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Abstract book

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Anticoagulation*inPractice*

Oral presentations Thursday

O1. VON WILLEBRAND FACTOR (VWF) DEFICIENCY CORRELATES WITH INCREASED ANGIOPOIETIN (ANG)-2 SYNTHESIS AND ANGIOGENESIS

Richard D Starke¹, Koval Smith¹, Koralia E Paschalaki^{1,2}, Thomas A J Mckinnon³, Carolyn M Millar³, Mike A Laffan³, and Anna M Randi¹

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Background: In endothelial cells (EC) VWF is stored within and directs Weibel-Palade body (WPB) formation. WPBs also contain the angiogenic regulator Ang-2 which binds to VWF. Decrease (type-1, partial, and type-3, total) or dysfunction (type-2) of VWF causes Von Willebrand disease (VWD). VWD can be associated with angiodyplastic vascular lesions causing intractable bleeding linked to dysregulated angiogenesis. We recently demonstrated that VWF-deficiency promotes angiogenesis, in vitro, in vivo and in blood outgrowth EC (BOEC) from VWD patients.

Aim: To investigate the regulation of Ang-2 by VWF in EC.

Methods: siRNA inhibited VWF in healthy-donor human umbilical vein EC (HUVEC). BOEC were cultured from blood of controls and VWD patients (4xtype-1, 3xtype-2M, 1xtype-2A) and 1xtype-3) to study Ang-2/VWF in relation to their previously characterised angiogenic phenotypes.

Results: Confocal microscopy demonstrated co-localisation of VWF/Ang-2 in HUVEC WPBs. Following PMA-stimulation, Ang-2 was released and localised to VWF-strings. VWF-deficient HUVEC showed a diffuse intracellular staining for Ang-2 protein and increased Ang-2 mRNA which correlated with a pro-angiogenic phenotype (proliferation/migration/capillary network on Matrigel). A similar correlation of increased Ang-2 expression with pro-angiogenic phenotype was seen in BOEC from a type-3 VWD patient. In healthy or type-2 VWD BOEC, the distribution and release of Ang-2 was similar to normal HUVEC; in type-2 BOEC, increased proliferation and migration were observed. Individual type-1 VWD patients showed different defects in VWF synthesis, WPB-formation and/or release. All showed normal Ang-2 mRNA, but 3/4 patients showed reduced PMA-stimulated release, consistent with their WPB phenotype. Type-1 VWD BOEC showed normal migration but elevated proliferation.

Summary/Conclusions: Increased Ang-2 expression correlated with increased angiogenesis in multiple

assays in VWF deficient cells (VWF siRNA-treated HUVEC and type-3 BOEC). No direct correlation was found between Ang-2 levels and angiogenic phenotypes in type-1 and 2 VWD BOEC.

O2. THE ROLE OF VON WILLEBRAND FACTOR IN FIBRIN CLOT FORMATION AND LYSIS

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Background Von Willebrand Factor (VWF) is known to interact with fibrin, however it is not fully understood if the presence of VWF alters the formation and structure of fibrin clots.

Aims To investigate the effect of VWF on fibrin clot formation

Methods Fibrin formation was assessed by the change in turbidity at 405nm and confocal microscopy. Purified clots were formed by incubating fibrinogen in the presence or absence of purified plasma VWF and thrombin. Fibrin formation in plasma was initiated by the addition of thrombin or sulphatides or tissue factor. For confocal microscopy fluorescently labelled fibrinogen was used. Incorporation of VWF into fibrin formation under shear stress was monitored in real-time by perfusing fluorescently labelled VWF over tissue factor coated surfaces.

Results Turbidity measurements of purified clots showed that VWF altered lag-time and maximum clot absorbance in a dose dependent manner. This was confirmed by confocal microscopy with the presence of VWF enhancing fibrin fibre formation and density. Similarly; the addition of purified VWF to VWF depleted plasma and initiation of fibrin formation with thrombin decreased lag-time and resulted in the formation of denser fibrin clots. Moreover the presence of VWF also affected clot lysis. This effect was more pronounced at lower thrombin and higher VWF concentrations. In plasma from a patient with severe type 1 VWD the lag-time of fibrin polymerisation initiated by thrombin was increased confirming the clinical importance of these observations. Interestingly, when clot formation was triggered with sulphatides (to activate FXII), the presence of VWF resulted in a denser clot structure; however in contrast, when clot formation was initiated with tissue factor, VWF still decreased the lag-time but resulted in the formation of a less dense clot structure. Finally, VWF was incorporated in fibrin clots formed under conditions of shear stress.

Summary/Conclusions Together these data demonstrate a crucial role for VWF in fibrin clot formation. Our data indicate that VWF has direct effects on fibrin polymerisation and that it is incorporated into the fibrin clot, modulating its structure and stability.

03. INTRADOMAIN HYDROPHOBIC INTERACTION OF N1574-GLCNAC GLYCAN STABILISES THE VWF A2 DOMAIN AND PREVENTS ADAMTS13 PROTEOLYSIS

Christopher Lynch and David A Lane. Centre for Haematology, Hammersmith Hospital Campus, Imperial College London.

Background: Shear forces in the blood trigger a conformational transition in the VWF A2 domain, from its native folded to an unfolded state, in which the cryptic scissile bond is exposed and is proteolysed by ADAMTS13. The conformational transition depends upon a Ca²⁺ binding site (CBS) and a vicinal cysteine disulphide bond (VicCC). N-linked glycosylation of VWF A2 domain at N1574 has also been shown to confer resistance to proteolysis, but with an uncertain mechanism as it is positioned away from known ADAMTS13 binding sites.

Aims: To investigate the mechanism by which N-linked glycosylation protects the VWF A2 domain from cleavage by ADAMTS13.

Methods: The VWF A2 domain and ADAMTS13 were expressed in mammalian cells. Thermal stability/unfolding (T_m) of VWFA2 was measured with SyproOrange. ADAMTS13 binding and cleavage were performed using established assays.

Results: Complete removal of glycan at N1574 (by mutagenesis, N1574Q, or PNGase treatment) destabilised the VWF A2 domain, ΔT_m 6-10°C. In contrast, mutation N1515Q, reduction and/or selective trimming using EndoH treatment of the VWFA2 glycan structure (to the residual GlcNAc residue) caused no reduction in stability. Modelling of N1574-GlcNAc linkage on the VWF A2 crystal structure indicated a potential interaction with the surface exposed, hydrophobic, Y1544. Substitution of the surface exposed 'hydrophobic patch' (Y1544D), recovered thermostability and prevented interaction with ADAMTS13 even in the absence of glycosylation. Destabilisation of the VWF A2 domain directly revealed ADAMTS13 binding site(s), with a strong correlation, r = -0.91, between T_m of VWF A2 variants and their solution binding (%).

Summary/Conclusions: Glycosylation at N1574 has previously been suggested to modulate VWF A2 domain interaction with ADAMTS13 through steric hindrance by the bulky carbohydrate structure. Here we show that only the first sugar residue (N1574-GlcNAc) is required to protect VWF A2 from ADAMTS13 proteolysis by providing stability to the VWF A2 domain. We propose that N1574-GlcNAc participates in an intradomain stabilising interaction with Y1544. This stabilising interaction helps to maintain the folded structure of VWF A2 and restricts ADAMTS13 binding and cleavage of the scissile bond.

04. THE SPACER-CUB DOMAIN INTERACTION OF ADAMTS13

Marta O Freitas, Kieron South, James TB Crawley and David A Lane. Centre for Haematology, Imperial College London.

Background – VWF cleaving protease, ADAMTS13, circulates in a “closed” conformation that is maintained by the interaction between its N-terminal spacer domain and C-terminal domains. ADAMTS13 requires conformational activation into an “open” form to become fully active. This conformational activation is mediated by the binding of VWF to ADAMTS13 C-terminal domains.

Aims - We aimed to characterize the interaction of ADAMTS13 spacer and its C-terminal CUB domains to further understand the molecular basis of ADAMTS13 conformational activation.

Methods – The affinity of the isolated ADAMTS13 CUB domains (CUB1, CUB2 and CUB1-2) to MDTCS (an ADAMTS13 N-terminal fragment lacking its C-terminal tail) was analysed by co-immunoprecipitation and SPR. The binding of ADAMTS13 and truncated versions lacking either CUB2 (ADAMTS13ΔCUB2) or CUB1-2 domains (ADAMTS13ΔCUB1-2) to the VWF-D4CK (the binding partner of the C-terminal domains of ADAMTS13) was analysed by SPR to establish which domains in ADAMTS13 bind VWF and in turn facilitate its conformational activation.

Results – Co-immunoprecipitation demonstrated that both CUB1 and CUB1-2 domains interact with MDTCS. SPR analysis shows that both CUB1 and CUB2 domains bind to WT-MDTCS but CUB1-2 exhibited a higher affinity, suggesting that both CUB domains mediate an interaction. CUB1 binding was abolished by mutation of ADAMTS13 spacer domain exosite, but this had no effect upon CUB2 binding. Finally, we show that the affinity of VWF-D4CK for ADAMTS13ΔCUB2 and ADAMTS13ΔCUB1-2 is similar (K_d of 176.9±31.6 nM and 181.8±25.3 nM, respectively) to its affinity for WT ADAMTS13 (K_d of 148.5±38.1 nM).

Summary/Conclusions – These results suggest that CUB1 domain mediates the interaction with the spacer domain that is further stabilised by CUB via a distinct binding site that allows ADAMTS13 to circulate in a “closed” conformation in the plasma. Conformational activation of ADAMTS13 by VWF-D4CK requires its binding to the non-CUB C-terminal domains (i.e the TSP2-8 domains), the identity of which is currently under investigation.

05. FACTORS AFFECTING THE CLEAVAGE OF VON WILLEBRAND FACTOR BY ADAMTS13 UNDER SHEAR STRESS

Tom McKinnon, Andria Demosthenous, Patricia Henne, Agata Nowak, Mike Laffan. Dept of Haematology, Imperial College London.

Background ADAMTS13 is the metalloproteinase that cleaves VWF in its A2 domain, regulating the multimeric size of VWF and thus its function. Many assays investigating the cleavage of VWF by ADAMTS13 are performed under static conditions that do not reflect the shear stress conditions under which this interaction physiologically occurs.

Aims To investigate the cleavage of VWF by ADAMTS13 in a shear stress based assay

Methods Plasma derived VWF was purified from Haemate P by gel filtration. Platelet derived VWF was obtained from lysed platelets. Recombinant (r) VWF and ADAMTS13 and their variants were expressed in HEK293T cells and purified by affinity chromatography. To assess ADAMTS13 cleavage under flow, plasma free blood supplemented with VWF with or without ADAMTS13 was perfused over collagen surfaces at high shear stress. Platelet capture was recorded in real-time and the extent of ADAMTS13 cleavage determined by the reduction in platelet surface coverage.

Results Addition of rADAMTS13 to whole blood or plasma free blood with VWF reduced the platelet surface coverage in a concentration dependent manner and the effect was more pronounced at higher shear rates. Interestingly and in contrast to static cleavage assays, the type 2A variants G1629E and E1638K and the VWF glycosylation variant N1574Q did not show enhanced cleavage by ADAMTS13 in this assay. However, consistent with static assays asialo-VWF lacking terminal sialic acid and platelet derived VWF was less susceptible to ADAMTS13 cleavage. To determine if ADAMTS13 was effective at cleaving a pre-formed thrombus; platelet rich or fibrin rich thrombi were formed under shear stress then ADAMTS13 perfused over. Significantly, ADAMTS13 was unable to reduce the size of a pre-formed thrombus even at high concentrations (200nM). Furthermore a gain-of-function ADAMTS13 variant lacking an N-linked glycan chain in the CUB-1 domain, although more effective than wild type ADAMTS13 at reducing VWF mediating platelet capture, was also unable to cleave pre-formed thrombi.

Summary/Conclusions This data demonstrates that cleavage data obtained under shear stress may not always correlate with static cleavage assays and moreover ADAMTS13 is effective at controlling the initial extent of platelet capture to VWF but is limited in its ability to reduce the size of a thrombus once it is formed.

06. A NOVEL ROLE OF ADAMTS13 IN THE REGULATION OF THROMBUS FORMATION AND STABILITY

Kieron South, Marta O Freitas, Christopher Lynch and David A Lane, Centre for Haematology, Imperial College London.

Background- ADAMTS13 is unusual amongst plasma proteases as it circulates with an active proteolytic domain and yet exhibits no known off target proteolytic activity. Recently we have shown that ADAMTS13 requires a substrate induced conformational change to attain full activity.

Aims—We aimed to establish whether ADAMTS13 substrate specificity is broadened on conformational activation and to identify any second proteolytic substrate. Furthermore, we aimed to ascertain how any new substrate might complement the VWF-ADAMTS13 axis.

Methods—Proteolysis by WT ADAMTS13 and the gain of function (GoF) spacer domain variant (R568K/F592Y/R660K/Y661F/Y665F) was determined by SDS PAGE. Binding of ADAMTS13 was determined by SPR. Polymerisation assays and flow assays of clot deposition were used to monitor ADAMTS13 proteolysis of clot formation and clearance.

