HAEMATOLOGY ASSOCIATION OF IRELAND

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Europa Hotel, Belfast
Friday 11 and Saturday 12 October 2024

Diagnostic Laboratory Haematology and Transfusion Session Abstract Book

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HAEMATOLOGY ASSOCIATION OF IRELAND

Diagnostic Laboratory Haematology and Transfusion Session Programme
Friday 11 October 2024, Europa Hotel, Belfast

TIME	TOPIC	SPEAKER
13.30-14.00	Tea/Coffee/Pastries	
CHAIRS: Dr Claire Wynne and Mr Paul Fitzsimons		
14.00-14.30	*Virtual Presentation* *Why we make mistakes in morphology reporting from the UKNEQAS CPD Digital morphology scheme*	Michelle Brereton, Lead Biomedical Scientist at Manchester University NHS Foundation Trust
14.30-15.00	*Laboratory diagnosis of VWD; understanding the importance of the phenotype-genotype relationship*	*Catriona Keenan Ph.D.* *Senior Clinical Scientist at the Haemostasis Molecular Diagnostic (HMD) Laboratory, part of the National Coagulation Centre, St. James's Hospital, Dublin* *Mairead Doyle* *Senior Medical Scientist at the National Coagulation Laboratory (NCL), part of the National Coagulation Centre, St. James's Hospital, Dublin*
15.00-15.30	*Major Haemorrhage Guideline and Major Haemorrhage Simulations*	*Conor McMahon,* *Medical Scientist,* *Beaumont/TU Dublin Practice Educator*
15.30-16.00	*Tea/Coffee/Poster Viewing*	
CHAIRS: Dr Irene Regan and Mr Paul Fitzsimons		
16.00-17.00	*Short presentations selected from abstract submissions*	
16.00-16.15	*DEVELOPMENT OF A HIGH-SENSITIVITY FLOW CYTOMETRY ASSAY FOR THE DETECTION OF GPI-DEFICIENT CLONES IN PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA AND RELATED BONE MARROW FAILURE SYNDROMES*	*Ciara Liptrot, Haematology , Mater Misericordiae University Hospital, Dublin*
16.15-16.30	*A REVIEW OF MALARIA DIAGNOSIS, SEVERITY AND MANAGEMENT 2019-2023 UNIVERSITY HOSPITAL GALWAY*	*Ellen Sugrue, Galway University Hospital*
16.30-16.45	*A CASE SERIES OF UNUSUAL HAEMOGLOBIN VARIANTS DETECTED AT THE SPECIALIST RED CELL LABORATORY, BELFAST CITY HOSPITAL*	*Colleen Williamson, Haematology Department, Belfast Health and Social Care Trust, Belfast*
16.45-17.00	*THREE-SITE COMPARISON OF CELL POPULATION DATA ON SYSMEX XN-SERIES ANALYSERS*	*James Harte presenting on behalf of Grace McMahon, Cork University Hospital*
17.00	*Close of Session* *Awards will be announced at Conference Dinner*	*Dr Claire Wynne*

This is a CPD Event

Please contact Sinead on sinead@sineadcassidy.com for further details.

Michelle Brereton DBMS, FIBMS, MSc, CSc.
Lead Biomedical Scientist in Haematological Cancer Diagnostic partnership (HCDP) in Haematology based at Manchester Foundation Trust. England, United Kingdom.

I trained and worked at Central Manchester in laboratory haematology including 12 years as a Senior Laboratory Scientist in stem cell transplantation and cell culture research. As a Chief Biomedical Scientist I managed the automation and morphology services and haemoglobinopathy testing, overseeing workflow and service management for our continuous processing service. During this time I was a member of the UK National Pathology Harmony Group and involved with working towards standardisation of reference ranges and units of measurement across the UK for haematology tests and also worked with the Council for the international society of Haematology on this issue. Supporting my profession I am on the Institute Of Biomedical Sciences advisory panel for Haematology and am the Institutes representative on the Board for LabTestsOnlineUK aimed at providing information about laboratory tests to the public.

I have a keen interest in blood cell morphology and am an advisor and key developer for the UK National External Quality Assurance Scheme for Digital Morphology. This provides a national, internet based, educational service to laboratory professionals who report and examine blood films as part of their daily work. I continue to work with UK NEQAS(H) to develop the Digital Morphology scheme and to expand this service to other interested professionals. My research in morphology reporting has centred on seeking explanation for common errors in reporting and using the information to improve how we teach and train the morphologists of the future to best inform clinical teams and serve the patients.

Since 2022 I have been working with the Haematology Clinical team examining and reporting bone marrow and blood cell morphology for the HCDP

Mairéad Doyle
Senior Medical Scientist, National Coagulation Laboratory, National coagulation Centre, St James Hospital, Dublin, Ireland
Mairéad is a Senior Medical Scientist at the National Coagulation Laboratory. She works as part of a team of medical scientists to provide a comprehensive testing service to aid the diagnosis and monitoring of inherited and acquired bleeding disorders for adults. Part of this involves maintaining accreditation status and adhering to best practice guidelines to ensure a quality service for the patients and clinicians who rely upon it.
Mairéad has a particular interest in development and implementation of new technologies within the laboratory, again working with her colleagues to update the repertoire of tests where a clinical need is identified. She is part of a multidisciplinary team supporting research and clinical trials in haemostasis.
Mairéad has a training role within the laboratory and enjoys this role, she feels teaching is the most effective learning tool of all. She has lectured at third level colleges in Dublin and Galway and has presented at haemostasis meetings.

