BSHT Annual Scientific Meeting 2017

Thursday 12 – Friday 13 October 2017
The Slate, University of Warwick, Coventry, UK

PROGRAMME + ABSTRACT BOOK

www.bsht.org.uk

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

PLATINUM:

GOLD:

SILVER:

BRONZE:
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12 October 2017

Dear Colleague

Welcome to the BSHT Annual Scientific Meeting 2017

It is a real pleasure for me to welcome you all to the 2017 Annual Scientific Meeting of the BSHT. This year, we are meeting in the well-equipped facilities of Warwick University Conference Centre. I hope that the intimate feel of the venue, together with the inclusive accommodation will increase interactions and future collaborations within the UK Haemostasis and Thrombosis Community, and beyond! After several years of joint meetings with other related societies and groupings, this year we again have a stand-alone meeting of the BSHT. Another novelty for the 2017 BSHT meeting is the involvement of a new conference organiser, Natasha Dougall from Wheldon Events, since the retirement of Jean Machin as the main BSHT conference organiser last year.

We have two days of new and emerging science from the Thrombosis and Haemostasis community, with an eclectic programme of invited speakers from the both overseas and the UK, and with many, what promise to be excellent abstract presentations. I hope that this meeting will be remembered in particular for the novel findings that will be presented for the first time here in Warwick. With a changing landscape of an increasing number of meetings available to us and with the ISTH changing to a yearly meeting from 2019, we as BSHT will have to re-evaluate how we run our meetings and coordinate these with the meetings of other societies and groups. However, I am convinced that there will always be a solid need and place for dedicated national meetings, which are relatively easy to attend, at a lower cost, and which provide more opportunities for our up-and-coming younger researchers to present their data and receive feedback on their work. 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 lots of inspiration in the new data and findings presented at this meeting!

On behalf of the BSHT Committee,

Professor Robert Ariëns, PhD
President of the BSHT
USEFUL INFORMATION

BADGES:

Delegates: CLEAR  
Speakers: RED  
Abstract presenters: YELLOW  
Sponsors: BLUE  
Committee: GREEN

CERTIFICATES OF ATTENDANCE:

Attendance certificates are available from the registration desk. 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 Ltd  
- Bayer plc  
- BMS Pfizer Alliance  
- CSL Behring  
- Hart Biologicals  
- INRstar - LumiraDx Care Solutions UK Ltd  
- LFB Biopharmaceuticals Limited  
- Quadratex Diagnostics Ltd  
- Stago UK Ltd  
- Sysmex UK Ltd  
- Werfen Ltd

MEETING DETAILS + ACCOMMODATION:

Meeting address: The Slate, University of Warwick (Lakeside Village), Coventry CV4 7AL  
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)  
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 18:00 – 19:30 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.
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<td>10:00 – 10:10</td>
<td>Welcome + Introduction</td>
<td>Robert ARIËNS</td>
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<td>Co-chairs: Yotis SENIS &amp; Catherine BAGOT</td>
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<td>Alternative splicing in coagulation</td>
<td>Elisabetta CASTOLDI (Netherlands)</td>
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<td>13:30 – 14:05</td>
<td>Flow analysis of hemostasis and thrombosis</td>
<td>Scott DIAMOND (USA)</td>
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<td>Scientist in Training 1: Fibrin produces a film that covers, protects and seals the clot. Fraser MACRAE (Leeds)</td>
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<td>Scientist in Training 4: Plasminogen activator inhibitor 1 is retained on the surface of activated platelets and enhances thrombus stabilisation. Gael MORROW (Aberdeen)</td>
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<td>Diabetes and thrombosis</td>
<td>Ramzi AJJAN (UK)</td>
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<td>The fibrin αC domain not only plays a role in lateral aggregation but also in longitudinal fibre growth. Helen MCPHERSON (Leeds)</td>
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"Fibrin produces a film that covers, protects and seals the clot" - Fraser MACRAE (Leeds)

"VWF-Mediated platelet ‘Priming’ potentiates novel leukocyte interactions under flow" - Adela CONSTANTINESCU-BERCU (London)

"Characterisation of a novel truncated VWF variant for the treatment of Von Willebrand’s Disease" - Robyn BELL (London)

"Plasminogen activator inhibitor 1 is retained on the surface of activated platelets and enhances thrombus stabilisation" - Gael MORROW (Aberdeen)
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| 17:20 – 17:25 | Efficacy of cryoprecipitate versus fibrinogen concentrate in clot formation and stability: implications for treatment of trauma induced coagulopathy  
Molly Carlier (Aberdeen) |
| 17:25 – 17:30 | Generating an antibody fragment to inhibit VWF function  
Mynhi DANG (London) |
| 17:30 – 17:35 | Evaluating the murine TFPI-protein S anticoagulant pathway  
Laura MEREWEATHER (London) |
| 17:35       | Meeting close day one                                                                              |
| 18:00 – 19:30 | Poster session + Drinks Reception  
The Slate |
| 19:30       | Dinner  
Radcliffe House Dining Room (purchased ticket holders only) |
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<td>Co-chairs: Mike LAFFAN &amp; Chris GARDINER</td>
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<td>08:30 – 09:05</td>
<td>The future of haemophilia care</td>
<td>Mike MAKRIS (UK)</td>
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<td>Crystal structure of Fab-bound ADAMTS13</td>
<td>Hyo Jung KIM (Nottingham)</td>
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<td>Why do antifibrinolytic lysine analogues cause bleeding?</td>
<td>Matthew LOCKE (Potters Bar)</td>
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<td>Co-chairs: Nicola MUTC &amp; Cedric DUVAL</td>
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<td>Do anticoagulants offer vascular protection?</td>
<td>Hugo TEN CATE (Netherlands)</td>
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<td>VWD to ACS: shifting the focus of VWF research</td>
<td>Dan HAMPSHIRE (Sheffield)</td>
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<td>Fibrin cross-linking and clot structure, role(s) in vascular disease</td>
<td>Cédric DUVAL (Leeds)</td>
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<td>12:00 – 12:20</td>
<td>Translating Whole Genome Sequencing into Bleeding and Platelet Disorders</td>
<td>Claire LENTAIGNE (London)</td>
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<td>Co-Chairs: Josephine AHNSTRÖM &amp; Clare WILSON</td>
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<td>Coagulation signalling and innate immunology</td>
<td>Wolfram RUF (Germany/USA)</td>
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Tissue factor de-palmitoylation, phosphorylation, de-encryption and release in microvesicles are related events  
*Camille ETTELAIE* (Leicester) |
| 14:15 – 14:20 | Discussion                                               |
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Trem-like transcript-1: a highly sensitive marker of early platelet activation  
*Christopher W SMITH* (Birmingham) |
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TTP autoantibody characterisation: recognition of ADAMTS13 conformations and associated IgG subclass  
*Mary I UNDERWOOD* (London) |
| 14:45 – 14:50 | Discussion                                               |
| 14:50 – 15:20 | Refreshments, networking + exhibition                    |
| 15:20 – 16:30 | SESSION SEVEN: Plenary 7 and Oral Communications 5  
Co-Chairs: *Robert ARIËNS* |
| 15:20 – 15:55 | Oral communication 16  
Extended D-E interactions near the classical knob-hole binding site play an important role in fibrin polymerisation and clot stability  
*Nathan ASQUITH* (Leeds) |
| 15:55 – 16:00 | Discussion                                               |
| 16:00 – 16:10 | Oral communication 17  
VWF and ADAMTS13 levels in early onset preeclampsia: prothrombotic mechanisms in mothers with elevated risk of venous thromboembolism  
*Ryan GRIFFIN* (London) |
| 16:10 – 16:15 | Discussion                                               |
| 16:15 – 16:25 | Oral communication 16  
Extended D-E interactions near the classical knob-hole binding site play an important role in fibrin polymerisation and clot stability  
*Nathan ASQUITH* (Leeds) |
| 16:25 – 16:30 | Discussion                                               |
| 16:30         | Meeting close                                            |

Today is World Thrombosis Day

**World Thrombosis Day**

13 October

[www.worldthrombosisday.org](http://www.worldthrombosisday.org)
Dr Ramzi AJJAN
FRCP, MMed.Sci, PhD
Associate Professor
University of Leeds, Division of Cardiovascular and Diabetes Research, Leeds, UK

**Diabetes and thrombosis**

Diabetes is associated with increased risk of cardiovascular events and one mechanism is related to an enhanced thrombotic milieu in this condition. Formation of the blood clot represents the final step in the atherothrombotic process leading to vascular occlusion and end organ damage, including myocardial infarction and stroke. The blood clot forms secondary to complex interactions between platelets and coagulation proteins culminating in the formation of the fibrin network, which forms the skeleton of the blood clot.

Diabetes is characterised by enhanced platelet activation as well as increased levels and activity of coagulation factors. Despite the increased thrombosis potential in diabetes, antithrombotic therapy remains largely similar in those with and without deranged glucose metabolism.

The current presentation will be divided into three main parts. In the first, current anti-thrombotic strategies for primary and secondary vascular protection will be briefly reviewed, including alternative anti-platelet therapies. In the second part, the talk will concentrate on uncovering non-platelet related mechanistic pathways that contribute to increased atherothrombosis risk in diabetes. This will include mechanisms for altered clot structure and impaired fibrin clot lysis that are diabetes-specific. The final part of the presentation will focus on the role of the fibrin network as a therapeutic target to reduce thrombosis risk in diabetes and will explore a novel methodology for modulation of fibrinolysis that may offer future diabetes-specific antithrombotic therapies.

Dr Elisabetta CASTOLDI
PhD
Associate Professor of Biochemistry
Maastricht University
Department of Biochemistry
Maastricht, The Netherlands

**Alternative splicing in coagulation**

The coagulation system is responsible for the formation of fibrin clots via the integrated and tightly regulated activity of numerous plasma proteins. Disruption of the delicate balance between pro- and anticoagulant factors is a major determinant of thrombotic and bleeding disorders. However, while the effects of quantitative variations of coagulation factors and inhibitors have been well documented, the role of qualitative variation has remained largely unexplored. A major source of qualitative variation in the human (coagulation) proteome is the process of alternative splicing, which generates structurally and often functionally different isoforms of the same protein. The expression of these splicing isoforms is influenced by genetic and acquired conditions, leading to considerable inter-individual variation. Recent studies have shown that some low-abundance splicing isoforms of coagulation factors have unique functional properties which make them important regulators of the haemostatic balance. For example, the fibrinogen γ' chain (4-8% of all fibrinogen γ chains) acts as a thrombin inhibitor and its relative expression is inversely correlated with the risk of venous thrombosis. Similarly, the recently discovered FV-short isoform (~5% of all plasma FV) binds the anticoagulant protein tissue factor pathway inhibitor (TFPI) with high affinity and causes bleeding when up-regulated. Since alternative splicing can be modulated using antisense molecules, these natural splicing isoforms represent potential therapeutic targets for the treatment of coagulation disorders.
SPEAKER SUMMARIES

Professor Scott L DIAMOND
PhD
University of Pennsylvania, Institute for Medicine and Engineering
1024 Vagelos Research Laboratories, Philadelphia, USA

Flow analysis of hemostasis and thrombosis

Hundreds of spatiotemporal reactions proceed within activating platelets and the polymerizing plasma as blood clots under flow. Microfluidic devices are ideal for recreating transport physics and hemodynamic forces. We have validated several devices to study hemophilia, combinatorial platelet receptor function, drug responses, platelet quorum sensing, or von Willebrand Factor (vWF) assembly in extreme stenotic flows. By careful control of anticoagulation, coagulation can be studied under diverse conditions of contact pathway activation and/or extrinsic pathway activation. We have measured the localization and regulation of thrombin in the core of developing clots. We have detected conditions where platelet polyphosphate accounts for about half of the fibrin formed during clotting. We have demonstrated that fibrin binds >80% of the thrombin produced in a clot. We have detected very unique conditions of shear-induced Nets (SINs) within sterile thrombotic occlusions. These microfluidic tools are now being applied to trauma patients, neonates undergoing congenital heart surgery, hemophiliacs, and patients with cardiovascular disease.

Professor Mike MAKRIS
MD
Professor of Haemostasis and Thrombosis
University of Sheffield
Department of Haematology, Royal Hallamshire Hospital, Sheffield, UK

The future of haemophilia care

There have been major advances in haemophilia care over the last 30 years that include home treatment with concentrates, introduction of recombinant concentrates and wide use of prophylaxis. A person with severe haemophilia on prophylaxis and without an inhibitor today can expect a normal life expectancy. In the last year we have seen the introduction of concentrates with extended half lives to the UK market which allow a reduced number of infusions. The next 5 years, however, are likely to see even more impressive developments including treatments not based on factor infusion such as anti-TFPI antibodies, si-RNA against antithrombin and the bispecific antibody against factors IX and X. Gene therapy which has been on the horizon for at least 20 years is finally yielding promising results and is likely to join the mainstream of treatments before too long.
**Professor Wolfram RUF**  
MD  
Scientific Director CTH  
Johannes Gutenberg University Medical Center  
Center for Thrombosis and Hemostasis, Mainz, GERMANY

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**Coagulation signalling and innate immunology**

Initiation of the TF coagulation pathway is directly coupled to innate immune sensing and inflammatory pathways. In addition, the hemostatic system and complement cascade are reciprocally amplified not only in sepsis, but also in venous thrombosis. The presentation will cover recent data on how TF’s procoagulant and hemostatic functions are controlled at the cellular level and contribute to acute and chronic inflammatory processes.

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**Dr Rachel E SIMMONDS**  
PhD  
Senior Lecturer in Immunopathogenesis  
University of Surrey, Microbial Science  
Guildford, UK

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**Disordered haemostasis - a second driver of infectious disease susceptibility?**

The neglected tropical disease Buruli ulcer is caused by subcutaneous infection with *Mycobacterium ulcerans*, and is characterised by chronic necrotizing skin lesions without immune activation. These pathogenic features are caused by a lipid-like exotoxin made by the bacteria, mycolactone, which diffuses through tissue in advance of the infection and can itself cause skin ulceration.

We have shown that mycolactone is a broad-spectrum inhibitor of Sec61-dependent protein translocation, the main entry mechanism for proteins into the ER. Mycolactone blocks translocation of most secretory and type I/II transmembrane proteins. This explains the lack of an immune response to the bacteria, since this would usually be orchestrated by the induction of secreted/membrane bound proteins.

The coagulation cascade is similarly mediated by Sec61-dependent proteins. In particular, endothelial cells play a vital regulatory role due to anticoagulant proteins such as thrombomodulin. We have found that thrombomodulin is rapidly depleted from cells exposed to as little as 3ng/ml mycolactone in a Sec61-dependent manner. Similar depletion was found in BU patient lesions, associated with a local coagulation defect commonly characterised by fibrin deposition in the absence of platelet deposition.

