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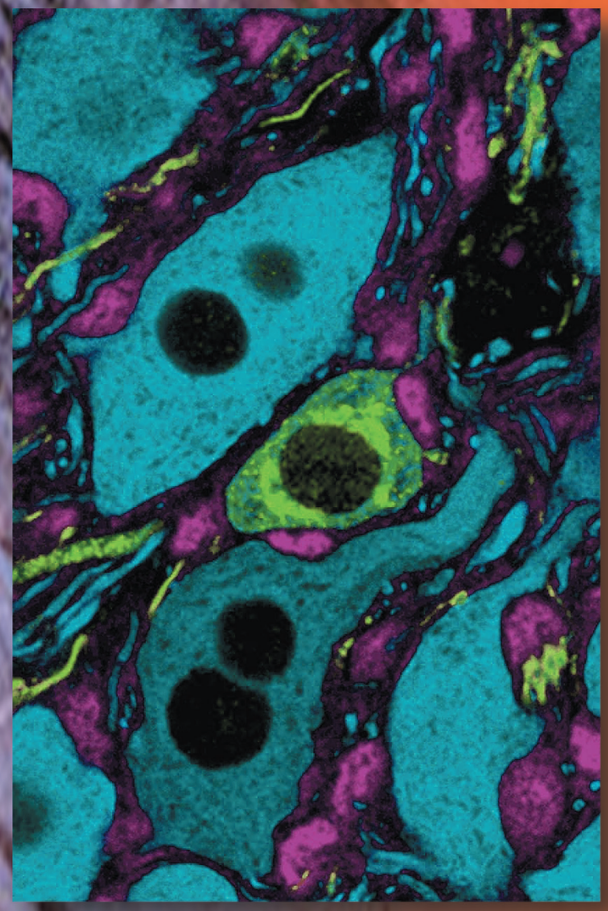

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Chief Executive: Mr Michael Nolan, BAppSc BA MPhil GradCertMgt FAIMS MASM

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

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ORIGINAL ARTICLE

A comparison of column agglutination and solid phase red cell adherence technologies for red cell antibody detection

Nora Daniel[1], Susan Finch[1], Thiru Vanniasinkam[2]

[1]PathWest Pathology, Department of Haematology, Transfusion Medicine Laboratory, Western Australia

[2]Charles Sturt University, School of Biomedical Science, New South Wales

Abstract

Pre-transfusion testing to determine patient blood group profiles and detect clinically significant antibodies is critical for transfusion. However, there is no single method for detecting red cell antibodies that all laboratories use in Australia. This study compared four widely used methods to identify the most sensitive and accurate platform.

Previously tested, 50 negative and 48 positive stored samples were used in this study. Samples were analysed for the presence of various red cell antibodies using the Ortho glass bead, BioRad gel, Grifols gel column agglutination technologies (CAT) and Immucor Echo Solid Phase Red Cell adherence assay (SPRCA). Results from all platforms were comparable (tetrachoric correlation co-efficient > 95%). There were a few samples with low titre antibodies that could not be detected by all platforms. The results of this study demonstrate that all four platforms perform equally well, however multi centre studies would be useful to further evaluate these technologies, and to compare protocols in various laboratories.

Keywords: Transfusion medicine, antibody detection, allo-antibodies, red cell antibodies, pre-transfusion testing

Introduction

Pre-transfusion red blood cell (RBC) testing to determine patient ABO and Rh(D) group profiles as well as detecting clinically significant RBC antibodies, is critical to selecting appropriate blood for transfusion (Poole and Daniels 2007). Generally, clinically significant antibodies are those that react at 37°C, are IgG in nature, and are able to mediate destruction of transfused red cells (Körmöczi and Mayr 2014). The Haemovigilance Report (National Blood Authority 2020) revealed that of the 488 adverse events reported between 2017 and 2018, 78.4% were associated with some level of morbidity. However, the number of patients or transfusion episodes resulting in these cases is not known. Importantly, of the 78.4% of adverse reactions reported, 66.2% were red blood cell related (National Blood Authority 2020).

Address correspondence to:
Dr Thiru Vanniasinkam
Charles Sturt University, School of Biomedical Science, New South Wales, Australia
E-mail: tvanniasinkam@csu.edu.au

In order to improve approaches to red cell antibody screening it is important to evaluate current methods widely used in transfusion laboratories.

Since 1984, based upon the American Association of Blood banks (AABB) recommendation, crossmatches could be omitted provided the antibody screen was negative. This was on the basis that if the antibody screen is negative, greater than 99.99% of RBC units electronically matched to ABO group and Rhesus D status will be compatible (Orlando *et al* 2018). Since electronic issue of blood units is routinely practised in transfusion medicine today, it is imperative to select antibody screening methods that are not only rapid, but sensitive without loss of safety and accuracy (Nance 2015). Considered the gold standard for many years, the tube agglutination technique has its limitations including time taken to perform, and unstable end points making grading and interpretation difficult (Rumsey and Ciesielski 2000). In the 1990s automated technologies such as column agglutination technology (CAT) and Solid Phase Red Cell Adherence assay (SPRCA) were introduced which significantly increased sensitivity and reproducibility of antibody identification (Garg *et al* 2017; Bajpai *et al* 2012).

Following automated screening most laboratories utilise additional methods to identify, confirm or exclude significant antibodies which are best detected by an

indirect antiglobulin test (IAT) (Dwyre *et al* 2006). It has been noted that although the column agglutination technology is favoured and widely used, the incidence of false positives requiring further investigation is higher compared with the traditional tube technique. In addition, Liu and Grossman (2013) demonstrated that although most of the non-specific reactions are of antibodies with little or no clinical significance, a small number serve as an indicator of future alloantibodies. Another study reported that the gel CAT method produced an inconclusive result which was later identified as Anti-Jka by SPRCA (Kay *et al* 2016). It is therefore important to compare methods in order to identify the technique that best serves the transfusion laboratory, leading to reduction of errors and to maintain a high degree of accuracy, sensitivity and specificity. To date, some comparisons have been made between different techniques, such as a study conducted in the United States comparing column technology against solid phase and glass bead versus gel CAT conducted in Germany (Schmidt *et al* 2013; Sawierucha *et al* 2018). However, there is limited published data on Ortho glass bead, BioRad gel, Grifols CAT and SPRCA. To the best of the authors' knowledge, this is the first study to compare these techniques to detect antibodies to RBC antigens in an Australian tertiary hospital setting.

Materials & methods

Samples selected for the study

Samples for the study were initially tested at either Fiona Stanley Hospital or Royal Perth Hospital between January 2020 and May 2020. Patient samples were selected based on positive and negative pre-transfusion RBC antibody screens performed by the Autovue Innova analyser. Fifty samples demonstrating negative and 48 samples with positive RBC antibody screen results were chosen. The following antibodies were detected in samples with positive results: anti-D, anti-C, anti-E, anti-e, anti-c, anti-K, anti-k, anti-Ch, anti-Fya, anti-Jka, anti-Jkb, anti-Lea, anti-Leb, anti-M and anti-Rg. After sample selection, plasma from whole blood EDTA was aliquoted, frozen and stored at minus 30° C for testing. All selected samples were de-identified and allocated a unique three digit and one letter identifier and corresponding initial results documented in an excel spreadsheet. Ethical clearance was granted by the Royal Perth Hospital Human Research Ethics Committee (RGS0000003062) and Charles Sturt University Ethics committee (H20119). All samples were tested using 4 platforms described below (2 automated and 2 manual methods).

Analysers used in the study

Ortho-Clinical Diagnostic Autovue

The Ortho-Clinical Diagnostics Autovue Innova is a fully automated system used as the routine analyser at Royal Perth Hospital and Fiona Stanley Hospital. Tests such as blood grouping, RBC antibody screens, phenotyping and crossmatch are performed using the Ortho Autovue. The principle of the test is column agglutination technology with the use of glass beads. Pipetting of liquid, cassette handling, incubation and centrifugation are fully automated, as well as grading, result interpretation, data management and transmission of results into a laboratory information system (Cheng and Wilkinson 2015). Result grading is by an inbuilt camera, which captures both the front and back of the cassette and result interpretation is performed using an internal image processing software and algorithm (Dada *et al* 2007). Images are captured and stored as a JPEG image. Ortho AHG cassettes are comprised of six columns and require 50 microlitres of 0.8% screening cells and 40 microlitres of plasma prior to incubation and centrifugation. In this study, Immulab Abtectcell III 0.8% screening cells were used.

Immucor Capture-R Echo Analyser (SPRCA)

The Immucor Echo SPCRA test consists of a monolayer of red cell membranes bound to the surface of a microtitre plate. When plasma and a low ionic strength solution (LISS) are added to the well and incubated at 37° C, antibodies, if present, adhere to the bound membrane antigens. To remove any unbound antibody, a washing step is necessary, followed by the addition of anti-IgG (a monoclonal anti human globulin) coated indicator cells to the plate. The final step of the assay is a 2min centrifugation before result interpretation (Ching 2012). The Capture-R ready screen strips were used in this study with a built-in positive control for each 3-cell screen performed. Results including visual well images were stored.