Catriona Keenan

Catriona Keenan Ph.D. is a Senior Clinical Scientist at St James's Hospital, Dublin. Catriona completed her Ph.D. in 2003 at the Department of Genetics, Trinity College Dublin, under the supervision of Professor Owen Smith on 'The Coagulation and Inflammation interface; the pathophysiological basis for disease states'. She then went on to set up the first accredited molecular testing laboratory in Ireland according to ISO 15189 Standards for Medical Laboratories; the Haemostasis Molecular Diagnostic (HMD) laboratory.
The HMD laboratory is part of the National Coagulation Centre (NCC), and the Department of Haematology at St. James's Hospital, Dublin. The laboratory

provides a national genetic screening service to patients and family members where there is a history of inherited bleeding and allied disorders such as Haemophilia and Von Willebrand's Disease (VWD). The HMD laboratory work closely with the National Coagulation Laboratory (NCL) at St. James's Hospital, providing a comprehensive integrated service to clinicians throughout Ireland.

Catriona has multiple publications and is an accomplished public speaker, having presented at national and international meetings. She lectures to both undergraduate and postgraduate courses on molecular diagnostics. Catriona is the deputy president of the Irish Association of Clinical Scientists, and is secretary of the U.K. Haemophilia Centres Doctors' Organisation (UKHCDO) Genetics Laboratory Network (GLN). She is a member of the Association for Clinical Genomic Science (ASCG), the British Society for Genetic Medicine (BSGM) and the British Society of Haematology (BSH).

Conor McMahon
Graduated TU Dublin B.Sc. Medical Science in 2021 and working in Blood Transfusion Department, Beaumont Hospital. Specialised as the department Training Officer. Completed TU Dublin M.Sc. Clinical Laboratory Science in 2024 researching Simulation Training & Massive Haemorrhage

DEVELOPMENT OF A HIGH-SENSITIVITY FLOW CYTOMETRY ASSAY FOR THE DETECTION OF GPI-DEFICIENT CLONES IN PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA AND RELATED BONE MARROW FAILURE SYNDROMES

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Introduction: Paroxysmal nocturnal haemoglobinuria (PNH) is a haematopoietic stem cell disorder caused by a somatic mutation of the PIG-A gene. Progeny of mutated cells have either a reduced expression or absence of glycosylphosphatidylinositol (GPI), resulting in the reduction or absence of GPI-anchored proteins. The absence of GPI is readily detected by flow cytometry, the gold standards laboratory diagnosis of PNH. Aplastic anaemia (AA) is a bone marrow failure syndrome characterised by pancytopenia and a hypocellular marrow. Approximately 60% of AA patients have a minor GPI-deficient clone at diagnosis. In cases of AA, the identification of a minor GPI-AP deficient clone, >1%, is of clinical significance. Expansion of clones may indicate disease progression and/or the need for intervention. Recent British Society for Haematology guidelines recommend that all patients diagnosed with AA should be tested for the presence of GPI-deficient clones at diagnosis using high-sensitivity flow cytometry. Expert groups, the ICCS and ESSCA published consensus guidelines on the detection of GPI-deficient clones using high-sensitivity flow cytometry. The purpose of this study was to validate a high-sensitivity flow cytometry assay for the detection of GPI-deficient cells, in accordance with these guidelines, enabling the MMUH laboratory to identify and monitor minor GPI-deficient clones.

Methods: The development of this high-sensitivity assay was achieved by optimising cytometer settings, determining optimal antibody concentrations required, and the development of sequential gating strategies. Analysis of results was performed using BD FACSDiva software. Sequential gating strategies were developed to isolate populations of interest; single red blood cells, neutrophils and monocytes, and to assess them for the presence or absence of corresponding GPI associated proteins. Cytometer was set up and compensated as per the method outlined by Sutherland *et al.,* (2018). For the validation of optimised PNH assays, spiking studies were performed as per the methods described by Illingworth *et al.,* (2018a). In brief, for both red and white cell assays, the LOB and LOD was determined by assessing 10 normal samples using optimised cytometer setting and antibody concentrations. To verify assay sensitivity (LLOQ) serial dilutions of a known PNH sample and a normal sample were performed, creating a range of GPI-deficient clones of assumed linearity. Serial dilutions were performed in triplicate to assess assay precision.

Results: Assay sensitivity (LLOQ) was assessed by performing spiking studies with a known PNH sample. Desired assay sensitivities were observed for the detection of GPI-deficient red cells, 0.01%, neutrophils, 0.03% and monocytes, 0.3%.

