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Poster abstracts

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Poster - 01

Characterisation of 17 Novel Von Willebrand Factor Missense Mutations

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Background:

Von Willebrand factor (VWF) is a large multimeric glycoprotein that plays an important role in haemostasis. Qualitative or quantitative abnormalities in plasma derived VWF is resultant in Von Willebrand disease (VWD). Previous studies have established a strong association between missense mutations found in the heterogeneous protein and disease-causing mechanisms. A recent cross-sectional familial VWD study identified 17 unrecognised missense mutations in suspected VWD patients.

Aim:

To investigate the impact of 17 unrecognised missense mutations on VWF expression and function.

Methods

Site directed mutagenesis was used to create 17-novel point mutations (R5K, G39K, D47V, E197K, V343M, A631R, C996S, C1031S, A1250D, E1292D, E1615D, D1691E, T1728S, R1830C, G1890E, T2023A, E2353K) in the pcDNA-FL-VWF expression vector that encodes for full length VWF. Recombinant VWF (rVWF) was expressed in HEK293T cells and VWF ELISA for quantification of protein levels in the media and cell lysate. The physiological function of rVWF was evaluated using static and shear-based assays.

Results:

The mutations G39R, D47V, A631T, C996S, G1890E all virtually abolished secretion of VWF, while mutations E1615D and E2353K reduced secretion. Interestingly co-transfection with wtVWF rescued expression to varying extents and mutant monomers could be incorporated into multimers and secreted. Endo H and immunofluoresce staining demonstrated retention of mutant VWF within the ER. Significantly, none of the secreted mutations had abnormal function under shear stress, despite the T1728S and R1830C mutations being located in the A3 domain which contains the major collagen binding site. While the E1615D mutation in the A2 domain was cleaved faster by ADAMTS13 indicative of VWD type 2A.

Conclusion:

The 17 novel mutations have varying effects on the VWF molecule and demonstrate the heterogenicity of VWD.

Poster - 02

Bleeding and thrombotic complications and their impact on mortality in patients supported with left ventricular assist device for cardiogenic shock

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Background: Bleeding and thrombotic complications are known to cause adverse outcome in patients supported with the HeartWare left ventricular assist device (HVAD)

Aims: To assess the incidence of bleeding and thrombosis in patients supported with HVAD, their predictive factors and the impact of bleeding and thrombosis on 1-year mortality.

Methods: A single centre retrospective observational study of patients supported with HVAD over 5 years from January 2015-October 2020.

Results: A total 139 patients (median age 52.5, 72.1% male) were included for analysis. The probability of 1-year survival was 73.1% (102/139). Advanced age (>60years) and EuroSCORE II score (>20%) were independently associated with reduced survival. Incidences of major bleeding and thrombotic complications were 46.8% and 35.3% respectively. Those supported with a secondary mechanical circulatory device (MCS) were more likely to experience a major bleed (HR: 3.06, 95% CI 1.8-5.3, p<0.0001) whilst patients receiving aspirin were protected from bleeding and thrombosis (HR; 0.34 95%CI 0.19-0.58, p<0.001). Pre-operative anaemia (HR 2.78, 95% CI: 1.2-6.3, p=0.014) and use of a secondary MCS device (HR 3.02, 95% CI: 1.6-5.7, p=0.001) were associated with an increased risk of thrombosis. Patients with any major bleed (with or without thrombosis) had a 7.68-fold (95% CI 3.5-16.8) increased risk of death compared to those without. In contrast, 'thrombosis only' patients had 4.23-fold (95% CI 1.8-10.2) increased risk of death compared those without thrombosis. The risk of mortality was increased in patients with any thrombosis and the risk of death was highest in patients with major bleed and thrombosis (HR 16.49 [95%7.7-35.3]) (Table 1).

Conclusions. Bleeding and thrombosis significantly increase the likelihood of mortality: patients with thrombosis with or without bleeding and those with combined thrombosis and bleeding complications had the highest 1-year mortality. Optimal perioperative haemostasis and anticoagulation remain crucial in patients supported with HVAD.

Table 1. Impact of bleeding and thrombosis on 1yr survival following insertion of Left ventricular assist device.

