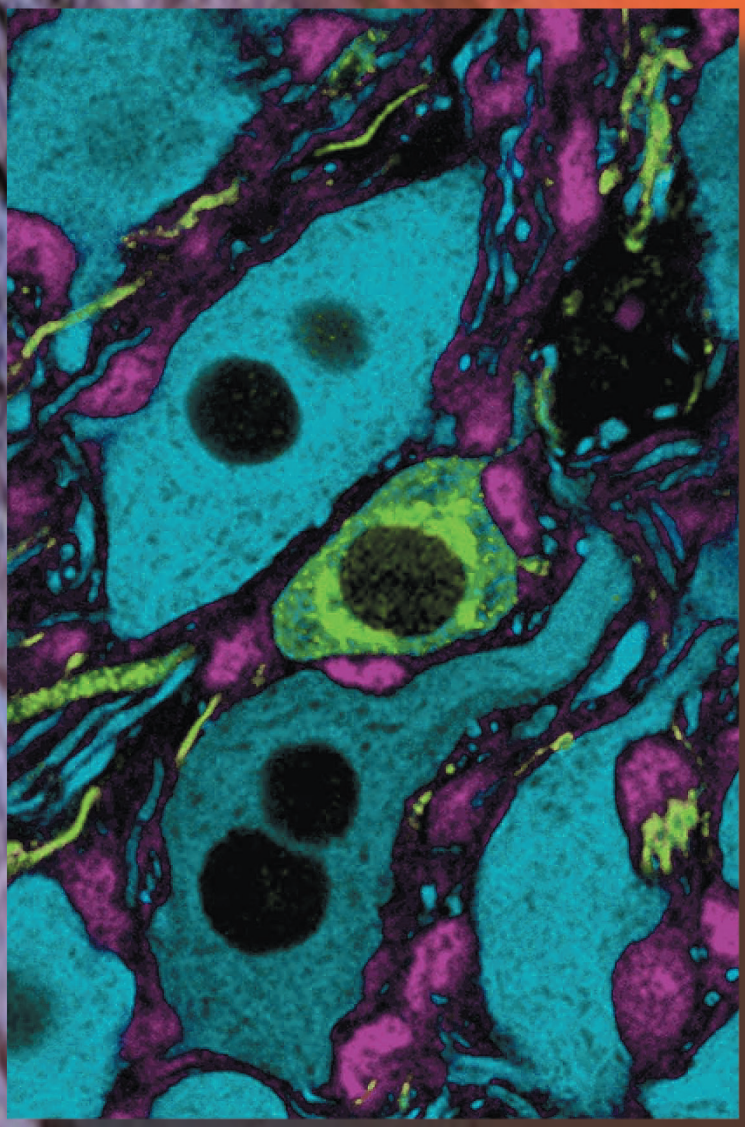
AUSTRALIAN JOURNAL OF
Medical Science
2024



ORIGINAL ARTICLES

CASE STUDY REPRINT

AIMS NATIONAL SCIENTIFIC MEETING "MOVING FORWARD" ABSTRACTS

REGULAR FEATURES

TECHNICAL NOTE

November 2024
Vol. 45
No. 4

UTS Online

Inspire Innovation

Unlock your potential with UTS Online's Master of Medical Science Leadership — a 100% online, tailored postgraduate degree designed for medical professionals.

- First Master's degree of its kind that combines leadership development with innovation in a medical science context
- Ranked top 150 globally for research
- Delivered by experts at the forefront of medical science and leadership
- 100% online

This groundbreaking master's course is your gateway to esteemed career paths such as Director, Manager, Advisor, Head of Laboratory Operations, or Lead Scientist.

- Elevate your leadership in cutting-edge medical fields
- Master effective communication, problem-solving, and adaptability
- Develop skills in analytical thinking, emotional intelligence, and ethical decision-making
- Build a strong network and relationships
- Embrace adaptability for innovation in medical science
- Tackle research, patient care, and healthcare management challenges with new skills

Join us and pioneer the future of medical science, gaining expertise and influence in your field.

Scan me for more info

CRICOS 00099F TEQSA PRV12060

Australian Journal of Medical Science

ADMINISTRATION

AIMS National Office

Chief Executive: Ms Sarah Just, BAppSc (MLS), GradCertMgt, MMedSc (Pathology), MAIMS

Design, formatting and management: AIMS National Office
Email: programs@aims.org.au
Website: www.aims.org.au
Telephone: 61 7 3876 2988
Address: PO Box 1911 Milton Qld 4064 Australia

The Australian Journal of Medical Science is the official publication of the Australian Institute of Medical and Clinical Scientists (AIMS).

Circulation 1500 per issue. The Journal is circulated to members in pathology laboratories, universities and research institutes throughout Australia and overseas.

Annual subscription rates are available from AIMS National Office.

Article reprints may be organised on request from AIMS National Office.

Advertising rates are available from AIMS National Office.

Abstraction of the Australian Journal of Medical Science is through the following serial catalogue listings: Australasian Medical Index, Chemical Abstracts, and EMBASE/Excerpta Medica.

The Australian Journal of Medical Science is included on the Australian Research Council ERA 2018 Journal List.

ISSN 1038-1643

Printed by Westminster Eagle Eye Printing, PO Box 161, Paddington Qld 4064.

Design Cover and layout design by Kim Brown, 23 Denman St Exeter SA 5019 kimbo@internode.on.net. Cover photograph courtesy of Prof Ian Gibbins, Flinders Medical Centre, Adelaide.

CONTENTS

November 2024 Vol. 45 No. 4

Original Articles

Technical Note

Case Study Reprint

AIMS National Scientific Meeting "Moving Forward"

Regular Features

Instructions to authors *are available on the AIMS website: www.aims.org.au*

ORIGINAL ARTICLE

Correlation between Beckman Coulter DxH900 and peripheral blood smear counts for nucleated red blood cell validation

Anna Di Cocco

Fellowship Dissertation
Monash Health Pathology, Dandenong Hospital, Dandenong, Victoria

Abstract

The aim of this study was to determine the correlation between automated haematology Beckman Coulter DxH900 nucleated red blood cell (nRBCs) counts and manual counts from peripheral blood smears with the intent to validate and transition to the automated method. This was done using the data that Monash Health acquires from the patient's manual count and comparing results provided by the Beckman Coulter DxH900 (DxH) automated analysers to correlate and compare the number of nRBC between these two methods. The manual count of nRBC via light microscopy by an experienced morphologist is the currently validated and implemented method providing results to health professionals. The correlation for all 190 tested samples was determined by considering a wide distribution of values and resulted in a value of $R^2 = 0.778$. The DxH analyses a large number of cells and categorises them appropriately but there are still interferences that are present that require a morphologist review. The gold standard therefore remains with the morphologist who can distinguish specific characteristics of cells and count them accordingly.

Key words: ***nucleated red blood cells, Coulter DxH900, manual differential, corrected white blood cell count***

Introduction

Red blood cells (RBCs) are non-nucleated, biconcave discs with a central pallor. Rodak *et al* (2012), explains that their main function is to carry oxygen to the tissues and replace it with carbon dioxide for exchange in the lungs. The active life span of a RBC is around 120 days with them subsequently removed by the spleen.

For RBCs to transport oxygen throughout the body they contain a protein known as a haemoglobin molecule, with each RBC containing approximately 640 million haemoglobin molecules (Hoffbrand *et al* 2006). The most dominant adult normal haemoglobin after six months of life is haemoglobin A (HbA) which is composed of two parts, a globin portion and a haem portion. The globin portion is made up of 4 polypeptide chains, two alpha (α) and two beta (β) globin chains and the haem structure contains iron in the ferrous Fe2+ state, in the centre of a protoporphyrin ring. The haem structure is situated in every one of the tetramer globin chains and that forms the haemoglobin molecule, 2,3-diphosphoglycerate (2,3-DPG) controls the affinity of each O_2 molecule, which is carried within each haemoglobin structure making a total of four O_2 molecules per haemoglobin.

Maturation of RBC occurs through differentiation from hematopoietic stem cells (HSC) to mature erythrocytes (Turgeon 2012; Hoffbrand *et al* 2006). RBCs are formed by a process known as erythropoiesis, regulated by the hormone erythropoietin (EPO) that interacts with tyrosine kinase signal transducers which are responsible for not only the early release of reticulocytes from the bone marrow but by preventing apoptotic cell death (Sirén *et al* 2001). EPO responds to the low oxygen tension levels in the kidney in cases such as hypoxia and hypovolemic shock. Eckardt and Kurtz (2005) highlighted that EPO production is dependent on its relationship with oxygen availability (Eckardt and Kurtz 2005). When oxygen levels in the body are high erythropoiesis is suppressed, but in times of hypoxia erythropoiesis is up-regulated to produce more RBCs to carry oxygen. Jelkmann (2011) found that oxygen deficiency is sensed in the peritubular cells of the kidney which stimulates erythropoiesis via EPO production and this results in the release of progenitor cells in the bone marrow (BM). The expression of erythroid-specific genes, late burst-forming unit erythroid (BFU_E) and colony-forming unit-erythroid (CFU_E) are also stimulated. Minior *et al* (2017) also mentions that EPO is well known to be

Address correspondence to:
Anna Di Cocco
Email: annabdicocco@gmail.com

the primary hormone mediating erythropoiesis and its secretion results in an increase in red blood cell mass by stimulating proliferation, differentiation, and maturation of erythroid precursors.

Carr and Rodak (2009) explain that the amplification and maturation sequence in the development of mature red cells begins with pronormoblasts with a diameter of 12 to 19µm, a nuclear to cytoplasmic ratio (N:C) of 4:1, a large, round appearing nucleus with zero to two nucleoli, and a fine chromatin pattern. Basophilic normoblasts decrease to 12 to 17µm in size but the N:C ratio is still 4:1. The cell shows maturity with nuclear chromatin becoming clumped and nucleoli are not seen, and basophilic cytoplasm is apparent. A polychromatic normoblast decreases further in cell size to approximately 11 to 15µm with the N:C 1:1, clumped chromatin and the cytoplasm is mixed with basophilic and pink colouration.

Orthochromic normoblasts – more commonly referred to as nRBCs – are smaller again (8 to 12µm) and the chromatin is condensed as the cell prepares for nucleus extrusion. The cytoplasm is acidophilic representing the increased levels of haemoglobin. Reticulocytes are the final stage of development that occurs in the bone marrow and these enter the circulation. Maturation of the RBCs is finalised and the cell size is 6 to 8µm (Carr and Rodak 2009).

Normally nRBCs reside in the bone marrow so when immature forms of RBCs are found in the peripheral circulation they pertain to different disease states and quantification allows the assessment of the severity of that disease state. They aid in diagnosing a variety of clinical and pathological conditions that coincide with increased erythropoiesis responding to low oxygen levels. Many pathophysiological conditions display nRBCs and these are discussed below (Carr and Rodak 2009; Valina *et al* 2020; Pikora and Krętowska-Grunwald 2023).

These disease states include acute and chronic hypoxia with varying lengths of hypoxia causing an increase in nRBC count in a rat model of intra-uterine growth restriction, similar to that seen in human neonates (Minior *et al* 2017; Davari-Tanha *et al* 2014). The Minior study exposed the rats to different duration times of 2, 6, 12, 24, 48, or 120 h in a severely hypoxic chamber titrating compressed air and nitrogen to produce the desired oxygen concentration (Minior *et al* 2017). Blood samples were collected and nRBCs were counted for correlation with decreased oxygen levels. Minior also states that in normal human pregnancy nRBCs decrease exponentially in circulation around 26–28 weeks of gestation, at which time the medullary erythropoiesis becomes more functional in the bone marrow (Minior *et al* 2017). It is therefore suggested that when there is a low-oxygen intra-uterine environment, an abundance of nRBCs will be present in the peripheral smear. The reason speculated for this outcome is a compensatory effect for the lack of oxygen, and utilizing erythropoiesis to its optimum capabilities, with visual evidence of nRBCs (Minior *et al* 2017; Bedrick 2014).

Nucleated red blood cells are also associated with rapid blood loss, hypovolemic shock, anaemia and haemolysis and are due to increased erythropoiesis in an attempt to compensate for acute anaemia (May *et al* 2019). Primary RBC numbers are rapidly decreased in blood loss and in haemolysis the number and structure of the RBCs are impaired due to their abnormal breakdown resulting in less oxygen-carrying capacity and compensatory increased erythropoiesis and nRBCs. In these conditions nRBCs are pushed out of the bone marrow due to inflammation, hypoxia, or both, causing increased hematopoietic stress to increase the number of peripheral RBCs. Kuert *et al* (2011) and Stachon *et al* (2005) also found that in these states, inflammatory cytokines such as interleukin 6 and interleukin 3 increase in concentration, paired with increased levels of erythropoietin in plasma and decreased oxygen, resulting in an increased number of circulating nRBCs (Kuert *et al* 2011; Stachon *et al* 2005).

β–thalassemia major is caused by an imbalance of the globin chain synthesis. This unequal production of chains leads to a decreased RBC survival, and thus oxygen capacity is minimised. This condition presents with increased extramedullary hematopoiesis, which is demonstrated by ineffective erythropoiesis and an increase in number in nRBCs which can number in the hundreds in peripheral films (Danise *et al* 2011; Danise *et al* 2009).

Megaloblastic anaemia is characterised by an impairment of DNA synthesis and defective nuclear maturation, which leads to anaemia via reduction of the number of red cell divisions and the presence of abnormally large RBCs in peripheral blood smears (Aslinia *et al* 2006). The impairment associated with macrocytic anaemia includes nutritional deficiencies such as Vitamin B12 and/or folate, and primary bone marrow disorders including myelodysplasia and leukemia. Aslinia *et al* (2006) also stated that macrocytosis due to Vitamin B12 or folate deficiency is a direct result of ineffective or dysplastic erythropoiesis and therefore compensatory erythropoiesis arises and with this the presence of nRBCs.

Normal newborn peripheral blood which is collected within the first 12 hours of birth can show the presence of nRBCs which can still be seen 3 to 5 days later, and then no nRBCs should be present (Constantino and Cogionis 2000). After this period the presence of nRBCs is associated with premature births, foetal hypoxia, malignant neoplasms, bone marrow diseases, and haemolytic disease of the newborn (Akhtar and Mahure 2015). McCarthy *et al*

(2005) and Valina *et al* (2020) also support that the increase of nRBCs in infants is an indicator of prolonged hypoxia, which may be linked with the length of time hypoxia mechanisms that occur pre-labour (McCarthy *et al* 2005; Valina *et al* 2020).

The hallmark of myelodysplastic syndrome (MDS) is ineffective haemopoiesis (Hoffbrand *et al* 2006), and it is characterised by a group of clonal disorders of multipotent haemopoietic stem cells, seen with increasing bone marrow failure in all three myeloid cell lines. Early detection can be made with the identification of non-lymphoid precursors, maturing neutrophil proliferation, and nRBCs with abnormal nuclear shapes or uneven cytoplasmic staining due to increased bone marrow proliferation (Matarraz *et al* 2012; Carr and Rodak 2009). The importance of detecting nRBCs has an impact on the overall survival and transformation to acute myeloid leukaemia.

Auto immune haemolytic anaemia (AIHA) is a disorder that shortens the survival rate of RBCs (Rodak *et al* 2012; Packman 2015). This is due to warm or cold auto-antibodies that bind to the surface of the RBC and cause its destruction and the BM will attempt to compensate for the loss, nRBC can be seen in the more severe cases of AIHA, as the disease can be mild to severe and can have either a gradual or acute onset.

Sickle cell anaemia (haemoglobin SS) is a haemoglobin disorder that occurs with the abnormal structure of the β chain at the sixth position when glutamic acid is replaced with a valine (Rodak *et al* 2012). This mutation causes the hallmark morphology of sickle cells, with the peripheral blood film also showing target cells and nRBCs. This is also a form of a haemolytic anaemia, where the life span for the RBC is shortened through haemolysis.

Microangiopathic haemolytic anaemia (MAHA) is characterised by RBC fragments, anaemia and haemolysis which can be intravascular, extravascular, or both (Pande *et al* 2023). MAHA includes thrombotic thrombocytopenia purpura (TTP) and haemolytic uremic syndrome (HUS) and in these severe cases nRBCS are present when the bone marrow responds to the anaemia.

Myelofibrosis is a clonal myeloproliferative neoplasm where bone marrow is replaced with fibrous connective tissue. Splenomegaly develops and ineffective and extramedullary haematopoiesis may occur and the peripheral blood will show tear drop cells, polychromasia, and nRBCs (Rodak *et al* 2012) .

To diagnose these disease states peripheral blood smears, which are flagged by the analyser for the presence of nRBCs need to be made and examined by an experienced morphologist. These smears are used to ascertain the correct number of nRBC and white blood cell (WBC) count may need to be corrected to obtain a result that is valid and can be provided to health professionals. This process does delay turnaround times and is labour intensive. This process could be improved with the utilisation of automation for the nRBC counts. The DxH can count the nRBC and provide an absolute count (nRBC#) stated by the analysis system manual provided by Coulter (2019).

Materials and methods

A total number of 190 samples were used, with an even distribution of low to high nRBC counts for comparison.

This data was obtained by Monash Health through blood specimens acquired from clinics and in-patients within the hospital (ethics approval ERM -106116). The samples are collected in a K_2EDTA tube. These samples are run for a complete blood count (CBC) profile provided by the DxH.

The nRBCs are measured by VCS 360 Technology where the analyser uses three measurements: individual cell volume (V), high-frequency conductivity (C) and laser-light scatter (S). The combination provides abundant cell-by-cell information translated into data plots. To identify individual cell volume, low-frequency current is used to differentiate cells by size.

The conductivity uses high-frequency current that can penetrate the cell to its interior, as the cellular walls act as conductors. This enables the characteristics of nuclear and granular constituents to be distinguished. Light scatter is a relationship between molecule size, cytoplasmic granularity/complexity and its refractivity to the angle of light from the laser beam (Coulter 2019; Serrando Querol and Nieto-Moragas 2021).

The VCS 360 module is responsible for the preparation and delivery of the sample to the flow cell for analysis of the WBC differential, reticulocytes and nRBC. The preparation occurs at the nRBC diff mix chamber where sample and reagents are added in a specific order: diluent, blood, additional diluent followed by an air mix. Cell lyse is added next, followed by a second air mix, and an incubation period. Once prepared, the sample progresses to the Multi-transducer Module (MTM) where cells are counted in an isometric sample stream. The algorithm analysis separates nRBC from WBC. The number of nRBC per 100 WBC is expressed as nRBC/100 WBC. This information can then be represented as data plots, as seen in Figure 1 which is provided by Coulter (2019).

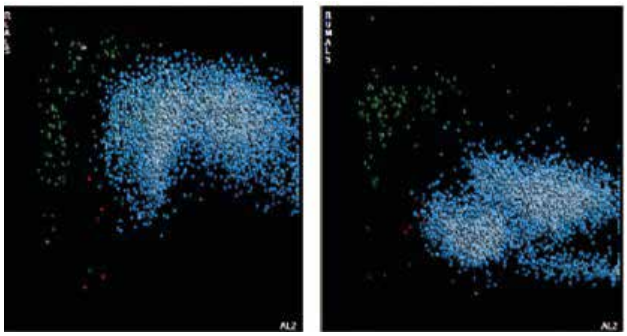


Figure 1. *The image shows the data plots as visual representations of the differential, nRBC, and reticulocyte population and density post-VCS analysis where the nRBC population is represented in red, seen in the bottom left quadrate (Coulter 2019).*

A flag will be displayed if the analyser reaches a predetermined number of events falling within the nRBC gating region of the data plot. This is designated as a 'trigger rule' for a peripheral blood film to be made from that sample, which on the default setting from the analyser is greater than 2%. If desired, a specific rule can be written for individual patients or a specific range.

Peripheral blood smears are made on the DxH slidemaker stainer II (automated haematology slide preparation) and stained with a Romanowsky stain (May Grunwald-Giemsa). This is followed by the smear being scanned manually by a morphologist for the presence of any nRBCs. The uncorrected white blood count (UWBC) is transcribed prior to a 100-cell differential count being performed by a morphologically trained scientist, who then performs a comparison of results before accepting the manual differential. If there is a significant number of nRBC then a corrected WBC will need to be calculated. This takes time and a delay in results being released to the treating clinician (Coulter 2019).

Data from the DxH900 that was collected was the nRBC count and the absolute nRBC count. Samples that had data extracted from the DxH also had the same data extracted from the laboratory information system (LIS). Microsoft Excel was used to compile data for comparison and attain graphs and regression lines for a correlation coefficient.

Results

The correlation for all 190 tested samples was determined by considering a wide distribution of values varying from low to high, between automated nRBC counts and manual nRBC counts.

Figure 2 compares nRBC (%) between automated nRBC count and the reference manual technique by linear regression for all samples. The demonstrated regression equation was $y = 0.326x + 7.398$ and the $R^2 = 0.778$.

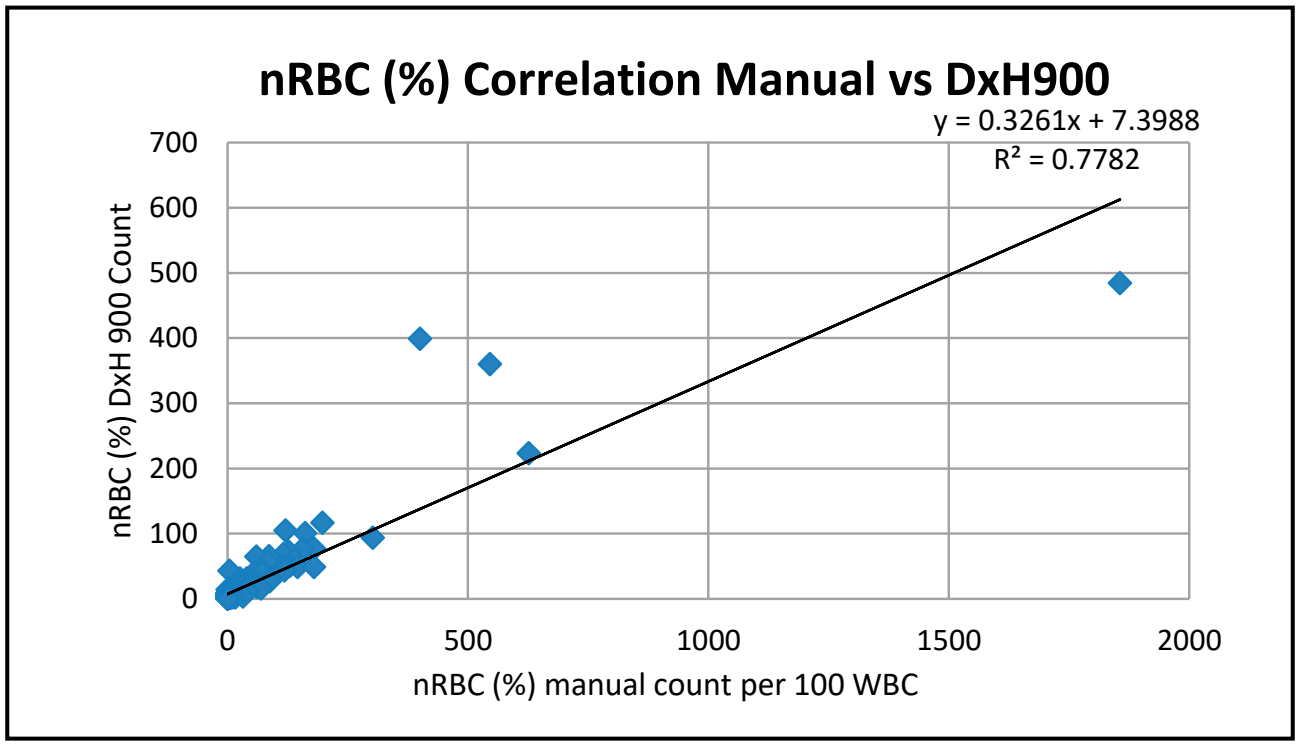


Figure 2. *nRBC (%) manual vs DxH determined by linear regression for all 190 samples .*

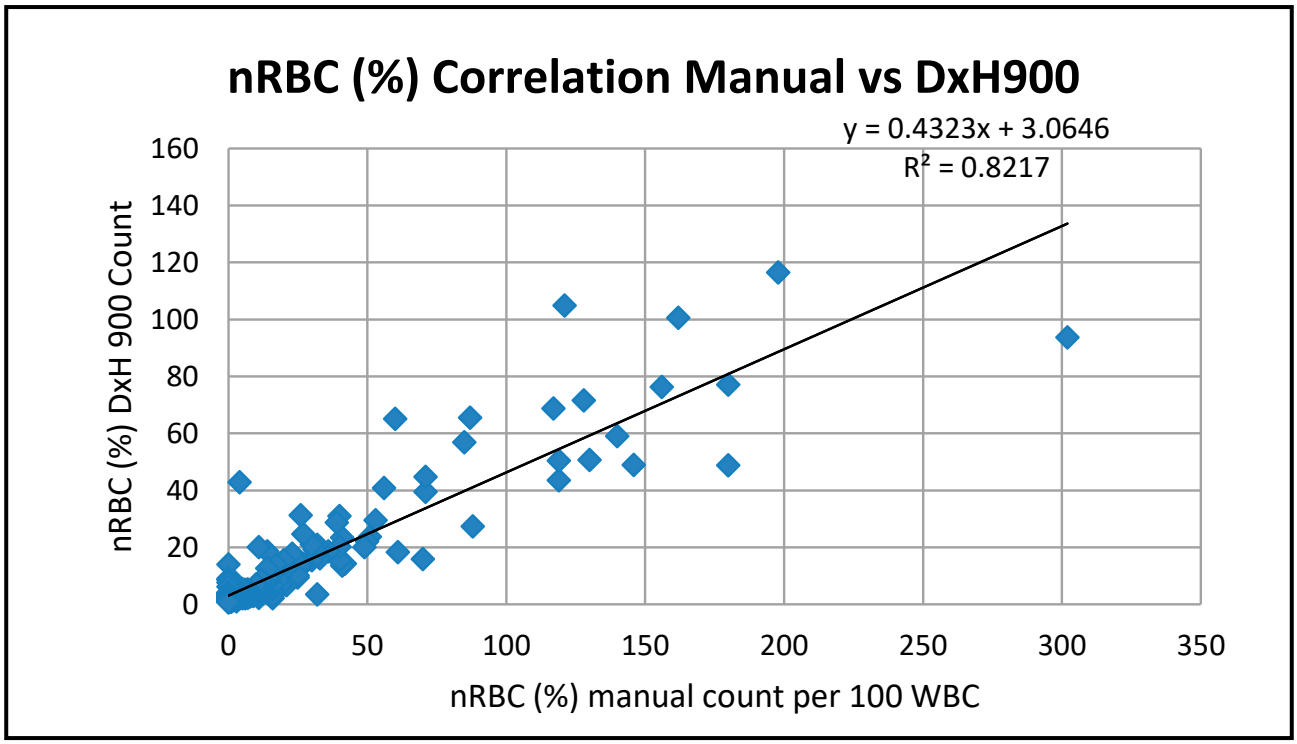


Figure 3. *nRBC (%) manual vs DxH determined by linear regression, for samples with <400 nRBC (186 samples from a total of 190 samples tested).*

Figure 3 compares nRBC (%) manual vs DxH determined by linear regression on a focused population of nRBC (under 400 nRBCs) which in this study was 186 out of a total of 190 samples tested. The demonstrated regression equation was $y = 0.432x + 3.064$, $R^2 = 0.821$.

When evaluating the data obtained from the morphologist, nRBC counts yielded a higher count than the analyser per 100 WBC. This is seen in 66% of the samples in Figure 4.

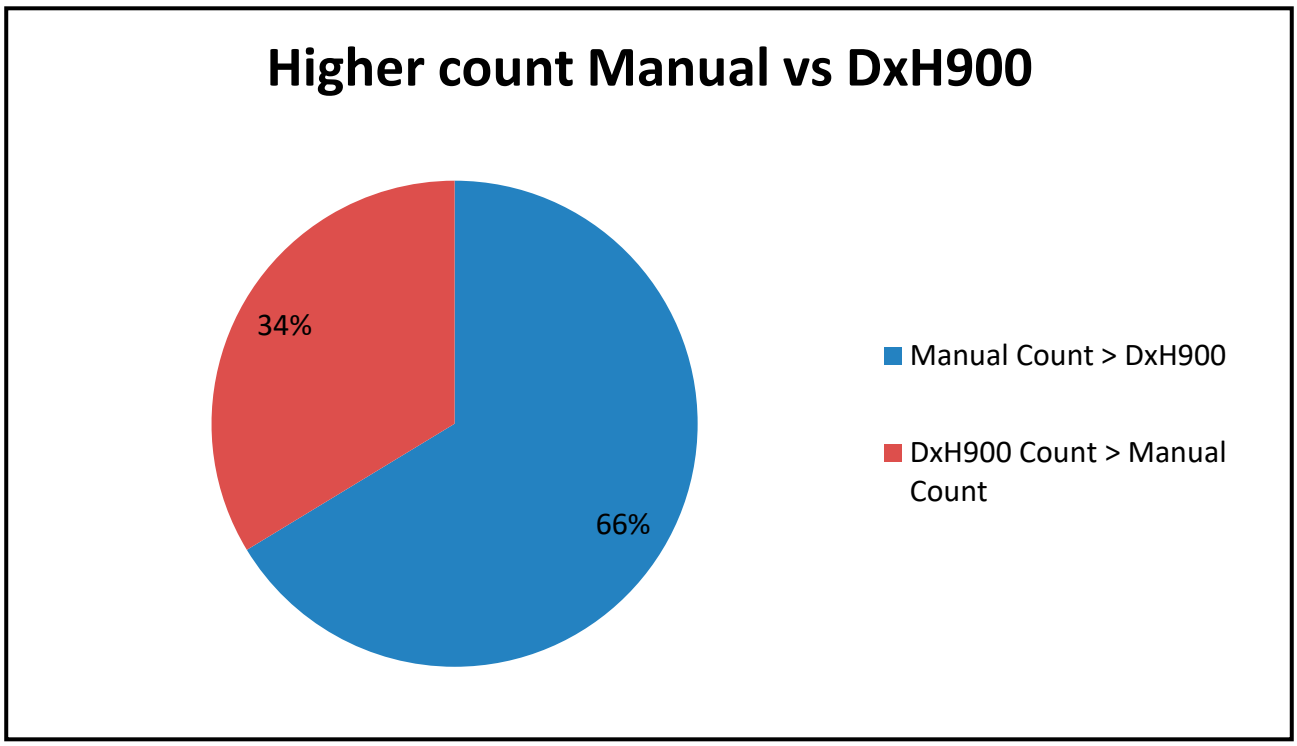


Figure 4. *Percent of samples that had a manual count higher than the DxH900 per 100WBC.*

Table 1. *Comparison of distribution of nRBC from manual counts to DxH 900 counts*

nRBC(%) range from peripheral film count	Number of samples	nRBC (%) range from the DxH900			
		≤1.0	1.0 – 10	10 - 100	>100
≤1.0	**43**	5	37	1	
1.0 - 10	61		60	1	
10 - 100	68		26	42	
>100	18			11	7

Table 1 demonstrates the distribution of nRBC counts from manual counts to the counts provided by the DxH900.

These 190 nRBC counts determined manually were within the range of 0–973% compared to the DxH, with only 10 samples within the range of 0–10% of percentage difference. For the results to be acceptable 95% of the sample population should to be within the 0-10%, as demonstrated in Figure 5.