Disease susceptibility is usually determined by the host’s immune response, and indeed for other mycobacterial infections (such as TB and leprosy) this is the case. However, the unique aetiology of Buruli ulcer, due to the action of mycolactone, now provides a model by which to study the role of variation in haemostasis as a driver of disease.
Do anticoagulants offer vascular protection?

Substantial experimental and limited clinical studies suggest that hypercoagulability accelerates atherosclerosis, while impaired clotting activity may diminish atherogenesis and even yield regression in vascular lesions. This evidence may provide a basis for a re-exploration of the rational of instituting anticoagulant therapy in patients with chronic arterial vascular disease, eg peripheral arterial vascular disease. While platelet inhibitory agents provide limited benefit, the combination with anticoagulants, or anticoagulants alone gets new interest as a possibly superior way of preventing atherothrombotic complications (Compass study). While thrombosis prevention is a predictable consequence of anticoagulation, I will discuss potential interactions of specific anticoagulants with mechanisms involved in atherogenesis and plaque stability. Such vascular protective effects may become an interesting target for clinical studies in patients with arterial vascular disease and at high risk of atherothrombotic mortality.
SPEAKER BIOGRAPHIES

**Dr Ramzi AJJAN**  
FRCP, MMed.Sci, PhD  
Associate Professor  
University of Leeds, Division of Cardiovascular and Diabetes Research, Leeds, UK

RA Ajjan (MD, FRCP, MMed.Sci, PhD) is an Associate Professor/Consultant in Diabetes and Endocrinology at Leeds University and Leeds Teaching Hospitals Trust. He runs a programme of basic, translational and clinical research, aiming to improve outcome in diabetes patients. His studies concentrate on reducing cardiovascular complications in this population and he has a particular interest in the relationship between glycaemia, thrombosis and vascular health. He is local lead for clinical Diabetes/Endocrine research and regional research network lead for endocrine/metabolic conditions.

**Dr Elisabetta CASTOLDI**  
PhD  
Associate Professor of Biochemistry  
Maastricht University  
Department of Biochemistry  
Maastricht, THE NETHERLANDS

Elisabetta Castoldi graduated in Biology from Ferrara University (Italy). After obtaining her PhD on the molecular genetics of coagulation factor V under the supervision of Prof. Francesco Bernardi, she moved to Maastricht (The Netherlands) for biochemical training in Prof. Jan Rosing's laboratory. A VIDI grant from the Dutch Organisation for Scientific Research allowed her to start her own research line, which focusses on the molecular genetics and functional characterisation of inherited coagulation defects predisposing to bleeding or thrombosis.

**Professor Scott L DIAMOND**  
PhD  
University of Pennsylvania, Institute for Medicine and Engineering  
1024 Vagelos Research Laboratories, Philadelphia, USA

Scott L. Diamond is the Arthur E. Humphrey Professor of Chemical and Biomolecular Engineering at the University of Pennsylvania. Dr. Diamond uses microfluidics, transport phenomenon, systems modelling, and biorheology to study blood coagulation under hemodynamic conditions. He has produced over 200 publications and patents. He has trained over 40 PhD and postdoctoral fellows. He is the recipient of the NSF National Young Investigator Award, the NIH FIRST Award, the AHA Established Investigator Award, and the AIChE Colburn Award.

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Rachel studied Molecular Biology at the University of Manchester before undertaking a PhD in Molecular Haematology at Imperial College under the supervision of David Lane. Her PhD and early post-doctoral there work focused on protein S deficiency and the EPCR. She later joined the Macrophage Biology group at the Kennedy Institute of Rheumatology, studying the anti-inflammatory effects of the Buruli ulcer exotoxin mycolactone. She was appointed Lecturer at the University of Surrey in 2011 and was awarded a Wellcome Trust Investigator Award in 2016.

**Professor Hugo Ten Cate**

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Hugo ten Cate is an internist-vascular medicine specialist with a longstanding interest in haemostasis and thrombosis. He directs the Maastricht Thrombosis Expert Center which combines translational; research and care for patients with complex thrombotic disorders or thrombophilia. He is chair of the board of the Dutch Federation of Anticoagulation clinics and Adjunct Professor at the Center for Thrombosis and Hemostasis, Mainz Germany. One of his main lines of research involves the role of coagulation proteins in atherothrombotic disease as well as other cardiovascular diseases, associated with hypercoagulability, sich as atrial fibrillation and stroke.
FIBRIN PRODUCES A FILM THAT COVERS, PROTECTS AND SEALS THE CLOT

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**Background:** Clot formation culminates in the conversion of fibrinogen to fibrin. Fibrin is known to polymerise into fibres that generate a network, constituting the clot backbone. However, the structures that determine the external boundary of a clot are unknown.

**Aims:** To investigate fibrin clot structure at the blood-air interface.

**Methods:** Electron, confocal and atomic force microscopy, immunohistochemistry and Langmuir–Blodgett trough were used to investigate the blood-air interface of blood clots in vitro, and in vivo in a murine model of dermal puncture wound injury. Recombinant fibrinogens were expressed in CHO cells. Bacteria migration through blood clots was investigated using Boyden chambers.

**Results:** Fibrin transfers to the blood-air interface through Langmuir film formation, producing a natural seal confining the clot. The film formed in plasma, whole blood, and in vivo. The film formed with purified fibrinogen +/- FXIII, showing that only fibrin is required. SDS-PAGE analysis of the sheet confirmed that it is composed of fibrin. Fibrinogen-deficient mice produced platelet-based clots not covered by film. Fibrin film formation occurred different thrombin concentrations (0.1-10 U/ml) and with different fibrinogen mutants ($\alpha_{220}$, $\alpha_{390}$, $\gamma'$, $\gamma_{3X}$). The film formed at different temperatures, but was thickest at skin temperature (31.5°C). Formation of the film was prevented by removing the air-blood interface with surfactants or oil. The fibrin film formed at the same rate as the fibre network, was connected to the clot network through tethering fibres, and lysed by plasmin at a similar rate to fibrin fibers. The film showed very few pores of <22nm diameter and slowed the movement of bacteria through the clot.

**Summary/conclusions:** These data show a novel unique mechanism by which fibrin produces a film at the blood-air interface that covers the entire clot. The fibrin film is a critical protecting mechanism for the external surface of blood clots.
**Scientist in Training 2:**

**VWF-MEDIATED PLATELET ‘PRIMING’ POTENTIATES NOVEL LEUKOCYTE INTERACTIONS UNDER FLOW**

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**Background:** Platelet-leukocyte interactions play important roles in both physiological and pathophysiological settings, particularly atherosclerosis, infection and DVT. Previously characterized interactions between these cells require the platelet to be fully activated. However, a recent study showed that platelets bound to von Willebrand Factor (VWF) (and not exposed to other platelet agonists) are able to capture leukocytes under flow. We hypothesized that the VWF A1-GpIba interaction ‘primes’ platelets, enabling them to form novel interactions with leukocytes under flow.

**Aims:** Characterise the A1-GpIba interaction and subsequent platelet-leukocyte cross-talk under flow.

**Methods:** Histagged VWF A1 and a mutant variant of A1 (Y1271C/C1272R, A1*) were expressed in insect cells and purified. A1/A1* or full-length VWF (FL-VWF) were coated onto microfluidic channels. Fluorescently-labelled whole blood or plasma-free blood were perfused through these channels at defined shear rates (1000s⁻¹ and 50s⁻¹). Platelet and leukocyte capture were recorded in real-time.

**Results:** The A1-GpIba interaction ‘primed’ the platelets by inducing intracellular Ca²⁺ signaling, activation of αIIbβ₃ and subsequent aggregate formation. Aggregation was abolished when plasma-free blood was used (i.e. in the absence of fibrinogen) or when αIIbβ₃ was blocked. ‘Primed’ platelets captured T cells and neutrophils under low shear. The number of platelet-leukocyte interactions was 3-5 fold increased in plasma-free blood compared to whole blood. Titrating plasma back into plasma-free blood resulted in a concentration-dependent decrease in platelet-leukocyte interactions, as did the addition of 50% fibrinogen. This indicates a competition between fibrinogen and leukocytes in binding the ‘primed’ platelets and implies that leukocytes can directly bind to activated αIIbβ₃. Platelet-leukocyte interactions were reduced in the presence of αIIbβ₃ blockers, but not influenced by P-selectin blockade. Leukocytes directly bound to activated αIIbβ₃ coated onto microchannels. This interaction induced phenotypic changes within neutrophils, culminating with the formation of neutrophil extracellular traps (NETs).

**Conclusion:** A1-GpIba ‘primes’ platelets leading to the activation of αIIbβ₃. For the first time, we show that this can directly interact with T cells and neutrophils and induce NETosis. Work is underway to determine the leukocyte receptor involved and the (patho)physiological implications of these findings.
ABSTRACTS ORAL – Scientists in Training

Scientist in Training 3:
Characterisation of a novel truncated VWF variant for the treatment of Von Willebrand’s Disease

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Background: Although the use of gene therapy for the treatment of Von Willebrand’s disease (VWD) is controversial, it is nonetheless an attractive option for the most severe forms of the disease. A gene therapy approach is however hampered by the size of the VWF cDNA (8.4kb) which makes it beyond the maximal packaging capacity of most viral vectors. To address this, we deleted various domains of Von Willebrand Factor (VWF) in an attempt to create a truncated protein that was fully functional but with a reduced cDNA size.

Aims: To generate a truncated and functional VWF protein

Methods: Truncated VWF variants were generated by inverse PCR and subsequently expressed in HEK293T cells. Recombinant proteins were characterised for expression, multimeric pattern, intracellular storage and static collagen and GPIbα binding and the ability to mediate platelet capture under shear stress. Clearance studies were performed in VWF deficient mice.

Results: While deletion of various combinations of the VWF C-terminal domains abolished or significantly impaired secretion, a VWF variant termed VWFTRUNC which lacked an ~2.7kb deletion, was expressed at ~2-3 fold high levels than wild type (wt) VWF. Immunofluorescent staining demonstrated that VWFTRUNC failed to form pseudo Webiel-Palade bodies but demonstrated a normal multimeric pattern. Under static conditions VWFTRUNC had normal collagen and GPIbα binding and susceptibility to ADAMTS13 proteolysis was not significantly altered. Interestingly, despite the large deletion, under shear stress VWFTRUNC was as effective as wtVWF at mediating platelet capture to collagen. Moreover, VWFTRUNC demonstrated comparable clearance to wtVWF.

Conclusion: VWFTRUNC was functionally normal and with its reduced cDNA size is more appropriate for cloning into common viral vectors. Furthermore, the enhanced expression levels and normal rates of clearance suggest that this VWF variant could be used as a standalone recombinant VWF product for the treatment of VWD.
PLASMINOGEN ACTIVATOR INHIBITOR 1 IS RETAINED ON THE SURFACE OF ACTIVATED PLATELETS AND ENHANCES THROMBUS STABILISATION

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Background: Plasminogen activator inhibitor 1 (PAI-1) is the principal physiological inhibitor of tissue-type plasminogen activator and urokinase. Platelet α-granules harbour the primary pool of PAI-1, which is secreted upon activation.

Aim: To define the contribution of the platelet membrane in retaining functional platelet-derived PAI-1.

Methods: Platelets were activated with collagen and thrombin and analysed for PAI-1 by ELISA and activity assays. In some cases, tirofiban was included to inhibit αIIbβ3 or fibrin polymerisation blocked with Gly-Pro-Arg-Pro (GPRP). Chandler model thrombus lysis and Hemacore analysis were performed with platelet-rich plasma (PRP) ± a neutralising antibody to PAI-1 and lysed with tPA. PRP clot lysis was monitored using confocal microscopy with inclusion of AF546 fibrinogen and AnnexinV AF647 to visualise platelets.

Results: The majority of PAI-1 antigen was detected in the platelet releasate, however ~20% remained associated with the cellular fraction. PAI-1 antigen was reduced in the cellular fraction on inclusion of tirofiban (73 ± 7%) or GPRP (64 ± 3%), and activity was completely abrogated. Real-time lysis of platelet-rich clots was significantly faster upon inhibition of PAI-1 (20 ± 13 min vs. >60 min), which was mirrored by a 3-fold increase in the lysis rate of whole blood thrombi. A clear PAI-1 dependant stabilisation of plasma thrombi was evident with both the releasate and cellular fraction, with maximal effect observed with whole activated platelets. Visualisation of platelet-rich plasma clots revealed rapid lysis upon inhibition of PAI-1 (5.7 ± 0.8 min) compared to the control (24 ± 1.5 min). Lysis was significantly slower around platelet aggregates in control clots suggesting platelet-associated PAI-1 attenuates lysis of platelet-associated fibrin.

Conclusion: Active platelet-derived PAI-1 is retained on the surface of stimulated platelets via a αIIbβ3 and fibrin dependent mechanism. Our data reveal a striking dependence for platelet-derived PAI-1 in stabilising platelet-rich thrombi against degradation.
Oral communication 1:

TRIGGERING RECEPTOR EXPRESSED ON MYELOID CELLS LIKE TRANSCRIPT-1 (TLT-1) TRIGGERS A NOVEL PLATELET/VWF INTERACTION

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**Background:** Von Willebrand Factor (VWF) is a large multimeric glycoprotein, which supports adhesive interactions between platelets and sites of vessel injury and plays an important role during haemostasis and thrombogenesis. It is known that under high shear forces VWF is able to induce the formation of stable platelet aggregates by binding to GPIbα and GPIIb-IIIa. Besides these well-defined interactions, our studies revealed that the novel platelet receptor TLT-1 acts as a ligand for VWF and participates in platelet adhesion and thrombus formation. To this end, we performed a binding screen of known platelet membrane proteins and identified Triggering receptor expressed on myeloid cells like transcript-1 (TLT-1) as a novel VWF binding partner. TLT-1 is a transmembrane protein, exclusively expressed in platelets where it is stored in α-granules and is presented on the platelet surface following thrombin induced platelet activation. A soluble form of TLT-1 (sTLT-1) is also released into circulation and sTLT-1 is known to enhance platelet activation. Furthermore, blockade of TLT-1 inhibits thrombin mediated platelet aggregation, suggesting an important role for TLT-1 during haemostasis and thrombosis. In this study we have examined the TLT-1/VWF interaction and its significance for thrombus formation.

**Aim:** To define the functional significance of the interaction of VWF with TLT-1.

**Methods:** Soluble TLT-1 (sTLT-1) and recombinant VWF fragments were expressed in HEK293T cells and purified by Nickel affinity chromatography. Binding assays were performed using plates coated with sTLT-1 and incubation with VWF and its fragments. Flow assays were carried out perfusing whole or plasma free blood over VWF or collagen surfaces in the absence or presence of an anti-TLT-1 antibody and high levels of sTLT-1.