Manual methods used in the study

The Bio-Rad System

The principle of the Bio-Rad system is column agglutination technology using six column cards containing a gel matrix with anti-human globulin or other antisera depending on the test requirements (Casina 2006). In this study, the assay is considered manual due to manual pipetting of Abtectcell III 0.8% screening cells as well as manual addition of plasma and result interpretation. Incubation and centrifugation were performed using corresponding incubators and centrifuges specific to the Bio-Rad system. The Bio-Rad testing system requires 50 microlitres of 0.8% screen cells with the addition of 25 microlitres of plasma followed by a 15min incubation and a 10min spin post incubation.

Grifols DG Gel System

The Grifols system, also using the principle of column agglutination technology, is comprised of a gel matrix with added anti-human globulin contained in an eight column card (Raos *et al* 2018) The Grifols system was also performed manually in this study with manual pipetting of Grifols Perfect screen 3 cell screening cells and plasma. Incubation and centrifugation were performed using corresponding incubators and centrifuges specific to the Grifols system. The Grifols system requires 50 microlitres of 0.8% screening cells and 25 microlitres of plasma followed by a 15min incubation and a 9min centrifugation before result interpretation.

Laboratory investigations

All assays were performed in the Transfusion Medicine laboratory at Royal Perth Hospital (RPH), Western Australia. The samples were stored at minus 30° C and thawed immediately before testing. All testing was performed according to manufacturer's instructions in small batches allowing for side-by-side comparison. Quality control using Immulab Securacell light 1 and 2 were used for all four platforms with all results within range. Samples were screened for the presence or absence of antibodies using Ortho Autovue glass bead CAT, Bio-Rad gel column agglutination, Grifols CAT and SPRCA in that order.

Interpretation of assay

CAT results were graded from 4+, 3+, 2+ and so forth down to 1+, depending on the strength and avidity of antibody with a grading of 4+ if all cells remain at the top of the column, indicating a high titre. For the solid phase red cell adherence method, results were graded according to the indicator red cell pattern on the base of the well in the test strip.

Statistical analysis

Tetrachoric correlation co-efficient and other metrics such as sensitivity and specificity were calculated using Microsoft Excel software for Windows 10. Of the 50 negative samples used in this study two were subsequently found to be positive for anti-E antibodies therefore only 48 samples were included in statistical calculation.

A total of 98 samples were tested with most platforms demonstrating a tetrachoric correlation coefficient of greater than 99%, with the exception of the SPCRA, demonstrating a slightly weaker correlation. Ortho glass bead CAT failed to detect anti- M, anti-K and anti-E alloantibodies in samples where the original reaction had been weak. All four platforms did not detect the M and Kantibodies in these samples. Bio-Rad CAT demonstrated comparable results to Ortho with a failure to detect the weak anti-M and anti-K alloantibodies, however the weak anti-E alloantibody was detected. Grifols demonstrated the highest sensitivity rate with only two false negative reactions associated with the same sample with known weak anti-M and anti-K, in line with both BioRad and Ortho column technologies. All CAT methods produced negative results against known negatives with no false positives, as outlined in Table 2. SPCRA failed to detect the aforementioned weak anti-M and anti-K antibodies, however this platform also failed to detect anti-Ch, -Rg, -Lea and -Leb alloantibodies. A further decrease in concordance with solid phase adherence method may be explained by the limitation of SPRCA detecting only IgG antibodies and since Lewis antibodies are primarily IgM, these antibodies were not able to be detected. Failure to detect weak anti-M and anti-K demonstrated by all four platforms may be explained by the initial low titre of antibody and potential compromise of antibody due to the storage and freeze/thaw process prior to testing. The two positive reactions produced by SPRCA, which were initially negative using the AutoVue Innova platform, were both identified to be anti-E alloantibodies, after further investigation.

Discussion

Accurate and sensitive pre-transfusion testing is fundamental for best practice in transfusion of compatible red cell units (National Blood Authority 2020).

Particular characteristics of an antibody such as class of immunoglobulin, thermal amplitude and antibody titre determine its significance and it is important to utilise robust antibody screening platforms that are effective in detecting clinically significant antibodies to prevent adverse events and to allow transfused red cells to have optimal survival rates *in vivo* (Flegel 2015).

The routine group and screen method used by the transfusion medicine laboratory at RPH is the Ortho Clinical Diagnostic Autovue Innova analyser supported by Bio-Rad CAT and/or tube methods. The study comparison was useful in identifying testing platforms that are able to detect clinically significant antibodies with similar turnaround times and ease of assay set up as compared with the AutoVue Innova analyser, with demonstrated tetrachoric correlation co-efficiencies of tested platforms being greater than 95% indicating comparable results. The results of Ortho, Bio-Rad and Grifols column technology were comparable with sensitivities of 91.7%, 91.7% and 95.8% respectively. Interestingly, although all platforms failed to detect a weak anti-K antibody in one sample, the SPRCA method missed three known Kell antibodies. A study by Schmidt *et al* (2013) described a decrease in the number of Kell antibodies detected using the SPRCA

Table1. *Results from testing the 48 positive samples using four platforms*

Antibody specificity	Number of samples* (n)	ORTHO results	BIO-RAD results	GRIFOLS results	SPRCA results
D	4	4	4	4	4
C	2	2	2	2	2
c	3	3	3	3	3
E	8	7	8	8	8
e	1	1	1	1	1
K	8	7	6	7	5
k	1	1	1	1	1
Fya	8	8	8	8	8
Jka	2	2	2	2	2
Jkb	1	1	1	1	1
M	5	3	3	4	4
Lea	2	2	2	2	1
Leb	1	1	1	1	0
Rg	1	1	1	1	0
Ch	1	1	1	1	0
Total	48	44	44	46	40

Table 2. *Results of red cell antibody screen tested using 4 platforms*

Samples	Method			
	ORTHO	BIO-RAD	GRIFOLS	SPRCA
Positive (48)	44	44	46	40
False negative	4	4	2	8
Negative (48*)	48	48	48	48
False positIve	0	0	0	0

**Of the 50 negative samples only 48 were included in statistical calculations as two were subsequently found to be positive for anti-E antibodies*

Table 3. *Comparison of diagnostic specificity and sensitivity of testing platforms**

	ORTHO	BIO-RAD	GRIFOLS	SPRCA
Sensitivity	91.7	91.7	95.8	85.1
Specificity	100	100	100	100
rtet	99.2	99.2	99.8	95.8
NPV	92.3	92.3	96	85.7
PPV	100	100	100	100

rtet – tetrachoric correlation co-efficient

NPV – Negative predictive value, PPV – Positive predictive value

** two samples that were initially negative for anti-E antibodies, which when subsequently tested using a papain enhanced BIO-RAD CAT system were positive, were excluded from the calculation of specificity and sensitivity*

method and noted that continued surveillance was needed to determine whether it would be of clinical significance. Another interesting finding in this study was the two positive screen results detected by the SPRCA analyser which had not been identified in the initial screen. This may be due to low levels of anti-E antibody in these samples which were below conventional column technology detection limits but were detected with enhancement by papain in the Bio-Rad column technique in this study. All other column methods gave negative results for the same samples, indicating that SPRCA may be a more sensitive method for detection of certain clinically significant antibodies compared to other technologies evaluated in this study. Similar findings have been reported by Kay *et al* (2016) who noted reactions of anti-Jka and -Jkb were stronger in the SPCRA technique when compared with column technologies. Another study reported that Rh antibodies appeared more reactive by gel CAT compared with the Ortho glass bead method (Sawierucha *et al* 2018). Similar findings have been anecdotally observed in the transfusion laboratory at RPH. The Grifols gel CAT method demonstrated the highest correlation coefficient and sensitivity at 99.8% and 95.8% respectively as compared with the other methods. A recent study performed by Blomme *et al* (2020) comparing Bio-Rad, Ortho and Grifols and produced similar findings. Based upon these results, one consideration is that some platforms may not be reliable for the detection of some antibodies such as anti-E and -Kell and further studies are required to investigate this finding.

Since there are no universally mandated pre-transfusion testing methods, it is essential to employ techniques that are easy to perform, accurate, sensitive and have acceptable turnaround times. In summary, the Ortho glass bead and BioRad gel CAT methods in this study are utilised in routine blood grouping and screening at RPH and in fact demonstrated similar results in the comparison study. The SPRCA platform, although having high affinity for Kidd antibodies, tended to miss antibodies with a weak titre, and in particular Kell antibodies, which are regarded as highly clinically significant, especially in pregnant women. Based upon the findings reported in this study, it would be advisable for transfusion laboratories to utilise additional methods that can supplement their workplace analysers, since each platform will yield different sensitivities of antibody detection. As diagnostic pathology becomes increasingly automated, the selection of accurate and cost-effective techniques is crucial in preventing significant adverse transfusion events. The limitations to this study were the small sample size and use of stored samples. All samples were selected for screening based upon results obtained by screening on the AutoVue Innova analyser with the expectation that similar results would be obtained at later testing on the same platform. It was demonstrated however that weaker anti-M and anti-K antibodies were not able to be detected on any of the four platforms in this study. This could have been due to the storage, freeze and thaw process that may have contributed to a drop in detectable red cell antibody titres in these samples. Ideally future studies should be conducted using fresh patient samples. A large multicentre study would be optimal as it is often difficult to obtain a large number of appropriate samples from one hospital at any given time.

