not monocytes and B-cells) under low shear. Leukocytes more strongly attached in regions of turbulent flow. The number of leukocytes binding was not influenced by blocking P-selectin, but was significantly reduced by $\alpha_{IIb}\beta_3$ blockade. Channels directly coated with activated $\alpha_{IIb}\beta_3$ captured neutrophils under low shear, leading to intracellular Ca^{2+}-release, and neutrophil extracellular traps (NETs) formation. NET release was dependent on the presence of shear, intracellular Ca^{2+}-release and NADPH oxidase. Given the importance of NETs in innate immunity, we are currently evaluating if 'primed' platelets could be involved in neutrophil recruitment and NETosis in infection settings. Under low shear, platelets could be captured in *Staphylococcus aureus*-coated microchannels and subsequently recruit leukocytes.

Conclusions:
VWF A1-GPIbα binding under flow 'primes' (but does not activate) platelets, leading to the activation of $\alpha_{IIb}\beta_3$ (but not degranulation). For the first time, we show that activated $\alpha_{IIb}\beta_3$ can directly capture neutrophils and induce NETosis. This novel interaction provides a mechanistic insight into how platelets function as immune cells. Work is underway to determine the leukocyte receptor involved and the (patho)physiological implications of these findings.

Scientist in Training – 4

CANCER CELLS RELEASE ACTIVE TF-fVIIa COMPLEX WHICH CAN BE DIRECTLY INHIBITED BY APIXABAN

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Background: Under normal conditions, coagulation factors are mainly produced in the liver. However, abnormalities in cancer cells can induce the expression of coagulation factors in various tumours. Cancer cells release microvesicles which contain tissue factor (TF) and are self-sufficient in procoagulant activity. However, the lack of a suitable synthetic substrate for factor VIIa (fVIIa) has hampered the mechanism of function of these microvesicles.

Aims: In this study, the expression and release of fVIIa and fXa in cancer cell-derived microvesicles was examined. In addition, by designing a novel substrate for fVIIa, we have discovered a novel function for selected direct oral anticoagulant (DOAC), Apixaban.

Methods: The expression of fVIIa and fXa was analysed in 4 cell lines using western blot and qRT-PCR. Microvesicles were then isolated from the cell lines, expressing both proteins, and tested for fVIIa and fXa. The activity of fVIIa was measured using a novel chromogenic substrate for factor VIIa (NH2-Asn-Leu-Thr-Arg-pNA). Microvesicle-associated fVIIa and fXa activites were measured and the presence of TF and fVIIa antigens determined by ELISA. Finally, the ability of the Apixaban and Rivaroxaban to inhibit the purified and microvesicle-associated fVIIa was examined.

Results: The designed chromogenic substrate was activated by fVIIa but not fXa. Analysis of the cell lines showed that the majority expressed fVIIa and fXa antigens. Furthermore, TF antigen and also fVIIa antigen and activity, but not fXa was detected in the microvesicles. Finally, Apixaban but not Rivaroxaban was capable of inhibiting the activities of both the isolated fVIIa, as well as the microvesicle-associated TF-fVIIa.

Conclusions: Cancer cell lines have the ability to produce and release fVIIa together with TF within microvesicles. Furthermore, Apixaban appears to be a selective inhibitor of TF-fVIIa.

Scientist in Training – 5

CROSS-TALK BETWEEN THE ALTERNATIVE PATHWAY OF COMPLEMENT AND THE INTRINSIC PATHWAY OF COAGULATION

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Background: Complement-coagulation crosstalk is now a widely accepted concept, however many interactions remain unexplored. The complement and coagulation cascades have evolved from the same ancestry and are implicated during systemic inflammation leading to detrimental effects if allowed to activate uncontrollably, as seen in disseminated intravascular coagulation. Complement and coagulation can activate both in plasma and on surfaces. Complement consists of three pathways: the classical pathway, mannose-binding lectin pathway and the alternative pathway. Coagulation is triggered via the intrinsic (via contact activation) and the extrinsic (via exposure of tissue factor) pathways. In this study we investigated interactions between the intrinsic pathway of coagulation and components of the complement (C)3 convertase of the alternative pathway with particular interest to properdin, C3b, and factor (F)XI.

Aims: To determine if components of the alternative pathway interact with the intrinsic pathway with any functional consequences.

Methods: C3b and properdin were immobilised on a sensor surface, with FXI and activated FXI (FXIa) as analytes, and interactions were analysed using surface plasmon resonance. Chromogenic assays were used to detect the effect of properdin on activation of FXI by dextran sulphate. SDS-PAGE was performed to determine if the enzyme FXIa could cleave properdin.

Results: FXI and FXIa both bound to C3b and properdin with very high (nM or pM) affinity. By employing a chromogenic assay we demonstrated that properdin resulted in reduced FXI activation via dextran sulphate. Properdin does not appear to be a substrate for FXIa using SDS-PAGE analysis.

Summary: These novel findings show a potential regulatory system of the contact pathway of coagulation by properdin and a new link for the serine protease cascades, further demonstrating the importance of complement-coagulation crosstalk.

1

CRYSTAL STRUCTURES OF THE RECOMBINANT β-FACTOR XIIa PROTEASE WITH BOUND PRO-ARG AND THR-ARG SUBSTRATE MIMETICS

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Centre for Biomolecular Sciences, School of Pharmacy, University of Nottingham, UK

Background: Coagulation factor XII (FXII) has role implicated in fibrinolysis, inflammation and thrombosis hence leading to a number of pathologies including angioedema and thrombosis disorders. FXII is a promising drug target for thrombotic diseases as it initiates both intrinsic pathway of coagulation and the contact pathway therefore involved in thrombosis but has non-essential role in haemostasis. Characterization of plasma protease inhibitors from plants present an exciting approach towards development of new drugs by providing a scaffold for structure based drug-design.

Aims: To perform crystallization studies on recombinant active form of FXIIa (βFXIIa).

Methods: βFXIIa protein was expressed in Drosophila expression system without (βFXIIa) and with (MBP-βFXIIa) MBP fusion tag, crystallized and their respective structure was solved using protein X-ray crystallography. Binding studies of βFXIIa with a kunitz type inhibitor EcTI, purified from *Enterolobium contortisiliquum* plant, was analysed on gel filtration chromatography and Surface Plasmon Resonance (SPR) assays.

Results: Recombinant βFXIIa forms obtained were active as analysed by substrate H-D-Pro-Phe-Arg-pNA 2HCl cleavage assays and their structure revealed characteristics of an active serine protease. The βFXIIa structure revealed active site occupied with substrate mimicking residues as found in physiological substrate of FXII. The MBP-βFXIIa crystal structure with bound inhibitor D-Phe-Pro-Arg chloromethyl ketone (PPACK) represent similar binding interactions as observed in other coagulation proteases. The EcTI inhibitor formed a complex at 1:1 molar ratio with βFXIIa protease; binding affinity K_D =3.38 × 10^{-7} M and, a model of their complex was obtained by molecular docking.

Summary: The βFXIIa structure revealed the S1 and S2 pockets were occupied with Arginine and Threonine residues, respectively from an adjacent molecule, which mimics the P1-P2 FXIIa cleavage site residues present in the natural substrates prekallikrein and FXII. A wider comparison of the βFXIIa structures with other serine proteases reveals different conformations of Tyr99 and the S2 pocket providing a structural basis for FXIIa substrate preferences at the P2 position. These crystal structures of βFXIIa provide a unique insight into a more open transient form of the S2 pocket and establishes a novel scaffold for structure guided drug design of antithrombotic agents targeting the contact pathway.

2

CHARACTERISATION OF A NOVEL GPIBα KNOCKIN MOUSE THAT LACKS THE LAST 24 AMINO ACIDS OF ITS INTRACELLULAR DOMAIN

Isabelle I. Salles-Crawley, Adela Constantinescu-Bercu, Kevin J Woollard & James TB Crawley
Centre for Haematology, Hammersmith Hospital Campus, Imperial College London, London, UK

Background

The platelet GPIbα-VWF A1 domain interaction is crucial in haemostasis for platelets to decelerate on unravelled VWF under high shear conditions. VWF engagement of GPIb-IX-V complex induces intracellular calcium fluxes and activation of tyrosine kinases that culminate in the activation of $\alpha_{IIb}\beta_3$. We recently demonstrated that platelets that are primed following binding to VWF under flow are able to recruit neutrophils via activated $\alpha_{IIb}\beta_3$.

Aims

To evaluate the (patho)physiological relevance of VWF/flow-dependent signalling through GPIbα.

Methods

GPIba$^{\Delta sig/\Delta sig}$ mice were generated via CRISPR-Cas9 technology to introduce a novel stop codon to delete the last 24 a.a. of the GPIbα intracellular tail that interacts with intracellular signalling partners. This aimed to generate a mouse with platelets with normal VWF-dependent platelet capture but abrogated GPIbα-A1 signalling. Platelet function was assessed by flow cytometry and platelet aggregometry. The ability of murine platelets to be primed on VWF under flow was also evaluated in *ex vivo* flow chamber.