Conclusion: The aim of the MMUH flow cytometry laboratory is to validate this assay for routine use in place of current methods. Not only is the developed assay capable of assessing minor GPI-deficient clones, as seen in AA and occasionally MDS, but it is also capable of accurately and reliably detecting larger GPI-deficient clones for the diagnosis of haemolytic PNH. Implementation of this assay would allow for the identification and quantitation of minor GPI-deficient clones in patients with AA and MDS, as well as monitoring clonal expansion and potential need for therapeutic intervention.

A REVIEW OF MALARIA DIAGNOSIS, SEVERITY AND MANAGEMENT 2019-2023 UNIVERSITY HOSPITAL GALWAY

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[1]University Hospital Galway, HSE, Galway , Galway

Introduction

Malaria is a potentially life threatening infection that is imported by travel in Ireland, with 53 cases reported nationally in 2022. Given its relative rarity and the serious consequences of delayed diagnosis and treatment, we reviewed the diagnosis of malaria from 2019- 2023. This review aims to identify parasitaemia burden presenting to this hospital and haematological markers of severity in this cohort. Current British Society for Haeamatology guidelines reccommend prompt quantification in cases of *P.Falciparum* due to effects of parasitaemia on clinical management (1).

Methods

All malaria testing requests submitted to UHG haematology lab January 2019- December 2023 were reviewed. For positive cases identified request forms, reference laboratory reports and clinical notes were reviewed.

Results

24 cases were identified. 4 in 2019, 0 in 2020, 4 in 2021, 5 in 2022, 11 in 2023. 1 case was excluded due to lack of information**.** All cases were unrelated.
17 of 23 (74**%**), cases were male. Mean age was 38 years (range 11-58),16/23 (70%) reported reason for travel was visiting friends and relatives (VFR) with most frequent country of travel being Nigeria (12/23, 52%). 5/23(22**%**) took malaria chemoprophylaxis with 3 reporting full compliance. The mean time from symptoms onset to presentation was 6 days. The most common presenting symptom was fever (22/23, 96%).

22/23, 96% had positive antigen test. *P.Falciparum* was identified in 20/23 cases (87%) and *P.Ovale* in 3/23 (13%), with no mixed infections. Parasitaemia on initial film was reviewed and results were >2% parasitaemia in 3 cases, 1-3% in 3, <1% in 17 cases.
20 cases were tested on admission with 1 tested in first 24 hours, 1 on Day 3 of admission and one on day 4. The average length of stay (LOS) was 5.7 days with 132 bed days used over 5 years. ICU Admission was required in 6 cases with mean ICU LOS being 4 days. 1 ICU admission patient had a parasitaemia >2%

Mean Hb on admission was 12.7 (range 9.9-15.0). Mean platelets on admission were 112. 17 patients had platelets of less than 150 on admission tests. No patient met DIC criteria at any point in admission. 18 patients had follow-up films through admission pre discharge.
Post discharge follow up films were carried out in 2 patients.

Discussion

Results are in keeping with national and international reported data on patient cohorts, risk factors and parasite species. There is a low burden of severe parasitaemia with no patients meeting haemoglobin count consistent with severe infection (2). Treatment guidelines report a parasitaemia >2% as severe (2). In keeping with current guidelines parasitaemia is reported on call for *P.Falciparum* species.

This is the first review of this cohort in this centre. Changing epidemiological patterns and increasing travel post lockdown necessitate an awareness of malaria in clinical encounters with patients returning from travel as well as knowledge of markers of severity of infection.

Future plans for this data include a quality improvement project with haematology lab scientists to assess on-call parasitaemia quantification staff requirements and service provision planning.

Reference: UK Malaria Guidelines 2016, British Society for Haeamtology Guidelines for diagnosis of malaria.

A Case Series of Unusual Haemoglobin Variants Detected at from the Specialist Red Cell Laboratory, Belfast City Hospital

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Introduction

Haemoglobinopathies are disorders of globin chain synthesis. They are spilt into two groups- haemoglobin variants and thalassaemia. Most are caused by mutations in the alpha or beta genes and can result in both qualitative and quantitative defects in globin chain production e.g.structurally abnormal globin chains in sickle cell disease, or reduced globin chains in thalassaemia.
5% of the world's population have haemoglobin mutations. Certain haemoglobinopathies are linked with certain ethnic groups i.e. a high incidence of HbS in Africa and alpha 0 thalassaemia in Asia.
The Red Cell Laboratory Belfast, processes ~1500 haemoglobinopathy samples a year. In 2023, the most significant abnormalities detected were 304 haemoglobin variants and 37 alpha 0 thalassaemia.
There are 35 adults and 19 children currently living in Belfast with a major haemoglobinopathy.

Laboratory Methods

The Sickle Cell and Thalassaemia Screening Program suggests haemoglobinopathy detection must be confirmed by two methods. The Belfast Trust employs capillary electrophoresis (CE) and Isoelectric Focusing (IEF). The results of both are compared, interpretated and reported together. Samples can be forwarded to Oxford red cell reference laboratory for confirmation. The patient's ethnicity and FBC results are also used to identify haemoglobinopathies.
Here we present a series of unusual haemoglobinopathies detected at the Red Cell Laboratory, Belfast City Hospital

Case Presentations

Case 1.