Conflicts of interest

The authors declare they have no conflicts of interest

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References

Bajpai M, Kaur R, Gupta E 2012. Automation in immunohematology. *Asian J Transfus Sci* 6(2): 140-144. doi:10.4103/0973-6247.98914.

Blomme S, De Maertelaere E, Verhoye E 2020. A comparison of three column agglutination tests for red blood cell alloantibody identification. *BMC Res Notes* 13(1), 129. doi:10.1186/s13104-020-04974-x.

Casina TS 2006. In search of the Holy Grail: comparison of antibody screening methods. *Immunohematology* 22(4): 196.

Cheng Y W, Wilkinson JM 2015. An experience of the introduction of a blood bank automation system (Ortho AutoVue Innova) in a regional acute hospital. *Transfus Apher Sci* 53(1): 58-63.

Ching E 2012. Solid Phase Red Cell Adherence Assay: A tubeless method for pretransfusion testing and other applications in transfusion science. *Transfus Apher Sci* 46(3): 287-291. doi:10.1016/j.transci.2012.03.018.

Dada A, Beck D, Schmitz G. Automation and Data Processing in Blood Banking Using the Ortho AutoVue® Innova System 2007. *Transfus Med Hemother* 34(5): 341-346. doi:10.1159/000106558.

Dwyre DM, Erickson Y, Heintz M, Elbert C, Strauss R G 2006. Comparative sensitivity of solid phase versus PEG enhancement assays for detection and identification of RBC antibodies. *Transfus Apher Sci* 35(1): 19-23. doi:10.1016/j.transci.2006.02.002.

Garg S, Saini N, Bedi, Basu S 2017. Comparison of micro column technology with conventional tube methods for antibody

detection. *J Lab Physicians* 9(2): 95-99. doi:10.4103/0974-2727.199627.

Kay B, Poisson J L, Tuma CW, Shulman I A 2016. Anti-Jka that are detected by solid-phase red blood cell adherence but missed by gel testing can cause hemolytic transfusion reactions. *Transfusion* 56(12): 2973-2979. doi:10.1111/trf.13782.

Körmöczi G F, Mayr W R 2014. Responder Individuality in Red Blood Cell Alloimmunization. *Transfus Med Hemother* 41(6): 446-451. doi:10.1159/000369179.

Liu C, Grossman BJ 2013. Antibody of undetermined specificity: frequency, laboratory features, and natural history. *Transfusion* 53(5): 931-938. doi:10.1111/trf.12070.

Nance S T 2015. Red cell antibody detection by serology USBT Science series 10[Suppl1]: 1-4.

National Blood Authority N B 2020. *Australian Haemovigilance Report, Data for 2017-2018*. Retrieved from https://www.blood.gov.au/system/files/Australian%20Haemovigilance%20Report%202017-18_FINAL%20v2.pdf accessed on 7th July 2021.

Orlando N, Bianchi M, Valentini C G, Maresca M, Massini G, Putzulu R, Zini G, Teofili L 2018. Red Cell Alloantibody Screening: Comparative Analysis of Three Different Technologies. *Transfus Med Hemother* 45(3), 179-183. doi:10.1159/000484570.

Poole J, Daniels G 2007. Blood Group Antibodies and Their Significance in Transfusion Medicine. *Transfus Med Rev 21*(1), 58-71. doi:10.1016/j.tmrv.2006.08.003.

Rumsey DH, Ciesielski DJ 2000. New protocols in serologic testing: a review of techniques to meet today's challenges. *Immunohematology* 16(4), 131-137.

Raos M, Novoselac J, Burnać IL, Lukic M, Leskovar I, Cepulic B G 2018. Evaluation of antibody screening and identification pre-transfusion tests using DG Gel cards. *J Appl. Health Sci* 4(2): 179-186.

Sawierucha J, Posset M, Hähnel V, Johnson C L, Hutchinson J A, Ahrens N 2018. Comparison of two column agglutination tests for red blood cell antibody testing. *PLoS One* 13(12): e0210099-e0210099. doi:10.1371/journal.pone.0210099.

Schmidt A, Bendix B J, Eapen, E K, Bryant S C, Subbs J R 2013. Single-center comparison of gel microcolumn and solid-phase methods for antibody screening. *Immunohematology* 29(3): 101-104.

The effectiveness of ultrasound guided fine needle aspiration cytology in detecting head and neck mass pathology

Norhafiza Mat Lazim,[1] Nurul Atikah Hamat[1], Rosdan Salim[1], Anani Aila Mat Zin,[2] Hashimah Ismail[3]

[1]Department of Otorhinolaryngology-Head and Neck Surgery, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, Kubang Kerian, Kelantan, Malaysia

[2]Department of Pathology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, Kubang Kerian, Kelantan, Malaysia

[3]Department of Otorhinolaryngology, Hospital Raja Perempuan Zainab II, Kota Bharu, Kelantan, Malaysia

Abstract

The ultrasound guided fine needle aspiration cytology (USFNAC) is an important test for diagnosis of benign and malignant tumours in head and neck. The aim of the study is to determine the accuracy, positive predictive value and association between benign and malignant tumours detected by USFNAC as compared to the conventional fine needle aspiration cytology (FNAC). Twenty one out of 30 cases (70%) were diagnosed with benign lesions and nine cases (30%) were diagnosed with malignant lesions from the USFNAC group. Based on pathological examination result, 18 cases were confirmed benign and eight cases were confirmed malignant. USFNAC yielded both greater accuracy and higher sensitivity compared to conventional FNAC. The USFNAC should be recommended as the primary diagnostic tool for a preoperative evaluation of patients presented with head and neck mass, especially when malignancy is suspected and the mass is less than 2.0 cm. This ensures an earlier correct diagnosis can be attained to facilitate a subsequent refined management plan.

Keywords: *Ultrasound, fine needle aspiration, ultrasound guided FNAC, cytology, head and neck mass*

Introduction

Head and neck masses represent a wide differential diagnosis from inflammation and reactive hyperplasia to benign and malignant neoplasia. Masses included in this region may arise in lymph nodes, the thyroid gland, major salivary glands, neurogenic or vascular tumours or from acquired abscesses and congenital cysts (El Hag *et al* 2003; Sharma *et al* 2011). Benign lesions accounting for 48% of these masses consist of pleomorphic adenoma, dermoid cyst, thyroglossal cyst, lipoma and branchial cyst. Infective and inflammatory lesions account for 38%, and include tuberculous lymphadenitis, reactive lymphadenitis and sialadenitis. Primary malignant lesions, including lymphoma, adenoid cystic carcinoma, synovial sarcoma, spindle cell carcinoma and mucoepidermoid carcinoma have been reported in up to 14% of cases (Sejal *et al* 2015).

Address correspondence to:
Norhafiza Mat Lazim
School of Medical Sciences, Universiti Sains Malaysia, Health Campus, Kubang Kerian, Kelantan, Malaysia
E-mail: norhafiza@usm.my

Fine needle aspiration cytology (FNAC) is a simple test, associated with minimal trauma and complications and results can be rapidly available. FNAC is a reliable method to differentiate between inflammatory and neoplastic lesions. It does not require anaesthesia, it is well tolerated, simple and cost effective (Piccioni *et al* 2011; Silas *et al* 2015). The difficulty for the performing pathologist to accurately determine where to insert the needle may however result in an unrepresentative sample (McIvor *et al* 1994). It is usually not performed for infectious and inflammatory conditions, because of the limitations of missing the target lesion, obtaining haemorrhagic smears or inadequate cellular material in these clinical conditions (Buckland *et al* 1999). This may lead to delay in definitive diagnosis and treatment with implications for the patient and overall management.

Ultrasound is a non-invasive procedure and can be very useful in the diagnosis of head and neck masses. It can detect smaller lesions in head and neck, can be repeated and has good patient tolerance (Poorey *et al* 2014). It is however operator dependent in that the experience of the radiologist plays a crucial role in the ability to detect and define the lesions.

The ultrasound can determine whether a lesion is cystic or solid. The nature, echogenicity, edge, presence of calcification and number of head and neck masses can

be assessed with ultrasound (Sharma *et al* 2011; Ahuja *et al* 2002). The ultrasound examination alone cannot satisfactorily differentiate between benign and malignant tumours as benign and malignant tumours are both hypoechoic. Other findings suggestive of malignancy includes irregular margin, posterior shadowing and heterogeneous internal echogenicity (Khan *et al* 2015). FNAC guided by ultrasound provides the opportunity of sampling impalpable lesions, targeting areas of interest within a mass, and avoiding necrotic or cystic areas and critical vascular structures like the carotid artery or jugular veins.

