BRITISH SOCIETY FOR HAEMOSTASIS AND THROMBOSIS

Annual Scientific Meeting 2023

Novel therapeutics & emerging technology in haemostasis & thrombosis

Wednesday 25 – Friday 27 January

Edgbaston Park Hotel & Conference Centre, Birmingham

Oral Communication abstracts 1-16

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OC 01

Impact of thromboprophylaxis on hospital acquired thrombosis following hospital discharge in patients admitted with COVID-19

Deepa J Arachchillage[1,2], Indika Rajakaruna[3], Zain Odho[4], Mike Laffan[1,2] on behalf of CA-COVID19 investigators

[1] *Department of immunology and inflammation, Centre for Haematology, Imperial College London, UK*
[2] *Department of Haematology, Imperial College Healthcare NHS Trust, London, UK*
[3] *University of East London, Department of Computer Science, London, University Way, London, UK.*
[4] *Department of Biochemistry, Royal Brompton Hospital, London, UK*

Background
Thrombosis is a major complication in patients admitted to hospitals with COVID-19. Thromboprophylaxis with either standard prophylactic or treatment dose low molecular weight heparin is given to all patients depending on the severity of the disease. However, post-discharge thromboprophylaxis remains controversial and guidelines suggested considering thromboprophylaxis in selected patients.

Aims
To assess the impact of thromboprophylaxis on hospital acquired thrombosis (HAT) in patients discharged from hospital following COVID-19 admission to hospitals compared to propensity matched cohort of patients discharged without hromboprophylaxis during the same period.

Methods
This is a multicentre observational study across 26 NHS Trusts. Data were collected both retrospectively and prospectively using a pre-designed standardised case record form. Adult patients (≥18 years) admitted with COVID19 between 1st of April 2020 and March 2021 were included.

Results
Overall, 8895 patients were included to the study. Of these, 971patients were discharged with thromboprophylaxis and propensity score matching (PSM) was performed using the nearest-neighbours method, with a desired ratio of 1:1 from patients discharged without thromboprophylaxis. Age, gender, body mass index, ethnicity, comorbidities including previous history of thrombosis, thromboprophylaxis and type during admission, treatment for COVID-19, intensive care treatment, thrombotic events, development of multiorgan failure, duration of admission and use of blood products and any other factors that may contribute to thrombosis following post-discharge were included in the PSM. Median age of patients discharged with thromboprophylaxis was 72years (range 18-84) vs 72years (18-83) without thromboprophylaxis and no difference in gender or duration of hospital stay. As expected by 1:1 PSM, no difference was observed in all other parameters between the two groups. Of patients discharged with thromboprophylaxis, 13/971(1.3%) developed HAT compared to 9 patients discharged without thromboprophylaxis (0.92%, 9/971), p=0.52.

Conclusions
Rate of HAT is low in patients with COVID -19, and thromboprophylaxis at discharge did not have a significant impact.

OC 02

HMB-001 – a novel bispecific antibody accumulating and targeting endogenous FVIIa to activated platelets supports enhanced haemostatic responses in models of Glanzmann thrombasthenia

Minka Zivkovic[1], Prafull S. Gandhi[2], Henrik Østergaard[2], Amalie C. Bonde[2], Catherine J. Rea[2], Benny Sørensen[2], Johan H. Faber[2], Roger E. G. Schutgens[1], Søren E. Bjørn[2], Rolf T. Urbanus[1]

[1] *Center for Benign Haematology, Thrombosis and Haemostasis, van Creveldkliniek, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX, Utrecht, the Netherlands.*

[2] *Hemab Therapeutics, Ole Maaløes Vej 3, 2200 Copenhagen, Denmark.*

Background
Glanzmann thrombasthenia (GT) is a severe platelet disorder caused by deficiency of fibrinogen receptor αIIbβ3. Acute bleeds in GT patients can be managed with recombinant factor VIIa (rFVIIa). HMB-001 is a novel bispecific antibody that binds to endogenous FVIIa with one arm and targets it to TLT-1 on activated platelets with its second arm. Multiple-dose subcutaneous administration of HMB-001 in cynomolgus monkeys showed accumulation of endogenous FVIIa. HMB-001 therefore has potential to treat and prevent bleeds in GT.

Aims
To evaluate the potentiation of FVIIa activity by HMB-001 in GT.

Methods
Platelet TLT-1 expression and plasma FVIIa levels were evaluated in GT patients and healthy controls. Targeting of rFVIIa to activated platelets by HMB-001 was evaluated with flow cytometry. Effects of HMB-001 were evaluated with light transmission aggregometry in αIIbβ3-inhibited (GT-like) platelets and in GT-like blood in a microfluidic flow chamber. Results were confirmed in blood of GT patients.

Results
TLT-1 expression on activated GT platelets was normal and plasma FVIIa levels in GT patients and healthy controls were similar. Flow cytometry showed increased binding of rFVIIa to activated platelets with HMB-001. Aggregation of activated GT-like platelets was absent but occurred when fibrin formation was initiated with rFVIIa. Aggregation onset shortened with increasing rFVIIa concentrations. HMB-001 potentiated effect of rFVIIa 10-fold and had no effect in absence of rFVIIa. Similar results were obtained with GT platelets. HMB-001 activity was TLT-1 dependent, as excess of soluble TLT-1 attenuated the HMB-001 activity. Perfusion of recalcified GT-like whole blood over a collagen surface resulted in platelet adhesion, but not fibrin formation. Addition of rFVIIa caused a dose-dependent increase in fibrin formation. HMB-001 strongly potentiated FVIIa-dependent fibrin formation on adhered platelets, which was confirmed in GT patient blood.

Summary/conclusions
HMB-001 potentiates FVIIa-mediated, fibrin-dependent platelet aggregation and enhances haemostatic responses in models of GT.