A 31 year old Irish female was offered testing after her newborn's heel prick was inconclusive. Her red cell indices were normal. CE detected a 16% peak in the HbD region (a clinically significant Hb variant), however IEF detected 2 variant bands not consistent with HbD. Hb Etobicoke was confirmed by molecular testing. Although Hb Etobicoke is an alpha gene mutation it has no known clinical significance or reproductive implications.

Case 2.

A 31 year old female was investigated as part of her antenatal booking bloods. Family origin questionnaire indicated patient was from Saudi Arabia (high risk area). The patient had a normal Hb and red cell indices. CE identified a peak in the HbS region of 18% but and IEF excluded HbS. The variant detected was not consistent with any common variants. Subsequent molecular testing confirmed the presence of Hb Setif a rare, mildly unstable haemoglobin. In these cases testing of the patient's partner is also offered to gauge prenatal risk. In this case, no variants were detected in the father.

Case 3

Routine FBC testing of a 22 year old Asian female detected microcytosis (Hb 127, MCH 20 pg). No abnormal peaks were detected on CE or IEF but the sample was forwarded for molecular testing due to high risk for alpha 0 thalassaemia. Although alpha 0 thalassaemia was suggested, εγδβ+ thalassaemia was detected. A deletion of a whole region of the beta globin gene, which affects the expression of beta, gamma and delta globin. This is the first time that this mutation has been described therefore effects can only be predicated to result in beta thalassemia. Further Actions – screening should be offered to any partner to assess the risks to a child. Family members should also be considered for testing.

THREE-SITE COMPARISON OF CELL POPULATION DATA ON SYSMEX XN-SERIES ANALYSERS

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Introduction:
To ensure that clinical haematology laboratories can provide the best quality service, it is essential to provide as much clinically relevant information as possible. Cell population data (CPD) parameters on Sysmex XN-series analysers are emerging haematological indices that provide novel quantitative information relating to the morphological and functional characteristics of leucocyte subpopulations, including leucocytic complexity, maturity and reactivity, and volumetric size. These advanced parameters have the potential to contribute to the diagnosis and prognosis of both haematological and non-haematological disorders.
However, CPD parameters are currently available for 'research use only' as there remains gaps in the understanding of these emerging parameters, in particular intra- and inter-laboratory comparability.

Materials and Methods:
The aim of this study was to address the current research gaps regarding the comparability of CPD parameters: firstly, an intra-laboratory comparability study was performed using three Sysmex XN-10 analysers; secondly, an inter-laboratory comparability study was performed using Sysmex XN-10 analysers at three different hospital laboratory sites. Venous whole blood samples were collected from 34 ostensibly healthy and consenting volunteers, and were analysed for routine haematology and CPD parameters; a sub-set of 20 whole blood samples were subsequently analysed at the three different hospital laboratory sites, to determine the intra- and inter-laboratory comparability.

Results:
The study demonstrated statistically and clinically significant biases in CPD parameters both between the XN-series analysers and between hospital laboratory sites, which exceeded the minimum permissible difference allowable according to analytical and biological variations. However, all biases were reduced – or eliminated entirely – following the implementation of an analyser-specific instrument alignment factor that corrected the measured parameters to achieve permissible precision.

Conclusion:
The overall intra- and inter-laboratory harmonisation of CPD parameters on Sysmex XN-series analysers is highly encouraging, and will likely support the potential translation of these parameters to clinical usage in the pursuit of providing a higher quality service to the patient.

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(Listed Alphabetically)

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109	Diagnostic Laboratory Haematology and Transfusion Session (Friday 11th October)	HOW BIOMEDICAL EXPERTISE IMPROVES PATIENT OUTCOMES : A DIRECT ANTIGLOBULIN POSITIVE ACQUIRED THROMBOTIC THROMBOCYTOPENIC PURPURA CASE STUDY.	Emily	Dinsmore
110	Diagnostic Laboratory Haematology and Transfusion Session (Friday 11th October)	Investigation of discrepant platelet counts in a cohort of haematology patients in Belfast City Hospital	Bronagh	Erwin
111	Diagnostic Laboratory Haematology and Transfusion Session (Friday 11th October)	COMMERCIAL APPT REAGENTS SIGNIFICANTLY DIFFER IN SENSITIVITY TO NOVEL FACTOR XIa INHIBITORS ASUNDEXIAN AND MILVEXIAN	James	Harte
112	Diagnostic Laboratory Haematology and Transfusion Session (Friday 11th October)	THE CLINICAL SCIENTIST PROFESSION - AN INSIGHT ON BEHALF OF THE NI TRAINEE CLINICAL SCIENTIST NETWORK	Lauren	Mc Connell
113	Diagnostic Laboratory Haematology and Transfusion Session (Friday 11th October)	EVALUATION OF THE EFFECTIVENESS OF PHOSPHATIDYLINOSITOL 4-KINASE BETA (PI4KB) INHIBITORS AS A TREATMENT OPTION FOR MULTIPLE MYELOMA.	Nhu Ngoc	Nguyen
114	Diagnostic Laboratory Haematology and Transfusion Session (Friday 11th October)	EVALUATION OF CAR-T MANUFACTURING STARTING PRODUCT CELL CONTENT USING A TBNK ASSAY AND INVESTIGATING THE RELATIONSHIP WITH PATIENT OUTCOME.	Jack	Priestley

A Comparative Study: Determining Statistical Significance In The Detection Of Clinically Significant RBC Antibodies using The Lone IAT Screen versus IAT and Enzyme Screen Combination.