Results

The loss of the intracellular tail of GPIbα was confirmed by Western blotting of washed *GPIba*$^{\Delta sig/\Delta sig}$ platelets while the extracellular domain remained unaffected. *GPIba*$^{\Delta sig/\Delta sig}$ mice exhibited a mildly reduced platelet count and slightly enlarged platelets compared to *GPIba*$^{+/+}$ littermates. All other haematological parameters were normal. Activation of *GPIba*$^{\Delta sig/\Delta sig}$ platelets with ADP and thrombin was largely unaffected; stimulation of *GPIba*$^{\Delta sig/\Delta sig}$ platelets with varying concentration of collagen-related-peptide (CRP) led to markedly decreased P-selectin exposure and $\alpha_{IIb}\beta_3$ activation on the platelet surface. Murine platelets captured on VWF under high shear were able to recruit mouse or human leukocytes under low shear conditions. We also confirmed that murine neutrophils similar to their human counterparts can bind activated $\alpha_{IIb}\beta_3$ under flow.

Summary/Conclusions

We successfully generated a novel transgenic mouse via CRISPR-Cas9 technology that have a truncated GPIbα cytoplasmic tail. Although the main filamin binding site has been preserved, *GPIba*$^{\Delta sig/\Delta sig}$ mice have slightly lower platelet counts and modestly enlarged platelets. The defect observed in *GPIba*$^{\Delta sig/\Delta sig}$ platelets after CRP stimulation confirm *in vitro* findings that GPIbα may be important for GPVI signalling. Flow assays to further assess platelet function and priming as well as thrombosis models are underway to characterise the physiological and pathophysiological role of the GPIbα-A1 signalling.

3

EFFICAY AND SAFETY OF PROTHROMBIN COMPLEX CONCENTRATE IN PATIENTS TREATED WITH RIVAROXABN OR APIXABAN COMAPARED TO WARFARIN PRESENTING WITH MAJOR BLEEDING

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Background

Prothrombin complex concentrate (PCC) is the standard treatment for warfarin related major bleeding events (MBE) including intracranial haemorrhage (ICH) and approved UK strategy for the same indications in rivaroxaban or apixaban.

Aim and Methods

In this respective review, efficacy and safety of PCCs for management of MBE in 344 patients receiving rivaroxaban, apixaban or warfarin were compared from January 2016 to April 2018.

Results

Median (range) dose of PCC was 2000 units (500-5000). ICH was the most common indication in all three groups (137/344, 39.8%) followed by gastrointestinal bleeding (93/344, 27%). ICH was more frequently the indication in patients receiving rivaroxaban (25/40, 62.5%) and apixaban (21/40, 52.5%) compared to warfarin (91/264, 34.5%), p=0.003. Patients on warfarin more frequently received PCC for musculoskeletal bleeding (12.9%) compared to rivaroxaban (5%) and apixaban (5%), p=0.003. Median rivaroxaban and apixaban levels were 230ng/ml (47-759) and 159ng/ml (45-255). Median INR pre- and post-PCC in patients on warfarin were 3.4 (1.9-15.4) and 1.2 (1.0-1.9). There was no difference in blood products used between groups. Thirty-day mortality rate was 24.7% (85/344) and did not differ between groups (p=0.17). Recurrent bleeding rates were warfarin 15.8%, rivaroxaban 20% and apixaban 30.8%, p=0.07. Thrombosis occurred in 4.1% (14/344) patients within 30 days with no difference between groups, p=0.83 (warfarin 4.2%, rivaroxaban 5.1% and apixaban 2.5%).

In patients with ICH, there were no differences in concomitant use of antiplatelet treatment, re-bleeding (overall 17.8%, warfarin 17.8%, rivaroxaban 20% and apixaban 15%, p=0.90) or 30-day mortality (overall 35%; warfarin 31.9%, rivaroxaban 44% and apixaban 38.1%, p=0.50). In this group, only 2/91 (2.2%) patients (on warfarin) had ischemic strokes within 30 days post PCC.

Summary/conclusions

There was no difference in the safety (thrombosis) and efficacy (30-day mortality and re-bleeding) in use of PCC to treat MBE in patients on warfarin, rivaroxaban and apixaban.

4

WHAT PHYSIOLOGICAL SURFACE IS RESPONSIBLE FOR THE THROMBIN FEEDBACK LOOP ON FACTOR XI?

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Background: Thrombin can activate Factor (f) XI in the presence of negatively charged surfaces such as dextran sulfate, however a physiological surface for this reaction is yet to be determined. It has been suggested that the thrombin feedback loop occurs at the platelet surface that is comprised of 60% PC, 20% PS and 20% PE.

Aims: To determine a physiological surface responsible for the thrombin feedback loop on FXI.

Methods: In order to examine thrombin activation of FXI and the physiological surface required we investigated the effect of different surfaces including dextran sulfate, phospholipid vesicles, sulfatides, and long- and short-chain polyphosphates on this reaction. Initial work was carried out in purified systems. Purified thrombin and FXI were used in chromogenic enzyme activity assays, turbidity experiments and SDS-PAGE gels. Normal Pooled Plasma and FXI-Deficient plasmas were used in turbidity and thrombin generation experiments.

Results: Chromogenic activity assay results confirm that the presence of dextran sulfate enhances FXI activation by thrombin and these were compared to more physiological surfaces such as phospholipid vesicles, sulfatides and polyphosphates. It was not possible to observe enhancement of FXI activation by thrombin in the presence of phospholipid whereas with sulfatides there was of enhancement of this feedback loop.

Summary/Conclusion(s): A physiologically relevant surface at which the thrombin feedback loop occurs is still yet to be identified. Whilst dextran sulfate, shows the most FXI activation by thrombin, the only physiological surface that enables detectable thrombin activation of FXI is sulfatides. The physiological relevance of the thrombin feedback loop remains to be determined.

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6

DISSIMILAR EFFECTS OF DIRECT ORAL ANTICOAGULANTS ON THE RELEASE OF TF-POSITIVE MICROVESICLES AND CANCER CELL PROLIFERATION

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Background: The activation of protease activated receptor (PAR)-2 by factor Xa (fXa) promotes the release of tissue factor-positive microvesicles (TF^+MV) and may contribute to proliferation in cancer cells.

Aims: This study examined the influence of direct oral anticoagulants (DOAC), Apixaban and Rivaroxaban, as inhibitors of the release of TF^+MV from cancer cell lines.

Methods: MDA-MB-231 breast cancer cell line and AsPC-1 pancreatic cancer cell line were seeded out (5×10^5) in DMEM and RPMI-1640 respectively. The cells were adapted to serum-free media and treated with Apixaban (0-1 µg/ml) or Rivaroxaban (0-0.6 µg/ml) in the presence or absence of fXa (10 nM). The release of TF^+MV into the media was then measured by determining the microvesicle density (Zymuphen kit) and TF antigen (Quantikine TF ELISA kit). Microvesicle-associated thrombin generation was also measured using a Calibrated Automated Thrombogram assay. In addition, cells were seeded out (2×10^4), treated as above and the rate of proliferation of the cells was monitored for up to 4 days using the crystal violet staining method.

Results: Activation of the cells with fXa (10 nM) enhanced the release of TF^+MV but was suppressed in the presence of either DOAC. Incubation of cell lines with Apixaban but not Rivaroxaban, in the absence of fXa, decreased the release of TF^+MV below that of resting cells, in a PAR2 dependent manner. Furthermore, incubation with Apixaban reduced the proliferation rate in both cells lines examined.

Conclusions: This study has established that the inhibition of PAR2 activation with DOAC reduces the release of TF^+MV and suppresses cell proliferation. Importantly, the presented data for the first time show that DOAC may selectively inhibit TF-fVIIa complex activity in addition to fXa.

7

MICRORNA-223 IN PLATELET-DERIVED EXTRACELLULAR VESICLES DOWNREGULATES TISSUE FACTOR EXPRESSION IN THP-1 MONOCYTIC CELLS

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Background: Extracellular vesicles (EVs) from activated platelets have been shown to contain microRNAs (miRNAs), the most abundant of which is miR223. Interestingly, miR223 in endothelial cells has been demonstrated to downregulate the expression of tissue factor (TF), the main initiator of the extrinsic coagulation pathway. However, the role of miR223 in regulating TF expression in monocytes is not known.

Aims: To examine whether miR223 from platelet derived-EVs alters TF expression in monocytes.

Methods: Vitamin D3-differentiated THP-1 monocytic cells were transfected with miR223 mimic or negative control miRNA (30nM) for 24h. TF protein expression was then analysed by flow cytometry and western blot analysis, and TF activity was determined using a TF-specific factor Xa generation assay. Washed platelets were activated with PAR1-agonist peptide (10µM) for 10 min and platelet releasate containing EVs was retained. Alternatively, EVs were isolated from the releasate by centrifugation at 100,000*g*. THP-1 cells were then incubated with platelet releasate or EVs (1000 vesicles/cell) for 24h and TF expression was measured. THP-1 cells were also co-transfected with an inhibitor of miR223, Antagomir223, or negative control miRNA, and TF expression analysed.

Results: Transfection of THP-1 cells with miR223 mimic for 24h resulted in a small but significant reduction in cell surface TF expression compared to cells transfected with the negative control miRNA, which was reflected in a reduction in total TF protein expression and TF-specific procoagulant activity. Confocal microscopy showed that platelet-derived EVs were taken up by THP-1 cells, and incubation with platelet releasate or EVs resulted in a decrease in TF expression. Co-transfection of miR223 together with Antagomir223 reversed the effect of miR223 on TF expression in THP-1 cells.

Summary: Both the miR223 mimic and miR223 from platelet-derived EVs downregulated TF expression in monocytes. This effect although small may serve to suppress any procoagulant activity in non-activated monocytes.