OC 03

Recombinant von willebrand factor (vonvendi) mediates reversal of platelet dysfunction induced by antiplatelet agents

Jo Mitchell,[1] Polly Whitworth,[2,3] Tanya Sage,[4] Neline Kriek,[4] Gemma Little,[4] Jonathan M Gibbins,[4] Michael J Desborough,[5,6] **Alex P Bye**[7]

[1]*Institute of Cardiovascular Sciences, College of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom*
[2]*Division of Cardiovascular Medicine, Radcliffe Department of Medicine, University of Oxford, Oxford, UK.*
[3]*Oxford Cardiovascular Clinical Research Facility (CCRF), John Radcliffe Hospital, Oxford, UK.*
[4]*Institute for Cardiovascular and Metabolic Research, Harborne Building, University of Reading, Reading*
[5]*Department of Clinical Haematology, Oxford University Hospitals NHS Foundation Trust, Oxford*
[6]*Radcliffe Department of Medicine, University of Oxford*
[7]*Institute of Molecular and Clinical Biosciences, St George's, University of London*

Background
Antiplatelet medications increase the risk of hemorrhage which can cause severe disability and death. There are no treatments available to reverse the effects of antiplatelet medication in cases of severe bleeding. Von Willebrand Factor (VWF) plays an important role in platelet adhesion to damaged tissue under high shear. Increasing plasma VWF above endogenous levels using recombinant VWF (rVWF/VONVENDI) could indirectly reverse the effects of antiplatelet agents.

Aims
Determine whether rVWF restores platelet function after antiplatelet treatment.

Methods
Blood samples from healthy donors treated *ex vivo* with aspirin (100µM), clopidogrel (1µM) or both (n=6) or cardiology patients receiving aspirin and/or a P2Y12 antagonist orally (n=20). Haemostasis was assessed using a thrombus formation assay and PFA-200, in the presence and absence of rVWF (0.5-5U/ml). Localisation of VWF and activation of intracellular signaling pathways in thrombi was assessed using immunocytochemistry. Addition of Alexaflour 647-labelled fibrinogen allowed measurement of fibrin formation and fibrinolysis using tissue-type plasminogen activator.

Results
Addition of rVWF to whole blood attenuated the reduction in thrombus volume caused by aspirin, cangrelor or both in healthy donors and in patients. VWF distribution throughout thrombi was increased upon addition of rVWF, indicating VWF enhances platelet-platelet interactions, in addition to increasing adhesion to collagen. Addition of rVWF enabled platelet adhesion without activation of platelet signaling molecules, PKC and tyrosine kinases, suggesting increased plasma VWF levels reduce the dependency of thrombus formation on platelet activation and signaling. Addition of rVWF altered fibrin structure and enhanced resistance of thrombi to fibrinolysis by forming more stable platelet-fibrin thrombi.

Conclusions
Increasing VWF above endogenous plasma levels indirectly reverses the effects of aspirin and P2Y12 inhibition on platelet function, this could provide an acute treatment option to limit hemorrhage.

OC 04

A microfluidic assay to investigate thrombogenesis in TTP patients

Adela Constantinescu-Bercu[1], Rens de Groot[1], Bertina Dragunaite[1], Sabina McCann[2], Raphael Sivera[1], Karen Vanhoorelbeke[3], Marie Scully[1]

[1]*Institute for Cardiovascular Science, University College London, London, UK*
[2]*Special Coagulation, Health Services Laboratories, UK*
[3]*Laboratory for Thrombosis Research, KU Leuven, Kortrijk, Belgium*

Background
Thrombotic thrombocytopenic purpura (TTP) patients with sustained low ADAMTS13 activity could benefit from an assay that more comprehensively monitors disease activity and risk of relapse.

Aim
To develop and optimise a dynamic assay for improved monitoring of TTP patients.

Methods
Microfluidic channels were coated with collagen or an antibody against VWF-A3 domain (82D6A). Blood was collected in citrate or lithium heparin from healthy volunteers, congenital (cTTP), or immune (iTTP) TTP patients with normal platelet counts, following informed consent. Fluorescently-labelled blood was perfused at $1800s^{-1}$, monitored in real-time and analysed using software developed in-house.

Results
125 patient samples (31 cTTP, 94 iTTP) and 52 healthy controls were analysed. Time-dependent platelet capture was observed in all conditions, forming thrombi on collagen, and small aggregates on anti-VWF A3. After 3 minutes of flow, platelet coverage was significantly increased in TTP samples with low ADAMTS13 activity (mean 22.5IU/dl) on both collagen and anti-VWF A3, with the latter correlating with VWF antigen levels. Thrombi forming on collagen presented different geometries in TTP patients with low ADAMTS13 activity, with significantly increased thrombi area mainly attributable to thrombi length. Thrombi length on collagen inversely correlated with ADAMTS13 activity and positively correlated with VWF antigen and activity levels, being a good indicator of disease activity in TTP patients close to relapse. Patients receiving Caplacizumab exhibited no platelet binding on either collagen or anti-VWF A3.

Conclusions
We present a new method to investigate VWF-dependent platelet recruitment under flow using an antibody against VWF-A3 domain to capture plasma VWF. Platelet surface coverage on this antibody, together with thrombi geometric parameters on collagen are good predictors for disease activity in TTP patients at risk of relapse. This assay could represent a new rapid and sensitive diagnostic tool to monitor TTP patients, and an important research tool to study thrombogenesis.

OC 05

Localisation and function of plasminogen within the thrombus milieu

Claire S. Whyte[1], Dean Kavanagh[2], Ausra S. Lionikiene[1], Steve P Watson[2], Robert J Parmer[3], Lindsey Miles[4], Nicola J. Mutch[1]

[1]*Aberdeen Cardiovascular & Diabetes Centre, Institute of Medical Sciences, University of Aberdeen, Aberdeen, UK*
[2]*Institute of Cardiovascular Sciences, College of Medical and Dental Sciences, University of Birmingham, Birmingham, UK*
[3]*Department of Medicine (9111H), University of California San Diego, and Veterans Administration San Diego Healthcare System, 3350 La Jolla Village Drive, San Diego, CA 92161.*
[4]*Department of Molecular Medicine, The Scripps Research Institute, 10550 N. Torrey Pines Rd., SP30-3020, La Jolla, CA 92037, USA.*

Background
Human and murine platelets express the transmembrane lysine-dependent plasminogen receptor, Plg-R_{KT}. This receptor functions to retain platelet-derived plasminogen on the activated platelet surface and can generate functional plasmin activity.