Complication	N = (139)	Alive (n=102)	Dead (n=37)	Crude time-adjusted HR (95%CI)	Time-adjusted HR* (95%CI)
No Bleed or Thrombosis	46 (33.1%)	44 (95.7%)	2 (4.3%)		
Any Bleed	65 (46.8%)	38 (58.5%)	27 (41.5%)	7.88 (3.8-16.5)	**7.68 (3.5-16.8)**
Bleed only	44 (28.1%)	32 (72.7%)	12 (27.3%)	1.90 (0.93-3.9)	**1.59 (0.75-3.3)**
Any Thrombosis	49 (35.3%)	26 (53.1%)	23 (46.9%)	12.76 (6.3-25.8)	**14.2 (6.8-30.0)**
Thrombosis only	28 (20.1%)	20 (71.4%)	8 (28.6%)	2.82 (1.2-6.4)	**4.23 (1.8-10.2)**
Bleed and Thrombosis	21 (15.1%)	6 (28.6%)	15 (71.4%)	17.84 (8.7-36.4)	**16.49 (7.7-35.3)**

Major bleeding is defined as >BARC 2

*Adjusted for patient age and Euroscore II. All HR's are shown relative to not having the condition of interest (HR 1.00)

Poster - 03

Long term outcomes of the use of statins for angiodysplasia in Von Willebrand disease

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Von Willebrand factor (VWF), although largely known for its role in haemostasis, has also been demonstrated to regulate angiogenesis confirming its role as a multifunctional vascular protein. This helps explain why recurrent gastrointestinal bleeding is a distinctive symptom of Von Willebrand disease (VWD) often due to associated angiodysplasia. Angiodysplasia associated bleeding is frequently refractory to conventional VWD replacement therapy.

We previously reported the benefit of atorvastatin in angiodysplasia and suggested that this was due to its anti-angiogenic effects. This biphasic cholesterol-independent effect of statins is well reported with proangiogenic effects at low therapeutic concentrations and angiostatic effects at high concentrations.

Here we report the 13 and 5 year follow up of two cases of VWD where high dose statins brought about efficacious termination of severe GI bleeding. The first is a 69 year old male with Type 1 VWD who was the first reported case in whom 40mg atorvastatin was successfully used to arrest intractable bleeding secondary to angiodysplasia in 2008. After 13 years on statin therapy the patient has had no significant GI bleeding requiring intensive intervention and remains stable on long term prophylaxis.

Our second patient is a 48 year old female with Type 2A VWD and severe angiodyplasia who required three units of blood per week for 5 months in 2016 despite VWF replacement. Commencing 80mg atorvastatin abolished her transfusion requirement and halted bleeding. 5 years on she remains stable on the same dose of statin and off prophylaxis with only one admission for PR bleeding in 2020 requiring 3 days of Voncento.

There have been no statin-induced side-effects observed in either patient. Successful therapy in this group is challenging and often not achievable. Long-term follow up of these patients treated with high-dose statins confirms they are largely bleed-free with no recurrence of their critical bleeding.

Poster - 04

Tranexamic acid has a direct impact on the structure of the fibrin network

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Background:

Tranexamic acid (TXA) is a lysine analogue with potent anti-fibrinolytic function. TXA binds to the lysine binding sites on plasminogen and plasmin, thus preventing their interaction with fibrin and thereby down-regulating fibrinolysis.

Aims:

To determine if TXA binding alters the structure of the fibrin network.

Methods:

Citrated plasma from healthy volunteers was spiked with a range of TXA concentrations (0-400 µM). A turbidity assay was performed to monitor clot formation and, fibrinolysis, initiated by tissue or urokinase type plasminogen activator (tPA and uPA, respectively). Confocal microscopy was used to monitor fibrin clot structure in the presence of Alexa Fluor 488 fibrinogen and Alexa Fluor 647 plasminogen.

Results:

Confocal microscopy revealed a fibrin network that was more dense and comprised of smaller fibres in the presence of increasing concentrations of TXA. Consistent with the confocal experiments, maximum absorbance was significantly increased in plasma clots formed in the presence of TXA ($p < 0.05$), suggesting an increase in clot density. Interestingly, we observed a decrease in binding of exogenous plasminogen to fibrin clots with increasing concentrations of TXA. Inhibition of fibrinolysis by TXA occurred significantly faster during uPA-mediated fibrinolysis when compared to tPA. Lysis was observed at 15 µM TXA with tPA, however, no fibrinolysis was detected at concentrations > 5 µM with uPA.