8

PREVALENCE OF ANTI-PROTEIN C ANTIBODIES AND ACQUIRED ACTIVATED PROTEIN C RESISTANCE IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: Systemic lupus erythematosus (SLE) patients have a higher risk for venous thromboembolism (VTE), often associated with antiphospholipid antibodies (aPL). The protein C (PC) pathway regulates haemostasis and inflammation. Acquired resistance to protein C (APCr) is associated with a high prevalence of anti-PC antibodies (anti-PC) and a more severe clinical phenotype in VTE patients with antiphospholipid syndrome, but their potential role in SLE is unknown.

Aim: To determine the prevalence of anti-PC and associations for APCr and thrombosis in patients with SLE.

Methods: Sixty-one patients with SLE have been enrolled and categorised by presence (+) or absence (-) of aPL and thrombotic history (aPL+/T+, n=15; aPL+/T-, n=17; aPL-/T+, n=8; aPL-/T-, n=21). Patients with heritable thrombophilia were excluded. Anti-PC levels were determined by ELISA and APCr by thrombin generation (TG) in the presence and absence of exogenous recombinant human activated PC (rhAPC) or Protac (to activate endogenous PC).

Results: 44% of all patients had anti-PC (aPL+/T+: 53%, aPL+/T-: 47%, aPL-/T-: 48%, aPL-/T+-: 25%; p=NS). aPL+ patients had higher median anti-PC levels than aPL- (37 vs 26U/mL, p=0.04). Anti-PC was associated with resistance to both rhAPC (p=0.01) and Protac (p<0.001). APCr prevalence was similar in aPL+ (54%) and aPL- (45%) patients. Resistance to rhAPC was associated with resistance to Protac (p=0.02), but was less frequently detected (30% vs 48% of all cases), especially in aPL- patients (14% vs 44% in aPL+; p=0.01). Resistance to rhAPC was higher in aPL+ compared to aPL- (p=0.02).

Conclusion: anti-PC were detected in patients with SLE even if aPL-. Resistance to activation of PC was more frequently detected than resistance to exogenous APC, suggesting a possible defect in PC activation. Predictive clinical-pathophysiological models based on anti-PC-profile and APCr assays might offer aPL independent tools for the identification and management of SLE patients at increased risk of thrombosis.

9

PATIENTS WITH RECURRENT VENOUS THROMBOEMBOLISM HAVE LESS ELASTIC CLOTS THAN THOSE WITH NON-RECURRENT DISEASE

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Objective – Venous thromboembolism (VTE) is associated with a high risk of recurrent events after withdrawal of anticoagulation. Plasma fibrin clots from VTE patients have been shown to be denser and poorly lysable, and these features can predict increased risk of VTE recurrences. The aim of this study was to determine the difference in plasma clot mechanical properties from patients having recurrent versus non-recurrent VTE.

Methods and Results – We previously developed a system for determining clot mechanical properties using an in-house magnetic tweezers system. This system was used to determine the mechanical properties of clots made from platelet poor plasma of 11 patients with recurrent VTE and 33 with non-recurrent VTE. Plasma samples were mixed with micrometre sized beads followed by thrombin and calcium to induce clotting, then placed in small capillary tubes and allowed to clot overnight. Bead displacements upon manipulation with magnetic forces were analysed to determine clot elasticity (G') and viscosity (G''). Recurrent VTE patients had nearly two-fold less elastic (0.78 (0.57 – 0.90) vs 1.43 (1.10 – 2.04), G' at 1 Hz, *P=0.0015*) and less viscous (0.74 (0.43 – 0.88) vs 1.37 (0.94 – 1.95), G'' at 1 Hz, *P=0.0008*) clots than those with non-recurrent VTE, regardless of male sex, unprovoked events, family history of VTE, fibrinogen concentration or body-mass index.

Conclusions – Using magnetic tweezers system for the first time in real-life patients, we found that plasma clots from recurrent VTE patients were less elastic and less viscous than those from non-recurrent VTE patients. These data indicate a possible role for fibrin clot elasticity in determining VTE recurrence.

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ERP44, A NEW THIOL ISOMERASE ENZYME THAT IS PRESENT IN PLATELETS

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Background: Thiol isomerases are endoplasmic reticulum (ER) resident proteins that modulate the formation, reduction and isomerisation of disulphide bonds, which are often rate-limiting reactions during protein folding. Recent evidence suggests that this family of proteins are surface presented in a number of different cell types where they are important in cell remodelling and recognition. Previous research has identified thiol isomerases within platelet surfaces including, PDI, ERp5, ERp57 and ERp72 which play pivotal roles in the regulation of platelet function. ERp44 is a thiol protein with one hydrophobic N-terminal thioredoxin domain containing a CRFS motif, followed by two thioredoxin inactive domains arranged in a clover-leaf-like structure. It is also acts as a multifunctional chaperone protein that regulates redox homeostasis and modulates thiol-mediated quality control and calcium and KDEL-receptor signalling. ERp44 is also implicated in the regulation and correct folding of various molecules including IgM within B lymphocytes, adiponectin, and formylglycine-generating enzyme.

Aims: The aim of this study is to investigate the expression and localisation of ERp44 in platelets to determine whether this thiol isomerase is also likely to be implicated in the regulation of platelet activation.

Methods: The presence of ERp44 in human/mouse platelets was examined by immunoblotting. Immunofluorescence and sucrose density gradient subcellular platelet fractionation were used to determine ERp44 location in human platelets.

Results: Immunoblotting and Immunofluorescence analysis confirmed that ERp44 is present in human and mouse platelets, as well as in the human megakaryocytic cell line (MEG01). The location, levels and subcellular associations of ERp44 with different organelles were therefore examined. Immunofluorescence microscopy (IFM) of permeabilised platelets revealed ERp44 to be dispersed throughout the cytoplasm in a punctate arrangement. Following activation, ERp44 partially translocated to a ring-like staining pattern towards the plasma membrane in confocal images and colocalised with proteins known to be present in the membrane proximal dense tubular system. Subcellular fraction of platelets by sucrose density gradient centrifugation revealed ERp44 to be principally located in low density fractions, consistent with interactions with the plasma membrane, and suggested that, like other platelet thiol isomerases, this enzyme is not located in □-granules. Indeed, cell surface localisation of ERp44 was detected on resting platelets by flow cytometry and found to increase in detectable levels following stimulation with 1U/mL thrombin. The mobilisation of ERp44 to the platelet surface was found to be dependent on actin polymerisation. It is possible that surface bound ERp44 may result from co-secretion with transmembrane proteins to which is it bound, or through binding to cell surface molecules following release as a soluble protein. ERp44 was detected in the supernatant of activated platelets suggesting that surface localisation is due, at least in part to protein release. **Conclusion:** These properties are shared with other functionally important platelet thiol isomerases, raising the possibility that ERp44 may also contribute to platelet regulation. We have generated antibodies to ERp44 and are currently exploring this question. This study has aided our understanding of subcellular DTS association of ERp44 and its release and mobilisation upon platelet activation. Therefore, affinity-purified function blocking anti-ERp44 antibodies will be used to determine the protein's role and its surface substrates. Further research will be undertaken to explore co-precipitation of ERp44 and investigate whether there is a direct association between ERP44 and other platelet surface proteins.

Keywords: platelets, megakaryocytic cell line (MEG01), thiol isomerase, platelet microparticles, Protein disulphide isomerase (PDI), Endoplasmic reticulum protein 57 (ERp57), Endoplasmic reticulum protein 44 (ERp44), Endoplasmic reticulum protein 5 (ERp5), Fluorescence-activated cell sorting (FACS), Dense Tubular System (DTS), immunofluorescence (IMF).

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CHARACTERISATION OF A GENETICALLY MODIFIED FXIII-A L34V MODEL AND POTENTIAL FOR VASCULAR STUDIES

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Background: Coagulation factor XIII (FXIII) is a key enzyme in stabilising blood clots by crosslinking fibrin molecules together, as well as crosslinking fibrinolysis inhibitors to fibrin. The human FXIII-A V34L variant leads to increased activation rates, forming clots with thinner fibrin fibres and smaller pores, and is protective against thrombotic disease.

Aim: To establish a murine FXIII-A V34L model for studying its role in vascular disease.

Methods: FXIII-A 34V and FXIII-A 34L (wild-type) mice were weighed weekly for 12 weeks to compare growth. Plasma FXIII activation was assessed by biotin incorporation assay, and antigen levels by western-blotting. Whole blood clot formation, strength and lysis was measured by ROTEM (EXTEM, EXTEM+tPA, FIBTEM), providing clotting time (CT), maximum clot firmness (MCF) and lysis time (LT). Clot contraction and erythrocytes extrusion (haemoglobin) were measured for 2h, before the final clot weight was quantified.

Results: No significant difference in animals' growth was observed between the groups. FXIII-A 34L plasma showed a 40.4% increase in activation rates compared to 34V, similar to comparison with human variants. No difference in plasma FXIII-A antigen levels was observed between the groups. ROTEM studies showed no difference between the groups for CT, MCF and LT, whether in the presence (EXTEM) or absence (FIBTEM) of functional platelets. The clot volumes and serum haemoglobin levels were not significantly different at each timepoint between the groups, and the final clot weight was similar.