Aim
To define the importance of Plg-R_{KT} in the localisation and function of plasminogen within the thrombus microenvironment.

Method
Blood was collected from wild type (WT) or Plg-$R_{KT}^{-/-}$ deficient mice. Thrombi were formed *ex vivo by* flowing blood over collagen and tissue factor coated microfluidic chambers at 250 – 1000 s^{-1}. Dylight633 (DL633)-labelled plasminogen and AlexaFluor488-fibrinogen were incorporated ± tissue plasminogen activator (tPA). Thrombus formation was imaged using a fluorescence microscope. Thrombosis was induced in the carotid artery using 30% $FeCl_3$ with prior infusion of AlexaFluor647-labelled fibrinogen and DyLight488-GP1bβ antibody to label platelets. After 5 min tPA was infused. Thrombus formation was imaged for 30 min in real-time using a Zeiss Examiner upright intravital microscope equipped with spinning disk confocal.

Results
Shear strongly impacts on plasminogen accumulation in thrombi formed *ex vivo* and was significantly more abundant at high shear (1000 s^{-1}). Thrombi formed from Plg-$R_{KT}^{-/-}$ deficient mice demonstrate reduced plasminogen accumulation compared to WT mice. Initial fibrinogen accumulation in $FeCl_3$ induced thrombi was similar in Plg-$R_{KT}^{-/-}$ and WT mice. However, post tPA infusion, Plg-$R_{KT}^{-/-}$ mice demonstrate a clear increase in fibrin(ogen) accumulation compared to WT. This indicates that in Plg-$R_{KT}^{-/-}$ mice the fibrinolytic potential was reduced tipping the balance in favour of fibrin formation. Interestingly, initial platelet accumulation was higher in Plg-$R_{KT}^{-/-}$ mice compared to WT.

Conclusion
These data indicate that under physiological flow conditions Plg-R_{KT} functions to incorporate and retain plasminogen into the growing thrombus. A novel i*n vivo* thrombolysis model revealed that deficiency in Plg-R_{KT} impacts on initial platelet accumulation and fibrin deposition. These data demonstrate the functional importance of thrombus-associated plasminogen in clot integrity and persistence *in vivo*.

OC 06

NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome hyperactivation in megakaryocyte lineage induces anemia and enhances the inflammatory response in mice.

Joshua H. Bourne[1], Joana Campos[1], Sophie Hopkin[1], Katharine Whitworth[1], Yotis Senis[2], Julie Rayes[1], Asif Iqbal[1] and **Alexander Brill**[1]

[1] Institute of Cardiovascular Sciences, College of Medical and Dental Sciences, University of Birmingham, Birmingham, B15 2TT, United Kingdom
[2] Etablissement Français du Sang, Inserm UMR-S1255 Strasbourg, Université de Strasbourg, Strasbourg, France

Background
NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome is a protein complex activating one of the major proinflammatory cytokines, interleukin-1 b (IL-1b). Recently, platelets have been reported to contain NLRP3 components, but the (patho)physiological role of the inflammasome in platelets remains elusive.

Aims
To characterize a novel mouse model, Nlrp3$^{A350C/+}$GPIb-Cre$^{+/KI}$, mimicking human Wuckle-Wells syndrome.

Methods
We created the Nlrp3$^{A350C/+}$GPIb-Cre$^{+/KI}$ mouse model and tested their blood count, platelet function and response to zymosan-induced peritonitis.

Results
Platelets from the mutant mice had elevated levels of both procaspase-1 and active caspase-1, which is an indicator of NLRP3 inflammasome assembly. Plasma levels of IL-1b in unchallenged mice were unchanged. No difference in the platelet counts, expression of major platelet receptors, GPIb□, CD41, CLEC-2 and GPVI, or platelet deposition on a collagen surface at arterial shear rate was observed. The release of □-granules (as judged by surface P-selectin expression) was comparable in mutant vs. control platelets in response to either CRP or thrombin as well as aggregation induced by CRP, thrombin, U46619 (a thromboxane A agonist) and histone 3. Mutant mice had reduced erythrocyte count and hemoglobin content, and splenomegaly. The numbers of Ter119^{+} (a pan-erythroid marker) cells in the bone marrow (BM) of mutant mice were strongly reduced. The levels of BM TGF-b were moderately increased suggesting that hyperactive NLRP3 could modulate erythropoiesis through TGF-□ signaling. In a zymosan-induced peritonitis model, peritoneal lavage fluid from mutant mice contained elevated levels of IL-1b, IL-2, IL-6, and MCP-1 as well as increased numbers of CD45^{+} leukocytes consisting predominantly of Ly6G^{+} neutrophils.

Conclusions
Hyperactive NLRP3 in the MK/platelet lineage does not affect platelet functions but promotes mild anemia and splenomegaly and enhances response to a proinflammatory challenge. These findings reveal a novel and unexpected role of MK/platelet NLRP3 in erythropoiesis and inflammation.

OC 07

Pim kinase: A novel regulator of platelet and megakaryocyte thromboxane A2 and C-X-C-R motif receptors

Sophie Nock[1], Lawrence Hutchinson[2], Khalid Naseem[3], Sarah Jones[1], Stuart Mundell[2], Amanda Unsworth[1]

[1]Thrombosis Group, Faculty of Science and Engineering, Manchester Metropolitan University, [2]School of Physiology, Pharmacology & Neuroscience, Bristol, UK, [3]Leeds Institute of Cardiovascular and Metabolic Medicine (LICAMM), School of Medicine, University of Leeds

Introduction
Pim kinases have recently been identified to play a role in the regulation of platelet function and thrombosis. Deletion or inhibition of Pim-1 results in reduced thrombus formation without altered hemostasis suggesting it is a desirable antiplatelet target. In other cell types, Pim kinases have been shown to regulate CXCR4 and we have previously described, Pim-1 regulates platelet function via regulation of TPαR signalling. The mechanism by which Pim-1 regulates TPαR and CXCR4 signalling in platelets and megakaryocytes has yet to be elucidated.