Results— ADAMTS13 proteolyzes fibrinogen in its A α -chain, resulting in cleavage products of ~25 kD and ~40 kD. Cleavage is inhibited by mAb directed against its protease domain. Proteolysis of fibrinogen by WT ADAMTS13 is inefficient, but is enhanced by conformational activation, i.e. in samples treated with GoF ADAMTS13. WT ADAMTS13 binds to fibrinogen with moderate affinity, a K_D of 153.3 \pm 39.4 nM, which results in saturation of the ADAMTS13 by the high concentration (~9 μ M) of fibrinogen. ADAMTS13 cleaving activity specifically impairs fibrin clot formation and under flow it can enhance clearance of fibrin clots. The EC₅₀ of the GoF variant in these assays is ~8 times lower than that of WT ADAMTS13 (1.7 \pm 0.7 nM and 13.2 \pm 0.1 nM, respectively).

Summary/conclusions— ADAMTS13 exhibits proteolytic activity against fibrinogen and this is enhanced upon conformational activation. As ADAMTS13 is saturated by fibrinogen, the latter may serve as a transport vehicle to the site of developing clot formation. This work suggests that the role of ADAMTS13 in the regulation of clot formation may extend beyond VWF cleavage. These findings may be important in the development of ADAMTS13 as an anti-thrombotic agent.

Oral presentations Friday

07. OPPOSING ROLES OF GLYCOGEN SYNTHASE KINASE-3 PHOSPHORYLATION IN PAR AND GPVI MEDIATED PLATELET FUNCTION AND THROMBOSIS

S.F. Moore, T.A. Blair, I. Hers. School of Physiology and Pharmacology, University of Bristol, Biomedical Sciences Building, University Walk, Bristol..

Background: A common event in platelet activation is the activation of phosphoinositide 3-kinase (PI3K) and its downstream effector Akt. One of the major substrates of Akt is the constitutively active Ser/Thr kinase glycogen synthase kinase-3 (GSK3 α/β). Akt phosphorylates GSK3 α/β at Ser21/9 in the NH₂-terminus resulting in a loss of GSK3 α/β activity against a subset of substrates. We hypothesise that GSK3 α/β is a negative regulator of platelet function and phosphorylation is required to promote/support platelet activation.

Aims: To identify the role of GSK3 α/β phosphorylation in platelet activation and thrombus formation.

Methods: Platelet function (aggregation, ATP-secretion, integrin activation (JON/A binding) and P-selectin exposure) and thrombosis (in vitro flow studies) were examined using platelets from wild-type (WT) and GSK3 α/β knock-in (KI) mice. GSK3 KI mice express a non-phosphorylatable form (GSK3 α Ser21Ala/GSK3 β Ser9Ala) of GSK3.

Results: Human and mouse platelets express both GSK3 α and GSK3 β with GSK3 β being the more abundant isoform. Both GSK3 α and GSK3 β are phosphorylated by a range of platelet agonists at Ser21/9. Phosphorylation induced by thrombin was dependent on both PKC α and PI3K/Akt activation whereas collagen-related peptide (CRP-XL) mediated phosphorylation required only PI3K/Akt activation. As expected, agonist-mediated phosphorylation of Ser21/9 was absent in GSK3 KIs, whereas total GSK3 α/β levels were unaltered.

Thrombin-mediated platelet activation was reduced in GSK3 KI platelets, which was rescued by treating platelets with the potent and selective ATP-competitive GSK3 inhibitor CHIR-99021. In contrast, collagen-related peptide (CRP-XL)-mediated platelet activation was dramatically enhanced in GSK3 KI platelets. This occurred with a parallel increase in phosphorylation of Akt at Ser473. Furthermore, thrombus formation on a collagen-coated surface was increased when using blood from GSK3 KI mice. Intriguingly inhibition of GSK3 activity with CHIR-99021 did not significantly alter CRP-XL-mediated platelet activation in both WT and KI.

Conclusions: GSK3 α/β phosphorylation supports PAR-mediated platelet function but restrains GPVI-mediated platelet activation and thrombus formation.

08. INHIBITION OF THROMBIN-ACTIVATABLE FIBRINOLYSIS INHIBITOR AFFECTS ANEURYSM DEVELOPMENT BUT NOT PROGRESSION IN THE ANGIOTENSIN II MODEL OF AAA

Katherine I Bridge^{1,2}, Charlotte Revill⁴, Marc A Bailey^{1,2}, Nadira Yuldasheva¹, Stephen Wheatcroft¹, Roger Butlin⁴, Richard Foster⁴, D Julian Scott^{1,2}, Ann Gils³, Robert Ariëns¹. 1. Theme Thrombosis, Division of Cardiovascular and Diabetes Research, University of Leeds. 2. The Leeds Vascular Institute, Leeds General Infirmary. 3. KU Leuven- University of Leuven, Department of Pharmaceutical and Pharmacological Sciences, Laboratory for Therapeutic and Diagnostic Antibodies, B-3000 Leuven, Belgium; 4. School of Chemistry, University of Leeds.

Background Thrombin-Activatable Fibrinolysis Inhibitor (TAFI) is an inhibitor of fibrinolysis and inflammation. Mice deficient in TAFI develop larger abdominal aortic aneurysms (AAA) after elastase infusion than controls.

Aims The aim of this study was to investigate the effect of TAFI inhibition on AAA formation *in-vivo*.

Methods Male, ApoE^{-/-} mice were infused with Angiotensin II (AngII) using subcutaneous mini-osmotic pumps, in combination with either MA-TCK26D6, an antibody which inhibits plasmin-mediated TAFI activation, or.396082, a direct small molecule inhibitor of the active site of TAFI. Incidence of AAA rupture was recorded. After 28 days, blood was collected from the IVC, and aortas photographed in situ. Aortic size was measured using Image J. A subset of animals received late treatment with MA-TCK26D6, 7 days after AngII infusion was initiated. AAA development in this group was monitored using Vevo2100 Ultrasound scanning.

Results Mice receiving either inhibitor developed normally, without increases in blood pressure or heart rate, and no episodes of spontaneous bleeding. Administration of either MA-TCK26D6 or.396082 led to a decrease in mortality secondary to aortic rupture (from 36.8% to 10.0% and 8.3% respectively). The incidence of AAA decreased with administration of MA-TCK26D6 (58.3% to 33.0%), but increased with.396082 (58.3% to 81.8%). Where AAA did occur, neither inhibitor resulted in a change in AAA size. Late treatment with MA-TCK26D6 had no effect on AAA progression in this model.

Summary/Conclusions Inhibition of TAFI *in-vivo* resulted in a decrease in mortality. Inhibition of plasmin-mediated activation of TAFI resulted in a decrease in the incidence of AAA likely due to localised fibrinolysis with unaltered inflammatory activity, whereas inhibition of all TAFI activity resulted in an increase in AAA formation, likely due to increased inflammatory response. Inhibition of TAFI after AAA development had no effect on progression.

09. ENDOTHELIAL BAMBI (BMP AND ACTIVIN MEMBRANE BOUND INHIBITOR) IS IMPORTANT FOR FIBRIN GENERATION AND THROMBUS STABILITY

Isabelle I. Salles-Crawley¹, James H. Monkman¹, Argita Zalli¹, David A. Lane and James T.B. Crawley¹. ¹ Centre for Haematology, Hammersmith Hospital Campus, Imperial College London.

Background BAMBI is a transmembrane protein related to the transforming growth factor- β superfamily, and is highly expressed in platelets and endothelial cells (EC). Using Bambi-deficient mice, we recently showed that BAMBI plays a role in haemostasis and also in thrombus stability. Using chimeric mice, we also demonstrated that it is most likely BAMBI present in the endothelium that influences the haemostatic response and thrombus stability.

Aims This study aims to delineate the mechanisms by which BAMBI exerts its function on the endothelium and how these influence thrombus formation and stability.

Methods Mouse plasma prostacyclin and nitric oxide (NO) levels were determined in vitro. EC were isolated from lungs or brains of Bambi^{+/+} and Bambi^{-/-} mice (MLEC and MBEC) and expression of adhesion molecules was assessed by flow cytometry. Mice were subjected to the laser-induced thrombosis model of the cremaster arterioles.

Results While no difference in levels of prostacyclin could be observed, plasma NO levels were significantly increased (~50%) in Bambi^{-/-} mice compared to wild-type (WT) littermates. Results from separate EC isolations showed that Bambi^{-/-} MLEC expressed lower levels of EC adhesion molecules PECAM1 and ICAM2 compared to Bambi^{+/+} MLEC. After laser injury of the endothelium, Bambi^{-/-} mice exhibited thrombus instability accompanied with a defect in fibrin(ogen) accumulation compared to Bambi^{+/+} littermates. Injection of hirudin prior to thrombus formation in WT mice recapitulated the Bambi^{-/-} thrombus instability phenotype while it had no effect in Bambi^{-/-} mice.

Summary/Conclusions We provide first evidence on how endothelial BAMBI may influence thrombus stability and hemostasis by modulating NO release and the expression levels of EC adhesion molecules. Bambi-deficient mice have a markedly diminished ability to accumulate fibrin during thrombus formation, most likely due to reduced thrombin generation. We are currently investigating how the level of endothelial and platelet activation as well as neutrophils recruitment into the thrombus can impact upon thrombus stability in Bambi-deficient mice.

010. NEXT GENERATION DNA SEQUENCING PANEL FOR HAEMOSTATIC AND PLATELET DISORDERS IN ROUTINE DIAGNOSTIC SERVICE

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Background: Next Generation sequencing (NGS) is transforming delivery of diagnostic molecular genetics. Gene panels are being utilised to analyse groups of related disorders and to extend diagnostic capability in comparison with previous Sanger sequencing.

Aims: To examine the utility of a 13 gene panel for diagnostic/research NGS analysis of haemostatic and platelet disorders.

Patients and Methods: SureDesign software (Agilent) was used to select clinically relevant areas of each gene to design 120mer biotinylated cRNA baits; the gene panel comprised *ADAMTS13*, *F5*, *F8*, *F9*, *F13A1*, *F13B*, *FGA*, *FGB*, *FGG*, *ITGA2B*, *ITGB3*, *MYH9* and *VWF*. *F9* was included for patients where haemophilia type was unclear. The service was introduced from January 2015 and patients were analysed for only those genes associated with their particular disorder. Sequencing was undertaken using Illumina MiSeq or HiSeq (≤ 96 samples/run). Common sequence variants were removed by filtering and remaining variants examined for possible pathogenicity.

Results: Requests were received for all but one (*F5*) genes on the panel. Candidate pathogenic mutations were identified in 24 of 39 patients (62%), including 15/16 males diagnosed with haemophilia A and 3/7 individuals with possible von Willebrand disease. Some analyses (e.g. *ADAMTS13*, *MYH9*, *VWF*) were requested to help exclude specific diagnoses. In addition to analysis of single genes, combinations were analysed simultaneously, e.g. *F8* & *F9* (possible carrier relative of deceased haemophilia patient; unknown type); *F8*, *F9*, *F13A1*, *F13B* and *VWF* (baby died of bleeding shortly after birth), *F8* & *VWF* (low FVIII:C).

Conclusions: NGS provides a single laboratory workflow for analysis of gene panels for related disorders as well as for whole genomes/exomes. Data analysis can include a single gene, such as *ADAMTS13*, or ≥ 1 gene for disorders such as those affecting fibrinogen. Use of NGS provides a single laboratory workflow for analysis of gene panels for many different disorders.

O11. STATINS ATTENUATE PRENYLATION OF RAB27B THEREBY IMPEDING RELEASE OF DENSE GRANULES FROM ACTIVATED PLATELETS

MM Jalal, FP Coxon and NJ Mutch. *Institute of Medical Sciences, University of Aberdeen, Aberdeen.*

Background: Statins inhibit HMGCoA reductase in the mevalonate pathway that is necessary for cholesterol synthesis. Inhibition of this pathway by statins also impairs prenylation of proteins by depleting cells of the lipid geranylgeranyl diphosphate (GGPP); prenylation involves the covalent attachment of GGPP to specific target proteins, enabling their association with target membranes. One such protein is Rab27b, a GTPase involved in regulating the secretion of platelet dense granules, which contain molecules important for platelet aggregation such as ADP, serotonin and polyphosphate.

Aims: Here we investigate whether statins directly interfere with prenylation of Rab27b in platelets and whether this inhibits dense granule release and platelet function.

Methods: Washed platelets were prepared from fresh blood obtained from healthy donors or were kindly provided by the blood bank. Unprenylated Rab27b was detected in platelet lysates by Western blotting. Light transmission aggregometry was used to investigate platelet aggregation. An ADP release assay was used to quantify dense granule secretion and flow cytometry to monitor binding of fibrinogen to activated platelets.

Results: Treatment of platelets for 24 h with atorvastatin (ATV) induced a dose-dependent accumulation of unprenylated Rab27b. Therapeutically relevant concentrations of ATV (1-10 μ M) significantly inhibited thrombin-induced platelet aggregation ($p < 0.005$) and ADP release ($p < 0.005$). Furthermore, pre-treatment with ATV for 24 h significantly reduced thrombin-stimulated binding of exogenous fibrinogen to platelets ($p < 0.05$). Co-treatment with GGPP rescued prenylation of Rab27b and restored ADP release in platelets treated with ATV, but surprisingly did not rescue the effect of ATV on platelet aggregation.

Conclusions: Statins impede dense granule release, fibrinogen binding and platelet aggregation *in vitro*, suggesting that these drugs may directly inhibit platelet function *in vivo*. It is likely that the effects on dense granule release are the result of inhibition of Rab27b prenylation.

