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P 01

Endothelial cells delay fibrin clot lysis under static conditions *in vitro*

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Background
Endothelial cells (ECs) regulate clot formation and lysis through the surface expression and secretion of factors that interact with components of the coagulation and fibrinolysis systems. Their dysfunction is thought to drive the hyperfibrinolysis that occurs in the early stages of traumatic injury.

Aims
The aim of this study was to determine whether cultured ECs influence tissue plasminogen activator (tPA)-induced clot lysis under static conditions.

Methods
Human umbilical vein ECs (HUVECs) were cultured in ECGM2 until confluent. HUVECs were trypsinised and seeded in 96-well plates at 10,000 cells/well. A mixture of 30% pooled normal plasma, 16 µM phospholipids and tPA (50-100 pM) in HBSS with 20 mM HEPES (pH 7.4) was added to the HUVECs. Clotting was initiated with 0.1 U/ml thrombin and 10.6 mM Ca^{2+} and clot formation and lysis monitored. Times to 50% clot lysis (CLT_{50}) were determined and reported as mean ± SD.

Results
In the absence of HUVECs, CLT_{50} were 291 ± 6, 111 ± 1 and 90 ± 2 min for 50, 75 and 100pM tPA, respectively. Contrastingly, in the presence of HUVECs, CLT_{50} were 153 ± 7 and 116 ± 2 min for 75 and 100 pM tPA, respectively, and clot failed to lyse in response to 50 pM tPA. HUVECs increased CLT_{50} by 43 ± 4 and 26 ± 1 min for 75 and 100 pM tPA, respectively ($p < 0.001$).

Conclusions
Unstimulated HUVECs considerably delayed clot lysis induced by 75 and 100 pM tPA and abolished that induced by 50 pM tPA, yielding an assay that could be used to study the contribution of endothelial dysfunction to trauma coagulopathy *in vitro*. The mechanisms underlying these effects may include thrombomodulin-mediated activation of thrombin activatable fibrinolysis inhibitor. Further investigations are required to unveil these mechanisms and to assess the effect of EC stimulation.

P 02

Altered fibrinolytic potential in antiphospholipid syndrome

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Background
Antiphospholipid syndrome (APS) is a highly prothrombotic acquired autoimmune disease caused by heterogeneous group of autoantibodies called antiphospholipid antibodies (aPL). The mechanisms underlying this highly prothrombotic state are poorly understood but may include augmented coagulation, endotheliopathy and derailment of fibrinolysis.

Aims
To assess the dysregulation of fibrinolysis in thrombotic APS.

Methods
Sixty-three patients with confirmed APS as per international consensus criteria for APS, on stable anticoagulation with warfarin and 20 age comparable healthy controls were analysed for levels of tPA, urokinase plasminogen activator (uPA), plasminogen activator inhibitor-1 (PAI-1) and thrombomodulin (TM) by ELISA. Fibrinolytic potential exogenous tPA was determined by turbidimetric clot lysis assay.

Results
Median age of APS patients was 50 years (range 22-65 years) with 69.8% (44/63) female. Of 63 patients (82.5%; 52/63) had thrombotic APS. The remainder (17.5%) had both thrombotic and obstetric APS. All patients were on long term stable anticoagulation (at least 6 months) and INR was within the target therapeutic range. Plasma tPA level was significantly lower in patients with APS (median 0.72 ng/mL [range 0.29-2.83 ng/mL]) than that in healthy controls (median 0.78 ng/mL [range .78-2.6 ng/mL]) ($P < 0.001$). There was no significant difference in levels of PAI-1, uPA, plasminogen, D-dimer, or fibrinogen between the two groups. There was no difference in the clot lysis time between patients with APS vs healthy controls: median 116 mins (range 41-405 mins) vs median 152.5 mins (range 40-368 mins), (p=0.12). Soluble TM was significantly higher in patients with APS than healthy controls: median 6.52 ng/mL (range 1.57-27.14 ng/mL vs 1.51 ng/mL (range 0.88-4.72 ng/mL) ($p < 0.00005$).

Conclusions
The reduction in tPA and significant increase in soluble TM in patients with APS suggests endothelial damage and may also impair fibrinolysis in patients with APS.