Conclusion:

These data show that TXA alters the structure of the fibrin network via reduced adherence of plasminogen and produces clots that have an increased number of fibres. Clots with higher density lyse at a slower rate, suggesting a second mechanism of action for TXA to inhibit fibrinolysis.

Poster - 05

Prior oral anticoagulation therapy and clinical outcomes in stroke patients with atrial fibrillation in Scotland: A national database study.

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Affiliations: The Grampian Data Safe Haven (DaSH).

Background:

Stroke risk is significantly increased in patients with atrial fibrillation (AF) and it has been shown to influence stroke outcomes. Oral anticoagulants (OAC) are recommended for stroke prevention in patients with AF. Despite the clinical benefits of therapy, many patients are still not prescribed an OAC, especially the elderly. This study analyses the impact of prior OAC therapy on stroke outcomes.

Methods:

This retrospective cohort study used four nationally linked datasets; the Scottish Stroke Care Audit, the Prescribing Information System, the Scottish Morbidity Record 01, and the National Records of Scotland. The study population included incident stroke patients with recorded atrial fibrillation between January 2010 and December 2015. Prior OAC therapy was defined as at least one prescription within six months before stroke. Multivariate logistic regression was used to calculate odds ratios, models were adjusted for basic demographics including age, sex and stroke type (haemorrhage or infarct). The main clinical outcomes analysed were initial stroke severity and short-term mortality post-stroke.

Results:

Of 9648 patients (mean age = 79.9 [± 9.6] years; 80 to 89 years age group = 42.1%), 2027 (21%) were receiving OAC before admission. 296 (14.6%) of these had haemorrhages, compared to 341 (4.5%) of the non-OAC group. Prior OAC independently increased the odds of being able to talk, orientated, lift arms and walk indicating milder initial stroke severity (adjusted odds ratio (aOR) 1.275; [95% Confidence Interval (CI), 1.127-1.443]) and was associated with more comorbidities: (aOR 1.251; [95% CI, 1.102-1.420]). Haemorrhage was associated with an almost two-fold increase in risk of death in the OAC group (aOR 1.9; [95% CI, 1.474-2.445]). After adjustment for stroke type, prior OAC therapy was associated with increased all-cause mortality at 30 days (aOR 1.240; [95% CI, 1.022-1.505]).

Conclusion:

Among this AF population, prior oral anticoagulation therapy appeared to be associated with less severe strokes but conversely increased odds of mortality at 30- and 90- days. This may be related to a higher burden of comorbidities in the OAC population and requires further exploration.

Poster - o6

Using CRISPR/Cas9 to investigate the structure/function relationship of PLCγ2 in platelets

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Background:

Phospholipase C gamma 2 (PLCγ2) is a key signalling hub, playing a role in a number of platelet signalling pathways. These include the collagen receptor GPVI which activates via an ITAM (immunoreceptor tyrosine-based activatory motif), and the podoplanin receptor CLEC-2, which activates through a similar mechanism known as a hemITAM. PLCγ2 is a large, multi-domain protein. The regulation of its function is through molecular interactions with a number of signalling proteins, although this is not fully understood.

PLCγ2 plays a similar role downstream of ITAM-linked receptors in other cells, namely B-cells. PLCγ2 mutations have been identified in 80% of patients who develop resistance to ibrutinib therapy. Some of these PLCγ2 mutations have been shown to cause a gain-of-function, but the effect of the others is unknown. It is also unknown whether these, or other mutations may arise in platelets, and what the functional effect on platelet activity might be.

Aim:

To characterise the effect of PLCγ2 mutations on PLCγ2 function downstream of (hem)ITAM receptors.

Methods:

CRISPR/Cas9 was used to generate PLCγ2 knock-in and knock-out models in DT40 cells. To characterise the functional effect of the mutations, calcium assays, NFAT assays, and tyrosine phosphorylation were used. A PLCγ2 inhibitor was used to further assess the impact of these mutations. This inhibitor was validated and used as a control in platelet-based calcium and aggregation assays.

Results:

PLCγ2 knock-out DT40s were successfully generated. Calcium responses and NFAT signalling in response to GPVI and CLEC-2 activation were abolished in these cells. Similar results were seen with a PLCγ2 inhibitor in both DT40 cells and platelets.

Conclusion:

Thus far these results confirm the essential role for PLCγ2 downstream of (hem)ITAM receptor signalling. Characterization of knock-in point mutations is in progress. Future work will move into zebrafish for in vivo analysis of PLCγ2 function.