O12. HAEMATOCRIT MODULATES CLOT FORMATION AND STABILITY AGAINST DEGRADATION

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Background: Red blood cells (RBCs) have been considered a relatively inert bystander in coagulation and thrombus formation, yet their sheer abundance in blood means that they are a dominant element in the resulting clot.

Aims: To examine the influence of haematocrit (HCT) on coagulation parameters, clot firmness and resistance of thrombi to fibrinolysis.

Methods: Whole blood drawn from healthy volunteers was separated into plasma and RBC constituents by centrifugation. Samples were reconstituted with 35% platelet rich plasma and RBCs to produce 20%, 40% and 60% final HCT. Thrombus lysis was assessed using the Chandler loop method and coagulation parameters using thromboelastography.

Results: Chandler model thrombi formed at both arterial and venous shear rates with 20% HCT were longer than those formed at 60% HCT ($p < 0.05$) and demonstrated increased resistance to lysis with tPA ($p < 0.005$). Inclusion of a transglutaminase (TG) inhibitor, to inhibit factor XIIIa, significantly augmented lysis of 20% HCT thrombi ($p < 0.001$) and to a lesser degree at 40% HCT thrombi ($p < 0.01$). In contrast inclusion of the TG inhibitor did not impact lysis of 60% HCT thrombi ($p = 0.113$). In the presence of the TG inhibitor equivalent lysis rates were observed at all HCT, suggesting that inhibition of factor XIIIa overcomes the stabilizing effect of HCT on lysis. Consistent with this western blots demonstrate an increase in cross-linked fibrin and $\alpha 2$ -antiplasmin in thrombi formed at 20% HCT. Thromboelastography indicated faster clot formation at low HCT and an increase in maximum clot firmness at 20% vs 60% HCT.

Summary/Conclusions: HCT has a dramatic impact on thrombus formation and stability, with lower HCT enhancing clot formation, resulting in thrombi with increased firmness and resistance to fibrinolysis. The impact of FXIII on stabilization of thrombi is dependent on RBC content of thrombi. These changes may help explain the increased risk of thrombosis in anaemia.

Scientists in Training Symposium Friday

O13. NEUTROPHIL EXTRACELLULAR TRAP (NET) FORMATION IN PATIENTS WITH BURN INJURY

Dinsdale RJ^{1,2}, Hampson P^{1,2}, Wearn CM^{1,2}, Hazeldine J^{1,3}, Bishop J³, Brill A⁴, Lord JM^{1,2}, Moiemmen N², Harrison P^{1,2}. ¹ Centre for Translational Inflammation Research, University of Birmingham. ² Healing Foundation Centre for Burns Research, University Hospitals Birmingham NHS Foundation Trust. ³ NIHR Surgical Reconstruction and Microbiology Research Centre, Birmingham University Medical School. ⁴ Centre for Cardiovascular Sciences, University of Birmingham.

Background: Major thermal injury results in immune dysfunction increasing the risk of sepsis. Central to host defence and innate immunity are neutrophils. Neutrophil extracellular traps (NETs) are an emerging area of innate immunity. NETosis results in the extrusion of nuclear material, decorated in granular proteins and modified histones e.g. citrullinated histone H3 (Cit H3), to the exterior of the cell. NETs primarily function to ensnare bacteria but uncontrolled NETosis is detrimental and can promote thrombosis.

Aims: We investigated the role of NETs in the sterile immune response to thermal injury and post injury sepsis. Additionally, we investigated how thermal injury and consequent septic episodes affected *ex vivo* NETosis.

Methods: Blood samples were collected from 74 burn injury patients (day 1-12 months following injury). Plasma cell-free DNA (cfDNA) levels were analysed using a Sytox[®] Green fluorometric assay. Citrullinated Histone H3 (Cit H3) was analysed by SDS-PAGE and Western blotting. To investigate *ex vivo* NETosis, neutrophils were stimulated with 25nM phorbol myristate acetate (PMA). NETs were quantified by cfDNA levels and fluorescent microscopy.

Results: Septic patients (n=46) have significantly (p>0.001) higher peak cfDNA levels compared to non-septic patients (n=28). Cit H3 was detectable in septic patient's plasma and coincided with episodes of sepsis. On day 14 following thermal injury c-statistic analysis for the association of plasma cfDNA and sepsis or multiple organ failure were 0.96 and 0.86 respectively. In addition, neutrophils from burn injury patients released significantly lower levels of DNA compared to healthy controls (p=0.0024). Fluorescence microscopy imaging confirmed that neutrophils isolated from burn injury patients are partially resistant to *ex vivo* NETosis induced by 25nM PMA.

Summary/Conclusions: NETosis occurs following human thermal injury. Elevated levels of cfDNA are associated with multiple organ failure and are a potential diagnostic marker of sepsis. Additionally, burn injury results in neutrophil dysfunction resulting in reduced *ex vivo* NETosis.

O14. LIPID RAFTS AND TRANSGLUTAMINASE ACTIVITY MEDIATE TRANSLOCATION OF FACTOR XIII-A FROM THE ACTIVATED PLATELET MEMBRANE TO BOUND FIBRIN

JL Mitchell, NJ Mutch. *Institute of Medical Sciences, University of Aberdeen, Aberdeen.*

Background We have previously shown that factor XIII (FXIII-A) is exposed on the activated platelet membrane and mediates extracellular cross-linking reactions¹.

Aims To analyse the mechanisms involved in FXIII-A exposure on stimulated platelets.

Methods FXIII-depleted plasma ± washed platelets were used to form static clots or model thrombi under flow ± transglutaminase (TG) inhibitor. In some cases platelets were pre-incubated with methyl-β-cyclodextrin (MβCD), cytochalasin D, eptifibatide and tirofiban or theophylline and prostaglandin E1 (PGE₁). Cross-linked α₂AP in thrombi was detected using Western blots. A FITC-labelled anti-FXIII-A antibody or N-(TAMRA) cadaverine were incorporated to detect antigen and activity respectively.

Results Addition of activated platelets to FXIII-depleted clots allowed visualisation of FXIII-A activity and antigen on surrounding fibrin. Inhibition of platelet activation prevented FXIII-A exposure and stabilization of FXIII-depleted thrombi. In the presence of MβCD, to disrupt lipid raft formation, FXIII-A was not detected on fibrin. Likewise, MβCD abrogated the ability of platelets to stabilise FXIII-depleted thrombi against fibrinolysis and cross-linked α₂AP was not detected in thrombi. These data suggest lipid rafts play a role in translocation of FXIII-A from the activated platelet surface onto the fibrin network. Similarly, TG activity was required for movement of FXIII-A from the platelet membrane onto adjacent fibrin fibres. Pre-treatment with tirofiban and eptifibatide, to block α_{IIb}β₃, attenuated the ability of platelets to stabilise FXIII-depleted thrombi, decreased levels of cross-linked α₂AP in thrombi and reduced FXIII-A on platelet-associated fibrin. Likewise, inclusion of cytochalasin D, to disrupt actin polymerisation, diminished the extracellular effects of FXIII-A. This suggests that α_{IIb}β₃ and the actin cytoskeleton are involved in translocation of FXIII-A from the platelet membrane to the fibrin network.

Summary/Conclusions Activated platelets expose functional FXIII-A on their membrane which translocates onto anchored fibrin via a lipid raft and α_{IIb}β₃-dependent mechanism requiring TG activity.

O15. TYROSINE PHOSPHORYLATION OF THE ITIM-CONTAINING RECEPTOR G6B-B IS ESSENTIAL FOR PLATELET HOMEOSTASIS

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Background: The immunoreceptor tyrosine-based inhibition motif (ITIM)-containing receptor G6b-B has been implicated as a critical regulator of platelet homeostasis. Tyrosine phosphorylation of the ITIM and immunoreceptor tyrosine-based switch motif (ITSM) in the cytoplasmic tail of G6b-B provides a docking site for the tyrosine phosphatases Shp1 and Shp2, which are thought to mediate downstream effects.

Aims: The aim of this study was to determine whether uncoupling of G6b-B from Shp1 and Shp2 abrogates G6b-B function in vivo.

Methods: We generated a mouse model in which tyrosine residues within the ITIM (Y212) and ITSM (Y238) of G6b-B were mutated to phenylalanines. Mice homozygous for the mutated G6b gene, referred to as G6b-B diY/F, were analysed for defects in platelet production and function.

Results: G6b-B diY/F mice exhibited a 75% reduction in platelet count and 38% increase in platelet volume, similar to that seen in G6b knockout (KO) mice. Extramedullary haematopoiesis and myelofibrosis was observed in both mouse models between 8-12 weeks of age. Platelet surface GPVI levels were similarly reduced by approximately 83% in both mouse models. In contrast, α IIb β 3 and GPIIb α levels were increased by 106% and 36%, respectively, in G6b-B diY/F mice, which was not seen in G6b KO mice. Interestingly, G6b-B was up-regulated 175% in platelets from G6b-B diY/F mice, possibly as a compensatory mechanism for reduced downstream signalling. Platelets from these mice did not respond to the GPVI-specific agonist collagen-related peptide, and showed an attenuated response to PAR-4 peptide.

Summary/Conclusions: Loss of tyrosine phosphorylation of the ITIM and ITSM of G6b-B disrupts platelet homeostasis and recapitulates many of the features of G6b KO mice. The lack of a complete phenocopy of the two mouse models suggests residual signalling by G6b-B diY/F or the presence of biologically active splice variants of G6b.

This work was funded by the British Heart Foundation and the Medical Research Council

O16. PLASMINOGEN ACTIVATOR INHIBITOR 1 IS RETAINED ON THE SURFACE OF ACTIVATED PLATELETS VIA A FIBRIN SPECIFIC MECHANISM

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Background: Plasminogen activator inhibitor 1 (PAI-1) is the principal physiological inhibitor of tissue-type plasminogen activator (tPA) and urokinase-type (uPA). Platelets contain the primary pool of PAI-1, which is secreted upon activation. It is now known that different populations of platelets exist which could differentially release PAI-1.

Aim: To analyse secretion of PAI-1 from platelets in response to different agonists and examine whether PAI-1 remains associated with the activated platelet membrane.

Methods: Platelets activated with collagen or convulxin (CVX) \pm thrombin or thrombin receptor activator peptide 6 (TRAP-6) were analysed for PAI-1 exposure using confocal microscopy and flow cytometry. In some cases platelets were incubated with Gly-Pro-Arg-Pro (GPRP), to inhibit fibrin polymerisation, or tirofiban, to block α IIb β 3. Phosphatidylserine (PS) positive platelets and PAI-1 were detected by Annexin V-AF647 and DyLight 550-labelled polyclonal antibody (R58) staining respectively. In some cases exogenous FITC-labelled fibrinogen was added.

Results: PAI-1 expression was significantly increased on platelets stimulated with collagen \pm TRAP-6 compared to unstimulated. Maximal PAI-1 exposure was observed upon activation with collagen and thrombin. PAI-1 was observed as a protruding "cap" on the surface of PS-positive platelets. In contrast, PAI-1 was located in the centre, over the granulome, of PS-negative platelets. Addition of tirofiban or GPRP dramatically reduced PAI-1 exposure on the platelet surface. In the presence of exogenous fibrinogen clear co-localization with PAI-1 was observed protruding from the "cap" of PS positive platelets. Flow cytometry data also revealed an increase in PAI-1 on CVX/thrombin stimulated platelets and a subsequent reduction in the presence of GPRP and tirofiban.

Conclusion: PAI-1 is exposed on the activated platelet membrane via an α IIb β 3 and fibrin dependant mechanism. This mechanism potentially localises PAI-1 at the site of fibrin formation thereby stabilising it against premature degradation by fibrinolytic proteases.

O17. A *F2RL3* VARIANT PREDICTING A Tyr157Cys SUBSTITUTION IN PROTEASE-ACTIVATED RECEPTOR 4 IS ASSOCIATED WITH REDUCED RECEPTOR FUNCTION IN PLATELETS

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Background Protease-activated receptors 1 and 4 (PAR1 and PAR4) mediate thrombin-induced platelet activation. Loss of function population variants in the PAR genes may influence platelet activation and the extent of platelet inhibition by anti-thrombotic drugs such as the PAR1 antagonist vorapaxar.

Aim To characterise the effect of a loss of function missense variant in *F2RL3* (the PAR4 gene) on platelet activation and inhibition by vorapaxar, and to determine the mechanism of loss of PAR4 function.

Methods Analyses were performed using platelets from a kindred with a heterozygous *F2RL3* c.471A>G transition predicting a p.Tyr157Cys (Y157C) substitution in PAR4, and using HEK293 cells transfected with expression constructs for CFP-tagged variant (PAR4-Y157C) or wildtype (PAR4-WT) PAR4.

Results Compared to controls, Y157C platelets showed reduced aggregation responses to PAR4 activating peptide (PAR4-AP) and to α -thrombin, but not to PAR1 activating peptide (PAR1-AP). P-selectin exposure, PAC-1 binding and Ca^{2+} mobilisation were also reduced in response to PAR4-AP, but not to PAR1-AP. Total PAR4 and PAR1 expression in Y157C platelets were similar to controls. However, the surface expression of PAR4 was reduced in Y157C platelets. Vorapaxar caused a greater reduction in aggregation responses to α -thrombin in Y157C platelets than in controls. Compared to PAR4-WT HEK293 cells, PAR4-Y157C cells showed reduced Ca^{2+} mobilisation, and IP3 accumulation in response to PAR4-AP and reduced surface PAR4 expression, consistent with the platelet phenotype. PAR4-Y157C co-localised in the HEK293 cell cytoplasm with the endoplasmic reticulum exit marker calnexin, but not the endosomal marker EEA1 suggesting defective trafficking to the cell surface.