Conclusion: Murine FXIII-A 34L variant increased activation rates over the 34V variant is comparable to that of the human variant (+68%), despite similar circulating levels. No difference were observed between the variants in *ex-vivo* studies of whole blood clotting, however *in-vivo* studies in murine thrombosis models may prove of interest. Future studies are required to investigate if this gain-of-function FXIII variant impacts on other aspects of vascular biology.

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BTK INHIBITION INHIBITS CLEC-2 MEDIATED PLATELET ACTIVATION

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Background

Thrombosis is the largest cause of death globally, with platelets playing a major role in the pathophysiology. Vessel inflammation increases risk of thrombosis due to upregulation of prothrombotic ligands, a phenomenon called thromboinflammation. The CLEC-2 receptor on platelets binds to upregulated podoplanin in thromboinflammatory conditions. Studies show that CLEC-2 deficient mice are protected from thrombosis in such conditions *without* an increase in bleeding. Unwanted bleeding is an issue in current thrombosis prophylaxis, making CLEC-2 an attractive therapeutic target.

Aims

This study aimed to investigate the potential of specifically inhibiting CLEC-2 to reduce platelet activation using ibrutinib; a tyrosine kinase inhibitor already used and well tolerated in clinical practice.

Methods

Light Transmission Aggregometry (LTA) was performed using healthy donor blood, treated *in vitro* with ibrutinib or vehicle, as well as blood taken *ex vivo* from consenting patients treated with ibrutinib or a control treatment or those with X-linked Agammaglobulinaemia (a genetic mutation in the Btk gene). Platelets were stimulated by the exogenous CLEC-2 ligand rhodocytin. Further to this, Capillary Flow Adhesion was performed to assess the binding of platelets in whole blood to a podoplanin-coated capillary at venous rates of shear. Again, this was done with healthy donor and patient blood treated *in vitro* and *ex vivo* ibrutinib respectively.

Results

Both *in vitro and* ex vivo treatment with ibrutinib reduced the size of platelet clusters bound to podoplanin in the flow adhesion assays by 50% when compared to controls ($p<0.05$ and $p<0.01$ respectively). Similarly, XLA and ibrutinib treated platelets did not aggregate during LTA when stimulated with rhodocytin.

Conclusion

In this study Btk inhibition was shown to significantly reduce CLEC-2 mediated platelet activation in two separate models. These results therefore warrant further investigation into the potential of ibrutinib for thromboprophylaxis in *in vivo* studies.

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INCIDENCE OF THROMBOCYTOPENIA AND HEPARIN INDUCED THROMBOCYTOPENIA IN PATIENTS RECEIVING EXTRACORPOREAL MEMBRANE OXYGENATION (ECMO) COMPARED TO CARDIOPULMONARY BYPASS (CPB) AND THE LIMITED SENSITIVITY OF PRE-TEST PROBABLITY SCORE

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Introduction

Heparin induced thrombocytopenia (HIT) is a special concern in patients receiving extracorporeal membrane oxygenation (ECMO) but the incidence of thrombocytopenia, reliability of pre-test probability score (PTPS) and the effect of HIT on 30-day mortality in patients receiving ECMO compared to CPB is unknown.

Aims and Methods

Single centre retrospective study from January 2016 to April 2018, 296 ECMO patients and 2998 CPB patients were studied (median duration in CPB was 4.6 [2-16.5] hrs compared to 7.1 [3-42] days in ECMO [<48hrs in ECMO were excluded]). PTPS (4Ts) and HIT screening were performed in all patients who showed a typical platelet drop in first 5-10 days after exposure to UFH with or without thrombosis. Confirmatory testing was done by ELISA.

Results

Platelet counts for ECMO and CPB over time are shown in Table 1. Severe thrombocytopenia and moderate thrombocytopenia were more common in ECMO compared to CPB throughout ($p<0.0001$). Thrombocytopenia increased in CPB patients on day-2 but by day-10 was normal in 83% compared to 42.3 % for ECMO.

A total of 96 patients had HIT screening tests (64/296 ECMO and 32/2988 CPB). Screening was positive in twenty patients (20/296, 6.8%) on ECMO and 18 (18/2998, 0.6%) on CPB ($p<0.001$). All positive screening tests were confirmed by ELISA. Median PTPS was 4 (3-7) on ECMO and 5 (4-7) on CPB. Four ECMO HIT patients had PTPS of 3 and would not normally been screened according to current guidelines. Mortality (overall 95/296, 32.1%) was not different with (6/20, 30%) or without HIT (89/276, 32.2%) in patients on ECMO.

Summary/conclusions

Thrombocytopenia is already common at ECMO initiation. CPB patients dropped their platelet count but recovered by day-10. HIT is more frequent in ECMO (both VV and VA-ECMO) compared to CPB. PTPS failed to detect HIT in 4/20 (20%) ECMO patients.

Table 1. Proportions of patients undergoing ECMO or CPB with different degrees of thrombocytopenia on days 1, 2, 5 and 10

Degree of thrombocytopenia		Severe (<50x109/L) %	Moderate (50-99x109/L) %	Mild(100-150x109/L) %	Normal platelet >150x109/L %
Day 1	CPB	0.2	14.5	50	35.36
	ECMO	4.4	40.0	23.2	32.4
Day 2	CPB	0.9	32.4	46.8	19.8
	ECMO	4.1	51.3	21.0	23.6
Day 5	CPB	2.0	14.0	23.6	60.5
	ECMO	9.4	46.3	22.5	21.7
Day 10	CPB	1.6	6.3	9.4	82.7
	ECMO	4.1	26.0	27.6	42.3

CPB= cardiopulmonary bypass, ECMO= Extracorporeal membrane oxygenation

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A PRACTICAL METHOD FOR REDUCING INTERFERENCE BY LIPAEMIA FOR COAGULATION TESTS

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Background

Plasma samples with lipaemia present a challenge for coagulation laboratories using optical analysers and to a lesser extent manual clotting methods. High speed centrifugation may be used to remove excess lipids but it is not known whether this affects haemostasis tests.

Aims

To determine whether removal of lipid by centrifugation affects PT, APTT, fibrinogen, D-dimer and von Willebrand factor antigen measurements.

Methods

Samples: 26 lipaemic samples (Triglyceride range 0.5-17.0 mmol/L; cholesterol 2.2-9.41 mmol/L). Ten normal plasmas spiked with 5μL/ per mL Intralipid 20 to produce a concentration of triglyceride known to interfere with coagulation measurements (>16 mmol/L). Lipaemic samples were tested before and after removal of the lipid layer formed by centrifugation at 10000**g** for 10 minutes. The spiked samples were tested before and after spiking, and after removal of the lipid layer. Testing was performed with the optical CS-5100 automated coagulometer (Sysmex), and mechanical clotting tests with a KC10 (Amelung). VWF antigen was performed by ELISA.

Results

Respectively, 4, 2 and 1 of the lipaemic samples, and all of the spiked samples failed to give PT, APTT and fibrinogen results on the CS-5100. Removal of the lipid layer reduced the cholesterol (median 0.6, range 0 - 3.3 mmol) and triglyceride concentration (1.5, range 0 - 6.0 mmol/L) sufficiently to obtain results for all tests. There were no statistically or clinically significant differences in any of the tests performed in lipaemic samples prior to and after lipid removal (where results were available) or between unspiked plasmas and the same plasmas after lipid removal.

Discussion/conclusions

Centrifugation of plasma at 10000**g** reduces lipaemia sufficiently to allow testing on an optical coagulation analyser without affecting PT, APTT, fibrinogen, D-dimer or VWF antigen results.
that reduces the risk of bleeding.

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PARTIAL RESCUE OF NATURALLY OCCURING ACTIVE SITE FACTOR X VARIANTS THROUGH DECREASED INHIBITION BY TFPI AND ANTITHROMBIN

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Background
Factor (F)X is activated by tissue factor (TF)/FVIIa into FXa. FXa is the serine protease component of the prothrombinase complex, the only known physiological activator of prothrombin. FX Nottingham (A404T) and Taunton (R405G) are two naturally occurring mutations, associated with bleeding tendencies in affected individuals. However, the reason for the phenotype is not known.

Aims
To characterise functionally FX Nottingham and Taunton mutations.

Methods
Recombinant wild-type FX, FX Nottingham and FX Taunton were generated and their procoagulant activities and inhibition were assessed in plasma. Using pure-component assays, the kinetic parameters for their activation by TF/FVIIa and peptide substrate and prothrombin cleavage were determined.

Results
The prothrombin times (PT) in FX-depleted plasma supplemented with the FX variants were increased to 104 and 55.5 sec for FX Nottingham and Taunton, respectively, compared to 13.7 sec for wild-type FX. Also in thrombin generation assays, wild-type FX showed higher procoagulant activity, compared to the mutants, at high (4pM) TF concentration. Surprisingly, at lower (1pM) TF stimuli, FX Nottingham and Taunton caused higher thrombin generation than wild-type FX. Detailed kinetic investigations showed reduced activities of FX Nottingham and Taunton, with k_{cat}/K_m reduced by ~55-fold and ~6-fold, respectively, for peptidyl substrate and prothrombin cleavage, explaining the prolonged PT time and decreased thrombin generation at high TF. Upon titrating Fondaparinux and tissue factor pathway inhibitor (TFPI) into plasma supplemented with the FX variants, the anticoagulant potential of Fondaparinux was decreased for FX Nottingham, whilst TFPI was only partially able to inhibit FX Taunton and showed little inhibitory effect on FX Nottingham.