Poster - 07

Impact of aspirin on bleeding and thrombosis in on-pump and off-pump coronary artery bypass graft

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Background:
Major bleeding is linked to poorer outcomes following cardiac surgery. Current guidelines (2016) recommend the continuation of aspirin prior to coronary artery bypass graft (CABG) and off-pump (OP) CABG. However, the effect of continuing aspirin on bleeding and blood product usage have not been systematically assessed since the 2016 introduction of the guidelines, particularly in OPCABG.

Aims:
To determine the effect of continuing aspirin prior to CABG or OPCABG on bleeding, thromboembolism (TE), blood product usage and overall clinical outcomes.

Methods: A single-centre, retrospective observational study comparing propensity matched patients who continued aspirin until the day of CABG or OPCABG to controls and to patients who discontinued aspirin 5-7days prior.

Results:
Length of hospital stay (LOHS), 30-day mortality and TE rate were similar in aspirin-continued patients in comparison to controls and aspirin discontinued patients for both CABG and OPCABG. The only difference in bleeding between controls and aspirin continued patients during CABG was a small increase in volume of cells salvaged among aspirin continued patients: 1592.5ml (0-6250) vs 1333ml (0-5336), p=0.01. With OPCABG, aspirin continued patients received more intraoperative red cell units (0.62units [0.39-0.85]) compared to controls (0.38units [0.17-0.58]), p=0.034, without difference in bleeding. Aspirin continued patients received more blood products intraoperatively and perioperatively and bled more than aspirin discontinued undergoing OPCABG.

Conclusions:
Current guidelines on continuation of aspirin prior to CABG and OPCABG are safe, including the decision to discontinue aspirin 5-7days prior to surgery. Aspirin continued patients had similar levels of bleeding to controls during conventional CABG. Any increase in blood product administration associated with continuing aspirin monotherapy prior to OPCAB, if required, is small. Any increase in bleeding should aspirin be continued prior to OPCABG was not associated with adverse outcomes. However, given low TE rates, this study was likely underpowered to definitively comment on TE.

Poster - o8

Severe COVID-19 is associated with endothelial activation and abnormal glycosylation of von Willebrand factor in patients undergoing hemodialysis

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Background:
A major clinical feature of severe coronavirus diease 2019 (COVID-19) is microvascular thrombosis linked to endothelial cell activation. Consistent with this, a number of studies have shown that patients with severe COVID-19 have highly elevated plasma levels of von Willebrand Factor (VWF) that may contribute to the prothrombotic phenotype. In the current study, we investigated the extent of endothelial activation in patients receiving hemodialysis who had either mild or severe COVID-19.

Methods:
Plasma VWF, ADAMTS-13, angiopoietin-2 (Ang2), and syndecan-1 levels were determined by ELISA. The sialic acid content of VWF was investigated using a modified ELISA to measure elderberry bark lectin, specific for sialic acid residues, binding to VWF.

Results:
Patients receiving hemodialysis with severe COVID-19 had significantly higher plasma levels of VWF and lower ADAMTS-13. VWF levels peaked and were sustained during the first 10 days after positive confirmation of infection. While Ang2 trended toward being higher in severely ill patients, this did not reach significance; however, severely ill patients had significantly higher soluble syndecan-1 levels, with high levels related to risk of death. Finally, higher VWF levels in severely ill patients were correlated with lower VWF sialic acid content.

Conclusions:
Severe COVID-19 in patients undergoing hemodialysis is associated with both acute and sustained activation of the endothelium, leading to alteration of the VWF/ADAMTS-13 axis. Lower VWF sialic acid content represents altered VWF processing and further confirms the disturbance caused to the endothelium in COVID-19.

Poster - 09

Analysis of Von Willebrands Disease causing mutations located in the D4 and C-domains

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Background:
Von Willebrand's disease is a common autosomal inherited bleeding disorder characterised by a quantitative (type 1 and type 3) or qualitative (type 2) deficiency of Von Willebrand Factor (VWF). While most of the repeating domains in the VWF molecule have ascribed roles, the functional role of the D4 and C-domains remain poorly understood, although we have previously shown that deletion of various C-domain regions abolishes VWF secretion. Analysis of the VWD database highlighted several putative type 1 and type 3 mutations in the D4 and C-domains that are yet to be characterised.