Conclusion We demonstrate that a novel naturally occurring *F2RL3* variant markedly reduced platelet PAR4 activation responses and increased the pharmacodynamic response to vorapaxar, most likely by causing mis-trafficking of PAR4 to the platelet surface. Population variants in *F2RL3* are a potential risk factor for bleeding in patients receiving PAR1 antagonists.

O18. HIGH RESOLUTION MICROSCOPIC CHARACTERISATION OF VWF BIOSYNTHESIS AND STORAGE IN TYPE 1 VWD PATIENTS WITH LARGE IN-FRAME VWF DELETIONS

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Background Type 1 von Willebrand disease (VWD1) results from partial quantitative deficiency of von Willebrand factor (VWF) caused by mutations in the VWF gene (*VWF*). Previous studies identified three novel in-frame VWD1 deletions, involving exons 3 (VWFdel3), 32-34 (VWFdel32-34) and 33-34 (VWFdel33-34), with index cases presenting VWF:Ag levels of 31, 12 and 32 IU/dL respectively and *in vitro* expression demonstrating reduced secretion of recombinant VWF (rVWF).

Aims To use high resolution fluorescent microscopy to investigate VWF biosynthesis and storage in Weibel-Palade bodies (WPB) of 3 VWF deletion variants.

Methods rVWF was transiently-transfected into HEK293 cells. 72h post-transfection, cells were stained with immunofluorescent antibodies to VWF, endoplasmic reticulum (ER) and trans-Golgi network (TGN). Parallel experiments stained for VWF and the mature WPB marker Rab27a. Imaging used a Nikon TiE inverted deconvolution microscope and an OMX Structured Illumination Microscopy (SIM) system.

Results WT rVWF expression produced pseudo-WPB at the TGN and in the cytoplasm that were Rab27a positive. Homozygous expression (Hom) of rVWFdel3 completely lacked WPB, with diffuse staining appearing to co-localise with ER, which was rescued following heterozygous expression (Het). Hom rVWFdel32-34 produced small, punctate VWF positive structures, clearly different to WT, but larger WPB structures were visible in Het rVWFdel32-34. Hom and Het rVWFdel33-34 both produced Rab27a positive WPB-like structures.

Summary/Conclusions Large exonic VWF deletions have profound effects on VWF maturation and WPB formation. Homozygous VWFdel3 abolishes WPB formation, possibly due to ER retention. VWFdel32-34 formed punctate VWF positive structures but these were smaller and rounder than WT, suggesting defective VWF tubule arrangement during WPB formation in the Golgi. VWFdel33-34 produced apparently normal Rab27a positive WPB. Co-transfection with WT rVWF restored some WPB formation for all mutations. This study demonstrates that large exonic VWD1 deletions may cause reduced VWF secretion via defects in WPB biogenesis.

Poster presentations

P01. THE EFFECT OF DDAVP ON THROMBIN GENERATION IN PATIENTS WITH MILD HAEMOPHILIA A.

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Background: Von Willebrand Factor (vWF) has a multifactorial role in hemostasis as it acts as a ligand for platelet aggregation, a carrier of factor VIII and it also facilitates the procoagulant role of platelets. The vasopressin analogue DDAVP causes a release of vWF and factor VIII from endothelial cells and a temporary rise in their plasma levels. Such rises can be effective in reducing the bleeding risk. There is heterogeneity in the clinical expression of bleeding diathesis in patients with mild Haemophilia A.

Aims: To investigate the relationship between thrombin generation and levels of factor VIII, in patients who received desmopressin (DDAVP) as a treatment trial.

Methods: We monitored 6 patients with mild Haemophilia A. We investigated the effect of subcutaneous DDAVP administration (0, 3 μ g/kg), during 4 time-points (0min, 90min, 4 hours, 6 hours) on parameters of thrombin generation in platelet poor plasma (PPP) and platelet rich plasma (PRP). We used tissue factor (TF) 1pm for low PPP reagent and TF 1.0 pm and 0.5 pm for PRP reagent.

Pre DDAVP patient samples are run together with quality controls (QC) samples with the corresponding concentrations of TF.

For thrombin generation measurements we used the Hemker method and calibrated automated thrombogram machine.

Results: Factor VIII (FVIII) increased in all 6 patients at all-time points. From baseline, the median increase was 293% at 90 minutes, 191% in 4 hours, and 158% after 6 hours. The median increase in chromogenic assay of FVIII was 422% after 90 minutes, 274% after 4 hours, and 216% after 6 hours. Regarding TGA parameters, ETP in PPP with 1.0 pm of TF increased in all patients at all-time points. The median increase was 91% after 90 minutes, 104% after 4 hours, and 64% after 6 hours compared to baseline. After 90 minutes the median increase in PRP 1.0 pm TF was 17% while for PRP 0.5 pm TF it was 28% compared to baseline. For 4 hours and 6 hours the increase was 23%, and 21% for PRP 1.0 pm TF and 31% and 21% for PRP 0.5 pm TF respectively.

Conclusion: Even though FVIII and ETP increased after the injection of subcutaneous DDAVP in all patients with mild Haemophilia A, ETP lagged behind the FVIII and peaked later.

P02. PLATELET FUNCTION IN PATIENTS WITH ACUTE STROKE OR TIA ADMITTED TO THE TARDIS STUDY ALREADY RECEIVING TREATMENT WITH ANTIPLATELET AGENTS

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Background. TARDIS is an international multicentre trial of patients with acute stroke or TIA, and is testing the safety and efficacy of intensive antiplatelet agents (combined aspirin, dipyridamole and clopidogrel) versus guideline (aspirin and dipyridamole, or clopidogrel alone) given for one month.

Aims. In a substudy, platelet function is measured remotely from the patient at baseline and after 7 days; some patients are already taking antiplatelet agents. We present here data obtained at baseline prior to randomisation to determine the effectiveness of existing antiplatelet therapies.

Methods. Platelet function testing was via measurement of P-selectin on platelets using the Aspirin and Clopidogrel Test Kits from Platelet Solutions Ltd. Blood samples were stimulated with arachidonic acid or adenosine diphosphate immediately after collection, stabilised with PAMFix (from PSL) and sent by post for flow cytometric analysis centrally.

Results. The Aspirin Test demonstrated that the group taking aspirin (without [n=124] or with dipyridamole [n=13]) had low platelet function (median 165.5 [IQR 130.5, 226.5] and 156 [137, 272] compared with 257 [153, 951] for 35 healthy controls (no drugs) (MWU p<0.001, p=0.049 respectively); only 1 aspirin patient had a level that remained high (MF>500). The Clopidogrel Test showed that patients taking clopidogrel (n=18) had lower platelet function at 639 [420, 963] than those taking aspirin (n=156) at 1018 [746.5, 1225] (p<0.001); additionally, results were much higher than those obtained after adding the effective P2Y₁₂ antagonist cangrelor to blood *in vitro* at 276 [226, 353]. 10 (56%) of the clopidogrel patients had a level that remained high (MF>500).

Summary/Conclusions. Patients who are already taking antiplatelet agents have variable platelet function. High residual platelet function ('resistance') might have contributed to the stroke or TIA. Platelet function testing via measurement of platelet P-selectin can be performed remotely in the context of a multicentre trial.

P03. COAGULATION TESTS IN AN ATYPICAL OVERDOSE OF RIVAROXABAN

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Background The monitoring of the direct oral anticoagulants (DOACS) is considered unnecessary routinely, however the degree of their effect on routine coagulation tests is of value in certain clinical situations.

Aims To investigate a disproportional rise in a patient's Prothrombin time ratio in a patient on a DOAC.

Methods A coagulation sample from a patient on Rivaroxaban showed a significant disproportionately prolonged PT ratio with a moderate APPT ratio. Mixing studies with normal plasma, one stage clot based factor assays for the extrinsic factors and modified anti-X(a) for Rivaroxaban were performed.

Results The initial PT ratio was 8.1 with an APTT ratio of 1.6, a repeat sample confirmed these and mixing studies with normal plasma did not correct the prolongation, consistent with the presence of an inhibitor. Low levels of the extrinsic factors and strong non-parallelism was seen in the assays. The Modified anti Xa for Rivaroxaban showed the patient had levels in excess of 1000 ug/L. After cessation of Rivaroxaban both the clotting tests and Rivaroxaban levels reduced to normal over four days.

Conclusion The results indicated a high level of Rivaroxaban. The APTT ratio behaved in an insensitive and unpredictable way. Although the patients eGFR was normal when started on Rivaroxaban and at representation she had (four weeks earlier) an acute kidney injury requiring renal filtration. Our hypothesis is that given her degree of malnourishment the eGFR was not an accurate reflection of renal function. Poor renal clearance caused high Rivaroxaban levels and the delay to normalise results. Caution must be shown when starting DOACS in malnourished patients with multiple comorbidities and apparent normal renal function. Care must be taken in the interpretation of routine clotting tests with the Direct Oral Anticoagulants due to their unpredictable effects.

P04. THE INTRODUCTION OF DIRECT ORAL ANTICOAGULANTS (DOACS) TO THE SOUTHERN TRUST

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Background The direct oral anticoagulants (DOACS) are a major breakthrough in oral anticoagulation. However as new, unfamiliar drugs with an inherent risk for pathological bleeding they present a hazard as well as a benefit for patients.

Aims An audit over 6 months of the inpatient prescription of direct oral anticoagulants demonstrated significant risks so the anticoagulant team published guidelines on the Trust intranet to promote safe practice in line with NPSA alert 18 and assist clinicians initiating these medicines. Following this, the aim was to extend DOAC use.

Methods Working with medical and nursing colleagues, the anticoagulant pharmacist managing the DVT clinic in the Emergency Department (ED) added the option of rivaroxaban to the DVT treatment pathway. By changing the treatment from enoxaparin and warfarin, patients would have the benefit of not having to return to ED for daily injections until their INR was therapeutic. This change began at the beginning of 2013.

Results This benefit was immediate with a drop in the number ED DVT clinic attendances, from 457 in 2012 to 229 in 2013 and to 134 in 2014. The average patient attendance dropped from 6.35 visits per patient 2012 to 2.46 visits in 2013 and 1.56 in 2014.

Summary/Conclusions This initiative has greatly relieved the burden on the Clinical Decision Unit in ED. The clinician and patient feedback was used to implement rivaroxaban as first-line treatment for DVT across the trust for all inpatients. This demonstrates the value of standardising patient treatment pathways, patient education, healthcare staff education and GP letters used for the transfer of care.

P05. EARLY OUTCOMES FOLLOWING ENDOLUMINAL DEEP VEIN STENTING USING DEDICATED VENOUS STENTS

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Background Post thrombotic syndrome (PTS) occurs in 25-60% patients with deep vein thrombosis (DVT), is associated with significant morbidity and carries a financial burden to the NHS. Anticoagulation and compression hosiery remain the cornerstone of treatment, but management options are expanding. Percutaneous interventional procedures including catheter directed thrombolysis (CDT) and stent insertion have shown promise in reducing long-term complications with minimal bleeding risk in acute DVT. In the chronic post-thrombotic limb, patients who have persistent pain, swelling or ulceration, invasive treatments may improve symptoms and enhance ulcer healing.

Aims Evaluate early outcomes following endoluminal stenting using venous stents.

Methods Patients undergoing deep vein reconstruction using venous stents between 2012-2015 were identified. Duplex ultrasonography was used to assess stent patency at 1d, 2wks, 3mths, 6mths and yearly following intervention. Venous Disability Scores (VDS) and Villalta Scores (VS) taken before and after intervention (6wks, 6mths, yearly) were calculated.

Results 347 stents were inserted in 140 patients (median age 39yrs, 80 female) with median follow-up 16mths (1-38mths). Overall 1yr primary, primary-assisted and secondary patency rates were 67%, 80% and 82% respectively. The 1yr re-intervention rate was 30% (16% within two-weeks). 57 had stenting of residual stenoses after CDT for acute iliofemoral DVT. Median post-operative VDS and VS were 0 ($P < 0.001$). 1 developed an ulcer at 6mths. 81 had stenting for chronic outflow obstruction. 77(95%) had occluded vessels prior to treatment. The median pre-operative VS was 14 (4-23) and 9 had an ulcer. Following intervention, the median VS was 4 (0-22, $P < 0.001$) and ulcer healing was achieved in 67% ($n=6/9$).

Conclusions Dedicated venous stents show promise at providing symptom relief in the short-term. Close surveillance is, required to maintain stent patency. Factors that influence occlusion warrant further investigation. Long-term evaluation of these stents is required to investigate whether these early results can be maintained.

P06. RESTORATION OF THROMBIN GENERATION IN INHERITED THROMBOMODULIN-ASSOCIATED BLEEDING DISORDER

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Background A novel bleeding disorder has recently been described in which a nonsense c.1161C>A variant in the thrombomodulin (TM) gene *THBD* results in truncation of the TM transmembrane and intracellular domains and increased plasma TM concentrations. It has been proposed that bleeding results from reduced protein C-dependent thrombin generation. There are no reports of effective therapies for this disorder.

Aims

To evaluate the effect of activated prothrombin complex concentrate (aPCC) and recombinant factor VIIa (rFVIIa) on thrombin generation in plasma samples from cases with *THBD* c.1161C>A and in healthy control plasmas spiked with recombinant soluble TM (sTM).