P 03

Real-world experience with a human fibrinogen concentrate: clinical data from adult and paediatric patients requiring fibrinogen for bleeding control and prevention

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Background: Fibrinogen is key to maintaining normal haemostasis. Fibrinogen deficiency can occur as a result of congenital fibrinogen disorders and acquired fibrinogen deficiency as a result of trauma or surgery. Fibrinogen may be administered on-demand or prophylactically for patients at risk of perioperative bleeding. Following a temporary approval period in France (2017–2020), human fibrinogen concentrate (HFC; Fibryga® Octapharma, AG) obtained full approval for congenital and acquired hypofibrinogenaemia.

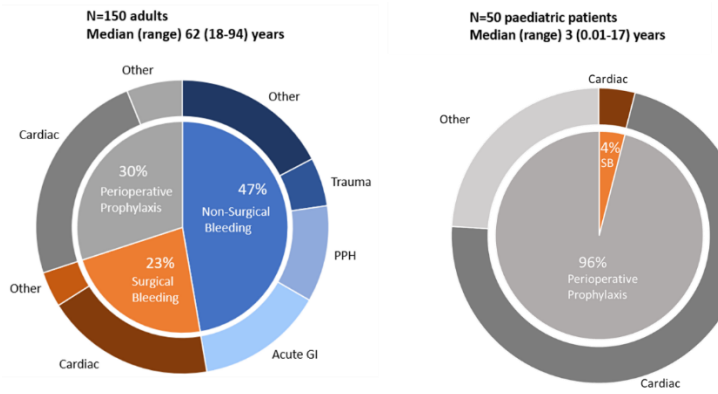
Aims: To evaluate real-world use of HFC for prophylactic and on-demand treatment of bleeding episodes to expand our knowledge of HFC as an option for fibrinogen replacement.

Methods: Data from six French hospitals was retrospectively collected from adult/paediatric patients with fibrinogen deficiency from December 2017 to February 2020. The primary endpoint was the indication for HFC administration, and the secondary endpoint was treatment success for on-demand treatment/perioperative prophylaxis (PP).

Results: Data from 150 adults (median [range] age 62 years [18–94]) and 50 paediatric patients (aged 3 years [0.01–17]) with acquired hypofibrinogenaemia were included. HFC was administered for non-surgical bleeding (NSB; adult only), surgical bleeding (SB), and PP, as detailed in Figure 1. Cardiac surgeries accounted for 79.5/75.0% of prophylactic use and 82.4/100.0% of SB use in adults and paediatric patients (Figure 1). Mean±SD total HFC doses were 3.06±1.69 g, 2.09±1.36 g, and 2.36±1.25 g for adult NSB, SB, and PP; 0.75±0.35 g and 0.83±0.62 g for paediatric SB and PP, respectively. Treatment success was 85.7% for NSB, 97.1% for SB and 93.3% for PP in adults, and 50.0% for SB and 87.5% for PP in paediatrics.

Summary/Conclusions: Efficacy and safety of HFC was favourable across age groups. This study contributes to the body of evidence supporting HFC use for bleeding control/prevention in real-world clinical practice, particularly with acquired fibrinogen deficiency.

Figure 1: HFC indications



P 04

Ligand-induced clu-stering of CLEC-2 is governed by ligand valency, receptor density and Syk

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Background
The platelet glycoprotein receptor CLEC-2 is considered a target for a new class of antiplatelet agent that will cause less bleeding than current drugs. CLEC-2 is activated by clustering which leads to phosphorylation of the conserved tyrosine in its tail and binding to the tandem SH2 domains in Syk, crosslinking two receptors. This initiates a signalling cascade that culminates in PLC□2 and platelet activation. Currently, we have a limited understanding on the regulation of clustering of CLEC-2 and its relation to signal strength.

Aims
The aims were to determine whether CLEC-2 is expressed as monomers or dimers in the membrane and investigate the variables that govern receptor clustering.

Methods
We used fluorescence correlation spectroscopy (FCS), single molecule photobleaching and non-detergent membrane extraction to determine the stoichiometry of CLEC-2 in cell lines and platelets. We further investigated the effect of ligands including novel nanobodies, receptor density and inhibition of Syk on CLEC-2 clustering using FCS.