**Results:** Under static conditions VWF binds to immobilised sTLT-1 with high affinity, depending on the presence of calcium ions and the multimeric structure of VWF. Binding was mapped to the A3D4 region of VWF and can be blocked with an antibody directed against TLT-1. Furthermore HEK293 cells stably expressing full length TLT-1 were able to bind multimeric VWF. In flow assays the anti-TLT-1 antibody marginally reduced platelet capture under shear stress, but a significantly reduced thrombus size and volume was observed, suggesting that targeting TLT-1 may be a novel, safe anti-thrombotic strategy. Furthermore spiking whole blood with high levels of sTLT-1 led to an enhanced thrombus formation on Collagen type I compared to VWF surface, indicating Collagen induced platelet activation is necessary to mediate TLT-1-VWF interaction.

**Conclusions:** Disruption of VWF binding to platelet TLT-1 showed a reduction in overall thrombus formation without ablating stable platelet capture, therefore targeting TLT-1 maybe a potentially safe route to preventing excessive thrombus formation.
Oral communication 2:

PARADOXICAL BLEEDING IN MICE LACKING THE KINASE-PHOSPHATASE PAIR CSK-CD148

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Background: Platelets contain high levels of Src family kinases (SFKs) that are essential for transmitting activation signals from a variety of receptors, including the VWF receptor complex GPIb-IX-V, the collagen receptor complex GPVI-FcRγ-chain and the integrin αIIbβ3. However, it remains unclear how SFKs are regulated in platelets.

Aims: To determine the role of the kinase-phosphatase pair C-terminal Src kinase (Csk) and CD148 in regulating SFK activity in platelets and their response to vascular injury.

Methods: Csk and CD148 conditional double-knockout (DKO) mice were generated by crossing Csk- and CD148-floxed mice with Pf4-Cre transgenic mice. Platelet function was measured in vitro, ex vivo and in vivo using standard assays. Platelet receptor expression and signal transduction was measured by flow cytometry, western blotting and capillary-based immunoassay.

Results: Deletion of Csk-CD148 in the megakaryocyte lineage in mice resulted in increased platelet SFK activity, but reduced platelet reactivity to collagen due to down-regulation of the ITAM-containing collagen receptor complex GPVI-FcR γ-chain. Interestingly, the immunoreceptor tyrosine-based inhibition motif (ITIM)-containing receptor G6b-B and Csk-homologous kinase (Chk) were concomitantly up-regulated, contributing to the reduced platelet response. In vitro, whole-blood thrombus formation under non-coagulant conditions on multiple surfaces was markedly impaired; however, tissue factor mediated thrombus formation on collagen under flow with high fibrin formation was normal. Consequently, DKO mice exhibited increased bleeding in the tail bleeding assay and thrombus instability following laser- and ferric chloride-induced injury of arterioles in the cremaster muscle.

Conclusions: This study establishes Csk-CD148 as a critical molecular switch controlling the thrombotic and haemostatic capacity of platelets, and reveals cell-intrinsic mechanisms that prevent pathological thrombosis from occurring.
Oral communication 3:

HYPERSENSITIVE PLATELET SUBPOPULATIONS DETERMINE COLLECTIVE BEHAVIOUR: RESULTS FROM A STUDY OF SINGLE PLATELET FUNCTION USING DROPLET MICROFLUIDICS

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Background: Platelets have long been known to be heterogeneous in size, volume and density, and functional heterogeneity has been suggested in several studies. However, there are currently no methods available to investigate platelet function on a single cell level. Single platelet research is needed to study intrinsic heterogeneity without the influence of adjacent cells and associated amplification mechanisms. Such a method should study platelets in isolation, and with high throughput, to detect potentially rare phenotypes, without interfering with normal platelet function.

Aims: The study aims to develop a high throughput droplet microfluidic protocol to investigate single platelet function and understand intrinsic functional diversity.

Methods: The innovative droplet microfluidic protocol involves compartmentalising platelets in 25-µm-diameter monodisperse (CV1-4%) and activating single platelets while excluding paracrine signalling. High throughput encapsulation (4 kHz) is coupled with flow cytometry for the high throughput quantification of platelet responses to multiple agonists using three endpoints; αIIbβ3 integrin activation, degranulation (P-selectin exposure) and membrane inversion (Annexin V exposure).

Results: In droplets, the absence of paracrine signalling and the presence of autocrine signalling produces a binary-like response. Single platelet responses to convulxin identify a small hypersensitive subpopulation of platelets that is 10-fold more sensitive than the main population. In platelet collective experiments, the hypersensitive subpopulation is sufficient to direct activation of the entire population via paracrine signalling. The hypersensitive subpopulation varies in number and sensitivity between agonists and individuals. Platelet functional heterogeneity in response to agonists therefore has a strong intrinsic component.

Conclusions: This study demonstrates the value of a high throughput droplet microfluidics and flow cytometry workflow for measuring system heterogeneity. Intrinsic, pre-programmed states were observed, with hypersensitive platelets driving global platelet activation in health and potentially in disease.
Oral communication 4:

THE HEPARAN SULFATE PROTEOGLYCAN PERLECAN IS A PHYSIOLOGICAL LIGAND OF THE INHIBITORY PLATELET RECEPTOR G6B-B

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BACKGROUND: The immunoreceptor tyrosine-based inhibition motif (ITIM)-containing receptor G6b-B is an essential regulator of platelet production and activation, loss of which results in severe macrothrombocytopenia and aberrant platelet function in mice and man.

AIMS: To identify the physiological ligand of G6b-B and determine its biological effects on platelets.

METHODS: Recombinant dimeric Fc-tagged mouse G6b-B ectodomain (mG6b-B-Fc) was used to identify tissues expressing the putative ligand of G6b-B by immunohistochemistry and to pull-down the ligand from tissue lysates. Following identification by mass spectrometry, the interaction between G6b-B and its binding partner was characterised in vitro. Effects of the ligand on platelets were assessed using standard functional and biochemical assays.

RESULTS: The most abundant protein identified in mG6b-B-Fc pull-downs from vena cava lysates was the heparan sulfate (HS) proteoglycan perlecan. This result was confirmed by in vitro binding assays, where mG6b-B-Fc bound robustly to purified perlecan, but not to other extracellular matrix proteins. Heparinase III treatment of immobilised perlecan almost completely abolished its interaction with mG6b-B-Fc, strongly suggesting that G6b-B binds to the HS side-chains of perlecan. This was supported by surface plasmon resonance measurements, revealing binding affinities of mG6b-B-Fc to perlecan, HS and heparin in the low nanomolar range. Adhesion of platelets to the perlecan protein core was observed only after removal of the HS side-chains, indicating their inhibitory function. However, soluble HS and heparin enhanced aggregation of human platelets to sub-threshold concentrations of collagen in platelet rich plasma, which may be a consequence of their inability to co-cluster G6b-B with activatory receptors. Intriguingly, heparin induced G6b-B phosphorylation in washed human platelets.

CONCLUSION: Findings from this study establish the HS side-chains of perlecan as physiological ligands of G6b-B. Ongoing studies are aimed at determining the optimal binding sequence of HS and analysing the biological consequences of this interaction. This work is funded by the British Heart Foundation (BHF) and the Deutsche Forschungsgemeinschaft (DFG, V2134-1/1 to Timo Vögtle).
Oral communication 5:

INHIBITION OF BTK DOES NOT BLOCK PLATELET ACTIVATION BY GPVI: STUDIES WITH IBRUNITIB, ACALABRUTINIB AND XLA PATIENTS

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Background: Ibrutinib and acalabrutinib, inhibitors of the protein tyrosine kinase Btk, are used to treat B cell malignancies. They bind irreversibly to Cys481 of Btk preventing autophosphorylation and phosphorylation of other substrates including PLCγ2. Patients treated with ibrutinib have increased bleeding events which correlate with inhibition of platelet aggregation by the collagen and fibrin receptor GPVI. However, patients treated with acalabrutinib, and patients with mutations in their Btk gene (X-linked Agammaglobulinaemia: XLA), do not bleed excessively.

Aims: We sought to investigate this discrepancy between the two Btk inhibitors and patients with XLA.

Methods: Aggregometry, tyrosine phosphorylation and Ca2+ mobilisation were performed in washed platelets. Aggregometry was also performed with platelet rich plasma (PRP) from patients taking ibrutinib 420mg daily, acalabrutinib 100mg twice daily, a control treatment and XLA patients.

Results: We have compared the effects of ibrutinib, acalabrutinib and XLA on platelet activation by the GPVI agonist collagen receptor peptide (CRP). Loss of activation of Btk, as verified by western blotting for autophosphorylation (Y223), and phosphorylation of PLCγ2 on Y759 and Y1217, had no effect on aggregation to CRP. 10-20 fold higher concentrations of ibrutinib and acalabrutinib blocked aggregation to CRP. Inhibition was independent of Tec inhibition since (i) it was reversible on washout; (ii) there was no further reduction in PLCγ2 phosphorylation; (iii) concentrations of ibrutinib and acalabrutinib that blocked phosphorylation of Tec did not block aggregation.

Conclusions: Our results confirm that ibrutinib blocks GPVI-induced platelet aggregation in vitro and ex vivo but show that, contrary to current understanding, this is due to an unidentified off-target effect. We hypothesise that reducing the dose of ibrutinib given to patients would abolish its off-target effects and therefore reduce bleeding. This is supported by the fact that patients with XLA, and those taking acalabrutinib, do not experience abnormal bleeding.
Oral communication 6:

THE FIBRIN αC DOMAIN NOT ONLY PLAYS A ROLE IN LATERAL AGGREGATION BUT ALSO IN LONGITUDINAL FIBRE GROWTH

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Background: The fibrinogen (FGN) α-chain is the largest FGN chain, two thirds extend from the D-region (221-610) to form a connector region (221-392) and a globular αC domain (392-610). The αC domain and its connector contain sites for α2-antiplasmin cross-linking and α-α chain cross-linking by FXIII. Previous data on FGN truncated at 251 (middle of connector region) indicated that the αC region is important for lateral aggregation of fibres.

Aims: To investigate the influence of the αC region components on clot structure and function with two new FGN variants deleting the connector region and/or the C-terminal globular domain.

Methods: Two recombinant FGNs truncated at residues 390 (loss of the αC domain) and 220 (removal of connector region + αC domain) and WT FGN were produced in CHO cells. FGNs were characterised by SDS-PAGE for homogeneity, clot structure was studied by turbidity, confocal microscopy with alexa-488 FGN variants and scanning electron microscopy (SEM), and mechanical properties were analysed by magnetic tweezers.

Results: Two FGNs were produced with truncated α-chains of expected sizes (α390 42kDa and α220 25kDa). Turbidity showed decreased maximum absorbance for α390 compared to WT clots, but similar absorbance for α220. Lag time was increased for α220 vs WT. Confocal microscopy showed that FGN α390 formed denser clots with thinner fibres and smaller pores, while α220 produced less dense clots containing thick, short and bundled fibres with large pores. SEM showed that α220 clot architecture has many fibre ends visible and very weak mechanical properties.

Conclusions: Our data show a clear role for the αC domain in lateral aggregation, but crucially indicate a new role for the αC connector region in longitudinal fibre growth and mechanical strength. These findings highlight the importance and complexity of the FGN αC region in clot structure and function.
Oral communication 7:

THE EFFECT OF ANTITHROMBOTICS ON THREE-DIMENSIONAL THROMBUS FORMATION

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Background: Various commercial and home-made microfluidic devices are currently being used to explore the effects of extensional and steady state shearing forces to assess in vivo thrombus formation. Largely the analysis of these images is limited to two-dimensional analysis.

Aim: To characterise the role of antithrombotics on the forming thrombus under flow conditions using confocal microscopy.

Methods: Channel slides were coated with 50 μg.ml⁻¹ type I collagen for 1 hour at 37°C and then blocked with a 1% w/v BSA solution in HBS buffer for 20 minutes. Blood samples were spiked with 1.25 μg.ml⁻¹ DiOC18(3) and 50 μg.ml⁻¹ Alexa Fluor 594 conjugated human fibrinogen. Samples were mixed with rivaroxaban in a DMSO vehicle and incubated for 10 minutes. Citrated blood samples were recalcified before perfusion. Blood was perfused through the coated chamber at 800 s⁻¹ and 5000 s⁻¹. Flow was terminated after 10 minutes and the channels were washed with a HBS buffer supplemented with 1 U.ml⁻¹ Heparin. Confocal z-stacks were recorded using a Zeiss LSM880 inverted microscope.

Results: We have generated confocal images that validate this approach to assessing the role of antithrombotics on the composition of the thrombus under flow conditions. We observed that rivaroxaban is more efficacious at reducing fibrin formation at lower shear conditions than higher shear. As expected, a greater proportion of platelets are incorporated into the forming thrombus at 5000 s⁻¹ but rivaroxaban was effective at reducing platelet surface area and volume in both shear conditions.

Conclusions: This study shows that for the first time it will be possible to determine the role of antithrombotic agents employing a more physiological approach to monitoring thrombus formation under flow conditions, and aid the development of the next generation of antithrombotic agents. The images concur with clinical observations that rivaroxaban is more efficacious at reducing platelet aggregation and fibrin formation at lower shear conditions.
Oral communication 8:

DEFECTIVE FIBRIN ACCUMULATION AND THROMBUS STABILITY IN BAMBI DEFICIENT MICE IS MEDIATED BY INCREASED APC ANTICOAGULANT ACTIVITY

Isabelle I. Salles-Crawley, Argita Zalli, James H. Monkman, David A. Lane, Josefin Ahnström and James T.B. Crawley
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Background: BAMBI is a TGFβ superfamily transmembrane protein that is highly expressed in platelets and endothelial cells. Bambi-deficient mice exhibit a mild bleeding phenotype and decreased arterial thrombus stability. Chimeric mice revealed that endothelial, rather than platelet BAMBI confers this phenotype.

Aims: We aimed to delineate the mechanisms by which BAMBI exerts its function on the endothelium and how it influences thrombus stability.

Methods: Bambi−/− mice, endothelial Bambi knockout mice (BambiEC−/−) and littermates were subjected to the laser-induced thrombosis model where platelets, neutrophils and fibrin(ogen) accumulation were assessed. Expression of adhesion molecules and thrombomodulin was assessed by flow cytometry in mouse lung endothelial cells (MLEC). Activated protein C activity in Bambi deficient endothelial cells was assessed in vitro and in vivo.

Results: No difference in the levels of plasma prostacyclin or nitric oxide could be observed between Bambi+/− and Bambi+/+ littermates. Incorporation of neutrophils in thrombi after laser injury of the endothelium was similar in Bambi+/− and Bambi−/− mice. Consistent with this, no difference in expression levels of PECAM-1, ICAM-1 or ICAM-2 was detected between Bambi+/− and Bambi+/+ MLEC. The increased number of emboli during thrombus formation present in Bambi+/− mice was accompanied with a defect in fibrin accumulation. Injection of hirudin prior to thrombus formation in Bambi+/− mice recapitulated the Bambi−/− thrombus instability phenotype while it had no effect in Bambi+/− mice. BambiEC−/− exhibited normal haemostasis but defective thrombus stability with decreased fibrin accumulation. Finally, we show that Bambi−/− MLEC have increased APC anticoagulant activity mediated by increased thrombomodulin levels. Inhibiting thrombomodulin function in vivo partially restored fibrin accumulation and thrombus stability in Bambi−/− mice.