Method Plasma from two cases with *THBD* c.1161C>A and control plasmas spiked with sTM underwent calibrated automated thrombography to quantify the endogenous thrombin potential (ETP). The analyses were repeated after addition of rFVIIa (NovoSeven; 5-100µg/mL) or aPCC (FEIBA; 0.25-1U/mL).

Results In the cases with *THBD* c.1161C>A, the ETP was 428.2 ± 20.1 nM/min (mean \pm SD) and 81.9 ± 10.3 nM/min (reference interval in 20 healthy controls 1092.4 - 2077.4), similar to previous descriptions of this disorder. Addition of 500ng/ml sTM reduced the ETP in control plasma to 548.5 ± 71.3 nM/min ($n=6$), thereby reproducing the defect in thrombin generation. Addition of rFVIIa or aPCC caused a dose-dependent increase in ETP in plasma from the cases sufficient to restore ETP to 889.2 ± 148.2 and 743.4 ± 107.0 nM/min ($n=3$) with 100µg/mL rFVIIa and 1995.8 ± 217.4 and 779.9 ± 38.0 nM/min ($n=3$) with 0.5U/mL aPCC. The magnitude of the responses to rFVIIa and aPCC were similar in sTM-spiked control plasma and in FVIII deficient and acquired haemophilia A plasmas samples not spiked with sTM.

Summary The defect in ETP associated with *THBD* c.1161C>A is partially restored *ex vivo* with rFVIIa or aPCC at therapeutically relevant concentrations. These data suggest a possible therapeutic role for bypassing agents in the treatment of TM-associated bleeding disorder.

P07. SINGLE CENTRE EXPERIENCE OF MANAGEMENT OF PATIENTS WITH ACQUIRED HAEMOPHILIA (AH) AND ACQUIRED VON WILLEBRAND DISEASE (AVWD). Dr P Chudakou & Dr B Myers, United Lincolnshire Hospitals

Background: Acquired coagulation inhibitors result from immune-mediated inhibition. Inhibitors are rare, usually directed against factor VIII (FVIII) and von Willebrand factor (VWF). AH has associated morbidity/mortality and most commonly occurs in the elderly.

Aims: Review local experience of AH and AVWD; comparison with national guidelines.

Methods: 14 patients with acquired coagulation inhibitors were identified from the haemophilia database, 11 AH, 3 AVWD. (11 diagnosed 2012-15, 3 patients 2001-11)

Results:

Mean age: 74 (30-90); M:F 4:10; Presenting symptoms: ecchymoses, (all), haematomas (1 retroperitoneal), haematuria, muscle bleed (psoas 1) GI bleeding (1), haemarthrosis (1).

AVWD: epistaxis (2); incidental pre-operative (1)

Underlying conditions not identified in most AH cases despite full malignancy and immune screening. One patient had a bladder tumour, one post-partum. All AVWD had IgG paraprotein and no other underlying condition.

7 patients required haemostatic agents (3 Novoseven; 4 FEIBA).

All AH patients had high dose steroids, 5 patients steroids only, without relapse.

4 patients had added cyclophosphamide with good response but poorly tolerated.

One patient received Rituximab due to poor initial response with excellent result.

All 3 patients with AVWD required monitoring only. Two had operations, managed with FVIII and IVIG with no complication and well-sustained levels

Three patients died following FVIII normalisation, 2 from bleeding (1 GI, 1 intracranial), 1 from pneumonia.

Summary/Conclusions Our survey results show a higher local incidence of these two rare conditions, likely due to high elderly population in Lincolnshire. They are otherwise in keeping with expected symptoms and prognosis, with a mortality rate following treatment of 27% in the AH patients, and all AVWD patients having a lymphoproliferative disorder. Our patients responded well to either bypassing factor, but only 25% responded completely to prednisolone alone; cyclophosphamide improved response rate but was poorly tolerated. Rituximab appears to be a good alternative second-line option.

P08. SAMPLE TRANSPORT ORIENTATION AFFECTS MICROPARTICLE RELEASE AND SPECIALIST HAEMOSTASIS RESULTS.

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Background: Pre analytical variables significantly influence laboratory assessment of haemostasis.

Aims: This project investigated the effects of sample transportation on routine and specialist haemostasis tests.

Methods: Following venepuncture, samples were transported by car, in either a vertical or horizontal orientation, for two, one hour periods. Samples for routine coagulation tests, factor assays, and thrombotic studies were processed on an ACL-TOP 700 analyser. Calibrated Automated Thrombography was performed on a Fluoroskan Ascent Microplate Fluorometer, using Thrombinoscope software, as described by Dargaud et al (2012).

Results: Dilute Russell's Viper Venom Screen was the only assay affected by transportation orientation. These ratios were significantly reduced ($p < 0.001$) when samples were transported horizontally compared to vertically- and non-transported (control) samples. Thrombin generation in horizontally transported samples produced significantly decreased results for Peak Thrombin ($p = 0.002$) and Velocity Index ($p = 0.005$) compared to vertically transported samples. These results, in combination, suggested a phospholipid dependent mechanism. Therefore, amended thrombin generation assays were performed and blood samples were filtered through a 0.2 μ m membrane. Thrombin generation with phospholipid only, tissue factor only, or working buffer only, generated an increase in Peak Thrombin and Velocity Index in horizontally compared to vertically transported samples.

Filtering samples decreased the Peak Thrombin and Velocity Index for all assays of thrombin generation. It also removed the reduction in Dilute Russell's Viper Venom Screen ratios in horizontally transported samples.

Summary/Conclusions: Differences in the Dilute Russell's Viper Venom Screen and thrombin generation may be the result of increased microparticle release in horizontally compared to vertically transported samples.

P09. USE OF OCTAPLEX AT A SOUTH THAMES DISTRICT GENERAL HOSPITAL: A CLOSED LOOP CLINICAL AUDIT

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Background Reversal of the anticoagulant effect of warfarin is achieved immediately and completely with use of Prothrombin Complex Concentrate (PCC). Octaplex is the PCC used by Kingston Hospital. Use of PCC is complicated by risks including anaphylaxis and prothrombotic complications. It is also very expensive. The British Committee for Standards in Haematology (BCSH) have produced extensive recommendations for its use.

Aims To audit how closely BCSH guidelines on use of PCC are being followed in Kingston Hospital, implement an intervention, and re-audit to assess any change.

Methods All cases where Octaplex was prescribed within the Trust from March to September 2014 were identified. Data was gathered from these 21 cases using a pro-forma to elicit key information including indication for warfarin reversal and administration of vitamin K. Our intervention was designed for maximal impact: firstly, results of the audit were presented at the Trust Transfusion Committee meeting to target the Haematology consultants. Secondly, a teaching session outlining the BCSH guidelines was delivered to the Trust FY1 doctors to target the juniors. A re-audit was carried out over a three month period beginning 2 weeks post intervention (n=10).

Results Octaplex was given appropriately in 76% of cases initially audited. This increased to 100% on re-audit. Vitamin K was used appropriately in 64% initially and 66% when re-audited. There was no statistically significant improvement noted when Chi squared analysis was performed ($p < 0.05$). **Summary/Conclusions** Octaplex is being used for indications not recommended by the BCSH. Whilst this improved following intervention, no statistical significance was noted and a larger sample size is required. Vitamin K is not being given enough, and this did not change post intervention. Further audit loops of intervention and re-audit are necessary to achieve greater concordance between use of PCC by the Trust and the BCSH guidelines.

P10. CHARACTERISATION OF A NOVEL VON WILLEBRAND FACTOR VARIANT CAUSING TYPE 2A VON WILLEBRAND DISEASE

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Background Type 2A Von Willebrand Disease (VWD) is characterised by loss of high molecule weight multimers caused by either defects in dimer or multimer formation, or enhanced susceptibility to ADAMTS13 proteolysis. Here we describe a novel VWF variant, Q1541R located in the A2 domain of VWF that results in a moderate to severe bleeding phenotype.

Aims To characterise the effect of the Q1541R mutation on VWF function.

Methods Expression of wild type (wt)VWF or VWF-Q1541R in HEK293 cells was assessed by VWF ELISA and pseudo Weibel-Palade body formation examined by confocal microscopy. Multimer formation, collagen binding, GPIb binding and static ADAMTS13 cleavage assays were performed using standard methods. Unfolding of the A2 domain was determined using optical tweezers. A shear based assay system was used to investigate the effect of the mutation on VWF function under shear stress.

Results VWF-Q1541R was expressed at a marginally lower level than wtVWF, but formed normal storage vesicles in HEK293 cells and exhibited a full range of multimers with normal collagen and platelet binding function. Under static conditions VWF-Q1541R was more susceptible to ADAMTS13 proteolysis and required less force to unfold the A2 domain. Under shear stress VWF-Q1541R mediated comparable VWF mediated platelet capture to collagen. Intriguingly, when VWF-Q1541R was perfused over collagen with ADAMTS13, although a reduction in platelet capture was observed the reduction was similar to that seen with wild type VWF. Similar observations were also made with two common type 2A variants G1629E and E1638K.

Summary/Conclusions The novel Q1541R variant results in VWD due to enhanced ADAMTS13 proteolysis. Interestingly, the increased ability of ADAMTS13 to proteolyse type 2A variants is lost when VWF is exposed to a collagen surface under shear stress.

P11. SIEMENS XPRECIA STRIDE™ VALIDATION STUDY USING THE ACL TOP® WITH HEMOSIL® RECOMBIPLASTIN REAGENT AND THE ROCHE COAGUCHEK® XS PRO

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Background As part of our anticoagulant service review, Leeds Teaching Hospitals are looking to utilize more point of care testing in both primary care and secondary care. We are investigating which handheld analyser would fulfill our needs the best and so performed a validation study on the new analyser from Siemens: Xprecia Stride Coagulation Analyser.

Aims To determine clinical substantial equivalence of capillary samples tested on Xprecia Stride with the existing system Coaguchek XS Pro and venous samples tested on ACL Top® with HemosIL® RecombiPlasTin reagent.

Methods This study was performed with subjects currently on warfarin therapy and those not receiving any anticoagulation therapy nor having a known coagulation disorder. Testing of capillary samples was performed on the Xprecia Stride and the Coaguchek XS Pro for INR. INR will also be performed on a venous sample from the same sampling occasion, analyzed on the Laboratory method ACL Top® with HemosIL® RecombiPlasTin reagent.

Repeated QC samples were run over 5 days to look at precision analysis for the Xprecia Stride Analyser.

Results Using Deming Regression analysis the coefficient of determination for the comparison of Stride v ACL Top is 0.92 (n=171)

Using Deming Regression analysis the coefficient of determination for the comparison of Stride v CoaguChek is 0.95 (n=167)

Precision on the QC material for Xprecia Stride: CV <4% (n=120)

Summary/Conclusions Xprecia Stride meets the acceptance criteria as stated in the validation protocol when compared to the ACL Top using RecombiPlasTin reagent as well as the CoaguChek XS Pro. The results are accurate and reproducible and therefore suitable for use in our reconfigured anticoagulant service.

P12. WHY IS SCREENING BY THE CURRENT STANDARD OF CASE FOR DVT OF LIMITED UTILITY IN SUSPECTED UPPER LIMB DEEP VENOUS THROMBOSIS?

Carrie Dinning, Andrew McSorley, Julie Blundell, Mr Michael Desmond Creagh. Royal Cornwall Hospital

Background Upper extremity deep venous thrombosis (UEDVT) accounts for about 10% of all deep venous thrombosis (DVT) with an estimated prevalence of two cases per 100,000 individuals. Screening using D-dimer testing for UEDVT is unvalidated, however health care professionals may utilise this test, understanding that it has high negative predictive value in acute venous thrombo-embolism (VTE).

Methods An audit was undertaken to determine the clinical presentation of confirmed UEDVT, the usage and utility of D-dimer screening. A D-dimer test with a cut-off level validated for possible lower limb DVT was used.

Results Methods and Twenty cases of UEDVT, diagnosed by contrast venography or dopplar ultrasound, were identified in a DVT Clinic between September 2013 and August 2015. The mean age of patients diagnosed with UEDVT was 46 years compared to 65 for lower limb DVT. Nine cases (45%) had a diagnosis of cancer, with UEDVT diagnosed in relation to a central venous catheter (CVC). Eleven cases had unprovoked UEDVT, of which two did not have a D-dimer screen, with five negative screens undertaken prior to imaging. Four cases had a positive D-dimer screen, two results were marginally elevated at 233 and 266 (local cut off 230ng/ml) in patients of 48 and 35 years of age.

Conclusions UEDVT was commonly identified in relation to cancer and CVC's, whilst in unprovoked cases the D-dimer screen was positive in only 44% of cases and of limited utility. Occlusion of the subclavian vein results in limited clot burden, which could in part explain the observed negative D-dimer screening. Patients with UEDVT presented at a younger age than for lower limb DVT. Given that the D-dimer reference range in the healthy population increases with age, an age adjusted cut-off level may be of greater utility. Further prospective study of D-dimer in suspected UEDVT should be undertaken.

P13. LUPUS ANTICOAGULANT TESTING IN CENTRAL MANCHESTER FOUNDATION TRUST – WHAT CAN BE IMPROVED?

Irina Earnshaw, medical student, Manchester Medical School and Manchester Royal Infirmary, Jecko Thachil, Department of Haematology, Manchester Royal Infirmary

Background Antiphospholipid syndrome (APS) is a hypercoagulable state caused by antiphospholipid antibodies (aPL), resulting in thrombosis and/or pregnancy-related complications. Diagnosis of APS involves identification of a clinical manifestation, and demonstration of an aPL, such as Lupus Anticoagulant (LA) or Anti-Cardiolipin Antibodies (ACA).