Results
Contrary to previous reports, we show that CLEC-2 is expressed as a mixture of monomers and dimers in platelets and cell lines. Using FCS, the multivalent CLEC-2 ligands, rhodocytin, AYP1 mAb, AYP1 $F(ab)_2$, the divalent nanobody (Nb) LUAS-2 and tetravalent Nb LUAS-4 significantly increased the molecular brightness of CLEC-2 and reduced the number of molecules, demonstrating receptor clustering, whereas monovalent AYP1 Fab and LUAS Nb had no effect. We show that CLEC-2 dimerisation/clustering increases with receptor expression and that ligand-induced clustering is blocked by inhibition of the tyrosine kinase, Syk.

Conclusions
We provide evidence that CLEC-2 is expressed as a mixture of monomers and dimers in the membrane. CLEC-2 forms higher order oligomers in response to multivalent but not monovalent ligands. We show that ligand valency, receptor density and Syk are critical variables that govern CLEC-2 clustering.

P 05

The potential of tumour-associate tissue factor mRNA measurement as a predictor of the risk of pulmonary embolism

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Introduction
Predicting the risk of thrombosis in cancer patients remains a challenge to effective thromboprophylaxis. The release of tissue factor (TF) as microvesicles (MV) can result in transient increases in the risk of embolism.

Aims
We hypothesise that the rapid increase in procoagulant potential is regulated at the protein-translation level and may be predictive of the risk of thrombosis.

Material & Methods
The release of TF-MV was assessed in three gastrointestinal cell lines (LoVo, CaCo2 and AsPC-1) with high TF-mRNA. Protein-translation, or mRNA-transcription were inhibited, using cycloheximide (10 nM) or actinomycin-D (1 µM) respectively. MV release was induced by activation of PAR2, MV collected and TF-antigen concentrations and activity measured in all samples. Furthermore, the incidence of Pulmonary Embolism (PE) in Gastrointestinal cancer patients (GI) was assessed in selected patient cohorts, following stringent exclusion criteria (colonic n=5; gastroesophageal n=4), which were type, gender and stage of cancer matched one-to-one with patients without PE (9+9 samples). Total-RNA was extracted from the samples using a FFPE-RNA extraction kit and TF-mRNA was quantified using a quantitative real-time PCR procedure along with a standard curve prepared using *in vitro*-transcribed TF-mRNA.

Results
Inhibition of protein-translation using cycloheximide prevented the incorporation of TF and suppressed TF activity, but not MV release. However, blocking of RNA-transcription did not prevent TF-MV release. Analysis of absolute amounts of tumour-associated TF-mRNA showed significant increases in patients who developed PE (mean=26.931±15.371 pg/100ng-total RNA; median=5.340 pg/100ng-total RNA; range=0.4-131.43 pg/100ng-total RNA) compared to those who didn't (mean=0.098±0.023 pg/100ng-total RNA; median=0.110 pg/100ng-total RNA; range=0-0.19 pg/100ng-total RNA).

Conclusions
This is a first demonstration of a correlation between the risk of PE in GI cancer and the levels of tumour-associated TF-mRNA, and supports the hypothesis that the process is driven by the rapid translation of mRNA into TF-protein, following stimulation. In conclusion, tumour-associated TF-mRNA may be a predictor for the risk of thrombosis.

P 06

Tissue factor acts as a gauge for the level of injury by regulating the balance of expression of $p16^{INKa}$ and $p21^{CIP1/WAF1}$ cell -cycle regulators

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Introduction
Following injury, the resultant cellular signals ensure the correct clearance and healing of the wound. The exposure of cells to tissue factor (TF) decide the fate of the cells by regulating apoptosis, survival and proliferation.

Aims
This study examined the mechanisms by which TF controls the G1/S checkpoint cell cycle progression.

Material & Methods
Human primary endothelial cells were incubated with various concentrations of recombinant TF (0.065-0.650 ng/ml), and in the presence of various inhibitory antibodies (10H10, HTF1, AIIB2 & SAM11). Samples were removed at various time intervals and the concentrations of $p16^{INKa}$ and $p21^{CIP1/WAF1}$ and cyclin D1 mRNA and protein were measured by RT-qPCR and western blot. Additionally, the transcriptional activity of E2F was measured *in situ* using an E2F-luciferase reporter.