Aim:
To characterise the impact of D4 and C-domains VWD mutations of VWF expression *in vitro.*

Methods
Mutations were generated by site directed mutagenesis and transiently expressed in HEK293/T cells alone or alongside wild type (wt) VWF and expression levels determined by ELISA. Co-transfections of mutant sequences alongside VWF lacking the A1 domain was performed to determine inclusion of mutant monomers into full length VWF. Endo H digests and immunofluorescence microscopy was performed to investigate cellular retention.

Results:
In-silico analysis revealed ten mutations C1950Y, G2044D, C2174G, E2233Q, R2287W, C2340R, G2343V, R2379C, S2497P and C2693Y reported as type 1 or type 3 with no functional analysis. Homozygous transfections resulted in abolished VWF secretion for all mutants which was rescued by co-transfection with wtVWF; however, for mutations C1950Y, C2174G, E2233Q, C2340R and R2379C the mutant monomer was not expressed indicating catastrophic effects on protein folding. Endo H digests confirmed ER retention and immunofluorescent imaging of transfected HEK293 cells showed that while most VWF was present in the ER, some pseudo Weibel-Palade bodies (pWPB) were formed for all mutants however more pWPB were formed with wtVWF.

Conclusion:
Mutations in the D4 and C-domain affect VWF secretion indicating a critical role for the D4 and C-domains for correct VWF folding.

Poster - 10

Splenectomy in refractory chronic immune thrombocytopenic purpura with equal sequestration of platelets in both liver and spleen on Indium labelled platelet survival study

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Acknowledgement: Dr Rowena Faulkner, *Consultant haematologist, Chesterfield Royal Hospital*

Case Presentation:

I want to present a case of 35 years old lady who initially presented with easy bruising in May,2017, found to have platelet count only 3.

After doing further investigation as inpatient, we concluded her diagnosis as Immune thrombocytopenic purpura and started her on oral prednisolone. But she showed only suboptimal response to steroids (highest platelet count was 59).

In October,2017 after completion of 4 cycles of Rituximab her platelet count remained at 20. We explored the option for splenectomy but at that time patient declined.

As 3rd line treatment patient was started on Eltrombopag in November,2017 along with steroid which did improve platelet count initially to 145 -244 but later showed variable platelet count with intermittent episodes of significant thrombocytopenia.

We again revisited the option for splenectomy and patient agreed but platelet survival study showed 55 hours platelet lifespan and 50%-50% splenic and hepatic sequestration, therefore not ideal for splenectomy.

Patient was started on mycophenolate along with Eltrombopag in April,19 but due to compliance issue it was stopped in July ,2019.

She was then started on Romiplostim which improved her platelet count initially but later became refractory.

She refused to be referred for clinical trial with Fostamatinib which is a splenic tyrosine kinase inhibitor.

In March,2020 patient was started on again MMF along with romiplostim 600mcg and low dose prednisolone. Whilst on this treatment she had intermittent course of pulsed dexamethasone when platelet count dropped to single figure.

In January,2021 despite being on maximum dose of MMF, her platelet count remained at 2. Therefore, she was finally referred for splenectomy in February,2021.

Then she had splenectomy in May 2021 and her platelet count started improving. Her platelet count is stable at 73 now.

Discussion: This case illustrates that we can still consider splenectomy for ITP patients who are refractory to all medical treatment options despite having equal platelet destruction in liver and spleen, as this would give at least partial remission.

Poster - 11

Is there a role for Anti-Xa monitoring to ensure safe DOAC efficacy in clinical practice?

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Background: Direct-acting oral anticoagulants (DOACs) are superseding warfarin for patients diagnosed with atrial fibrillation (AF). A benefit from DOACs therapy is no routine monitoring or dose adjustments. However, studies have shown high plasma DOAC levels may be associated with bleeding, and low levels with increased cardioembolic stroke rates. Factors effecting DOAC plasma levels are multifaceted and still cannot be fully elucidated by clinical trials, such as extremes of body weight (BW) (<50kg - >120kg) or co-prescribed interacting drugs.

Aims: Identifying patients in a pharmacist-led AF clinic with extremes of BW or co-prescribed interacting drugs and measure Anti Xa levels to ensure safe DOAC efficacy.