Methods
HEK293 cells transfected with a Flag tagged TPαR or GFP-Tagged TPαR and MEG01 cells (expressing CXCR4) were treated with Pim kinase inhibitors (AZD1208, LGH447). Receptor levels (surface and total) were then assessed using both flow cytometry and microscopy. TxB2 generation was measured by ELISA and calcium mobilisation was measured to determine effects on downstream signalling.

Results
No alteration in TxB2 generation indicates Pim kinase regulates platelet TxA2 receptor signalling independently of COX1 regulation. Pim kinase inhibition causes internalisation of both the CXCR4 and TP receptors when assessed using flow cytometry and fluorescence microscopy. Consistent with the receptors being internalised, a reduction in TxA2 and SDF1a mediated calcium mobilisation, and phospho-PKC signalling was observed following treatment of platelets, HEK293 and MEG01 cells with Pim kinase inhibitors.

Discussion
Inhibition of Pim kinase attenuates TPαR and CXCR4 signalling via receptor internalisation. CXCR4 and TP receptor share similar consensus sequences, containing homologous serine residues within their intracellular loops suggesting that Pim could phosphorylate TPαR in a similar mechanism to CXCR4, regulating receptor expression at the platelet and megakaryocyte surface.

Reduction of platelet and megakaryocyte TPαR and CXCR4 receptor levels and signalling offers a novel platelet targeting strategy, especially in inflammatory conditions, such as rheumatoid arthritis and SLE that are associated with increased circulating plasma levels of thromboxane A2 and SDF-1a.

OC 08

Analysis of the Protein S - TFPIα Anticoagulant Pathway *In Vivo*

Parvathy Sasikumar*, Anastasis Petri *, Patricia Badia Folgado, David Jones, Josefin Ahnström, Isabelle I. Salles-Crawley & James TB Crawley

Centre for Haematology, Hammersmith Hospital Campus, Imperial College London, London, UK
** Authors contributed equally to this work*

Background
We recently developed an *in vivo* mouse thrombosis model that is highly sensitive to the concentration and anticoagulant function of human TFPIα (hTFPIα). This was achieved by blocking the endogenous murine TFPI (mTFPI) with an inhibitory monoclonal antibody (mAb) and co-injecting recombinant hTFPIα to restore the anticoagulant pathway. Our *in vitro* data support the hypothesis that TFPIα anticoagulant is highly dependent upon its endogenous cofactor protein S (PS). However, the importance of the TFPIα-PS pathway has not been assessed *in vivo*.

Aims
To explore the contribution of murine PS (mPS) enhancement of hTFPIα anticoagulant function *in vivo,* we used two strategies: 1) we used a recombinant C4BP β-chain to specifically block the endogenous mPS cofactor function. 2) we analysed the anticoagulant effect of the recombinant hTFPIα R199Q/E226Q variant, which has severely compromised ability to be enhanced by PS.

Methods
C4BP β-chain CCP1-CCP2 was expressed in insect cells, purified, and its affinity for recombinant mPS derived. Thereafter, its ability to inhibit the mPS cofactor enhancement of hTFPIα was assayed using thrombin generation assays. Recombinant hTFPIα R199Q/E226Q was expressed, purified and characterised using FXa inhibition and thrombin generation assays. Laser-induced thrombus formation was used to study the mPS cofactor function *in vivo* using these two complementary strategies.

Results
The C4BP β-chain bound to mPS with high affinity (K_D=0.21±0.04nM) and completely reversed the mPS enhancement of hTFPIα in thrombin generation assays. In mice where endogenous mTFPI was blocked, injection of recombinant hTFPIα dose-dependently reduced fibrin deposition. This was significantly reversed when the C4BP β-chain was co-injected with hTFPIα, revealing the important contribution of mPS in the enhancement of TFPIα anticoagulant pathway *in vivo*. *In vitro* assays revealed the hTFPIα R199Q/E226Q variant could not be enhanced by mPS. Comparison of WT and R199Q/E226Q hTFPIα was also performed in our mouse model which confirmed the importance of PS in augmenting TFPI function *in vivo* by modulating fibrin accumulation during laser-induced thrombus formation.

Conclusions
In this study, we developed the first mouse model that allows analysis TFPIα-PS pathway and show for the first time that PS can act as a cofactor for hTFPIα *in vivo* and plays a major role in controlling fibrin accumulation.

OC 09

Analysis of TFPIα Anticoagulant Function *In Vivo*

Anastasis Petri*, Parvathy Sasikumar*, Patricia Badia Folgado, David Jones, Isabelle I. Salles-Crawley, Josefin Ahnström & James TB Crawley

Centre for Haematology, Hammersmith Hospital Campus, Imperial College London, London, UK
** Authors contributed equally to this work*

Background
Tissue factor pathway inhibitor (TFPI) regulates coagulation through inhibition of the initiating TF-FVIIa complex. The most effective isoform in human plasma, TFPIα, contains three Kunitz (K) domains where K1 and K2 inhibit the TF-FVIIa and FXa, respectively, and K3 facilitates binding to its cofactor, protein S (PS). Understanding the role/importance of the TFPI anticoagulant pathway *in vivo* is challenging due to interspecies differences and the embryonic lethality of both *Tfpi*- and *Pros1*-deficient mice.

Aims
To develop a strategy to study the key role of human TFPIα (hTFPIα) anticoagulant function in mice.

Methods
To completely block TFPI anticoagulant function in mice, we generated and functionally characterised a monoclonal antibody (mAb) against the murine TFPI (mTFPI) K2 domain (14D1 mAb). To explore the role of plasma TFPIα, mice were injected with the 14D1 mAb (to block endogenous mTFPI) together with hTFPIα to restore the TFPI anticoagulant function in mice. Species compatibility between hTFPIα, the murine coagulation factors and, importantly, murine PS, was assessed using FXa inhibition assays and thrombin generation assays. The influence of injecting 14D1 mAb +/- hTFPIα on laser-induced thrombus formation was measured.