Summary/Conclusion
Two naturally occurring variants, FX Nottingham and Taunton, both display decreased proteolytic activity. However, their lack of activity in plasma can be partially rescued by decreased inhibition by the inhibitors, TFPI and antithrombin.

16

ASSESSING THE IMPACT OF NICE GUIDELINE NG89 ON HOSPITALISED MEDICAL PATIENTS AT RISK OF VENOUS THROMBOEMBOLISM

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Background: Hospitalised medical patients (HMP) are at increased risk of VTE. Updated NICE guidance on VTE prevention (NG89) recommends pharmacological VTE prophylaxis for a minimum of 7 days when the risk of VTE outweighs their risk of bleeding. Current standard of care is to stop at discharge. NG89 allows the use any recognised VTE risk assessment tool rather than the current universal DH tool.

Aims: To assess the likely resource impact of implementing NICE guideline NG89 in HMP's in view of the requirement for post discharge LMWH prophylaxis and also the impact of using the validated PADUA VTE risk assessment tool instead of the current DH tool.

Methods: A retrospective analysis of data from an electronic VTE risk assessment and prescribing tool used for all HMPs admitted to a large teaching hospital in England between January 2016 and March 2018. Patients historical VTE risks were used to generate a PADUA score to determine the proportion of patients who would have been eligible for pharmacological thromboprophylaxis in comparison with the current DH VTE risk assessment. Results: 90,000 non-surgical admissions were analysed. 56% of patients were recommended LMWH thromboprophylaxis with no contraindications. 67.5% of patients were admitted for <7 days and would have required post discharge LMWH - 15,000 patients per year from a single hospital. The PADUA score was calculated with only 2/11 data points missing (representing <5% risks). Only 43% of patients with at least one risk on the DH tool had a score of ≥4 requiring pharmacological prophylaxis.

Summary/conclusions: There are > 7 million non-surgical hospital admissions per year in England. The resource implications for delivery of extended thromboprophylaxis as per NICE NG89 are substantial and potentially undeliverable however the option to use alternative VTE risk assessments eg. PADUA could significantly reduce the numbers of patients who are eligible for LWMH and offset the resource impact.

17

THE CRITICAL ROLE OF TISSUE FACTOR PATHWAY INHIBTOR (TFPI) UNDER FLOW AND *IN VIVO*

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Background

Tissue factor pathway inhibitor (TFPI) is a Kunitz-type inhibitor that is essential for haemostatic regulation. It functions through inhibition of the initiating TF-FVIIa complex in a FXa-dependent manner. Full length TFPI circulates in plasma at very low concentrations (<0.2nM), meaning that current plasma-based assays underestimate the importance of TFPI due to depletion of the inhibitor. In both humans and mice, complete TFPI-deficiency is incompatible with life making analysis of the role of TFPI *in vivo* difficult, and limiting analysis of its role in thrombosis.

Aims

To develop a novel assay to monitor TFPI function under flow, that diminishes the effects of TFPI depletion, and to determine the contribution of TFPI to thrombosis *in vivo*.

Methods

TFPI function was analysed under static conditions using the thrombin generation assay. Citrated whole blood was perfused over VWF and TF coated microchannels to capture platelets. Thereafter, recalcified plasma containing fluorescently-labelled fibrinogen was perfused at low shear, and real-time fibrin deposition was monitored. Recombinant TFPI or anti-TFPI blocking antibodies were added to analyse the role of TFPI in this model. To determine the role of TFPI to *in vivo* thrombus formation, inhibitory anti-murine TFPI or isotype control antibodies were injected into mice prior to laser-induced thrombosis model.

Results

In static thrombin generation assays, endogenous plasma TFPI only moderately influenced the initiation phase of coagulation, by prolongation of the lag time and decreased rate of thrombin generation. However, we found that addition of recombinant TFPI in our novel flow-based assay markedly increased the onset of fibrin deposition. Conversely, inhibition of endogenous plasma TFPI significantly shortened the time to initiation of fibrin deposition, revealing the importance of the plasma TFPI pool. *In vivo*, inhibition of TFPI in mice lead to a profound increase in fibrin deposition at the site of injury revealing the major importance of TF-dependent coagulation in this model.

Summary/Conclusions

We show that depletion of TFPI in static assays leads to underestimation of the contribution of TFPI to coagulation. The use of flow-based assays in which TFPI is not depleted enables a clear and more profound effect of TFPI to fibrin deposition that underscores the critical anticoagulant role for this inhibitor. We show for the first time, that TFPI deficiency *in vivo* leads to a profound increase in fibrin deposition in the laser-induced thrombosis model.

18

ANTIBODY-MEDIATED CLEARANCE OF ADAMTS13 IN ACQUIRED THROMBOTIC THROMBOCYTOPENIC PURPURA: A ROLE FOR MONOCYTES?

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Background

Patients with immune-mediated thrombotic thrombocytopenic purpura (iTTP) have ADAMTS13 deficiency caused by anti-ADAMTS13 IgG autoantibodies. These antibodies most commonly bind to the ADAMTS13 Spacer domain. Anti-ADAMTS13 antibodies can exert their pathogenic effects by inhibiting ADAMTS13 function and/or inducing clearance. Antibody-mediated clearance is likely the primary disease mechanism as iTTP patients most frequently present with severely reduced antigen levels at presentation. Moreover, low antigen levels are associated with increased mortality. Despite its pathogenic importance, the mechanisms through which ADAMTS13 clearance occurs are unknown. Phagocytic cells such as monocytes play an important role in the clearance of antibody-antigen immune complexes in some diseases and may be important in removing ADAMTS13-IgG immune complexes from the circulation in iTTP.

Aims

To investigate mechanisms of clearance of ADAMTS13 immune complexes using iTTP patient antibodies

Methods

Immune complexes were made using a biotinylated recombinant ADAMTS13 fragment, MDTCS, and IgG purified from normal plasma or from 3 patients with iTTP and low ADAMTS13 antigen levels at presentation (<2% normal). Immune complexes were incubated with cultured monocytes (THP-1 cells). Cells were lysed and MDTCS detected by Western blotting and ELISA.

Results

MDTCS was detected in monocyte cell lysates by both Western blotting and ELISA when MDTCS was incubated with iTTP patient IgG, whereas little/no MDTCS was detected in cells incubated with MDTCS and normal IgG. The role of other cell-types involved in clearance of immune complexes is currently under investigation.

Summary/conclusions

We have shown for the first time that ADAMTS13 is more readily internalised by monocytes in the presence of autoantibodies isolated from iTTP patients with low ADAMTS13 antigen levels. The precise mechanisms by which immune complex uptake occurs remain to be determined. Characterisation of these mechanisms may provide novel ways in which IgG-mediated ADAMTS13 clearance may be reduced in iTTP.

P-1

DESIGN AND ANALYSIS OF SMALL COMPOUNDS TO RESTRAIN THE FUNCTIONS OF TISSUE FACTOR WITHIN TUMOUR CELLS

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Background: The restriction of prolyl-protein *cis*/*trans* isomerase 1 (Pin1) activity has been shown to prevent the release of tissue factor (TF) leading to the accumulation of the latter protein within the cell. The accumulation of TF within the cell in turn leads to nuclear localisation of p53, upregulation of Bax protein and cellular apoptosis.

Aim: This study aimed to design and test novel small molecules that are capable of inhibiting Pin1, and investigate the outcome on TF activity and cellular apoptosis.

Methods: A set of four compounds were designed based on 5-(p-methoxyphenyl)-2-methylfuran-3-carbonyl amide and synthesised with modification to potentially enhance the activity. The compounds differed in the amino acid termini to contain A) D-tyrosine B) D-tryptophan, C) D-phenylalanine and D) 3-(2-naphthyl)-D-alanine. MDA-MB-231 cells were treated with each compound (100 µM) or vehicle control. TF activity was measured by fXa-generation assay. Cellular apoptosis was measured at 18 h using a chromogenic TUNEL assay. In addition, the nuclear localisation of p53 was also examined by fluorescence microscopy and bax mRNA and Bax protein expression was measured by RT-PCR and western blot analysis.

Results: Treatment of cells with compound A resulted in a reduction in cell numbers and TF activity, in conjunction with increased nuclear localisation of p53 and Bax expression, compared to the control sample. In addition, compounds B and D displayed reduced function towards the measured criteria in the cells, while compound C was largely ineffective.

Conclusions: In conclusion, we have designed and produced functional small compounds to regulate the function of TF within cells. These compounds may prove to be beneficial in moderating TF procoagulant function and restraining the resulting increased tumour cell growth.