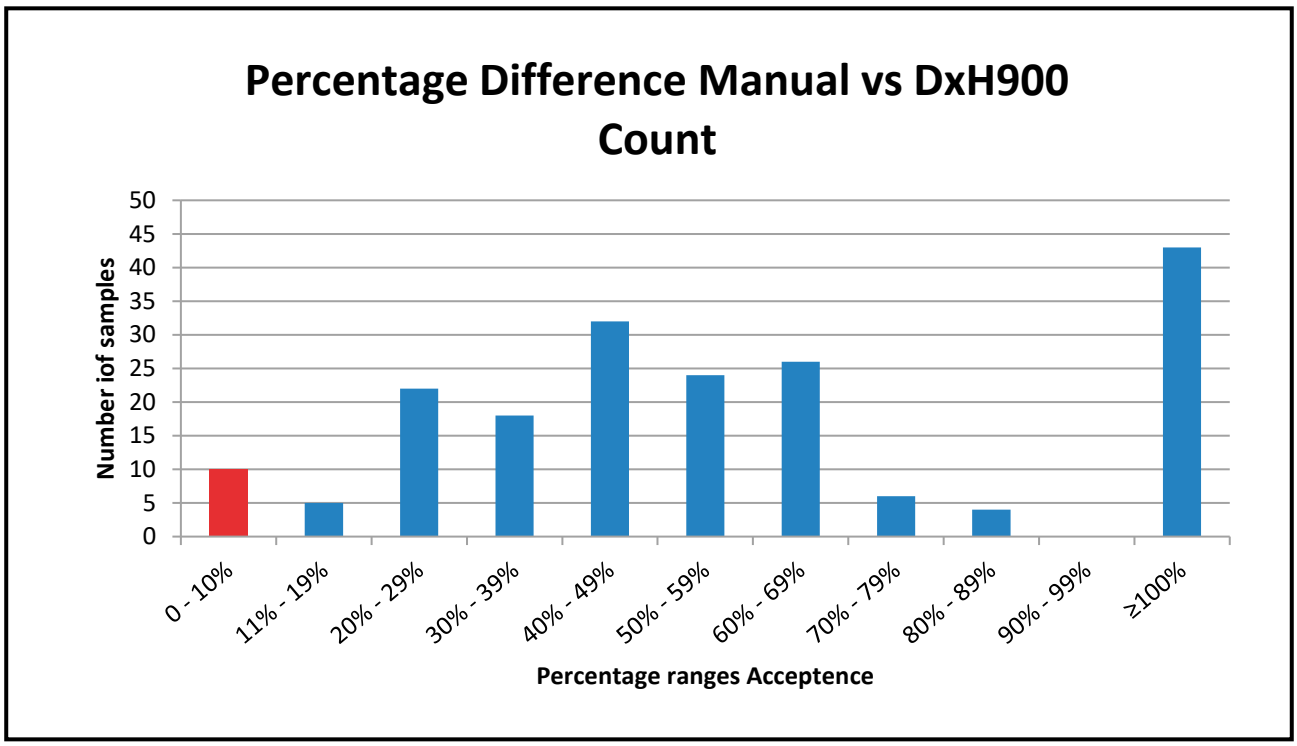


Figure 5. *Percentage difference and acceptance for DxH900 compared to the manual count.*

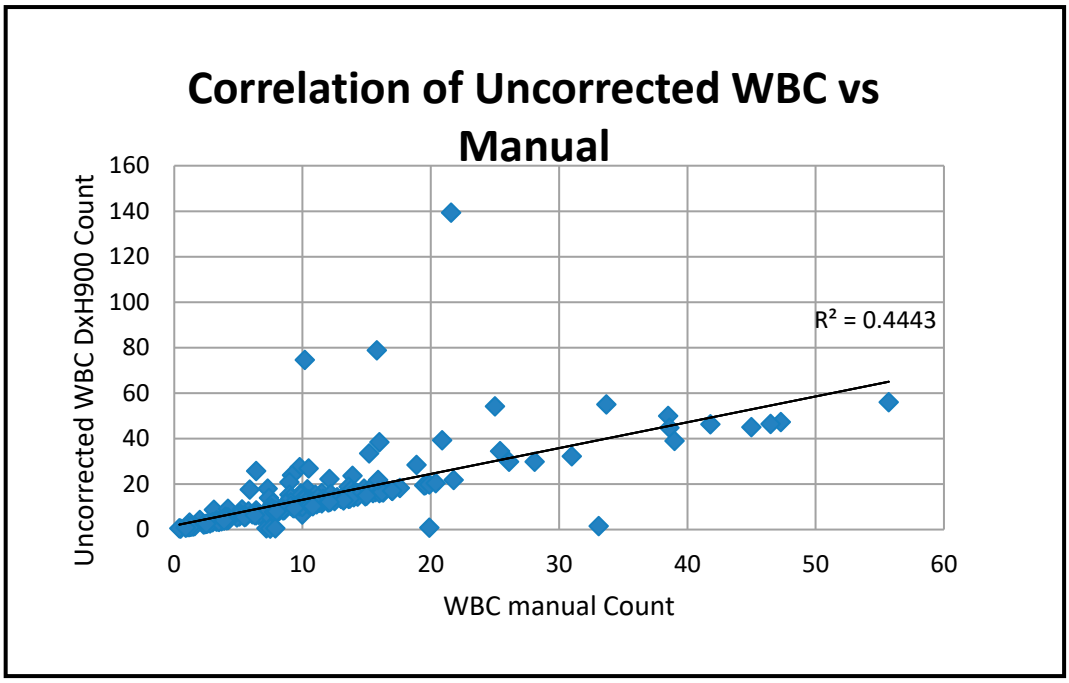


Figure 6. *Uncorrected WBC correlation of manual vs DxH900 determined by linear regression for all 190 samples.*

Figures 6 and 7 demonstrate the uncorrected and corrected WBC correlation of manual vs DxH determined by linear regression for all 190 samples respectively. This represents how the nRBC count affects the WBC.

Figures 8 demonstrates lymphocyte correlation of manual vs DxH determined by linear regression for all 190 samples where the R^2= 0.591. This represents how the presence of nRBC can affect the lymphocyte percentage in a differential count.

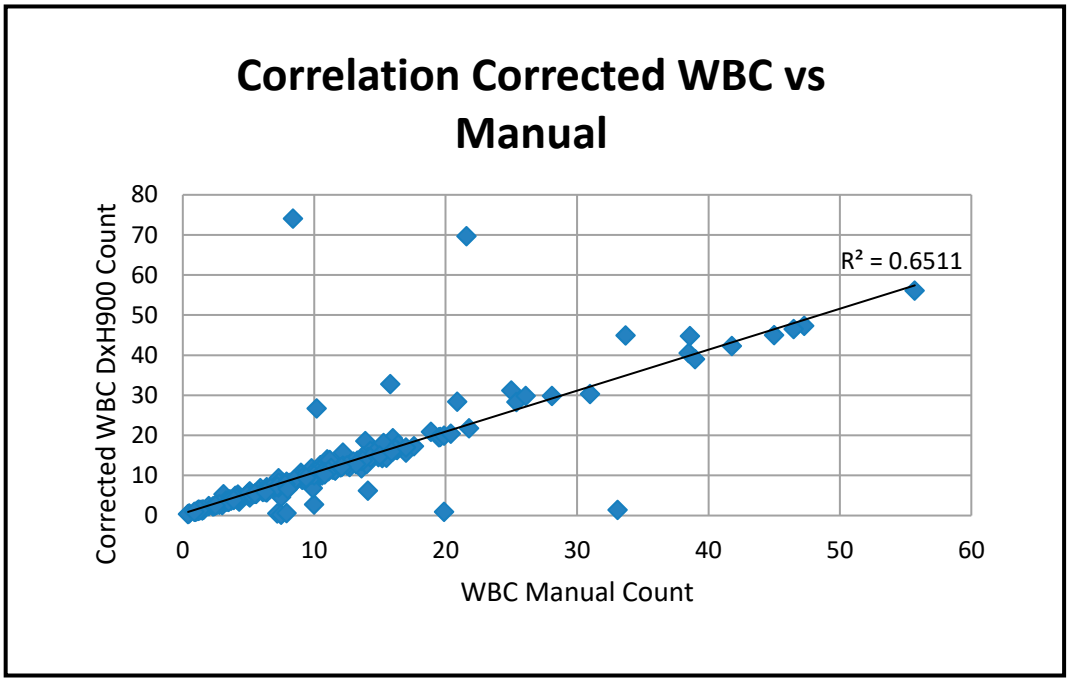


Figure 7. *Corrected WBC correlation of manual vs DxH determined by linear regression for all 190 samples.*

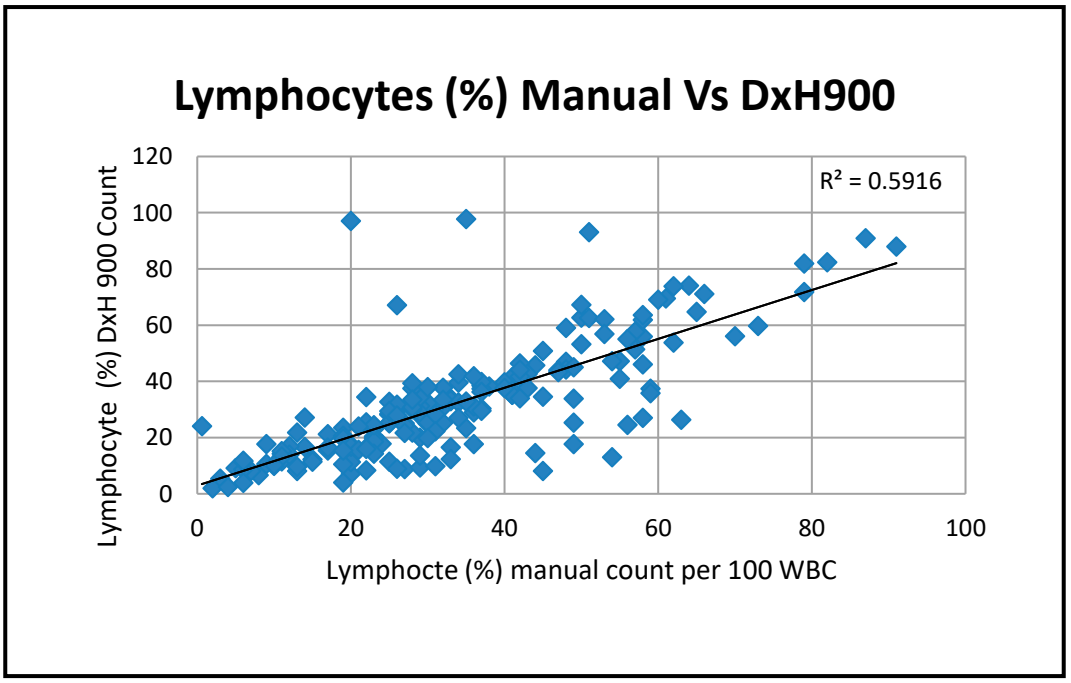


Figure 8. *Lymphocyte (%) correlation of manual vs DxH determined by linear regression for all 190 samples.*

Discussion

This study looked at the nRBC results provided by the DxH and determined if these values could be used without the need to perform a manual differential for nRBC. The data evaluated were over a wide range of the nRBCs that were present in the samples.

The data show reasonable correlations between nRBC percent values (%) with R^2 = 0.778. Although data were collected over a wide distribution, the vast majority of samples had counts under 400 nRBC/100 WBC with only four samples above 400 nRBC/100 WBC. Figure 3 shows those 186 samples under 400 nRBC, and the R-value shows an improvement to 0.821. The correlation is still below the limits of acceptance, with the acceptable R-value being greater than 0.90.

Figure 4 represents that 66 % of the sample's manual count yielded a higher nRBC count than that of the analyser. These counts were then performed by multiple morphologists and still have showed an increased nRBC count bias compared to the analyser.

A closer look at how the counts are distributed is compared in Table 1 where the number of samples manually counted nRBC within a range versus how the DxH counted the nRBC. The most comparable results were seen within the 1.0-10 nRBC counted range for the manual count and DxH with 60 samples falling within that category.

When resulting a peripheral blood film, morphologists look at the entire blood picture to diagnose the patients' condition. When nRBCs are detected they are not the only factor taken into consideration, noting, that a normal reference range, for nRBC is 0/100WBC in adults and 3-10/100WBC in normal full-term infants (Rodak *et al* 2012; Constantino and Cogionis 2000).

Currently, the nRBC correction from the DxH900 analyser does not meet the criteria to be accepted therefore the results must be confirmed morphologically.

Due to the heterogeneous morphology of nRBCs they can be misidentified as another cell type, most commonly lymphocytes caused by the impedance and VCS technology (Hedley *et al* 2011; Hwang *et al* 2016). Coulter (2019) acknowledges there are limitations of nRBCS on the DxH which are taken into consideration when looking at the data gathered (Coulter 2019). These interferences effect WBC and total nucleated cell count (TNC) and are seen with nRBCs, giant platelets, platelet clumps, malarial parasites, precipitated elevated proteins, cryoglobulin, micro-lymphoblasts, very small lymphocytes, fragmented white cells, agglutinated white cells, lyse resistant red cells and un-lysed particles > 35 fL in size.

These interferences occur as the un-lysed RBCs, such as the ones infected by malarial parasites, enter the WBC counting chamber and increase the total WBC and both the numbers of lymphocytes and monocytes. This results

in the under counting of the nRBCs and over estimating whichever the cell that is classified by the analyser (Muthunatarajan and Basavaiah 2021). This in turn affects the WBC which is measured directly, and multiplied by the calibration factor. This is then corrected for nRBCs if identified by the analyser.

Due to the analyser's limitations and known interferences, a manual differential count is currently performed to account for those interferences and provide a more accurate representation of the WBCs to correct for the nRBC interference. The UWBC is measured directly using the Coulter Principle and cannot be edited once the analyser provides this result. It is this value that is used as the total WBC count to complete a manual differential with the WBCs as seen by the morphologist and whether nRBCs are present or not.

In many instances, the WBC is corrected automatically when the instrument has a high confidence in the accuracy of the WBC however a nRBC film flag will also be triggered if nRBCs are suspected, and these samples will be checked on a peripheral blood film for confirmation. The analyser provides the UWBC as an estimate of the magnitude of correction on the final WBC, based on the assumption that all the nRBCs have been categorised correctly and accounted for (Coulter 2019).

Figure 6 concludes that the UWBC count is higher than the manual corrected WBC count. This same trend is also seen in the corrected counts in Figure 7. Only when the morphologist performs a manual differential a true representation is shown of the WBC count and nRBC.

Figure 8 shows the correlation of lymphocyte (%) between manual and DxH with the R-value of 0.591, which demonstrates how a cell can be incorrectly categorised, and can affect the differential and the white cell count.

If the peripheral film is not spread evenly, this can lead to a section of the film having a greater number of nRBCs than other areas. This could be because the section where the 100 WBC differential count was performed had a bias in nRBCs population hence skewing the results. McCarthy *et al* (2005) suggests using a 200 differential cell count, particularly when the nRBC count is lower, to provide a more accurate count for nRBCs. They also found that automated analysers have more accurate nRBCs counts at low values than manual counts. The 190 films that were reviewed by a team of experienced morphologists showed that the nRBC were predominately higher than the analyser. As demonstrated in Figure 5, 190 samples showed the percentage difference between 0–973% compared to the analyser count, with only 10 samples within the range of 0–10% and were acceptable for the automated nRBC count. This underlines the limitation of the DxH and nRBC being detected but not categorised appropriately.

Similar studies have been conducted evaluating the correlation between automated haematology analysers and manual peripheral blood smears. However, their investigations differ in multiple aspects from this study (outlined below) although they provide insight into how other analysers and laboratories approach the accuracy, efficiency, and workflow of nRBC counts.

Research in this area by McCarthy *et al* (2005) also made comparisons between an analyser and nRBC manual counts. That study used a different analyser, the Cell-DYN 4000 instrument from Abbott Laboratories, but only compared nucleated red blood cells in umbilical cord blood. The samples collected were from women who presented at the labour department for delivery. The manual nRBC count was expressed per 100 WBC for comparison to the Cell-DYN 4000 which showed a high level of agreement with R values >0.9.

An investigation by Valina *et al* (2020) predominantly focuses on the comparison between the Sysmex XN analyser and manual peripheral nRBC counts, expressed per 200 WBC, to attain a more efficient and accurate method to report nRBC without the need to perform a film. The population that was targeted was a sample size of 121 neonates. Good correlation was seen over a wide range and showed accurate and effective enumerated nRBC counts, implying that the automated count provided by the Sysmex XN could be used to replace the manual microscopic nRBC count and lead to increased turnaround times.

This is the first study that has focused on this spectrum of data which is not synonymous with a specific age group nor disease type, but purely compares the number of nRBCs attained by the Beckman coulter analyser DxH900 and manual peripheral nRBC count.

Conclusion

Although the DxH900 analyses a large number of cells and categorises them appropriately at low numbers, there are still interferences that are present that require a morphologist review. The gold standard therefore remains that of the morphologist who can distinguish specific characteristics of cells and count them accordingly.

References

Akhtar S, Mahure S 2015. Nuance of nucleated rbcs (normoblastemia) in peripheral blood film. *Panacea Journal of Medical Sciences*, 5, 7-13.

Aslinia F, Mazza JJ, Yale SH 2006. Megaloblastic anemia and other causes of macrocytosis. *Clin Med Res*, 4, 236-41.

Bedrick AD 2014. Nucleated red blood cells and fetal hypoxia: a biologic marker whose 'timing' has come? *J Perinatol*, 34, 85-6.

Carr J, Rodak FB 2009. *Clinical Hematology Atlas*, Saunders Elsevier.

Constantion BT, Cogionis B 2000. Nucleated RBCs—significance in the peripheral blood film. *Laboratory Medicine*, 31, 223-229.

Coulter B 2019. *UniCel DxH 900 Series with System Manager Software. Coulter Cellular Analysis System*.

Danise P, Amendola G, Di Concilio R, Cillari E, Gioia M, Di Palma A, Avino D, Rigano P, Maggio A 2009. Nucleated red blood cells and soluble transferrin receptor in thalassemia syndromes: relationship with global and ineffective erythropoiesis. *Clin Chem Lab Med*, 47, 1539-42.

Danise P, Maconi M, Barrella F, Di Palma A, Avino D, Rovetti A, Gioia M, Amendola G. 2011. Evaluation of nucleated red blood cells in the peripheral blood of hematological diseases. *Clin Chem Lab Med*, 50, 357-60.

Davari-Tanha F, Kaveh M, Nemati S, Javadian P, Salmanian B 2014. Nucleated red blood cells count in pregnancies with idiopathic intra-uterine growth restriction. *J Family Reprod Health*, 8, 77-81.

Eckardt KU, Kurtz A 2005. Regulation of erythropoietin production. *Eur J Clin Invest*, 35 Suppl 3, 13-9.

Hedley BD, Keeney M, Chin-Yee I, Brown W 2011. Initial performance evaluation of the UniCel® DxH 800 Coulter® cellular analysis system. *Int J Lab Hematol*, 33, 45-56.

Hoffbrand AV, Moss PAH, Pettit JE 2006. *Essential Haematology*, Blackwell.

Hwang DH, Dorfman DM, Hwang DG, Senna P, Pozdnyakova O 2016. Automated Nucleated RBC Measurement Using the Sysmex XE-5000 Hematology Analyzer: Frequency and Clinical Significance of the Nucleated RBCs. *Am J Clin Pathol*, 145, 379-84.

Jelkmann W 2011. Regulation of erythropoietin production. *J Physiol*, 589, 1251-8.

KuertS. Holland-Letz T, Friese J, Stachon A 2011. Association of nucleated red blood cells in blood and arterial oxygen partial tension. *Clin Chem Lab Med*, 49, 257-63.

Matarraz S, Teodosio C, Fernandez C, Albors M, Jara-Acevedo M, López A, Gonzalez-Gonzalez M, Gutierrez ML, Flores-Montero J, Cerveró C, Pizarro-Perea M, Paz Garrastazul M, Caballero G, Gutierrez O, Mendez GD, González-Silva M, Laranjeira P, Orfao A 2012. The proliferation index of specific bone marrow cell compartments from myelodysplastic syndromes is associated with the diagnostic and patient outcome. *PLoS One*, 7, e44321.

May JE, Marques MB, Reddy VVB, Gangaraju R 2019. Three neglected numbers in the CBC: The RDW, MPV, and NRBC count. *Cleveland Clinic Journal of Medicine*, 86, 167-172.

McCarthy JM, Capullari T, Spellacy WN 2005. The correlation between automated hematology and manually read smears for the determination of nucleated red blood cells in umbilical cord blood. *J Matern Fetal Neonatal Med*, 17, 199-201.

Minior VK, Levine B, Ferber A, Guller S, Divon MY 2017. Nucleated Red Blood Cells as a Marker of Acute and Chronic Fetal Hypoxia in a Rat Model. *Rambam Maimonides Med J*, 8.

Muthunatarajan S, Basavaiah SH 2021. Discriminant value of automated leucocyte VCS parameters in the detection of tropical infections. 35, e23723.

Packman CH 2015. The Clinical Pictures of Autoimmune Hemolytic Anemia. *Transfus Med Hemother*, 42, 317-24.

Pande A, Kumar A, Krishnani H, Acharya S, Shukla S 2023. Recent Advances in the Management of Microangiopathic Hemolytic Anemias (MAHA): A Narrative Review. *Cureus*, 15, e47196.

Pikora K, Krętowska-Grunwalk A 2023. Diagnostic Value and Prognostic Significance of Nucleated Red Blood Cells (NRBCs) in Selected Medical Conditions. 12.

Rodak BF, Fristsma GA, Keohane EM 2012. *Hematology Clinical Principles and Applications*, Elsevier.

Serrando Querol M, Nieto-Moragas J 2021. Evaluation of the New Beckmann Coulter Analyzer DxH 900 Compared to Sysmex XN20: Analytical Performance and Flagging Efficiency. 11.

Siren AL, Fratelli M, Brines M, Goemans C, Casagrande S, Lewczuk P, Keenan S, Gleiter C, Pasquali C, Capobianco A, Mennini T, Heumann R, Cerami A, Ehrenreich H, Ghezzi P 2001. Erythropoietin prevents neuronal apoptosis after cerebral ischemia and metabolic stress. *Proc Natl Acad Sci U S A*, 98, 4044-9.

Stachon A, Bolulu O, Holland-Letz T, Krieg M 2005. Association between nucleated red blood cells in blood and the levels of erythropoietin, interleukin 3, interleukin 6, and interleukin 12p70. *Shock*, 24, 34-9.

Turgeon ML 2012. Clinical Hematology Theory and Procedures. Lippincott Williams and Wilkins.

Valina O, Inssen JL, Rutsche J, Leibundgut K 2020. Evaluation of the Automated Nucleated Red Blood Cell (NRBC) Enumeration on Sysmex XN Analyser in Preterm and Term Neonate. *Sysmex Journal International*, 30.

ORIGINAL ARTICLE

The contribution of NSW Health Pathology to medical research: the first 10 years

Emmanuel J Favaloro PhD FFSc (RCPA)[1,2,3]

[1] *Lead Scientist, Thrombosis and Haemostasis Research, Sydney Centres for Thrombosis and Haemostasis, Institute of Clinical Pathology and Medical Research (ICPMR), NSW Health Pathology, Westmead Hospital, Westmead, NSW*

[2] *Faculty of Science and Health, Charles Sturt University, Wagga Wagga, NSW*

[3] *School of Medical Sciences, Faculty of Medicine and Health, University of Sydney, Westmead Hospital, Westmead, NSW*

Abstract

Medical research is integral to improving diagnosis and treatment of disease. NSW Health Pathology is the largest publicly funded pathology organisation in Australia and also undertakes research related activities, but no formal broad analysis of these activities is available.

A PubMed search of publications was conducted looking for authors who have ascribed their affiliation as NSW Health Pathology (or variations therein).

The final analysis identified a total of 2345 publications citing at least one affiliation as NSW Health Pathology (or variations therein). These publications appeared in a wide range of journals, and from a broad spread of geographical locations and fields of practice.

To the author's knowledge, this provides the most comprehensive analysis of publications arising from researchers citing a NSW Health Pathology affiliation and highlights a broad spread of publications arising from several geographical locations and service streams of NSW Health Pathology.

Key words: ***Medical research, NSW Health Pathology, PubMed.***

Introduction

Medical research is integral to improving diagnosis and treatment of disease. NSW Health Pathology (NSWHP) is the largest publicly funded pathology organisation in Australia. The NSWHP website (https://pathology.health.nsw.gov.au/) indicates that 70% of medical decisions rely on pathology and that the organisation performs 100,000+ clinical and scientific investigations each day, employs 5,000+ staff throughout NSW, has 60+ laboratories, and has 150+ collection services in NSW. Established in 2012, the organisation currently provides comprehensive pathology services; these are organised into several service streams (namely the 'clinical streams' of Anatomical Pathology, Chemical Pathology, Haematology, Immunology, Microbiology, Pre- and post-analytical, and Transfusion), which manage the delivery and performance of these pathology services (https://pathology.health.nsw.gov.au/services/pathology/). In addition to pathology, NSWHP operates several additional state-wide services: Biobanking, Public Health, Point of Care Testing, Genomics, Forensic Medicine, Forensic & Environmental Toxicology, Criminalistics, and Perinatal Postmortem (https://pathology.health.nsw.gov.au/services/).

Medical research is also one of NSWHP's core activities. The importance of medical research to the operations of NSWHP is noted in several publications from the organisation, which aspires to "lead the way in teaching, training and research in diagnostic and forensic pathology to deliver better outcomes and experiences for the community" (NSW Health Pathology Clinical Services Plan 2019 – 2025). NSWHP also has a research strategy outlined in the document titled "Research Strategy NSW Health Pathology Towards 2025" (https://pathology.health.nsw.gov.au/research/research-strategy/), and has also published several annual 'Research Activity Reports'

Address correspondence to:
Emmanuel J Favaloro
Haematology, ICPMR, Westmead, NSW 2145.
Ph: +612 8890 6618; Fax: +612 9689 2331
email: Emmanuel.Favaloro@health.nsw.gov.au

(2017 to 2020 inclusive https://pathology.health.nsw.gov.au/research/our-research/research-activity-reports/.

No recent formal evaluation of medical research performed within NSWHP has been undertaken to the author's knowledge. The current report therefore aims to provide a snapshot of the output of NSWHP researchers, as undertaken and published in the scientific and medical literature, and also noting NSWHP as at least one of the authors' affiliation(s). Since NSWHP was initially established in 2012, the current report essentially assesses data captured within the subsequent 10 or so years.

Materials and methods

As noted above, NSWHP was first established in 2012. The PubMed database is a database managed by the US National Institute of Health (NIH) National Library of Medicine. Available at https://pubmed.ncbi.nlm.nih.gov, the website provides simple and advanced search features. The PubMed database was chosen because it is a freely available database and is well respected and utilised by medical researchers. The database is also the one used by NSWHP to document publications from NSWHP staff on its website (https://pathology.health.nsw.gov.au/research/our-research/research-publications/). Although the database does not cover all journals publishing medical research, it does comprise "more than 37 million citations for biomedical literature from MEDLINE, life science journals, and online books" (https://pubmed.ncbi.nlm.nih.gov). Notably the AJMS is not represented in the PubMed database, and so publications from this journal have been missed in the current data. There are several other well-established databases, but these tend to be available only to subscribers (i.e. exist behind some kind of paywall), and thus are not freely available. This includes some databases in which the AJMS is represented (e.g. Embase).

The recommended affiliation citation for NSWHP researchers according to an internal document is 'NSW Health Pathology'. Researchers may however use variations of this affiliation. An initial scoping review was performed on April 1 2024, using a search of 'NSW Health Pathology' as well as the abbreviation 'NSWHP' in the affiliation field of the advanced search site of PubMed. This uncovered several additional affiliation formats used by NSWHP researchers, primarily 'New South Wales Health Pathology', so a second search was performed using 'New South Wales Health Pathology' in the affiliation field of the advanced search site also on the same day. An initial evaluation of search results was then undertaken to assess for any other unexpected patterns for the affiliation. Finally, a subsequent search was performed using the search string "((New South Wales (NSW) Health Pathology[Affiliation]) OR (NSW Health Pathology[Affiliation]) OR (New South Wales Health Pathology[Affiliation]) OR (NSWHP[Affiliation]))" on May 11 2024. The search data was downloaded to a personal computer and formal data analysis then undertaken.

Ethics

As this is a retrospective review of publication citations available from a freely accessible database, not otherwise involving patients or non-freely available information, specific ethics approval for the study was deemed to not be required.

Definitions and assignments

As indicated in the introduction, NSWHP provides both pathology services and additional services including state-wide services. Pathology services are organised into both service streams (also called 'clinical streams') and separate pathology networks according to geographic locality and use of specific laboratory information systems (i.e. separated as 'West', 'Rural and Regional', 'South', 'North' and 'East') (Favaloro *et al* 2023). Other service streams have also been outlined in the introduction. Research activity within NSWHP may occur within any of these services, fields or geographic locations. The 'locations' often cited by authors using an NSWHP affiliation will not specify a given network or service steam, but the affiliation location and department will usually provide this information indirectly. For example, the author to this report works within the Haematology Department at the Institute of Clinical Pathology and Medical Research (ICPMR) located at Westmead Hospital. His affiliation can therefore be linked both to the Haematology clinical stream (or service stream) as well as to the 'West' network of laboratories (Favaloro *et al* 2023). Similarly, an author citing a department of 'Anatomical Pathology' or else 'Tissue Pathology' can be ascribed to the 'Anatomical Pathology' clinical (service) stream; if they cite Royal North Shore Hospital as their location, they will also be ascribed to the 'North' network, but if they cite Royal Prince Alfred Hospital as their location they will be ascribed to the 'South' network, and so on.

Results

The search strategy and summary data are shown in Figure 1. The initial search identified 1796 publications, with highest usage of the organisation's recommended affiliation citation as "NSW Health Pathology", but also identifying secondary usage of "New South Wales Health Pathology", "NSWHP" and "New South Wales (NSW) Health Pathology". The secondary search using "New South Wales Health Pathology" identified 536

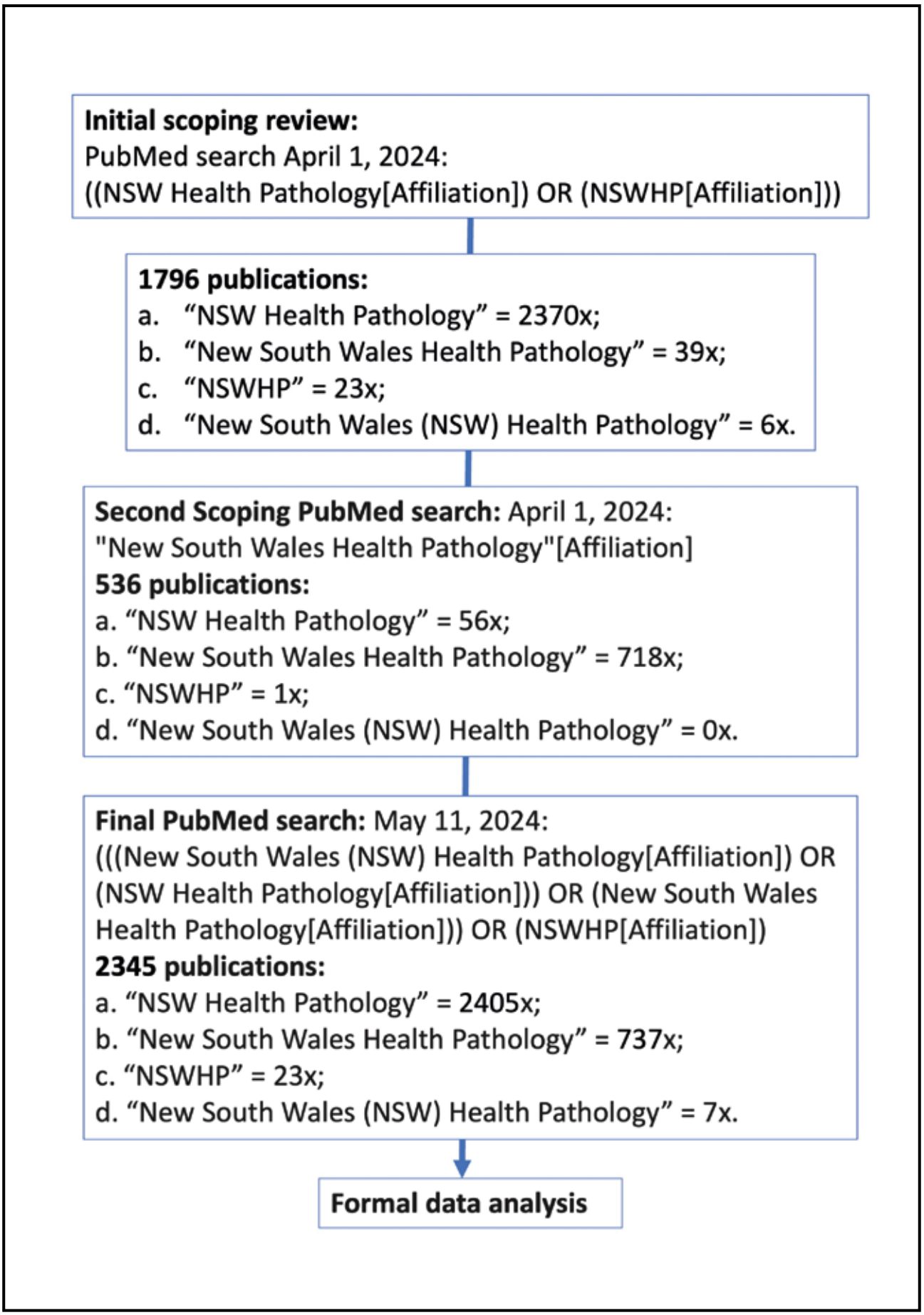


Figure 1. *Study design.*

publications, many of which were missed in the first search. After checking the output for any other possible affiliation citation variations, a final search was performed using the four identified affiliation citation variations and no further affiliation citation variations were identified. This final search identified a total of 2345 publications citing at least one of these affiliation variations.

The yearly publication rate of NSW Health Pathology researchers captured by the search and according to year, up to the final complete year of 2023, is shown in Figure 2A. Surprisingly, there were two publications cited in 2012 (the year the organisation was created) followed by four and nine publications respectively in the next two years. It is unclear if the low initial publication counts reflected "uncertainty" around using a NSWHP affiliation, or a lack of author knowledge around the 'correct' affiliation citation to use, but publications ascribed to NSWHP grew over subsequent years. NSWHP researchers published in excess of 400 PubMed listed papers/year in the last four full years of data capture (2020-2023 inclusive). The most "popular" PubMed listed journals utilised by NSWHP researchers is shown in Figure 2B. The most popular journal was perhaps unsurprisingly the Journal 'Pathology'. Forty-six NSWHP affiliated researchers published in excess of 20 papers each over the data capture period (Figure 2C), with the lead author publishing nearly 250 publications over this time. An analysis was also performed for NSWHP affiliated researchers contributing to these publications as first, second or last author. In general, first and second listed authors are either the major contributors of the study (for original studies) or take the lead for writing duties, inclusive of reviews, or might also act as the 'corresponding' author. In contrast last authors are often the 'senior' authors, who may take overall responsibility for the study or for a research group or who might otherwise act as the 'corresponding' author. In summary, nine authors published ≥10 papers each over the data capture period as a first author (Figure 2D), 11 authors published ≥10 papers each over the data capture period as a second author (Figure 2E), and 18 authors published ≥10 papers each over the data capture period as a last author (Figure 2F).