Recommendations vary regarding the use of ultrasound in improving the accuracy of fine needle aspiration. Ultrasound guided FNAC (USFNAC) has an added advantage of better visualization of lesions, improved accuracy of needle placement, and hence potentially fewer unrepresentative samples as compared to the routine palpation-guided method (Knappe *et al* 2000). The USFNAC is a safe procedure with low recollection rates (Sattar *et al* 2016). A study of USFNAC in retro-jugular lymph nodes found that the diagnostic accuracy of the procedure was 85.4% based on comparison with the surgical biopsy diagnosis (Islam *et al* 2015). For parotid masses, USFNAC can avoid injury to the facial nerve or major vessels and avoid unnecessary delay in management (Panneerselvam *et al* 2013). The diagnostic accuracy of USFNAC of head and neck masses can also reduce the overall number of neck mass surgeries (Lee *et al* 2009).

Patients and methods

The study was a comparative cross-sectional study between USFNAC and conventional FNAC that was performed from February 2017 until January 2018. The study was conducted at the Otorhinolaryngology (ORL) clinic of Hospital Raja Perempuan Zainab II (HRPZII) and Hospital University Sains Malaysia (HUSM). The study was supported by USAINS grant HUSM and approved by the institutional ethical review board (USM/JEPeM/16120549).

A control group of patients who underwent conventional FNAC was identified for the period of one year (January 2016 until December 2016). The data was retrospectively retrieved from medical and laboratory records with permission from hospital director. Sixty patients with a head and neck mass in the parotid gland, submandibular gland thyroid gland or neck nodes that had undergone conventional FNAC, followed by subsequent surgical biopsy were included in the control group. The cytological diagnosis and histological diagnosis were also recorded.

Patients who came after February 2017 with any head and neck mass in the parotid gland, submandibular gland, thyroid gland and neck nodes who were clinically indicated for FNAC were selected for USFNAC. Demographic characteristics including age, gender and ethnic origin were obtained. The study patients were all 18 years of age and older. All the patients were subjected to detailed history taking, general examination and thorough clinical ear, nose and throat examination. The details of the study and procedures were given to the patients and informed consent was obtained. Thirty patients recruited in this study underwent USFNAC and this was performed in the radiology suite by the attending radiology and pathology team one week after they were first seen at the ORL clinic. During the procedure, the mass site was scanned by using Siemens Acuson X300 ultrasound machine and the location, size, and the number of the lesions was noted. USFNAC was performed with an 18- or 22-gauge needle attached to 10- or 20-ml syringe. The aspirated material was then spread onto clean numbered slides which were immediately immersed in 95% alcohol for fixation or air-dried and were sent to the pathology laboratory.

The result of the USFNAC report was interpreted focusing on the adequacy of the sample tissue and the final diagnosis. USFNAC results were obtained one to two weeks after the procedure. The FNAC result obtained were categorized as either a benign or a malignant neck mass. Any inconclusive or indeterminate results were excluded from the study. The results of conventional FNAC and USFNAC were compared for the detection of head and neck tumour pathology. The histopathological examination (HPE) results from surgical biopsy specimens were recorded for confirmation and comparison as a gold standard.

Patients with inadequate or inconclusive aspirate, missing FNAC or HPE results, patients with non-palpable head and neck masses and patients who were previously confirmed to have metastatic head and neck tumour were excluded from the study.

Data from the study was imported to IBM SPSS version 23 from Excel file. Coding for each categorical variable was properly managed before statistical analysis was done. Statistical analysis was done by using IBM SPSS version 23 and STATA 14.0. Data exploration and cleaning was done and was properly checked for any duplicate entry, wrong entry or missing value. Descriptive analysis also took place to describe the variables. The frequency and percentage was reported for all categorical variables and presented in Table 1. Statistical tests were performed to look for percentage of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

Results

From February 2017 until January 2018, 40 cases underwent USFNAC of head and neck masses. Thirty cases satisfied the inclusion criteria in the study. Ten cases were excluded

due to inconclusive USFNAC results and no histopathology examination for comparison as a gold standard. The cases in the study included 15 female and 15 male patients aged above 18-years-old. The mean age of patients who underwent USFNAC was 50 years. The ethnic mix was 25 Malay, four Chinese and one Siamese nationalities. The control group comprised of 60 cases, 24 male and 36 female patients and the nationalities were 56 Malay , two Chinese, one Siamese and one Indian patient (Table 2).

Site of the head and neck mass

The head and neck masses involved in the study are from the thyroid gland, the salivary glands (parotid gland or submandibular gland), neck lymph nodes and other cystic or congenital neck masses. The site of head and neck masses from USFNAC showed 50% were from the thyroid mass, 23% from salivary gland and 16% from neck nodes. In conventional FNAC 40% were from the thyroid gland, 30% from salivary glands and other 30% was from the neck nodes and other neck masses. The proportion of benign head and neck masses detected by conventional FNAC and USFNAC was 78.3% and 70.0%, respectively. Malignant head and neck masses detected by conventional FNAC was 21.7%. while USFNAC detected 30.0% malignant head and neck mass (Table 2).

Analysis of ultrasound guided FNAC with histopathological findings

After USFNAC of head and neck masses, 21 out of 30 cases were diagnosed as benign lesions and 9 cases were malignant. Based on HPE results, 18 cases were confirmed benign and 8 cases were confirmed malignant. Three cases of benign pathology on USFNAC were interpreted as malignant on HPE, while one malignant case by USFNAC was interpreted as benign on HPE. The proportion of benign to malignant of the study group was therefore 19: 11 (63.3%;36.7%). Most of the head and neck masses were from the thyroid gland, followed by the neck nodes and salivary glands. USFNAC results of benign and malignant head and neck mass are shown in Table 3.

For the conventional FNAC of head and neck masses, 47 cases (78.3%) were diagnosed as benign lesions and 13 cases (21.7%) were malignant. Based on the HPE result, 40 cases were confirmed benign and 10 cases were confirmed malignant. Seven cases of benign FNAC were interpreted as malignant, while 3 malignant cases by FNAC were interpreted as benign on HPE. The results of USFNAC and conventional FNAC in comparison with HPE findings are shown in Table 4.

This study shows that USFNAC yields 86.7% overall accuracy which is higher than overall accuracy yield by conventional FNAC (83.3%) in detecting benign and malignant tumours. USFNAC also demonstrates higher sensitivity compared to conventional FNAC (72.7% and 58.8% respectively). USFNAC also shows higher PPV compared to conventional FNAC; 88.9% and 76.9% respectively. Conventional FNAC and USFNAC does not show much difference in specificity and NPV. Specificity of conventional FNAC and USFNAC is 93.0% vs 94.7%, while NPV of conventional FNAC and USFNAC is 85.1% vs 85.7% (Table 5).

Discussion

Head and neck masses have complex differential diagnoses and non-specific clinical presentation. The most accurate diagnostic methods are therefore crucial in differentiating an inflammatory disease from a benign or malignant neck mass. The treatment of head and neck masses is largely based on the findings of fine needle aspiration cytology which determines whether it is a benign or malignant lesion. Further imaging investigation is necessary in malignant cases and in selected benign lesions which required surgical intervention as part of the treatment. The definitive management will depend on the site of the mass, its size, the effects of the mass on adjacent structures, the presence of airway compromised or advanced metastasis (Riaz *et al* 2014).

Demographic of the patients in the study

In this study the majority of the patients were Malay (83%). This could be due to the majority of the Kelantan populations being of Malay ethnicity. The second commonest ethnic group was Chinese, accounting for 13%. The ethnic group has no association with the USFNAC findings in this study. Some head and neck malignancies were thought to have association with genetic defects and there were a few studies that suggested the prevalence of specific head and neck malignancy in a specific ethnic group (Filion *et al* 2010; Jalan *et al* 2017).

The female to male ratio was equal (1:1) and there was no significant association noted between gender and USFNAC findings. Previous studies reported that in head and neck malignancy, males were more commonly affected than females because males were more likely to indulge in major risk habits like alcohol consumption and smoking (Batool *et al* 2017).