J Cheshire[1]

[1]Blood Transfusion, Belfast Health & Social Care Trust, Belfast, Northern Ireland

The current BSH guidelines (2012) advise that pre-transfusion testing must contain ABO and Rh grouping, as well as an Indirect Antiglobulin Technique (IAT) antibody screen (followed by an IAT and enzyme identification panel if positive). The aim of this study is to determine the relevance of providing an additional three cell antibody screening in the form of enzyme (ficin or papain) treated cells to pre-transfusion testing to aid in increasing the detection of clinically significant red cell antibodies. This additional enzyme treated 3-cell screen is currently utilised by various Blood Transfusion laboratories across the globe. A total of 4291 routine blood samples, collected in 6 mL Ethylenediaminetetraacetic Acid (EDTA) vials, were examined for atypical antibodies using a combination of the IAT and enzyme techniques on the automated IH-500 and IH-1000 BIO-RAD analysers. Antibody identification was carried out within the Blood Transfusion Laboratories of the Belfast Health & Social Care Trust (BHSCT) via IAT 11-cell panel and enzyme treated 11-cell panel. A total of 13.7% (n=590) of samples returned positive antibody results via the IAT, enzyme assay or both. Of these 590 samples, 69% (n=407) of those results were via enzyme assay only. The introduction of enzyme screens has led to detection of 'enzyme only' antibodies, both specific and non-specific. Determining whether these antibodies are clinically significant (potential to induce a haemolytic transfusion reaction (HTR)) is vital for ensuring patient safety. A total of 90.4% of enzyme-only antibodies were autoantibodies (specific or non-specific) and therefore, not clinically significant. The remaining 9.6% (n=40) enzyme-only antibodies were alloantibodies from blood group systems: Rh 9.3% (n=38), Kell 0.25% (n=1) and Kidd 0.25% (n=1). Confirmation via the reference laboratory of the Northern Ireland Blood Transfusion Service (NIBTS) detailed these antibodies as enzyme-only, not low-titre alloantibodies, therefore, non-clinically significant. Statistical analysis via a Chi Squared test showed that the combined protocol of IAT and enzyme screens led to a statistically significant increase in antibody detection ($p<0.01$). However, analysis of each antibody revealed none of the enzyme-only antibodies were clinically significant. This study highlights that the significant increase in the detection of non-clinically significant antibodies via the additional enzyme screen, greatly overshadows any benefit of detecting enzyme-only antibodies. The majority of such antibodies are a hindrance to blood transfusion laboratories, with their detection being of no benefit to the patient, and potentially causing delays in transfusion due to greatly increased antibody identification times. Furthermore, this study carried out cost-benefit analysis on the implementation, and inclusion of the enzyme screen for the BHSCT. Calculations revealed that, with regards to reagents and consumables alone, the continued use of the enzyme screen as part of routine pre-transfusion screening had projected minimum additional costs of approximately £124,000 per annum. This is a significant financial undertaking, especially when considering that the majority of antibodies this assay is detecting are non-clinically significant. Therefore, implementation of the enzyme screen in a high throughput hospital blood transfusion laboratory is not advised.

HOW BIOMEDICAL EXPERTISE IMPROVES PATIENT OUTCOMES : A DIRECT ANTIGLOBULIN POSITIVE ACQUIRED THROMBOTIC THROMBOCYTOPENIC PURPURA CASE STUDY.

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[2]School of Biomedical Sciences, Ulster University, Coleraine, Northern Ireland

Thrombotic thrombocytopenic purpura (TTP) is a rare, life-threatening haematological disorder with an estimated prevalence of 3.7 cases per million annually and a mortality rate exceeding 90% if untreated[1][2]. TTP is characterised by a pentad of conditions: microangiopathic haemolytic anaemia, thrombocytopenia, pyrexia, and commonly, renal and neurologic dysfunction. The disorder results from either a congenital or acquired deficiency of the ADAMTS13 enzyme, which cleaves von Willebrand factor (VWF). Without this enzyme, large VWF multimers cause spontaneous platelet aggregation in the microvasculature, leading to ischemic damage, particularly in the kidneys and central nervous system.