The geographic work site location of authors with a NSWHP affiliation was also assessed and summary data shown in Figure 3. It should be noted that this data captures all authorships ascribed to a NSWHP affiliation publication and a given geographic location (e.g. Westmead Hospital, Royal North Shore Hospital, Royal Prince Alfred Hospital and so on). Since multiple authors appear in most publications, the counts in these figures will exceed the total publication counts shown in Figure 2A. Figure 3A shows the authorship counts in the capture period by geographic work site location. Sixteen work site locations contributed authorships in excess of 20 each over the data capture period. Figure 3B shows the authorship counts in the capture period according to the NSWHP 'service stream'. Ten 'service streams' contributed authorships in excess of 60 each over the data capture period. Figure 3C shows the authorship counts in the capture period by 'service network'. Seven 'service networks' contributed authorships in excess of 25 each over the data capture period. Finally, Figure 3D shows the authorship counts in the capture period by both work site location and 'service fields', to provide better context around authorships.

Discussion

To the author's knowledge, this is the first detailed analysis of medical research output from researchers citing a NSWHP affiliation. The analysis has focussed on journals listed within the PubMed database, a freely available database and therefore the veracity of the data can be easily checked. It will additionally permit future comparative analyses of other publicly funded pathology organisations or research facilities.

The author was involved in a separate similar analysis published in 1998, which comprised an analysis of medical research in New South Wales (NSW) from 1993-96, as also assessed by Medline publication capture (Favaloro 1998). In that publication, it was found that NSW hospitals were very much involved in medical research and indeed accounted for the majority of publications identified - generally in excess of those arising from medical research institutions.

No comparative analysis of publication output from NSWHP vs established medical research institutions has occurred on this occasion, since the intention here is to provide a summary of NSWHP affiliated publications in the first 10 years (or so) of existence. As NSWHP associated research is also expected to have a different focus to that of established medical research institutions, the utility of such a comparison may be limited. Further comparative exercises may be performed in the future. The author is not aware of any similar comparative analysis of medical research undertaken by any other organisation where that research is freely available in the literature. There are however likely to be annual research reports from a wide variety of organisations that list publications from their researchers in that yearly data capture period. NSWHP has indeed released several such research reports in the past (NSW Health Pathology Research Activity Reports; 2017 to 2020 inclusive (https://pathology.health.nsw.gov.au/research/our-research/research-activity-reports/).

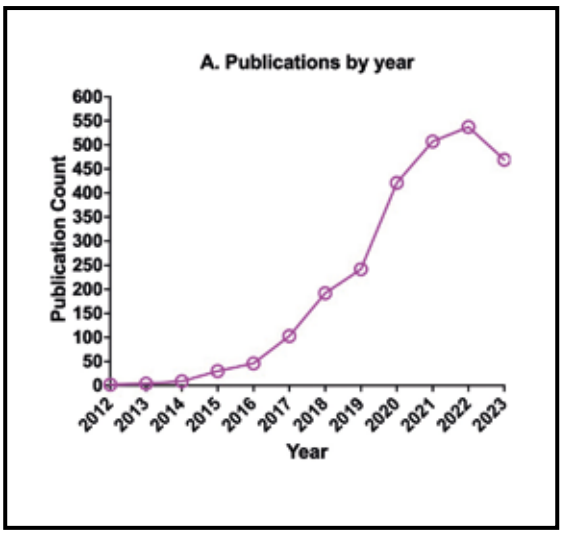


Figure 2A. *Publications ascribed to authors citing a NSW Health Pathology affiliation and extracted from the PubMed database as published over the years 2012 to 2023 inclusive, comprising the full years available at study completion date.*

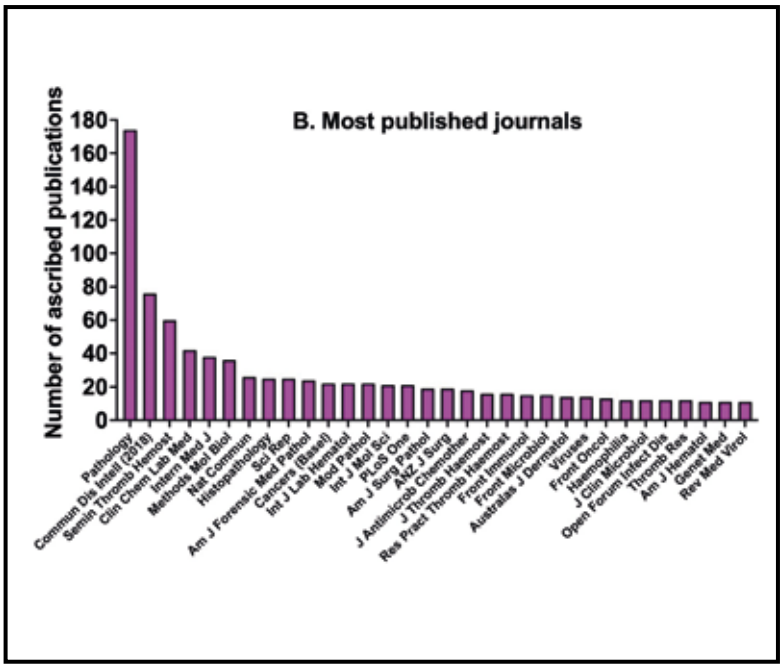


Figure 2B. *Most published journals as pertaining to data shown in Figure 2A (>10 publications).*

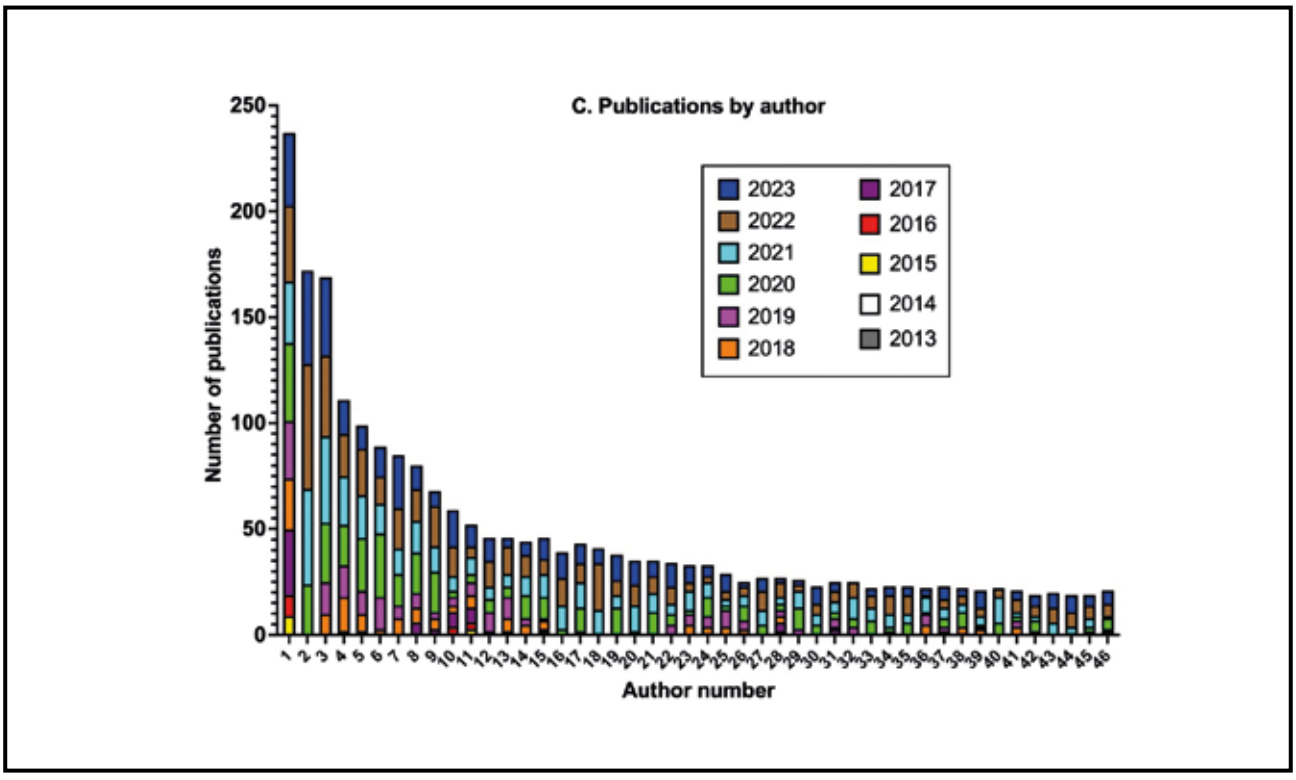


Figure 2C. *Publications ascribed to authors citing a NSW Health Pathology affiliation, as related to data in Figure 2A, and where a NSW Health Pathology affiliated author can be identified. Author identity has been kept anonymous.*

Figure 2. *Study metrics - Outcomes Part 1.*

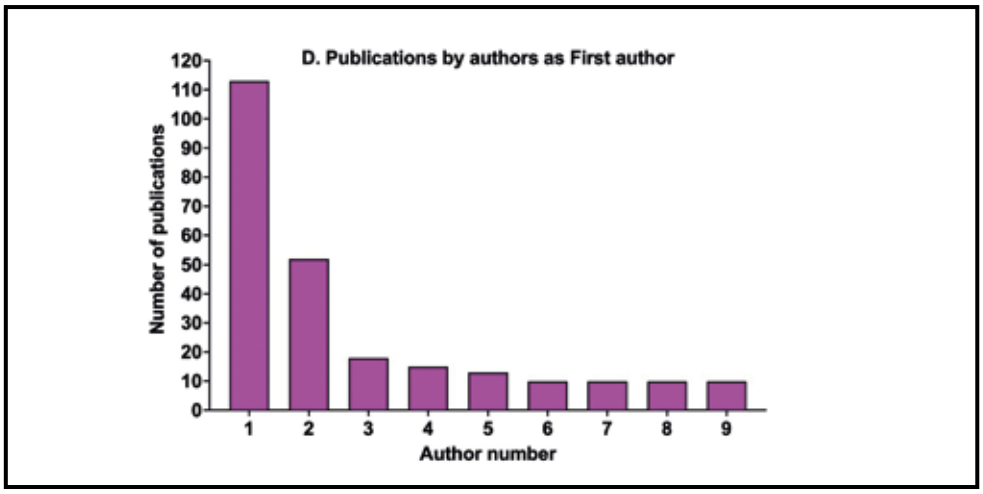


Figure 2D. *Publications ascribed to authors citing a NSW Health Pathology affiliation, as related to data in Figure 2A, and where a NSW Health Pathology affiliated author is listed as the first (or lead) author (≥10 publications). Author identity has been kept anonymous.*

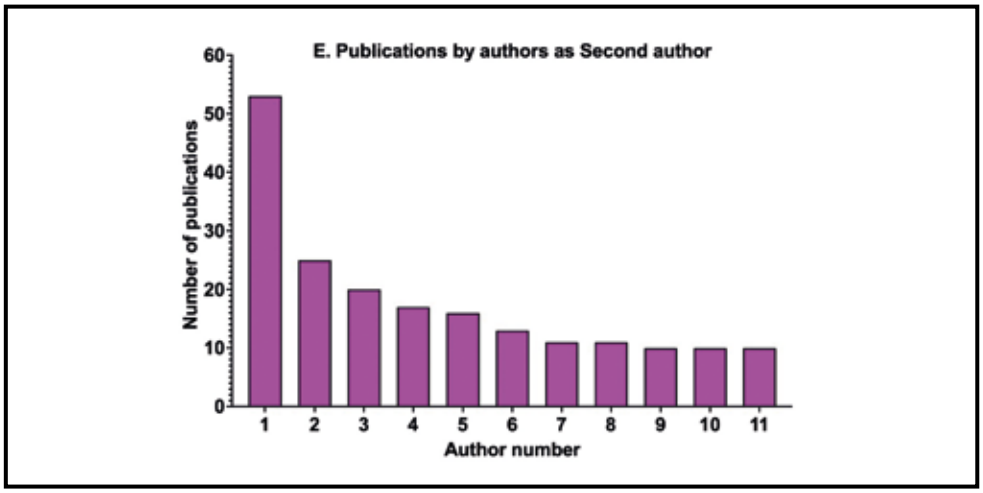


Figure 2E. *Publications ascribed to authors citing a NSW Health Pathology affiliation, as related to data in Figure 2A, and where a NSW Health Pathology affiliated author is listed as the second author (≥10 publications). Author identity has been kept anonymous.*

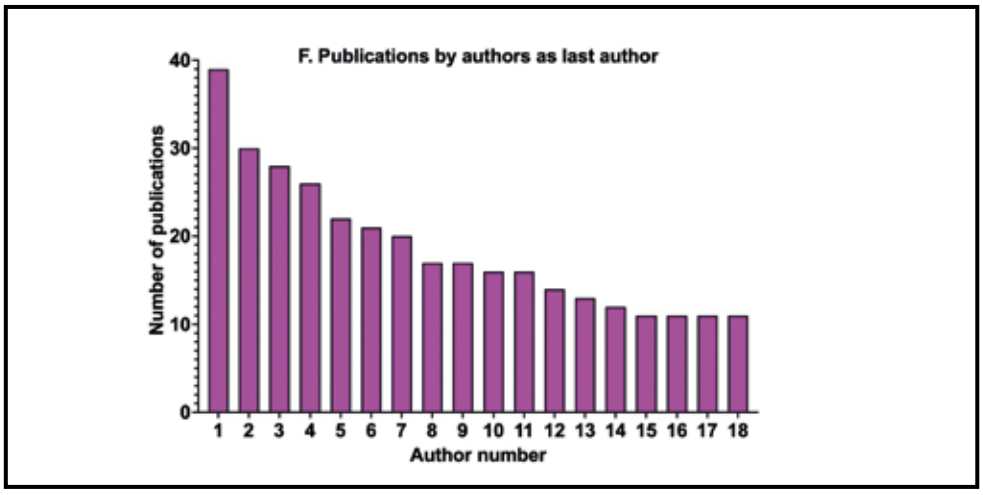


Figure 2F. *Publications ascribed to authors citing a NSW Health Pathology affiliation, as related to data in Figure 2A, and where a NSW Health Pathology affiliated author is listed as the last (or senior) author (≥10 publications). Author identity has been kept anonymous.*

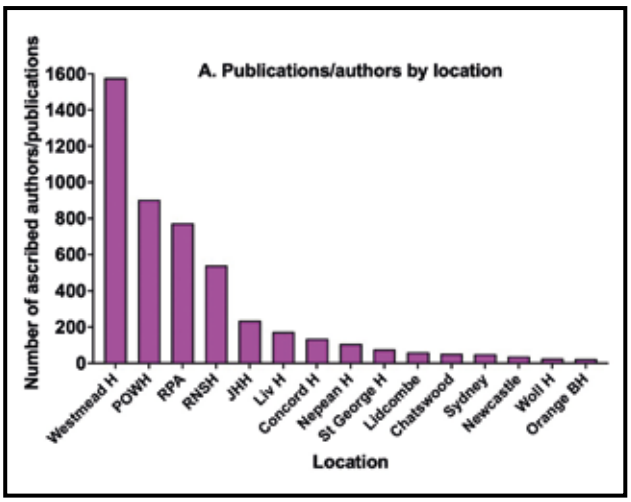


Figure 3A. *Publications/authors as ascribed to data in Figure 2A listed according to affiliation cited location (where >20 publications/authors listed).*

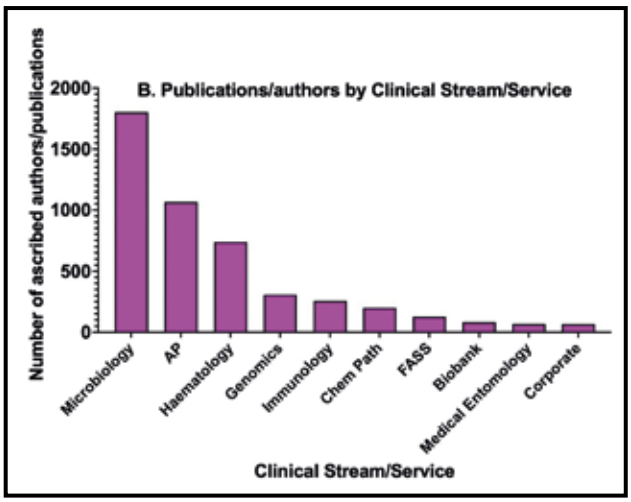


Figure 3B. *Publications/authors as ascribed to data in Figure 2A listed according to Clinical Stream or other NSW Health Pathology Service (where >60 publications/authors listed).*

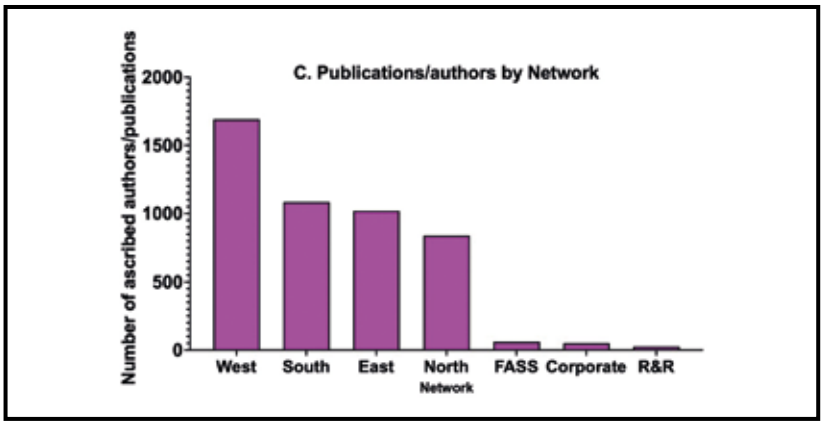


Figure 3C. *Publications/authors as ascribed to data in Figure 2A listed according to NSW Health Pathology Network Location (where >25 publications/authors listed).*

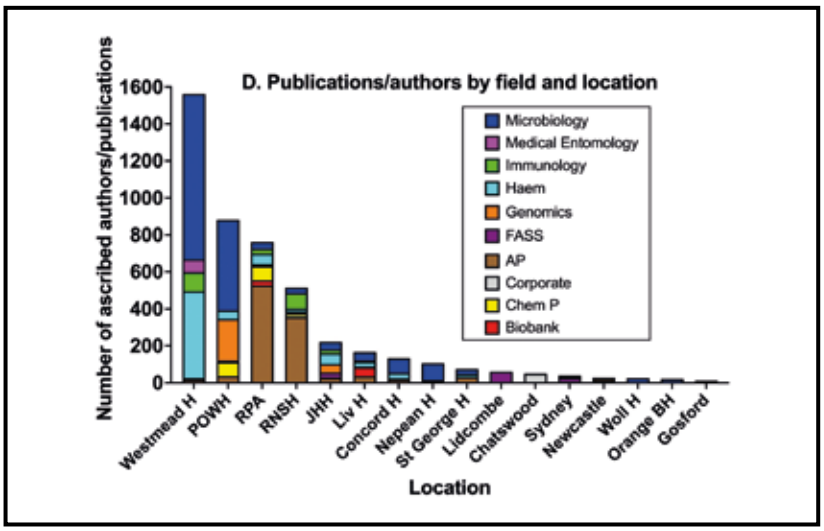


Figure 3D. *Publications/authors as ascribed to data in Figure 2A, and according to location and Clinical Stream or NSW Health Pathology Service ('field').*

Figure 3. *Study metrics - Outcomes Part 2.*

Study limitations and strengths

There are various study strengths and limitations that could be highlighted and are summarised in Table 1. The main limitation is that data available from only a single database has been assessed. Additional data bases are available and could also be assessed, assuming there is availability. On the other hand, the PubMed database is freely available, well respected, and contains in excess of 37 million citations for biomedical literature from MEDLINE, life science journals, and online books (PubMed database - US National Institute of Health (NIH) National Library of Medicine). Future assessments are planned using other databases and additional approaches when time permits.

Conclusion

To the author's knowledge, this is the most comprehensive analysis of publications arising from researchers citing a NSWHP affiliation. It highlights a broad spread of publications arising from several locations and service streams of NSWHP.

Conflict of interest statement

The author was, and remains, an employee of NSW Health Pathology during the data capture period. He has attempted to remain otherwise impartial during the analysis. The views expressed in this paper are those of the author, and not necessarily those of NSW Health Pathology or other institutions to which the author is affiliated.

Table 1. *Strengths and limitations of current study*

Study Limitation	Study Strength	Additional Comments
Assesses output from single database	PubMed database freely available, well respected, and has extensive listing. Ability to search author 'affiliations' using free text. Use of free database permits check of data veracity.	Addition databases that could be assessed in the future, but with caveats are: (a) Embase – but requires access (paywall), which author does not have (b) Google Scholar – freely available, but does not permit easy retrieval of captured data; also, difficult to filter captured data to publications associated with a particular organisation (c) Web of Science – author has access via University affiliation, but database does not list NSW Health Pathology as an Affiliation for search purposes (d) Scopus - author has access via University affiliation; although database does not list NSW Health Pathology as an organisation for 'organisation' search purposes, a free-text search of "NSW Health Pathology" is possible within an affiliation field.
Assesses publication number, but does not assess publication type or quality	Publication number is a major used metric.	Future analyses can be undertaken assessing publication type, journal impact factors, and other available metrics.
No comparison made with other 'comparable' organisations	Use of free database permits check of data veracity.	Direct comparison of publication metrics is limited given limitation of analysis to publication number without further assessment of publication type and quality metrics. Different organisations may target different journals, with different capture within any given database. Different organisations have different research focus.

References:

Favaloro EJ. Medical research in New South Wales 1993-96 assessed by Medline publication capture. Med J Aust. 1998 Dec 7-21;169(11-12):617-22.

Favaloro EJ, Mohammed S, Vong R, Pasalic L. Harmonization of Hemostasis Testing Across a Large Laboratory Network: An Example from Australia. Methods Mol Biol. 2023;2663:71-91

NSW Health Pathology Website. https://pathology.health.nsw.gov.au/. Accessed Sept 27, 2024.

NSW Health Pathology - Pathology Services. https://pathology.health.nsw.gov.au/services/pathology/. Accessed Sept 27, 2024

NSW Health Pathology - Services. https://pathology.health.nsw.gov.au/services/. Accessed Sept 27, 2024

NSW Health Pathology Clinical Services Plan 2019 – 2025. Available at: https://pathology.health.nsw.gov.au/wp-content/uploads/2022/10/Clinical-Services-Plan.pdf. Accessed Sept 27, 2024

NSW Health Pathology Research Activity Reports; 2017 to 2020 inclusive. Available at: https://pathology.health.nsw.gov.au/research/our-research/research-activity-reports/. Accessed Sept 27, 2024

PubMed database- US National Institute of Health (NIH) National Library of Medicine. Available at https://pubmed.ncbi.nlm.nih.gov. Last accessed Sept 27, 2024.

PubMed database- US National Institute of Health (NIH) National Library of Medicine Advanced Search. Available at https://pubmed.ncbi.nlm.nih.gov/advanced/. Last accessed Sept 27, 2024.

Research Strategy NSW Health Pathology Towards 2025 (available at: https://pathology.health.nsw.gov.au/research/research-strategy/. Accessed Sept 27, 2024

ORIGINAL ARTICLE

Human endogenous retroviruses – for better or worse

Robert W. Tindle [1,2]

[1]*Emeritus Professor (Immunology), Faculty of Science, University of Queensland, Brisbane, Qld*

[2]*Sir Albert Sakzewski Research Centre and Clinical Medical Virology Centre (Director, (rtd.) Royal Children's Hospital, Brisbane, Qld*

Abstract

This brief review examines the origin, and biological and medical significance of retrovirus-derived DNA sequences inserted in the human genome.

Approximately 40% of human DNA is identical or similar to viral genetic material, much of which is derived evolutionarily from human endogenous retroviruses (hERVs) and is capable of copying itself to multiple locations in the genome.

Retrovirus transposons ('retrotransposons') have contributed substantially to heritable genomic diversity in human DNA by multiple mechanisms including regulation of human gene expression and introduction of genetic instability. Retrotransposon-DNAs encoding useful functions have spread through the human population by natural selection but conversely, retrotransposons are associated with various clinical disorders.

Cumulative retrovirus-induced anarchic cellular replication may be a necessary consequence of ageing and a prelude to neoplastic disease.

Retrotransposons portend a re-evaluation of genetic control of the human phenotype and how future therapeutic strategies might be planned. Therapies for retrotransposons are in their infancy.

Key words: ***retrovirus, transposon, gene expression, human phenotype, cancer, genetic mutation, provirus, population genetics, forensics.***

Introduction

'You may have a virus' is a remark that a general practitioner might make to a patient early in the diagnostic process.

This remark could not be more true! Since the Human Genome Project in the early 2000's, scientists have been seeking to understand the significance of the approximately 40% of human DNA that is identical or similar to viral genetic material. Much of this DNA has been derived from retroviruses termed human endogenous retroviruses, (hERVs) (Mills *et al* 2007).

Origin of retrovirus derived DNA

It is likely that viruses have infected all cellular organisms since life first arose on earth. Humankind has most probably weathered numerous viral epidemics during the 6 million or so years that Homo species have walked the planet. Of course, the precise identity of the virus invaders remains unknown.

While some viral invaders would have used the same raw material, (DNA), as us to make up their genes, retroviruses use RNA for their genetic material. Retroviruses almost certainly accounted for some (possibly many) epidemics.

Human retrovirus incorporation into human DNA

Retroviruses fuse with the cell they invade via cell surface receptors, releasing into the cytoplasm viral RNA genome which uses viral reverse transcriptase enzyme to synthesise a double-stranded DNA copy of itself. Using viral integrase, this 'proviral' DNA integrates into specific sites in the host cell DNA, from whence it can be transcribed by the host cell's machinery to produce viral proteins and potentially new viral particles.

Retrovirus transposons (retrotransposons) can copy and paste (transpose) themselves within the host cell

Address correspondence to:
Robert Tindle
r.tindle@uq.edu.au

genome, but unlike retroviruses they cannot produce infectious particles, and therefore cannot spread between organisms. Millions of retrotransposons are scattered around the human genome. They may be full-length, but most are fragmented and inactive. Most are flanked by long terminal repeats (LTRs) left over from the reverse transcription process, which sometimes act as regulatory elements, influencing nearby genes. Long interspersed nuclear elements (LINEs) are autonomous retrotransposons that can transpose themselves; short interspersed nuclear elements (SINEs) (including Alu sequences) are deficient and rely on LINEs and other transposable elements for transposition.

Not all transposon-mediated DNA transposition requires retroviral involvement. Cut-and-paste transposition of DNA transposons, which encode a transposase enzyme, may occur (Hickman and Dyda 2015). Most DNA transposons are inactive and are less common than retrotransposons in the human genome but can still play a role in genetic variation and disease.

While retroviruses can insert themselves almost anywhere in the genome, there are some constraints. LTR elements, for example, favour adenine and thymine (AT) rich sequences in human DNA. The cellular machinery controlling the insertion process also determines that movement of retrotransposons is not entirely random. Furthermore, mechanisms exist (e.g. DNA methylation, histone modifications, and others (Mita *et al* 2016; Goodier 2016)) which help maintain a balance between retrotransposon activity and gene function, thereby suppressing potentially disruptive insertions within genes. Correspondingly, retrotransposons have evolved to evade cellular control mechanisms - by mutation and shuffling their DNA sequences are examples of this. Some retrotransposons exist in high copy number throughout the genome thus enhancing their chances of escape mutants arising. A balance between the host response to retrotransposition and corresponding retrotransposon evasion allows the benefits of transposon activity to exist in the host alongside its potential harms.

The human genome contains retrotransposon fragments from around 30-50 retrovirus families (Frank *et al* 2022) a fossil record, so to speak, of waves of ancestral viral epidemics. In addition, humans have inherited viral genetic material from the epidemics endured by our mammalian ancestors. The majority of retrotransposons that occur in the human genome are ancient and inactivated by mutation. Transposition of nucleic acid sequences alters the cell's genetic identity.

Influence of retrotransposons on the human phenotype

Retrotransposons have several different effects on the human genome (Elbarbary *et al* 2016). Some act as promoters or suppressors driving or turning off the expression of nearby human genes. Others can be processed into 'microRNAs' that regulate gene expression ensuring that the right genes are expressed in the right place at the right time. Some retrotransposons shuffle and recombine with other retrotransposons and/or undergo mutations. It is estimated that perhaps 25% of human gene expression is regulated in this way (Rebollo *et al* 2012). Where it has been integrated into the germline (eggs or sperm) viral genetic material has contributed substantially to heritable genomic diversity in human DNA (Cordeau and Batzer 2009).

Retrovirally derived DNA encoding for functions useful in human evolution are subject to positive natural selection; the viral-derived DNAs spread through the human population just like any other 'useful' human genes (Cordeau and Batzer 2009). Examples occur in the human placenta, the immune system, the brain and elsewhere.

On the other hand, retrotransposons are associated with various clinical disorders. Genetic variation resulting from retrotransposon activity may predispose to disease susceptibility (Singh 2007).

No retroviruses known to infect humans today actively insert new genetic material into the genome. HIV has the potential to do this but this is thought to be a relatively rare event, further reduced significantly by anti-retroviral therapy (Mahé *et al* 2020).

Overall, retrotransposons are a complex and fascinating group of genetic elements that have had a profound impact on human evolution and biology. They have played a significant role in shaping the human genome and continue to influence our biology today— for better or worse.

Examples of the influence of retrotransposons on the human phenotype

Retrotransposons contribute to genetic variability and can provide additional regulatory elements (Giménez-Orenga *et al* 2021). They can help with expression of viral proteins in the genome.

The syncytin gene has been sequestered to serve an important function in placenta physiology (Mi *et al* 2000). This gene, encoding proteins syncytin-1 and syncytin-2, is the envelope gene of the human endogenous defective retrovirus, HERV-W (Blond *et al* 1999). Syncytin is essential in multinucleate placental syncytiotrophoblasts,

derived from foetal trophoblasts which have a critical role in nutrient transport and waste exchange between the mother and foetus. Mutations in the syncytin genes lead to placental abnormalities e.g. those mutations that cause preeclampsia (Bu *et al* 2022).

Retrotransposons help to protect the body from infection in a variety of mechanisms affecting both the innate and adaptive immune systems. For example, APOBEC3G is a retrovirus-derived protein that can mutate viral RNA, making it less likely to be translated into viral proteins (Grandi and Tramontano 2018; Salter *et al* 2019) and retrotransposon - derived HLA-C presents viral peptides to T cells (Di Marco *et al* 2017), a prelude to T-cell mediated killing of infected cells. Retrotransposons have contributed to the formation of the HLA class 1 family of genes (Dawkins *et al* 1999; Doxiadis *et al* 2008) who's polymorphic immunohaplotypes are the basis of the adaptive immune system.

Retrotransposons are expressed at high levels in the developing brain regulating the expression of genes that are involved in brain development (Ferrari *et al* 2021). Retrotransposons also contribute to the formation of neural networks and promote the survival of neurons.

There are further numerous instances of 'useful' viral DNA in the human genome (Ryan 2004).

The consequences however of retrotransposon activity in the genome are not always beneficial. Proteins produced by retrotransposon activation may be perceived as 'foreign' by the immune system leading to autoimmune diseases (Alcazer *et al* 2020). Retrotransposons are associated with a number of autoimmune and inflammatory diseases e.g. multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjögren syndrome (SS) (Gröger and Cynis 2018).

Retrotransposons may activate oncogenes by multiple mechanisms, including disrupting tumour suppressor genes, inducing chromosome breakages, transcription of chimeric proteins (Jansz and Faulkner 2021; McKerrow *et al* 2022) and generally causing genomic instability (Mills *et al* 2007). The outcome is dysplastic/neoplastic cell proliferation. Retrotransposon-driven activation is known to occur in some twenty types of cancer (McEwen 2021). Retrotransposon-envelope proteins function as tumour-specific antigens which elicit cytotoxic T-cell responses in patients with various types of cancer (Rycaj *et al* 2015).

Retrotransposon-induced anarchic cellular replication may be a necessary consequence of ageing (Liu *et al* 2023a), and the inevitable consequence of being a complex multicellular organism (Smith 2023; Breivik 2023).

An emphasis is needed in basic research and drug development to include the concept of retrotransposon-induced neoplasia. Not all cancers occur of course because of retroviral induced replicative dysfunction of cells. Oncogenes can be activated via other exogenous and endogenous pathways in which direct retrotransposon-involvement has not been reported (Jang *et al* 2019.

Retrotransposons have also been implicated in some neurodegenerative diseases; Alzheimer's disease, Parkinson's disease, and MS (Liu *et al* 2023a; Römer 2021; Licastro and Porcellini 2021; Sankowski *et al* 2019) by promoting brain inflammation, disrupting the expression of genes involved in neuronal survival, and promoting the formation of protein aggregates (Küry *et al* 2018).

Putative retrotransposon-associated disruption of brain development and interference with neurotransmitter signalling (Küry *et al* 2018) is implicated in schizophrenia, bipolar disorder, and major depressive disorder.

Retrotransposons are also reported to have a modest effect on cognitive function through chronic inflammation of the brain inducing their activation during the aging process and leading to cognitive decline (Sankowski *et al* 2019).

There is evidence that quiescent retrotransposons in the genome can be reactivated by adventitious viral infections e.g. influenza A virus, hepatitis C virus (HCV), severe acute respiratory syndrome coronavirus-2 (SARSCoV-2); herpes simplex virus type-1 (HSV) and HIV (Li *et al* 2023b).