The age of the study cohort ranged from 18 to 79 years with a mean age of 50 years. Benign neck masses were noted to occur in the patients from second to fourth decade of life while the malignant neck masses were noted to occur in patients in the fourth to sixth decade. A study by Riaz *et al* (2014) stated that age alone cannot be considered as a factor to determine the pathological outcomes of the tissue diagnosis either it is a benign or malignant neck mass (Riaz *et al* 2014). The other factors that can influence the diagnosis of a benign or malignant neck mass were the comorbidity, a previous history of irradiation and the

Table 1. *Descriptive analysis conventional FNAC and USFNAC*

Variable	FNAC (n=60)	USFNAC (n=30)
Type of neck mass		
Thyroid	24 (40.0%)	15 (50.0%)
Salivary gland	18 (30.0%)	7 (23.3%)
LN/others	18 (30.0%)	8(26.7%)
Diagnosis		
Benign	47 (70.0%)	21 (78.3%)
Malignant	13 (21.7%)	9 (30.0%)
Size		
Less than 2 cm	8 (13.3%)	5 (16.7%)
More than 2 cm	52 (86.7%)	25 (83.3%)

Table 2. *Association of ultrasound guided FNAC results (benign vs malignant) with demographic characteristics*

Variable	Ultrasound guided FNAC results		*p*-value[a]
	Benign n (%)	Malignant n (%)	
Race			
Malay	18 (72.0)	7 (28.0)	0.622
Non-Malay	3 (60.0)	2 (40.0))	
Gender			
Male	12 (80.0)	3 (20.0)	0.427
Female	9 (60.0)	6 (40.0)	
Age category			
≤ 40 years	7 (77.8)	2 (22.2)	0.681
> 40 years	14 (66.7)	7 (33.3)	
Smoking			
No	12 (63.2)	7 (36.8)	0.419
Yes	9 (81.8)	2 (18.2)	
Comorbidity			
No	11 (73.3)	4 (26.7)	> 0.95
Yes	10 (66.7)	5 (33.3)	

***Table 3**. USFNAC results of benign and malignant head and neck mass*

USFNAC	Number of cases
Nodular hyperplasia	4
Benign neoplasm/ benign thyroid nodule	2
Follicular neoplasm/ benign follicular nodule/ benign follicular hyperplasia	3
Reactive lymphadenopathy	3
Warthin tumour	2
Reactive lymphoid cells	1
Granulomatous lymphadenitis	1
Branchial cyst/ squamous lined cyst	2
Thyroglossal cyst	1
Papillary neoplasm	1
Colloid goitre	1
Follicular lesion of undetermined significant	1
Papillary thyroid carcinoma	2
Metastatic Carcinoma/ metastatic papillary thyroid carcinoma/ metastatic squamous cell carcinoma	5
Adenomatous nodule with degenerative changes	1

***Table 4**. USFNAC and conventional FNAC findings in comparison with HPE*

		HPE result		Total
		Benign	Malignant	
USFNAC result	Benign	TN = 18	FN = 3	21
	Malignant	FP = 1	TP = 8	9
Total		19	11	30
Conventional FNAC result	Benign	TN = 40	FN = 7	47
	Malignant	FP = 3	TP = 10	13
Total		43	17	60

***Table 5.** Diagnostic test on conventional FNAC and ultrasound guided FNAC in detecting benign and malignant tumour*

Diagnostic test	FNAC (n = 60)	USFNAC (n = 30)
Sensitivity (%)	58.8	72.7
Specificity (%)	93.0	94.7
Positive predictive value (%)	76.9	88.9
Negative predictive value (%)	85.1	85.7
Overall accuracy (%)	83.3	86.7

lifestyle of the patient such as smoking and nutritional status. This study showed that comorbidity of the patient and smoking habit had no significant association with the USFNAC findings. A study done by Filion *et al* (2020) documented that the established risk factors that have been associated with the development of head and neck cancers include tobacco consumption (both smoked and chewed), alcohol use, and the human papillomavirus (HPV) (Filion *et al* 2010; Jalan *et al* 2017).

Characteristics of the neck masses in the study

Out of the 30 patients who underwent USFNAC, benign head and neck mass was more commonly encountered, accounting for 70% of cases, while 30% were malignant. This figure was slightly higher than found in a study by Chen *et al* (2010) who had reported 64% benign lesions and 36% of malignant lesions (Chen *et al* 2010). Many patients with head and neck masses do not seek medical help unless the mass is increasing in size or it causes compression and/or obstructive symptoms, as the patients fear it is malignant (Batool *et al* 2017).

The most common head and neck mass in this study was of thyroid origin (50%) followed by the salivary gland (23.3%) and neck nodes (26.7%). The findings were similar to a study by Riaz *et al* (2014). However, no significant association was found between the origin or type of the neck mass with the USFNAC findings in the study by Lee *et al* (2009) (Lee *et al* 2009).

When the size of the lesion was more than 2cm, malignancy needs to be excluded. The study showed no association between the size of the mass and malignancy. In addition, a study by Dongbin *et al* (2015) revealed that there was no significant effect of the size of neck mass (≥10 mm or <10 mm) on the adequacy of USFNAC (Dongbin *et al* 2015). For a malignant neck mass, the significant clinical features are a painless and fixated mass, multilobulated, irregular surface/margin and a hard consistency. In this study, the findings of a malignant mass were associated with head and neck mass fixation and mobility, and cases showing these features were papillary thyroid carcinoma and metastatic lymphadenopathy from papillary thyroid carcinoma and metastatic squamous cell carcinoma.

The two most common benign tumours were nodular hyperplasia of thyroid (16.7%) and follicular adenoma of thyroid (13.3%) and the most common malignant tumour was papillary thyroid carcinoma which accounted for 20% of cases.

The study results showed that thyroid ultrasound combined with FNAC is a useful strategy for the initial evaluation of thyroid nodules. A study by Chen *et al* (2010) revealed up to 18.7% of indeterminate cases of thyroid nodules were diagnosed as malignant using this approach (Chen *et al* 2010). It is important to consider the existence of an occult malignancy if cystic changes or microcalcification in a thyroid nodule is associated with neck lymphadenopathy (Van den Brekel 1933). The accuracy of FNAC, and also the inadequacy rates are influenced by operator technique and experience, and the reliability of inserting the needle into the solid part of the mass which is determined by characteristics such as cystic nodule and calcification (Dongbin *et al* 2015).

A concordant benign and malignant head and neck diagnosis was obtained in 26 out of 30 cases. The other four cases had false positive and false negative results. Three cases with false negative diagnosis were cases of colloid goitre and follicular lesion of undetermined significance on cytology and turned out to be papillary thyroid carcinoma and follicular variant of papillary thyroid carcinoma on histopathological examination. The other case was an adenomatous nodule with degenerative changes on cytology, which turned out to be anaplastic thyroid carcinoma. There was one case of false positive diagnosis in patient with papillary thyroid carcinoma who had left cervical lymph node metastasis on cytology, but the histopathology examination result was reactive lymphadenopathy.

On conventional FNAC, 47 out of 60 cases were diagnosed as benign (78.3%) and with the remaining 21.7% malignant. Out of these results, three cases showed false positive result and seven cases showed false negative results with respect to histopathology examination findings. The reason why false positive and false negative results occurred can be due to an operator of ultrasound FNAC techniques with limited experience.

The role of ultrasound in the diagnosis of head and neck mass has been discussed extensively in the literature. It is considered safe, faster and repeatable (Krishnappa *et al* 2013). Although ultrasound is used as the first imaging modality for patient with head and neck mass, it is challenging to use ultrasound alone to differentiate between benign and malignant lesion. Combination therefore of both of ultrasound with FNAC is a better assessment modality for a definitive diagnosis. Ultrasound can provide visualisation and guidance of the FNAC to aim the aspiration at the preferred target site. The USFNAC is efficient in detecting head and neck mass which is multinodular, cystic, large, and a deep-seated mass.

The USFNAC has been proven to improve the accuracy of the preoperative diagnosis of a parotid gland mass, it can avoid injury to the facial nerve or major vessels and avoid unnecessary delay in the diagnosis and management of parotid mass (Panneerselvam *et al* 2013). The USFNAC certainly improved the diagnostic efficiency in our study with diagnostic accuracy of 86.7%, with conventional FNAC

showing a diagnostic accuracy of 83.3%. The results were similar to a study by (Krishnappa *et al* 2013) which showed diagnostic accuracy of guided FNAC was 88% against the 76% accuracy of free-hand FNAC.

This result is also comparable with the results of previous studies who reported a decrease in the number of inadequate specimens with USFNAC (Knappe *et al* 2000; Panneerselvam *et al* 2013; Lee *et al* 2009; Cai *et al* 2006). The inadequacy rate was higher with conventional FNAC (10.42%) than that of the ultrasound guided FNAC (2.78%). The sensitivity, specificity and diagnostic accuracy was 71.43%, 90.91%, 83.33% respectively with conventional FNAC and 100%, 92.31%, 95.45% respectively with USFNAC (Jalan *et al* 2017).

In USFNAC, as described earlier, fewer of our cases that were diagnosed as benign on the USFNAC showed discordant result on histopathology examination. The discordant results shown in a study by Khurshid *et al* (2012) involved masses such as pleomorphic adenoma and basal cell adenoma, that were diagnosed as adenoid cystic carcinoma on histopathology examination. It is a common error because both have uniform epithelial like cells and may have fibrillary myxoid stromal component. The other example is in the diagnosis of Warthin tumour on cytological result, but histopathology examination revealed lymphoepithelial cyst. This is due to the fact that both lesions can present as cystic lesions and may associate with neoplasms, and this can lead to a false positive result.

It is debated in some articles that USFNAC is less sensitive than CT scan and MRI in detecting head and neck mass (Krishnappa *et al* 2013). This could explain some of the false positive and false negative results in this study, which were 3.33% false positive and 10% false negative results. The cases with false positive and false negative result also can be due to several reasons such as huge neck mass, long standing neck mass, history of neck inflammation and history of previous irradiation. As mentioned earlier, the other factors of false positive and false negative results are operator dependent. The limitation of experience can be improved with frequent practice. The creation of a 'one-stop' clinic for head and neck lump for assessment and investigation in providing rapid diagnosis of head and neck malignancy would be beneficial (Ganguly *et al* 2010).