Summary/Conclusions: BambiEC−/− similar to Bambi−/− mice display increased thrombus instability accompanied by a markedly diminished ability to accumulate fibrin during thrombus formation, confirming the important role of endothelial BAMBI in thrombus formation. Our data further suggest that BAMBI modulates thrombomodulin levels in the endothelium that influence APC anticoagulant activity and thrombus stability.
Oral communication 9:

FIBRINOGEN AND FIBRIN BIND GPVI-MONOMER WITH NANOMOLAR AFFINITY, BUT NOT GPVI-DIMER

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Background: Glycoprotein VI (GPVI) is expressed on the surface of platelets in monomeric form, associated with an Fc Receptor γ-chain (FcRγ). Collagen binding to GPVI leads to platelet activation via clustering of GPVI DIMERS around the ligand, permitting cross-phosphorylation of the coupled FcRγ ITAM motifs. GPVI was originally proposed to function as a haemostatic initiator following vessel damage and collagen exposure. GPVI has been shown to bind: laminins; adiponectin; Emmprin and Fibrin, in addition to collagen, suggesting a role for GPVI in thrombus propagation.

Aims: We aimed to determine the role of binding of fibrin/ogen to the GPVI receptor in thrombus propagation.

Methods: Recombinant monomeric GPVI (mGPVI) consisted of the collagen binding domain (CBD). Recombinant dimeric GPVI (dGPVI) consisted of the CBD with a C-terminal IgG1-Fc sequence. Both proteins were expressed and purified from a transient HEK293T cell line by Amir Babar, Jeannette Miller and Andrew Herr. SPR binding studies were performed on a Pioneer platform (PALL® FortéBio®) on a COOH-V chip. Fibrinogen was immobilised to 3825RU on flow cell (FC)-1 and 3423RU on FC-3 using amine coupling, with FC-2 as a reference channel. FC-3 fibrinogen was converted to fibrin using 10U/mL thrombin for 2 hours at 37°C. mGPVI and dGPVI binding to fibrinogen and fibrin was performed at 37°C using dynamic inject up to a final concentration of 1µM.

Results: Monomeric GPVI binds to fibrin with a Kd of 302±5nM and to fibrinogen with a Kd of 336±1nM. Dimeric GPVI did not bind to fibrinogen or fibrin.

Summary/Conclusion: Collagen preferentially binds dimeric GPVI whereas fibrin/ogen preferentially binds monomeric GPVI. Inactive platelets with the majority of GPVI in monomeric form will preferentially bind to fibrin/ogen in a growing thrombus. The relative concentrations of collagen or fibrin/ogen in the surrounding environment could dictate the ratio of monomeric/dimeric GPVI present on platelets, and therefore regulate thrombus propagation.
Oral communication 10:

CRYSTAL STRUCTURE OF Fab-BOUND ADAMTS13

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Background: ADAMTS13 is a plasma metalloprotease that regulates the platelet-tethering function of von Willebrand factor (VWF). The mechanisms involved in ADAMTS13-mediated proteolysis of VWF are unique. This first involves the shear-dependent unfolding of VWF, which exposes cryptic exosites and the cleavage site. Only then can the N-terminal metalloprotease (MP) to spacer domains of ADAMTS13 (termed MDTCS) engage with, and proteolyse, the VWF A2 domain. More recent biochemical studies suggest that the ADAMTS13 MP domain is allosterically regulated and exists in distinct latent and active conformations.

Aims: To understand how ADAMTS13 functions at a molecular level, we aimed to resolve the crystal structure of the N-terminal domains of ADAMTS13 (MDTCS).

Methods & Results: We expressed and purified ADAMTS13 MDTCS with a C-terminal His tag in S2 insect cells. MDTCS was purified to homogeneity by affinity and size exclusion chromatography. We isolated a stable complex between MDTCS and an anti-MP domain Fab. This complex was successfully crystallised, from which we determined the structure of the complex to a resolution of 2.8Å. For the first time, we reveal the structure of the MP domain and its conformation relative to the adjacent domains. We define the architecture of the Zn²⁺-binding active site and two distinct Ca²⁺-binding sites within the MP domain. We also resolve the positions and orientation of the VWF-binding exosites within the MP, Disintegrin-like, Cys-rich and Spacer domains, and suggest the mode of interaction between ADAMTS13 and VWF. Closer inspection of the MP domain, suggests that the structure may represent the inactive/latent conformation, and provides insights into the mechanisms underlying the allosteric activation of ADAMTS13.

Conclusion: Our structural study of functional domains of ADAMTS13 provides novel insights into the remarkable selectivity and specificity of ADAMTS13.
Oral communication 11:

ALLOSTERIC ACTIVATION OF ADAMTS13

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Introduction: ADAMTS13 proteolytically regulates von Willebrand factor (VWF) platelet-tethering function. Highly unusually, ADAMTS13 is predicted to circulate as an active enzyme. Despite this, ADAMTS13 is resistant to inhibition by plasma inhibitors, it exhibits a very long active plasma 1/2-life (3-7 days), and yet appears completely specific for VWF. How these characteristics are manifest remains unclear. We propose a model in which ADAMTS13 circulates with its metalloprotease (MP) domain in a latent conformation. Only once ADAMTS13 engages its substrate (VWF) through one or more of its specific exosite interactions (MP, Dis, Cys-rich or spacer domains) does the MP domain become allosterically activated and capable of proteolysing VWF.

Aims: To understand ADAMTS13 allosteric activation at a molecular level.

Methods & Results: Kinetic analysis of ADAMTS13 proteolysis of a recombinant 96 amino acid VWF A2 domain fragment, and mutants thereof in which the MP, Dis, Cys-rich or spacer domain binding exosites were ablated, confirmed that the Cys-rich and spacer domain exosites are involved in substrate binding. The Dis domain exosite also binds VWF, and in so doing enhances the $k_{cat}$ for proteolysis by ~50-fold, suggesting that this exosite is involved in allostery activation of the ADAMTS13 MP domain. To explore conformational changes in ADAMTS13 following binding to the VWF A2 domain, we performed isothermal titration calorimetry (ITC) to measure binding affinity, stoichiometry and energetics of binding. Using ITC, the affinity ($K_D = 450nM$) of ADAMTS13 for VWF was much lower than determined using plate binding assays (~40nM). Moreover, the energetics were consistent with a conformational change in ADAMTS13. To further explore this, we examined changes in intrinsic tryptophan fluorescence upon binding of ADAMTS13 to VWF. These data also supported a conformational change in ADAMTS13 in response to VWF binding.

Conclusion: Taken together this study provides evidence for a functional conformational change in ADAMTS13 upon substrate binding that may serve to activate the enzyme prior to proteolysis.
Oral communication 12:

WHY DO ANTIFIBRINOLYTIC LYSINE ANALOGUES CAUSE BLEEDING?

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Background. Lysine analogues, aminohexanoic acid (AHA) or tranexamic acid (TXA) are widely used to reduce bleeding. However, data analysis from the CRASH-2 trial on trauma (2011) and from the WOMAN trial on post-partum haemorrhage (2017), show TXA given after 3 hours increased risk of death due to bleeding.

Aims. To understand the circumstances under which antifibrinolics promote bleeding.

Methods. Methods included clotting and fibrinolysis assays with tPA, uPA and TXA covering a therapeutic range. Microtitre plate plasminogen activation kinetic assays were performed with clots made from purified fibrinogen, plasma or euglobulin and whole blood (Halo assays). Thromboelastometry (ROTEM) was used in parallel with plasma, platelet rich plasma (PRP) and whole blood.

Results. TXA from 5 to 1000 µM was a potent inhibitor of tPA-catalysed fibrinolysis in all systems. However, with uPA, TXA between 100 and 1000 µM stimulated plasmin release from clots made from fibrinogen, plasma or euglobulin. Aprotinin (Trasylol) was an effective plasmin inhibitor in all systems, IC50 =650 nM, within its therapeutic range. ROTEM assays with plasma and uPA+TXA resulted in weak clots that could be stabilised by aprotinin. Significantly, with whole blood or PRP, TXA was able to stabilise clots even in the presence of uPA, also confirmed in Halo assays.

Conclusions. Previous animal trauma models demonstrate an initial spike of tPA release followed by uPA after 2-3 hours. Early administration of TXA blocks fibrinolysis with tPA and stabilises clots. Without early TXA, ongoing coagulation and fibrinolysis depletes inhibitors and platelets and subsequently uPA is released into a pro-fibrinolytic environment. Late TXA stimulates uPA activity by opening the conformation of plasminogen and protects plasmin from alpha-2-antiplasmin (a2AP). Current trauma treatment recommends replacing haemostatic proteins and platelets, which may be enhanced by aprotinin. Furthermore, maintaining circulating TXA <100 µM would prevent pro-fibrinolytic side effects on plasminogen conformational and blocking a2AP inhibition.
Oral communication 13:

TISSUE FACTOR DE-PALMITOYLATION, PHOSPHORYLATION, DE-ENCRYPTION AND RELEASE IN MICROVESICLES ARE RELATED EVENTS

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Background: One enigma in the regulation of TF cofactor activity has been the ability of this protein to exist on the surface of cells in an encrypted form without triggering coagulation. However, following cellular activation TF becomes de-encrypted and is also released within microvesicles, augmenting the procoagulant activity. TF de-palmitoylation of Cys-245 and phosphorylation of Ser-253 are interrelated modifications of TF which orchestrate this augmentation in TF procoagulant activity.

Aims: In this study we have examined the mechanism by which de-palmitoylation can induce the phosphorylation and subsequent release of TF in microvesicles.

Methods: The pCMV-Ac-TF-tGFP plasmid was mutated to express TF with various mutations of the amino acids within the membrane-proximal region of the cytoplasmic domain of TF (residues 243-250). Cell-surface TF activity, FVIIa binding potential, the phosphorylation state of Ser-253 within TF and the release of TF within microvesicles were examined by FXa generation assay, FVIIa-binding assay, immunoprecipitation/western blot and TF-ELISA, respectively.

Results: Alanine-substitution of selected positive amino acids within the membrane-proximal region of the cytoplasmic domain of TF abolished the observed phosphorylation at Ser-253 and significantly reduced the release of TF within microvesicles. Moreover, substitution of Cys-245 with Phe to mimic the palmitoylation of Cys-245 reduced the phosphorylation of TF and its incorporation into microvesicles. Furthermore, alteration of the amino acids linking the positive-cluster to the phosphorylation site, particularly Val-250, also hindered TF-phosphorylation and release.

Conclusion: De-palmitoylation of TF de-restricts the membrane-proximal amino acids including the positively charged Arg and Lys residues, from the inner leaf of the membrane. Moreover, the hydrophobic interactions of Val-250 alter the structure of TF, bringing the two flanking peptides together. This resultant approximation of the positive amino acids and the Ser-253 domain creates a PKC-recognition motif which is essential for the phosphorylation of TF and therefore, its subsequent incorporation into cell-derived microvesicles.
Oral communication 14:

TREM-LIKE TRANSCRIPT-1: A HIGHLY SENSITIVE MARKER OF EARLY PLATELET ACTIVATION

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Background: Recent findings suggest thrombi can be divided into a core of tightly packed, highly activated P-selectin positive platelets, and a shell of loosely packed, P-selectin negative platelets. TREM-like transcript-1 (TLT-1) is an immunoreceptor tyrosine-based inhibition motif (ITIM)-containing receptor expressed in α-granules of platelets and megakaryocytes, which is rapidly upregulated to the surface upon activation. TLT-1 has been proposed to bind fibrinogen and facilitate platelet activation. However, TLT-1 function in thrombus formation and stability has yet to be defined.

Aims: To determine the expression of TLT-1 relative to P-selectin during platelet activation in vitro and thrombus formation in vivo.

Methods: TLT-1 and P-selectin surface expression were analysed in activated mouse platelets and megakaryocytes in vitro by flow cytometry and confocal microscopy, and during thrombus formation following laser injury of cremaster arterioles in mice.

Results: TLT-1 is more rapidly and robustly upregulated to the surface of mouse and human platelets compared with P-selectin upon activation with thrombin and collagen-related peptide. Collagen stimulation of megakaryocytes also resulted in a more robust upregulation of TLT-1 compared to P-selectin. Similarly, TLT-1 more rapidly translocated to the surface of activated platelets compared with P-selectin during laser-induced thrombus formation, with TLT-1 detected throughout thrombi, compared to P-selectin which was restricted to a highly localized region directly adjacent to the site of injury. In both platelets and megakaryocytes, TLT-1 colocalized with P-selectin in α-granules. However, some staining did not colocalize, suggesting differential distribution within α-granules or localize to other as yet unidentified compartments.

Conclusions: Findings from this study demonstrate more rapid upregulation and peak surface expression of TLT-1 compared to P-selectin in human and mouse platelets in vitro, and during thrombus formation in mice following laser injury of arterioles. Moreover, they suggest platelet activation in the shell region of growing thrombi occurs more rapidly and to a greater extent than previously thought.
Oral communication 15:

TTP AUTOANTIBODY CHARACTERISATION: RECOGNITION OF ADAMTS13 CONFORMATIONS AND ASSOCIATED IgG SUBCLASS

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Background: Patients with immune mediated thrombotic thrombocytopenic purpura (iTTP) have an ADAMTS13 deficiency due to anti-ADAMTS13 IgG autoantibodies. ADAMTS13 can exist in two distinct conformations. In the circulating ‘closed’ form, the CUB domains shield the Spacer domain, a domain frequently recognised by antibodies in iTTP. Once bound these antibodies exert their pathogenic effects by inhibiting ADAMTS13 function and/or inducing clearance. Antibody-mediated ADAMTS13 clearance is an important disease mechanism as severely reduced ADAMTS13 antigen at presentation is associated with increased mortality. The ability of IgG to effect target antigen clearance is determined by IgG subclass and so may play an important role in TTP pathogenesis.

Aims: To investigate whether iTTP patient autoantibody binding to ADAMTS13 is dependent upon ADAMTS13 conformation, and to measure the IgG subclass of these antibodies in relation to ADAMTS13 antigen levels.

Methods: Autoantibody binding to ‘open’ or ‘closed’ ADAMTS13 in 7 iTTP presentation samples was analysed. Recombinant ADAMTS13 was captured in its ‘closed’ form via its 6xHis tag to Ni2+ coated plates. ADAMTS13 was specifically ‘opened’ using an anti-ADAMTS13 antibody that alters ADAMTS13 conformation. After plasma incubation, bound patient antibodies were detected using an anti-human IgG antibody. Subclass specific anti-human antibodies i.e. IgG1-4 were used to measure IgG subclass.