Results
Lower concentrations of TF promoted equal increases in $p16^{INKa}$ and $p21^{CIP1/WAF1}$, permitting E2F transcriptional activity, together with upregulation of cyclin D1, leading to cell proliferation. The increase in $p16^{INKa}$ expression was mediated though β1-integrin signalling and was prevented following inhibition of β1-integrin using AIIB2 antibody or blocking the exosite within TF using the 10H10 antibody. However, this was independent of PAR2 activation. Upregulation of $p21^{CIP1/WAF1}$ was also dependent on β1-integrin signals but was significantly increased on blocking of PAR2 activation with SAM11 antibody, or by inhibiting the protease function of TF-fVIIa complex using HTF1 antibody. Exposure of cells to higher concentrations of TF resulted in disproportionate increases in $p16^{INKa}$ and $p21^{CIP1/WAF1}$ expression, together with lower detectable E2F transcriptional activity, leading to cell apoptosis.

Conclusion
We propose that the balance of the stoichiometry between $p16^{INKa}$ and $p21^{CIP1/WAF1}$ expression is regulated by the strength of the arising signals, following the exposure of cells to differing amounts of TF. This acts as a means for quantifying the level of cellular damage during injury or trauma.

P 07

Tissue Factor (TF) and factor VII modulate the cell phenotype and are potential indicators of malignancy in pancreatic cystic lesions

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Introduction
Premalignant pancreatic cellular genotype may remain stable for many years, but the rapid onset to malignant phenotype is often associated with the presence of inflammation. Tissue factor (TF) and coagulation factor VIIa (fVIIa) are known to influence the cellular malignant properties including those associated with epithelial to mesenchymal transition (EMT).

Aims
The potential of TF and fVII within pancreatic cyst fluid as indicators of malignant transformation was assessed. Additionally, the influence on pancreatic epithelial cells were examined.

Methods
Cyst fluid was prospectively collected from 31 patients with pancreatic cystic lesions (REC 18/LO/0736) and analysed in a blinded fashion. The level of TF and fVIIa proteins were measured by ELISA, and the fVIIa:TF ratios calculated. Additionally, human pancreatic nestin-expressing cells (HPNE) and AsPC-1 cell line were treated with combinations of recombinant TF (0-0.65 ng/ml) and factor VIIa (5 nM), and the expression of vimentin mRNA and protein measured.

Results
Significant histological stage-dependent increases in TF level were detected, which corresponded to the progression of the normal ductal epithelium to invasive adenocarcinoma. The mean TF concentration was significantly higher (p=0.006) in the high-risk group (high-grade dysplasia & malignant; 1.17 ng/ml, 95% CI 0.68, 1.67) vs the low-risk group (benign & low-grade dysplasia; 0.27 ng/ml, 95% CI 0.1, 0.44). The cut-off value for TF was (0.75 ng/ml, AUC 0.877, p= 0.002). FVIIa:TF ratio was lower in the high-risk group (mean= 84.82 [95% CI 0, 185.04]) vs the low-risk group (mean= 437.46 [95% CI 0, 901.02]), (p= 0.274). Incubation of pancreatic cells *in vitro*, with similar concentrations of recombinant TF resulted in increased expression of vimentin. This influence was moderated by supplementation with fVIIa in HPNE, but not AsPC-1 cells.

Conclusion
Cyst-associated TF levels correlate with the cytological progression to the malignant phenotype, and appear to be moderated by the presence of fVIIa.

P 08

Investigating endothelial cell Pim kinase as a novel anti-thrombotic target

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Background
Venous thrombosis, the formation of a blood clot in a vein, is driven by a combination of blood stasis, plasma hypercoagulability and endothelial dysfunction. An activated or damaged endothelium favours thrombosis, by promoting coagulation, platelet activation, and reducing fibrinolysis. Elevated plasma levels of inflammatory markers IL-6, IL-8, or TNFα, which activate the endothelium, are associated with a 2 to 6-fold increase in the risk of deep vein thrombosis. Targeting the thrombo-inflammatory properties of the endothelium could provide an effective anti-thrombotic therapeutic approach for the prevention of venous thrombosis.
Pim kinases (Pim-1, -2, and -3), have been shown to modulate platelet function, and whilst shown to be expressed in human umbilical vein endothelial cells, the role of Pim kinase in the thrombotic properties of the endothelium remains unknown.