There is some bias and limitations while completing this study. The first one is operator competence of ultrasound and FNAC technique by the radiology and pathology team. with the adequacy of samples is influenced by the technique of aspiration and number of sampling attempts. Secondly, FNAC does not allow evaluation of the histomorphology. As a result, false negative and false positive results can occur. Third, HPE is a gold standard for definitive diagnosis. This was not possible however as many benign head and neck masses are not indicated for surgical excision unless indicated due to such symptoms as compression.

Conclusion

This study results strongly suggested that ultrasound guided FNAC should be used as the primary diagnostic tool for the preoperative evaluation of patients with head and neck masses, especially when the mass is small, located near the neurovascular structures, or when malignancy is suspected. By using this technique, it can provide an earlier correct diagnosis to facilitate a subsequent refined management plan.

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References

Ahuja A, Ying M 2002. An overview of neck node sonography. *Investigative Radiology* 37(6): 333-42.

Batool QU, Khaliq BA, Kirmani OS, Lone Z, Khaliq BI, Mir A, Qadir W, Makhdoomi O 2017. USG Guided FNAC vs. Blind FNAC in Evaluation of Thyroid Swellings:A Comparative Study. *IJIR* 3(3): 899-902.

Buckland JR, Manjaly G, Violaris N, Howlett DC 1999. Ultrasound-guided cutting-needle biopsy of the parotid gland. *J Laryngol Otol* 113(11):988-92. https://doi.org/10.1017/S0022215100145785.

Cai XJ, Valiyaparambath N, Nixon P, Waghorn A, Giles T, Helliwell T 2006. Ultrasound-guided fine needle aspiration cytology in the diagnosis and management of thyroid nodules. *Cytopathology* 17(5):251-6. https://doi.org/10.1111/j.1365-2303.2006.00397.

Chen PY, Chiou SC, Yeh HY 2010. Correlation of Ultrasonography with Fine Needle Aspiration Cytology and Final Pathological Diagnoses in Patients with Thyroid Nodules. *Chin J Radiol* 35: 1-7.

Dongbin A., Heejin Kim, Jin Ho Sohn, Jae hyuk Choi, Kyung Jin Na 2015. Surgeon performed US guided FNAC of head and neck mass lesions: sampling adequacy and diagnostic accuracy. *Ann Surg* 22: 1360-1365. https://doi.org/10.1245/s10434-014-4119-2.

El Hag IA, Chiedozi LC, Al Reyees FA, Kollur SM 2003. Fine Needle Aspiration Cytology of Head and Neck Masses, Seven Years' Experience in a Secondary Care Hospital. *Acta Cytologica* 47:387-392. https://doi.org/10.1159/000326538.

Filion EJ, McClure LA, Huang D, Seng K, Kaplan MJ, Colevas AD, Gomez

SL, Chang ET, Le QT 2010. Higher Incidence of Head and Neck Cancers Among Vietnamese American Men in California. *Head Neck* 32(10): 1336–44. https://doi.org/10.1002/hed.21330.

Ganguly A, Giles TE, Smith PA, White FE, Nixon PP 2010. The benefits of on-site cytology with ultrasound guided fine needle aspiration in a one-stop neck lump clinic. *Ann R Coll Surg Engl* 92: 660–4. https://doi.org/10.1308/rcsann.2010.92.8.660.

Islam MO, Sarker MZ, Mahmud M, Ahmed MU, Bhuiyan AR, Chowdhury AA 2015. Comparative Study of Ultrasound Guided FNAC and Conventional FNAC in the Diagnosis of Parotid Tumour. *Bangladesh J Otorhinolaryngol* 21(1):5-16. http://dx.doi.org/10.3329/bjo.v21i1.24290.

Jalan S, Sengupta S, Ray R, Mondal R, Phukan J, Bardhan J, Ghosh T 2018. A comparative evaluation of USG-guided FNAC with conventional FNAC in the preoperative assessment of thyroid lesions: A particular reference to cyto-histologically discordant cases. *Bangladesh Journal of Medical Science* 16(2):274-80.

Khan N, Afroz N, Agarwal S, Khan MA, Ahmad I, Ansari H, Chauhan S, Rabindranath D, Khan AA 2015. Comparison of the efficacy of the palpation versus ultrasonography-guided fine-needle aspiration cytology in the diagnosis of salivary gland lesions. *Clin Cancer Investig J* 4:134-9. doi: 10.4103/2278-0513.152717.

Khurshid A, Johar I, Raza M, Muneeb K, Bakht Z 2012. Differentiating Malignant from Benign Lesions- The value of Preoperative Cytology in the Management of Parotid Neoplasm. *Ann Pak Inst Med Sci* 8(1): 70-74.

Knappe M, Louw M, Gregor RT 2000. Ultrasonography-guided fine-needle aspiration for the assessment of cervical metastases. *Arch Otolaryngol Head Neck Surg* 126(9):1091-1096. doi:10.1001/archotol.126.9.1091.

Krishnappa P, Ramakrishnappa S, Kulkarni MH 2013. Comparison of free hand versus ultrasound-guided fine needle aspiration of thyroid with histopathological correlation. *J Environ Pathol Toxicol Oncol* 32 (2): 149-55. doi:10.1615/JEnvironPatholToxicolOncol.2013006980.

Lee EW, Chen C, Sauk S, Ragavendra N 2009. How Diagnostic Is Ultrasound-Guided Neck Mass Biopsy (Fine-Needle Capillary Sampling Biopsy Technique)? Evaluation of 132 Non-thyroid Neck Mass Biopsies with Pathologic Analysis Over 7 Years at a Single Institution. Journal of Ultrasound in Medicine. *Jour Ultrasound Med* 28:1679-84. https://doi.org/10.7863/jum.2009.28.12.1679.

McIvor NP, Freeman JL, Salem S, Elden L, Noyek AM, Bedard YC 1994. Ultrasonography and ultrasound-guided fine-needle aspirat ion biopsy of head and neck lesions: a surgical perspective. *Laryngoscope* 104: 669–74. https://doi.org/10.1288/00005537-199406000-00005.

Poorey VK, Tyagi A 2014. Accuracy of fine needle aspiration cytology in head and neck masses. *Indian J Otolaryngol Head Neck Surg* 66(2):182-186. doi:10.1007/s12070-014-0709-3.

Panneerselvam S, Vaithianathan RK, Santhanam R 2013. Accuracy of ultrasound guided FNAC in evaluation of thyroid nodules. *Int J Cur Res Rev* 5(2);94-100.

Piccioni LO, Fabiano B, Gemma M, Sarandria D, Bussi M 2011. Fine needle aspiration cytology in the diagnosis of parotid lesions. *Acta Otorhinolaryngologica Italica* 311:1-4.

Riaz N, Morris LG, Lee W, Chan TA 2014. Unraveling the molecular genetics of head and neck cancer through genome-wide approaches. *Genes Dis* 1(1): 75–86. https://doi.org/10.1016/j.gendis.2014.07.002.

Sattar A, Shamim SH, Wahab S, Javed A 2016. Yield and Safety Profile of Ultrasound Guided Fine Needle Aspiration Cytology (FNAC) of Lymph Nodes. *J Col Pysicians Surg Pak* 26(50):357-60.

Sejal N Mistry, Mukesh N Dodia, Manish R Mehta, Paresh J Khavdu, Alpesh D Fefar 2015. Role of FNAC in Non-Metastatic Non-Hormonal Neck swellings. *Journal of Research in Medical and Dental Science* Vol. 3, Issue 4:266-68.

Sharma G, Jung AS, Maceri DR, Rice DH, Martin SE, Grant E 2011. US-guided Fine Needle Aspiration of Major Salivary Gland Masses and Adjacent Lymph Nodes: Accuracy and Impact on Clinical Decision Making. *Radiology* 259 (2): 471-478.

Silas OA, Ige OO, Adoga AA, Nimkur LT, Ajetunmobi OI 2015. Role of Fine Needle Aspiration Cytology (FNAC) as a Diagnostic Tool in Paediatric Head and Neck Lymphodenopathy. *J Otol Rhinol* 4(1). doi: 10.4172/2324-8785.1000211.

Van den Brekel MW, Castelijns JA, Stel HV, Golding RP, Meyer CJ, Snow GB 1933. Modern imaging techniques and ultrasound-guided aspiration cytology for the assessment of neck node metastases: a prospective comparative study. *Eur Arch Otorhinolaryngology* 250:11-17. doi: 10.1007/BF00176941.

ORIGINAL ARTICLE

Review of coagulation testing in liver disease

Rohit Chadha

Monash Health Pathology, Victoria

Abstract

Routine laboratory tests such as the international normalized ratio (INR), activated partial thromboplastin time (APTT), fibrinogen and platelets are frequently abnormal in liver disease, often pointing towards a hypocoagulable state. As shown by various studies though these patients may be in haemostatic balance. In multiple studies, routine coagulation tests could not predict when this balance gets disturbed and these tests do not measure *in vivo* coagulation activity. This unpredictability is demonstrated clinically by some patients with liver disease undergoing surgery without any appreciable need for blood products and others having either bleeding or thrombotic complications. Both of these sets of patients may have similar routine coagulation results. It has been seen that this balance is more accurately assessed with viscoelastic tests and thrombin generation tests, which are affected by both pro-haemostatic and anti-haemostatic factors.