For humans, porcine ERVs (P retrotransposons) illustrate a concern when using porcine tissues and organs in xenotransplantation (Galka *et al* 2022). P retrotransposons can integrate into the human genome (and be passed on to the recipients' offspring) with possibly dangerous consequences.

Most transposons have accumulated mutations or deletions over time that render them incapable of producing infectious virions. While some retain the necessary genes and regulatory elements to express the viral proteins needed for virion assembly and release, their activity is suppressed by the host cell preventing widespread replication and infection.

Some retroviruses capable of producing infection have acquired genes from their hosts during their evolution (Ploegh 1998). Those captured genes whose products provide a selective advantage to the virus spread through the virus population and furnish a mechanism of human gene horizontal transfer. The clinical relevance, if any, of horizontal gene transfer through retroviruses is unclear.

A paradigm shift in thinking

It is fair to say that the human species could not exist without its quota of transposable elements of viral origin. The idea that every one of us has virus genes irreversibly embedded in our DNA is challenging enough, but that the virus genes influence who we are and what might befall us is existentially daunting. The traditional mindset that human providence in all its complexity is read from a set of inherited exclusively 'human' genes expressed in a predetermined way requires re-examination in the light of what contemporary retroviral research tells us. The concept that our DNA is the set-in-stone masterplan for our metabolic and physical outcome needs reappraisal. 'Human' genes must at least in part delegate control to a retrotransposon-influence that confers change on cells and tissues.

The fluidity in timing and location of gene transcription, the interaction among transcription initiation events and mutations incurred by retrotransposon (and other transposon) activity, illustrates a decentralisation of control of phenotypic outcomes at many levels.

Looking forward

What can clinical practice do to curb our bodies being usurped by harmful retrotransposons in our genomes? Not a lot at present apart from addressing the medical conditions the retrotransposons produce. Therapies for retrotransposons are in their infancy. Researchers are trying several routes e.g. reverse transcriptase inhibitors to block retrovirus integration and replication, genetic techniques (including CRISPR/Cas9) to disable retrotransposons, and immunotherapies to boost the immune response to retrotransposon-infested cells.

Transposons may eventually be helpful as novel biomarkers for diagnosis, prognosis, and prediction of response to therapy, particularly those which are detected in body fluids and therefore are easily accessible (Giménez-Orenga and Oltra 2021).

One might envisage injectable transposon elements as having a role as therapeutic 'bullets'.

LINES, SINES, and especially Alu elements inserted in the genome at sites and copy number specific for a given individual, provide a genetic 'fingerprint' which is passed down to the descendants of that individual. This gives a useful population genetics tool for tracing ancestral lineages and migration events.

Similarly, analysis of Alu sequence location and polymorphism in DNA samples from crime scenes can help exclude, or at least narrow down, suspects (though short tandem DNA repeats are more reliable forensic markers).

An understanding of retroviruses is in its infancy. Continuing research will likely provide further insights into human evolution and disease mechanisms.

Meanwhile, retroviruses provide an important vector in the human gene therapy clinic, particularly for monogenic diseases (Kurian *et al* 2000; Bulcha *et al* 2021).

References

Alcazer V, Bonaventura P, Depil S 2020. Human Endogenous Retroviruses (HERVs): Shaping the Innate Immune Response in Cancers. *Cancers (Basel)*: 12 (3) 610. doi:10.3390/cancers12030610. PMC 7139688. PMID 32155827. Accessed July 1, 2024

Blond J L, Besème F, Duret L, Bouton O, Bedin F, Perron H, Mandrand B, Mallet F 1999. Molecular characterization and placental expression of HERV-W, a new human endogenous retrovirus family. *J Virol* 73, 1175–1185 DOI: 10.1128/JVI.73.2.1175-1185.1999 Accessed February 8, 2024

Breivik J, 2023. Making Sense of cancer. Publisher: River Grove Books ISBN: 978-1-63299-761-6 (Paperback) ISBN: 978-1-63299-762-3 (eBook)

Bu C, Wang Z, Ren Y, Chen D, Jiang, S-W 2022. Syncytin-1 nonfusogenic activities modulate inflammation and contribute to preeclampsia pathogenesis. *Cell Mol Life Sci* 79(6):290 DOI: 10.1007/s00018-022-04294-2 Accessed February 8, 2024

Bulcha JT, Wang Y, Ma H, Tai PWL, Gao G. 2021.Viral vector platforms within the gene therapy landscape. *Sig Transduct Target Ther* 6 (53) https://doi.org/10.1038/s41392-021-00487-6 Accessed February 8, 2024

Cordaux R, Batzer, M. 2009. The impact of retrotransposons on human genome evolution. *Nat Rev Genet* 10, 691–703 https://doi.org/10.1038/nrg2640 Accessed February 8, 2024

Dawkins R, Leelayuwat C, Gaudieri S, Tay G, Hui J, Cattley S, Martinez P, Kulski J. 1999 Genomics of the major histocompatibility complex: haplotypes, duplication, retroviruses and disease. *Immunological Reviews* 167; 275–304. doi: 10.1111/j.1600-065x.1999.tb01399x.PMID: 10319268 R

Di Marco M, Schuster H, Backert L, Ghosh M, Rammensee H-G, Stevanović S 2017. Unveiling the Peptide Motifs of HLA-C and HLA-G from Naturally Presented Peptides and Generation of Binding Prediction Matrices. *J Immunol*: 199 (8): 26392651. doi:10.1111/j.1600065X.1999.tb01399.x. PMID 10319268. S2CID 9924684.https://doi.org/10.40/jimmunol.1700938 Accessed February 8, 2024

Doxiadis GG, de Groot N, Bontrop RE 2008. Impact of Endogenous Intronic Retroviruses on Major Histocompatibility Complex Class II Diversity and Stability. *J Virol*: 82 (13) 6667–6677. doi:10.1128/JVI.00097-08. PMC 2447082. PMID 18448532 Accessed February 8, 2024

Elbarbary RA, Lucas BA, Maquat LE . 2016 Retrotransposons as regulators of gene expression. Science. 351(6274):aac7247. doi: 10.1126/science.aac7247. Epub 2016 Feb 11. PMID: 26912865; PMCID: PMC4788378. Accessed July 7, 2024

Ferrari R, Grandi N, Tramontano E, Dieci G. 2021 Retrotransposons as Drivers of Mammalian Brain Evolution. *Life (Basel)*: 11(5):376.

Frank JA, Singh M, Cullen HB, Kirou RA, Benkaddour-Boumzaouad M, Cortes JL, Garcia Pérez J, Coyne CB, Feschotte C. 2022. Evolution and antiviral activity of a human protein of retroviral origin. *Science*. 378(6618):422-428. PMID: 36302021; PMCID: PMC10542854 https://doi.org/10.1126/science.abq7871 Accessed February 8, 2024 https://doi.org/10.3390/life11050376 Accessed February 8, 2024

Gałka S, Nowak E, Bednarek I 2022. Selective human cells in vitro transduction with porcine endogenous retrovirus (PERV). *Acta Virol*: 66(2) 110-126. https://doi.org/10.4149/av_2022_201 Accessed February 8 2024

Giménez-Orenga K, Oltra E 2021. Human Endogenous Retrovirus as Therapeutic Targets in Neurologic Disease. *Pharmaceuticals (Basel)*: 14(6):495. DOI: 10.3390/ph14060495 Accessed February 8 2024

Goodier JL 2016 Restricting retrotransposons: a review. 7:16 Mob DNA https://doi.org/10.1186/s13100-016-0070-z Accessed July 7 2024

Grandi N, Tramontano E 2018. Human Endogenous Retroviruses Are Ancient Acquired Elements Still Shaping Innate Immune Responses. *Front Immunol:* 10(9) 2039. DOI: 10.3389/fimmu.2018.02039 Accessed February 8, 2024

Gröger V, Cynis H 2018 Human Endogenous Retroviruses and Their Putative Role in the Development of Autoimmune Disorders Such as Multiple Sclerosis. *Front Microbiol:* 9 265. doi:10.3389/fmicb.2018.00265. PMC 5826199. PMID 29515547. Accessed February 8, 2024

Hickman AB, Dyda F 2015. Mechanisms of DNA Transposition. *Microbiol Spectr:* 3(2):MDNA3-0034-2014 PMID: 26104718; PMCID: PMC7422641. Accessed February 8, 2024

Jang HS, Shah NM, Du AY 2019. Transposable elements drive widespread expression of oncogenes in human cancers. Nat Genet 51(4):611-617. https://doi.org/10.1038/s41588-019-0373-3 Erratum in: *Nat Genet*. May;51(5):920. PMID: 30926969; PMCID: PMC6443099. Accessed February 8, 2024

Jansz N, Faulkner, GJ 2021. Endogenous retroviruses in the origins and treatment of cancer. *Genome Biol* 22,147 https://doi.org/10.1186/s13059-021-02357-4 Accessed February 8, 2024

Kurian KM, Watson CJ, Wyllie AH 2000. Retroviral vectors. *Mol Pathol*: 53(4):173-6 PMID: 11040938; PMC1186965 DOI: 10.1136/mp.53.4.173 Accessed February 8, 2024

Küry P, Nath A, Créange A, Dolei A, Marche P, Gold J, Giovannoni G , Hartung H-P, Perron H 2018. Human Endogenous Retroviruses in Neurological Diseases. *Trends Mol Med* 24/4, 379-394. DOI:https://doi.org/10.1016/j.olmed.2018.02.007 Accessed February 8, 2024

Licastro F, Porcellini E 2021. Activation of Endogenous Retrovirus, Brain Infections and Environmental Insults in Neurodegeneration and Alzheimer's Disease. *Int J Mol Sci* 22(14):7263. https://doi.org/10.3390/ijms22147263 Accessed February 8, 2024

Liu X, Liu Z, Wu, Z (plus 32 others) 2023a. Resurrection of endogenous retroviruses during aging reinforces senescence. Cell 186 (2): 287–04.e26. doi:10.1016/j.cell.2022.12.017. ISSN 0092-8674. PMID 36610399. S2CID 232060038. Accessed February 8, 2024

Liu S, Heumüller SE, Hossinger A, Müller S, Buravlova O, Lichtenthaler SF, Denner P, Vorberg IM 2023b. Reactivated endogenous retroviruses promote protein aggregate spreading. *Nat Commun*;14(1):5034. https://doi.org/10.1038/s41467-023-40632-z Accessed February 8, 2024

Mahé D, Matusali G, Deleage C, Alvarenga RLLS, Satie AP, Pagliuzza A, Mathieu R, Lavoué S, Jégou B, de França LR, Chomont N, Houzet L, Rolland AD, Dejucq-Rainsford N 2020. Potential for Virus Endogenization in Humans through Testicular Germ Cell Infection: the Case of HIV. J Virol. 94(24):e01145-20. PMID: 32999017 PMC7925188 DOI: 10.1128/JVI.01145-20 Accessed February 12, 2024

McEwen GK, Alquezar-Planas DE, Dayaram A, Gillett A, Tarlinton R, Mongan N, Chappell KJ, Henning J, Tan M, Timms P, Young PR, Roca AL, Greenwood AD 2021. Retroviral integrations contribute to elevated host cancer rates during germline invasion. *Nat Commun* 12(1):1316. https://doi.org/10.1038/s41467-021-21612-7 Accessed February 9, 2024

McKerrow W, Wang X, Mendez-Dorantes C, Fenyö 2022. D LINE-1 expression in cancer correlates with p53 mutation, copy number alteration, and S phase checkpoint. PNAS 19 (8) e2115999119https://doi.org/10.1073/pnas.2115999119, Accessed February 9, 2024

Mi S, Lee X, Li, XP, Finnerty H, Racie L, LaVallie E, Tang XY, Edouard P, Howes S, Keith JC Jr, McCoy JM 2000. Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. *Nature* 403, 785–89 https://doi.org/10.1038/35001608 Accessed February 9, 2024

Mills RE, Bennett EA, Iskow RC. Scott E Devine 2007. Which transposable elements are active in the human genome?. *Trends in Genetic* 23 (4): 183-91. doi:10.1016/j.tig.2007.02.006. PMID 17331616. Accessed February 9, 2024

Mita P, Boeke JD. How retrotransposons shape genome regulation. Curr Opin Genet Dev. 2016 Apr;37:90-100. doi: 10.1016/j.gde.2016.01.001. Epub 2016 Feb 6. PMID: 26855260; PMCID: PMC4914423. Accessed July 7, 2024

Ploegh HL. Viral strategies of immune evasion 1998. Science, 280 248–253 DOI: 10.1126/science.280.5361.248 Accessed February 9, 2024

Rebollo R, Romanish MT, Mager DL. Transposable elements: an abundant and natural source of regulatory sequences for host genes. Annu Rev Genet. 2012;46:21-42. doi: 10.1146/annurev-genet-110711-155621. Epub 2012 Aug 16. PMID: 22905872. Accessed February 9, 2024

Römer C 2021. Viruses and Endogenous Retroviruses as Roots for Neuroinflammation and Neurodegenerative Diseases. *Front. Neurosci* 15 https://doi.org/10.3389/fnins.2021.648629 Accessed February 9, 2024

Ryan FP 2004 Human endogenous retroviruses in health and disease: a symbiotic perspective. J R Soc Med.; 97(12):560-5 https://doi.org/10.1177/014107680409701202 Accessed February 9, 2024

Rycaj K, Plummer JB, Yin B, Li M, Garza J, Radvanyi L, Ramondetta LM, Lin K, Johanning GL, Tang DG, Wang-Johanning F 2015. Cytotoxicity of human endogenous retrovirus K-specific T cells toward autologous ovarian cancer cells. *Clin Cancer Res* 21 (2) 471–483. https://doi.org/10.1158/1078-0432.CCR-14-0388 Accessed February 9, 2024

Salter JD, Polevoda B, Bennett RP, Smith, HC 2019. Regulation of Antiviral Innate Immunity Through APOBEC Ribonucleoprotein Complexes. In: Harris, J., Marles-Wright, J. (eds) *Macromolecular Protein Complexes II: Structure and Function*. *Subcellular Biochemistry* 93. Springer, Cham. https://doi.org/10.1007/978-3-030-28151-9_6. Accessed February 9, 2024

Sankowski R, Strohl JL, Huerta TS, Nasiri E, Mazzarello AN, D'Abramo C, Cheng KF, Staszewski O, Prinz M, Huerta PT, Al-Abed Y 2019. Endogenous retroviruses are associated with hippocampus-based memory impairment. PNAS 116 (51) 25982-25990. PMID: 31792184 PMCID: PMC6925997 DOI: 10.1073/pnas.1822164116 Accessed February 9, 2024

Singh SK 2007. Endogenous retroviruses: suspects in the disease world. *Future Microbiology* 2 (3): 269–275. doi:10.2217/17460913.2.3.269. PMID 17661701 Accessed February 9, 2024

Smith R 202., What is the meaning of cancer? BMJ 383 2510 doi: https://doi.org/10.1136/bmj.p2510 Accessed February 9, 2024

Preferred terminology for the description of 'ripped erythrocytes': good professional practice for the Australian Standard AS ISO 15189:2023 accredited medical laboratory

Dennis Mok[1], Rana Nabulsi[2], Sharfuddin Chowdhury[3], Naira Eloyan[4], Sharita Meharry[5], Georgi Koutin[6], Tai The Diep[7], María del Rocío González Guerrero[8], Cilia Maria Katharina Sindt[9], Arisina Chung Yee Ma[10], Donna Marie Gillespie[11], Larysa Sokolova[12]

[1]*Medical Management Consulting, Birkdale, Queensland, Australia*

[2]*Department of Laboratory Medicine and Pathology, Dubai Health, Dubai, United Arab Emirates*

[3]*King Saud Medical City, Riyadh, Saudi Arabia*

[4]*Armenian Medicinal Products Regulatory Authority, Ministry of Health, Yerevan, Armenia*

[5]*School of Science, Faculty of Health and Environmental Sciences, Auckland University of Technology, Auckland, New Zealand*

[6]*Role 2 Base Medical Treatment Facility, Camp Film City, Pristina, Kosovo*

[7]*School of Biosciences, Faculty of Health and Medical Sciences, University of Surrey, Guildford, United Kingdom*

[8]*Rio Tinto Hospital, Huelva, Spain*

[9]*University College South Denmark, Esbjerg, Denmark*

[10]*Queen's Medical Centre, Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom*

[11]*Chicago, United States*

[12]*Kyiv City Maternity Hospital No. 1, Kyiv, Ukraine*

The contemporary issue in describing ripped erythrocytes

In the medical laboratory accredited to Australian Standard AS ISO 15189:2023 all examination reports must contain accurate results with unambiguous interpretation, as specified in Subclause 7.4.1.1 a). This can act as both a preventive and a protective tool to minimise risks to serious adverse healthcare events. The medical laboratory supports this objective by providing reporting terms that are in alignment with current good practice. This also applies to light microscopic analysis of erythrocytes in peripheral blood smears (Figure 1 and Figure 2). The terms used for 'ripped erythrocytes' vary: three different terms are used by national and international organisations; and ten terms are used by authoritative or established textbooks (n = 22) (Table 1). This technical note aims to provide a justified recommendation for the AS ISO 15189:2023 accredited medical laboratory.

Address correspondence to:
Sharfuddin Chowdhury
Email: s.chowdhury@ksmc.med.sa

Declared descriptions from international and national organisations

Ripped erythrocytes are currently termed differently by six relevant organisations: three international and three national organisations. The terms include 'cell fragment', 'fragments', and 'schistocyte(s)'. The Regenstrief Institute Logical Observation Identifiers, Names and Codes (LOINC) Committee in the United States, which develops terminologies for medical laboratories, has termed ripped erythrocytes as 'fragments' and 'schistocytes' in the latest LOINC version (Version 2.78) (Baorto *et al* 2022). The LOINC codes are used as the preferred terminology in examination reporting according to the Royal College of Pathologists of Australasia (RCPA) best practice guideline (RCPA 2024a). It is important to note that the terms 'fragments' and 'schistocytes' are not in the preferred term list in Standards for Pathology Informatics in Australia (SPIA), therefore they cannot be found in the RCPA SPIA Haematology and Transfusion Medicine Reference Set (Version 4.0) (RCPA 2024b). The RCPA Quality Assurance Programs, which operates independently from but in alignment with the RCPA, released a guidance document

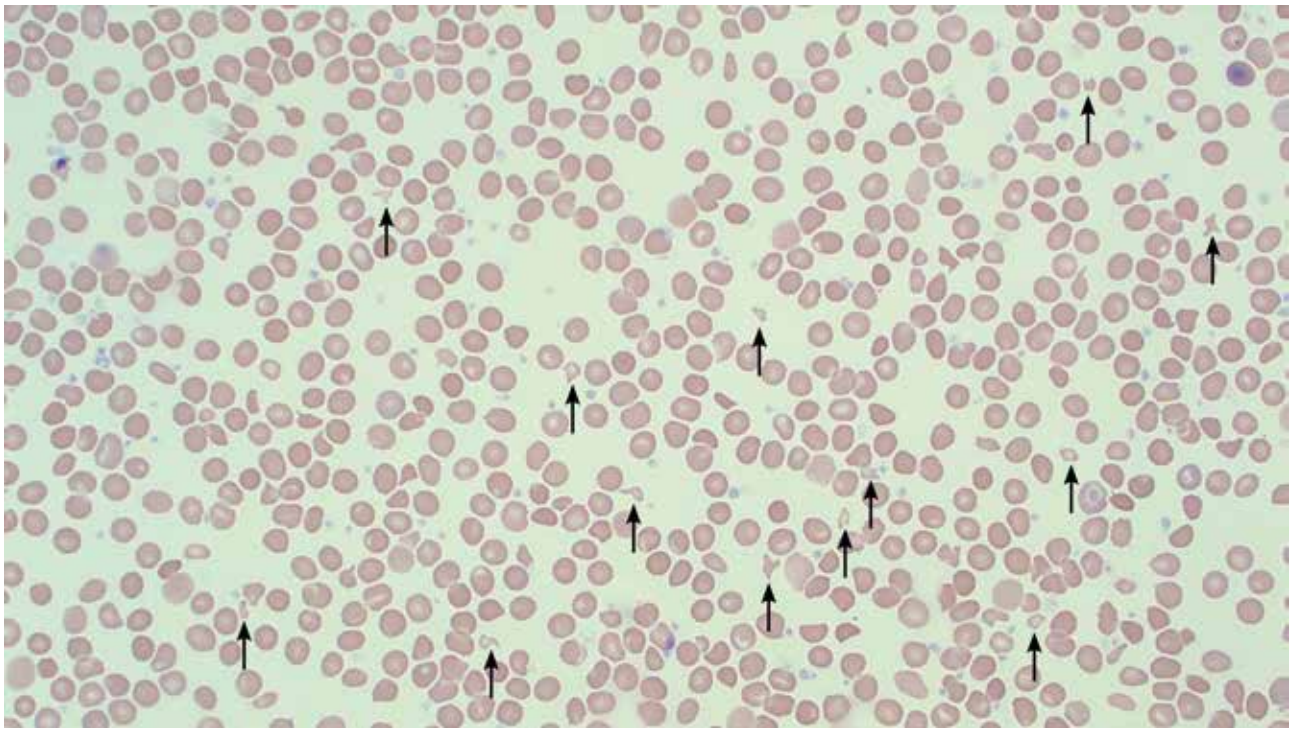


Figure 1. Light microscopic analysis of schistocytes (magnification of 1000×). *A case of third-degree burn showing the presence of schistocytes (indicated by arrows). Schistocytes are ripped erythrocytes produced by a microangiopathic process. Wright-stained peripheral blood smear at pH 6.8 (Neill 2019).*

on erythrocyte nomenclature (RCPA Quality Assurance Programs 2004) and the RCPA Quality Assurance Programs-preferred term is 'schistocyte'. The New Zealand Institute of Medical Laboratory Science (NZIMLS), which is the professional body for medical laboratory professionals in New Zealand, has released a guidance document for reporting of blood film morphology and the NZIMLS-preferred term is 'cell fragment' (NZIMLS 2011). The Subcommittee on Nomenclature for Properties and Units (NPU) of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Committee on Nomenclature, Properties and Units and the International Union of Pure and Applied Chemistry (IUPAC) Human Health Division released the IFCC-IUPAC coding system named 'NPU Terminology' to support the communication of examination results (Petersen *et al* 2012; Hansen *et al* 2023). The international version of NPU Terminology is distributed by the Danish Health Data Authority; thus, the NPU Terminology-preferred term is 'schistocytes'. Finally, the International Council for Standardization in Haematology (ICSH) Committee on Standardization of Peripheral Blood Cell Morphology has published recommendations for the standardisation of nomenclature of peripheral erythrocyte morphological features and the ICSH-preferred term is 'schistocyte' (Palmer *et al* 2015). In summary, there are three options available for the description of ripped erythrocytes.

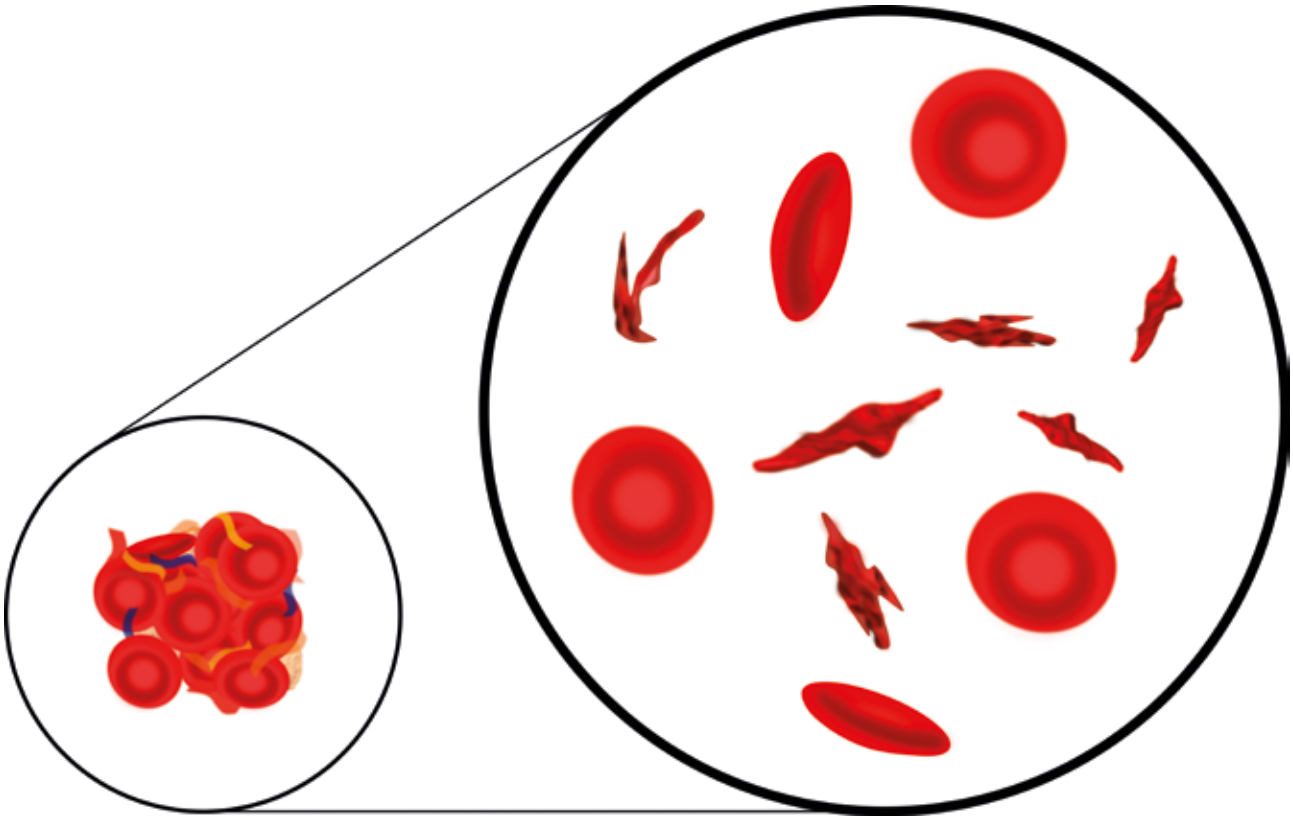
Analysis of justified courses of action

Course of action 1 (COA 1)

The medical laboratory adopts the nomenclature recommended by the RCPA by using LOINC-preferred terms and thus uses the terms 'fragments' and 'schistocytes'. COA 1 is in partial alignment with the medical laboratory's commitment to good professional practice, as specified in Clause 5.5 a) of AS ISO 15189:2023. The implementation of COA 1 fulfils the RCPA recommendations.

Course of action 2 (COA 2)

The medical laboratory adopts the nomenclature recommended by the RCPA selectively and thus uses the term 'schistocytes' only. COA 2 supports the nomenclature recommended by the ICSH. The implementation of COA 2 is in alignment with the International Laboratory Accreditation Cooperation mutual arrangement to which the National Association of Testing Authorities, Australia is a current signatory. COA 2 fulfils the RCPA best practice guideline by using the LOINC-preferred term and the ICSH recommendation by using ICSH-preferred term of 'schistocyte'. Haematology reports issued by the medical laboratory that adopts COA 2 by referring to 'schistocytes' are internationally identifiable and in full



a) *Thrombi in blood vessels contain tough fibrin filaments that can damage erythrocytes by fracturing the fragile erythrocyte membrane.*

b) *Schistocytes are presented in the peripheral blood until they undergo haemolysis or removal by macrophages in the spleen.*

Figure 2. The formation of schistocytes from passing across filaments of fibrin in thrombi. *Schistocytes can be formed when normal erythrocytes are cytoskeletally fractured by fibrin filaments in damaged and sclerosed small blood vessels.*

alignment with the medical laboratory's commitment to good professional practice, as specified in Clause 5.5 a) of AS ISO 15189:2023.

Implications for medical laboratory professionals

Medical laboratory professionals must be able to identify such 'ripped erythrocytes' and should report them as 'schistocytes', not 'fragments' in routine light microscopic analysis of peripheral blood smears. The presence of schistocytes is associated with microangiopathic haemolytic anaemia that can result from the following potential life-threatening disorders (Rozenberg 2024).

Thrombotic thrombocytopenic purpura (TTP) — Eli Moschcowitz first described TTP at the Beth Israel Hospital in 1924 (Moschcowitz 1924). TTP is a rare and life -threatening condition. TTP is considered a haematologic emergency due to the mortality rate at ≤ 20% even with appropriate treatment. The consumption coagulopathy and the partial or complete obstruction of the vessel lumina by microthrombi result in a microangiopathic haemolytic anaemia. The diagnosis of TTP can be challenging due to the significant overlap of the clinical presentation with other thrombotic microangiopathies. Most cases of TTP are acquired and are due to the deficiency of liver-derived ADAMTS13 von Willebrand factor-cleaving protease. TTP associated with anti-ADAMTS13 immunoglobulins is characterised by severe functional deficiency of ADAMTS13 and associated with transient specific anti-ADAMTS13 immunoglobulins. TTP associated with genetic deficiency of ADAMTS13 is characterised by homozygous or compound heterozygous mutation in the ADAMTS13. Overall, the lack of ADAMTS13 activity results in microthrombi formation and end-organ damage (Noris *et al* 2024). TTP is often associated with the pentad

Table 1. Terms used in the description of 'ripped erythrocytes' in authoritative or established textbooks.

Terms (*n* = 10)	**Textbook titles** (*n* = 22)	**References** (*n* = 22)
Cell fragment(s)	*Hoffbrand's essential haematology*	Hoffbrand *et al* 2024
Comet cell(s)	*Wintrobe's atlas of clinical hematology*	Hayden 2017
Fragmented red blood cell(s)	*Atlas of diagnostic hematology*	Bansal and Crane 2020
Fragmented red cell(s)	*Clinical hematology and fundamentals of hemostasis*	Harmening *et al* 2024
	Color atlas of clinical hematology: molecular and cellular basis of disease	Hoffbrand *et al* 2019
	Color atlas of hematology: an illustrated field guide based on proficiency testing	Bhargava *et al* 2018
	Haematology	Hay *et al* 2023
	Williams manual of hematology	Lichtman *et al* 2022
Fragment cell(s)	*Clinical hematology and fundamentals of hemostasis*	Harmening *et al* 2024
	Color atlas of hematology: an illustrated field guide based on proficiency testing	Bhargava *et al* 2018
	Haematology case studies with blood cell morphology and pathophysiology	Singh *et al* 2017
Red blood cell fragment(s)	*Wintrobe's atlas of clinical hematology*	Hayden 2017
	100 cases in clinical pathology and laboratory medicine	Shamil *et al* 2023
Red cell fragment(s)	*Clinical haematology: illustrated clinical cases*	Mehta and Gomez 2017
	Clinical hematology and fundamentals of hemostasis	Harmening *et al* 2024
Schistocyte(s)	*Atlas of hematopahology with 100 case studies*	Naeim 2024
	Blood cells: a practical guide	Bain 2021
	Clinical hematology and fundamentals of hemostasis	Harmening *et al* 2024
	Clinical hematology atlas	Carr 2021
	Guide to paediatric haematology morphology	Rozenberg 2024
	Haematology	Hay *et al* 2023
	Haematology and the Asian patient: 51 clinical cases	Leung and Ma 2024
	Hematology: 101 morphology updates	Bain 2023
	Hematology board review: blueprint study guide and Q&A	Reynolds and Ahmed 2023
	Lanzkowsky's manual of pediatric hematology and oncology	Niss and Quinn 2021
	Rodak's hematology: clinical principles and applications, seventh edition	Manchanda 2024
	Wintrobe's atlas of clinical hematology	Hayden 2017
	Wintrobe's clinical hematology	Smock 2023
Schizocyte(s)	*Williams hematology: the red cell and its diseases*	Narla 2021
Triangular cell(s)	*Wintrobe's atlas of clinical hematology*	Hayden 2017

of fever, thrombocytopenia, transient neurologic deficits, and acute kidney injury (Kumar *et al* 2022; Harmening *et al* 2024).

Haemolytic uraemic syndrome (HUS) — Conrad von Gasser first described HUS in 1955 (Gasser *et al* 1955). HUS is a rare but serious illness that can be life-threatening. HUS is also associated with thrombocytopenia, but is distinguished from TTP by the absence of neurologic symptoms and the dominance of acute kidney injury. HUS is a condition that mainly affects children younger than five years, and approximately 90% of cases are associated with infection by Shiga toxigenic *Escherichia coli* (STEC) and less frequently with sepsis by *Shigella dysenteriae*. *Escherichia coli* O157:H7 remains the most common type of STEC (Kumar *et al* 2022; Noris *et al* 2024). *Escherichia coli* O157:H7 contains shigatoxin-1 or shigatoxin-2 or both and the mode of action involves binding to the intestinal mucosa and subsequence release of a *N*-glycosidase ribosome-inactivating enzyme that cleaves a specific adenine from 28S RNA resulting in gastroenteritis (Kozlovac and Hawley 2017).

Disseminated intravascular coagulation (DIC) — Dupuy first described pathological observations related to DIC in 1834 (Levi and van der Poll 2014). DIC is a life-threatening syndrome characterised by systemic disseminated and often uncontrolled activation of coagulation and results in the formation of thrombi throughout the microcirculation (Papageorgiou *et al* 2018). DIC occurs as a complication of a wide variety of conditions. It is most often associated with sepsis, trauma, organ destruction, malignancy, obstetrical calamities, vascular abnormalities, liver disease, and severe toxic or immunological reactions. In the final stage of DIC, the consumption coagulopathy contributes to the haemostatic failure (Kumar *et al* 2022; Harmening *et al* 2024).