Aims
To investigate the role of the Pim kinase isoforms in endothelial cell biology and their contribution to venous thrombosis using human umbilical vein endothelial cells (HUVECs) to determine the anti-thrombotic potential of Pim kinase inhibitors.

Methods
The regulatory role for Pim kinase in endothelial cell control of thrombus formation in response to TNFα, an inflammatory initiator of endothelial cell damage was determined using HUVECs and techniques including immunofluorescence microscopy, qPCR, Western Blotting, and ELISA.

Results
We confirmed mRNA expression of all three Pim kinase isoforms, and protein expression of Pim-1 in HUVECs. TNFα-induced HUVECs treated with a pan Pim kinase inhibitor, AZD1208 demonstrated a decrease in gene expression of VWF, a pro-coagulant mediator, and release of inflammatory markers, IL-6, and IL-8.

Summary
Collectively, our findings identify a potential role for Pim kinase in venous thrombosis and indicates that Pim kinase inhibitors could be repurposed for use alongside other anti-thrombotic agents for the prevention of venous thrombosis.

P 09

Generation of Multivalent Nanobodies to Probe Cluster-Induced Platelet Receptor Activation

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Background – The platelet surface receptors GPVI and CLEC-2 stimulate powerful activation of platelets through interaction with their endogenous ligands collagen and podoplanin respectively. They are considered as targets for a new class of antiplatelet drug for prevention of arterial and venous thrombosis. Clustering of these platelet receptors is a pre-requisite for platelet activation and aggregation. Current ligands used to activate these receptors have a relatively undefined valency. Thus, there is a need for a standardised multivalent ligand for GPVI and CLEC-2 mediated platelet activation. Nanobodies (nbs), single variable chains V_{HH} fragment antibodies, have previously been raised against the ligand binding domains of GPVI (54 nbs, 33 structural classes) and CLEC-2 (48 nbs, 12 structural classes).

Aims – To modify two of the most potent nbs against GPVI and CLEC-2 to create divalent, trivalent and tetravalent variants with two/three/four equivalent binding epitopes. Followed by characterisation of the multivalent nbs binding to their respective receptors.

Methods – Receptor binding affinity was determined for the mono/multivalent GPVI and CLEC-2 nbs using surface plasmon resonance. Platelet aggregometry was used to investigate the effect of nb binding on platelet activation in the presence and absence of agonist ligands.

Conclusions – A high level of clustering, at least 3 receptors, is required to produce enough downstream signalling for GPVI and CLEC-2 mediated platelet activation. Divalent nbs are potent inhibitors of activation while trivalent/tetravalent nbs fill the need for a standardised multivalent agonist, both have applications in academia and clinical studies. Moreover, these nanobodies can be produced at high yield and low cost.

P 10

FIX is localised in the vessel wall and can generate thrombin when in complex with collagen IV

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Introduction
Haemophilia B is a blood clotting disorder caused by deficiency in coagulation Factor IX (FIX). Patients are treated by infusion of FIX concentrates based on their plasma concentration. However, FIX also binds reversibly to collagen IV in the basal lamina of blood vessels and may establish and important extravascular store.

Aim
To determine if the FIX-collagen IV complex participates in extravascular coagulation.

Methods
Flat-bottomed plates were coated with collagen IV prior to application of FIX. Thrombin generation (TG) was performed in FIX deficient plasma (FIX-DP) or pooled normal plasma (PNP), initiated with 1 pM tissue factor (TF) and measured using the Calibrated Automated Thrombogram. Different preparations of FIX were compared; Alprolix and Replenine. FIX distribution *in vivo* was studied in sections of synovial tissue from controls and Haemophilia B patients using immunofluorescence.