This review will commence with a description of the effect of liver disease on pro-haemostatic and anti-haemostatic factors. It will then continue to discuss the factors that impact routine coagulation tests, viscoelastic tests, and thrombin generation assays. The standard lab tests will be compared and contrasted with viscoelastic tests and thrombin generation assays in the context of liver disease. Hyperfibrinolysis is one of the pathologies of liver disease and tests for fibrinolysis and their clinical importance in liver disease will also be discussed in this review.

Keywords: Primary haemostasis, secondary haemostasis, liver disease, haemostasis testing, haemostasis abnormalities, rebalanced haemostasis

Introduction

The liver has a major role in haemostasis as it synthesizes the majority of plasma proteins involved in primary haemostasis, secondary haemostasis, and fibrinolysis. It is also responsible for the clearance of activated haemostatic protein or protein-inhibitor complexes from the circulation. These proteins which are both pro-haemostatic and anti-haemostatic are balanced in a normal individual. Liver disease may cause a rebalance of the pro and anti-haemostatic protein which may be progressive with the severity of the disease. Child–Pugh and the Model for End-stage Liver Disease (MELD) scores have been widely used for the assessment of prognosis in liver cirrhosis.

Address correspondence to:
Rohit Chadha
Monash Health Pathology, Victoria
E-mail: rohitchadha33@gmail.com

The Child-Pugh score is used for assessing the prognosis — including the required strength of treatment and necessity of liver transplant — of chronic liver disease, mainly cirrhosis. The score is determined by scoring five clinical measures of liver disease. A score of 1-3 is given to each measure, with 3 being the most severe. The five clinical measures are total bilirubin, serum albumin, prothrombin time or INR, ascites, and hepatic encephalopathy. Chronic liver disease is classified into Child-Pugh class A to C, employing the added score from above (Child and Turcotte 1964; Pugh *et al* 1973; Cholongitas *et al* 2005).

The MELD score, although initially created to predict survival in patients with complications of portal hypertension undergoing elective placement of transjugular intrahepatic portosystemic shunts, has become a metric by which the severity of the liver disease can be accurately described. The MELD score consists of serum bilirubin, creatinine levels, INR or prothrombin time, and aetiology of liver disease. The major use of the MELD score has been in the allocation of organs for liver transplantation. However, the MELD score has also been shown to predict survival in patients with cirrhosis who have infections, variceal bleeding, as well as in patients with fulminant hepatic failure and alcoholic hepatitis. MELD may be used in the selection of patients for surgery other than liver transplantation and

in determining the optimal treatment for patients with hepatocellular carcinoma who are not candidates for liver transplantation (Malinchoc *et al* 2000; Kamath *et al* 2001; Kamath *et al* 2007).

Alteration of haemostasis in liver disease

Primary haemostasis

Platelet number

Thrombocytopenia is the most common haematological abnormality encountered in patients with chronic liver disease (CLD) (Qamar et *al* 2009; Gianni and Savarino 2008). The factors contributing to the thrombocytopenia in liver disease can be broadly divided into a reduced production rate, splenic sequestration, and increased rate of destruction.

Direct bone marrow suppression along with depressed thrombopoietin levels in CLD cause a decreased platelet production. Thrombopoietin regulates both platelet maturation and production and is impaired in CLD (Koike *et al* 1998). Other significant reasons for bone marrow suppression can be viruses, alcohol, iron overload, and medications (Mitchell *et al* 2016). Splenic sequestration results from hypersplenism (Aster *et al* 1966). The increased rate of platelet destruction in liver disease occurs through increased fibrinolysis, increased shear stress, infections, and bacterial translocation. These result in increased platelet aggregation, while autoimmune disease and raised titres of anti-platelet immunoglobulin result in the immunologic destruction of platelets (Mitchell *et al* 2016).

Platelet function

There are many contradictory reports associated with platelet dysfunction. Most earlier studies demonstrated clear hypoaggregability based on *in vitro* platelet function tests. A decreased response to collagen, thrombin, arachidonic acid, adenosine diphosphate (ADP), epinephrine, and ristocetin have been reported (Rubin *et al* 1979; Ingeberg *et al* 1985; Laffi *et al* 1987; Laffi *et al* 1988; Laffi *et al* 1992; Laffi *et al* 1993; Sa´nchez-Roig *et al* 1994; Laffi *et al* 1996; Pantaleo *et al* 2004; Jüttner *et al* 2009).

Various causes described for impaired aggregation are defective platelet signal transduction mechanisms (Laffi *et al* 1993), an acquired storage pool deficiency (Laffi G *et al* 1992), and decreased amount of arachidonic acid (which is required for thromboxane A2 production) in the platelet membrane (Owen *et al*). Fibrin degradation products also competitively inhibit the ADP receptors on platelets (Thorsen *et al* 1986) and platelet-vessel wall interaction examined under flow conditions is impaired (Ordinas 1996). Increased production of prostacyclin and nitric oxide (both potent platelet inhibitors) by endothelial cells may additionally contribute to impaired platelet function *in vivo* (Guarner *et al* 1992; Albornoz *et al* 1999). Finally, platelet and vessel wall interaction may be defective in patients with liver disease due to platelet receptors' proteolysis by plasmin (Michelson and Barnard 1990; Pache *et al* 1994) or due to reduced haematocrit (Turitto and Baumgartner 1975). There are however many contradictory reports. The conflicting reports are mainly because of the different inclusion criteria, different types, and degrees of liver disease, or the small sample size of various studies. There was a report of normal adhesion to collagen and fibrinogen underflow at standardised platelet count and haematocrit (Lisman, Caldwell *et al* 2006).

Contrary to these findings, Raparelli *et al* in 2017 demonstrated enhanced platelet aggregation in cirrhotic patients after stimulation with subthreshold concentrations of collagen or ADP. There was no difference in aggregation between patients and healthy controls with threshold concentrations of collagen. The addition of an inhibitor of the lipopolysaccharide acid (LPS) receptor (Toll-like receptor 4 binding protein), inhibited platelet aggregation only in cirrhotic patients. This study concluded that the presence of high circulating levels of Toll-like receptor 4 binding protein, shifted in the systemic circulation, may be the cause of enhanced platelet activation in cirrhotic patients.

Thromboxane B2, a prothrombotic molecule, is increased in liver cirrhosis as determined by increased urinary 11-Dehydrothromboxane B2 (Davi *et al* 1998) and *in vivo* Thromboxane B2 in decompensated patients with a transjugular intrahepatic portosystemic shunt procedure (Queck *et al* 2019). The same study found increased portal hepatic venous pressure gradient as an indirect sign of thrombogenic potential.

There has also been considerable research in identifying activated platelets and soluble markers of platelet activation, which may be predictive of *in vivo* thrombotic tendencies. In recent times flow cytometry has been used to study platelet activation markers on platelet surfaces such as membrane p-selectin (basal and post-stimulation), platelet monocyte complexes, platelet leucocyte complexes, GP IIb and IIIa (post- stimulation). Also, soluble markers of platelet activation such as CD40L and soluble p-selectins have been studied. Even when the markers are increased, they should be carefully interpreted since their elevation could also be due to decreased clearance by the liver.

Analysis of the literature on assays of platelet function reveals no consensus on the degree of platelet activation. Details are in Table 1 which elucidates the various studies of platelet activation.

Table 1. *Studies on platelet activation*

Platelet activation normal/decreased

Study	Population	Methodology/Tests	*In vivo* Activation	*Ex vivo* Activation
Laffi *et al* 1987	Severe liver cirrhosis (n=10)	Platelet count-adjusted PA using washed platelets		Reduced aggregation with thrombin and collagen and receptor dependent activation of Phospholipase C, A2 and cyclooxygenase/thromboxane synthetase.
Jüttner *et al* 2009	Tumor (n = 9) and cirrhosis (n = 25, mixed aetiology)	LTA, mPs	Decreased mPs in all patients	Decreased LTA in cirrhosis with ADP and epinephrine (cholestatic > non-cholestatic), decreased mPs in all patients
Alkozai *et al* 2015	HCC (n = 22) and cirrhosis (n = 16, viral)	mPs Flow cytometry (basal and stimulation), sPs	Normal mPs exposure on resting platelets. Increased sPs (Due to decreased clearance by liver)	Decreased mPs exposure after activation.
Potze *et al* 2016	NAFLD (n = 68, steatosis, NASH, NASH cirrhosis), ASH cirrhosis (n = 15)	Flow cytometry	Normal mPs exposure on resting platelets. Normal PF4. Increased sPs.	Slightly decreased mPs exposure after activation.
Vinholt *et al* 2017	Cirrhosis (n = 27, ASH)	Flow cytometry		Decreased GPIIbIIIa post activation compared to control. Decreased platelet aggregation for collagen-related peptide and ADP
Eyraud *et al* 2018	HCC (n = 24) and cirrhosis (n = 26, mixed aetiology)		no concomitant increase of circulating markers of platelet activation: platelet micro vesicles, platelet-leukocyte complexes, soluble CD40L and soluble CD62P. Normal mPs	Normal mPs in response to peptide agonist of thrombin receptor (TRAP). Decreased LTA pre-graft versus post-graft (D28, Plt adjusted)