Results: In all patients, a reduced titre was detected against ‘closed’ ADAMTS13, when compared to ‘open’, suggesting important epitopes are concealed when ADAMTS13 is ‘closed’. In 3/7 <25% of those antibodies that bound ‘open’ also bound ‘closed’ ADAMTS13, in 4/7, 25-50% could bind to ‘open’ and ‘closed’. The IgG subclass distribution recognising ‘open’ and ‘closed’ ADAMTS13 was similar, suggesting this was not influenced by conformation.

Summary/conclusions: Although iTTP patient ADAMTS13 autoantibody binding is highly dependent upon conformation, we detected no clear correlations between ADAMTS13 antigen levels, conformational specificity and IgG subclasses in this small group of iTTP patients.
ABSTRACTS ORAL - Friday

Oral communication 16:

EXTENDED D-E INTERACTIONS NEAR THE CLASSICAL KNOB-HOLE BINDING SITE PLAY AN IMPORTANT ROLE IN FIBRIN POLYMERISATION AND CLOT STABILITY

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Background: Molecular simulations indicate the presence of an extended binding interface beyond the traditional knob-hole interactions that occur when thrombin converts fibrinogen to fibrin (Kononova, JBC 2013). Within this extended binding interface, γAsp297, γGlu323 and γLys356 in the D-region of one fibrin molecule interact with βLys58, βAsp61 and βHis67 in the E-region of another, respectively. The effects of these novel electrostatic interactions on fibrin polymerisation and clot structure are unknown.

Aims: To study the role of the extended knob-hole interface in polymerisation kinetics, clot structure and clot mechanics.

Methods: Recombinant human fibrinogen γDEK variant (γD297N/E323Q/K356Q) and wild-type (WT) were produced in CHO cells and purified by affinity chromatography. Clot polymerisation kinetics were studied by turbidity. Clot visco-elastic properties were determined by magnetic tweezers. Confocal microscopy was used to study clot formation, clot structure and clot lysis. Single γD297N, γE323Q and γK356Q fibrinogen mutants were also produced and characterised.

Results: γDEK showed extended lag phase (+30%), slower clotting rate (-45%) and lower maximum OD (-41%) compared to WT. This variant produced a denser clot network (+41%) in hydrated conditions compared to WT, which resulted in slower lysis rates (-37%). Frequency dependent moduli were calculated and G’ (elastic modulus), was similar at 0.1Hz but higher at 1 and 10Hz, compared to WT. G” (energy loss modulus) was increased at 0.1Hz 1Hz and 10Hz, compared to WT. The loss tangent (tanδ, visco-elasticity) was increased at 0.1Hz and 1Hz but similar at 10Hz. Preliminary turbidity data for γD297N γE323Q and γK356Q fibrinogen variants show reduced maximum OD similar to γDEK.

Conclusions: The abolition of electrostatic interactions responsible for the extended binding interface results in altered polymerisation kinetics (prolonged protofibril formation), clot structure and viscoelastic properties. Our findings support previous molecular simulations and demonstrate that the D-E binding interface extends beyond the classical knob-hole interaction to reinforce fibrin polymerisation.
Oral communication 17:

**VWF AND ADAMTS13 LEVELS IN EARLY ONSET PREECLAMPSIA: PROTHROMBOTIC MECHANISMS IN MOTHERS WITH ELEVATED RISK OF VENOUS THROMBOEMBOLISM**

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**Introduction:** Preeclampsia (PE) is a serious complication of pregnancy with potentially life-threatening consequences for both mother and baby. Early onset preeclampsia (EOPE; onset <34 gestational weeks), is associated with higher maternal and fetal risks than late onset PE. The risk of venous thromboembolism is increased, particularly in severe PE. Consequently, mothers may be considered for thromboprophylaxis. However, risk assessment may be challenging due to competing bleeding risks such as placental abruption, post-partum haemorrhage, or renal impairment.

**Aim:** To characterise the relationship between VWF and ADAMTS13 levels in EOPE patients in order to better understand parameters that may modulate thrombotic and bleeding risk.

**Methods:** Plasma samples obtained from healthy pregnancy (HP) or PE were compared to healthy control (HC) females. Plasma VWF and ADAMTS13 levels were determined by VWF and ADAMTS13 ELISA. ADAMTS13 activity was measured using FRETS73.

**Results:** Mean plasma VWF levels were significantly elevated in pregnancy and further significantly increased in PE. In contrast, plasma ADAMTS13 levels were significantly decreased in pregnancy and PE when compared to HCs. While severity of PE did not correlate with VWF levels, the lowest ADAMTS13 levels and activity was observed in patients with severe PE. Moreover the VWF/ADAMTS13 ratio was calculated to be lowest in patients with severe PE. Lectin analysis of VWF glycans demonstrated reduced sialic acid content. Interestingly, plasma levels of Angiopoietin-2 (Ang2) that is stored in Weibel-Palade bodies alongside VWF, were found to rise in healthy pregnancy but were decreased in severe PE.

**Conclusions:** PE is associated with highly elevated plasma VWF and decreased ADAMTS13 levels and activity. Furthermore, the VWF protein in PE patients shows altered glycosylation which may affect its normal processing and lower plasma Ang2 levels may be an interesting marker for severe PE disease.
EFFICACY OF CRYOPRECIPITATE VERSUS FIBRINOGEN CONCENTRATE IN CLOT FORMATION AND STABILITY: IMPLICATIONS FOR TREATMENT OF TRAUMA INDUCED COAGULOPATHY

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Background: Post-trauma haemorrhage is the major cause of mortality and is exacerbated by depletion of coagulation proteins especially plasma fibrinogen.

Aim: To determine the optimal fibrinogen concentration to achieve adequate clot stability and to compare the effects of cryoprecipitate and fibrinogen concentrate on clot formation, firmness and stability.

Methods: Various concentrations of fibrinogen concentrate (RiaSTAP) or cryoprecipitate were added to fibrinogen depleted plasma. Thromboelastometry was performed to determine clotting time and maximum clot firmness when stimulated by extrinsic and intrinsic pathways. Clot turbidity and fibrinolysis were measured by an absorbance based assay ± tPA with varying thrombin concentrations. FXIII and α2AP levels were measured using ELISA and clot structure visualised by confocal microscopy.

Results: No significant differences were observed in the clotting time with RiaSTAP and cryoprecipitate, when induced by extrinsic or intrinsic activators, over the range of fibrinogen concentrations. Maximum clot firmness was augmented by increasing fibrinogen concentration, with RiaSTAP producing firmer clots. Higher maximum absorbance was observed for both sources with increasing fibrinogen concentration. Increasing fibrinogen concentration using cryoprecipitate correlated with an increase in 50% lysis times, surprisingly there was a lack of correlation with RiaSTAP. Cryoprecipitate contained significantly higher concentrations of α2AP and FXIII concentration than RiaSTAP. Fibrin structure was markedly different when clots were formed using the two preparations of fibrinogen; with cryoprecipitate showing a homogenous uniform distribution of fibers while RiaSTAP produced dense clusters of fibers interspersed by large pores.

Conclusion: Restoring fibrinogen concentration to normal circulating levels improves clot strength and stabilises clots against fibrinolysis. The impact of cryoprecipitate on time to 50% lysis times was dose dependent and it was significantly more effective than RiaSTAP. The presence of additional proteins in cryoprecipitate, such as α2AP and FXIII potentially facilitate the formation of more stable clots that are protected against premature degradation.
GENERATING AN ANTIBODY FRAGMENT TO INHIBIT VWF FUNCTION

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Background
Von Willebrand Factor (VWF) plays an essential role in haemostasis by mediating platelet capture to sites of vessel injury and acting as the carrier molecule for factor VIII. Elevated plasma VWF is associated with an increased risk of cardiovascular disease making it a possible therapeutic target. Several studies have attempted to design inhibitors of VWF with varying degrees of success; however these have normally focused on targeting the A1 domain of VWF to inhibit platelet capture function, although this presents the risk of causing excessive bleeding. To overcome this we have identified that the C-terminal domains of VWF can be safely inhibited to reduced but not abolish function. In the current study we have prepared an antibody fragment library against VWF that will be used to generate antibody fragments against specific regions of VWF.

Methods
The human domain antibody fragment phage library was prepared and panned against purified plasma derived VWF. Following three rounds of panning individual clones were isolated and their ability to bind VWF and inhibit function determined.

Results
After three rounds of selection >5000 individual clones were obtained. To date 560 clones have been analysed and 25 strong VWF binders have been isolated and expanded for further testing. Re-panning of the final selection round against other proteins produced minimal clones suggesting that the majority of the isolated library was against VWF.

Conclusion
We have successfully created an antibody fragment library against full length VWF protein and have begun to analyse single clones for specificity and inhibitory activity.
EVALUATING THE MURINE TFPI-PS ANTICOAGULANT PATHWAY

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Background
Tissue factor pathway inhibitor (TFPI) is a Kunitz-type inhibitor that is essential for haemostatic regulation. In humans, protein S (PS) functions as a cofactor for TFPI, although the molecular mechanisms that underpin this are yet to be fully defined. Although both PS and TFPI exist in mice, evidence suggests that protein S may not function as a cofactor for murine (m) TFPI, suggesting that species differences in PS and or TFPI may account for this. Therefore, we aimed to compare the function of the human and murine TFPI-PS anticoagulant pathways.

Methods
His-tagged human (h)TFPI, mTFPI and mPS were expressed in HEK293T cells and purified to homogeneity. TFPI and PS function were assayed using FXa inhibition assays. To assess the function of recombinant mPS and hPS as a cofactor for human activated protein C (APC), CAT assays were performed in protein S-deficient plasma.

Results
Both recombinant hTFPI and mTFPI reduced FXa activity in a dose dependent manner. Antibodies against human or murine TFPI ablated TFPI inhibitory function. As previously demonstrated, hPS efficiently enhanced hTFPI anticoagulant function. Interestingly, although mPS enhanced hTFPI function in an identical manner to hPS, mTFPI was only minimally enhanced by mPS; and not at all by hPS. Both hPS and mPS augmented APC anticoagulant function CAT assays, suggesting (contrary to a previous report) that mPS can effectively function as a cofactor for human APC.

Conclusion
Recombinant mTFPI is not efficiently enhanced by hPS or mPS. The reason for this is likely due to species differences in the mTFPI Kunitz 3 domain that interacts with PS. Both mTFPI and mPS are being used for generation of inhibitory mAbs to facilitate future in vivo studies that target this anticoagulant pathway. The assays that we have optimised will facilitate screening of these mAbs.
P1
ASSESSMENT OF COAGULATION PROFILE, FIBRINOGEN, PROTEIN C, PROTEIN S, ANTITHROMBIN AND VITAMIN K LEVELS AMONG SUDANESE NEONATES WITH PROVEN SEPSIS IN MATERNITY HOSPITAL, SUDAN


Background: Neonatal sepsis represents one of the most common causes of neonatal morbidity and mortality worldwide, non specific clinical remarks makes earlier diagnosis difficult. In neonatal sepsis, generalized inflammatory status occurs, and coagulation alterations results as consequence to inflammation due to the tight link between inflammation and coagulation. Most of neonatal sepsis mortalities results from disseminated intravascular coagulation (DIC). DIC represents end outcome of several coagulation changes and gives a bad progression of a septic neonate. The aim of the study was to assess the platelet counts, prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time (TT), fibrinogen, Protein C, protein S, antithrombin (AT), and vitamin K in Sudanese neonates with sepsis and compare them with healthy neonates. Testing of coagulation studies among septic neonates helps to get a better management and better progression especially when clinical manifestations, severity of sepsis, onset of sepsis, causative agents, and demographic data have been considered.

Methods: the study was a prospective case control study conducted in Omdurman maternity hospital in the period from June 2013 to April 2015 to a total of 100 samples divided into case and control group (50 for each) selected by non probability sampling, blood culture was done routinely for all neonates with suspected sepsis, the first fifty neonates with a positive culture were taken and analyzed as case group by the analyst, and these was done under clinical supervision and acceptance from Dr.Abdulhalim Merghani (senior consultant neonatologist). Platelets were counted by the cell counter (haematology analyzer sysmex KX-21). PT, APTT, TT, fibrinogen, protein C and S were assessed by the clotting procedure by use of semi automated coagulometer (Stago). AT was assessed spectrophotometrically by the turbimetric method by semi automated chemistry analyzer (Mindray BA-88A). Vitamin K was assessed by the HPLC (Schmitzu. 10 ADVP).

Results: the gender distribution was 23, 27 and 24, 26 males and females for test and control respectively, in the case group; 17 neonates with early onset sepsis (from birth-7 days), and 33 with late onset (7-28 days). Ten from test group died (20%) due to neonatal sepsis, of them; four cases (40%) with early onset neonatal sepsis, and six (60%) with late onset (7-28 days). Blood culture distributions were; 23 Pseudomonas (46%), 9 Salmonella (18), 7 Klebsiella (14%), 3 Staph.epidermidis (6%), 3 Strep.fecailis (6%), 2 E.coli (4%), 2 Staph.aureus (4%), and 1 Streptococci (Non group B) (2%). Platelets count was decreased, PT and APTT were prolonged, TT was shorted, fibrinogen was increased, PC, PS, AT and vitamin K were decreased (means 60,289c/mm³, 16.6sec, 47.8sec, 18.6sec, 482.2mg/dl, 34.4%, 33.4%, 183.9Mg/ml, and 0.86ng/ml. And 212,030c/mm³, 13.9sec, 37.5sec, 20.6sec, 393.7mg/dl, 36.8%, 34.7%, 221.5Mg/ml, and 1.23ng/ml for case and control respectively).

Conclusion: it has been concluded that platelets count significantly decreased (P.value 0.000), both PT and APTT significantly prolonged (0.018, and 0.000), TT significantly shortened (0.009), fibrinogen was significantly increased (0.003), and AT significantly decreased (0.003) in neonatal sepsis. APTT and protein C showed significant correlation with the outcome (0.00 and 0.04) respectively, so both can predict early mortality, PT and TT showed significant correlation with early onset sepsis (0.02 and 0.00) respectively. Demographic data (gender, gestational age, mode of delivery, and Gram stain typing had no effect on haemostatic parameters.
POSTERS

P2
LINKING THE ELECTRONIC VENOUS THROMBOEMBOLISM (VTE) RISK ASSESSMENT OUTCOME DIRECTLY TO PRESCRIBING – WAS IT USEFUL?

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Background: Hospital associated thromboses (HATs) remain a significant cause of morbidity and mortality. In many Trusts the electronic-VTE risk assessment (RA) is separate to e-prescribing within electronic patient records (EPR). Root cause analysis of HATs in Oxford University Hospitals (OUHFT) suggested that some might have been prevented if the recommended outcome of the eVTE RA was directly linked to e-prescribing of thromboprophylaxis (TP). OUHFT EPR team updated the VTE RA to achieve this in December 2016.