An 18-year-old male presented to the emergency department with persistent epistaxis, intermittent sensory disturbance on his right side, general malaise, fatigue, and a brief episode of dysarthria earlier that day. Initial investigations involved a head CT however no haemorrhagic or ischemic damage was detected. A full blood count revealed anaemia and thrombocytopenia: erythrocyte count of $2.01x10^{12}$/l (normal: $4.5\text{-}5.5x10^{12}$/l), haemoglobin of 64 g/l (normal: 130-170 g/l), haematocrit of 0.197 l/l (normal: 0.4-0.5 l/l), RBC distribution width of 27.9% (normal: 11.6-14.0%), reticulocyte count of 18.8% (normal: 0.5-2.5%), and a platelet count of $21x10^{9}$/l (normal: $150\text{-}410x10^{9}$/l). Biochemical tests showed elevated lactate dehydrogenase (LDH) at 1614 u/l (normal: 135-225 u/l), creatinine at 129 µmol/l (normal: 53-92 µmol/l), total bilirubin at 33 µmol/l (normal: 1-21 µmol/l), and aspartate aminotransferase (AST) at 63 u/l (normal: 10-40 u/l. Such measurements consolidate the patient had haemolytic anaemia while the elevated creatinine indicated a degree of renal impairment.

The identification of schistocytes, spherocytes, keratocytes, anisocytosis, polychromasia, and thrombocytopenia on a peripheral blood film suggested TTP. Recognising the severity of the condition and importance of prompt clinical intervention, the biomedical scientist, promptly informed the clinical team. This was key as the patient hadn't yet been seen by a doctor. A rare and unexpected finding was a positive IgG direct antiglobulin test (DAT), unusual in TTP since erythrocyte destruction is mechanical rather than immune-mediated. There have been documented cases where a positive DAT delayed TTP diagnosis and treatment, leading to fatal outcomes including mortality[3][4]. In this case, however, the antibody was later identified as a non-specific warm autoantibody. Fortunately, the patient had already been transferred for specialist care with TTP still the most probable diagnosis.

TTP was confirmed after monitoring ADAMTS13 activity levels at the onsite laboratory. The patient received several rounds of plasma exchange therapy, corticosteroids, rituximab, and a one-unit transfusion to replenish erythrocytes. He now attends routine reviews with ADAMTS13 monitoring to avert the occurrence of relapse.

This was a unique and challenging case to diagnose due to its idiopathic origin, the patient's young age, sex, and the presence of a positive DAT. It underscores the critical role of biomedical scientists in patient care, highlighting the importance of recognising and utilising their expertise to enhance clinical practice and improve patient outcomes.

[1]National Organization for Rare Disorders. (2023) Thrombotic Thrombocytopenic Purpura. Available at: https://rarediseases.org/rare-diseases/thrombotic-thrombocytopenic-purpura/ [Accessed Aug 27, 2024].
[2]Scully, M. (2022) Advances in thrombotic thrombocytopenic purpura. Available at: https://www.rcpath.org/profession/publications/annual-reports/annual-report-2021-2022/advances-in-thrombotic-thrombocytopenic-purpura.html [Accessed Aug 27, 2024].
[3]Ghrewati, M., Mahmoud, A., Beliani, T., Zakharia, K. and Kumar, M. (2023) How Should Complicated Cases of Thrombotic Thrombocytopenic Purpura with Positive Coombs Test Be Treated? *Cureus,* 15(12)
[4]Koduru, K., Hamieh, T., Koduru, P., Abd-Alnoor, N. and Maroules, M. (2009) Thrombotic Thrombocytopenic Purpura and Positive Coombs Test — Not Mutually Exclusive! Journal of Hospital Medicine, 4(1)

Investigation of discrepant platelet counts in a cohort of haematology patients in Belfast City Hospital

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Introduction

Within the BHSCT, platelet counts are determined using impedance and optical methods on the Sysmex XN-20 automated analyser. In line with laboratory SOP's, morphological assessment is performed on patients with a platelet count of <100 x 109/L on first presentation and/or if there is a discrepancy between current and previous results.

From May 2021 - October 2023, the laboratory had experienced intermittent discrepancies in platelet counts from haematology patients in BCH, who, as confirmed by medical staff, had no explanation for these differences. The aim of this investigation was to elucidate the cause of discordance in platelet counts in patients within the BHSCT.

Methods

A retrospective analysis was performed on the data collected between May 2021 and October 2023. Our objective was to discount analytical sources of error such as technicalities between impedance and optical methods for platelet quantification across multiple sites in conjunction with manual platelet counts.

Results

A total of 228 samples were investigated; 166 were from the haematology inpatient unit at BCH, 62 were from other sources. 106/166 inpatient samples were identified as inaccurate. A "reticulocyte abnormal scattergram" was flagged on 81% of the inaccurate platelet counts (8 samples did not flag as abnormal, and 12 where the flag was not documented). Twenty two samples had significant haemolysis visually. 17 repeat specimens were obtained, taken within 8 hours of the original, all of which returned results that were in keeping with the patient's previous history. Allowing for previous transfusion, the increase in the patient count was remarkable (>30 x 109/L), sparking concern.

Discussion

Having sought advice from Sysmex UK, they confirmed that a platelet count with a flag "retic abnormal scatterplot" was not robust and an alternative method of establishing the platelet count should be explored, namely morphology. Following analysis, it was determined that there was no laboratory component to the discrepancy.