Aims This report set out to look at the use of LA in the Central Manchester Foundation Trust over six months, to find if its request, and use of results, were appropriate.

Methods The hospital database was searched, returning 914 requests for LA testing over the six-month period. There were 85 positive results, and the case notes of these patients were looked at to find the reason for referral, ACA results, any anticoagulation, and any clinical manifestations of APS suffered.

Results Four specialties had referred the most patients: clinical haematology, gynaecology, obstetrics and rheumatology. Of these, gynaecology returned the least positive LA (<1%), and looking into reason for referral revealed that many of their patients were being tested inappropriately. Looking into the clinical backgrounds of all LA-positive patients showed that many qualified for but did not have a diagnosis of APS, and were not necessarily receiving the correct treatment.

Summary/Conclusions Recommendations include educating gynaecology on when referral for LA testing is appropriate, and encouraging the diagnosis of APS where present, so that patients can be treated correctly and included in research. Further research into patients with negative LA results (such as ACA results and reasons for referral) will provide further data, and will be looked into in future.

P14. REVERSAL OF THE HEPARIN EFFECT WITH PROTHROMBIN COMPLEX CONCENTRATE – AN *IN VITRO* STUDY USING THE THROMBIN GENERATION ASSAY

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Background LMWH are common anticoagulants. Despite this, they do not have an effective antidote. Clinicians therefore face a dilemma when presented with a patient who is bleeding or requires surgery and who has been treated with a LMWH.

Aims The aim of this study was to assess the reversibility of suppression of thrombin generation in samples of normal pooled plasma (NPP) spiked *in vitro* with a range of anticoagulants and subsequently with a 4 factor prothrombin complex concentrate (PCC).

Methods Thrombin generation (TGA) was performed as per Hemker et al using 5pM tissue factor as a trigger. NPP samples were initially spiked with unfractionated heparin (UFH, 0-0.5 IU/ml), tinzaparin (0-0.8 IU/ml), enoxaparin (0-0.8 IU/ml) and fondaparinux (0-0.8 µg/ml). Samples were also spiked with increasing doses of a 4 factor PCC (0.3, 0.6 (~25 IU/kg), 0.9 IU/ml). All samples spiked with an anticoagulant were then subsequently spiked with the range of concentrations of PCC, in addition to protamine sulphate (PS, 2.5 µg/ml) with and without PCC.

Results The samples spiked with an anticoagulant demonstrated dose-dependent suppression in thrombin generation. As expected, those spiked with PCC showed the reverse. UFH served as a control with complete reversal seen with addition of PS alone and little reversal with PCC. With decreasing anti-IIa activity, e.g. with enoxaparin and fonaparinux (a specific anti-Xa inhibitor), there was increasing dose-dependent reversal of the suppression of thrombin generation.

Conclusions This study demonstrates reversal of LMWH by PCC in an *in vitro* spiking study using TGA. The anti-Xa activity appears to be more reversible than the anti-IIa activity. These experiments need to be replicated on patient samples however, as the anti-IIa effect is disproportionate in spiked samples. *In vivo*, this effect is lost after the first few hours with clearance of the high molecular weight units.

P15. COMPARISON OF THE EFFECT OF FRESH FROZEN PLASMA PRE-AND POST-TRANSFUSION IN ITU PATIENTS ON ROUTINE AND GLOBAL HAEMOSTATIC ASSAYS

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Background FFP is widely used in ITUs, often as prophylaxis or pre-procedure. Routine PT/INR are poor at predicting bleeding risk and FFP fails to correct abnormal results in most patients. Point-of-care global haemostatic assays such as ROTEM® are now widely available and are increasingly being incorporated into transfusion algorithms with little evidence to support this, particularly in determining the efficacy of FFP.

Aim To compare results at baseline, 30 minutes & 2-4 hours post transfusion using PT, INR, APTT, fibrinogen, factor assays and global haemostatic assays (ROTEM®, thrombin generation (TGA)) in patients with acquired coagulopathy on ITU transfused with FFP.

Methods Citrated whole blood samples were collected from 13 patients on ITU both pre- and post-FFP. Data was obtained from 16 transfusion episodes. Ethical approval was gained from the National Research Ethics Committee. Standard and clotting factor assays were run as per routine laboratory practice. NATEM, EXTEM, INTEM and FIBTEM tests were run on the ROTEM®. TGA was performed as per Hemker et al. on the Calibrated Automated Thrombogram.

Results 7/16 baseline INRs were ≤2. No abnormal INR corrected into the normal range. Using a paired t-test, significant improvement (p<0.05) in PT/INR was seen at 30 minutes but not at 2-4 hours. Factor assays did show significant initial improvements, specifically II, V, VII and X. TGA showed significant change in lagtime and peak at 30 min. NATEM - of the 4 ROTEM® tests – only demonstrated significant improvement in MaxVel, tMaxVel and angle at the first time-point.

Conclusions Factor assays are most sensitive to FFP transfusion. Changes detected in all assays are short-lived and are reduced at 2-4 hours post transfusion. The findings in this small study do not suggest superiority of global assays over PT/INR.

P16. ADAMTS-13 STABLE IN CITRATED WHOLE BLOOD FOR 48 HOURS

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Background: If ADAMTS-13 activity is lowered, ULvWF may accumulate within the blood causing thrombosis due to platelet aggregation, which may lead to

Thrombotic Thrombocytopenic Purpura (TTP), a life-threatening disorder. It is difficult to distinguish TTP from other thrombotic microangiopathies such as atypical Haemolytic Uraemic Syndrome (aHUS). In aHUS ADAMTS-13 activity is normal whereas low results (<5.0%) are diagnostic of TTP in the correct clinical setting. It is important to diagnose acute TTP as treatment is urgently required, usually with plasma exchange.

Aims: Accurate ADAMTS-13 activity results are required quickly to diagnose TTP and rule out aHUS. Lack of information about the changes in the interval between collection and testing has been a problem. This study was carried out to assess sample stability over a 48 hour period.

Methods: Blood samples from 8 healthy volunteers (P1–P8) and 15 patients (P9–P23) were collected into Becton Dickinson citrated vacutainer tubes (0.109M). Blood was separated into 0, 24 and 48 hour aliquots. Each aliquot was left at room temperature and centrifuged at 2000G at the relevant times. Plasma was obtained by double centrifugation and stored at -70°C prior to analysis. Technozyme ADAMTS-13 Activity ELISA was used (Pathway Diagnostics.), a chromogenic test for the determination of ADAMTS-13 activity.

Results: Statistical analysis used the Friedman Test (Nonparametric Repeated Measures ANOVA). P value = 0.3813 (not significant).

ADAMTS-13 activity results (%)				
Normals	0 hours	24 hours	48 hours	Average result
P1	93	85	83	87
P2	69	85	85	80
P3	89	89	87	88
P4	68	76	76	73
P5	82	84	79	82
P6	102	95	91	96
P7	85	93	96	91
P8	71	84	84	80
Mean	82	86	85	

Statistical analysis used the Friedman Test (Nonparametric Repeated Measures ANOVA). P value = 0.7860 (not significant).

ADAMTS-13 activity results (%)				
Patients	0 hours	24 hours	48 hours	Average result
P9	50	54	53	52
P10	45	32	38	38
P11	32	33	34	33
P12	12	12	14	13
P13	57	65	52	58
P14	46	41	42	43
P15	78	77	60	72
P16	61	60	70	64
P17	29	28	29	29
P18	43	68	69	60
P19	4	5	4	4
P20	87	69	68	75
P21	59	78	72	70
P22	37	51	44	44
P23	68	75	74	72
Mean	47	50	48	

Summary/Conclusions:

Results show no clinically relevant change; the P value is not statistically significant therefore samples requiring ADAMTS-13 activity assays by Technozyme are stable up to 48 hours at room temperature. Samples requiring baseline ADAMTS-13 levels can be tested on samples received within 48 hours of collection and samples can be transported as whole blood without cooling/ freezing the plasma and requiring dry-ice.

P17. FUNCTIONAL CHARACTERISATION OF A NOVEL SPLICE MUTATION, C.7887+2T>A, ASSOCIATED WITH TYPE 1 VON WILLEBRAND DISEASE.

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Background Mutations causing qualitative or quantitative deficiency of von Willebrand factor (VWF) result in von Willebrand disease (VWD), the most prevalent inherited bleeding disorder. Splice mutations are commonly associated with a mild (type 1 VWD; VWD1) or severe (type 3 VWD) quantitative deficiency because they result in a null VWF allele.

Aims To investigate the disease mechanism associated with a novel splice mutation, c.7887+2T>A, correlated with moderate VWF reduction in a heterozygous VWD1 patient and severe reduction in a compound heterozygous VWD1 patient.

Methods Due to a lack of patient RNA, bacterial exon trap plasmid pET01 was used to assess *in vitro* effect of c.7887+2T>A on VWF splicing. Bacterial pCI-neo plasmids expressing either wild-type VWF cDNA (WT) or predicted mutant cDNA were transfected or co-transfected into HEK293T cells and quantity of mutant VWF expressed relative to WT only was assessed using ELISA. Multimer analysis of secreted VWF was performed via electrophoresis on 1.6% (w/v) SDS-agarose gels. High-resolution microscopy of transfected HEK293 cells was performed to assess pseudo-Weibel-Palade body (WPB) formation.

Results c.7887+2T>A was predicted to cause complete skipping of VWF exon 47 resulting in in-frame deletion p.Pro2591_Leu2629del. Expression of the deletion mutant caused significant reduction in VWF secretion compared to WT in both the homozygous (Hom; 58%) and heterozygous (51%) state, but with no significant effect on intracellular retention. In addition, expressed multimers were normal and microscopy highlighted no

observable differences in WPB-like structures compared to WT following Hom expression.

Summary/Conclusions c.7887+2T>A causes an in-frame deletion of VWF exon 47 resulting in a viable allele that when expressed causes reduced VWF secretion but normal multimer and WPB formation, correlating with disease phenotype. Functional characterisation of splice mutations is important to fully understand their impact on VWF and their mode of pathogenicity.

P18. COMPARISON OF POINT OF CARE AND LABORATORY DDIMER MEASUREMENTS

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Background Ddimer is used in combination with 2 level Wells score to investigate patients with suspected DVT. In patients that are low risk, a negative ddimer test effectively excludes the need for further investigations for DVT. Point of Care (POC) devices are increasingly being used in both primary and secondary care. Proficiency testing data show that Roche cobas h232 POC ddimer and Innovance laboratory methods are both widely used with a cut-off of 0.5 µg/ml (500 ng/ml) for DVT exclusion

Aims Comparison of DDimer results obtained using Roche cobas h232 cardiac ddimer (POC) and Siemens Innovance /Sysmex CS5100 laboratory method (Lab).

Methods.

Blood samples from 22 healthy normal subjects and 26 subjects presenting to ED with possible DVT were collected in Lithium heparin and 0.109m trisodium citrate (both Vacutainer BD) for Ddimer by POC and Lab methods respectively.

Citrated samples were centrifuged at 2000g for 10 mins for lab method analysis.

Results Results obtained by cobas POC method were significantly lower than those obtained with Innovance lab method in normal subjects (means 0.13 µg/ml and 0.32 µg/ml, p, 0.0001, r = 0.77) and in possible DVT patients (means 0.93 µg/ml and 1.46 µg/ml, p<0.05, r = 0.58). Amongst possible DVT cases results were in concordance in 19/26 cases (both below cut-off in 8 and both above cut-off in 11). In the other 7 cases lab results were above the cut-off whereas POC results were below it. 2 of these patients had ultrasound investigations reported as suggesting possible DVT.

Summary/Conclusions Innovance and cobas DDimer results were significantly different. Depending on the local protocols in use our data predicts that the proportion of patients undergoing ultrasounds for DVT diagnosis would be lower when DDimer was determined using the cobas POC system compared to use of the Innovance laboratory assay.

P19. THE EFFECT OF VON WILLEBRAND FACTOR ON THE INVASIVE POTENTIAL OF BREAST CARCINOMA CELL LINES

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Background Conflicting roles for Von Willebrand Factor (VWF) in the pathogenesis of cancer have been described. Whilst VWF released from endothelial cells can promote metastasis of melanoma cells; a previous study showed that VWF deficient mice demonstrated increased metastatic potential when injected with lung carcinoma cells. Moreover it has also been suggested that VWF can induce apoptosis in some lung and breast cancer cell lines.

Aims The aim of the current study was to investigate the effect of VWF on the growth, proliferation, migration and invasion of breast carcinoma cells

Methods The interaction of breast cancer cell lines MCF7 and MDA231 with VWF was assessed under static and flow conditions. Cell proliferation was determined using a tetrazolium salt based assay; cell migration was assessed using scratch assays and cell invasive potential was determined using a modified matrigel based method. Apoptotic cells were detected with annexin-V staining.

Results Both MCF7 and MDA231 cells bound to VWF under static conditions and significantly under conditions of shear stress with MCF7 cells showing the greatest binding potential. Furthermore MCF7 cells demonstrated enhanced interaction to VWF released from stimulated endothelial cells. Binding to VWF was specific since the interaction was prevented with polyclonal anti-VWF antibodies. Addition of soluble VWF marginally inhibited proliferation of MCF7 but not MDA231 cells, but had a significant effect on the migration of MCF7 cells and was able to induce a degree of apoptosis in the same cell line. Interestingly, both soluble VWF and VWF incorporated into a basement membrane inhibited the invasive potential of MCF7 cells and to a lesser extent, the invasiveness of MDA231 cells.