Aims: To assess the impact of linking the e-VTE RA recommended outcome directly to e-prescribing.

Method: A retrospective analysis of the VTE RA and e-prescribing of TP of 138 inpatients (152 admissions) at OUHFT: 81 admissions in 4.5 month period before the change in eVTE RA, and 71 admissions in the 4.5 months subsequent. Patients were randomly sampled from those with HATs, as completed HAT forms detailed whether or not TP was considered appropriate retrospectively. Information was obtained from HAT forms and EPR. The appropriateness of prescription and congruence between RA and prescription were reviewed.

Results: Appropriate eVTE RA and correct e-prescribing: 55.6% pre, 85.9% post-intervention. Appropriate eVTE RA but prescription incorrect* or omitted: 13.6% pre, 5.6% post-intervention. Inappropriate eVTE RA but correct prescription: 9.9% pre, 8.5% post. Inappropriate eVTE RA and incorrect prescription: 2.5% pre, 0% post. eVTE risk assessment not done: 18.5% pre, 0% post.

*defined as not matching the risk assessment outcome

Summary: This EPR initiative has resulted in increased relevance of the eVTE RA and increased congruence of VTE RA recommended outcome with e-prescribing; supporting improvements in patient safety.

P3
PREVALENCE OF ACTIVATED PROTEIN C RESISTANCE WITH HIGH AVIDITY ANTI-PROTEIN C ANTIBODIES IN VARIOUS ANTIPHOSPHOLIPID SYNDROME CLINICAL PHENOTYPES

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Background: Acquired activated protein C resistance (APCr) in association with anti-protein C antibodies (anti-PC) are associated and may provide a marker for a more severe thrombotic phenotype in antiphospholipid syndrome (APS) patients with venous thromboembolism (VTE).

Aims: To determine the prevalence of anti-PC in association with APCr in a larger cohort of and in different APS clinical phenotypes: VTE or arterial thrombosis (AT) ± pregnancy morbidity (PM), PM only; and asymptomatic antiphospholipid antibodies (aPL).

Methods: Patient samples were collected locally and from the international APS ACTION cohort. Anti-PC and avidity were determined by in-house ELISA. APCr was measured as % inhibition of ETP by thrombin generation (5 pM TF and 4 µM phospholipids +/- exogenous APC (rh-APC; NR 56-132%) or endogenous PC (using Protac; NR 63-141%).

Results: In this ongoing study we tested 418 patients: 196 VTE, 116 AT (27 AT + VTE), 36 PM, 70 aPL. 39% tested positive for anti-PC with a higher prevalence in PM (53%) compared to VTE (39%), AT (38%), aPL (34%) (ANOVA p=0.003). High avidity anti-PC were detected in 58% of the patients with anti-PC; observed in 65%,61%, 63%, and 33% of VTE, AT, PM and aPL positive for anti-PC patients, respectively. A higher proportion of patients showed APCr after activation of endogenous PC (62%) compared to addition of exogenous APC (39%; p<0.0001). 39% (112/289) were resistant to both, with high avidity anti-PC present in: 70% of VTE, 78% AT, 92% PM, but only 44% aPL patients with dual APCr (fisher’s exact test, p <0.0001).

Conclusions: APS (VTE, AT and PM) and aPL patients showed a high prevalence of APCr (using activation of endogenous PC or exogenous APC), which in association with high avidity anti-PC may provide a pathogenic marker. Associations with severity and course of disease in various APS clinical phenotypes remain to be established.
**P4**

**IS ACTIVATED PROTEIN C (APC) THE MISSING LINK BETWEEN ENDOTHELIAL MICROVESICLES (EMVS) AND ALTERED THROMBUS FORMATION?**

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**Introduction:** Endothelial microvesicles (EMVs) are small plasma membrane fragments (0.1-1μm) released into the circulation following physical stress or stimuli. High levels of EMVs have been linked to increased risk of thrombosis. EMVs promote coagulant activity and are also involved in anticoagulant pathways, due to the proteins and lipids they carry. Despite this, to date, the exact role of EMVs in thrombus formation or breakdown is unknown. APC is an anticoagulant pathway that inhibits FVa and FVIIIa.

**Aim:** To investigate the effect of adding EMVs (isolated from cultured endothelial cells) to a developing thrombus by modulating the generation of APC.

**Method:** Thrombi similar to in vivo were formed by the Chandler Loop. Different components from blood tubes were collected i.e., (whole clot), (chopped clot), (clot plasma), (plasma from the tube), and centrifuged. Plasma of which was collected and added to wells at 1:1 ratio with the APC-specific chromogenic substrate, plate reader was used to measure APC production.

**Results:** EMVs appeared to increase the production of APC. Clot in the presence of EMVs vs no EMVs (3.36 +/- SD 1.22 vs. 2.39 +/- SD 0.83, p=0.05). Chopped clot in the presence of EMVs vs no EMVs (3.45 +/- SD 0.08 vs 3.25 +/- SD 0.12, p=0.02).

**Conclusion:** EMVs appear to increase the production of APC inducing anticoagulant. This suggests EMVs may upregulate anticoagulant activity.

**Acknowledgment:** Grateful thanks to The Gerald Kerkut Charitable Trust and University of Southampton for funding.

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**P5**

**TISSUE FACTOR INDUCED ENDOTHELIAL CELL APOPTOSIS IS MEDIATED BY SRC1, FAK, AND B1-INTEGRIN PROTEINS**

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**Background:** Accumulation of TF within endothelial cells has been shown to lead to cellular apoptosis, mediated through p38 activation.

**Aims:** This study examined the roles of Src1, Fak and β1-integrin to act as signalling intermediaries associating increased cellular levels of TF with apoptosis.

**Methods:** To promote the accumulation of TF, the pCMV6-AC-TF-tGFP plasmid was mutated to express TF with (Ser253→Ala) which cannot be released efficiently. Human dermal blood endothelial cells (HDBEC; 4 × 10⁴) were transfected to express TFWt-tGFP, TFAla253-tGFP or tGFP proteins. Cells were pre-incubated with pp60c Src inhibitor (TSTEPQpYPGENL; 500 µM) or a control peptide (TSTEPQWpYPGENL) prior to activation with PAR2-agonist peptide (SLIGKV-NH; 20 µM). Cellular apoptosis was then measured following 24 h incubation, using the TUNEL assay. Other sets of cells were co-transfected to express pCMV6-AC-TF-tGFP, together with a specific Src1 siRNA or alternatively, a control siRNA. The cells were then activated and the levels of cellular apoptosis determined at 24 h post-activation. In addition, transfected cells were incubated with the Fak inhibitor (1,2,4,5-Benzenetetraamine 4HCl; 100 µM), or a-β1-integrin blocking antibody (AIIB2) prior to activation with SLIGKV-NH. The cells were incubated for 90 min and Src1 activity was measured using a src-kinase activity assay kit.

**Results:** While activation of cells expressing TFAla253-tGFP resulted in increased cellular apoptosis in HDBEC. However, pre-incubation of the cells with the Src1 inhibitor, but not the control peptide reduced the rate of apoptosis. Similarly, suppression of Src1 expression using siRNA prevented the TF-induced cell apoptosis. In addition, pre-incubation of HDBEC with β1-integrin inhibitory antibody prior to activation inhibited the level of Src1 activation. Finally, Src1 activity was also reduced on pre-incubation of the cells with the Fak inhibitor.

**Conclusions:** Taken together, these data suggest that Src1 is required for TF-mediated cellular apoptosis in endothelial cells. Furthermore, Fak protein and β1-integrin mediate the pro-apoptotic TF signalling.
P6
TINZAPARIN REDUCES ANGIOGENESIS AND VASCULOGENESIS IN A CHORIO-ALLANTOIC MEMBRANE MODEL

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Background: The beneficial influences of low molecular weight heparin (LMWH) in the treatment of cancer patients have been reported previously and are thought to extend beyond anticoagulation. It has been suggested that LMWH may also act as an anti-angiogenic agent limiting the supply of blood to the growing tumour.

Aim: To explore the anti-angiogenic potential of Tinzaparin using a Chorio-allantoic membrane (CAM) based model.

Methods: The influence of Tinzaparin on vascular proliferation was examined using a CAM model. Gelfoam pads (Pfizer) were soaked with a range of concentrations of Tinzaparin (0-5 IU/ml) or the VEGF-receptor blocker, Bevacizumab (0-12.5 µg/ml). The CAM (11 day old) were accessed through an opening made at the top of the egg and the pads were placed on the CAM of the developing chick embryos. The vascularisation of the CAM tissue was monitored over 72 h by photographing the area surrounding the pads, and assessed by visual scoring of the vascular density, length and thickness.

Results: Treatment of the CAM with Tinzaparin at concentrations of 1.25 IU/ml or higher, resulted in reduction in vessel density. Furthermore, there was a noticeable reduction in the formation of both large vessels and capillaries following treatment with Tinzaparin whereas treatment with Bevacizumab only reduced the density of capillaries.

Conclusions: Our studies indicate that Tinzaparin is capable of reducing the rate of angiogenesis and vasculogeneis, in an in vivo model. Such activity may in turn limit the blood supply to a growing tumour and may provide an explanation for the observed anti-cancer properties associated with LMWH.

P7
THE PRESENCE OF BIPHASIC CLOT WAVEFORMS IN CRITICALLY ILL PATIENTS IS REAGENT-SPECIFIC

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Background: The APTT coagulation curve on optical coagulometers, sometimes shows a decrease in light transmittance prior to clot formation, manifesting as a biphasic clot waveform (BCW). This is frequently observed in patients who subsequently develop disseminated intravascular coagulation. This may be a useful clinical tool to identify at risk patients but can also cause instrument error flags. Anecdotal evidence suggests that the BCW may be reagent specific.

Aims: To determine whether BCW is a reagent specific phenomenon.

Methods: APTT testing was performed on plasma sample from 40 normal subjects and anonymised residual samples from 78 patients with critical illness. Normal plasma was also spiked with VLDL and C-reactive protein. Three APTT reagents were studied using a Sysmex CS5100 coagulometer: A (soy phosphatides/ellagic acid); B (vegetable phospholipids/silicon dioxide); and C (synthetic phospholipids/ellagic acid). The initial slope of the precoagulation phase was calculated thus: Δ% transmitted light/time to start of clot formation.

Results: In all normal plasmas, the initial slope was <0.06. A BCW (initial slope >0.1) was observed in 45% and 28% of patient samples with reagent A and C respectively but none with reagent B. 5% of patient samples produced a strong BCW (initial slope >0.5) with reagents A and C. The instrument could not identify the clotting time in 2 and 3 samples respectively. The addition of CRP (25 – 300 µg/mL) in the presence of 100 µg/mL exogenous VLDL, to normal plasma produced a dose-dependent increase in the initial slope with reagents A and C but not reagent B. There was no significant correlation between the steepness of the initial slope with D-dimer, platelet count or PT.

Summary/Conclusions: We have shown that the presence or otherwise of a BCW in the APTT of critically ill patients is highly reagent specific. This has implications if BCW is used to predict DIC, because values may vary between different hospitals.
P8

MAPPING THE INTERACTION OF VWF WITH FXIIa AND IST POTENTIAL AS A NEW ANTI-COAGULANT TARGET

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Background: Both Von Willebrand Factor (VWF) as well as Factor XII (FXII) have distinct but established roles in haemostasis. While VWF is important for the mediation of platelet capture to sites of vascular injury under high shear stress, FXII is known as the initiator of the intrinsic coagulation pathway. Interestingly, studies have shown that absence or inhibition of FXII does not impair haemostatic function but has a thrombo-protective effect, rendering it a potentially safe anticoagulant target.

Aims: To characterise the nature and role of FXIIa and VWF interactions.

Methods: In this study, we have expressed recombinant FXII in a human embryonal kidney cell expression system and subsequent to His-trap purification have investigated a novel binding interaction between VWF and Factor XII.

Results: Using static plate binding and FXII activation assays, we demonstrated that activated FXII (FXIIa) but not FXII is a previously unrecognised binding partner of VWF. Data show that FXIIa binds to plasma derived VWF and recombinant VWF but not to VWF lacking the A1 domain suggesting that the FXIIa binding site lays within the VWF A1 domain. In addition, initial flow assays demonstrated that the presence of FXIIa leads to enhanced platelet capture by VWF, indicating that the VWF-FXIIa interaction may play an essential role in thrombus formation and growth.

Conclusion: Based on these results, we hypothesise the interaction between VWF and FXII is an important component of thrombus formation and that inhibition of this interaction might be a novel and safe way to prevent thrombosis without increasing the risk of bleeding side effects.

P9

ADAMTS13 REQUESTS AND FINAL DIAGNOSIS: A REVIEW OF SAMPLES SENT TO QEHB LABORATORY

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Background: The ADAMTS 13 activity and inhibitor assays are performed to confirm or exclude a diagnosis of Thrombotic Thrombocytopenic Purpura (TTP). Since 2015, the Queen Elizabeth Hospital Birmingham (QEHB) laboratory has offered these assays to NHS Trusts in the West Midlands. This study is a review of samples that were referred for diagnosis from July 2015 to February 2017.

Aims: To describe the case mix and the laboratory parameters in patients whom ADAMTS13 requests are made in the West Midlands.

Methods: Cases were identified by interrogation of QEHB telepath for ADAMTS13 assays. Clinical diagnoses and outcome were confirmed by review of the clinical notes from referring centres, alongside laboratory parameters.

Results: Excluding surveillance of patients with known TTP, a total of 59 samples from nine NHS Trusts were processed during the time period. Twelve new cases of acquired TTP were identified. The four most common alternative diagnoses were: autoimmune cytopenias (n=6); pregnancy associated hypertensive disorders (n=5); sepsis related DIC (n=5); and severe haematinic deficiency (n=5). In four cases, no clear underlying diagnosis was identified. In non-TTP cases, the range of ADAMTS 13 activity was 26 to 107%, with a median value of 73 %. The median platelet count in patients with TTP was 11 (4-34), compared to 48 for alternative diagnoses (1-39). The median serum creatinine in patients with TTP was 77 (69-384), and in non-TTP patients this was 149 (40-986).

Conclusions: In our centre, 80% of acute requests for ADAMTS13 activity were within the normal limits. The cornerstone of TTP management is rapid institution of plasma exchange, a time and cost intensive treatment. The range of alternative diagnoses illustrates the importance of confirming or refuting TTP in a timely manner.
P10 STATINS INHIBIT PLATELET FUNCTION AND MODULATE CLOT FORMATION STRUCTURE AND STABILITY

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Statins inhibit the mevalonate pathway by impairing prenylation of proteins by depleting cells of lipid geranylgeranyl diphosphate (GGPP). Such proteins are Rab27b and Rap1a, small GTPase involved in the secretion of platelet granules and integrin activation. The aim of this study was to investigate the impact of statins on prenylation of small GTPase in platelets and downstream effects on fibrin clot stability.