The samples in question were mainly those taken early in the morning for ward rounds. There were no issues with repeat samples or samples taken later in the day (subsequent samples were in keeping with patient history and transfusion expectation). In addition, the issue was not limited to specific patients on the ward, or indeed those patients on sequential days. It also appeared that these were restricted to particular days of the week suggesting the possibility of a pre-analytical issue at source, such as storage of sample bottles before or after venepuncture, or the methodology of venepuncture employed at source level.

Conclusion

Any issue whether they be analytical or pre-analytical must be investigated swiftly and as a matter of urgency. This is imperative as decisions such as patient discharge based on an erroneous result could lead to a catastrophic consequence.

COMMERCIAL APPT REAGENTS SIGNIFICANTLY DIFFER IN SENSITIVITY TO NOVEL FACTOR XIa INHIBITORS ASUNDEXIAN AND MILVEXIAN

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Introduction:
Small-molecule inhibitors of factor XIa (FXIa) – including asundexian and milvexian – are currently in clinical trial for the prevention and treatment of thromboembolism. The activated partial thromboplastin time (APTT) may be suitable for the pharmacodynamic assessment of FXIa inhibitor concentrations *ex vivo*: however, limited information is currently available on the responsivities of commercial APTT reagents, which may exhibit differential sensitivities to factor XIa inhibitors similar to current factor IIa and factor Xa inhibitors [1-4].

Therefore, we evaluated the sensitivity of commercial APTT reagents to asundexian and milvexian using *in vitro* plasma-based systems.

Materials and Methods:
Standard human-derived plasma was incubated with ascending concentrations of asundexian or milvexian (0 ng/mL – 4,000 ng/mL), representative of the concentration maxima observed in pharmacokinetic studies[5,6]. APTT-based clotting times were measured on CN-6000 analysers with five commercial reagents from Sysmex (Kobe Japan), containing different contact activators and phospholipids.

Results:
Asundexian and milvexian exhibited concentration-dependent prolongations of APTT-based assays with all commercial reagents. However, differential sensitivities to ascending concentrations were observed depending on the composition of the reagent: reagents containing ellagic acid were more sensitive than reagents containing silica, and reagents containing synthetic phospholipids were more sensitive than reagents containing natural phospholipids.

Conclusions:
Although APTT-based assays are sensitive to factor XIa inhibitors, asundexian- and milvexian-associated prolongations are not comparable between commercial reagents. Clinical laboratories should be aware of the composition of individual APTT reagents, and the sensitivity of any given reagent must be carefully considered by both clinicians and laboratorians when interpreting investigations for factor XIa inhibitors.

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THE CLINICAL SCIENTIST PROFESSION - AN INSIGHT ON BEHALF OF THE NI TRAINEE CLINICAL SCIENTIST NETWORK

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Introduction:
Clinical Scientists are healthcare experts who support clinical staff in their work with patients as well as conducting research and developing techniques to help prevent, diagnose and treat illness. The role of a Clinical Scientist (CS) can include laboratory work, testing and analysis, research, teaching, leadership and management. They are often described as the bridge between science and medicine and range from trainee, senior, principal and consultant positions.
In Northern Ireland (NI) and the UK, the title of 'Clinical Scientist' is protected by law and can only be used following Health and Care Professions Council (HCPC) registration. To become registered, trainees must complete a relevant training route in their specialty and pass examination. This study explores the background, training experience and higher specialist education in a cohort of trainee and qualified Clinical Scientists from a range of specialties in NI.

Materials and methods:
A structured 15-question survey was circulated by email to 50 participants either training for HCPC registration or registered as a CS on behalf of the NI Trainee CS Network. A total of 32 participants responded and completed the survey for anonymous review.

Results:
Nineteen respondents are currently training to become a HCPC Registered CS and 13 have already qualified as such. The majority of respondents are affiliated with Life Sciences 26 (81%), specialising in Genomics (61.5%), Haematology (19%), Biochemistry (11.5%), Virology (4%) and Genetics (4%). Six respondents (19%) are affiliated with the physical sciences and specialise in Medical Physics.
Of the 32 respondents, 30 (94%) had a post graduate qualification such as a MSc (53%) or PhD (47%) prior to training and only five (16%) were state registered scientists e.g. Biomedical Scientists. A total of 16 (50%) respondents are training/have trained through the National School of Healthcare Science Scientific Training Programme (STP), seven (22%) through the Academy of Healthcare Science STP Equivalence, eight (25%) through The Association of Clinical Scientists (ACS) Route 2 training program and one (3%) through ACS Route 1. The average time to complete training in this cohort, irrespective of training route is three years and the majority of participants (53%) had over 5 years relevant work experience prior to commencing CS Training.

Of the 19 current trainees surveyed, 16 (84%) are in a formal training position yet only seven trainees (37%) are guaranteed a substantive post upon registration. Of the 13 registered Clinical Scientists, 46% have additional post-registration qualifications such as Fellowship of the Royal College of Pathologists and are in Principal and Consultant CS positions.

Conclusions:
There are several training programmes in place for many clinical areas and disciplines in NI to support HCPC Registration as Clinical Scientists with the national STP route being the most common pathway to qualification in this cohort. Interestingly, no respondents availed of the IBMS equivalence route. Qualification as a CS is often a highly competitive process and requires highly skilled, qualified and motivated individuals. In summary, this study highlights the emerging role of Clinical Scientists in healthcare throughout NI with 97% of respondents recommending training to become a registered CS.