Method: Blood samples were taken at Tmax and analysed in the lab using hemosil chromogenic liquid Anti-Xa assay. This assay measures direct FXa inhibitor concentrations. DOAC concentration is analysed as a complex with endogenous antithrombin present in the sample. When these complexes form reactions take place. The paranitroaniline released from the reactions result in a colour change which is monitored by the ACL-TOP analyser kinetically at 405nm. The colour change produced is inversely proportional to the amount of DOAC level in the patient sample.

Results:

DOAC	Reason for level	Anti Xa Level
Rivaroxaban 20mg	143 kg	461.2 ng/ml
	142 kg	198.4 ng/ml
	140 kg	202.7 ng/ml
	135 kg	185.5 ng/ml
	Levetiracetam	469.4 ng/ml
	Levetiracetam	317.3 ng/ml
	Enzalutamide	190.0 ng/ml
Apixaban 5mg BD	135kg	186.0 ng/ml
	43.2kg	236.2 ng/ml
	130kg	231.5 ng/ml
Apixaban 2.5mg BD	Rifampicin	83.3 ng/ml
Edoxaban 60mg OD	138kg	143.0 ng/ml
Edoxaban 30mg OD	38kg	356.9 ng/ml

Figure 1: Anti Xa level results of each DOAC, peak plasma levels used in patients for stoke prevention. Reference ranges as per EHRA (J. Steffel et al. 2021) Apixaban 69-321 ng/ml, Edoxaban 101-288 ng/ml, Rivaroxaban 178-343 ng/ml

Summary/conclusion: 3 (23%) patients Anti Xa level were higher than the recommended therapeutic range. None of these patients reported any bleeding side effects. Therapy was not changed for the patients on rivaroxaban, and risks explained. Edoxaban therapy was changed to apixaban, pending repeat levels. All 3 patients had declining renal function, suggesting this may be an important factor in efficacy. Critically, the Anti Xa reference ranges are very broad for each drug and frequency of monitoring would need to be reviewed.

Poster - 12

UK NEQAS (Blood Coagulation) Genetics of Heritable Bleeding and Thrombotic disorders programme - a review of External Quality Assessment (EQA) Scheme exercises 2021

Ian Jennings[1], **Christopher Reilly-Stitt**[1], Marian Hill[2], Andrew Page[3], Ruth Wheeler[4], Megan Sutherland[5], Steve Kitchen[1], Isobel Walker[1]

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Background: The programme blends whole blood sample distribution, to assess laboratory methods for variant analysis, with paper exercises to scheme participants. The latter are a useful tool to assess data interpretation and reporting processes for rare bleeding disorders, without the need to analyse whole blood.

Results : Exercises distributed in 2021 and outcomes are described in table 1.

Patient details	Exercise format	Genetic variant present	% obtaining full marks
Case 1. *F8*	Whole blood	hemizygosity for c.6440G>T, p.(Gly2147Val)	63% (14/22) Marks deducted if: "risk to offspring" was absent in advice given or reports did not use recommended nomenclature.
Case 2. *F8*	Whole blood	*F8* intron 22 inversion	100% (21/21)
Case 3. *F5*	Paper	c.5044G>T, heterozygous p.(Glu1682) c.6419G>A, heterozygous p(Gly2140Asp)	73% (8/11)
Case 4. *PROS1*	Paper	c.1063C>T, heterozygous p.(Arg355Cys) c.1501T>C, heterozygous p.(Ser501Pro)	80% (8/10)

Summary: For whole blood exercises, all centres correctly identified the genetic variant in case 1; marks were lost for omissions in the interpretative reports. In case 2, all participants investigating a *F8* Intron 22 inversion achieved full marks. This demonstrates improvement in methodologies used to perform genetic analysis compared to an exercise in 2011 when 4/22 centres failed to identify the variant. For the paper exercises, the variant classification for *F5* had 100% of participants reporting that the variant was either pathogenic or likely pathogenic for c.5044G>T and 80% made the same conclusion for c.6419G>A. For *PROS1,* 100% of participants reported the variant either pathogenic or likely pathogenic for c.1063C>T and 70% reported the variants either a disease associated polymorphism/variant of uncertain significance for c.1501T>C. The scheme offers a blended approach to participants from genetics laboratories, testing platforms used for variant analysis and encouraging use of recommended guidelines for clinical reporting procedures.