Results
The 14D1 mAb specifically bound to recombinant mTFPIα with high affinity (K_D=0.40±0.07nM) and inhibited its anticoagulant activity against FXa (IC_{50}=0.75±0.10nM). hTFPIα was efficiently enhanced by murine PS in FXa inhibition assays and thrombin generation assays, indistinguishably from human PS. In the laser-induced thrombosis model, injecting the 14D1 mAb alone inhibited the endogenous TFPI in mice and resulted in a profound increase in fibrin deposition at the site of injury. Co-injection of recombinant hTFPIα (0-4nM) in this thrombosis model dose-dependently reversed the increase in fibrin deposition, demonstrating the profound anticoagulant effect of plasma TFPIα *in vivo*.

Conclusions
We have, for the first time, developed an *in vivo* model in mice that is sensitive to the anticoagulant properties of hTFPIα in plasma. This approach reveals the importance of the plasma pool of TFPIα *in vivo.* Our results will pave the way for assessing its involvement in physiology/pathophysiology of thrombosis, its therapeutic potential, as well as the contribution of other cofactors like PS and FV-short, to the TFPI anticoagulant pathway.

OC 10

Cell-based high throughput screening to identify compounds that inhibit the endothelial pro-thrombotic switch during cytokine storms

Daisy Jones[1]; Gaye Saginc[1]; Claire Peghaire[1]; Daniela Pirri[1]; Edoardo Schneider[3]; Luca Braga[3]; Michael Appiah[2]; Tom McKinnon[2]; Ryan Thwaites[1]; Graeme Birdsey[1]; Mauro Giacca[3]; Anna M. Randi[1]

[1] *National Heart and Lung Institute; Imperial College London*
[2] *Department of Immunology and Inflammation, Imperial College London*
[3] *King's College London, British Heart Foundation Centre of Research Excellence, School of Cardiovascular Medicine & Sciences, London UK*

Background
Systemic inflammatory reactions with excessive release of pro-inflammatory cytokines ("cytokine storm") are linked to severe conditions such as ARDS, sepsis and COVID19. Cytokines are powerful activators of the endothelium; plasma of severe COVID19 was shown to activate endothelial cells (EC) *in vitro*. EC activation results in a pro-coagulant release of Von Willebrand Factor (VWF). High levels of plasma VWF in severe COVID19 patients indicate systemic endothelial activation and increased risk of thrombosis.

Aims
To identify drugs that inhibit endothelial activation (ICAM1 expression) and VWF release, which may have a therapeutic impact in patients with systemic inflammation and cytokine storm.

Methods
We established an *in vitro* model of endothelial activation to perform a high-content imaging screen of libraries containing 3049 molecular compounds. A cocktail of 6 cytokines, increased in COVID19 patients' plasma, was selected to treat cultured human umbilical vein EC (HUVEC) for 24hr. We investigated the effect of compounds on ICAM1 and VWF expression and release, as readouts of endothelial activation, by RT-qPCR, immunofluorescence (IF) and ELISA. We studied the functional consequences of VWF release using a platelet capture flow-based assay.

Results
Treatment with the 6-cytokine cocktail induced VWF release from EC and VWF-mediated platelet adhesion to HUVEC. We identified multiple compounds that partially restored intracellular VWF levels. We validated 4 "hit" compounds using IF, ELISA and tested their ability to reduce cytokine induced VWF release. We excluded compounds based on cellular toxicity. Top hits were from therapeutic classes including anti-inflammatory, anti-viral and hormones. We focused on a compound that inhibited cytokine-mediated VWF release, partially restored Weibel-Palade Bodies structures and VWF-mediated platelet adhesion formation *in vitro.*

Conclusions
This study identified candidate compounds that target endothelial dysfunction caused by a "cytokine storm", which might be beneficial in reducing pro-thrombotic endothelial activation in COVID19 patients.

OC 11

The TFPIα C-terminal tail is essential for the synergistic enhancement of TFPIα mediated inhibition of FXa by protein S and FV-short

Magdalena Gierula[1], Victoria M. Noakes[1], Isabelle I. Salles-Crawley[2], James T.B. Crawley[1], Josefin Ahnström[1]

[1]Centre for Haematology, Imperial College London, UK; [2]Molecular and Clinical Sciences Research Institute, St George's, University of London, UK

Background
TFPIα anticoagulant function is highly dependent on its synergistic cofactors, protein S (PS) and factor (F)V-short for efficient inhibition of FXa. Both cofactors interact with TFPIα, PS through Kunitz 3 residues Arg199 and Glu226 and FV-short with the TFPIα C-terminal tail. While the TFPIα-PS interaction is required for enhancement by PS alone, how FV-short further augments TFPIα function remains unclear.

Objective Aims
Determine the importance of the TFPIα-PS and TFPIα-FV-short interactions for synergistic enhancement of FXa inhibition.

Methods
To investigate how lack of the TFPIα-PS and TFPIα-FV-short interactions affects enhancement of TFPIα, two TFPIα variants were used: ΔPS, (R199Q/E226Q) or ΔCT (aa 1–249) that are unable to bind to PS or FV-short, respectively. The ability of the variants to bind FV-short and enhance TFPIα function was studied by plate binding and FXa inhibition assays in the presence and absence of PS and FV-short.

Results
WT TFPIα and TFPIα ΔPS bound to FV-short with high affinity (K_d in low nM range). As expected, TFPIα ΔCT did not bind to FV short. Both TFPIα ΔPS and TFPIα ΔCT inhibited FXa, with ΔCT showing moderately reduced activity (<2-fold) compared to WT. In the absence of FV-short, PS enhanced WT TFPIα with an EC50 of 11.95±0.92nM, with only minimal enhancement of TFPIα ΔPS. However, when FV-short was titrated in the presence of PS (5nM), FXa inhibition by TFPIα ΔPS was efficiently enhanced by PS (EC50: 0.46±0.1 and 0.68±0.04 for WT and TFPIα ΔPS, respectively), suggesting that FV-short rescues the synergistic enhancement when the TFPIα-PS interaction is absent. The inhibition of FXa by TFPIα ΔCT was not enhanced by increasing concentration of FV-short (0-4nM). In contrast to TFPIα ΔPS, the TFPIα ΔCT-mediated inhibition of FXa was not enhanced even in the presence of both cofactors.