P-2

IDENTIFICATION AND FUNCTIONAL INVESTIGATION OF GENES IN PATIENTS WITH INHERITED BLEEDING DISORDERS

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Inherited bleeding disorders (IBDs) comprise a heterogeneous group of disorders that result from a wide range of genetic defects in blood vessels, coagulation proteins and platelets, and are associated with a variable bleeding tendency. Although many genetic causes of bleeding disorders are known primarily for the coagulation factor genes, the underlying genetic cause of rare platelet-based bleeding disorders are clinically challenging and difficult to diagnose due to the absence of a gold standard platelet function test that has the capability to diagnose these disorders efficiently, as well as the presence of variable phenotypic presentations. As a consequence, using next-generation sequencing (NGS) including (whole genome and exome sequencing) coupled with a traditional Sanger sequencing and bioinformatics analysis can be used to investigate these rare genetic mutations, and subsequently to identify the likely causative gene in patients where the aetiology is presently not known. Whole exomes of five patients, recruited in the UK-GAPP study, have been analysed to determine candidate variants from WES data. Six candidate variants have been selected for further analysis including *FCER1G* c.173A>G; p.Tyr58Cys and *FHOD1* c.1525C>G; p.Arg509Gly in patient 1, *NBEAL2* c.7369C>T; p. Arg2457Trp in patient 2, *DNM3* c.1072G>A; p. Gly358Ser in patient 3, *RAI1* c.311A>G; p. Asp104Gly in patient 4 and *CD36* c.975T>G; p. Tyr325Ter in two related patients of the same family. Sanger sequencing has confirmed these variants. Further functional analysis such as platelet spreading analysis, light transmission aggregometry, flow cytometry, CRISPR mediated genomic editing technology, and western blot can be applied to study these variants further and confirm/refute causality. In conclusion, NGS and genotype and phenotype data from the UK-GAPP study play a key role in the studying of IBDs, and can provide valuable information about the genes involved in platelet formation, regulation and removal.

P-3

CORRELATION BETWEEN ELISAS AND AUTOMATED CHEMILUMINESCENCE PANELS TO DETECT ANTI-BETA2-GLYCOPROTEIN I ANTIBODIES IN LABORATORY DIAGNOSIS OF ANTIPHOSPHOLIPID SYNDROME

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Background

Presence of IgG and/or IgM anti-beta2glycoprotein I (anti-β2GPI) antibodies (in titre >99th centile) on two or more occasions at least 12 weeks apart is one of the three laboratory criteria for diagnosis of antiphospholipid syndrome (APS). Inter-laboratory variability remains a problem in the performance of anti-β2GPI enzyme-linked immunosorbent assays (ELISAs) due to the lack of uniformity in reference materials for calibration.

Aims and methods

The agreement between our current assay for IgG anti-β2GPI (Orgentec Anti-B2GP1 IgG assay ELISA) with two other commercial ELISA kits (QUANTA Lite β2 GPI IgG ELISA and Biorad Autoimmune EIA Anti-beta2 Glycoprotein I IgG Test Kit) and the ACL Acustar (Instrumentation Laboratory, A Werfen, UK) which uses chemiluminescence principle, was assessed. A total of 100 samples (43 APS patients positive for anti-β2GPI, 37 patients negative for anti-β2GPI but with clinical features associated with APS and 20 healthy controls) were tested as per manufacturers' instructions. Of the 43 IgG anti-B2GP1 positive patients, 19 had positive LA, 28 had positive IgG aCL and 12 had positive IgM aCL and 18 had triple positive aPL.

Results

The rate of anti-β2GPI positivity of patients' samples varied from 60.4% (26/43) to 69.7% (30/43) depending on the kit and assay. Complete agreement between all assays occurred only in 44.2% (19/43) of samples from patients with APS. However, a strong agreement of 86.5% (32/37) was observed in negative results with both ELISAs and ACL Acustar. Performance against the reference Orgentec ELISA is shown in Table 1. Three patients (triple positive aPL) with strongly positive anti-β2GPI by ELISA but negative by ACL Acustar is a major concern.

Summary/conclusions

This study confirms the discrepancies between different kits for IgG anti-β2GPI detection. Of particular concern are negative results for patients with triple positive APS by other methods. Development of reference materials could overcome this issue.

Table 1

	Hemosil ACL Acustar	Quantalite ELISA	Biorad ELISA
Manufacturer's cut-off	>20 U/mL	>20 SGU	>20 G
Sensitivity in APS% (95% CI)	69.7% (53.7 – 82.3)	62.79 % (46.73 – 77.02)	65.1% (49 – 78.5)
Specificity % (95% CI)	86.5% (70.4 – 94.9)	100% (90.51 – 100)	86.4% (70.4 – 94.9)
Positive predictive value (PPV) (95% CI)	85.71% (72.18 – 93.28)	100	84.8% (67.3 – 94.2)
Negative predictive value (NPV) (95% CI)	71.1 (60.57 – 79.7%)	69.81 (61.07 – 77.32)	68.0% (52.7 – 80.4)

P-4

AN EVIDENCE REVIEW TO DETERMINE THE EFFICACY OF NOVEL ORAL ANTICOAGULANTS (NOACS) COMPARED TO WARFARIN AT REDUCING VENOUS THROMBOEMBOLISM RECURRENCE IN ADULT PATIENTS

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Background: Venous thromboembolism (VTE) is responsible for 25,000 deaths a year in the UK. Treatment of patients with previous VTE is vital to prevent recurrence. Anticoagulants form the basis of therapy, with warfarin the mainstay of secondary prevention. However, newly developed drugs not requiring regular monitoring exist. These Novel Oral Anticoagulants (NOACs) are beginning to see use as a substitute for warfarin.

Aims: This review aims to determine the difference in VTE recurrence in adult patients, without chronic coagulopathies, receiving a single NOAC (dabigatran,rivaroxaban, apixaban,edoxaban) versus those receiving standard warfarin therapy as a preventative measure, following initial anticoagulation for the acute VTE.

Methods: The NICE Evidence portal was used to look for guidelines and evidence summaries. Search strategies combined free-text and index terms to search bibliographic databases (MEDLINE, EMBASE, CENTRAL and Web of Science) for systematic reviews(SR) and randomised control trials(RCTs). A strict eligibility criteria was applied, following the population in question, intervention, comparator and outcomes to include recurrent VTE.

Results: 1184 records were screened for suitability and of these 17 were appraised. Records were appraised using either the AGREE II or CASP checklist. Guidelines and evidence summaries were reviewed but did not contain sufficient detail regarding NOACs. The remaining records comprised of 1 SR and 6 RCTs reported NOACs as non-inferior to conventional therapy (low molecular-weight heparin followed by warfarin) in reducing VTE recurrence.

Conclusions: This review has found that all NOACs are non-inferior to warfarin in preventing VTE recurrence. Despite limitations with cost and the limited reversibility of their anti-coagulant effect, the comparative convenience of NOACs versus warfarin and safety in the reduction of major bleeding events may provide a more clinically acceptable alternative for patients. A review of evidence directly comparing NOACs to one another is an important subsequent step for the management of VTE recurrence.

POSTERS

P-5

P-6
EFFECTS OF WEIBEL-PALADE BODY PLASTICITY ON VWF FUNCTIONING.

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Background
Von Willebrand factor (VWF) plays a key role in vascular physiology. While there is a large pool of circulating plasma VWF, activated endothelial cells release a localised bolus of highly multimerised VWF in response to secretagogue action. This material is produced by exocytosis of Weibel-Palade bodies, the secretory granules of endothelial cells. We have discovered that the granules are found in a range of sizes, and that the size distribution affects the functioning of VWF in haemostatic assays.

Aims
To explore the effect of WPB size on VWF function using in vitro flow assays.

Methods
We use flow chambers and high-throughput imaging coupled to high-content automated microscopy analysed by automated morphometry, to determine the effects of WPB size on the ability of newly released VWF to form strings, to recruit platelets and to recruit plasma VWF.

Results
The size of WPB can be manipulated using a range of approaches, and the length of these organelles correlates with the endothelial ability to carry out pro-haemostatic functions.

P-7

LOW MOLECULAR WEIGHT HEPARIN REDUCES TUMOUR FORMATION, INVASION AND VASCUARISATION

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Background:

It has been suggested that the treatment of cancer patients with low molecular weight heparin (LMWH) may have additional benefits that extend beyond anticoagulation.

Aims:

This study aimed to explore the potential of Tinzaparin and Dalteparin to influence tumour formation, growth and invasion *in vitro*, and to examine the influence on tumour vascularisation using a Chorio-allantoic membrane (CAM) based model.

Methods:

WM-266-4 melanoma and AsPC-1 pancreatic cancer cell lines were treated with LMWH, Tinzaparin and Dalteparin (0-2 IU/ml), and the rate of tumour formation, growth and invasion were measured *in vitro*. In addition, the influence of pharmacological concentrations of the anticoagulants on the growth, invasion and vascularisation of tumours derived from WM-266-4 and AsPC-1 cells was also measured using the CAM model.

Results:

Tinzaparin and Dalteparin (2 IU/ml) significantly reduced tumour formation and invasion by the cell lines *in vitro*. In addition, treatment of CAM with LMWH reduced the local vascular density and the average vessel diameter beyond that achievable with Bevacizumab (12.5 µg/ml). Treatment of CAM-implanted WM-266-4 and AsPC-1 tumours with Tinzaparin (2 IU/ml) also reduced tumour invasion and vascularisation.

Conclusions:

In conclusion, LMWH may have anti-cancer properties by reducing the formation, invasion and vascularisation of the tumours.

P-8

VARIATIONS IN BINDING OF GPVI VARIANTS TO DIFFERENT FIBRIN(OGEN) FRAGMENTS

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Background: GPVI is a glycoprotein receptor that plays a critical role in platelet adhesion and thrombus formation through collagen binding. Recent studies have shown that fibrin(ogen) is also a ligand for GPVI, pointing to a binding site in the fibrin D-region. However, there are discrepancies as to what form of GPVI (dimeric/monomeric) binds to fibrin(ogen) and its fragments.

Aims: To explore the binding ability of monomeric/dimeric GPVI using a variety of commercial and in-house purified fibrin(ogen) fragments, in addition to in-house recombinant wild-type fibrinogen and a recombinant fibrinogen variant that cannot polymerize due to mutations in the knobs involved in polymerization.