HELLP syndrome — Louis Weinstein first described the HELLP syndrome in 1982 in pregnant patients (Weinstein 1982). HELLP syndrome is associated with 'haemolysis, elevated liver enzymes and low platelet count' and is a type of pregnancy-associated thrombocytopenia. HELLP syndrome is a rare life-threatening pregnancy complication associated with eclampsia that mainly affects blood and the liver (Harmening *et al* 2024).

Aortic valve disease by Marfan syndrome (MFS) — Antoine Bernard and Jean Marfan first described the MFS in 1896 in a patient with arachnodactyly (Khan 2011). MFS is an autosomal dominant-inherited multi-systemic connective tissue disorder. Myxomatous degeneration of the mitral (left atrioventricular) valve is a common feature of MFS which can be life-threatening (Loukas 2020). MFS is associated with the deficient production of fibrillin-1 from the *FBN1* that is an essential component of microfibrils and is a major constituent of microfibrils that form elastic fibres in tissues of the middle wall of the aorta and arteries. This type of aortic disease causes aortic valve regurgitation (Neill 2017; Mitchell 2022).

Implications for medical laboratory internal auditors

The medical laboratory internal audit process must ensure laboratory personnel are able to report light microscopic analysis results relating to schistocytes qualitatively and quantitatively according to the medical laboratory's own requirements, as specified in Subclause 8.8.3.1 a) of AS ISO 15189:2023. The laboratory internal auditor should note that the ICSH has published an update of the 2012 recommendations for schistocyte identification, diagnostic value, and quantitation of schistocytes (Zini *et al* 2021). The laboratory internal auditor must first evaluate laboratory personnel competence in light microscopic analysis of schistocytes in stained peripheral blood smears, as specified in Subclause 6.2.1 b) of AS ISO 15189:2023. Secondly, the laboratory internal auditor must also evaluate laboratory personnel competence in light microscopic quantitative analysis of schistocytes and determination of whether results are within established critical decision limits, and performance of necessary actions to notify laboratory users or other authorised personnel, if required, as specified in Subclause 7.4.1.3 (Critical result reports) of AS ISO 15189:2023.

Acknowledgements

The authors would like to thank Gillian Rozenberg, FAIMS FFSc (RCPA), Principal Scientist, South Eastern Area Laboratory Services, Prince of Wales Hospital, Randwick, Australia, for providing the light micrograph, and the following reviewers for their professional criticisms and recommendations to the manuscript: Helen Neilson, BSc (Hons) CQP MCQI RCT, Quality Manager, NHS Greater Glasgow and Clyde, Glasgow, United Kingdom; and Clarita Dontogan, BSc MPH DPA, Chief of the Allied Health Professionals, Region II Trauma and Medical Center, Bayombong, Philippines.

References

Bain BJ 2021. *Blood cells: a practical guide.* 6th ed. Hoboken: John Wiley & Sons, Wiley-Blackwell. Chapter 3, Morphology of blood cells; 64-197.

Bain BJ 2023. *Hematology: 101 morphology updates.* Hoboken: John Wiley & Sons, Wiley-Blackwell. Chapter 90, Schistocytosis is not always microangiopathic hemolytic anemia; 120.

Bansal MG, Crane GM 2021. Hypochromic and hemolytic anemias. In: Salama M, Teruya-Feldstein J, Kremyanskaya M, editors. *Atlas of diagnostic hematology.* Philadelphia: Elsevier; 22-46.

Baorto D, Baroni R, Deckard J, Hafeza E, Lumakovska E, Mitchell B, Owens F, Rallins M 2022. *LOINC mapping guide: hematology and serology.* Version 1.0. Indianapolis: Regenstrief Institute.

Bhargava P, Etzell J, Finn W 2018. Erythrocytes. In: Glassy EF, editor; Bhargava P, Etzell J, section editors. *Color atlas of hematology: an illustrated field guide based on proficiency testing.* 2nd ed. Northfield: College of American Pathologists; 48-127.

Carr JH 2021. *Clinical hematology atlas*. 6th ed. St. Louis: Elsevier. Chapter 11, Variations in shape and distribution of erythrocytes; 93-106.

Gasser C, Gauthier E, Steck A, Siebenmann RE, Oechslin R 1955. Hämolytisch-urämische syndrome: bilaterale nierenrindennekrosen bei akuten erworbenen hämolytischen anämien. *Schweiz Med Wochenschr* 38: 905-919.

Hansen YBL, Toska K, Lund A, Flatman R, Ceder R 2023. Properties and units in the clinical laboratory sciences. Part XXVII: online dynamic NPU manual (IUPAC Technical Report). *Pure Appl Chem* 95: 125-131.

Harmening DM, Rhees JR, Green R 2024. Hemolytic anemias: extracorpuscular defects. In: Harmening DM, editor; Hutson LM, associate editor; Kirk DE, Lansink S, editorial associates. *Clinical hematology and fundamentals of hemostasis.* 6th ed. Philadelphia: F.A. Davis Company; 283-311.

Hay D, King A, Desborough M 2023. *Haematology.* 11th ed. Chichester: John Wiley & Sons, Wiley-Blackwell. Haemolytic anaemias; 39-53. (Lecture notes).

Hayden JA, Weksler BB 2017. Anemia. In: Weksler BB, Schechter GP, Ely SA, editors. *Wintrobe's atlas of clinical hematology.* 2nd ed. Philadelphia: Wolters Kluwer; 140-241.

Hoffbrand AV, Chowdary P, Collins G, Loke J 2024. H*offbrand's essential haematology.* 9th ed. Hoboken: John Wiley & Sons, Wiley-Blackwell. Chapter 6, Haemolytic anaemias; 65-78. (Essentials).

Hoffbrand AV, Vyas P, Campo E, Haferlach T, Gomez K 2019. *Color atlas of clinical hematology: molecular and cellular basis of disease.* 5th ed. Hoboken: John Wiley & Sons, Wiley-Blackwell. Chapter 8, Hemolytic anemias; 89-105.

Khan MG 2011. *Encyclopedia of heart diseases.* 2nd ed. New York: Springer Science+Business Media, Springer. Marfan syndrome; 603-607.

Kozlovac JP, Hawley RJ 2017. Biological toxins: safety and science. In: Wooley DP, Byers KB, editors. *Biological safety: principles and practices.* 5th ed. Washington: American Society for Microbiology, ASM Press; 247-268.

Kumar Y, Abbas AK, Aster JC 2022. Hematopoietic and lymphoid systems. In: Kumar Y, Abbas AK, Aster JC, Deyrup AT, editors. *Robbins and Kumar basic pathology.* 11th ed. Philadephia: Elsevier; 345-399.

Leung AYH, Ma ESK 2024. *Haematology and the Asian patient: 51 clinical cases.* Boca Raton: Taylor & Francis Group, CRC Press. Chapter 43, Atypical haemolytic uraemic syndrome; 177-180.

Levi M, van der Poll T 2014. A short contemporary history of disseminated intravascular coagulation. *Semin Thromb Hemost* 40: 874-880.

Lichtman MA, Kaushansky K, Prchal JT, Levi MM, Burns LJ, Linch DC 2022. *Williams manual of hematology.* 10th ed. New York: McGraw Hill. Chapter 19, Fragmentation hemolytic anemia; 98-103.

Loukas M 2020. Heart. In: Standring S, editor-in-chief; Tunstall R, section editor. *Gray's anatomy: the anatomical basis of clinical practice.* 42nd ed. Philadephia: Elsevier; 1068-1096.

Manchanda N 2024. Anemias: red blood cell morphology and approach to diagnosis. In: Keohane EM, Butina MM, Mirza KM, Walenga JM, editors. *Rodak's hematology: clinical principles and applications, seventh edition.* 7th ed. St. Louis: Elsevier; 262-274.

Mehta AB, Gomez K 2017. *Clinical haematology: illustrated clinical cases.* 2nd ed. Boca Raton: Taylor & Francis Group, CRC Press. Case 87; 283-284.

Mitchell R 2022. Heart. In: Kumar Y, Abbas AK, Aster JC, Deyrup AT, editors. *Robbins and Kumar basic pathology.* 11th ed. Philadephia: Elsevier; 308-344.

Moschcowitz E 1924. Hyaline thrombosis of the terminal arterioles and capaillaries: a hitherto undescribed disease. *Proc NY Pathol Soc* 24: 21-24.

Naeim F, Rao PN, Song SX 2024. *Atlas of hematopathology with 100 case studies.* Cambridge: Elsevier, Academic Press. Chapter 4, Disorders of red blood cells; 83-111.

Narla M 2021. Structure and composition of the erythrocyte. In: Prchal JT, Lichtman MA, editors. *Willams hematology: the red cell and its diseases.* New York: McGraw Hill; 3-19.

Neill AL 2017. *The A to Z of the heart.* 3rd ed. Pencaitland: Handspring Publishing. Valves; 70-71.

Neill AL 2019. *The A to Z of blood and iron.* 5th ed. Pencaitland: Handspring Publishing. Stains- Giemsa, Wright's stains; 174.

New Zealand Institute of Medical Laboratory Science 2011. *Standardised reporting of blood film morphology.* 4th ed. Rangiora: New Zealand Institute of Medical Laboratory Science. Cell fragment; 8.

Niss O, Quinn CT 2021. Classification and disgnosis of anemia in children and neonates. In: Fish JD, Lipton JM, Lanzkowsky P, editors. *Lanzkowsky's manual of pediatric hematology and oncology.* 7th ed. London: Elsevier, Academic Press; 37-59.

Noris M, Ruggenenti PL, Remuzzi G 2024. Thrombotic microangiopathies, including hemolytic uremic syndrome. In: Patel RM, Landman J, editors. *The Netter collection of medical illustrations.* 3rd ed. Vol. 5, Urinary system. Philadelphia: Elsevier; 350-362.

Palmer L, Briggs C, McFadden S, Zini G, Burthem J, Rozenberg G, Proytcheva M, Machin SJ 2015. ICSH recommendations for the standardization of nomenclature and grading of peripheral blood cell morphological features. *Int J Lab Hematol* 37: 287-303.

Papageorgiou C, Jourdi G, Adjambri E, Walborn A, Patel P, Fareed J, Elalamy I, Hoppensteadt D, Gerotziafas GT 2018. Disseminated intravascular coagulation: an update on pathogenesis, diagnosis, and therapeutic strategies. *Clin Appl Thromb/Hemost* 24: 8S-28S.

Petersen UM, Dybkær R, Olesen H 2012. Properties and units in the clinical laboratory sciences. Part XXIII: The NPU Terminology, principles, and implementation: a user's guide (IUPAC Technical Report). *Pure Appl Chem* 84: 137-165.

RCPA Quality Assurance Programs 2004. *Red cell nomenclature*. Westmead: RCPA Quality Assurance Programs. Schistocytes; 6.

Reynolds SB, Ahmed A 2023. Platelet and megakaryocyte disorders. In: Khoriaty RN, Jones M, Worden FP, editors. *Hematology board review: blueprint study guide and Q&A*. 2nd ed. New York: Springer Publishing Company, Demos Medical Publishing; 115-141.

Royal College of Pathologists of Australasia 2024a. *Best practice guideline for rendering pathology reports* V2.2. Surry Hills: Royal College of Pathologists of Australasia. Section 5, Guiding principles; 7-8.

Royal College of Pathologists of Australasia 2024b. *Standardised pathology informatics in Australia (SPIA) guidelines.* Version 4.1. Surry Hills: Royal College of Pathologists of Australasia. Section 13, Safe pathology reporting; 50-59.

Rozenberg G 2024. *Guide to paediatric haematology morphology.* Boca Raton: Taylor & Francis Group, CRC Press. Microangiopathic haemolytic anaemia; 36-37.

Shamil E, Ravi P, Chandra A, editors. 2023. *100 cases in clinical pathology and laboratory medicine.* 2nd ed. Boca Raton: Taylor & Francis Group, CRC Press. Case 70: headache and a rash; 217-219. (Rymer J, series editor. 100 cases).

Singh I, Weston A, Kundur A, Dobie G 2017. *Haematology case studies with blood cell morphology and pathophysiology.* Cambridge: Elsevier, Academic Press. Chapter 7, Immune haemolytic disorders; 103-115.

Smock KJ 2023. Examination of the blood and bone marrow. In: Means RT Jr, Arber DA, Glader BE, Appelbaum FR, Rodgers GM, Dispenzieri A, Fehniger TA, Michaelis LC, Leonard JP, editors. *Wintrobe's clinical hematology.* 15th ed. Vol. 1. Philadelphia: Wolters Kluwer; 1-17.

Weinstein L 1982. Syndrome of hemolysis, elevated liver enzymes, and low platelet count: a severe consequence of hypertension in pregnancy. *Am J Obstet Gynecol* 142: 159-167.

Zini G, d'Onofrio G, Erber WN, Lee S-H, Nagai Y, Basak GW, Lesesve J-F 2021. 2021 update of the 2012 ICSH recommendations for identification, diagnostic value, and quantitation of schistocytes: impact and revisions. *Int J Lab Hematol* 43: 1264-1271.

Acute mast cell leukaemia case study

Hyeon Jeong Min

NSW Health Pathology, Department of Haematology, Prince of Wales Hospital, Randwick, NSW

Abstract

Mast cell leukaemia (MCL) is a rare subtype of systemic mastocytosis (SM), characterised by clonal proliferation of the mast cells in the bone marrow and other organs. According to the World Health Organization (WHO), MCL must meet SM diagnostic criteria and possess additional features such as the presence of atypical mast cells in bone marrow, peripheral blood and extracutaneous organs. Acute mast cell leukaemia has a significantly worse prognosis than other subtypes of SM. Some treatment options are available for acute MCL cases including tyrosine kinase inhibitors (TKIs), chemotherapy, and allogenic stem cell transplants.

The following case study describes the diagnostic and therapeutic approaches for a patient with acute MCL who completed consolidation therapy for FMS-like tyrosine kinase 3 (FLT3)-positive acute myeloid leukaemia (AML). Neoplastic mast cells were observed on the peripheral blood film and in the bone marrow (BM) nine months after the initial FLT3-positive AML diagnosis. Several tests were conducted including a BM examination, immunohistochemistry, molecular test and serum tryptase level analysis, in accordance with WHO criteria for acute mast cell leukaemia diagnosis. Due to the absence of a *KIT* D816V mutation and the patient's age, the treatment options were limited. As targeted MCL treatment was not feasible, the patient was provided supportive care.

Keywords: ***Systemic mastocytosis, mast cell leukaemia, KIT D816V mutation, tyrosine kinase inhibitors***

Introduction

A mastocytosis is a clonal proliferation of mast cells in one or more organs (Georgin-Lavialle *et al* 2013; Zheng *et al* 2018; Jafari *et al* 2019). Based on the clinical and pathological presentation, mastocytosis is classified into systemic mastocytosis (SM), cutaneous mastocytosis (CM), and mast cell sarcoma (Joris *et al* 2012; Jafari *et al* 2019). A rare disease, SM has an estimated incidence rate of 13 cases per 100,000 people and generally occurs in adults (Galura *et al* 2020). The release of mediators and infiltration of mast cells causes symptoms of the condition. These symptoms include hypertension, rash, pruritus, musculoskeletal pain, and fever (Jafari *et al* 2019; Galura *et al* 2020).

Address correspondence to:
Hyeon Jeong Min
Prince of Wales Hospital
320-346 Barker St, Randwick NSW 2031
E-mail: mina1405@hotmail.com

A diagnosis of SM is confirmed by the presence of mast cell infiltration in at least one extracutaneous organ, most commonly the bone marrow (Bae *et al* 2013; Galura *et al* 2020; Zanelli *et al* 2023). SM is further classified according to its disease-specific features, including indolent SM (ISM), smouldering SM (SSM), aggressive SM (ASM), SM with an associated hematopoietic neoplasm (SM-AHN), and MCL (Georgin-Lavialle *et al* 2013; Valent *et al* 2014; Jafari *et al* 2019; Zanelli *et al* 2023). MCL is a rare form of SM that accounts for less than 0.5% of all SM cases (Georgin-Lavialle *et al* 2013; Zheng *et al* 2018; Galura *et al* 2020). The disease is characterized by the leukaemic spread of mast cells across multiple organs, including the liver, peritoneum, spleen, bones, and bone marrow (Valent *et al* 2014; Zanelli *et al* 2023). A diagnosis of MCL requires the presence of SM diagnostic criteria and at least 20% atypical/immature mast cells in a bone marrow as defined by the World Health Organization in 2022 (Khoury *et al* 2022; Zanelli *et al* 2023) (Table 1).

Depending on the presence of organ damage (C-findings), MCL is divided into acute and chronic variants (Galura *et al* 2020; Zanelli *et al* 2023).

***Table 1**. Diagnostic criteria for systemic mastocytosis (Adapted from Zanelle* et al *2023).*

2022 WHO: The diagnosis of SM can be made when the major criterion plus one minor criterion or at least three minor criteria are fulfilled.
Major criterion
• Multifocal dense infiltrates of MCs (≥ 15 MCs in aggregates) in BM biopsy and/or other extracutaneous organs.
Minor criteria
• > 25% immature or atypical or immature MCs in BM biopsy or BM smear or other extracutaneous organs • Any kind of *KIT* mutation in BM, blood or other extracutaneous organs. • MCs expressing CD25 with or without CD2 (in addition to normal MC markers). • MCs expressing CD30. • Persistently elevated serum tryptase level (> 20 ng/mL), unless there is an associated myeloid neoplasm, in which case, this parameter is not valid.

WHO: World Health Organization; **SM:** Systemic mastocytosis; **BM:** Bone marrow; **MCs:** Mast cells; **CD:** Cluster of differentiation

Most patients (60-90%) experience the acute form of MCL which develops rapidly and aggressively, resulting in massive organ damage. The chronic form of MCL has a prolonged course without rapid organ damage, but progression to acute MCL appears to occur over a variable period (Valent *et al* 2014; Zanelli *et al* 2023).

Acute MCL has a poor prognosis, with a median survival of less than six months (Bauer *et al* 2017; Zheng *et al* 2018). There are limited treatment options for acute MCL and little data on treatment (Joris *et al* 2012; Bauer *et al* 2017; Zanelli *et al* 2023). A molecular analysis of the *KIT* Proto-Oncogene (*KIT*) gene is essential for selecting the appropriate treatment (Joris *et al* 2012; Georgin-Lavialle *et al* 2013; Galura *et al* 2020). This case report describes the laboratory findings and prognosis of a patient with acute MCL without *KIT* D816V mutation.

Case report

A 64-year-old male was admitted to the hospital with a history of completing consolidation therapy for FLT3-positive AML in December 2021. The patient commenced maintenance treatment with ongoing transfusions and in February 2022, a BM aspiration was performed due to abnormal laboratory results. The laboratory findings were notable for a decreased white blood cell count (1.40 x 10^9/L; RR 3.5-11.00 x 10^9/L), neutropenia (0.60 x10^9/L; RR 1.7-7.0 x10^9/L), anaemia (haemoglobin 94 g/L; RR 130-180 g/L), and thrombocytopenia (11 x 10^9/L; RR 150-450 x 10^9/L). A microscopic examination of the peripheral blood film revealed some abnormal mononuclear cells with heavily granulated cytoplasm, with granules overlying the eccentric nucleus and with cytoplasmic budding (Figures 1A and 1B). This patient had a marked increase in serum tryptase 515.0 µg/L (RR 0.0-11.4 µg/L).

Bone marrow examination demonstrated a hypocellular marrow with reduced three lineage haematopoiesis. Approximately 44% of cells were intermediate to large atypical round granular cells, favoured to be abnormal mast cells. These cells had a single round or bilobed eccentric nucleus with variable granules in the cytoplasm (Figures 2A and 2B). Approximately 5% of cells were typical blasts with some having monoblastic appearance. About 50% of the abnormal granular cells showed metachromatic staining of cytoplasmic granules with toluidine blue stain (Figure 3). Flow cytometry analysis showed approximately 36% of the total nucleated cells were intermediate to large immature myeloid cells, and their immunophenotype was CD45 +/HLA-Dr+(high)/CD 117 +(bright)/CD 34-/CD10+/CD2+ (dim)/CD 25+/CD4+.

A cytogenetic analysis of bone marrow cells revealed a normal male karyotype (46, XY) with no abnormalities. No mutations were detected in molecular analysis, including

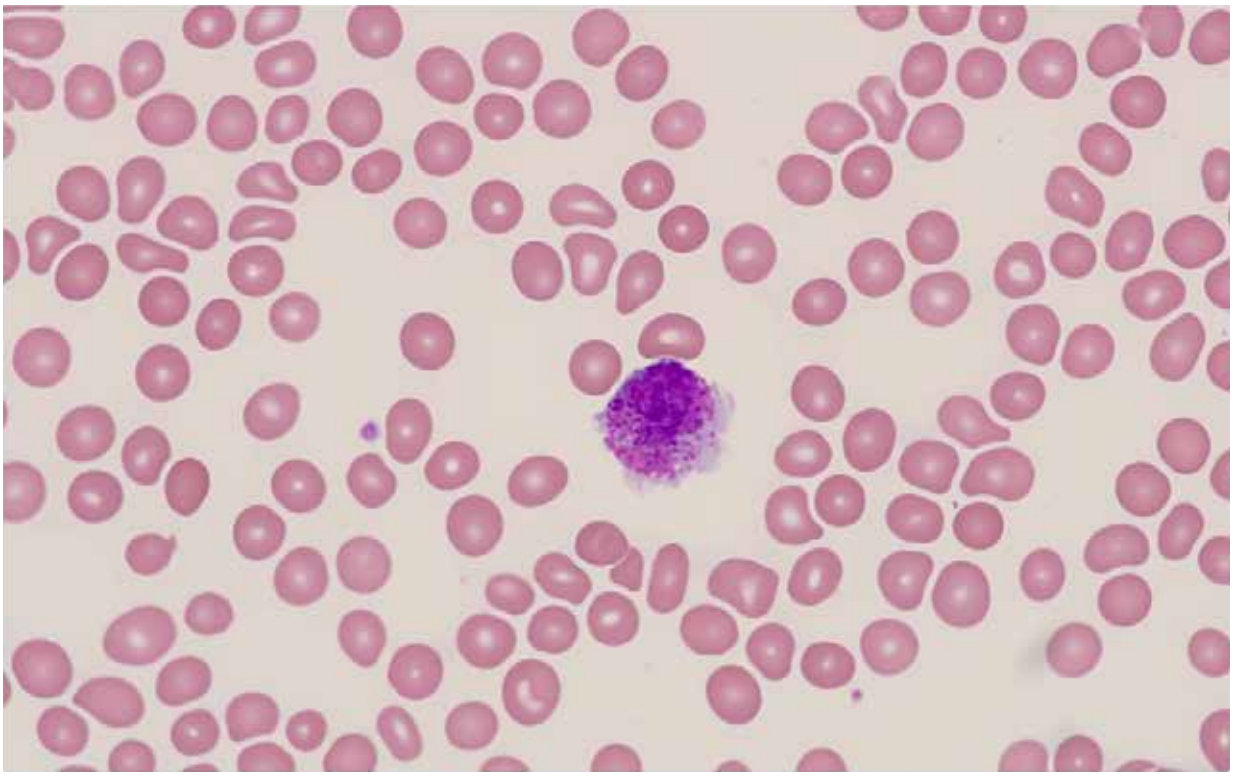


***Figure 1A**. Peripheral blood film showing a circulating atypical mast cell with cytoplasmic budding. Wright stain, original magnification x 1000*

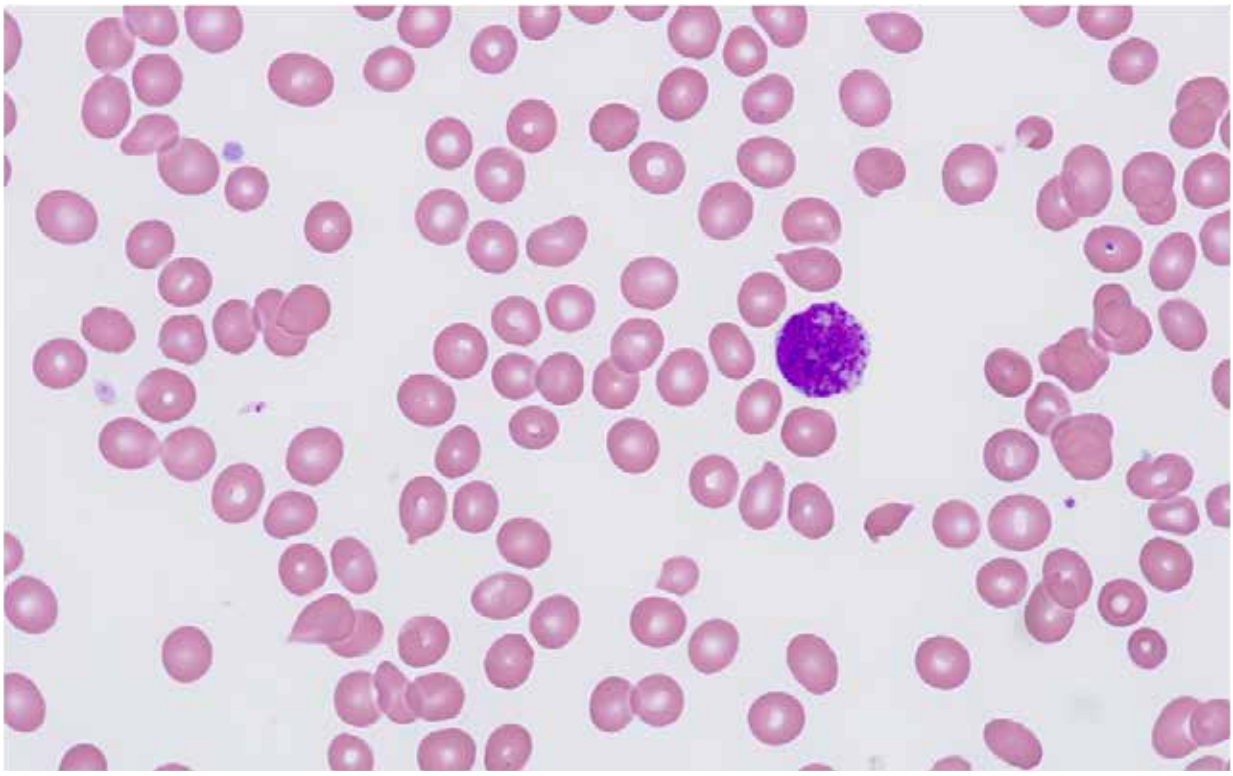


***Figure 1B**. Peripheral blood film showing an atypical mast cell with metachromatic granules. Wright stain, original magnification x 1000*

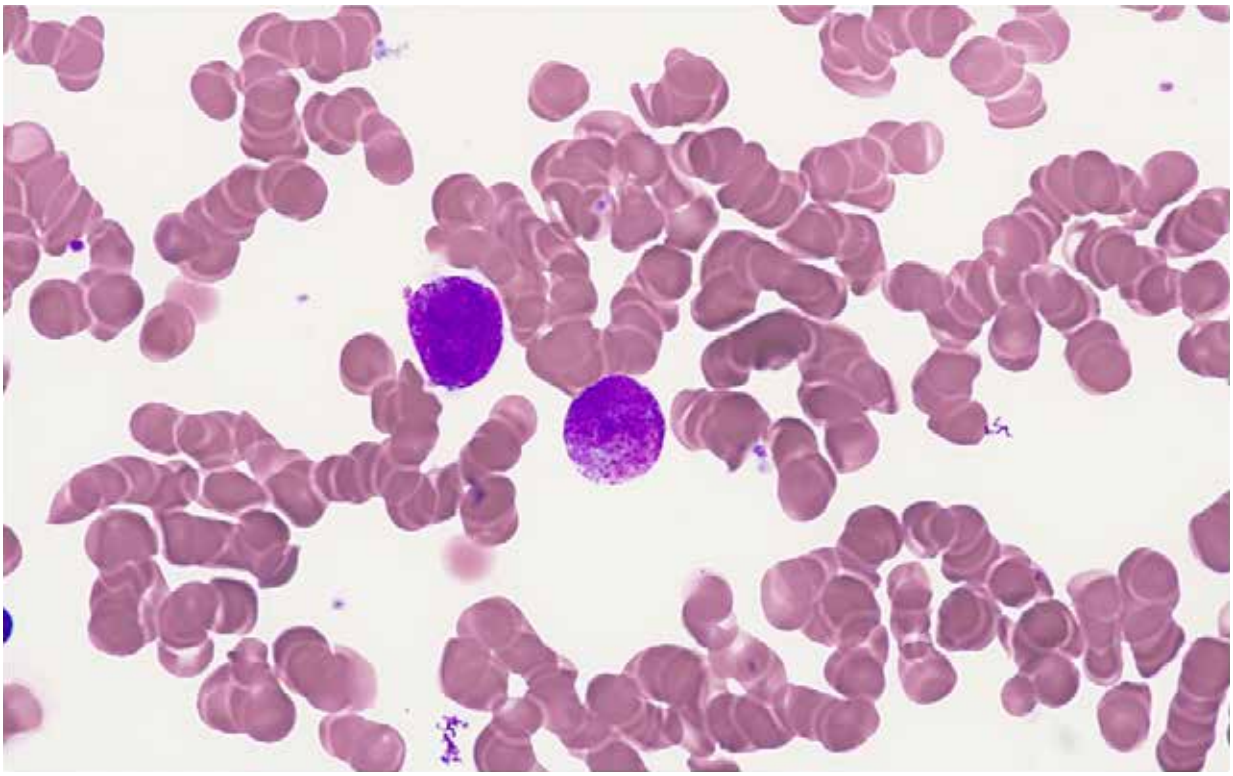


***Figure 2A**. Bone marrow aspirate showing atypical mast cells with a bilobed eccentric nucleus and variable granules in their cytoplasms. Wright stain, original magnification x 1000*

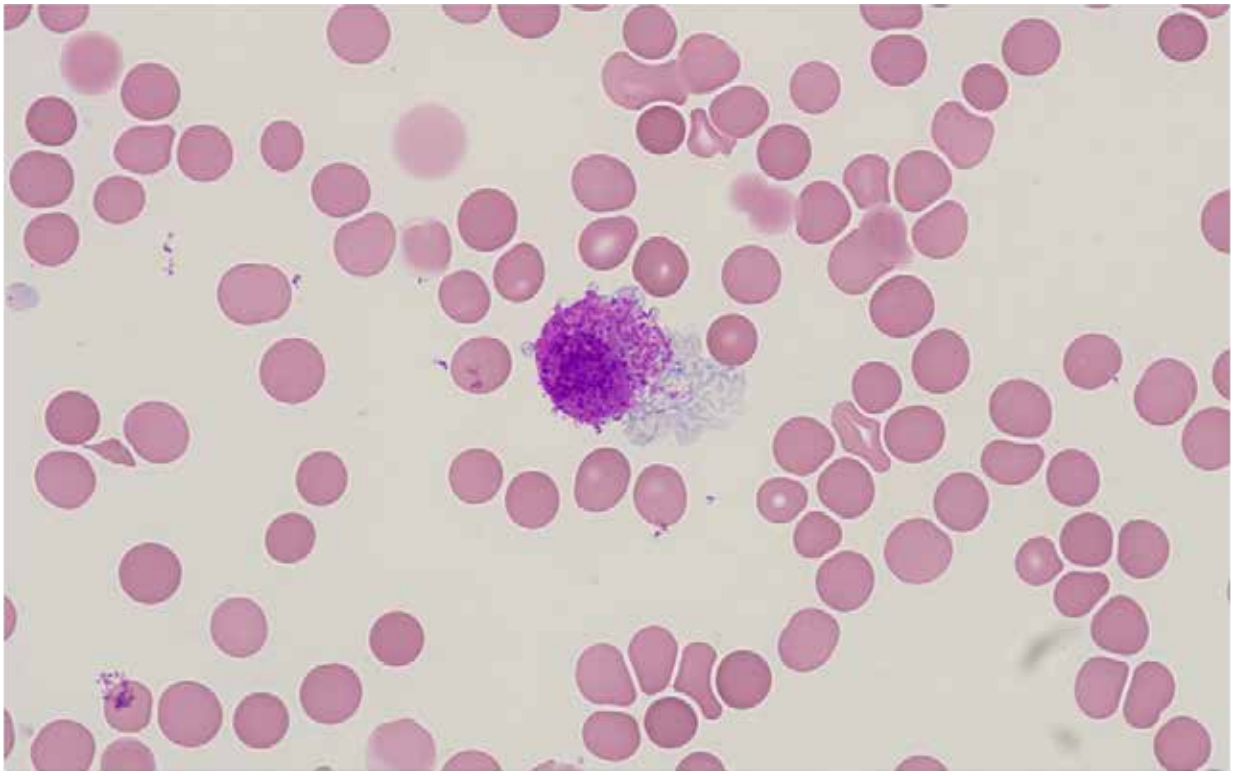


***Figure 2B**. Bone marrow aspirate smear showing a neoplastic mast cell with a single round eccentric nucleus moderately large purple-pink granules in the cytoplasm with long cytoplasmic tendrils. Wright stain, original magnification x 1000*