Conclusions
The interaction between TFPI Kunitz 3 and PS is essential for PS-dependent enhancement of TFPIα function. However, in the presence of FV-short, this interaction is dispensable. Furthermore, the C-terminal tail of TFPIα is essential for the full anticoagulant function of TFPIα, for the interaction between FV-short and TFPIα and for the synergistic enhancement by PS and FV-short – highlighting the critical role of this region in regulation of coagulation.

OC 12

Phenotypic features and genetic analysis of heterozygous and homozygous variants in glycoprotein Ib platelet alpha subunit (*GP1BA*), glycoprotein Ib platelet beta subunit (*GP1BB*) and glycoprotein IX (*GP9*) genes in inherited macrothrombocytopenia patients

Catarina Monteiro[1,2,3,4], Ana Gonçalves[2,3,4], Mónica Pereira[1,3,4], Marta Gonçalves[3,4,5], Catarina Lau[3,4,5], Eugénia Cruz[1], Neil V. Morgan[6], Sara Morais[1,3,4], Rosário Santos[2,3,4]

[1]Unidade de Trombose e Hemostase, Serviço de Hematologia Clínica, Centro Hospitalar Universitário do Porto (CHUPorto), 4099-001 Porto, Portugal; [2]Unidade de Genética Molecular, Centro de Genética Médica Jacinto de Magalhães (CGM), CHUPorto; [3]UMIB - Unidade Multidisciplinar de Investigação Biomédica, ICBAS - Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, (UMIB/ICBAS/UP), 4050-313 Porto, Portugal; [4]ITR - Laboratory for Integrative and Translational Research in Population Health, Porto, Portugal; [5]Unidade de Diagnóstico Hematológico, Serviço de Hematologia Clínica, CHUPorto [6]Institute of Cardiovascular Sciences, College of Medical and Dental Sciences, University of Birmingham, Edgbaston,
Birmingham B15 2TT, United Kingdom.

Background: Platelet GPIb-IX-V is a surface adhesion receptor that mediates haemostasis by initiating platelet adhesion upon vascular damage. It consists of GPIbα (*GP1BA*), GPIbβ (*GP1BB*), GPIX (*GP9*) and GPV (*GP5*) subunits linked in a 2:2:2:1 ratio. Loss-of-function variants in *GP1BA*, *GP1BB* or *GP9*, affecting the complex assembly, can cause monoallelic or biallelic Bernard-Soulier syndrome (BSS), resulting in either absent, low expression or dysfunctional GPIb-IX-V. Gain-of-function (GoF) *GP1BA* variants are associated with an increased binding affinity for von Willebrand factor, resulting in platelet-type von Willebrand Disease (PT-VWD).

Aim: From a cohort of patients with presumed inherited macrothrombocytopenia, our study aimed to characterize the phenotypic and genetic basis of a group suspected of having *GP1BA*, *GP1BB* or *GP9* involvement.

Methods: The diagnostic suspicion was based on clinical data (family history, bleeding score), platelet counts and indexes [mean platelet volume (MPV) and immature platelet fraction (IPF)] and functional studies (ristocetin-induced platelet agglutination and glycoprotein quantification by flow cytometry). The recognition of clinically relevant variants was based on allele frequency, bioinformatics predictions and disease/family co-segregation analysis.

Results: Genetic studies allowed the identification of 11 BSS families, 2 associated with a *GP9* variant, 3 with *GP1BB* variants and 6 with *GP1BA* variants. Genotype-phenotype correlations shows differences in MPV, platelet counts and function between monoallelic and biallelic patients, with a more pronounced dysfunction in biallelic forms. An additional new GoF variant in *GP1BA* was identified in a case presenting as PT-VWD.

Summary: Upon clinical and genetic evaluation, we were able to obtain a diagnosis in a total of 24 cases, from 12 families, and 8 new variants (5 in *GP1BA*, 3 in *GP1BB*) were identified and presumed as causative. Mutagenesis studies in CHO cell lines are in course to assess the pathogenic nature of new *GP1BA* variants and the potential modifications in GPIb-IX-V assembly and/or function.