Methods: ELISA plates were coated with fibrinogen or fibrin(ogen) fragments. Fibrinogen was converted to fibrin by addition of thrombin, FXIII and calcium. Plates were blocked before incubation with monomeric/dimeric human GPVI constructs in solution. Bound GPVI was detected using HRP-conjugated anti-His (GPVI-monomer) or HRP-conjugated goat anti-human IgG-Fc (GPVI-dimer) antibodies.

Results: Immobilized commercial fibrin D-dimer bound to monomeric GPVI, as previously shown. The same was observed for in-house purified D-dimer, obtained from fibrin digestion with either plasmin or trypsin. Monomeric GPVI also bound to commercial D-fragment, with increased binding to purified D-fragments when fibrinogen digestion was performed in the presence of EDTA. The binding of both monomeric and dimeric GPVI to fibrin(ogen) was not as strong as to its fragments. Nonetheless, results showed a similar pattern of binding to commercial and recombinant fibrinogen, as well as fibrin derived from these.

Conclusions: GPVI binding to distinct fibrin(ogen) fragments is variable. This could contribute to the inconsistencies observed between groups when using ELISA-style binding assays. Other biophysical techniques should be explored to bring clarity as to which binding interactions are physiologically relevant. Similarities in the binding profiles of commercial and recombinant fibrin(ogen) to GPVI are promising, and encourages the use of fibrinogen mutants in subsequent GPVI binding investigations.

P-9

THE PROTHROMBOTIC PHENOTYPE INDUCED BY NILOTINIB AND PONATINIB TYROSINE KINASE INHIBITORS IS REVERSED BY BLOCKADE OF CALCIUM FLUX

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Background: Nilotinib is a second generation tyrosine kinase inhibitor (TKI) used in the treatment of chronic myeloid leukemia (CML) which have previously demonstrated an association with thrombotic state. Ponaitnib is the third generation of TKI used in patient resistance to second generation of TKIs. Both TKIs have been associated with an increase in arterial occlusive events in CML patients.

Aims: To determine the effect of nilotinib and ponatinib on platelet function and *ex vivo*. To assess the acute effect of TKI treatment on primary haemostasis, platelet and endothelial activation and thrombus formation *in vivo* using a C57BL/6 mouse model. To determine strategies to ameliorate the prothrombotic effects of nilotinib and ponatinib *ex vivo* and *in vivo* thrombus formation in mouse models (calcium channel blocker, diltiazem).

Methods: Analysis of ferric chloride-induced vascular injury model of mesenteric arterioles by intravital microscopy (IVM) and carotid arteries by laser Doppler imaging. Determine *in vitro& ex vivo* analysis of murine platelet adhesion and thrombus formation using whole blood from human or C57BL/6 mice under arterial shear flow. Determine the effects of TKIs on the level of inflammatory markers by Enzyme linked immunosorbent assay (ELISA).

Results: *In vitro* treatment of human blood with nilotinib or ponatinib increased PAR-1 induced p-slectin exposure compared with vehicle control. In addition, treatment of wild type C57BL/6 mice with either nilotinib or ponatinib or vehicle control for 4 hours increased the thrombus formation with mesenteric arterioles. For *ex vivo* treatment, the growth of thrombus increased with nilotinib or ponatinib treatment compared to vehicle control. In addition, the amount of plasma level of inflammatory markers such as soluble P-selectin, TNF-alpha and IL-6 was increased compared vehicle control. Pretreatment of C57BL/6 mice with the calcium channel antagonist diltiazem, prior to ponatinib or nilotinib reversed the prothrombotic phenotype and prevented the increase in cytokine levels.

Conclusion: based on these results, ponatinib and nilotinib have a prothrombotic effect that leads to thrombotic complications which can be reversed by pretreatment with diltiazem.

P-10
INVESTIGATION OF MECHANISM OF TISSUE FACTOR-MEDIATED CELL APOPTOSIS

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Background: We have previously shown that tissue factor-positive microvesicles (TF+MV) can induce cellular apoptosis in endothelial cells through a mechanism that involves the activation of protease activated receptor 2 (PAR2).

Aims: In this study the mechanism for the induction of endothelial cell apoptosis by TF+MV was examined.

Methods: TF+MV were isolated from BxPC-3 pancreatic and 786-O kidney cancer cell line and the density of the TF+MV was determined with the Zymuphen assay. The associated TF antigen and activity were measured using ELISA and a chromogenic thrombin-generation assay, respectively. Coronary-artery endothelial cells (HCAEC) were treated with a range of concentrations of TF+MV. In some experiments the PAR2 was blocked on HCAEC using the anti-PAR2 antibody (SAM11; 20 µg/ml) prior to treatment. Other cells were treated with anti-TF antibodies HTF1 (20 µg/ml) to block fVIIa binding, or 10H10 (20 µg/ml) to inhibit TF signalling. TF+MV were also pre-incubated with an inhibitory anti-factor VIIa antibody (10 µg/ml) prior to supplementation to cells. Finally, samples of TF+MV were incubated with Apixaban (1 µg/ml) or Rivaroxaban (0.6 µg/ml) prior to addition to cells. Apoptosis was assessed in HCAEC using a chromogenic TUNEL assay and cell numbers were determined by crystal violet staining.

Results: TF+MV from both cell lines were capable of inducing apoptosis in HCAEC with the maximal rate observed using 0.05 nM of TF+MV from 786-O cells. Pre-incubation of HCAEC with anti-PAR2 antibody (SAM11) significantly reduced the rate of HCAEC apoptosis. Furthermore, the pre-incubation of TF+MV with an inhibitory anti-fVIIa antibody, or with HTF1 anti-TF antibody, but not with 10H10 anti-TF antibody prevented HCAEC apoptosis. Finally, pre-incubation of the microvesicles with Apixaban but not Rivaroxaban blocked the HCAEC apoptosis.

Conclusions: The induction of cell apoptosis by high levels of TF+MV is mediated through the activation of PAR2 by TF-fVIIa complex, but not by fXa.

P-11

THE CELLULAR LOCATION OF TISSUE FACTOR IS CONSTRAINED BY MAGI-1 AND IS RELEASD BY PAR2 ACTIVATION

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Background: Membrane associated guanylate kinase inverted (MAGI) are a family of scaffolding proteins which are involved in tight-junction assembly and cell-attachment. MAGI proteins modulate the activity and stability of various membrane proteins via the interaction with various PDZ domains. The cytoplasmic domain of tissue factor contains a putative PDZ-binding domain and may therefore be anchored by MAGI proteins.

Aim: In this study the interaction of tissue factor with the three forms of MAGI (1-3) and the regulation of TF release following protease activated receptor 2 (PAR2) activation was examined.

Methods: The cellular localisation of TF with each of the three MAGI proteins was examined using the proximity ligation assay (PLA) in MDA-MB-231 breast cancer cell line. Sets of cells were also incubated with PAR2-activating peptide (SLIGKV; 20 µM) for up to 30 min before examination. Additionally, the interaction of TF and MAGI1 was examined by immunoprecipitation and western blot, before and after PAR2 activation.

Results: Analysis of cells using PLA indicated a high level of association between TF and MAGI1 with lower levels detectable with MAGI2 and MAGI3. The interaction between TF and MAGI1 was confirmed by co-immunoprecipitation of TF using anti-MAGI1, as well as co-immunoprecipitation of MAGI1 with anti-TF antibody. Furthermore, the activation of PAR2 within cells resulted in significant dissociation of the TF-MAGI1 complex as measured by PLA at 20 min post-activation. This was further confirmed by the reduction in the amount of co-immunoprecipitated proteins following 20 min incubation of cells with PAR2-AP.

Conclusions: Our study is the first to show an interaction between TF and MAGI1 which dissociate upon PAR2 activation. In addition, the localisation of TF by MAGI1 constitutes an unprecedented mechanism in the regulation of TF function.

P-12

LONG TERM EXPOSURE TO TISSUE FACTOR INDUCE CELL PROLIFERATION BY LOWERING PTEN PROTEIN LEVELS

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Background: Exposure to tissue factor (TF) has been shown to promote cell proliferation at low levels and apoptosis at higher concentrations. PTEN is a key regulator of the PI3K-Akt pathway which determines the susceptibility of cells to apoptosis and therefore can influence cell proliferation. Previously we showed that short-term exposure of cells to TF can induce rapid increases in PTEN activity. However, it is known that PTEN is also destabilised following activation.

Aim: To examine the influence of prolonged exposure of cells to TF, on the levels of PTEN antigen, Akt activity and on cell proliferation.

Methods: Three cell lines expressing wild-type PTEN; MDA-MB-231 (breast cancer), LoVo and Caco-2 (colorectal cancer) were treated with low levels of rec-TF (Innovin; 0.5 U/ml) over a period of 5 days. The levels of PTEN antigen and Akt activity were monitored using the PTEN-ELISA (abcam) and Akt-kinase activity (Enzo) kits, respectively. In addition, the cell numbers were determined using the crystal violet staining procedure and compared.

Results: Prolonged incubation of cells with rec-TF induced significant reduction in the level of PTEN antigen in all treated cell lines compared to respective untreated controls. This alteration in PTEN was proportionally replicated in the higher Akt kinase activities, particularly in LoVo and MDA-MB-231 cells. These patterns were also reflected in the rates of cellular proliferation in all cell lines.