EVALUATION OF THE EFFECTIVENESS OF PHOSPHATIDYLINOSITOL 4-KINASE BETA (PI4KB) INHIBITORS AS A TREATMENT OPTION FOR MULTIPLE MYELOMA.

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Background: Multiple Myeloma (MM) is a haematologic malignancy characterised by the proliferation of malignant plasma cells, posing significant challenges in treatment due to relapse and resistance issues. Golgi apparatus dysregulation has emerged as a potential target for therapy, particularly through the inhibition of phosphatidylinositol 4-kinase beta (PI4KB). Despite promising preclinical data, the efficacy of golgi inhibitors in MM remains largely unexplored.

Methods: Firstly, the MTT assay was employed to assess cell viability and determine the IC50 values for PI4KB inhibitors (N314, N377, and N399). Moreover, western blot analysis was utilised targeting ubiquitin, GM130, and p62 to investigate stress pathways in myeloma cell lines RPMI-8226 and U226, both untreated and treated with N399 for 6 hours. Additionally, the expression levels of PI4KB and p62 were examined in untreated MM cell lines RPMI-8226, OPM2, OPM2 BTZ, EJM, JJN3, MOLP8, U226, INA6, and KMS12BM BTZ using western blotting techniques.

Results: Dose-response curves were generated to determine IC50 values for three PI4KB inhibitors. RPMI-8226 cells exhibited the highest sensitivity, while U226 cells showed resistance to all drugs. Western blot analysis revealed differential expression of PI4KB, p62, and ubiquitin across MM cell lines. Treatment with N399 induced p62 accumulation in U226 cells, while GM130 expression was stronger in RPMI-8226 lysates. These findings suggest potential therapeutic targets and differential drug responses in MM.

Conclusion: The study provides valuable insights into the potential of PI4KB inhibitors as novel therapeutic agents for MM and underscores the need for further investigation to translate these findings into clinical applications.

EVALUATION OF CAR-T MANUFACTURING STARTING PRODUCT CELL CONTENT USING A TBNK ASSAY AND INVESTIGATING THE RELATIONSHIP WITH PATIENT OUTCOME.

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Introduction

Chimeric Antigen Receptor T-cell (CAR-T) therapy has emerged as an effective ground-breaking treatment for some patients with CD19 positive haematological malignancies. The success of CAR-T therapy is influenced by multiple clinical and technical factors, including obtaining good quality starting material for commercial CAR T manufacture. There is limited data on the immune profile of patients at the time of harvest and any impact starting cellular material may have on clinical success.

Materials & methods

This project firstly validated a new method to evaluate the cellular composition of MNC (A) apheresis products which are CAR-T manufacturing starting material using a commercial CE IVD (BD Bioscience) for TBNK (T-cell, B-cell, NK cell) enumeration. Due to an international supply shortage of commercial kits it was decided to develop an in-house assay for use as contingency. An FC0624 commercial multi-test control (R&D systems) was analysed using both assay methods for reproducibility and repeatability. The flow cytometry analysis was performed using the FACS DIVA software on the FACS CANTO II flow cytometer. MNC(A) /CAR T starting product samples from Biobank patients (n=14) were assessed for absolute (cell / µl) and % values of T-cells (CD3/CD4/CD8), B-cells (CD19), and NK cells (CD56/CD16). The patients PET scans determined whether the patient had entered complete metabolic remission (CMR) or had persistent disease (PD). We investigated the relationship with overall clinical response and the occurrence of Cytokine Release Syndrome (CRS).

Results

The BD 6-colour TBNK IVDD assay is superior for routine patient testing. The IVDD compliant assay shows better reproducibility values and a lower r^2 value being obtained during repeatability testing. The 6-colour BD TBNK assay also shows higher quality results across all test parameters as illustrated by the CV% obtained using control samples. We have proven the reproducibility and repeatability of the assay with control and clinical samples.

Chart review showed that 6 patients were in remission (CMR) while the remaining 8 patients experienced persistent disease (PD). Within the 14 patients (n=14), 10 displayed symptoms of CRS, while 4 did not display any signs of CRS. The findings showed that there was no statistical significance (P value >0.05) in the CD3, CD4, CD8, CD16/56, CD19 levels or CD4/CD8 ratio present in both patient cohorts.

Conclusions

The patient sample multi-parameter results showed that higher CD4/CD8 ratio was associated with improved clinical response post CAR-T and with the occurrence of CRS. While these findings are not currently statistically significant, further numbers are required to determine the clinical significance of this trend. The acquisition of prospective patient data, with the goal of building a substantial database of test results to monitor the trends occurring regarding CRS and CMR is necessary.. The newly validated TBNK analysis of the CAR T starting material enables a ‘snapshot’ of the patient’s immune status at this point of treatment. While we have not identified a link with overall outcome, identifying a favourable immune environment in a larger patient group will provide a valuable base line to assess the introduction of a new treatment options.