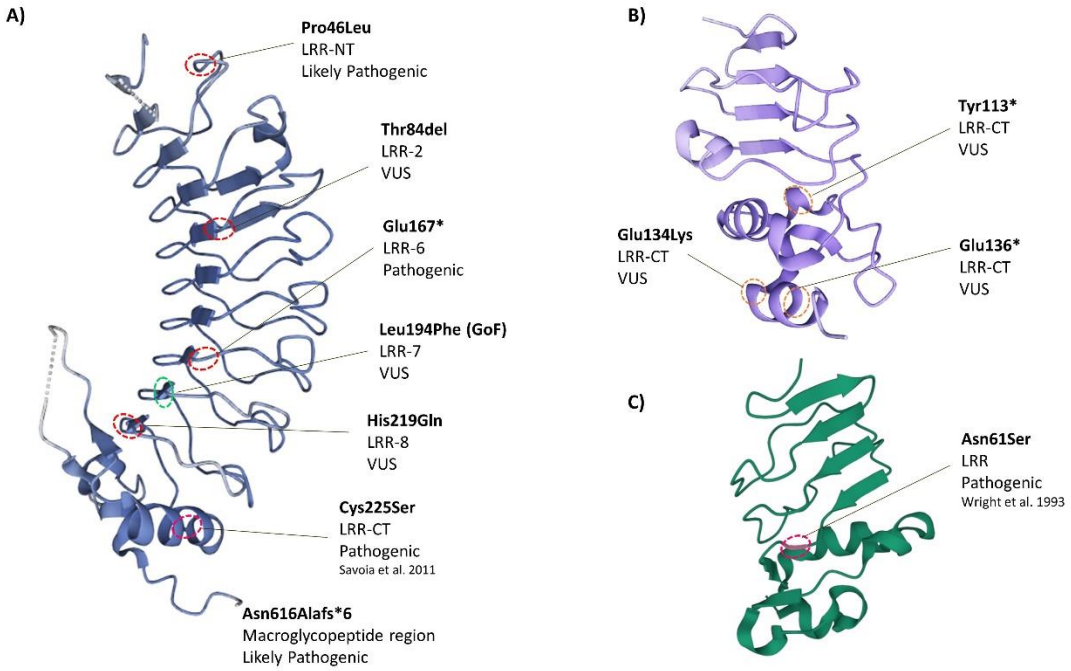

Figure 1: A) Ribbon drawing showing the crystal structure of the platelet glycoprotein Ibα (GPIbα) N-terminal region (Protein data bank code 1GWB). The location of the mutated residues is highlighted with a red doted circle for newly identified *GP1BA*, pink doted circle for the already known variant Cys225Ser (Savoia et al. 2011), and green doted circle representing the PT-VWD- associated new variant. A frameshift variant in the macroglycopeptide region was also found. **B)** Crystal structure of the platelet glycoprotein Ibβ ectodomain (GPIbβ) (Protein data bank code 3RFE). The location of the mutated residues is highlighted with an orange doted circle for newly identified *GP1BB* variants, all in the LRR-CT domain. **C)** Ribbon diagram of the crystal structure of the platelet glycoprotein IX (GPIX) (Protein data bank code 3REZ). The variant identified in two families was reported by Wright et al. 1993.

OC 13

A high degree of concordance in platelet activation between FcγRIIA stimuli.

Samantha J Montague[1], Ying Di[1], Eleyna M Martin[1], Richard Buka[1], Adam Cunningham[2], Jérôme Rollin[3], Caroline Vayne[3], Claire Pouplard[3], Yves Gruel[3], Phillip LR Nicolson[1,4], Steve P Watson[1]

1. *Institute of Cardiovascular Sciences, University of Birmingham, Birmingham, UK*
2. *Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, UK*
3. *Department of Haemostasis, Regional University Hospital Centre Tours, Tours, France*
4. *Institute of Cancer and Genome Sciences, University of Birmingham, Birmingham, UK*

Background

Activation of the low affinity immune receptor FcγRIIA on platelets underlies thrombosis and thrombocytopenia observed in immune-complex driven pathologies, including heparin-induced thrombocytopenia (HIT) and vaccine-induced immune thrombotic thrombocytopenia (VITT). Immune complexes are formed of platelet-factor-4 (PF4) and anti-PF4 antibodies and in HIT, with heparin, which bind, cluster and activate FcγRIIA. Despite similar exposure to heparin or vaccine, only a low proportion of patients develop HIT/VITT. Recently, monoclonal anti-PF4 antibodies 5B9 (HIT-like antibody) and 1E12 (VITT-like antibody) were generated to investigate mechanisms of platelet activation in HIT/VITT. Aside from these, there are limited FcγRIIA ligands. Further, only a proportion of individuals have platelets that are activated by immune/antibody complexes and HIT/ VITT serum (containing immune complexes), suggesting heterogeneity.

Aim

To explore whether individuals can be classified into responders or non-responders to different FcγRIIA stimuli. Platelet activation after stimulation with 5B9, 1E12 and isolated VITT antibody (6Ab) was compared in numerous individuals, to understand differences in responsiveness to FcγRIIA ligands.

Results

1E12 stimulated full aggregation and increased CD62P expression of washed platelets in all donors tested (n=5-7). 5B9 stimulated aggregation in 4/6 donors and CD62P expression in 5/5 donors in the presence of 0.5 U/mL heparin. 6Ab (isolated VITT antibody) stimulated aggregation in 6/8 donors and CD62P expression in 8/11 donors. We have also generated nanobodies raised against FcγRIIA and investigated their binding capacity in whole blood, in a FcγRIIA-transfected cell line and with a FcγRIIA ELISA. Four out of twenty nanobodies showed strong binding, with the strongest nanobody chosen for the generation of a tetravalent FcγRIIA nanobody.

Summary/Conclusions

The high degree of concordance between the FcγRIIA stimuli in most individuals regardless of platelet preparation indicates that a platelet-related factor(s) may determine whether an individual is a responder. Future work will explore the molecular basis of platelet-associated differences between responders and non-responders.

This study is funded by the Department of Health and Social Care (DHSC) and supported by the National Institute for Health Research (NIHR) (NIHR135073).

OC 14

Mechanisms of platelet accumulation in an endothelium-coated elastic vein valve model of deep vein thrombosis

Hosam Alden Baksamawi 1, *, Alessio Alexiadis 1, Daniele Vigolo 1,2,3 and Alexander Brill 4,*.

1. *School of Chemical Engineering, University of Birmingham, Birmingham B15 2TT, UK.*
2. *School of Biomedical Engineering, The University of Sydney, Sydney, NSW 2006, Australia.*
3. *The University of Sydney Nano Institute, University of Sydney, Sydney, NSW 2006, Australia.*
4. *Institute of Cardiovascular Sciences, College of Medical and Dental Sciences, University of Birmingham, Birmingham B15 2TT, UK;*

Background
Deep vein thrombosis (DVT) is a hectic medical problem taking the lives of millions of people around the world. DVT designated together with its main complication, pulmonary embolism (PE) as venous thromboembolism (VTE) develops in about 900,000 individuals in the US annually.

Aims
Given both technical and ethical issues in using animals in research, it is necessary to develop an appropriate in vitro model that would recapitulate the conditions of thrombus development in the vein.

Methods
We have developed a unique microfluidics chamber with moving valve leaflets to mimic the hydrodynamics of a large vein. The chamber was coated with a human umbilical vein endothelial cell (HUVEC) monolayer under constant flow of the medium. The monolayer was stable and did not denude under venous flow. A “back and forth” flow pattern, typical for the veins, was used in experiments.