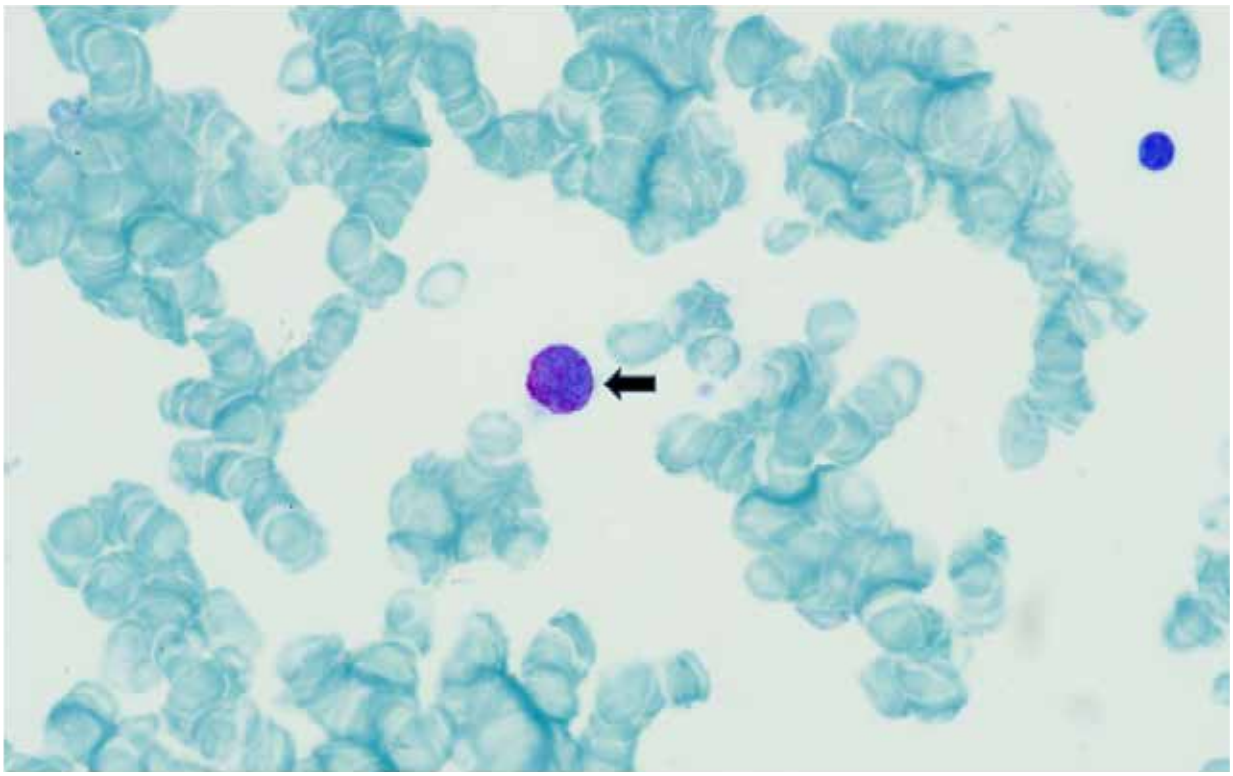


Figure 3. *Toluidine blue stain showing medium-sized round or oval mast cell with densely packed purple metachromatic granules in the cytoplasm (black arrow). Original magnification x 400*

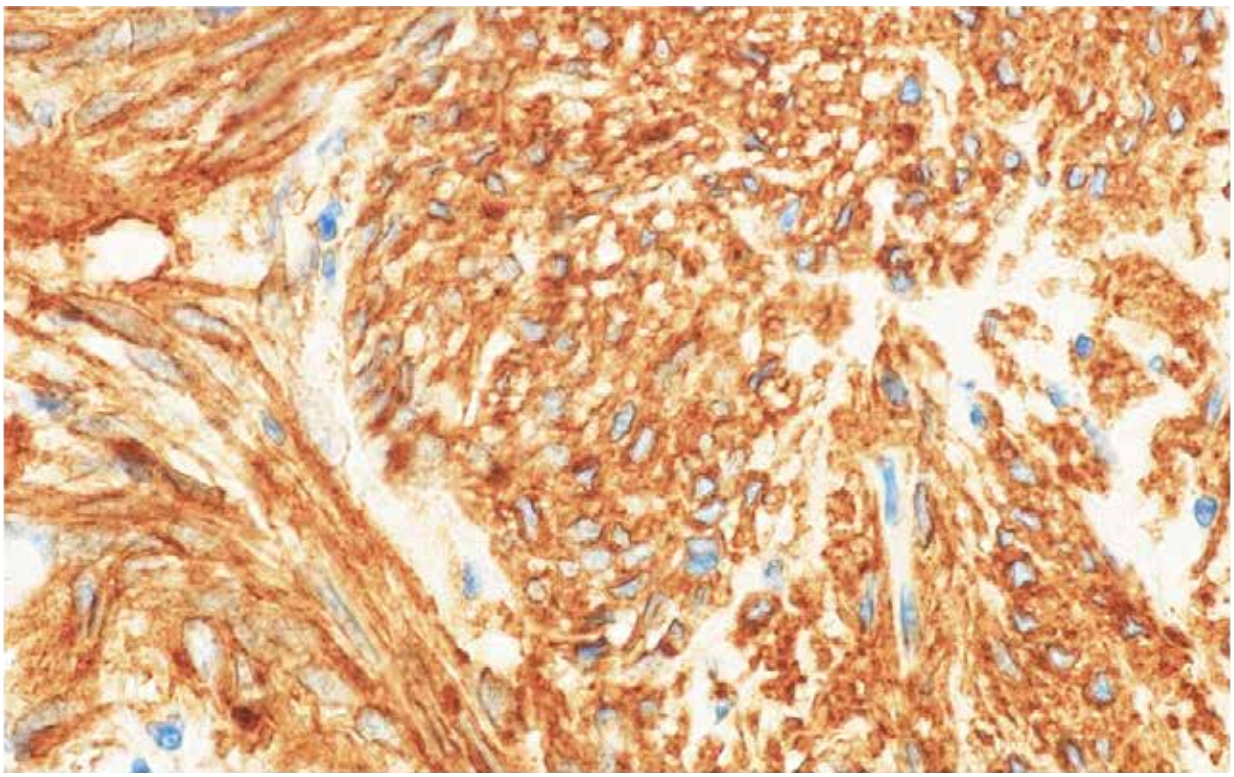


Figure 4A. *Immunohistochemical staining of bone marrow biopsy in which mast cells are positive for CD117. Original magnification x 400*

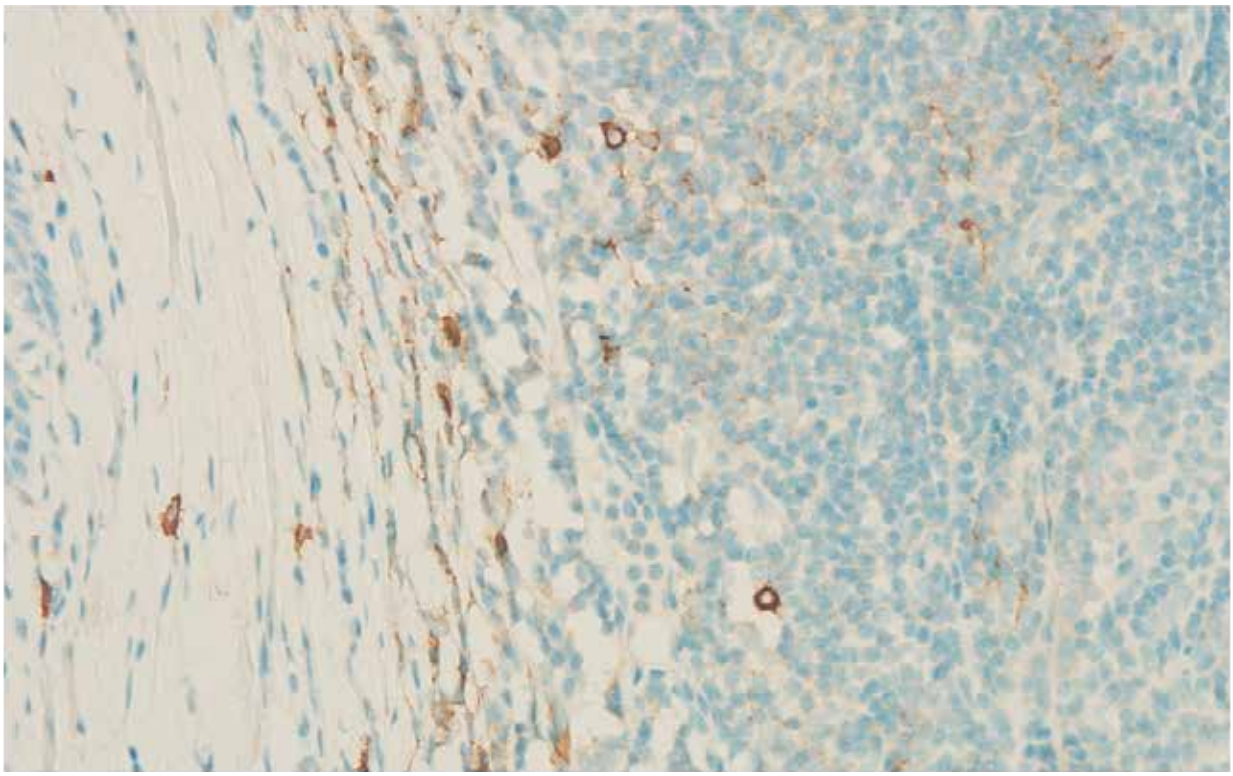


***Figure 4B**. Immunohistochemical staining of bone marrow biopsy showing a weekly positive reaction for mast cell tryptase. Original magnification x 400*

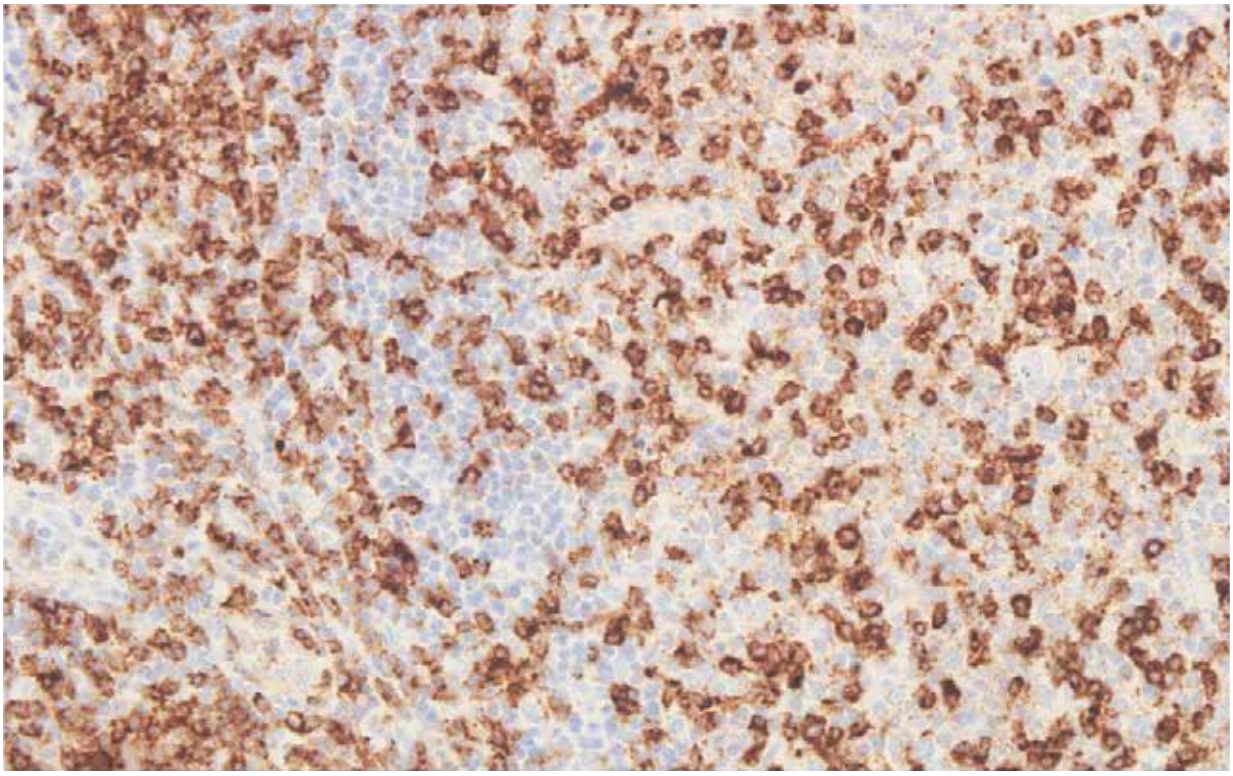


***Figure 4C**. Immunohistochemical staining of bone marrow biopsy showing an increased number of neoplastic mast cells positive for CD2. Original magnification x 400*

the most frequently detected mutation, *KIT* D816V. Immunohistochemistry revealed a strong positive of CD117, CD 2 and weekly positive in mast cell tryptase (Figures 4A-C).

The laboratory results of this patient fulfilled the diagnostic criteria of SM, with approximately 44% of atypical immature mast cells in bone marrow and elevated serum tryptase level (> 20 ng/mL). Cytopenia caused by infiltration of neoplastic mast cell was identified as a C-finding of acute MCL.

Discussion

As an aggressive form of mastocytosis, acute MCL is a very rare disease. It is characterized by the proliferation of atypical mast cells and frequently affects multiple organs. Most acute MCL cases present with cytopenia, hepatic dysfunction, hypersplenism, malabsorption, osteolytic lesions, and weight loss, known as C-findings (Galura *et al* 2020; Zanelli *et al* 2023). For its diagnosis, acute MCL must meet the criteria for SM and organ function impairment, as well as leukemic involvement of the bone marrow. (Galura *et al* 2020; Pardanani 2021; Zanelli *et al* 2023). The heterogeneity of acute MCLs clinical and morphological features often makes diagnoses difficult and the clinical course of the disease unpredictable (Valent *et al* 2014).

Mast cells may not always be distinguishable by standard staining, particularly when they exhibit an immature morphology and are hypogranulated or if there is an associated haematological malignancy, such as AML, overlying the mast cell infiltration in the BM (Pardanani 2021; Zanelli *et al* 2023). In this case study initial indicators for the diagnosis were cytopenia and the presence of circulating atypical mast cells with coarse metachromatic granules in the blood film.

Atypical mast cells are classified into three subtypes based on their morphological characteristics: metachromatic blasts and atypical mast cells I and II. A metachromatic blast exhibits a blast-like nuclear morphology with numerous metachromatic granules (Bae *et al* 2013; Valent *et al* 2014). The atypical mast cell type I has elongated cytoplasmic projections, oval eccentric nuclei, and hypogranulation. Unlike type I mast cells, type II mast cells normally exhibit bilobed or polylobed nuclei (Bae *et al* 2013; Valent *et al* 2014). As shown in this case study, MCL and aggressive forms of SM commonly display more pronounced cellular atypia, such as metachromatic blasts or atypical mast cell type II (Bae *et al* 2013; Zanelli *et al* 2023).

It is essential to perform immunophenotyping in order to identify abnormal mast cells (Galura *et al* 2020). The key markers for identifying abnormal mast cells in SM are tryptase, CD117+, CD25+ and CD2+. Despite this, tryptase and CD117 expression alone do not indicate the neoplastic nature of mast cells (Georgin-Lavialle *et al* 2013; Galura *et al* 2020; Zanelli *et al* 2023). The immunohistochemical expression of CD25 and/or CD2 is currently recognised as a minor diagnostic criterion for SM, as the expression of these markers is indicative of clonal infiltrates of mast cells (Joris *et al* 2012; Zanelli *et al* 2023). Neither of these antigens is expressed in normal mast cells, myeloid precursor cells or immature mast cells from other myeloid neoplasms (Galura *et al* 2020; Zanelli *et al* 2023).

Mutations in the *KIT* gene are also hallmarks of the disease (Joris *et al* 2012; Pardanani 2021). The deregulation of the *KIT* gene, including overexpression and gain of function mutations, has been discovered in several types of cancer in humans (Cruse *et al* 2014). Mutations in codon 816 of *KIT* cause constitutive activation of KIT kinase in adult-type mastocytosis (Joris *et al* 2012; Georgin-Lavialle *et al* 2013). There are several types of activating *KIT* mutations, which respond differently to KIT inhibitions based on the site and type of mutation (Joris *et al* 2012; Georgin-Lavialle *et al* 2013). The *KIT* D816V mutation occurs in more than 80% of adult patients with SM, particularly in the aggressive forms, with a frequency greater than 95% in patients with MCL (Joris *et al* 2012; Zanelli *et al* 2023). However, several studies have found that *KIT* D816V mutations are not always positive, as illustrated in this case study (Joris *et al* 2012; Galura *et al* 2020; Zanelli *et al* 2023). This information is useful in predicting drug resistance and in supporting individualisation of therapy based on the response of specific mutant proteins to specific drugs (Joris *et al* 2012; Georgin-Lavialle *et al* 2013; Pardanani 2021).

The treatment options for acute MCL include TKIs, chemotherapy and allogeneic stem cell transplantation (allo-SCT) (Georgin-Lavialle *et al* 2013; Galura *et al* 2020; Pardanani 2021). Allogenic stem cell translation may also be an effective treatment option for MCL (Georgin-Lavialle *et al* 2013; Galura *et al* 2020; Pardanani 2021). According to some reports, the graft reduces the mast cell burden after transplantation. Due to the limited ability of this treatment to completely eradicate the disease, allo-SCT is yet to be proven in MCL patients and, as of now, requires further evaluation (Georgin-Lavialle *et al* 2013; Bauer *et al* 2017). One of the most promising therapeutic options is the use of TKIs, such as avapritinib or midostaurin, which have been shown to possesses a potent inhibitor of the *KIT* D816V mutation (Galura *et al* 2020). Unfortunately, the patient in this case study did not have the *KIT* D816V mutation and therefore could not be treated with TKIs. Another possible treatment option was cladribine, but it has only been utilized in a very small number of acute MCL cases and has demonstrated relatively low efficacy

(Joris *et al* 2012). After careful discussion with the patient and his family, supportive care was provided, including platelet and red cell transfusions every one to two weeks.

In conclusion, it can be challenging to diagnose acute MCL due to its rarity. An early diagnosis may increase the chances of successfully administering treatment and extending patient survival time.

A thorough understanding of the morphology and classification of acute MCL based on the novel criteria is essential for appropriately guiding diagnosis. In addition, molecular analyses of the *KIT* gene are critical in determining the appropriate treatment. An appropriate drug selection can facilitate effective treatment of subsequent relapses and prevent the occurrence of resistance.

References

Bae MH, Kim HK, Park CJ, Seo EJ, Park SH, Cho, YU, Jang S, Chi HS, Lee KH 2013. A Case of Systemic Mastocytosis Associated with Acute Myeloid Leukemia Terminating as Aleukemic Mast Cell Leukemia after Allogeneic Hematopoietic Stem Cell Transplantation. *Annals of Laboratory Medicine*, 33(2), pp.125–129.

Bauer J, Longo W, Yang D 2017. Mast Cell Leukemia: Review of a rare disease and case report of prolonged survival after Allogeneic Stem Cell transplant. *Human Pathology: Case Reports*, 10, pp. 46–49.

Cruse G, Metcalfe DD, Olivera A 2014. Functional Deregulation of KIT. *Immunology and Allergy Clinics of North America*, 34(2), pp.219–237.

Galura GM, Cherukuri SV, Hakim N, Gaur S, Orazi A 2020. Acute aleukemic mast cell leukemia: Report of a case and review of the literature. *Leukemia Research Reports*. http:// https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7725938. Accessed September 1, 2023.

Georgin-Lavialle S, Lhermitte L, Dubreuil P, Chandesris M-O, Hermine O, Damaj G 2013. Mast cell leukemia. *Blood*, 121(8), pp.1285–1295.

Jafari E, Hadipour A, Khandani BK, Abolhasani F 2019. Mast Cell Leukemia with Ascites and Multiple Organs Damage. *Iranian Journal of Pathology*, 14(03), pp.265–269.

Joris M, Georgin-Lavialle S, Chandesris M.-O, Lhermitte L, Claisse J-F, Canioni D, Hanssens K, Damaj G, Hermine O, Hamidou M 2012. Mast Cell Leukaemia: c-KIT Mutations Are Not Always Positive. *Case Reports in Hematology*. http:// https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3444844. Accessed October 2, 2023.

Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF, Bejar R, Berti E, Busque L, Chan JKC, Chen W, Chen X, Chng WJ, Choi JK, Colmenero I, Coupland SE, Cross NCP, De Jong D, Elghetany MT, Takahashi E 2022. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. *Leukemia*, 36(7), pp.1703–1719.

Pardanani A 2021. Systemic mastocytosis in adults: 2021 Update on diagnosis, risk stratification and management. *American Journal of Hematology*, 96(4), pp.508–525.

Valent P, Blatt K, Eisenwort G, Herrmann H, Cerny-Reiterer S, Thalhammer R, Müllauer L, Hoermann G, Sadovnik I, Schwarzinger I, Sperr WR, Mannhalter C, Horny, HP 2014. FLAG-induced remission in a patient with acute mast cell leukemia (MCL) exhibiting t(7;10)(q22;q26) and KIT D816H. *Leukemia Research Reports*, 3(1), pp. 8–13.

Valent P, Sotlar K, Sperr WR, Escribano L, Yavuz S, Reiter A, George TI, Kluin-Nelemans HC, Hermine O, Butterfield JH, Hägglund H, Ustun C, Hornick JL, Triggiani M, Radia D, Akin C, Hartmann K, Gotlib J, Schwartz LB. Verstovsek S 2014. Refined diagnostic criteria and classification of mast cell leukemia (MCL) and myelomastocytic leukemia (MML): a consensus proposal. *Annals of Oncology*, 25(9), pp.1691–1700.

Zanelli M, Quintini M, Magnasco S, Aprile L, Palicelli A, Zizzo M, Sanguedolce F, Ricci S, Pancetti S, Zuccalà V, Martino V, Broggi G, Caltabiano R, Cavazza A, Parente P, Mecucci C, Martino G, Ascani S 2023. Mast Cell Leukemia: An Update with a Practical Review. *Cancers*, 15(6), pp.1664–1664.

Zheng Y, Nong L, Liang L, Wang W, Li T 2018. De novo mast cell leukemia without CD25 expression and KIT mutations: a rare case report in a 13-year-old child. *Diagnostic Pathology*, 13(1), pp.1-5.

This case study has been republished as formatting issues led to significant omissions when originally published in the November 2023 edition. The AJMS accepts full responsibility for this error.

AIMS National Scientific Meeting “Moving Forward”

Adelaide Convention Centre 21-23 August 2024

Oral and poster presentation abstracts

O1: 'TRANSFUSION LABORATORY ESSENTIALS': BUILDING TRANSFUSION KNOWLEDGE FOR NEW AND RETURNING LABORATORY SCIENTISTS

S. Kowalski[1], S. Benson[2]

[1] *Australian Red Cross Lifeblood, SA, 5000*

[2] *Australian Red Cross Lifeblood, NSW, 2000 Email: Skowalski@redcrossblood.org.au*

Introduction

Australian Red Cross Lifeblood's Clinical Education Team (CET) is creating an e-learning course of microcredentials called 'Transfusion Laboratory Essentials'. It is designed for new and returning laboratory scientists to build their knowledge of transfusion laboratory practice. The course has been developed to complement existing laboratory training programs and will be in the form of online interactive modules, utilising contemporary instructional design and adult learning techniques. By completing the course, the learner will have gained the background knowledge to understand their role in providing safe and appropriate transfusions. Method

Lifeblood commenced the development of this course in 2022 after a gap analysis identified a need for transfusion education resources for new scientists. The concept was supported by laboratory managers who acknowledged this would assist with training new staff members. The multi-disciplinary project team consists of laboratory and clinical transfusion subject matter experts, an instructional and graphic designer, and communications experts and is overseen by a transfusion medicine specialist.

There are three modules currently available: 'The Australian Transfusion Community', 'Pretransfusion testing', and 'Pretransfusion labelling requirements'. Upcoming modules include: 'Blood group systems', 'ABO and Rh discrepancies', and 'Antibody investigations'. Each module will cover an essential laboratory element of the transfusion process. Downloadable resources will be available for learners to use as job aids. A series of assessment questions concludes each module, with a certificate being provided on successful completion.

Future modules will cover crossmatching, blood components and storage, massive transfusions, and adverse transfusion reactions.

Results

The course has so far been positively received by participants, with enrolment numbers at a pleasing number of 324 (as of 6/5/24).

Conclusion

This online course aims to provide new and returning laboratory scientists with essential knowledge to confidently undertake transfusion-related laboratory activities.

O2: A CASE OF BABESIOSIS

Wells R[1], Naomi Peake[2]

[1] *AIMS*

[2] *Haematology Department, Sullivan Nicolaides Pathology, Brisbane, Qld*

This case study describes a Babesia sp infection in a 60-year-old woman who had travelled to North America and Canada. On her return she presented with fevers, headaches and myalgia. Tests were performed for respiratory viruses and influenza and a full blood count (FBC) and electrolytes and liver function tests (ELFTs) were also requested.

Abnormalities were detected in the ELFT results and the FBC gave an abnormal lymphocyte flag and high monocyte count. On examination of the blood film, intra- and extracellular parasites were seen and these were then diagnosed as Babesia sp. She was also found to have either an active or past Lyme disease (borreliosis) infection.

She was hospitalised and treated but went into respiratory distress. After further treatment, she was cleared of the infection and discharged.

O3: ADVANCEMENTS IN LABORATORY OPERATIONAL CAPABILITIES FOR EMERGENCY MEDICAL TEAMS (EMT'S)

L Jones[1], M Morrow[1], M Cherian[1], N Walsh[1], K McDermott[1]

[1] *National Critical Care and Trauma Response Centre, Eaton, NT, 0820, Australia*

Introduction

The National Critical Care and Trauma Response Centre (NCCTRC) plays a vital role in the Australian Government's disaster and emergency medical preparedness and response capability framework. At the core of this capability is the Australian Medical Assistance Team (AUSMAT), a multi-disciplinary medical team that deploys to health emergencies of national and international significance.

Integral to AUSMAT's effectiveness is its laboratory

component, designed to operate in austere environments, where resources are limited and conditions are unpredictable, aligning to the World Health Organization's (WHO) Classification and Minimum Standards for Emergency Medical Teams.

The aim was to pinpoint the areas for improvement in compliance with WHO EMT Minimum standards.

Method

A comprehensive assessment focusing on policy, technology, capability and capacity was conducted to evaluate the operational effectiveness of the AUSMAT laboratory.

Results

A multidisciplinary pathology technical working group was established to provide expert advice and recommendations. Key areas of enhancement for WHO EMT operation were identified including the development of specialised protocols tailored to the challenges of austere environments, implementation of quality control measures, and improvement in laboratory design and infrastructure. To address the future health emergency demands, initiatives such as immersive simulation platforms for realistic training scenarios, utilisation of innovative technologies like BioFire, and the establishment of a walking blood bank were recommended.

Conclusion

Through continuous innovation and adaptation, AUSMAT has attained re-verification as a WHO EMT Type 1 Mobile, Type 1 Fixed, and Type 2 Surgical deployable hospital. The NCCTRC and AUSMAT remain committed to advancing laboratory capabilities, ensuring readiness for evolving health emergencies.

O4: FAST? NORMAL? SLOW? CASE STUDIES INVOLVING A PARTICULARLY TOUGH TEST; ACTIVATED PARTIAL THROMBOPLASTIN TIME (APTT). THE SPECIALISED HAEMOSTASIS LAB PERSPECTIVE.

O Yacoub

SA Pathology, Adelaide, South Australia, 5000

APTT: Fast? Normal? Slow?

Join me for a discussion on some fascinating 'bleeding and clotting' case studies from our laboratory in Adelaide. We've all heard of it, but have you ever wondered what testing is involved when this 'screening' test requires investigation? This presentation will take you on a journey from the patient's arm (preanalytical variables), to specialised haemostasis lab testing 'logic' (including factor assays and interpretation) and end with clinical interpretation and relevance.

O5: TITLE: CHALLENGE ACCEPTED - NAVIGATING A NEW NON-PARALLEL UNIVERSE

T. Stanton [1], S.J.McKeague[1], R.Coleman[1], R.Adams[1]

[1]*Sullivan Nicolaides Pathology, Haematology Department, Bowen Hills, QLD, Australia*

Abstract:

Introduction:

Traditionally, one-stage coagulation factor assays are performed at multiple dilutions to assess for the presence of non-parallelism, a phenomenon linked to the presence of inhibitors including Lupus anticoagulant and some anticoagulant medications. In a 2022 Research and Practice in Thrombosis and Haemostasis Forum, a challenge to this practice was issued by Emmanuel J. Favaloro and Leonardo Pasalic. In the interest of moving forward and not resting on tradition when better practices are available, a study was conducted assessing the frequency, causes and clinical impact on non-parallelism in our patient population to construct a model that combines single and multi-dilution testing that maximises cost savings and maintains diagnostic accuracy.

Methods:

1324 routine factor levels performed with multi-dilution analysis (MDA) in a single laboratory were retrospectively reviewed. 50 samples with known lupus anticoagulant (LAC) and 50 samples with known rivaroxaban use were analysed. An optimal pathway for reflex MDA testing was developed by examining different thresholds for MDA and their impact on efficiency and diagnosis.

Results:

Non-parallelism is rare, and is most common in Factor XI assays. The most common causes are unknown, drug and LAC. The degree of non-parallelism is strongly related to rivaroxaban concentration but showed low correlation to LAC. A model that only performs MDA on levels outside of a predetermined threshold results in significant cost savings, does not impact lab efficiency, and retains diagnostic accuracy.

Conclusion:

Utilising single dilution factor levels, with reflex MDA in select patients, represents a feasible and cost-effective alternative that could allow laboratories to move forward from the traditional paradigm of universal MDA, whilst still identifying patients with clinically significant pathology.

O6: EMBEDDING EMPLOYABILITY IN MEDICAL LABORATORY SCIENCE (MLS) PROGRAMS: RATIONALE AND PROTOCOL

Prajwal Gyawali PhD[1], Edward Bliss PhD[1], Prashant Regmi PhD[2], Natalie Milic PhD[2], Eliza Whiteside PhD[1], Elizbeth Cardell PhD[1]

[1] *University of Southern Queensland (UniSQ)*

[2] *Charles Darwin University (CDU)*

Aim and Rationale: Accredited pathology laboratory services periodically undergo a paradigm shift. This imposes challenges to medical laboratory scientists because they are required to embrace, cope with, and effectively manage these changes to meet the dynamic nature of the pathology industry. To overcome the challenges, medical laboratory scientists must engage in life-long learning and develop career-building skills. Hence, it is important that undergraduate medical laboratory science (MLS) programs embed employability best practice into their curriculum. Employability best practice not only enables the graduates to seek a desired career but, more importantly, recognises and promotes those attributes that are required to engage in life-long learning and career development. Currently, there is a lack of research regarding best employability practice in MLS program curricula. As a result, MLS program curricula often lacks an appropriate employability framework and may not necessarily meet industry demands. Hence, this project aims to address this gap by investigating the perceptions of MLS students, graduates, academics, and employers on employability best practice, employability framework and essential graduate attributes.

Method: This is a cross sectional mixed methods study utilising data collection via a survey tool and collaborative workshop. The questions within the survey are adapted from the DkIT Embedding Employability study with some modifications. The proposed research will be undertaken by UniSQ in collaboration with CDU. However, UniSQ researchers are willing to extend this collaboration to all interested AIMS accredited universities. The study will be rolled out from September this year.

Outcomes: The findings will be used to develop and incorporate employability best practice into MLS pedagogy and curriculum.

O7: ENHANCING EMPLOYABILITY OF MEDICAL LABORATORY SCIENCE GRADUATES

Dr I. Cassady[1], Dr A. Hicks[1], Dr B. King[1], Dr A. Kundur[1], Prof I. Singh[1]

[1] *Griffith University, Gold Coast, QLD 4222, Australia*
Ian.cassady@griffith.edu.au

Introduction: Griffith University (GU) is in the south-east Queensland region with an enrolment of approximately 46,000 students. Its major campus at the Gold Coast is the site of the Medical Laboratory Science (MLS) program. The GU-MLS program was developed collaboratively with industry professionals with a breadth of disciplines that are valuable in regional multidisciplinary laboratories. The curriculum includes all diagnostic MLS disciplines as well as genetics and molecular diagnostics and is accredited with the Australian Institute of Medical and Clinical Scientists (AIMS) and the UK Institute of Biomedical Sciences. Students attend a mandatory 560-hour placement in their fourth year and undertake two research projects. To increase student preparedness for placement the GU-MLS program has delivered the Enhancing Employability Workshop (EEW) in 2022 (2), 2023 (1) and 2024 (1). The micro- credentialled EEW focuses on industry specialist material such as pre-analytical error training, 3D virtual pathology simulations, telepathology, understanding NATA requirements and ISO15189 standards.

Aims & Approach: This study aimed to (i) assess the student experience and respond to student evaluation and (ii) assess the experience of industry supervisors of placement students regarding student preparedness. Students were surveyed before and after completion of the EEW. Industry supervisors were surveyed at the mid-placement meeting during student placement.

Results & Conclusions: Specific EEW topics were laboratory information systems, central specimen reception and pre-analytical errors, work-based competencies, compliance with NATA standards, discipline-specific automation tasks as well as employability training. Analysis of survey data indicated a positive impact of the EEW on student preparedness for placement from both student and industry supervisor responses.

We acknowledge funding of this project by AIMS grant REGS-29704-2022.

O8: EVALUATION OF HELENA CASCADE ABRAZO POINT-OF-CARE PT/INR AND APTT TESTING.