Conclusions: The exposure of cells to TF induces the activation of PTEN. However, the activation of PTEN also leads to its destabilisation and degradation. Therefore, prolonged exposure of cells to low levels of TF results in a cumulative reduction in the levels of cellular PTEN through increased degradation. This results in higher Akt activity making the cells less susceptible to apoptosis and resistant to therapy, with enhanced proliferative properties.

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CLINICAL MANAGEMENT OF AUTOIMMUNE ACQUIRED FACTOR XIII DEFICIENCY DURING PREGNANCY

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Background Women with a factor XIII deficiency can experience meno-menorrhagia and repeated miscarriages. During pregnancy, regardless of etiology, factor XIII deficiency can cause uterine bleeding as well as poor formation of cytotrophoblastic shell with increased risk of placental detachment.

Aims To present the case of acquired factor XIII deficiency during pregnancy and our approach in this case.

Methods The patient has undergone detailed anamnesis and objective exam and paraclinical investigations, including abdominal ecography, hemoleucogram, coagulation profile and the determination of factor XIII levels and the positivity of antinuclear antibodies.

Results Our patient is a 33-year old woman, pregnant in week 13, presented at our clinic for spontaneous vaginal spotting. The abdominal ultrasound showed normal evolution of fetus, but also a posterior uterine wall hematoma of 3 x 3 cm. The patient had no history of bleeding disorders. She had no previous miscarriages. She wasn't under any medication. There is no familial history of bleeding disorders or miscarriages. The patient was in good general status and the clinical examination revealed no problems. The hematological work-up showed a correct platelet count. The standard laboratory tests such as PT, aPTT, fibrinogen level and bleeding time were all normal. We performed factor XIII evaluation and revealed a low level of 59% (normal range 80-150%). Together with the gynecologist, we started a treatment with progesterone and advised bed rest for our patient. We started a search for autoimmune diseases with the results showing positive antinuclear antibodies. At week 16, there is no additional bleeding and the fetus is growing normally.

Summary/Conclusions The optimal clinical management of pregnancy in women with factor XIII deficiency is dependent on a multidisciplinary team formulating a prospective individualized management plan.

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ORAL SURGERY FOR COMBINED HAEMOPHILIA A AND B. THE DOS AND DON'TS PRESENTED IN A CLINICAL SCENARIO

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Background Patients diagnosed with an inherited blood clothing deficiency are at high risk of developing complications, following oral surgery. A very common genetic bleeding disorder is haemophilia. The severity of clinical manifestations is directly correlated with the clotting factor deficiency and it is depicted by various haemorrhagic episode, at different anatomical regions.

Aims To present our approach in a case of combined haemophilia A and B requiring oral surgery.

Methods The patient has undergone anamnesis and objective exam, followed by surgical intervention and management of complications as will be presented.

Results A 30-year-old Caucasian man was referred for dental extractions. The medical history of the patient presented severe haemophilia A. Earlier on the day of surgery, the patient was transferred to the haematology department where he received 1000 U.I. of VIII factor concentrate. Teeth extraction was performed. Four hours following dental surgery, the patient presented moderate bleeding in the pterygomandibular space. He received another 2x1000 U.I. factor VIII concentrate and compressive gauze. Twenty-four hours later, the patient presented with widely spread right laterocervical haematoma. Due to the risk of asphyxia, an emergency temporary tracheostomy was performed. On day 4 after the surgical intervention, the patient experienced an episode of tonico-clonic seizures. After clinical management, the patient's laboratory tests improved partially. Laboratory tests were repeated and showed that levels of both clotting factors VIII and IX were less than 1%. Systemic treatment was changed as according to these new findings. The patient received 2.000 U.I. factor VIII, 2500 U.I. factor IX and 2 units of fresh frozen plasma/ day. The patient recovered gradually within 5 days.

Summary/Conclusions It is crucial to identify patients with bleeding disorders before performing dental extractions. Managing these patients requires a close cooperation between haematologists and oral surgeons.

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DIETARY ZINC INATKE MODULATES PLATELET AGGREGATION, CLOT FORMATION AND FIBRINOLYSIS

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Background

Zinc acts as an important cofactor in several haemostatic reactions. Platelets are the major reservoir of zinc in blood. It is housed in the α-granules and is released following platelet stimulation thus augmenting local concentrations of this biomolecule. Several studies have shown a direct impact of *ex vivo* manipulation of zinc in haemostasis. In this study, we directly address the impact of limited dietary zinc on platelet aggregation, clot formation and fibrinolysis.

Aims

To investigate the role of dietary zinc intake in platelet aggregation, clot formation and lysis during haemostasis.

Methods

Healthy volunteers were recruited and dietary intake of zinc was restricted for 2 weeks (1 mg/day), following a repletion period for 2 weeks (4.5-18 mg/day). Blood was collected from three phases and PRP was isolated to assess haemostatic parameters.

Results

Maximal platelet aggregation to collagen was significantly decreased during the depletion phase (54.86 ± 8.1 %) compared to habitual diet phase (108.5 ± 7.5 %). Visualisation of real-time clot formation and lysis were observed using a Hemacore Thrombodynamics Analyzer. Rate of clot growth was significantly slower in depletion phase (21.58 ± 3.7 um/min) compared to the rate in habitual phase (43.60 ± 3.4 um/min). Clots formed from PRP did not retract as efficiently following a zinc depletion diet (clot weight 511.7 ± 66.8 mg) compared to habitual zinc intake (365 ± 15.8 mg). Lysis of PRP clots by tissue plasminogen activator (tPA) from the depletion phase (44.17 ± 3.5 min) was significantly delayed compared to the habitual phase (40.33 ± 2.3 min).

Summary/Conclusions

These data indicate that subtle changes in dietary zinc impact its bioavailability and uptake by platelets. Attenuated levels of zinc in platelets has a direct impact on platelet aggregation, clot formation and fibrinolysis thus highlighting the crucial role of zinc in haemostasis.

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LARGE-SCALE SCREENING FOR MEMBRANE PROTEIN INTERACTIONS INVOLVED IN PLATELET-MONOCYTE INTERACTIONS

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Background:

Beyond the classical roles in haemostasis and thrombosis, platelets are important in the initiation and development of various thromboinflammatory diseases. In atherosclerosis and deep vein thrombosis, for example, platelets bridge monocytes with endothelium and form heterotypic aggregates with monocytes in the circulation. This can alter monocyte phenotype by inducing their activation, stimulating adhesion and migration. These interactions involve cell surface receptor-ligand pairs on both cells. This list is likely incomplete as new interactions of importance to platelet biology are continuing to be discovered as illustrated by our discovery of PEAR-1 binding to FcεR1α.

Results:

We have developed a highly sensitive avidity-based assay to identify novel extracellular interactions among 126 recombinantly-expressed platelet cell surface and secreted proteins involved in platelet aggregation. In this study, we will use this method to identify novel platelet-monocyte interactions. We aim to identify ligands for orphan receptors and novel partners of well-known proteins. Identified interactions will be studied in preliminary functional assays to demonstrate relevance to the inflammatory processes supporting atherogenesis.

Conclusions:

Platelet-monocyte interactions are essential for the development of thromboinflammatory disease. Up until relatively recently, technologies only allow us to limit our studies on each individual protein interaction at a single time. These studies propose for the first time to study the cell surface platelet-monocyte interactions in a systematic large-scale approach using a reliable screening method we have developed. If successful, this will likely to identify previously unknown ligands for important receptors that will be investigated in details and also provide a list of novel interactions for the field. This should stimulate studies on developing alternative therapeutic strategies to treat vascular inflammatory disorders such as atherosclerosis, DVT and sepsis and other clinically important inflammatory conditions.

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ONE-STEP PURIFICATION OF FIBRIN(OGEN) FRAGMENTS AND THEIR BINDINGS TO GPVI IN SOLUTION

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Background: GPVI is an immunoglobulin (Ig)-like receptor for collagen expressed on the surface of platelets. Recent papers have shown that GPVI also binds to fibrin in the D-region, playing an important role in thrombus growth and stability. Nevertheless, there are discrepancies in regard to the binding data published in different laboratories mainly based on solid-state binding assays.

Aims: To produce fibrin(ogen) fragments in large scale for structural studies, investigate the binding of fibrin(ogen) fragments to monomeric GPVI in solution, and search for conditions to stabilise GPVI-fibrin fragments complexes in suspension.

Methods: Fibrin(ogen) was subjected to tryptic digestion, followed by purification using size exclusion chromatography from which the D-fragment and D-dimer were purified. The molecular weight and purity of the purified fragments were confirmed by SDS-PAGE and mass-spectrometry. The binding affinity was measured using microscale thermophoresis by titration of fibrin(ogen) fragments added to FITC labelled GPVI monomer.

Results: Fibrin(ogen) fragments were produced in large scale using a simple one-step purification method. The molecular weight of the fragments is in good agreement with those reported in the literature. Binding was observed for D-fragment and D-dimer to GPVI monomer in solution, with an EC50 of 119±24 nM for D-dimer.

Conclusions: Despite variations in binding between GPVI and fibrin(ogen) fragments reported recently, measured mainly by immobilized or solid state binding assays, this work further detected their binding in solution. Based on the binding affinity determined, future work should focus on the assembly of the GPVI/D-dimer complexes in suspension to reveal the molecular details of the interaction, which can be used in the future to design potential anti thrombotic drugs.

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