Poster – 13

Measurement of fibrinogen using two concentrations of Thrombin with patients receiving Argatroban therapy or normal plasma spiked with Argatroban: a review of UK NEQAS (Blood Coagulation) External Quality Assessment Scheme exercises 2021

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Background: Measurement of fibrinogen with the Clauss assay is a thrombin time-based method that can be affected by Direct Thrombin Inhibitors. Argatroban is a direct thrombin inhibitor used in patients being treated for HIT and VITT.

Methods: A supplementary exercise for Argatroban and fondaparinux included samples containing argatroban from a pool of treated patients and from a spiked normal plasma. Participants were requested to return Fibrinogen assay results. In addition, a regular Laboratory Programme exercise (survey 249) included a sample for fibrinogen assay from a patient undergoing plasma exchange and receiving argatroban for VITT.

Results: Samples distributed as part of an argatroban exercise (A21:01-05) and as part of the laboratory programme (249 21:31) in 2021 are detailed in table 1.

Sample details	Argatroban level	Fibrinogen with 35NIH/ml	Fibrinogen with 100NIH/ml	Ratio of median fibrinogen result 100NIH/35NIH
A21:01	0.05ug/ml	Median 2.5g/L Range 2.15-2.54 g/L	Median 2.19 g/L Range 2-2.37 g/L	0.88
A21:02	0.95ug/ml	Median 0.4 g/L Range 0.21-0.88 g/L	Median 4.21 g/L Range 3.65-4.64 g/L	10.52
A21:03	1.54ug/ml	Median 0.4 g/L Range 0.12-0.47 g/L	Median 3.5 g/L Range 2.83-3.9 g/L	8.75
A21:04	0.63ug/ml	Median 1.38 g/L Range 1.13-1.38 g/L	Median 2.95 g/L Range 2.54-3.3 g/L	2.14
A21:05	0.92ug/ml	Median 0.4 g/L Range 0.2-0.87 g/L	Median 2.89 g/L Range 2.54-3.3 g/L	7.23
249 21:31	0.2ug/ml	Median 0.51 g/L Range 0.4-0.84 g/L	Median 0.7 g/L Range 0.4-1.09 g/L	1.37

Table 1

Discussion: Commercial reagents for Fibrinogen quantitation have a range of thrombin concentrations and it is clear that the level of argatroban in patient or spiked samples can interfere with the measurement of fibrinogen. The data from the samples distributed highlight the fibrinogen underestimation with some assays in samples with levels of Argatroban at therapeutic levels. Manufacturer's inserts do not describe any assay limitation with regard to Argatroban or other direct thrombin inhibitor.

Poster - 14

Monitoring Emicizumab-data from a World Federation of Haemophilia International External Quality Assessment Scheme exercise

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Background:
Emicizumab is a bispecific monoclonal to replace Factor VIII for treatment of Congenital Haemophilia with and without Factor VIII inhibitors present. This study was to assess laboratory testing methods for accurate measurement of emicizumab plus to inform participants monitoring patients on emicizumab of the sensitivity of other laboratory tests employed for the treatment of Haemophilia A.

Methods:
FVIII deficient plasma was spiked with emicizumab to mimic a patient receiving a prophylactic treatment dose at a maintenance level. Participants were encouraged to report an APTT and any assay detecting the presence of emicizumab.

Results:
45 /140 centres returned results. Eleven different APTT reagents were employed by participants with a median APTTR of 0.74 (n=42). Factor assays by un-modified APTT assays gave a median of 410 IU/dL (n=15, range 1.05-708.8 IU/dL). There were 20 sets of results for chromogenic FVIII assays with bovine FX (and either human or bovine FIXa), with an overall median of 0.5 IU/dL. 11 participants returned results for emicizumab measurement with an overall median of 57.5µg/ml.

Summary:
Monitoring emicizumab may be required in Haemophilia A patient management. The APTTR and APTT un-modified factor assay results for spiked emicizumab samples are effected by emicizumab at maintenance doses and cannot be used by clinical teams to indicate the drug level. The emicizumab results from the modified assays using emicizumab as a calibrator are offered in 24% of the laboratories submitting results for this exercise. Chromogenic assays with bovine FX (and either human or bovine FIXa) are insensitive to emicizumab thus allowing for accurate measurement of endogenous Factor VIII.

Conclusion:
Emicizumab measurement may be required to reassure clinical teams of patient compliance and confirm drug is present at expected levels. WFH guidance on assays to use is available with the reference Srivastava et al, Haemophilia 2020; Suppl 6:1-158.