S Abraham[1], E Duncan[1]

[1] *SA Pathology, Adelaide, South Australia, 5000.*

The Prothrombin Time/ International Normalised Ratio (PT/INR) and Activated Partial Thromboplastin Time (aPTT) are global screening tests used to detect coagulation factor deficiencies and monitor anticoagulant therapy. The Cascade Abrazo Point-of-care (POC) PT/INR (human placental thromboplastin) and aPTT (ellagic acid activator) tests citrated whole blood and has a fibrinogen clot endpoint.

We evaluated POC precision using provided QC material and compared patient results to plasma tests using our routine laboratory (LAB) Stago STA R-MAX analyser with reagents Neoptimal (rabbit brain thromboplastin) and TriniCLOT aPTT S (micronized silica activator). Spare blood from unselected samples was used for POC tests and included patients on anticoagulants and for thrombophilia screening.

Using Normal (L1) and Abnormal (L2) QC material over the study days, the between-run CV% of PT L1 and L2 was 1.71% and 6.72% respectively. The aPTT L1 between-run CV was 2.84%; an aPTT L2 QC material was not available.

The POC-PT compared well to the LAB-PT (n 35, 6 on warfarin) with r^2 0.88 and a Passing Bablok (PB) slope 0.99. Similarly, POC-INR compared to LAB-INR had an acceptable r^2 0.89, PB slope 1.00 and mean bias 0.05. aPTT results were sorted into two categories – All (n 41) and aPTT <40 sec (n 29, most on Direct Oral Anticoagulant therapy). The correlation was moderate with PB slopes 1.19, 1.43; r^2 0.81, 0.58; mean bias 2.93, 2.56 seconds respectively. However, as the POC-aPTT had a reported normal reference range of 22-40 seconds, slightly higher than the LAB-aPTT of 24-38 seconds, this was consistent and diagnostic agreement was 88%.

These results show that the Cascade Abrazo has potential as a suitable device to measure POC PT/INR and aPTT. Further studies are required to compare to other reagents/ analysers and to assess the sensitivity and specificity to detect factor deficiencies and monitor warfarin therapy.

O9: EXAMINATION OF PHOTOPERIOD AND GLUCOSE CONTROL IN AUSTRALIAN ADULTS: A PILOT STUDY

P Regmi[1], G Minigo[1]

[1] *Charles Darwin University, Casuarina, Northern Territory, 0810 Australia*

Introduction

Photoperiod, a principal regulator of circadian rhythms, is the duration of light exposure each day. Naturally it is limited to a daylength, however, exposure to artificial light at night has extended photoperiod in humans. Long photoperiod impairs circadian rhythm leading to poor glucose control in animals. This study aims to examine the photoperiod and its association with glycosylated haemoglobin (HbA1c) in Australian adults.

Methods

Participants wore ActLumus wristband (Condor Instruments, Brazil) for seven days to record light exposure, body temperature, activity, and sleep in real time. Anthropometric measurements were taken, HbA1c was measured by point of care device (A1cNow®, PTS Diagnostics, USA), and participants completed Pittsburgh sleep quality index and depression anxiety sleep questionnaires during first clinic visit. Photoperiod was calculated as duration between first and last (>5 lux) light exposure in the day.

Results

This pilot study is still recruiting until May 2024. Interim analysis showed that the participants had mean±SD age 30.7±4.9 years, SBP 112±11 mmHg, DBP 75±9 mmHg, BMI 25.8±6.1 kg/m2, waist-hip ratio 0.88±0.11, and HbA1c 5.1±0.5%. Participants spent 58-73% duration of their day during the light condition and artificial light accounted for 28.9±5.1% of total photoperiod. As the study is still ongoing, statistical analyses have not yet been performed; results will be available in August 2024.

Conclusions

Photoperiod accounted for nearly 2/3rd of the day and nearly 1/3rd of which was artificial light after sunset. Extended exposure of artificial light after sunset may delay circadian rhythm which may impact glucose control in long-term.

O10: HAEMOGLOBINOPATHY DETECTION ON ROUTINE HBA1C TESTING

D. Lovelock

Sullivan Nicolaides Pathology, Bowen Hills, QLD, 4006, Australia.

Introduction

HbA1c testing is routinely used to both monitor and diagnose diabetes mellitus. Haemoglobin separation techniques, such as high-performance liquid chromatography and capillary zone electrophoresis, are commonly used to determine HbA1c concentrations. The presence of a variant haemoglobin, or elevated levels of HbA_2 and/or HbF are often an incidental finding on HbA1c testing. Some authors have suggested that HbA1c testing may be a method of screening for haemoglobinopathies in the general patient population.

Methods

Retrospective case study and literature review.

Results

The HbA1c results from a single week of testing were reviewed for the presence of variant peaks, or elevated HbA_2 and/or HbF. Of the 18820 results obtained, 124 results showed either an elevated HbA_2/HbF, or a variant peak. Presumptive identification of variant peaks performed based on review of electrophoregram data. No further identification of peaks was performed on these samples. Of the 63 patients with a variant peak identified, 40 patients demonstrated the presence of presumed HbS, HbE, HbD or HbS. An elevated HbA_2 peak, characterised as a $HbA_2 > 3.3\%$, was the sole abnormality in 50 cases. A combined elevated HbA_2/elevated HbF was identified in 3 patients, and 7 patients demonstrated an elevated HbF.

Conclusions

While some authors have suggested that HbA1c testing could be used to screen for haemoglobinopathies, there are some important ethical considerations which should be considered, the most important of which is patient consent for screening of a genetic disorder. However, with appropriate laboratory guidance, HbA1c testing may highlight those patients who require further follow-up testing for haemoglobinopathy diagnosis.

O11: INVESTIGATING THE IMPACT OF GUIDELINE ADHERENCE AND COVID-19 ON THE MANAGEMENT OF TYPE 2 DIABETES IN REGIONAL QUEENSLAND

A.Kundur[1], N.Dasi[1], B.Rajan[1], P.Jose[2], I.Singh[1]

[1] *School of Pharmacy and Medical Sciences, Griffith University, Gold Coast, Queensland, 4211 and Australia.*

[2] *School of Medicine and Dentistry, Griffith University, Gold Coast, Queensland, 4211 and Australia.*

Background: Guidelines for the management and treatment of Type 2 Diabetes Mellitus (T2DM), a chronic metabolic disorder, were introduced in Australian health care system to optimize health outcomes for individuals with T2DM. These guidelines include lifestyle modifications, pharmacotherapy, and regular monitoring. Adherence to clinical practice guidelines stresses the importance of routinely monitoring glycated haemoglobin A1c (HbA1c) levels in patients with T2DM, typically recommending testing every six months. However, there is a scarcity of comprehensive research assessing the clinical consequences of the suggested testing frequency. This gap in the literature underscores the need for in-depth studies to establish the significance of adhering to these guidelines.

Methods: This retrospective cohort pilot study utilized the data gathered from January 2019 - December 2021 from general practice in Gold Coast, Queensland, Australia. Guideline adherence rates for each patient were determined by the proportion of HbA1c tests conducted within the intervals recommended by Australian guidelines. Adherence levels were then categorized as low (≤41%), moderate (42%–64%) and high (>65%). To evaluate the impact of COVID 19 on patient health parameters repeated measures one way ANOVA with Tukey's post hoc test used.

Results & Discussion: The results from this study have shown that Patients with low adherence may have a gradual or sustained poorly controlled HbA1c levels, while those with high adherence maintained or improved levels. High guideline adherence group correlated with 100% of patients showing improved lipid profile over three years, indicating the reduced risk factors for cardiovascular risk when compared to low adherence.

Conclusion: Greater guideline adherence and frequency of testing for HbA1c was associated with improved health outcomes for individuals with T2DM. The results from this study may provide further evidence to support the use and adherence to T2DM clinical guidelines.

O12: URINARY NEPHRIN: POTENTIAL TO BECOME A PREDICTOR FOR EARLY GLOMERULAR INJURY DURING PREGNANCY

B. Mesfine[1], D. Vojisavljevic[1], R. Kapoor[1], Y. Kandasamy[1,2], D.Watson[1,2], **D. Rudd[1]**

[1] *James Cook University, Townsville*

[2] *Townsville University Hospital*

Introduction: Pregnancy-related medical complications (gestational diabetes and preeclampsia) may cause renal damage. Urinary nephrin has previously been shown to provide early identification of PE in high-risk pregnancies, however, the role of urinary nephrin for determining early glomerular injury is yet to be explored. This study aimed to investigate the use of urinary nephrin as a predictor for early glomerular injury in a large cohort study (KIDMIN) conducted at the Townville University Hospital.

Methods and Materials: Pregnant women (n=273) were classified into 3 categories according to urinary albumin/creatinine ratio (ACR): normo, micro and macroalbuminuria. The urinary nephrin/creatinine ratio (NCR) cutoff value which could predict the stage of albuminuria was determined as an indirect indicator of early glomerular injury. The percentages of pregnant women with elevated nephrinuria were calculated for each of the ACR categories.

Results: NCR positively correlated with urinary ACR ($r=0.29$, $p<0.0001$) and women with PE had markedly higher NCR. NCR increased comparably in women with normo, micro and macroalbuminuria. Using a cutoff value of 14ng/mg, nephrinuria was detected in 64.9% of women with normoalbuminuria, 94.7% with microalbuminuria, and 100% with macroalbuminuria. Of the women in the normoalbuminuric group demonstrating nephrinuria; 77.8% had a hypertensive disorder and 62.7% had diabetes in pregnancy. NCR was able to predict glomerular injury with a sensitivity and specificity of 93% and 42% respectively when related to PE and 97% and 36% respectively when related to all pregnancy complications. Conclusion: This study found that NCR was elevated in the absence of albuminuria and may indicate early glomerular injury. NCR for early detection of glomerular injury could become a useful tool for monitoring renal function during pregnancy.

O13: LOCAL VALIDATION OF A METHOD TO MEASURE EMICIZUMAB IN BLOOD.

E M Duncan

SA Pathology, Adelaide, SA 5000 Australia.

Emicizumab (Hemlibra) is a novel drug therapy for prophylaxis in haemophilia A. It is a monoclonal antibody which mimics factor VIII (FVIII), serving as a co-factor for human factor IXa to activate factor X. In clinical trials and in real-world experience, emicizumab significantly reduces annual bleeding rates in severe haemophilia A including in those patients with FVIII inhibitors. The drug is given by subcutaneous injection in standard doses and is expected to reach a steady-state level of 22 to 85 ug/mL. There is no requirement to monitor emicizumab, but a drug level is helpful 1. to confirm the expected steady-state level is achieved; 2. to check compliance especially if bleeding; 3. to assess when the patient is also treated with FVIII for surgery; 4. to determine if emicizumab is unexpectedly low, raising suspicion of an anti-drug antibody.

An activity assay for emicizumab is available based on the 1-stage FVIII clotting protocol and using a commercial standard, control material and test samples at a starting dilution of 1/40. This has been set up at SA Pathology and shows satisfactory between-run precision (CV 7.5% - 8.0%), wide linear range (9-125 ug/mL), good accuracy in a between-laboratory comparison (<2% difference to therapeutic targets), and within acceptance in external Quality Assurance surveys. Thirty-nine patients on emicizumab have had steady-state levels measured, with the majority of results 20-76 ug/mL (93 samples tested). The assay has been helpful during surgery when FVIII therapy is also given, although high plasma FVIII can interfere with drug measurement. Emicizumab can interfere with the 1-stage FVIII assay, and if FVIII measurement is also needed it must be measured by a chromogenic method with bovine factors IXa and X. In our experience the emicizumab assay is robust and assists clinical decisions, making it of value to haemophilia treatment centres.

O14: MULTIDISCIPLINARY CASES – EXPECT THE UNEXPECTED

David Gillis

Immunopathologist , Pathology Queensland

Many patients with immunological and inflammatory conditions present with symptoms and signs which require testing across many of the disciplines of pathology.

Several cases of immunology and inflammatory conditions are presented which required testing across chemistry,

haematology, immunology, and microbiology to arrive at a diagnosis. Not infrequently the testing in one specialty will only go so far in obtaining a diagnosis and it is only by putting all tests altogether that appropriate management based on testing can be achieved.

The final diagnosis in each of these cases was somewhat unexpected.

O15: PRE-ANALYTICAL ERRORS AND THEIR PREVENTION IN AN EMERGENCY DEPARTMENT SETTING

C Morias[1,2] G Palmer[2], **A Santhakumar[1]**

[2] *SA Pathology Noarlunga, Noarlunga, SA, 5168, Australia*

[1] *School of Dentistry and Medical Sciences, Charles Sturt University, Wagga Wagga, NSW, 2650, Australia*

Introduction: The pathology laboratory plays a key role in a patient's healthcare journey and the results from pathology testing can influence medication, hospital admission status and differential diagnoses. Errors in the pre-analytical phase may negatively impact the total testing process (TTP) and therefore patient outcomes. The pre-analytical phase involves multiple healthcare professionals from different disciplines and departments.

Methods: An observational study of pre-analytical errors was conducted over two months at an Australian hospital emergency department (ED) and associated laboratory. This study was used to assess the type and number of pre-analytical errors occurring at this site. A Likert-Like survey targeting the ED staff was used to form a basis for the intervention phase of the experiment which aimed to reduce the overall number of pre-analytical errors occurring at this site. An intervention study focusing on educating the ED staff on pre-analytical errors from four bases (request-based, specimen-based, transport-based, and laboratory-based) was conducted over two months.

Results: The observational study found 12159 unique pre-analytical errors over four different categories. The intervention was not successful in significantly reducing the number of pre- analytical errors at this site; however multiple suggestions have been made to help reduce the number of pre-analytical errors in the future. These include introducing a pneumatic tube system, running a monthly/bi-monthly training program, and implementing a training program targeting laboratory staff.

Conclusion: An extended study with more frequent interventions is needed to assess the benefits of running an intervention training program focusing on pre-analytical errors.

O16: RECOMBINANT PROTEIN TECHNOLOGY APPLIED TO INVESTIGATE WHETHER ALLOANTIBODIES TO A NOVEL RH VARIANT IN MATERNAL SERUM, ASSOCIATED WITH HDFN, BOUND TO A AN RHCE CONSTRUCT

Shen Zhao[1], Filip Radenkovic[1,2,] **Robert Flower[2],** Lucia Zacchi[1], Martina Jones[1], Brett Wilson[3], Yew Wah Liew[3], John-Paul Tung[2,4,5,6,] Catherine Hyland[2,4]

Australian Institute for Bioengineering and Nanotechnology, University of Queensland, St Lucia, Queensland 4067, Australia Research and Development Laboratory, Strategy & Growth, Australian Red Cross Lifeblood, Kelvin Grove, Queensland 4059, Australia Red Cell Reference Laboratory, Clinical Services and Research, Australian Red Cross Lifeblood, Kelvin Grove, Queensland 4059

Introduction: A discordant pairing between maternal blood group and the fetal blood group is associated with risk of the formation of alloantibodies and Haemolytic Disease of the Fetus and Newborn (HDFN) in future pregnancies. The RBC panels used to detect antibodies to RBC in the maternal circulation are designed for variants found in Mendelian ratios mainly in European populations low-frequency variants and variants found in other ethnic groups will not be detected. A novel low-frequency variant, CETW (c.486C>G), in the Rh blood group system has been reported to be associated with HDFN. As cells of this phenotype are rare the use of recombinant technology to express the antigen was investigated.

Methods: Full length WT (RHCE*Ce) and CETW (RHCE*CeTW; c.486C>G) RHCE gene constructs were cloned into a mammalian expression vector (pEGFPn1) with a linked C- terminal fluorescent protein (GFP). Following transfection and antibiotic selection, a stable pool of RHCE expressing clones was selected using fluorescence activated cell sorting (FACS). RHCE expression in stable pools was investigated using a commercially available anti-Rh antibody (BRIC69) via flow cytometry. Binding of maternal serum, reported to contain anti-CETW, against these stable pools was also investigated using flow cytometry.

Results: While an expressed RhCE protein (expressing C and e variants) was detected using the developed method, alloantibodies specific to the CETW variant RhCE protein did not react differentially between the CETW variant and wild type RhCe construct at the concentrations tested.

Conclusion: While recombinant technology presents an alternative to standard red blood cell serology, further work is required to optimise presentation of these constructs particularly those with single nucleotide variants.

O17: SKILLS DEVELOPMENT PATHWAY

Maureen Jacobsen, Tony Woods, Robyn Wells, Sarah Just

Australian Institute of Medical and Clinical Scientists

Introduction

Part of being a professional is taking responsibility for your own skills, recognising when they need to be improved and updated and then undertaking formal or other learning pathways. These enhanced skills and knowledge must be applied and shared. Continuing professional development (CPD) is ongoing, career long, systematic and planned.

Aim

This presentation will outline some of the pathways for all working in the laboratory to undertake CPD, be enrolled in a CPD program such as APACE, increase the range of CPD activities and be recognised through certification for that continuing education and professional development.

A/Prof Tony Woods, AIMS Fellowship Committee, will outline the prerequisites and the process of obtaining the formal qualification of the AIMS Fellowship.

Ms Robyn Wells, Editor of AJMS, will present on passing on your knowledge by publishing your case studies, work projects and dissertations.

The medical laboratory science profession in Australia now has its own national professional certification scheme, CMLS. The CEO of AIMS, Sarah Just, will outline the requirements and how to apply for CMLS

Conclusion

By actively engaging in CPD, being enrolled in a CPD program, either certified or are working towards CMLS certification, a medical scientist will not only develop skills and knowledge but enhance their career prospects.

O18: THROMBOTIC MICROANGIOPATHY INDUCED BY THE EXPOSURE TO OXALIPLATIN: A CASE REPORT.

H. Legimun[1], G. Rowley[1], V. Nguyen[1]

[1] Peter MacCallum Cancer Centre, Parkville, Victoria, 3052, Australia

Introduction

Oxaliplatin is a platinum-based chemotherapy that is widely used as part of standard treatment for metastatic colorectal cancer. Although rare, the occurrence of thrombotic microangiopathy (TMA) due to the exposure of this agent has been previously reported and may present as a life-threatening event. Timely diagnosis is critical for the administration of appropriate clinical management. Here, we present a case of acute TMA development after oxaliplatin infusion. Method

Clinical data and pathology results were obtained through institutional electronic medical records and laboratory information systems. Data collection spans from pre-chemotherapy until 25 days after the onset of symptoms. Results were reviewed and compared to other reported cases in the literature.

Results

Post oxaliplatin treatment, the patient exhibited symptoms of TMA with microangiopathic haemolytic anaemia, thrombocytopenia and acute kidney injury. These were demonstrated by the precipitous drop of haemoglobin from 129g/L to 88g/L. Haptoglobin was critically low at >0.1g/L with serum creatinine and lactate dehydrogenase elevated at 306umol/L and 908U/L respectively. Within 24 hours, platelets fell from 120×10^9/L to 45×10^9/L. Haemoglobin and platelet count normalised 7 days after the incident while creatinine took about 25 days to reach normal levels. Coagulation profile of the patient remained normal except for D-Dimer at >20 mg/L. Normal ADAMST-13 activity and the absence of history of gastrointestinal infection further guided the diagnosis of this TMA towards atypical haemolytic uraemic syndrome (aHUS).

Conclusion

Through various laboratory testings and clinical observations, the rapid onset of TMA development post chemotherapy was demonstrated in this case. As the patient was undergoing haemolysis, this further substantiated the need for efficient and prompt care. The role of timely and reliable laboratory testings and efficient supportive clinical care resulted in the recovery of the patient. Oxaliplatin was excluded from patient's future chemotherapy regiments.

O19: TWIN TO TWIN TRANSFUSION AND TWIN ANAEMIA POLYCYTHEMIA SEQUENCE

Morris J[1]

[1] Eastern Health Pathology, Box Hill Hospital

Introduction

In monochorionic diamniotic (MCDA) gestations the twins share a placenta within which there are multiple vascular anastomoses, which run on the surface of the chorionic plate, and allow the blood to flow between the twins in

foetal life or during delivery. Although the blood flow is balanced in most cases, in up to 15% of cases1, net blood flow is toward one of the twins. When this occurs before birth, this is known as either twin to twin transfusion (TTTS) or twin anaemia polycythaemia sequence (TAPS), depending on the size of the vascular anastomoses and associated clinical symptoms. TTTS is caused by imbalanced blood flow through relatively large placental anastomoses from donor to recipient, while TAPS is caused by unbalanced slow transfusion of red blood cells through a few small placental ateriovenous anastomoses. When bleeding between the twins occurs during delivery, it is known as peri partum or acute TTTS. In both TTTS and TAPS, this can lead to large intertwin haemoglobin differences resulting in anaemia of donor twin and polycythaemia in recipient twin. In this case presentation of TAPS, we reviewed clinical and laboratory features of Twin 1 (Donor Twin) and Twin 2 (Recipient twin).

Method

Haematology results were obtained using Sysmex haematology XN10 analyser, and pre transfusion testing from Bio-Rad IH500 analyser.

Results

Donor twin showed a haemoglobin of 66g/L, reticulocyte 22.9% and recipient twin showed a haemoglobin of 247 g/L and reticulocyte count of 4.01% which correlates with the diagnosis of TAPS, in addition to an abnormal ultrasound showing selective intrauterine growth restriction and TAPS leading to emergency Caesarean delivery.

Conclusion

Antenatal ultrasound, postnatal laboratory results, placental pathology play a key role in the diagnosis of TAPS.

1. Tollenaar Lisanne S.A. et al. 2021. TAPS Knowledge and insights after 15 years of research. MFM Maternal-Fetal Medicine 3(1):p 33-41 January 2021

O20: THE UTILITY OF MOLECULAR TESTING IN CYTOLOGY

Alisa Williams

Laboratory Manager, Cytopathology, SA Pathology, FMC

ABSTRACT

It is the era of personalised medicine for cancer diagnosis, management and treatment. This is largely due to rapid development of molecular techniques, such as Next Generation Sequencing, and understanding of cancer genomics. Molecular testing plays a part in diagnosis, treatment and prognosis of cancer with histology and cytology specimens routinely used for testing. Cytology specimens are an ideal specimen as they are obtained via minimally invasive methods and offer a reduced turnaround time for diagnosis. Specimens include fine needle aspiration and serous cavity effusions. Cellular material can either be fresh, alcohol- fixed or formalin-fixed providing multiple options for preparation and laboratory protocols. Cell blocks (formalin-fixed paraffin embedded) are often the specimen of choice.

Molecular testing can be used to identify a wide range of somatic mutations in solid tumours and haematological malignancies, as well as infectious agents. Commonly encountered tumours for molecular testing include malignant melanoma and non-small cell lung carcinoma (NSCLC). More than 50% of malignant melanomas contain BRAF mutations, with the most common variant V600E (up to 90% of cases). Melanoma patients with tumours harbouring mutations in BRAF may benefit from BRAF kinase inhibitor testing. NSCLC is the most common type of lung cancer with diagnosis, management and treatment options rapidly evolving. It is a heterogeneous disease with numerous genomic subtypes. Molecular testing is the standard of care for advanced stage NSCLC.

Cytology is a useful and efficient tool as a source of material to aid in the diagnosis of malignancies and identification of molecular mutations. The identification of molecular markers for diagnosis, prognosis and treatment is an evolving landscape and highlights the importance of a multi-disciplinary approach to patient management.

P1: ANALYZING LIPAEMIC SPECIMENS IN A HOSPITAL LABORATORY – A CASE STUDY

E. Cheung[1]

[1] *Sullivan Nicolaides Pathology, Brisbane, QLD, 4120, Australia*

Introduction

Lipaemia is a well-known interference that affects testing across different disciplines. In a hospital laboratory, various techniques are employed for the correction or removal of interfering lipids. We report a case of lipaemia and describe methods to circumvent this interference.

A 47-year-old female with immune-mediated thrombocytopenia, currently treated with steroids, presents for a routine check-up. Her medical history is otherwise unremarkable.

Method

Biochemistry results were obtained from the analysis of serum on the Abbott Alinity C and Abbott Architect c16000. Hematology results obtained from the analysis of whole EDTA blood on the Siemens XN1000. Coagulation results were obtained from the analysis of sodium citrate plasma on the Siemens CN3000 and Stago STart Max. Ultracentrifugation was performed using the Beckman Coulter Airfuge.

The referring haematologist was interviewed for additional medical history and treatment details.

Results

Lipaemic index of 8 on the Abbott Alinity C. Haemoglobin (Hb) corrected by 24%. (29g/L) There was no significant difference in Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) between using spectrophotometric and electromagnetic detection methods. Total protein corrected by 24%. (26g/L) Sodium corrected by 4%. (5mmol/L)

Conclusion

This case illustrates the effective application of multiple analytical techniques to overcome the challenges posed by lipaemia in a hospital laboratory setting.

P2: BIOCHIP IMMUNOFLUORESCENCE AS A NEW DIAGNOSTIC TOOL FOR AUTOIMMUNE BLISTERING SKIN DISEASES

W Melbourne[1], YZ Chiang, CY Zhao Yang[1], R.Xuan, A Wijayanti, K Tran, J Kim, K Legaspi , N Ishii , H Koga, E Schmidt, D Zilliken ,T Hashimoto, DF Murrell

[1] *Anatomical Pathology. St George Hospital, Sydney, Australia.*

[2] *University of New South Wales, Sydney, Australia*

[3] *Kurume University, Kurume, Fukuoka, Japan*

[4] *University of Luebeck, Luebeck, Germany.*

Abstract

Autoimmune blistering skin diseases are a heterogenous group of disorders characterised by autoantibodies targeting the important structural proteins in the skin that mediate cell-cell or cell-matrix adhesion. The conventional method for diagnosing autoimmune bullous disorders is a multi-step procedure, involving histopathology and direct immunofluorescence, followed by indirect immunofluorescence and enzyme-linked immunosorbent assay (ELISA). Indirect immunofluorescence uses patient's serum and a substrate to visualize circulating autoantibodies. Recently, the Biochip mosaic-based indirect immunofluorescence technique has been made available to allow polyvalent immunofluorescence tests and provide antibody profiles in a single incubation, Sera from patients with provisional diagnoses of autoimmune blistering skin diseases were collected prospectively and sent to the pathology laboratory for indirect immunofluorescence using the Biochip method. The Biochip mosaic contained 6 test substrates which included monkey oesophagus, human salt-split skin, bullous pemphigoid (BP)180, desmoglein (Dsg) 1, Dsg 3, and BP230. The results showed The BIOCHIP mosaic showed a sensitivity and specificity of 86.8%, 85% for BP180 or BP230 being positive in BP and 75%, 97.7% for Dsg1 in PF and 60.9%,73.6% for Dsg3 in PV.

The BIOCHIP mosaic-based immunofluorescence technique presents a novel and faster way of diagnosing autoimmune blistering skin diseases. This test potentially a simple, time and effort saving test that can aid in the diagnosis and screening of BP, PV and PF. Our preliminary results showed that the Biochip method has a high diagnostic accuracy for PV and BP. However, more studies need to be carried out to minimise interpretation error.

Biography

Doctor Wei Melbourne (MSc) has been a snr hospital scientist at immunohistochemistry Anatomical pathology St. George Hospital (Sydney, Australia) since 1997. Wei graduated in Medicine at An Hui Medical College in China, trained as a surgeon and plastic surgeon in Shanghai, and went on to study medical science in Australia; graduated Degree in Health Science 1997; Masters of Public Health in 2006; Master of Science in Medicine at UNSW Australia in 2013. Wei has worked in diagnostic EB laboratory since 2000.

Contact email : wei.melbourne@health.nsw.gov.au

P3: DEVELOPMENT OF AN ONLINE LEARNING MODULE TO ENHANCE MEDICAL LABORATORY SCIENCE UNDERGRADUATE STUDENTS' PROFESSIONAL CONNECTEDNESS AND IDENTITY

D.M. Hanafy[#], G.K. John, L. Pemberton, J. Rae, A.B. Santhakumar[*]

Charles Sturt University, Wagga Wagga, NSW, 2678, Australia

Introduction

Graduate employment metrics, used to measure student and institutional success, puts pressure on higher education providers to explore opportunities for better

preparing students for the workforce. The demand for flexible education platforms has introduced further challenges in designing a curriculum that promotes connectedness with profession. The aim of the study was to understand student perceptions of their future profession and related continuing professional development (CPD) activities, and to identify opportunities that improve student connectedness to the workforce.

Methods

The study consisted of three phases. An anonymous survey was advertised to undergraduate students enrolled in AIMS accredited programs (phase 1) to understand their perception of workforce. These results assisted in developing an online learning module (phase 2) that attempted to consolidate key areas of workforce connectedness. Usefulness of the module was evaluated through an anonymous questionnaire (phase 3).

Results

The majority (79%) of participants noted that they were not members of any professional organisation, 59% were not aware of CPD activities and 41% noted that it would be useful to have a module that hosts job advertisements, information about professional bodies and associated CPD activities. Therefore, in phase 2, a free online module was developed using Brightspace learning management system. The questionnaire in phase 3 highlighted the importance of the module in presenting information in a user-friendly platform.

Conclusion

Many professional bodies offer memberships and CPD activities that undergraduate students are often not aware of. The module provided an easy to access platform, consolidated key information that promotes workforce integration, enhancing students' sense of professional identity

P4: ENHANCING RURAL DIABETES MANAGEMENT: EVALUATING THE IMPACT OF HBA1C POINT-OF-CARE TESTING (POCT) ON CLINICAL OUTCOMES

I. Ferreira[1], L. Matteucci[1], J. Kite[1], C. Boddington[1], K. McLaren[1], J. Denton[1]

[1] Integrated Cardiovascular Clinical Network, Rural Support Service, 5042, South Australia, Australia

Introduction

This study examines the agreement between Point of Care Testing (POCT) and conventional pathology for HbA1c levels in rural diabetes management. Additionally, the study evaluated whether POCT can streamline clinical pathways while maintaining precision and accuracy of traditional pathology, ultimately improving outcomes for patients with TDM-1 and TDM-2, as well as those with impaired fasting glucose.

Methods

A multi-centre evaluation involved 243 HbA1c tests across four rural centres using the Cobas b101 device. Healthcare professionals received training. Patient demographics, test platform, and clinical outcomes were collected. The precision and accuracy were compared using statistical tests, including the Shapiro-Wilk test for data normality, Mann-Whitney U test for significance, and logistic regression to assess predictor variables.

Results

The Bland-Altman plot revealed strong agreement between POCT and conventional pathology, with only one outlier attributed to medication changes. Both datasets exhibited non-normal distributions ($p < 0.001$). Despite this, there was no significant difference between POCT and conventional pathology ($p > 0.05$). Additionally, logistic regression analysis identified HbA1c as a significant predictor for changes in care plans, with 12% of patients undergoing treatment adjustments. Furthermore, 97% of patients expressed satisfaction with the POCT intervention. Conclusion

POCT demonstrates comparable results to conventional pathology, facilitating timely clinical decisions and improving diabetes management in rural areas. Its use can enhance patient engagement, streamline processes, and promote health equity, especially in regions with limited healthcare access.

P5: ERYTHROCYTE MICROVESICULATION HEALTH AND DISEASES.

U. Maluze

Aim: To demonstrate (using Guava Easy flow-cytometer) that micro vesicles are released from erythrocyte membrane naturally in normal and disease conditions, without inducing with calcium chloride; and also, to know how many erythrocytes makes a micro vesicle.

Methods: 15mls of blood samples from the stored in CPDA or SAG-M was provided by NHS blood and transplant, and 10 ml of this blood were mixed thoroughly with 30ml of phosphate buffered saline in a 50ml centrifuge tube, and centrifuge immediately at 600 x g at 4 degrees centigrade for 10 minutes to remove soluble plasma proteins. The supernatant was removed and discarded after centrifugation. This process was repeated three times in order to remove any erythrocyte bound plasma proteins.

Erythrocytes were then counted with haemocytometer using x10 objective lenses and x40 for magnification). The number of red blood cells countered were recorded and documented. Flow cytometry analysis was conducted using 10ul of the eMV stock sample which were diluted in 190ul (1:20) of phosphate buffer saline.Biuret protein assay and Agarose gel electrophoresis were performed. Haemolysed red blood cell was used as a negative control method.

Results: Erythrocyte cell counts was performed using haematology cell counting chamber and analysed with Guava flow cytometer on three consecutive times. The number of cells counted was greater than 400cells. About 3446666 million of micro vesicles were released by 1ml of the isolated erythrocyte. The unknown protein component of micro vesicles was determined as 2.5mg/ml and the protein bands separation were identified using agarose gel electrophoresis.

Conclusion: The isolated micro vesicles were analysed using Guava Easy flow cytometer. The protein concentration was quantified using Biuret assay method, and the protein band separation was analysed using Agarose gel electrophoresis.

P6: EVALUATING CLINICAL EQUIVALENCE BETWEEN DIFFERENT CENTRIFUGAL CONDITIONS

T.B.Q. Goh[1], F.M.F. Chay[1],G.B. Tan[1], L.F.K. Yiap[1], A.Omar[1], K.Lim[1], L.Z. Ong[1]

[1] Department of Laboratory Medicine, Alexandra Hospital, Singapore 159964, Singapore

Introduction

To improve the turnaround for analyte testing, we investigated if the centrifugal speed and time of the Rotina 380 Benchtop Centrifuge at 1680 RCF for 8 minutes can achieve equivalent performance as its recommended setting of 1000-1300 RCF for 10 minutes.

Method

22 sets of donor blood samples were collected in BD Vacutainer Gel Serum Separator Tube (SST) with one evaluation and one control sample each. After standing at room temperature for 45 to 70 minutes, each donor evaluation sample was centrifuged at 1680 RCF for 8 minutes while control sample was centrifuged at 1300 RCF for 10 minutes concurrently using the same slot position. Platelet counts, gamma-glutamyltransferase, creatinine, sodium, lipase, urea, hemolysis index, icterus index, lipemic index, lactate dehydrogenase, potassium and high sensitivity troponin I were subsequently analysed on the Sysmex XN-10 and Abbott Alinity ci platforms.