Results
No thrombin was generated by FIX-DP ± 1 pM TF on uncoated or collagen coated surfaces. However, on surfaces with the FIX-collagen complex present, FIX-DP generated a peak thrombin concentration comparable to PNP. FIX binding was confirmed by a chromogenic activity assay and an increase in FIX coating concentration (1-1000 U/ml) resulted in an increase in peak thrombin concentration. Collagen plates coated with extended half-life concentrate, Alprolix, generated a significant increase in the endogenous thrombin potential (ETP), when compared to short acting concentrate, Replenine (* $p < 0.05$). FIX was located in the vessel, within the vessel wall and extravascular space in synovial tissue sections from controls.

Conclusion
Our data confirm that FIX binds to collagen IV and raises the possibility that the intrinsic tenase may assemble on the collagen surface. Localisation of FIX in the vessel wall and extravascular space may provide an extravascular reservoir of FIX at a haemostatic and functional location, such as the joints.

P 11

Mathematical models of thrombin generation: A unified approach.

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Background
Mathematical models of thrombin generation have long been used to explore the mechanisms of the coagulation cascade. However, the use of these models as predictive tools is made challenging, in part, by the choice of reaction rates which can vary across orders of magnitude between different laboratories. The effect of this variation on model predictions has yet to be explored.

Aims
To develop a new process for building models of thrombin generation that allows investigation into, and quantification of, the effects of uncertainty in reaction rates and to implement this process to build a new model of thrombin generation that is validated against a large data set.

Methods: The reactions, rates and literature sources of current models of thrombin generation were investigated. Reaction rates were found to be based on multiple, often differing, sources. These were used to form realistic values for each reaction. Using both gradient descent and Bayesian fitting methods the revised model's predictions were compared to laboratory data from a large dataset comprising coagulation factor levels and optical density thrombin generation curves from 333 individuals.

Results
This systematic investigation generated a new model comprising 86 reactions that utilised 160 experimentally measured reaction rates across 59 different sources. This model showed significant improvements in predicting Optical Density curves compared with previous models and gave minor improvements in predicting ETPs. Uncertainty in the predictions is quantified point to where further experimentation can improve future models.

Summary/Conclusions
The use of a single source for rates for coagulation reactions is common among existing mathematical models. Systematic fitting methods helps overcome the experimental variation to produce a more predictive model of thrombin generation that is able to more accurately align with experimental data from large cohorts of donors, thereby increasing the applicability of these models for personalised medicine.

P 12

Post-infusion monitoring of Afstyla FVIII replacement therapy – data from the UK National External Quality assessment for Blood Coagulation (UK NEQAS BC) programme 2022

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Introduction

Monitoring of Afstyla replacement therapy for Persons With Haemophilia (PWH) is required and needs laboratories to use appropriate methods for accurate measurement. In May 2022 NEQAS BC. performed an Afstyla exercise using samples containing pre and post treatment doses. For this product there are recommendations from the manufacturer to double the one stage factor assay results. There were 6 participants out of 95 that commented on the validity of one stage assay results.

Method

Samples were constructed by addition of Afstyla FVIII concentrate at two levels to FVIII deficient plasma from a patient with severe haemophilia A (<1IU/dL FVIII) with normal VWF level in order to mimic a low level and post -treatment levels (2 samples distributed).. 104 participants in 15 countries took part. Participants were requested to perform the FVIII assays they would employ for measurement of Afstyla, regardless of whether they have patients on Afstyla.

Results

Method specific analysis showed median FVIII assay results for Afstyla 'pre' treatment levels for one stage assays= 4.55IU/d L and chromogenic assays = 7.0IU/dL. The post treatment sample showed one stage assays median 33.25IU/dL (n=88), chromogenic assays median 70.9 IU/dL (n=58).
Discussion: Manufacturer recommend a doubling of one stage assay results. Applying this to the median results would result in a 94% recovery of one stage assay level/ chromogenic assay level. From the data returned and applying the multiplication step to one stage assay results recoveries of between 84% and 126% compared to the chromogenic assay results were achieved.