ADP release from dense-granules of thrombin-stimulated platelet was significantly (P<0.001) impeded following overnight treatment with (10-40 µM) atorvastatin (ATV). Addition of GGPP rescued ADP release from dense-granules by 60% suggesting this mechanisms occurs via interference in the mevalonate pathway and the inhibition of Rab27b prenylation, as confirmed by Western blots analysis. ATV significantly attenuated α-granules release in thrombin-stimulated platelets, which was visualised as impaired accumulation of endogenous P-selectin, PAI-1 and fibrinogen on the activated membrane by confocal microscopy. Changes in αβγ integrin activation on the stimulated platelet surface, observed as defective binding of exogenous fibrinogen and PAC-1 by flow cytometry analysis, were also evident following treatment of platelets with ATV. Statins were also found to significantly inhibit thrombin-induced platelet aggregation by (20%, P<0.01) and (50%, P<0.001) following incubation of platelets overnight with 20 and 40 µM ATV respectively. Not surprisingly GGPP did not rescue platelet aggregation indicating that multi-mechanisms are involved in inhibition of platelet responses by statins.

PRP clots formed post-treatment with ATV were visualised by confocal microscopy and revealed significant alterations in clot structure; observed as thinner fibrin fibres and fewer platelet aggregates. Furthermore, PRP clots and whole blood Chandler model thrombi formed from overnight treated samples with ATV revealed faster lysis by tPA compared to the untreated samples.

Statins attenuate fibrinogen-binding to activated platelets, clot retraction, α-granule and dense-granule release. Statins were also found to alter fibrin clot characteristics in vitro, suggesting an alternative cardiovascular protective mechanism in vivo.

P11 THE IMPACT OF INTRODUCTION OF NOVEL ORAL ANTICOAGULANTS (NOACS) INTO A GENERAL PRACTICE AND A HOSPITAL ANTICOAGULANT SERVICE: TWO LOCAL SERVICE EVALUATIONS

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Background: Several NOACs have been licensed in the UK and they do not require routine anticoagulation monitoring. However, little has been reported on the impact of NOACs on UK anticoagulation services.

Aims: To evaluate the impact of NOACs on anticoagulation service resource use, capacity and patient experience in primary and secondary care.

Methods: Two local service evaluations were conducted: One hospital (service A) and one general practice anticoagulation service evaluation (service B). Evaluations included: Service mapping interviews describing the structure of service; retrospective medical records review describing treatments and resource use in two 6 month periods, one before (Jan-June 2012; pre-NOAC) and one after (Jan-June 2015; post-NOAC) NOAC introduction; and a patient experience survey.

Results: In service A: Post-NOAC, 87.6% of 508 anticoagulants initiated were NOACs. Post-NOAC, the service ran four fewer clinics per week vs pre-NOAC and, at the same time, the approximate number of patients managed by the service increased from 6000/year in 2012 to 9000/year in 2015. There were 1212 anticoagulation clinic contacts/month pre-NOAC (n=492) vs 207 post-NOAC (n=500) for newly initiated patients. There were 62 anticoagulation-related hospital inpatient admissions (n=494 patients) pre-NOAC vs 21 (n=500 patients) post-NOAC. 45.0% of 40 surveyed NOAC-treated patients had no questions/concerns vs 17.8% of 45 non-NOAC-treated patients.

In service B: Post-NOAC, 23.6% of 547 anticoagulants initiated were NOACs. Service structure and staffing remained constant post-NOAC vs pre-NOAC but n=509 patients were treated post-NOAC vs n=478 pre-NOAC. Mean visits per patient were 14.2 pre-NOAC (n=50) but post-NOAC, 0.8 for NOAC-treated and 16.1 for non-NOAC-treated patients (n=49). 71.9% of 32 surveyed NOAC-treated patients had no questions/concerns about their anticoagulant vs 15.9% of 44 non-NOAC-treated patients.

Conclusions: Introduction of NOACs in these two very different anticoagulation services was associated with an increase in patient throughput, reduction in healthcare resource use and fewer patient concerns/questions.
**P12**

**LACK OF INFORMATION WHEN PRESCRIBING DIRECT ORAL ANTICOAGULANTS (DOACS) COULD BE LEADING TO PATIENT HARM**

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**Background:** Direct oral anticoagulants (DOACs) are a major breakthrough in oral anticoagulation. They are more convenient for patients, more stable and cost-effective, when compared to warfarin. The indications and duration of treatment with DOACs is changing rapidly. But, how well is information being communicated with patients and other healthcare professionals once they are started on DOACs.

**Aims:** Our study looked at the information provided on discharge paperwork of inpatients discharged on direct oral anticoagulants, within our trust across all specialities.

**Methods:** Over a month period at a teaching hospital, the discharge summaries of all inpatients discharged on a direct oral anticoagulant were reviewed. We looked at if the indication, duration and review information was provided.

**Results:** 71 patients were discharged on direct anticoagulants during the month from our hospital. Of these 15 (21%) had no indication and 57 (80%) had no duration documented on the discharge summary or prescription section. Only 11 (15%) had details with regards to review information and appropriate investigations to be performed in the future.

**Summary/Conclusions:** The audit identifies a clear issue found with our discharge policy with regards to direct oral anticoagulants. Clearly this could lead to confusion in the community and lead to a possible significant event. We hope to improve the discharge process by educating doctors, pharmacists and implementing compulsory sections to the discharge summary. Without doubt DOACs provide a fantastic alternative to warfarin. However, the reduced amount of information provided compared to warfarin on discharge could lead to significant events for patients. We feel that this is something that needs to be addressed as use of DOACs is only likely to continue increasing.

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**P13**

**NEXT-GENERATION SEQUENCING IN PATIENTS WITH NO APPARENT LABORATORY PLATELET DEFECT DESPITE HAVING AN EXTENSIVE BLEEDING HISTORY**

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**Background:** Inherited bleeding disorders comprise a heterogeneous group of diseases that reflect abnormalities of blood vessels, coagulation proteins and platelets. Next-generation sequencing (NGS) technologies have been used previously for the rapid analysis of genes implicated in bleeding disorders and genes known to have a key role in haemostasis.

**Aims:** To determine the genetic aetiology of disease in a UK-wide cohort of patients with no apparent laboratory platelet defect despite having an extensive bleeding history leading to a clinical diagnosis of a platelet disorder.

**Methods:** Patient blood was obtained and analysed using a unique approach combining platelet phenotyping by lumaggregometry and genotyping by whole exome sequencing (WES), as outlined by the UK-Genotyping and Phenotyping of Platelets (GAPP) study.

**Results:** In the last 10 years, nearly 1000 patients have been recruited to the UK-GAPP study. Approximately 60% have no platelet defect despite significant bleeding consistent with platelet dysfunction. To date, patients with more than one affected family member in the ‘no defect’ group have undergone WES (n=17). We have identified and confirmed the genetic basis of disease in two related patients; a heterozygous genetic variant (c.1611 C>A) was found in THBD which encodes the protein thrombomodulin and results in a stop codon and truncation of the protein (p.Cys537Stop) showing proof-of-principle of the workflow employed. In addition, novel variants in genes not previously associated with haemostasis have been discovered through our bioinformatic pipeline. The function of these genes and their encoded proteins is under investigation.

**Conclusions:** WES combined with platelet phenotyping is an efficient method of determining the genetic cause of disease in patients with inherited bleeding disorders. In addition, it aids in the discovery of novel genetic variants and genes not previously implicated in haemostasis helping further our knowledge of haemostasis.
P14
THE SYNERGY BETWEEN TISSUE FACTOR-CONTAINING MICROVESICLES AND PAR2 ACTIVATION IN THE INDUCTION OF APOPTOSIS IS DEPENDENT ON THE PROPERTIES OF THE CANCER-DERIVED MICROVESICLES

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Background: We have previously shown that tissue factor (TF)-rich microvesicles (MV) can induce cellular apoptosis in endothelial cells. The induction of apoptosis appears to be dependent on the accumulation of TF, and is dissimilar in MV derived from different cells. Furthermore, escape from apoptosis appears to be associated with the ability of the recipient cells to dispose of excess TF, following activation.

Aims: To compare the MV derived from two cell lines and to contrast the potential of the MV to induce endothelial cell apoptosis.

Methods: MV were isolated from the conditioned media of pancreatic (BxPC-3) and kidney (786-O) cancer cell lines, by ultracentrifugation. MV were collected from resting cells, and following 30 min treatment with PAR2-activating peptide (SLIGKV; 20μM). The density of the MV was determined using the Zymuphen assay, and the associated TF antigen and activity measured by ELISA and thrombin-generation assay, respectively. Coronary-artery endothelial cells (HCAEC) were treated with a range of concentrations of TF-MV and cells apoptosis assessed by TUNEL assay. Moreover, in some cases HCAEC were pre-incubated with MV prior to activation and the rate of cell apoptosis determined.

Results: Measurement of MV density indicated greater release from BxPC-3 cells compared to 786-O cells and was reflected in the TF-MV antigen content and the associated thrombin generation. Following activation of PAR2, the TF release increased from 786-O cells but decreased in BxPC-3 cells. MV derived from BxPC-3 cells induced HCAEC apoptosis in a TF-concentration dependent manner. In contrast, maximal rate of HCAEC apoptosis was observed with 0.65 nM MV from 786-O cells and higher concentrations were less effective. Activation of HCAEC following incubation with MV, from either cell further enhanced the rate of apoptosis.

Conclusion: While the uptake of cancer-derived MV can overload ability of cells to manage TF, additional MV-associated factors appear to determine the pro-apoptotic potential of these MV.

P15
LOSS OF AGDV SEQUENCE AND INTERACTION WITH PLASMA FACTOR(S) CONTRIBUTE TO THE EFFECT OF FIBRINOGEN γ’ ON CLOT STRUCTURE AND FUNCTION

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Background: Fibrinogen (FGN) γ’ is a splice variant in which the C-terminal 4 residues of the γ-chain are replaced with 20 residues that are negatively charged. We previously showed that plasma-purified γA/γ’ FGN influences polymerisation and prototfibril formation in clots with reduced prototfibril packing and heterogeneous networks.

Aims: Determine the mechanism by which γ’ influences fibrin structure

Methods: Recombinant FGN with γ’-chain truncations (γ’0, 0-12 and 0-16: deleting all, 8 and 4 residues from the C-terminus respectively), full-length FGN γ’ and γA were produced in CHO cells as homodimers. Homogeneity was confirmed by SDS-PAGE. Clot structure was studied by turbidity (formation and fibrinolysis), confocal and scanning electron microscopy, and mechanical properties were investigated by magnetic tweezers.

Results: SDS-PAGE confirmed different γ’ lengths were produced with intact α and β-chains. γ’0 showed reduced maximum absorbance compared to γA (–FXIII). Average clotting rate was slower with increasing γ’ length, both +/- FXIII, compared to γA. No differences were seen with fibrinolysis for any truncations +/ FXIII compared to γA. Confocal microscopy showed no differences in the number of fibres. However, the truncated γ’ fibres were less straight compared to γA, and fibre straightness correlated negatively with γ’ length. With increasing γ’ extension a reduction of clot stiffness was observed. Turbidity with plasma deficient FGN incubated with the truncated FGN showed reduced maximum absorbance for all truncated FGN in comparison to γA.

Conclusion: Differences in clot structure for plasma purified γA/γ’ are not fully reproduced in this recombinant protein in a purified system but when truncated FGN was introduced to plasma deficient FGN a reduction in maximum absorbance was observed for all the truncations. These data indicate that the difference seen in γ’ could either be due to loss of the AGDV site or potential interactions occurring within the plasma.
P16
Tissue Factor Attenuates PI3K/Akt Pathway Through Upregulation of PTEN.

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Background: The PI3K/Akt pathway is crucial in cell survival, proliferation, and apoptosis. Tissue factor (TF) has been shown to promote endothelial cell proliferation at low concentrations and apoptosis at pathological concentrations. PTEN is the key regulator of the PI3K/Akt pathway and dephosphorylation of PTEN increases its lipid-phosphatase activity resulting in the disruption of PI3K/Akt function.

Aim: To examine the influence of TF on PTEN activation, and Akt activity.

Methods: Seven cell lines expressing PTEN and various levels of TF (LoVo, ASPC-1, MDA-MB-231, MCF-7, PANc-1, T47-D and Caco-2) were treated with low (0.5 U/ml) and high concentrations (10 U/ml) of recombinant TF (Dade-Innovin) for 60 min. Other sets of cells were treated with PAR2-activating peptide (SLIGKV; 20 μM) for up to 30 min. The cells were then lysed and the ratio of PTEN phosphorylation determined by western blot. Furthermore, the lipid-phosphatase activity of PTEN and the kinase activity of Akt were analysed using the PTEN-activity (echelon) and Akt-kinase activity (Enzo) kits, respectively.

Results: At lower concentrations (0.5 U/ml) of rTF the ratio of phospho-PTEN:total-PTEN was lowered in all cell lines tested. In agreement with this, the phosphatase activities of PTEN increased and was reflected in the lower Akt kinase activities. In contrast, incubation of cells with high concentrations of rTF (10 U/ml) resulted in PTEN phosphorylation in ASPC-1, MDA-MB-231, PANc-1 and Caco-2 cell lines, but remained similar to those with low rTF in LoVo, MCF-7 and T47-D cells. However, these were not proportionally reflected in the observed PTEN phosphatase activities, and Akt kinase activities. Moreover, activation of PAR2 differentially altered PTEN phosphorylation increasing PTEN activity in all cell lines except ASPC-1 and PANc-1 cells.

Conclusion: Exposure of cells to TF enhances the lipid-phosphatase activity of PTEN in short term, which results in lowered Akt kinase activity.

P17
The GP1BA-CRE Transgenic Mouse: A More Megakaryocyte-Specific Deleter Strain Than the PF4-CRE Mouse

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Background: Conditional knockout (KO) mouse models are invaluable tools for studying platelet production and function. However, it has emerged that the deleter strain of choice for generating megakaryocyte (MK)-specific KO mice, namely the Pf4-Cre transgenic mouse is leaky, casting ambiguity on some of the phenotypes that have been reported.

Aims: To provide an alternative and more lineage-specific means of generating MK-specific KO mice.

Methods: We produced a Gp1ba-Cre transgenic mouse, in which expression of Cre recombinase is driven by the endogenous Gp1ba locus. Tissue-specificity of the Gp1ba-Cre transgene was assessed by crossing the Gp1ba-Cre mouse with the mT/mG double-fluorescent Cre reporter mouse. The deletion efficiency the Gp1ba-Cre and Pf4-Cre transgenes was assessed by crossing mice with Csk- and Shp1-floxed mice and measuring platelet Csk and Shp1 levels by quantitative capillary-based immunoblotting (ProteinSimple Wes). Phenotypes of Gp1ba-Cre- and Pf4-Cre-generated KO mice were compared using standard platelet assays.