Poster - 15

Zinc dietary intake in humans regulates zinc platelet pool and impacts on haemostatic parameters

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Background

A large pool of zinc, released following activation, is found within platelets. Zinc has been implicated as a cofactor in haemostatic reactions, but the direct impact of dietary zinc on haemostasis has not been investigated.

Aims

To determine whether dietary zinc intake alters the platelet pool and assess the impact on haemostatic parameters.

Methods

Thirty-six healthy volunteers followed a diet depleted in zinc for two weeks (1 mg/day) followed by a two-week repletion phase (≥4.5 mg/day). Blood was collected at baseline (habitual diet), depletion and repletion phases. Platelet rich plasma (PRP) was isolated and various haemostatic parameters quantified.

Results

Zinc levels in platelet lysates, quantified by Inductively Coupled Mass Spectrometry, revealed a significant reduction during dietary depletion compared to habitual diet (6.78 ± 0.52 vs. 9.63 ± 1.18 µM; $p<0.05$). Levels were normalised following repletion of zinc (9.96 ± 0.65 µM; $p<0.05$). Lagtime to thrombin generation was delayed by zinc depletion compared to habitual diet (16.39 ± 4.05 vs. 13.07 ± 1.07 min $p<0.05$) and thrombodynamic analysis revealed attenuated clot growth (37.52 ± 3.02 µm/min vs. 51.55 ± 2.35 µm/min; $p<0.001$). Platelet aggregation was significantly attenuated by depletion of zinc (45.06 ± 6.9 % vs. 90.80 ± 6.62%; $p<0.005$) as was clot retraction (459 ± 17.1 mg vs. 382 ± 9.73 mg; $p<0.001$). Lysis of PRP clots by tPA was delayed by zinc depletion (99.46 ± 5.19 vs. 78.30 ± 3.85 min; $p<0.005$). All haemostatic parameters returned to baseline levels upon dietary repletion of zinc.

Conclusion

Our data show for the first time that changes in dietary zinc impact on the pool of this ion within platelets. Depletion of platelet zinc manifests as changes in platelet activity and clot dynamics, indicative of an impaired haemostatic response. These data have implications in populations and individuals susceptible to zinc deficiency.

Poster - 16

Changing haematocrit in patients with autoimmune haemolytic anaemia affects clot formation and resistance to fibrinolysis

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Background:
The prothrombotic profile in anaemic individuals is multifactorial, however a direct pathogenic role of red blood cells (RBCs) through abnormal clot structure has not been investigated.

Aims:
To examine the role of RBCs in promoting thrombosis in anaemic individuals.

Methods:
Ex-vivo manipulation of the HCT was used to produce reconstituted samples containing 20%, 40% and 60% final HCT, with 35% PRP. Serial whole blood samples from patients with autoimmune haemolytic anaemia (AIHA) were obtained. Coagulation parameters were assessed using thromboelastometry and whole blood thrombin generation. Chandler model thrombi were formed under continuous flow from reconstituted blood in the presence of FITC-labelled fibrinogen and lysis induced by bathing thrombi in 1 µg/ml tPA.

Results:
Thromboelastometry revealed shorter clot time (CT) (p<0.01), clot formation time (CFT) (p<0.001) and an increase in the α-angle at 20% HCT vs 60% HCT (p<0.05); indicating faster clot formation at lower HCT. Increased maximum clot firmness (MCF) (p<0.05) at low HCT was also observed. HCT did not impact whole blood thrombin generation. Chandler model thrombi formed at 20% HCT vs 60% HCT were longer (p<0.05) and demonstrated increased resistance to lysis with tPA (p<0.001). In patients with AIHA a HCT ≤ 30% resulted in a 2-fold faster CFT (p<0.0001) with a significantly steeper α-angle (p<0.001) compared to samples from a HCT > 30%. MCF values demonstrated firmer clots with a HCT ≤ 30% compared to HCT > 30% (p<0.01). Thrombi formed from serial samples from the same patients with AIHA demonstrated a clear link between rising HCT and reduced resistance to lysis.

Conclusion:
Augmented clot formation and resistance to lysis is observed at low HCT in experimental manipulated samples. These data mimic the situation in clinically anaemic AIHA patients, with low HCT promoting increased thrombus formation and resistance to lysis, which improves with rising HCT.