Conclusion

Mathematical manipulation of factor assay for the data in this exercise appear to confirm the manufacturers recommendation. There is a risk that laboratories may not be aware of the EHL being prescribed. Afstyla levels by a chromogenic assay, do not require post analytical manipulation recommended by UKHCDO guideline, DOI: 10.1111/hae.13907.

P 13

NEQAS Blood Coagulation trials platelet pools to launch a platelet EQA programme for Light Transmission Aggregometry

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Introduction: Light transmission aggregometry testing on patients being investigated for bleeding disorders is a valuable diagnostic clinical service. These assays are complicated by the short timeframes for testing, within 4 hours of bleed and the difficulty with providing abnormal controls. Currently internal quality controls (IQC) for LTA are limited to normal healthy donors and although EQA is available it does not audit the entire process. NEQAS BC in conjunction with the Welsh Blood Service (WBS), has provided cold-stored pooled platelets for a pilot EQA exercise for LTA. The first phase of the pilot study was to assess the stability of LTA on three different pools of platelets. Following phase one, an expanded phase two exercise was completed that sent platelets to five testing sites.

Method: Pooled platelets were manufactured at the WBS (denoted as day 0) with platelet counts ranging from 720-993 $X10^9$/L. Aliquots (15mls) were dispatched to testing sites along with fresh frozen plasma (FFP), in temperature regulated transport boxes, to arrive within 24 hour time window. Upon receipt the testing sites, each site is colour coded in figure 1, were required to adjust the platelet count on the aliquot, as required by their specific assay, by diluting with the FFP. LTA was performed on day 1 or 2 using several different platforms: Helena Aggram; PAP-8 and Chronolog 700. The platelet count at the five sites was adjusted to 143-330$X10^9$/L before aggregation was performed with agonists including: ADP, arachidonic acid, collagen, epinephrine and ristocetin.