Summary/Conclusions Together these data suggest a role for VWF in inhibiting the invasive potential of breast cancer cells. Further studies are required to understand the mechanisms behind this and how some breast cancer types evade the inhibitory effect of VWF.

P20. THE EFFECTS OF RIVAROXABAN ON SPECIALIST COAGULATION TESTS

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Background Rivaroxaban is a direct FXa inhibitor with predictable pharmacokinetics and pharmacodynamic properties with no required laboratory monitoring in clinical trials.

We have evaluated the effects of rivaroxaban on specialist coagulation tests using a range of drug concentrations.

Methods Blood samples from 10 normal healthy volunteers were centrifuged at 2000g for 10 minutes at room temperature to achieve platelet poor plasma. Plasma samples were pooled and spiked with varying concentrations of rivaroxaban to attain 8 different concentrations (0-1000ng/ml).

Spiked plasma was evaluated by performing One-Stage factor II, V, VII & X using Innovin® reagent and assays of VIII, IX & XI using Actin FS®, as well as DRVVT and Thrombophilia tests. All tests were performed on the Instrumentation Laboratory ACL TOP analyser and the Sysmex Haemostasis CS series analysers.

Results Factors VIII, IX and XI show a very similar relationship with concentration dependant underestimation occurring (approximately 25% under estimation at 150ng/ml). The PT based extrinsic assays of FII, V, VII & X were largely unaffected. The DRVVT ratio was falsely elevated with a dose dependant association. The Thrombophilia tests of Clot S, Clot C & APC-R show the greatest amount of interference from rivaroxaban concentration with an overestimation of Protein C and Protein S and prolongation of APC-R displayed beyond 50ng/ml of drug. This prolongation could potentially lead to an undiagnosed factor V leiden. Chromogenic Antithrombin, Protein C and free Protein S antigen were unaffected by increasing rivaroxaban concentrations.

Summary/Conclusions Factor VIII, IX and XI tests were underestimated in the presence of rivaroxaban at concentrations in excess of 50mg/ml and therefore be preferable to test a patient sample for specialist coagulation assays that did not have any rivaroxaban present in the sample.

P21. THE FIRST YEAR OF THE POINT OF CARE D-DIMER EQA PROGRAMME, INCLUDING ASSESSMENT OF TESTING AND POST ANALYTICAL INTERPRETATION OF RESULTS

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Background: Point of Care (POC) D-dimer testing is increasingly being used in evaluation of patients with suspected thromboembolism (VTE). A D-dimer test result below the stated cut off value together with a low clinical evaluation score would suggest VTE was unlikely and allow the clinician to avoid referring the patient for complex and expensive investigations. The devices used are portable desktop analysers well suited to non laboratory areas but still require quality control to ensure reliable test results. A specific External Quality Assessment (EQA) programme for POC devices has been introduced.

Methods: 4 surveys per year are distributed, each comprise one sample. A period of 17 days is given to complete the test and return the result. 5 surveys have been completed to date with in total 81 Cobas h232 (Roche) users, 26 Triage (Alere) users - both quantitative methods and the qualitative Clearview Simplify kits (Alere, n=10users?). Participating centres were asked to test the samples and, using the short patient scenario provided, state whether DVT was unlikely or DVT not excluded.

Results: Cobas results showed reasonable between-centre agreement, with percentage CVs ranging from 12-18%. Interpretations showed full agreement in none of the 5 samples. In survey five 3 centres stated "DVT unlikely" despite results well above the cut off value. Triage results showed %CV of between 0-32% and interpretations were in agreement in 4 of the 5 samples. In survey five 1/11 centres stated "DVT unlikely" with a result well above the cut off. For the Clearview, overall results showed 4 samples to be positive and one negative. Of the 5 samples 3 showed total agreement in both results and interpretations.

Conclusions: These data identify a clear need not only for quality assessment but also for education to improve the post analytical interpretation of test results.

P22. EVALUATION OF THE POINT-OF-CARE XPRECIA STRIDE COAGULATION ANALYZER* FOR PT/INR FOR WARFARIN MONITORING

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Background The Xprecia Stride™ Coagulation Analyzer from Siemens Healthcare (SH) is a novel, handheld Point of Care (POC) device that generates rapid PT/INR results from fingerstick samples for OAT monitoring, specifically Warfarin, a vitamin K antagonist. This external validation study assessed the clinical correlation of the Xprecia Stride analyzer PT/INR test against an established laboratory hemostasis method (BCS® XP System) as well as an alternate point-of-care device (CoaguChek® XS system).

Aims The aim of the study was to demonstrate strong correlation between Xprecia Stride Coagulation Analyzer test results and a central laboratory assay as well as an alternative point-of-care system.

Methods Test methods were based on CLSI guidelines. Evaluation of Precision Performance of Quantitative Measurement Methods (EP05-A2), Measurement Procedure Comparison and Bias Estimation Using Patient Samples (EP09-A3), and Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory (EP28-A3c).

Results Passing Bablok regression analysis yielded a slope of 1.00 and an intercept of 0.10, with $R^2=0.91$ across the range of 0.8 to 7.7 INR when the Xprecia Stride was compared to the BCS XP. Passing Bablok regression analysis yielded a slope of 0.94 and an intercept of -0.02, with an $R^2=0.94$ across the range of 0.9 to 7.7 INR when the Xprecia Stride was compared to the CoaguChek XS. Repeatability using whole blood demonstrated %CVs were <5.8 across the reportable range. LQC at two levels demonstrated repeatability precision % CVs that were <3.6 and within laboratory %CVs that were <7.0. The Expected Range for the PT/INR on the Xprecia Stride analyzer was 0.9 to 1.1 for subjects not on OAT.

Summary/Conclusions The Xprecia stride analyzer PT/INR test results demonstrated a strong correlation to the BCS XP system as well as the CoaguChek XS system.

*Under FDA review. Not available for sale in the U.S. and product availability varies by country

P23. THE EFFECTS OF APIXABAN ON SPECIALIST COAGULATION TESTS.

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Background Apixaban is a direct FXa inhibitor with predictable pharmacokinetics and pharmacodynamic properties with no required laboratory monitoring in clinical trials.

Aims We have evaluated the effects of Apixaban on specialist coagulation tests using a range of drug concentrations.

Methods Blood samples from 10 normal healthy volunteers were centrifuged at 2000g for 10 minutes at room temperature to achieve platelet poor plasma. Plasma samples were pooled and spiked with varying concentrations of Apixaban to attain 8 different concentrations (0-1000ng/ml).

Spiked plasma was evaluated by performing One-Stage factor II, V, VII & X using Innovin® reagent and assays of VIII, IX & XI using Actin FS®, as well as DRVVT and thrombophilia tests. All tests were performed on the Instrumentation Laboratory ACL TOP analyser and the Sysmex Haemostasis CS series analysers.

Results Both Intrinsic Factor Assays IX and XI show a very similar relationship to that of Factor VIII with a concentration dependant underestimation occurring beyond 70ng/ml of added drug. The PT based Extrinsic assays of FII, V, VII & X were largely unaffected. The DRVVT ratio was falsely elevated with a dose dependant association.

The Thrombophilia tests of Clot S, Clot C & APC-R show the greatest amount of interference from Apixaban concentration with an overestimation dependant relationship of Protein C and Protein S and prolongation of APC-R displayed beyond 200ng/ml of drug. Chromogenic Antithrombin, Protein C and free Protein S antigen were unaffected with increasing Apixaban concentrations.

Summary/Conclusions Factor VIII, IX and XI tests were underestimated in the presence of Apixaban at concentrations in excess of 70mg/ml and therefore in light of this it would be preferable to test a patient sample for specialist coagulation assays that did not have any Apixaban present in the sample.

P24. ROLE OF FIBRINOGEN γ' IN AAA AND THE FUNCTIONAL EFFECTS OF FIBRINOGEN Γ' LEVELS ON CLOT STRUCTURE

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Background Abdominal aortic aneurysm (AAA) represents a chronic inflammatory disease. Elevated fibrinogen can predict a greater risk of thrombosis and is higher in AAA patients. A fraction of fibrinogen called the γ' chain differs from the γA chain at its C-terminus where it has a unique 20-amino acid extension. γ' can influence clot structure in vitro, causing thinner fibres, increased branching and reduced pore size.

Aims Investigate levels of Fibrinogen and Fibrinogen γ' in AAA patients and controls and the effects of High and Low fibrinogen γ' on clot structure in patient plasma

Methods 609 AAA Patients and 559 age matched controls were recruited. Fibrinogen levels and Fibrinogen γ' levels were measured by Claus method and ELISAs. Fibrinogen depleted plasma was repleted with different ratios of purified $\gamma A/\gamma'$ Fibrinogen (3%, 10%, 40%). 23 samples with High γ' and 31 samples with Low γ' were selected. Clots were formed and viewed under a laser scanning confocal microscope.

Results Fibrinogen was higher in AAA than controls (3.68 g/L (3.18-4.31), 3.50 g/L (3.09-4.08), p=0.001). There was no difference in γ' levels between AAA and controls (334.53 μ g/ml (212.16 – 508.02), 331.00 μ g/ml (211.28 – 510.54), p=0.747). There was a lower percentage of γ' in AAA than in controls but this did not reach significance (9.26% (5.64 – 13.74) and 9.44% (6.04 – 14.89), p=0.173).

In fibrinogen repleted plasma an increase in γ' resulted in plasma clots with increased areas of agglomeration. This was reflected in patients, with increased areas of agglomeration in patients with high γ' (37%) compared to low γ' (4%).

Summary/Conclusions The raised levels of fibrinogen in the AAA patients may reflect the underlying inflammatory process that is associated with cardiovascular disease. There is no association between AAA and fibrinogen γ' levels, this is different to other arterial diseases. Increased agglomerations may be due to increased branching or the negative charge of γ' .

P25. POLYPHOSPHATE AUGMENTS THE PLASMINOGEN ACTIVATOR FUNCTION OF FACTOR XIIA AND CO-LOCALISE ON PLATELET-BOUND FIBRIN

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Background Platelet polyphosphate (polyP) binds factor XII (FXII) and acts as a surface for autoactivation and activation by kallikrein¹. Activated FXII (α FXIIa) is a known weak activator of plasminogen.

Aims Here we analyse the role of polyP in stimulating α FXIIa-mediated plasminogen activation

Methods α FXIIa-mediated plasminogen activation and fibrinolysis \pm polyP₇₀ were assessed by chromogenic assay and clot lysis respectively. Native gel electrophoresis was used to detect polyP binding. Washed platelets were analysed for binding of DL488-labelled FXII or DAPI to detect polyP by confocal microscopy and flow cytometry. Plasma clots were formed \pm washed platelets \pm DAPI and DL488-FXII.

Results PolyP₇₀ (average polymer chain length) significantly augments α FXIIa-mediated fibrin clot lysis (105 ± 6.5 min vs. 238 ± 14.4 min; $P < 0.0001$) and plasminogen activation ($P < 0.0001$). α FXIIa did not directly influence plasmin activity. Native gels illustrate binding of polyP₇₀ to α FXIIa but not β FXIIa that lacks the surface binding domain. In line with this, β FXIIa could not stimulate plasminogen activation. A similar enhancement (2.6-fold) of α FXIIa-mediated clot lysis by polyP₇₀ was observed with Glu- and Lys-plasminogen; suggesting α FXIIa does not facilitate transition of the closed (Glu) to open (Lys) form. An additional enhancement of α FXIIa-mediated plasminogen activation was observed with soluble fibrin. However, other surfaces that activate FXII, specifically RNA and collagen, did not augment α FXIIa-plasminogen activation. PAI-1 and PAI-2 did not modulate α FXIIa-mediated clot lysis or plasminogen activation \pm polyP₇₀. In contrast, C1 inhibitor and histidine-rich glycoprotein down-regulated the plasminogen activator function of α FXIIa. DL488-FXII bound clearly to platelets and the adjacent fibrin network. Similarly, platelet-derived polyP was found to be associated with the platelet surface and on surrounding fibrin fibres where it co-localised with FXII.

Summary/Conclusions In the presence of platelet polyP and fibrin α FXIIa is a potent plasminogen activator which may be relevant *in vivo*.

P26. DEFECTIVE α_2 ANTIPLASMIN CROSS-LINKING AND THROMBUS STABILITY IN A CASE OF ACQUIRED FXIII DEFICIENCY

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Background Activated factor XIII (FXIIIa) cross-links fibrin and incorporates α_2 antiplasmin (α_2 AP) thereby stabilising clots mechanically and against fibrinolytic degradation¹. Acquired FXIII deficiency is a rare condition associated with autoimmune disorders, infections, drugs and malignancy. A 72 year old woman presented with haematuria, unprovoked extensive bruising and a splenic haematoma.

Aims To analyse the mechanisms underlying the development of an idiopathic case of acquired FXIII deficiency.

Methods FXIII activity assays and urea clot stability were performed. Model thrombi were formed under flow from patient samples, commercial FXIII-depleted or normal plasma. Cross-linked α_2 AP in thrombi and FXIII-B in plasma were detected by Western blot. FXIII-A activity and antigen were measured by in-house assay and ELISA, respectively.