Results: Platelet count and function were normal in Gp1ba-Cre heterozygous mice. Virtually all platelets from these mice were GFP+, indicative of highly efficient recombination. Very few other haematopoietic lineages were GFP+, demonstrating the tissue-specificity of the Gp1ba-Cre transgene. Csk was deleted 93 and 99 percent in platelets when using the Gp1ba-Cre and Pf4-Cre deleter mice, respectively. Similar deletion efficiencies were observed with Shp1. As a consequence, platelet phenotypes of Gp1ba-Cre-generated KO mice were milder than those of Pf4-Cre-generated mice. However, additional inflammatory and immunological anomalies were observed in Pf4-Cre-generated KO mice, due to non-specific deletion of Csk and Shp1 in myeloid and lymphoid lineages. These inflammatory phenotypes were however not observed in Gp1ba-Cre-generated KO mice, demonstrating the superior specificity of this deleter strain.

Summary: The Gp1ba-Cre mouse provides a more specific means of generating MK-specific KO mouse models with fewer side-effects than the Pf4-Cre mouse.
P18

EXPRESSION OF THE VON WILLEBRAND FACTOR C-DOMAINS IN E.COLI

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Background: Von Willebrand Factor (VWF) is a large multidomain protein that performs essential roles in normal haemostasis. While crystal structures of the A1, A2 and A3 domains and the cysteine knot domain have been resolved, limited structural data exists on the rest of the molecule that is comprised of D and C domains. Such studies are hampered in part due to the large number of cysteine residues located in the D and C-domains. Recently the FVIII binding region in the VWF D’ domain was expressed in e.coli and used to derive structural NMR data suggesting that other cysteine rich domains of VWF could be produced in this way.

Aims: To express and purify the individual VWF C-domains

Methods: The cDNA for the C1, C2, C3, C4, C5 & C6 domains was cloned into the bacterial expression vector pET32-TRX creating fusions proteins with an N-terminal thioredoxin tag and a C-terminal His-tag. Proteins were expressed in E.coli Origami cells following induction with IPTG and proteins were purified by Nickel affinity and ion-exchange chromatography.

Results: With the exception of the C5 domain, all the VWF C-domains could be expressed at high levels in E.coli after 6 hours of expression and were found in both soluble and insoluble fractions. In vectors lacking the thioredoxin tag no protein was found in the soluble fraction and only minimal protein was observed in inclusion bodies. Proteins were purified by two passages over a nickel affinity column and following cleavage of the TRX tag, were purified to homogeneity using ion-exchange. All proteins demonstrated good expression levels ~1mg/100ml of culture. Finally the C4 domain which contains the RGD sequence was able to bind to immobilised GPIIbIIia confirming that the proteins were properly folded.

Summary/Conclusions: The isolated VWF C-domains can be expressed at high levels in e.coli cells and purified to homogeneity. Using this system we can now obtain structural information about this region of the VWF molecule.

P19

ANOMALOUS FIBRIN AMYLOID FORMATION IN THE PRESENCE OF FERRIC IRON, LIPOPOLYSACCHARIDE AND LIPOTEICHOIC ACIDS: ASSESSMENT WITH NOVEL AMYTRACKER™ STAINS AND THIOFLAVIN T

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Background: Most chronic diseases, including cardiovascular, neurodegenerative, or autoimmune diseases, are accompanied by long-term inflammation. Although typically mediated by ‘inflammatory’ cytokines, the origin of this inflammation is unclear. In recent work, we discovered that the presence of highly substoichiometric amounts (10^{-8} molar ratio) of lipopolysaccharide (LPS) from Gram-negative bacteria caused fibrinogen clotting to lead to the formation of an amyloid form of fibrin.

Aims: Here, we show that the broadly equivalent lipoteichoic acids (LTAs) from two species of Gram-positive bacteria have similarly (if not more) potent effects.

Methods: We added ferric iron, LTAs (from Staphylococcus aureus and Streptococcus pyogenes) and LPS (from E. coli) to healthy human plasma and detected amyloid formation using fluorescent signals and confocal microscopy. The fluorescent markers were thioflavin T (ThT) fluorescence, as well as luminescent conjugated oligothiophene dyes (LCOs), marketed under the trade name Amytracker™, which is also known to stain classical amyloid structures.

Results: We show all three bacterial wall inflamagens and iron, give very large fluorescence enhancements when clotting is initiated in plasma via thrombin. The staining patterns differ significantly as a function of both the amyloidogens and the dyes used to assess them, indicating clearly that the nature of the clots formed is different. This is also the case when clotting is measured viscometrically using thromboelastography.

Conclusion: Over all, the data provide further evidence for an important role of bacterial cell wall products and also increased iron, that are all observable in chronic, inflammatory diseases and the accompanying coagulopathies. The assays may have potential in both diagnostics and therapeutics.
P20
AN AUDIT OF URGENT CLOTTING REQUESTS FROM A LARGE METROPOLITAN EMERGENCY DEPARTMENT TO DETERMINE THE TRUE BURDEN OF CLINICALLY URGENT REQUESTS

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Background: RCPath A&E Key Performance Indicator (KPI) recommends a target for completion of 90% of clotting investigations within 1 hour of receipt by the laboratory. The Royal London Hospital A&E requests approximately 50 clotting screens per day. Local A&E and Haematology consultants’ consensus identifies limited situations when a clotting screen is clinically urgently indicated; active bleeding; trauma patient and/or suffering from a head injury, or if currently taking an anticoagulant. We hypothesise that there is over requesting of urgent clotting screens outside these 3 clinical scenarios.

Aims: review clinical indications for urgent clotting screen requesting on four separate days across the 4 quarters of 2016, stratifying into the 3 clinical situations justifying urgent clotting screening : 1) Active Bleeding 2) Head Injury or Trauma 3) concurrent Anticoagulant.

Methods: A&E clotting screens for one day of each quarter of 2016 was extracted from Barts Health NHS Trust PDW (Pathology Data Warehouse). Contemporaneous, A&E electronic patient records were cross-referenced to ascertain the reason for A&E attendance, with binary outcomes (‘Yes’ or ‘No’) for the 3 urgency criteria for requesting. It was then determined whether the patient was discharged or not, and if admitted whether under medical, surgical or obstetrics and gynaecological care.

Results: 214 patient records were reviewed. 52 patients (24.3%) were classifiable into one of the 3 clinically urgent criteria: 23 trauma, 17 head injury, and 24 concurrent anticoagulant. 140/214 (65.4%) patients were admitted: 69% medical; 27% surgical; 2% gynaecology; 2% other. Only 67 (31.3%) patients were discharged.

Summary/Conclusions: 75.7% of urgent A&E clotting screens did not meet the predetermined clinical scenarios justifying an urgency of an hour turnaround to support clinical management. We propose to work with A&E staff to share this, and further regular evaluation data, to influence assay-requesting behaviour and consequent laboratory KPI performance.

P21
EFFECT OF MUTATIONS IN THE FIBRINOGEN αR95G96D97 SEQUENCES ON CLOT STRUCTURE AND ON THE INTERACTION OF FIBRINOGEN WITH RED BLOOD CELLS

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Background: Recent data suggest a role for red blood cells (RBCs) in haemostasis and thrombosis through interactions of RBCs with the clot. RBCs are shaped into polyhedorocytes in the clot, RBC retention in the clot is dependent on FXIII, and fibrinogen has been reported to bind to RBCs. However, the binding site for RBCs on fibrinogen is unknown.

Aims: The aim of this study is to investigate the role of the fibrinogen α-chain R95G96D97 sequence, which is located in the coiled coil region, in clot structure and binding of fibrinogen to RBCs.

Methods: Three new mutations in the RGD sequence were produced: R95E, G96V and D97K. These mutations were stably transfected into βy-CHO cells, expressed in roller bottles and purified by IF1 affinity chromatography. SDS-PAGE and CD-spectra were performed to test the proteins for their primary and secondary structure compared to WT. The binding between fibrinogen and RBCs was tested using a plate binding assay. Turbidity and confocal microscopy was used to study the effect of the mutations on clot structure. Flow cytometry was used to confirm the binding of RBCs with fibrinogen.

Results: SDS-PAGE analysis showed high integrity and purity of the proteins, but the mobility of the α-chains appeared affected, indicating differences in polypeptide folding for all mutations. CD spectra showed the same pattern of α-helical content for each mutation as for the WT. Turbidity analysis showed reduced maximum absorbency for R95E and G96V and to a lesser extent for D97K compared to the wild type, indicative of thinner fibres. Flow cytometry confirmed specific interaction between RBCs and fibrinogen. Preliminary results of RBC binding indicate a reduction of D97K fibrinogen binding to the RBC.

Summary/Conclusions: Mutations in the fibrinogen αR95G96D97 sequence influence fibrin formation and clot structure and may be able to change RBC binding.
POSTERS

P22
VENOUS THROMBOEMBOLISM PREVENTION - CRUCIAL ROLE OF PHARMACY IN QUALITY IMPROVEMENT

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Hospital associated thromboses (HATs) are a potentially preventable cause of morbidity and mortality. Appropriate venous thromboembolism (VTE) prevention measures reduce the risk of HAT. The key patient safety audit for VTE prevention is whether inpatients receive appropriate thromboprophylaxis (TP).

Collaboration with pharmacy enabled development of a new robust quarterly audit of VTE prevention from July 2016 at Oxford University Hospitals (OUHFT). Ward pharmacists were trained in VTE risk assessment (RA) and undertook an audit of 10 patients per clinical area/ward across the Trust (approximately 400 patients). The audit evaluated whether a patient was prescribed and receiving ‘appropriate TP’ according to pharmacist assessment (thus independent of the clinical team and electronic VTE RA outcome). The details of any patient who was not considered to be receiving appropriate TP were immediately feedback to the clinical team responsible.

The audits demonstrate continuous improvement in patients receiving appropriate TP: overall appropriate TP was 94.2% July 2016, 94.8% October 2016, 96.7% January 2017, and 98.0% April 2017.

Strengths of this audit include the robustness of the data collected; immediate feedback to the responsible clinical team with regard to any patient not considered to be on appropriate TP; and upskilling pharmacists in VTE prevention providing additional daily safety net on the wards.

The improvement in appropriate TP is likely due to: feedback of robust data around TP; upsckilling pharmacists in VTE prevention; linking the ‘outcome recommendations’ of updated electronic VTE RA directly to electronic prescribing (implemented December 2016); and increased awareness and education.

These audits have provided robust high quality data, allowing assurance to the Trust on the percentage of inpatients receiving appropriate TP and crucially helping to drive quality improvement in patient safety. Improved appropriate TP at OUHFT should translate to fewer HATs and a reduction in associated morbidity and mortality.

P23
ANTICOAGULATION OPTIMISATION PROJECT – GP SURVEY

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Background: NICE guidance on atrial fibrillation (AF) recommends that patients on anticoagulation with warfarin should have time in therapeutic range (TTR) assessed. Patients with TTR <65% should be reviewed to try and improve control; for some direct oral anticoagulants (DOACs) may be suitable.

Oxford University Hospitals (OUHFT) provides a ‘dose and post’ warfarin service, managing 8000 patients (5600 with AF). Whilst this service benchmarks well (mean TTR 72%), analysis in 2016 showed that 2125 patients had TTR <65% (1500 with AF). Oxfordshire GPs are responsible for review of anticoagulation control and DOAC initiation. Informal feedback suggested not all GPs felt confident to do this.

Aim: To determine the anticoagulation educational and service needs of Oxfordshire GPs.

Method: OUHFT and Oxford Academic Health Science Network (AHSN) sent a questionnaire to all Oxfordshire GP practices in April 2016.

Results: 76 responses were received from individual GPs. 43.2% of GP respondents did not feel confident in assessing anticoagulation control on warfarin; 66.7% did not feel confident in knowledge of DOACs; 52.7% did not feel confident in prescribing DOACs. Additional services considered useful: email support (77.0%), telephone advice (48.7%), education (48.7%), specialist pharmacist outreach support (29.7%), centralised DOAC initiation (44.6%). 37 respondents provided comments which were thematically coded, some containing multiple codes. Themes included: education/outreach (13), centralised information resource and local guidance (11), time pressures (9), resources and cost (9), safety concerns (2).

Summary: These results led to collaborative project between OUHFT, Oxford AHSN and Oxford Clinical Commissioning Group to encourage anticoagulation optimisation. We have funding for 1.5 pharmacists for 1 year to provide an email/telephone service, outreach support and centralisation of guidelines. Feedback of the service so far is positive and TTR data will be reviewed regularly to analyse impact.

Supported by Medical Educational Goods Services (MEGS) grants from Pfizer and Daiichi-Sankyo.
POSTERS

P24
CLINICAL EVALUATION OF A 40 GENE INHERITED COAGULATION BLEEDING DISORDERS (ICBD) AND 57 GENE INHERITED PLATELET DISORDERS (IPD) NEXT GENERATION SEQUENCING (NGS) PANELS

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Introduction: Inherited bleeding disorders refer to disorders in coagulation factors, fibrinolytic system or platelet abnormalities. Whereas some are easily diagnosed through a combination of careful clinical assessment and variably complex laboratory analyses, some remain unclassified. Although improved phenotypic assays have enhanced diagnostic yield, genotyping now offers more accurate and complete diagnosis for some of these conditions. The advent of next generation sequencing technology enables routine analysis of many clinically relevant genes.

Aim: To technically validate two new IPD and ICBD targeted gene panels, screening 93 disease-implicated genes for the diagnosis of IBDs, and to assess diagnostic utility.

Methods: The validation cohort comprised 133 IBD patients, of whom 44 were undiagnosed. The diagnosed patient cohort was used to confirm the sensitivity and specificity of the NGS targeted panels. IPD and ICBD panels were designed to target 57 and 40 genes respectively. The library used Illumina TSCA kits v1.5. NGS was performed on the Illumina MiSeq, 2x150 cycles PE reads. Three validation runs for both panels were performed to determine technical performance and assay reliability. Basespace TruSeq® Amplicon App v1.1 was used to generate the BAM and VCF using the GATK pipeline with Refseq annotation using hg19. Coverage analysis was performed using CoverMi v1.3, Variant annotation used VariantStudio v2. Variant interpretation followed the ACMG guidelines.

Results: NGS panel variants pick up rate, validated using Sanger sequencing, demonstrated a 100% concordance. NGS panel approach increased the diagnostic yield of class 5 and 4 variants in previously undiagnosed patients especially patients with platelet disorders. The panel resolved one family who had unexplained macrothrombocytopenia, and found to have an AD, frameshift mutation in the TUBB1 gene.

Conclusion: The technical validation and increased diagnostic yield of the IBD targeted gene panels can be utilised as a rapid high-throughput NHS genomic service to improve diagnosis of IBDs.