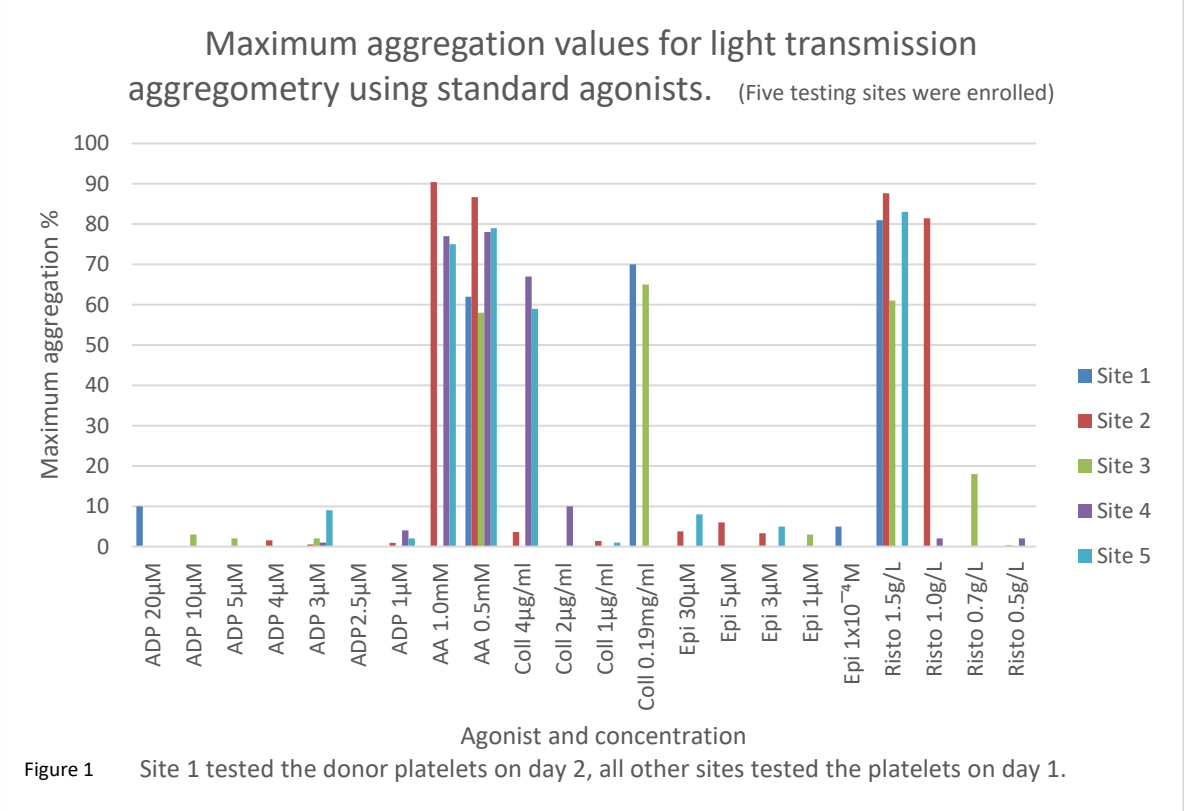

Figure 1 Site 1 tested the donor platelets on day 2, all other sites tested the platelets on day 1.

Results: The LTA reported normal responses to: arachidonic acid in 5/5; collagen in 4/5 and ristocetin in 4/5 centres. Abnormal responses to ADP and epinephrine at all concentrations in all centres.

Discussion: This pilot exercise explored the logistics required for delivery of cold stored platelets to laboratories. The interpretation of the results suggests platelets, delivered at 4-8°C, are a viable source of EQA material for assessment of LTA. Four of the five sites performed the testing on the day the packs arrived (day 1), with one site performing the testing the next day (day 2). The consensus with the interpretative data returned has shown that the testing time window of 1-2 days, when the platelets are stored at 4-8°C, does not affect agonist response on the aggregometer. The exercise has proved that a larger cohort will be part of the next phase plus ISTH or BSH concentrations of agonists for platelet EQA exercises.

Conclusion: Platelet EQA currently available focuses on the post analytical phase of platelet function testing. This pilot EQA programme demonstrates the possibility of material for multicentre testing and comparison of LTA results that is closer to auditing the entire analytical process on conform to the ISO standard 15189 required by clinical laboratories.

P 14

Comparison of coagulation parameters associated with fibrinogen concentrate and cryoprecipitate for treatment of bleeding in patients undergoing major cytoreductive surgery: Results from a randomised, controlled Phase 2 study

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Background
The FORMA-05 study compared the efficacy and safety of human fibrinogen concentrate (HFC) versus cryoprecipitate for maintaining haemostasis in bleeding patients undergoing cytoreductive surgery for pseudomyxoma peritonei (PMP).

Aims
This *post-hoc* analysis aimed to explore coagulation parameters in patients who received HFC versus cryoprecipitate, with a focus on seven patients in the cryoprecipitate group who developed thromboembolic events (TEEs).

Methods
FORMA-05 was a prospective, randomised, controlled Phase 2 study. Patients undergoing PMP surgery with predicted blood loss ≥2 L received HFC (4 g) or cryoprecipitate (2 pools of 5 units), repeated as needed. Plasma fibrinogen, platelet count, factor (F) XIII, FVIII, von Willebrand Factor (VWF) antigen and ristocetin cofactor activity levels, EXTEM A20, FIBTEM A20 and endogenous thrombin potential (ETP) were measured perioperatively.

Results
Patients were randomised to receive cryoprecipitate (N=23) or HFC (N=21). Fibrinogen, platelet count, EXTEM and FIBTEM A20, FXIII, FVIII, VWF levels and ETP were maintained throughout surgery in both groups. No patients in the HFC group developed TEEs. Two patients in the cryoprecipitate group developed deep vein thromboses (DVT), and appeared to have a procoagulant status preoperatively, with distinctively higher fibrinogen level, FIBTEM A20, and platelet levels which persisted perioperatively. Five patients in the cryoprecipitate group developed pulmonary embolism (PE), with these patients showing a disproportionate increase in VWF levels intraoperatively (post-cryoprecipitate administration) which was retained postoperatively.

Summary/Conclusions
Patients treated with HFC versus cryoprecipitate showed broad overlaps in coagulation parameters, however some differing patters were observed in patients who developed TEEs. Patients who developed PE experienced a disproportionate rise in VWF following cryoprecipitate administration, while patients who developed DVT displayed a procoagulant status before and following surgery. Preoperative testing may allow these patients to be identified and thus minimise risk of TEEs.