Results PT, APTT, fibrinogen and platelet count and function were normal. Defective clot stability was not improved by addition of normal plasma. FXIIIa activity levels (0.09 U/ml) were reduced and a modified Bethesda inhibitor titre of 1.6 BU/ml indicated the presence of a FXIII inhibitor. A single dose of FXIII concentrate and immunosuppression with prednisolone induced remission. A relapse, detected by abnormal urea clot stability occurred and prednisolone and Rituximab treatment induced a second and persistent remission

Subsequent laboratory analysis revealed rapid lysis of model thrombi (presentation 194.6 FU/min⁻¹; relapse 183.5 FU/min⁻¹) relative to normal plasma (17.7 FU/min⁻¹) and the absence of cross-linked α_2 AP. Likewise, no FXIIIa activity was detected in the presentation sample and only minimal levels upon relapse. Low FXIII-A antigen was measured at initial presentation (6.7 %) and relapse (38.8 %). Western blots confirmed the presence of FXIII-B indicating only the A-subunit and A₂B₂ complex were absent. Analyses of remission samples confirm normal activity (0.429 U/ml) and antigen (80.1 %) levels.

Summary/Conclusions These data suggest a non-neutralising inhibitor of FXIII caused defective α_2 AP cross-linking and reduced thrombus stability resulting in excessive bleeding.

P27. SINGLE NUCLEOTIDE VARIANTS C.2365A>G AND C.2385T>C ARE ASSOCIATED WITH INCREASED VWF PLASMA LEVELS AND VWF:FVIII BINDING

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Introduction: von Willebrand factor (VWF) plasma levels vary considerably; between 50-200IU/dL in 95% of the general population. Coagulation factor VIII (FVIII) is protected from degradation by VWF, which is thus a determinant of FVIII level. Factors that have been associated with this variation include ABO blood group and VWF single nucleotide variants (SNV), including rs1063856 (c.2365A>G; p.Thr789Ala) and rs1063857 (c.2385T>C; p.Tyr795=). These two variants co-segregate.

Aims: To further investigate the association between SNV rs1063856/rs1063857 and VWF/FVIII level and elucidate the mechanism(s) involved.

Methods: SNV were genotyped in ~1100 healthy controls (HC) recruited by the MCMDM-1VWD study. *In vitro* expression of VWF containing the SNV was performed in HEK293T cells, followed by measurement of VWF mRNA expression using TaqMan quantitation and VWF:Ag via ELISA. mRNA half-life was measured after inhibiting transcription using 5µg/ml actinomycin D at 0, 2, 3 and 4 hours post treatment. VWF:FVIII binding (VWF:FVIII B) was measured using ELISA.

Results: Non-reference alleles for both SNV were significantly associated with ~10% higher VWF levels. *In vitro* expression (n=3) demonstrated significantly increased VWF levels of 38% for c.2365A>G (p=0.0009), 55% for c.2385T>C (p=0.006) and 92% for both SNV *in trans* (p=0.03). mRNA expression (n=3) was also increased; 50% for c.2365A>G (p0.005), 50% for c.2385T>C (p=0.02) and 75% for both SNV (p<0.001). RNA half-life (n=3) increased by 7% for c.2365A>G, 8% for c.2385T>C and ~10% for both SNV *in trans*.

c.2365A>G was significantly associated with a 40% reduction in VWF:FVIII B while c.2385T>C and both SNV *in trans* were associated with significantly increased VWF:FVIII B (50% and 90% respectively).

Conclusion: SNV c.2365A>G and c.2385T>C independently influence VWF:FVIII B but are both associated with increased VWF mRNA and protein expression. This increase is due to longer mRNA half-life for the non-reference allele, leading to increased protein production.

P28. NEW POINT OF CARE TESTING (POCT) INR EQA ONE STEP TESTING PROCESS FOR COAGUCHEK XS, XS PLUS AND XS PRO USERS

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Background: NEQAS (BC) provides an EQA programme for POCT INR testing, including for CoaguChek XS, XS Plus and XS Pro devices. Surveys for XS users comprise 2 lyophilised samples; participants are required to add one diluent to lyophilised plasma and after 10 minutes reconstitution, a further diluent is added to re-calcify before testing. This process is repeated for the second sample; the whole process takes approximately 30 minutes to complete. Participants consider this a time consuming and in some cases complicated process. To try to resolve this, a new one step testing process was investigated.

Method: 400 centres received sample packs containing Sample 1 (0.5 ml lyophilised plasma), Diluent 1 (1.3 ml of calcium chloride), Sample 2 (0.25 ml lyophilised plasma), Diluent 2 (0.65 ml of calcium chloride) and 2 pipettes. To test the samples, Diluent 1 was added to Sample 1, gently swirled for 15 seconds and left to reconstitute for 2 minutes. The INR was then measured by addition of a drop of plasma to the XS test strip. The same procedure was followed for Sample 2. Participants recorded their test strip batch number, device type and date the test was performed.

Results: For Sample 1, 337 participants returned results with a median INR 3.7 and CV of 9%. For sample 2, 339 participants returned results, with a median INR 3.8 and CV of 8.5%. Seven participants reported errors with sample 1, and 6 with sample 2. In comparison with the existing testing method this one step process produced similar CVs and numbers of reported errors. Participants preferred this new system of testing, stating that it was quicker and easier to perform.

Conclusion: The new one step method is suitable for use and will be introduced into the EQA programme in October 2015.

**P29. OUT OF THE LAB AND INTO THE COMMUNITY:
NEW POINT-OF-CARE PT/INR COAGULATION
ANALYZER PROVIDING PATIENT BENEFITS FROM
COMPLIANCE TO CONVENIENCE**

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Background Since the time Oral Anticoagulation Therapy (OAT) began in the 1940s, Warfarin has remained the dominant oral anticoagulant. The aim of OAT is to maintain anticoagulation levels that are capable of preventing thromboembolic events without increasing the risk of hemorrhagic complications. At our hospital, OAT is currently evaluated by laboratory monitoring of the International Normalised Ratio (INR); however, these patients are usually tested every 4/6 weeks and are in therapeutic range 40-60% of the time. We believe that increasing the frequency of testing should result in increased time in the therapeutic range. A great advance has been the use of point-of-care (POC) devices allowing INR determinations in capillary blood, alongside computerized decision support systems (CDSS).

Aims To compare the INR measurement taken with the Xprecia™ Stride Coagulation System* using capillary blood with the conventional lab plasma method on the BCS XP.

Methods: 862 samples of venous and capillary blood were collected from in-patients on warfarin/acenocoumarol, who had been admitted for INR monitoring from January 11th to February 13th 2015.

Results Pearson's Correlation coefficient (r) for the overall performance was 0.93 ($p < 0.0001$, 95% CI 0.92 to 0.94). Weighted Deming regression yielded a slope of 0.80 and intercept of 0.19. Bias statistics analysis for INR values between 1.5 and 3.5 and between 3.6 and 4.5 resulted in median bias of -0.3 with 96.5% of samples being within $\pm 30\%$ and median bias of -0.6 with 90.0% of samples being within $\pm 30\%$ respectively.

Conclusions POCs systems have great potential for faster clinical decision making and offer less invasive testing. The POC Xprecia Stride™ Coagulation Analyzer is a reliable alternative to the laboratory method to monitor INR in patients on oral anticoagulants and has a good adherence on therapeutic values.

*Under FDA review. Not available for sale in the U.S. and product availability varies by country

**P30. DUTY OF CANDOUR APPLIED TO HOSPITAL
ACQUIRED THROMBOSIS**

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Since November 2014, NHS Trusts are subject to statutory duty of candour. Providers are required to inform a patient as soon as possible of a notifiable incident (moderate-severe harm). NHS England requires hospitals to perform root cause analysis (RCA) for hospital acquired thrombosis (HAT). The combination of these responsibilities raises a number of difficulties in delivering duty of candour for HAT. For example, HAT RCA is often a slow process (up to 6 months); an RCA is reported as 'potentially preventable' if there is any deviation from local VTE guidelines, which vary between Trusts; and there is no evidence that a limited error, such as single missed dose of thromboprophylaxis, would result in actual harm. On 27th April 2015 we held an open multi-disciplinary meeting* with patient involvement to discuss these issues. After discussion, the group arrived at an algorithm. We felt that if RCA found no errors in hospital care then it was not necessary to inform the patient of the results. However, it was agreed that best practice would be that patients are made aware that a VTE within 90 days of admission results in an investigation, and that they would be informed if there were any significant omissions in their care (recommend information added to patient VTE leaflets). It was agreed that for 'potentially preventable' HATs that an expert group should make a judgement as to whether on the balance of probabilities the error(s) resulted in significant clinical harm. The group unanimously agreed that if errors were potentially causal then this must be told to the patient. The group was split approximately 50:50 on whether the patient should be informed if an error had been made which was unlikely to be causal, highlighting the difficulty and potential inconsistency between Trusts of applying duty of candour to HAT.

P31. BLEEDING INCIDENCE AND PHENOTYPE IN HAEMOPHILIA A CARRIERS AND HAEMOSTATIC MEASURES USED.

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Background: Haemophilia A carriers show wide ranges of Factor VIII levels. Studies have shown that they bleed more than normal population.

Aim: To look at the bleeding incidence and phenotypes in haemophilia A carriers and to clarify whether factor VIII levels are correlated to bleeding incidence and how these carriers were haemostatically managed.

Methods: This is a retrospective observational study of haemophilia A carriers registered in haemophilia Centre in central Manchester university hospitals. We monitored their Factor VIII levels and bleeding phenotype and treatment modalities as well as presence of associated bleeding disorders.

Results: Of 108 haemophilia A carriers, mean factor VIII level was 45.06 U/dL, 77 (71.3%) have levels below 50 U/dL and 31(28.7%) have levels above 50U/dL. 69 (63.8%) carriers had bleeding history, 48 (44.44%) had menorrhagia, 19 (17.59%) had post dental extraction bleeding, 15 (13.88%) had easy bruising, 13 (12.03%) had post surgical bleeding (tonsillectomy, hysterectomy, thyroidectomy, appendectomy), 4 (3.7%) had post partum haemorrhage, 5 (4.62%) had epistaxis and 3 (2.72%) had other bleeding histories (hematuria, hematochezia and haemarthrosis). 56 (72.72%) of low level carriers (<50U/dL) and 13(41.93%) of normal level carriers(>=50U/dL) have bleeding history. 68 (62.96%) of carriers required treatment including Tranexamic acid for menorrhagia and epistaxis {23 (21.29%)}, OCP for menorrhagia {10(9.25%)}, Merina coil {6(5.55%)}, DDAVP nasal spray for menorrhagia {3(2.77%)}, Tranexamic acid and DDAVP cover for surgeries and procedures {43 (39.81%)} and factor VIII cover before surgeries {12(11.11)}. 59 (76.62%) low level carriers required treatment and 9 (29.03%) of normal level carriers required treatment. Five (4.62%) carriers did not have family history and diagnosed because of their bleeding history. Seven (6.48%) carriers had associated bleeding disorders including von Willibrand disease and factor XI deficiency.

Conclusion: Haemophilia A carriers commonly bleed, specially low level carriers and treatment options should be considered specially before operations and procedures. Other bleeding disorders may be thought if presentation is not correlating with factor VIII level.

P32. OUTCOMES IN PATIENTS WITH ISCHAEMIC STROKE OR TRANSIENT ISCHAEMIC ATTACK WHO WITH OR WITHOUT NEW ATRIAL FIBRILLATION: DATA FROM THE 'TRIPLE ANTIPLATELETS FOR REDUCING DEPENDENCY AFTER ISCHAEMIC STROKE' (TARDIS) TRIAL

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Background Atrial fibrillation (AF) is a common antecedent of ischaemic stroke (IS) and TIA, and requires anticoagulation to reduce recurrence. Whether this intervention normalises medium-term outcomes is unclear and is examined here using data from the ongoing TARDIS trial.

Aims The impact of newly diagnosed atrial fibrillation (AF) on outcome at day 90 was assessed in the ongoing TARDIS trial.

Methods TARDIS is assessing the safety and efficacy of intensive vs guideline antiplatelet agents in 4,100 patients with acute non-cardioembolic IS or TIA. Baseline characteristics and outcome at day 90 (recurrent events, functional outcome, including modified Rankin Scale, mRS) are recorded. Information on a new diagnosis of AF development is collected up to day 90. Data are odds ratio (OR) or mean difference (MD) with 95% confidence interval (CI) relative to no AF; day 90 outcome models were adjusted by age, sex, pre-morbid mRS, history of previous stroke, qualifying event (IS/TIA), NIHSS and cortical syndrome.

Results Of 2635 recruited patients, 92 suffered AF. In comparison with other patients, AF patients were 4.4 years older, and were more likely to have had a qualifying event of stroke, OR 3.2 (95% CI, 1.7, 5.9). AF patients were also more likely to have been taking antiplatelets prior to their qualifying event, OR 1.8, (1.2, 2.7). AF patients had a lower baseline heart rate, MD -3.8 (-6.5, -1.2). By day 90, AF patients were more likely to have had a recurrent IS/TIA (6 occurred before diagnosis, 7 afterwards) than patients without AF, OR 2.9 (1.6, 5.4). In adjusted analyses, there were no significant differences in death, dependency, disability, quality of life, cognition or mood between patients with or without AF.

Summary/Conclusions Stroke/TIA recurrence was more common in patients with AF than in other TARDIS patients.