Results
Unstimulated platelets reconstituted with the whole blood demonstrated moderate accumulation at the leaflet tips, which directly correlated with leaflet flexibility. Platelet activation by thrombin induced robust platelet accrual over the luminal side of the leaflets, but the conglomerate was unstable and gradually destroyed during a 12-min experiment. Inhibition of glycoprotein (GP) IIb-IIIa did not affect platelet accumulation. In contrast, blockade of the interaction between GPIba with A1 domain of von Willebrand factor completely abolished platelet deposition. Stimulation of the endothelium with histamine, a known secretaqoque of Weibel-Palade bodies, promoted platelet accrual at the basal side of leaflets, where human thrombi are usually observed.

Conclusions
Blood flow hydrodynamics plays a pivotal role in DVT initiation. Accumulation of activated platelets at the valve leaflets is mediated by GPIba-VWF interaction and occurs at the leaflets tips, whereas endothelial activation results in platelet accrual behind the leaflets.

OC 15

Fibrinolytic dysregulation in vaccine-induced immune thrombocytopenia and thrombosis (VITT)

Megan Simpson[1], Claire S Whyte[1], Anuj Narwal[1], Steven Humphreys[1], Andrew Page[2], Catherine N Bagot[3], Henry Watson[1], Nicola J Mutch[1]

[1]Aberdeen Cardiovascular & Diabetes Centre, Institute of Medical Sciences, School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Aberdeen, United Kingdom
[2] Royal Infirmary Edinburgh, Edinburgh, UK
[3]Department of Haematology, Glasgow Royal Infirmary, Glasgow, UK

Background

VITT clinically resembles autoimmune heparin-induced thrombocytopenia (HIT), in which antibodies against platelet factor 4 (PF4) provoke platelet activation. VITT is characterised by the presence of thrombosis, thrombocytopenia, hypofibrinogenemia and high D-dimer.

Aim

To identify the impact of VITT on the fibrinolytic arm of the haemostatic response.

Methods

Platelet-poor plasma (PPP) or serum was collected from 17 patients diagnosed with VITT. Whole blood (WB) or platelet-rich plasma (PRP) from healthy donors was spiked with VITT patient or control sera. Spiked PRP clots were formed with $CaCl_2$ ± Alteplase or Tenecteplase. PAI-1 activity and antigen, factor XIII, CRP (C-reactive protein) and cytokines were quantified by automated ELLA or ELISA and Western blots performed to probe for fibrinogen, plasminogen and PAI-1.

Results

Consistent with an inflammatory state, CRP and inflammatory cytokines (IL-1β, IL-6, IL-8 and TNFα) were elevated in VITT patients. Low fibrinogen, high D-dimer (35,000 ng/mL [IQR 8,965 – 41,078 ng/ml] and PAI-1 levels were observed. Spiking sera from VITT patients into PRP significantly delayed lysis in several patients compared to control sera. Delayed lysis could be attenuated by lysing with, Tenecteplase, a PAI-1 resistant form of recombinant tPA. Spiking healthy platelets with VITT sera increased PAI-1 activity and antigen when compared with control sera. Consistent with reduced fibrinogen observed in VITT patients there was a significant reduction in factor XIII and evidence of fibrinogenolysis.

Conclusions

VITT induces an inflammatory state that in several patients gives rise to elevated levels of PAI-1, perhaps reflecting platelet activation or endothelial release. In addition to elevated D-dimer there is evidence of fibrinogenolysis, indicative of dysregulated plasmin activity and reduced factor XIII. These data suggest that a feature of this heterogenous syndrome is derailment of fibrinolysis exacerbating the complex clinical phenotype of these patients.

OC 16

Thrombi from patients with acute myocardial infarction show fibrin film that increases with ischaemia duration

Cedric Duval, McPherson HR, Greenwood JP, Ariëns RAS

Leeds Thrombosis Collective, Discovery & Translational Science Department, Leeds Institute of Cardiovascular And Metabolic Medicine, University of Leeds, Leeds, United Kingdom.

Introduction

We have shown that fibrin makes a film on the surface of blood clots. Fibrin films are also present on thrombi extracted from patients with acute myocardial infarction and ischaemic stroke, although these observations were retrospective and descriptive respectively. Clinical and biochemical factors affecting fibrin film formation within the vasculature are unknown.

Aims

The aims of this study were to quantify the presence of fibrin films on thrombi from patients with acute myocardial infarction (MI), and to determine which clinical and laboratory parameters influence film coverage.

Methods

Patients (n=52) with acute ST-elevation MI were recruited after admission to Leeds Teaching Hospitals NHS Trust. Thrombi were obtained through aspiration thrombectomy, immediately washed in saline, fixed in 2% glutaraldehyde, dehydrated and subjected to critical point drying, prior to imaging by scanning electron microscopy. Peripheral plasma samples were taken from the antecubital vein and were used for turbidimetric analysis of clotting and lysis, analysis of clot fibre density by confocal microscopy, and determination of clot pore size by permeation. Data are expressed as median (range) and correlation was assessed by Spearman rank analysis.

Results

The majority (84.6%) of patients were male. Median age was 61 (31-88) and BMI was 27.1 (21-49). Film coverage ranged from 0.6-60.8% on all thrombi, with a median of 5.5%. Correlation of clinical data and film coverage showed a significant positive correlation of film coverage with ischaemia duration (between call for help and thrombus extraction (r=0.282 p=0.045). For the laboratory data, film coverage significantly correlated with lag-phase (r=0.489 p=0.005), time to 25% (r=0.430 p=0.014), 50% (r=0.555 p=0.001), 75% (r=0.508 p=0.003), 100% (r=0.404 p=0.022) MaxOD, and with time to reach Vmax (r=0.544, p=0.001).

Conclusions

These data show the presence of variable coverage with fibrin film on all acute MI thrombi. Film coverage increased with ischaemia duration and slower ex-vivo clotting.