Results

Platelet counts of all 22 evaluation samples were within acceptable range of 0-1 x 109/L (Clinical and Laboratory Standards Institute (CLSI) guidelines). Result differences between the evaluation and control samples of 19 donors passed the Royal College of Pathologists Australian's (RCPA) 2021 Analytical Performance Specifications (APS) criteria for all analytes. Differences in potassium levels obtained for three donor samples were out of acceptable criteria and two of the samples contained presence of fibrin strands that could have led to the differences.

Conclusion

For the eight routine chemistry analytes, clinical equivalence was demonstrated between the evaluated centrifugal condition of 1680 RCF for 8 minutes and the recommended centrifugal condition of 1300 RCF for 10 minutes. Therefore, the evaluated centrifugal condition can be used for analyte testing without compromising its accuracy while improving turnaround time.

P7: EVALUATING POINT-OF-CARE CRP TESTING FOR ANTIBIOTIC PRESCRIPTION GUIDANCE IN REGIONAL AND REMOTE SOUTH AUSTRALIAN GENERAL PRACTICES

L. Matteucci[1], I. Ferreira[1], J. Kite[1], C. Boddington[1], K. McLaren[1], J. Denton[1]

[1] Integrated Cardiovascular Clinical Network, Rural Support Service, South Australia, Australia

Introduction

The study objective was to investigate the primary reason for antibiotics prescription when using Point of Care testing (POCT) in rural and remote medical centres in South Australia, focusing on the potential roles of CRP levels, reasons for GP visits, and other contributing factors.

Methods

The study involved 273 CRP tests conducted with the Cobas b101 device at four different rural centres. The multi-centre evaluation included healthcare professionals at these centres, each receiving training on using the device. During the study period, the following data was recorded: CRP results, patient demographics, reasons for GP visits, the need for antibiotic prescriptions, subsequent hospitalizations, and patient satisfaction.

Results

The results showed that 67% of participants were female, with ages ranging from 9-97 years. CRP levels

varied widely, with a mean of 23 mg/L and a median of 6 mg/L. Among the participants, 37% received immediate antibiotics, while 38% required hospitalization.

The thematic analysis revealed that conditions such as respiratory issues, gastrointestinal problems, and infection and inflammatory diseases had higher rates of antibiotic prescriptions at elevated CRP levels. This suggests that CRP levels can be a determinant for antibiotics in certain contexts, but reasons for GP visits still play a critical role in guiding treatment decisions.

Conclusion

CRP measurements using POCT can indicate when antibiotics are needed, particularly if other clinical signs support it. However, GP visit reasons still play a significant role in guiding medical interventions. The results underscore the importance of effective CRP screening while considering other clinical factors to avoid overprescribing antibiotics and to combat antibiotic resistance.

P8: IDENTIFICATION OF GENETIC VARIANTS ASSOCIATED WITH DEEP VEIN THROMBOSIS (DVT) IN A COHORT OF PATIENTS AFFECTED WITH DVT IN SRI LANKA.

Hasaranga KLJ[1], Noordeen N[1], Wetthasinghe TK[1], Dissanayake VHW[1]

[1] *Centre for Genetics and Genomics, Faculty of Medicine, University of Colombo, Sri Lanka.*

Introduction:

Deep Vein Thrombosis (DVT) is a thromboembolic manifestation that occurs due to various genetic and non-genetic etiologies. The aim of this study was to design and implement a molecular assay to identify genetic variants suggestive of causing DVT in a cohort of patients affected with DVT in Sri Lanka.

Methods:

A comprehensive literature review was conducted to identify genetic variants associated with DVT. Using online tools Tetra-Amplification-Refractory-Mutation-System Polymerase Chain Reaction (T-ARMS-PCR) protocols were designed, developed and optimized to genotype the extracted DNA samples of the subjects with prior informed consent. The genotype results were validated by gold-standard Sanger sequencing for accuracy before sample genotyping.

Results:

110 subjects comprising 62 (56.4%) females and 48 (43.6%) males ranging 2 to 73 years of age were genotyped. There were 14 (12.7%) normal for the variant (CC), 39 (35.5%) heterozygotes (CA) and 57 (51.8%) homozygotes (AA) for *CYP4V2* c.775C>A (rs13146272) variant, with an ancestral (C) allele frequency of 0.3045 and variant (A) allele frequency of 0.6955 at Hardy Weinberg's equilibrium. For *F5* c.2573A>G (rs4524) variant 37 (33.6%) were normal for the variant (AA) while 73 (66.4%) heterozygotes (AC) and nil homozygotes (GG) were detected with a calculated allele frequency of 0.6651 and 0.3349 for both ancestral (A) allele and variant (G) allele respectively.

Conclusion:

The developed and optimized T-ARMS-PCR assay of this study can be implemented to screen the *CYP4V2* c.775C>A (rs13146272) variant in DVT diagnosed patients. Allele frequencies of both *CYP4V2* and *F5* variants were consistent with published South-Asian population allele frequencies suggesting the reliability of our finding.

P9: MODIFICATION OF AN EXPERIENTIAL, LEARNING-BY-DOING APPROACH TO CLINICAL FLOW CYTOMETRY TRAINING AT THE UNIVERSITY OF WESTERN AUSTRALIA

Linden M[1], Fuller K[1], Hui H[1], Santos C[1], Fernandez S[1]

[1] *University of Western Australia*

We developed a pedagogical approach to flow cytometry training, grounded in experiential, problem-based learning-by-doing. In this approach, students perform compensation, calculate staining index, and collaboratively design a gating strategy to analyse clinical flow cytometry data sets using FlowJo software. The approach is implemented in postgraduate programs designed to train specialist medical scientists, including the Master of Infectious Disease and the AIMS accredited Master of Clinical Pathology, where it leverages unique active and collaborative eLearning spaces at The University of Western Australia. The approach allows students to achieve learning outcomes not previously possible with traditional teaching formats, with favourable perceptions and improved student confidence in performance of cytometry data analysis and interpretation.

Since it was first implemented and published in 2016, our approach has been modified to include a self-directed offering (adapting to restrictions on face-to-face learning during the pandemic), updated for changes in consensus guidelines for clinical flow cytometry published by the Australasian Cytometry Society, and modified to

accommodate changes in audiovisual, information technology and software licensing agreements as part of a switch to Bring Your Own Device (BYOD) approaches at The University of Western Australia.

We will present data and reflections on how our approach has been adapted in the changing landscape of medical scientist training in the pandemic and post-pandemic setting. We will present data on how these adaptations have impacted student confidence and competence in the analysis and interpretation of clinical cytometry data.

P10: MULTICENTRE EVALUATION OF POINT-OF-CARE NT-PROBNP TESTING FOR CARDIAC RISK ASSESSMENT AND CLINICAL OUTCOMES IN RURAL SOUTH AUSTRALIA

J. Kite[1], I. Ferreira[1], L. Matteucci[1], T. Wilson[1], C. Boddington[1], K. McLaren[1], J. Denton[1]

[1] *Integrated Cardiovascular Clinical Network, Rural Support Service, 5042, South Australia, Australia*

Introduction

This research seeks to assess the effectiveness of Point-of-Care NT-ProBNP testing in evaluating cardiac risk, and how its incorporation affects clinical pathways for cardiac care in rural South Australia. The study specifically explores variations in NT-ProBNP levels based on demographic factors and patient history.

Methods

A multicentre evaluation of the Cobas h232 for NT-ProBNP was conducted at four general practice clinics in rural South Australia. NT-ProBNP levels were cross-analysed with themes emerging from a thematic analysis. The study examined demographic data, trends in NT- ProBNP, and the relationship between NT-ProBNP levels, patient demographics, and clinical outcomes.

Results

Data from 84 tests were analysed, with a mean age of 79 years and a median age of 82 years. The mean NT-ProBNP level was 1,554 pg/mL, with a median of 671 pg/mL. 71% of the of patients tested for NT-ProBNP with unknown history of heart failure met the criteria for specialists follow up due to NT-ProBNP >300 pg/mL. The remaining 29% triggered a cardiologist referral or ECHO referral for unknown reasons.

The linear regression analysis suggests that NT-proBNP levels increase with age in both genders, more noticeably in males. This suggests a need for further research and supports age and gender-stratified clinical guidelines.

Conclusion

Point-of-care NT-ProBNP testing is valuable for cardiac risk assessment in rural settings. Considering clinical themes, gender, and age in NT-ProBNP thresholds enhances clinical pathways and patient satisfaction, supporting the broader adoption of POCT NT-ProBNP testing for comprehensive cardiac risk assessment and tailored treatment strategies.

P11: PERSPECTIVES AND EXPERIENCES OF STUDENTS AND STAFF OF A MICROSESSION MEDICAL LABORATORY SCIENCE (PATHOLOGY) UNDERGRADUATE COURSE.

B.P. Budiono, A. Street, D.M. Hanafy, L. Pemberton, A.B. Santhakumar

Charles Sturt University, Wagga Wagga, NSW, 2678, Australia

Introduction

In 2021, a microsession model was implemented for the Bachelor of Medical Laboratory Science (Pathology) course at Charles Sturt University. This condensed course allows a student to graduate within 3 years full-time (via six 8-week microsession blocks per year) instead of the usual 3.5-years (in a conventional 16-week block trimester per year).

Method

An anonymous, cross-sectional online survey was disseminated to all enrolled students and teaching staff within the program. Likert-scale questions relating to student & staff satisfaction and overall learning and teaching experience was collected and analysed using SPSS (v.29). Open-ended comment questions regarding facilitators and barriers to student success were thematically analysed using Nvivo (v.13) software.

Results

Overall themes included whether students felt disadvantaged in a condensed mode of learning compared to the conventional course. Would students report higher stress in this format? Would this be balanced by certain benefits such as quicker completion, and a more efficient intensive school for practical classes? Academics had experience of both types of programs, and although they might have individual preferences, were staff readily able to adapt? Were they able to implement their pedagogical strategies optimally in a condensed session?

Conclusion

Our study provides a unique insight into our student population and teaching staff, by highlighting the needs

and priorities that students and staff valued in their educational experience. This data is useful for both educators and policy makers in subject and course design. Although certain adaptations in pedagogical methods have to be considered when teaching in a microsession model, both staff and students alike have expressed numerous benefits.

P12: VERIFICATION OF THE ABBOTT ALINITY HQ ANALYSER FLAGGING (VERSION 5.6)

G. Rowley, C. Durkin, V. Nguyen

Peter MacCallum Cancer Centre, Melbourne, Victoria, 3051, Australia

Introduction

Analyser flagging is an essential technology that alerts the laboratory that abnormal scatter plots are present and is suspicious of atypical cells. Version 5.6 will enable us to set the flagging sensitivity, from level 1 through to 4, for Blasts (BL) and Variant Lymphocytes (VL). Flagging must ensure abnormalities are identified but non-specific flagging is minimalised.

Methods

A 3-step process was adopted for flag setting determination and confirmation. Fresh patient samples (N=13) with true positive BL or VL flags were selected, the gating level was adjusted to determine at what point the sample ceased to flag.

Then further samples (N=24) which flagged were correlated with the blood film report. The flagging setting was adjusted as above.

Finally, a larger cohort of samples (N=100) with the flagging from step-2 were locked in and correlated with the blood film.

Results

The patients were separated as Lymphoid or Myeloid or non-haematological disease cohorts. Sensitivity, specificity and accuracy truth tables were compiled.

The flagging settings with V5.6 software upgrade have shown a slight improvement. However, the sensitivity and specificity were still sub-optimal, especially the degree of False positivity for non-haematological patients.

The frequency with which a blood film review is mandated is based on the diagnosis. Initial investigations with the Alinity, at installation, required our review frequency to be reduced for lymphoid diseases. Version 5.6 has enabled a review of these criteria.

Conclusion

The flagging settings has not substantially reduced the number of blood films requiring review. Together with the implementation of a lower frequency of a blood film review, as determined by diagnosis, and with the frequency of review requirements from the reoccurrence of flagging, morphology efficiencies will be achieved. Additionally, our “Rules based” middleware algorithms (AMS) provide us with confidence that patients with significant changes are reviewed.

Australian Professional Acknowledgement of Continuing Education (APACE)

3 APACE credits per set of questions will be awarded if at least 8 out of 10 questions are answered correctly.

Journal-based CPD No. 102
Page 1 of 2

Questions relating to the article *'Correlation between Beckman Coulter DxH900 and peripheral blood smear counts for nucleated red blood cell validation'* at page 210 of this issue.

1	This study looked at the nRBC results provided by the DxH and determined if these values could be used without the need to perform a manual differential for nRBC.	True/False
2	Manual count of nRBC via light microscopy by an experienced morphologist is the currently validated and implemented method providing results to health professionals.	True/False
3	A red blood cell contains approximately 640 million haemoglobin molecules.	True/False
4	Orthochromic normoblasts are 8 to 12µm in size.	True/False
5	Normally nRBCs reside in the bone marrow so when immature forms of RBCs are found in the peripheral circulation they pertain to different disease states.	True/False
6	β–Thalassemia Major presents with increased extramedullary hematopoiesis.	True/False
7	VCS 360 Technology analyser uses three measurements: individual cell volume (V), low-frequency conductivity (C) and laser-light scatter (S).	True/False
8	The number of nRBC per 100 WBC is expressed as 100 WBC/nRBC.	True/False
9	When evaluating the data obtained from the morphologist, nRBC counts yielded a higher count than the analyser per 100 WBC.	True/False
10	Interferences may result in the under counting of nRBCs.	True/False

Name: _____

Email: _____

Please photocopy this page or print it from the electronic AJMS which is stored in the AIMS 'Member Centre' under the heading 'Journal' at www.aims.org.au. ***Circle your answers, then scan, and enter them as activities in the APACE diary under 'My CPD Record'.***

https://aims.org.au/apace

Australian Professional Acknowledgement of Continuing Education (APACE)

3 APACE credits per set of questions will be awarded if at least 8 out of 10 questions are answered correctly.

Journal-based CPD No. 103
Page 2 of 2

Questions relating to the article *'Human Endogenous Retroviruses – for better or worse.'* at page 229 of this issue.

1	Approximately 40% of human DNA is identical or similar to viral genetic material.	True/False
2	Retrotransposons can transpose themselves within the host cell genome, but unlike retroviruses they cannot produce infectious particles.	True/False
3	The majority of retrotransposons that occur in the human genome are ancient and inactivated by mutation.	True/False
4	Retroviruses known to infect humans today, actively insert new genetic material into the genome.	True/False
5	APOBEC3G is a retrovirus-derived protein that can mutate viral RNA, making it less likely to be translated into viral proteins.	True/False
6	Proteins produced by retrotransposon activation may be perceived as 'foreign' by the immune system leading to autoimmune diseases.	True/False
7	Retrotransposons help to protect the body from infection in a variety of mechanisms affecting both the innate and adaptive immune systems.	True/False
8	Retrotransposons may activate oncogenes.	True/False
9	DNA transposons are inactive and are more common than retrotransposons in the human genome.	True/False
10	The human genome contains retrotransposon fragments from around 30-50 retrovirus families.	True/False

Name: _____

Email: _____

Please photocopy this page or print it from the electronic AJMS which is stored in the AIMS 'Member Centre' under the heading 'Journal' at www.aims.org.au. ***Circle your answers, then scan, and enter them as activities in the APACE diary under 'My CPD Record'.***

https://aims.org.au/apace

The APACE (Australasian Professional Acknowledgement of Continuing Education) scheme is a voluntary programme that recognises continuing education, formal courses and a wide range of professional activities which contribute to your professional growth.

The healthcare industry is undergoing rapid change. We are expected to keep our knowledge and skills up to date to enable us to perform to the highest professional standard. The APACE scheme provides a method by which your professional activities are recognised.

Changes to APACE due to COVID-19 pandemic UPDATE

An APACE Certificate is usually awarded on attaining 100 CEU credits within a two year period.

As webinars and online conferences, meetings and workshops are all interactive, it was considered that this is the same as attending in person, therefore the same number of points will be awarded for attendance either virtually or face-to-face. This should enable more members to attend as no travelling time, costs and in some cases the online attendance with be without cost to the attendee.

Therefore, the extended time frame due to Covid-19 will no longer apply.

https://www.aims.org.au/apace

Australian Institute of Medical Scientists

Immunohaematology Quality Assurance Program

Meet your accreditation requirements with the AIMS Immunohaematology Quality Assurance Program (QAP)

- Designed to suit both small and large laboratories;
- Includes blood grouping, antibody screening/identification and compatibility testing;
- Runs bi-monthly starting in July at the beginning of each financial year;
- The reports provide participants' own results along with graphical representation of results of their peers (de-identified) allowing for easy comparison and analysis.

For enrolment enquiries please contact:
aimsqap@aims.org.au

Instructions to authors

The following instructions are based on the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals", also known as the Declaration of Vancouver, and on the *Australian Government Style manual: for authors, editors and printers*, 6th edition, 2002. URLs were correct on September 29th, 2008.

Manuscripts that do not fully comply with the following 'Instructions to Authors' may be returned for revision before they are considered for publication.

The *Australian Journal of Medical Science (AJMS)* will consider for publication any paper relevant to the field of Medical Science. Disciplines include blood banking, clinical biochemistry, haematology, histopathology, immunology, microbiology and molecular biology. Areas of general interest to medical laboratory scientists, including toxicology, epidemiology, public and community health, and professional and management issues will also be considered.

Papers published in the *AJMS* are in the form of:

- Review Articles
- Original Articles
- Brief Communications
- Technical Notes
- Case Studies
- Letters to the Editor
- Book Reviews

Articles submitted for publication are understood to be offered only to the *AJMS* and those accepted become the property of the *AJMS*.

All individuals listed as authors must have made a substantial contribution to the conception and design of the study, the acquisition of data or the analysis and interpretation of data; the drafting of the article or revising it critically for important intellectual content; and final approval of the version to be published. The corresponding author must take responsibility for obtaining permission from all the authors for the submission of any version of the manuscript and for any changes in authorship.

When the manuscript is submitted the authors must disclose any potential conflict of interest and/or commercial support.

Requirements & preparation of manuscripts

General

Articles should be submitted in electronic format to programs@aims.org.au. If an article is too large to be submitted by email, it should be submitted on an or USB stick.

Number pages consecutively commencing with the title page.

Arrange the article in the following sequence:

- Title page
- Abstract and key words
- Main Text
- Acknowledgements
- References
- Tables - each table, complete with title and footnotes, on a separate page
- Legends for illustrations.

Authors should ensure that their manuscript communicates their ideas and concepts simply and clearly so that the article is easily read and understood. Authors are strongly recommended to refer to the recommendations on reporting standards as outlined in the statements and checklists of the CONSORT group (see: http://www.consort-statement.org/) and similar groups such as STARD (see: http://www.stard-statement.org/). The principles outlined in these standards may be used as general guidelines and not just as applied to clinical trials and diagnostic studies.

Title page

The title of the article should not exceed three lines (40 characters per line), including punctuation and spacing. All authors must be identified on the title page (e.g. William Smith, Susan Yeo, ...). Where applicable, the title page should also include the name of the institution with which each author is affiliated and to which the work should be attributed. In the case of multiple authors, the name, postal address, email address, telephone and facsimile number of the author responsible for correspondence relating to the manuscript should be indicated.

Abstract & keywords

The abstract should be approximately 150 words and should make sense when read alone or in conjunction with the article. The abstract should be a concise overview that describes the important details of the article including the purpose of the study/investigation, basic procedures (study subjects/experimental animals/observational and analytic methods) and the results and principal conclusions. New and important aspects of the work and its implications may also be included. References should not be included.

Three to ten keywords may be listed. Authors are advised to comply with the terms from the Medical Subject Headings (MeSH) list from Index Medicus (see http://www.nlm.nih.gov/mesh/). Keywords should be given below the Abstract.

Text

The style of writing should conform to acceptable English usage. Do not use slang, medical jargon or unnecessary abbreviations. Accepted spelling is the first choice given in the latest edition of the Macquarie Dictionary.

Wherever possible, observational or experimental articles should be divided into sections headed:

- Introduction
- Materials and methods
- Results
- Discussion
- References

For other types of articles such as commentaries, reports and reviews, use an appropriate format or consult the Editors for guidance. Do not include a separate section for conclusions, these should be given in the discussion.

Introduction

Clearly state the purpose of the article leading the reader from the known to the unknown. Summarise the rationale for the study and state the question to be answered as appropriate. Give only strictly pertinent references, and do not review the subject extensively.

Materials & methods

Present the materials and methods in a logical sequence. Describe the selection of the observational or experimental subjects (patients or experimental animals, including controls) clearly. Notification of ethics approval must be given where relevant. Identify the methods, apparatus and procedures in sufficient detail to allow other workers to reproduce the results. Give references to established methods, including statistical methods. Adequately describe new or substantially modified methods. Identify precisely all drugs and chemicals used, including generic name(s), dosage(s), and route(s) of administration. Do not identify patients or hospitals without consent.

Results

Present the results in the same sequence as given in the Materials and methods; use tables and illustrations where these will help the reader understand the work being presented. Do not repeat in the text all the data in the tables or illustrations.

Discussion

Indicate the new and important aspects of the study and emphasise the conclusions that follow. Do not repeat in detail data given in the Results section and do not add new data. Include in the Discussion the implications of the findings and their limitations and compare the observations to other relevant studies. Recommendations may be included if appropriate. Link the conclusions with the goals of the study and answer the experimental question stated in the Introduction. However, avoid unqualified statements and conclusions not completely supported by your data. Avoid claiming priority and alluding to work that has not been completed. State new hypotheses when warranted, but clearly label them as such.

Acknowledgements

Acknowledge individuals who have made substantial contributions to the study including technical work and financial support. Authors are responsible for obtaining consent from all the individuals acknowledged by name as inclusion may be interpreted as an endorsement of the article's contents.

References

The AJMS uses a modified Harvard System (author-date system).

Throughout the body of the manuscript cite the author/s name and the publication year in parentheses as in the following examples:

(i) Research in this area (Jones 1999) …

(ii) It has been successfully demonstrated that (Smith and Brown 1981; Auteur 1995; Scienziato *et al* 2007).

(iii) Following further investigation, Wetenschapper (2002 highlighted the difficulties inherent in…

Where there are three or more authors, acknowledge only the first author, e.g. (Smith *et al* 2007). For two authors the following style should be used: (Smith and Brown 2007).

The reference list should be in the format described below. Journal titles should be abbreviated in Index Medicus format (see: ftp://nlmpubs.nlm.nih.gov/online/journals/ljiweb.pdf) using standard abbreviations from the ISSN List of Title Word Abbreviations (see: http://www.issn.org/en/node/344) All authors should be given in the reference list.

Do not use abstracts as references. "Unpublished observations" and "personal communications" may not be used as references, although references to written, not verbal, communications may be cited (in parentheses) in the text. Include in the references manuscripts accepted but not yet published, designate the journal followed by "in press" (in parentheses). Information from manuscripts submitted but not yet accepted should be cited in the text as "unpublished observations" (in parentheses).

Examples of the correct form for references are given below:

Journal Reference:

Stein MK, Downing RW, Rickels K 1978. Self-estimates in anxious and depressed outpatients treated with pharmacotherapy. *Psychol Rep* 43: 487-492.

Personal Author(s) of a book:

Osler AG 1976. *Complement: mechanisms and functions*. Englewood Cliffs: Prentice-Hall.

Editor, Compiler, Chairman as Author:

Rhodes AJ, Van Rooyen CE, comps. 1968. *Textbook of virology: for students and practitioners of medicine and the other health sciences*. 5th ed. Baltimore: Williams and Wilkins.

Chapter in Book:

Weinstein L, Swartz MM 1974. Pathogenic properties of invading microorganisms. In: Sodeman WA Jr, Sodeman WA, eds. *Pathologic physiology: mechanisms of disease*. Philadelphia: WB Saunders; 457-472.

Online documents:

National Center for Biotechnology Information. OMIM: online Mendelian inheritance in man. http://www.ncbi.nlm.nih.gov/omim. Accessed February 25, 2007.

Tables

Number tables consecutively with Arabic numerals and supply a brief title for each. Give each column a short or abbreviated heading. Place explanatory matter in footnotes, not in headings. Explain in footnotes all non-standard abbreviations used in each table.

For footnotes, use the following symbols in this sequence:

* † ‡ § ¶ ** ††

In preparing tables, consideration should be given to the page width of the Australian Journal of Medical Science. All tables should be prepared for publication vertically. In the text, cite each table in consecutive order, and mark in the margin of the text its approximate location.

If data from another published or unpublished source is used, written permission must be obtained and a copy must accompany the manuscript.

Illustrations

Colour illustrations may be submitted on a USB stick. Images should be scanned at a minimum of 300 dpi.

When plotting points, the following symbols are preferred:

In most instances, figures will be reduced to one column in width. All letters and numbers should be drawn to be at least 1.5 mm high after reduction, symbols at least 1.0 mm. Titles for illustrations belong in the legends for illustrations and not on the illustrations themselves.

Photomicrographs must have internal scale markers and the magnification must be stated. Symbols, arrows, or letters used in the photomicrographs should contrast with the background.

Cite each figure in the text in consecutive order, e.g. "Figure 1 illustrates …" or "… as shown (Figure 2)". If a figure has been published, acknowledge the original source and submit with the manuscript written permission from the copyright holder to reproduce the material. Permission is required, regardless of authorship or publisher, except for documents in the public domain.

Legends for illustrations

When symbols, arrows, numbers, or letters are used to identify parts of illustrations, identify and explain each one in the legends. The figure legend must contain a boldface (a) name ("Figure" + arabic figure number) and (b) substantive title.

Abbreviations

Use only standard abbreviations (see list of commonly used abbreviations).

Avoid abbreviations in the title. The full term for which an abbreviation stands must precede its first use in the text unless it is a standard abbreviation for a unit of measurement.

Report measurements in the units in which the measurements were made. In most countries the International System of Units (SI) is standard.

Commonly used abbreviations

Abbreviation or Symbol	Standard Units of Measurement
g	gram
g	gravity
Hz	hertz
h	hour
IU	international unit
K	kelvin
kg	kilogram
L	liter, litre
m	meter, metre
min	min
M	molar
mL	millilitre
mol	mole
N	newton
nm	nanometre
p	probability
rpm	revolutions per min
s	second
wk	week
yr	year

Additional information

The following are useful sources of information. The first two publications are used by the AJMS as standard references.

Style Manual Committee. Council of Biology Editors. *Scientific style and format: the CBE manual for authors, editors, and publishers.* 6th ed. Cambridge University Press, 1994.

Style manual for authors, editors and printers. 6th ed. John Wiley & Sons Australia Ltd, 2002.

O'Connor M, Woodford FP. *Writing scientific papers in English: an ELSE-Ciba Foundation guide for authors.* Amsterdam, Oxford, New York: Elsevier-Excerpta Medica, 1975.

Day RA. *How to write and publish a scientific paper.* Philadelphia, Institute for Scientific Information Press, 1979.

Zeiger M. *Essentials of writing biomedical research papers*. 2nd ed. New York, McGraw-Hill, 2000.

Matthews JR, Matthews RW. *Successful scientific writing: a step-by-step guide for the biological and medical sciences*. 3rd ed. Cambridge, Cambridge University Press, 2007 [Also available in eBook format.]

Medical Training Solutions

Online Training and Competency Assessment for the Clinical Lab and Point of Care

Features/Benefits

- Streamlined tracking and documentation;
- High quality multimedia content developed with the University of Washington;
- Over 50 hours of Continuing Education (CE) credit;
- Create your own tests;
- Upload and track your existing documents;
- Used by over 1,500 laboratories.

As an AIMS member you have exclusive access to the Medical Training Solutions (MTS) online clinical laboratory continuing education programme. Please note only current financial members of AIMS are eligible to access MTS. Free Student members do not have access to this resource.

Overview

AIMS purchases a yearly subscription to the Medical Training Solutions (MTS) online clinical laboratory continuing education programme as one of your member benefits. AIMS members receive unlimited access to the laboratory training and competency assessments from MTS.

MTS is accessible online from work and at home so it is most convenient to you. The laboratory training and competency assessment provides training, continuing education and rotating competency assessment tests on clinical laboratory procedures in an engaging format.

MTS adds new training courses periodically. At the moment there are more than 60 courses in the Training Library and over 90 presentations in the Lecture Library.

https://www.aims.org.au/Web/Web/Member-Centre/Medical-Training-Solutions-MTS.aspx

http://www.medtraining.org/

The *Training Library* covers topics in:

- Safety
- Specimen Collection
- Microscopy
- Molecular Techniques
- Chemistry
- Haematology
- Coagulation
- Microbiology
- Body Fluids
- Blood Banking
- Immunology
- and more!!

Australian Institute of Medical and Clinical Scientists

Fellowship of AIMS

The AIMS Fellowship is an attractive and highly competitive option to academic post graduate degrees. The Fellowship is recognised by the Department of Health for meeting the requirements for supervision of category GX and GY laboratories.

TRANSFUSION SCIENCE
CLINICAL BIOCHEMISTRY
HAEMATOLOGY
ANATOMICAL PATHOLOGY
IMMUNOLOGY
MICROBIOLOGY
GENERAL (including Core Laboratory)

Qualification for the Fellowship is by EXAMINATION in one of the eight disciplines.

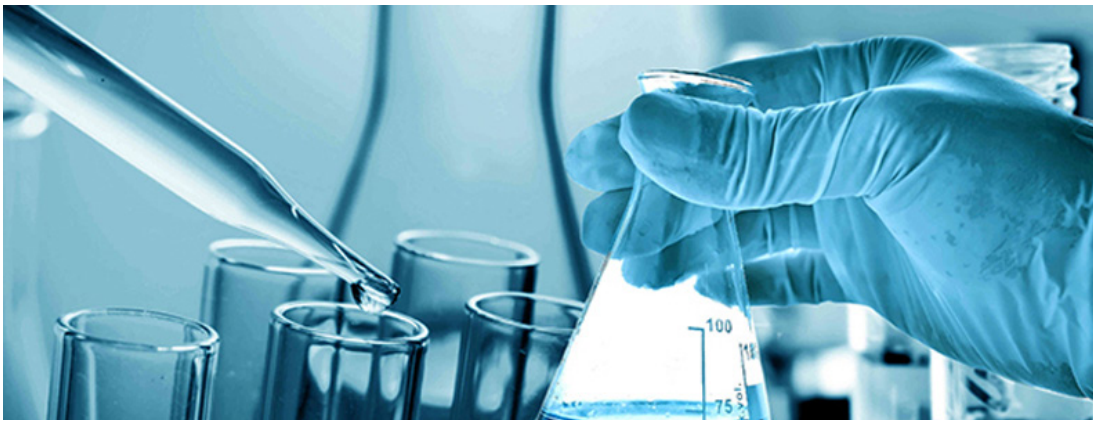
Candidates for the Fellowship must have been members for a minimum of two years and must meet certain other criteria.

The Fellowship program is modular - candidates must complete:

- two compulsory modules
- two elective modules
- a viva voce examination
- a scientific diseration OR a successful relevant research degree thesis completed within the last two years (eg Honours, Masters, PhD); OR a relevant paper published in a peer reviewed journal.

To enrol in the Fellowship program or for further information please contact the AIMS National Programs Manager:

Ph: +61 7 3876 2988
E mail: programs@aims.org.au



Australian Institute of Medical and Clinical Scientists

cmls
Australian Council for the Certification of the Medical Laboratory Scientific Workforce

qualified ✓
competent ✓
certified ✓

Changes to Certification arrangements for the Medical Laboratory Science Profession

From April 2023, the Australian Council for the Certification of the Medical Laboratory Scientific Workforce (CMLS) Board are no longer accepting applications for certifications directly. Instead, professional bodies operating CMLS approved CPD schemes will be able to issue certification on behalf of the Council for their members who meet the requirements for certification as detailed on the CMLS website.

What this means for AIMS members utilising APACE

AIMS Members using the APACE scheme to track their professional development activities can now apply to be certified through the AIMS National Office.

AIMS National Office will now issue Certification to APACE users who have:

- Completed their required CPD activities;
- Been issued their APACE certificate;
- Provided a competency assessment signed by your employer **as part of your AIMS Membership**.

AIMS Members will have access to their APACE record and submission system in the AIMS Members' Area. To get started, follow the step-by-step guide detailed at: https://www.aims.org.au/Web/Web/APACE/Certification-CMLS.aspx

Why become Certified?

Your status as a certified medical laboratory professional is a public guarantee that you are qualified, competent, and continuing your professional development.

If you would like more information on Certification, contact the AIMS National Office via email at: programs@aims.org.au.

CALL FOR SUBMISSIONS

SUBMIT YOUR ARTICLE TO THE AJMS TODAY

Submit your article to our journal to be shared with the Medical Science community.

The **Australian Journal of Medical Science** (AJMS) will consider for publication any paper relevant to the field of Medical Science.

Disciplines include:

- Blood Banking
- Clinical Biochemistry
- Haematology
- Histopathology
- Immunology
- Microbiology
- Molecular biology

Areas of general interest to medical laboratory scientists will also be considered, including:

- Toxicology
- Epidemiology
- Public and Community Health
- Professional and Management Issues

Papers published in the AJMS are in the form of original articles, case studies, discipline updates, opinion pieces and review articles.

All successful articles will be peer reviewed and published online and print.

We look forward to reading your work!

EMAIL YOUR ARTICLE TO

programs@aims.org.au