Platelet Activation increased

Study	Population	Methodology/Tests	*In vivo* Activation	*Ex vivo* Activation
Panasiuk *et al* 2005	Liver Cirrhosis (n=40)	Flow cytometry	Increased mPs expression, on resting platelets but similar platelet–monocyte aggregates. As cirrhosis develops and plt count diminishes, plt aggregates with monocytes increase.	
Ogasawara *et al* 2005	ASH and hepatitis C(n=20)	Flow cytometry	Increased p-selectin (ASH and hepatitis C) and PAC-1 positivity (hepatitis C) on resting platelets	
Sayed *et al* 2010	Hepatitis B and C Cirrhosis (n=60)	Flow cytometry	Substantially increased p-selectin exposure and platelet–monocyte aggregates on resting platelets	
Basili *et al* 2011	Cirrhosis (n = 51, mixed aetiology)	sNOX2dp, sPs, sCD40L, isoprostanes (urinary and platelet)	Increased sNOX2dp, sPs, sCD40L, isoprostanes (urinary and platelet)	
Egan *et al* 2016	Compensated cirrhosis (n = 46, mixed aetiology)	Immunoassay	Soluble GPVI increased	
Raparelli *et al* 2017	Cirrhosis (n = 69) Mixed	Flow cytometry, platelet count–adjusted aggregation, sPs, sCD40L, LPS, zonulin,	Increased number of platelet–monocyte Aggregates. Increased sPs, sCD40L, LPS, zonulin	Enhanced activation with subthreshold doses of activator (Collagen). LPS was implicated for the enhanced activation as Toll-like receptor 4 inhibitor blunted this effect.
Stoy *et al* 2018	Cirrhosis (n = 19, mixed aetiology)	Platelet–leukocyte complexes	Increased Platelet-Neutrophil complexes	

Platelet Activation normal

Study	Population	Methodology/Tests	*In vivo* Activation	*Ex vivo* Activation
Lisman *et al* 2006	Mixed (n=16)	Adhesion to collagen and fibrinogen under flow at standardized platelet count and haematocrit		Normal adhesion to both surfaces

FC = flow cytometry, HCC= hepatocellular carcinoma, LPS = lipopolysaccharide acid, LTA = light transmission aggregometry, mPs = membrane P-selectin, PA = platelet aggregation, PF4 = platelet factor 4, PLT = platelet, PVT = portal vein thrombosis, sNOX2dp = soluble NADPH oxidase derived peptide, sPs = soluble P-selectin, PAC-1 is an antibody specific to the activated form of alphaIIbbeta3

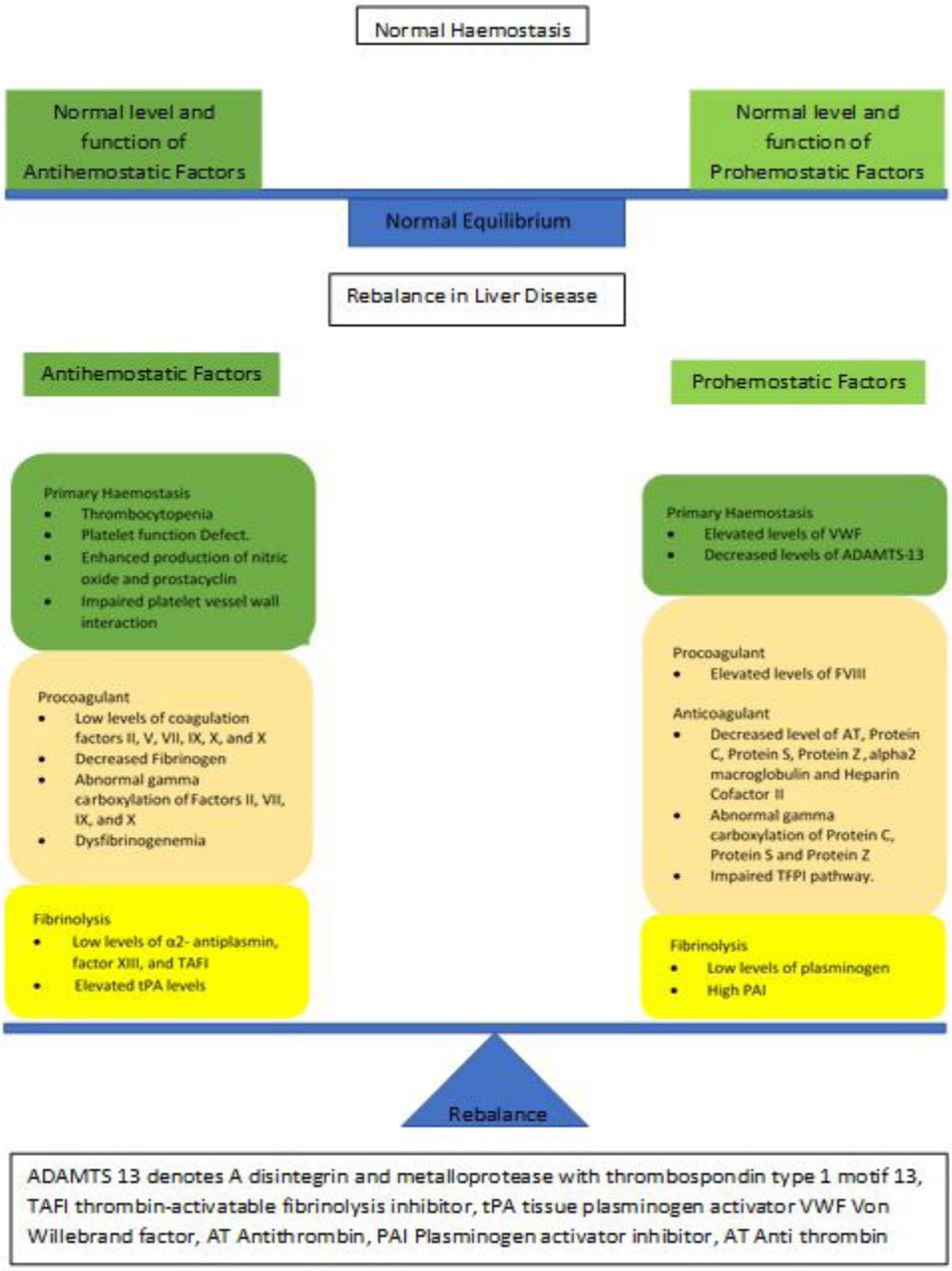

Figure 1. *Balance of prohaemostatic and antihaemostatic factors in normal haemostasis and liver disease*

The contradictory reports in platelet dysfunction are partly due to the technical difficulties in studying platelets. Platelets are fragile and easily activated *in vitro*. Platelet assays are not standardised with varying concentrations of agonists. Variations between studies can also be explained by the CLD population's heterogeneity with different degrees of severity and other aetiologies. Other factors causing changes in primary haemostasis are plasma levels of von Willebrand Factor (vWF) which are considerably increased in patients with liver failure (Ferro *et al* 1996; Lisman *et al* 2006), and the plasma a disintegrin and metalloproteinase with a thrombospondin type 1 motif member 13 (ADAMTS13) activity and antigen levels decreased as the severity of cirrhosis increases (Feys *et al* 2007; Uemura *et al* 2008).

Effect of changes in primary haemostasis in liver disease

Although the combined effect of thrombocytopenia, platelet function defects, impaired platelet vessel wall interaction, and enhanced production of nitric oxide and prostacyclin may cause bleeding, very high levels of vWF, commonly present in chronic liver disease, may normalise platelet adhesion to the subendothelium in the case of vascular injury as evidenced by *in vitro* experiments carried out under flow conditions mimicking those that occur *in vivo* (Lisman T *et al* 2006). An imbalance between the decreased ADAMTS13 activity and its substrate's increased levels may reflect a state that predisposes the patients with advanced liver cirrhosis to platelet thrombus formation (Feys *et al* 2007; Uemura *et al* 2008). One of the roles of platelets is to support thrombin generation by assembling activated coagulation factors on their surfaces. A platelet count as low as 60×10^9/l in platelet-rich plasma from patients with cirrhosis is usually sufficient to preserve thrombin generation at a level equivalent to the lower limit of the normal range in healthy subjects.

Secondary haemostasis

Procoagulant effect: Since the liver is assumed to be the site of synthesis for all proteins (except for factor(F) VIII) necessary in thrombin generation, decreased plasma concentrations for all proteins except for FVIII are observed in patients with liver failure. Clotting factor concentration usually falls in parallel with the progression of liver disease, although levels vary considerably (Tripodi *et al* 2009). FVIII levels are often increased many times in patients with stable cirrhosis. The increase in FVIII levels is associated with increased hepatic biosynthesis of vWF and decreased expression of low-density lipoprotein receptor-related protein (LRP), rather than increased FVIII synthesis (Hollestelle *et al* 2004). vWF acts as a carrier of FVIII, and this association protects FVIII from rapid proteolysis (Lenting *et al* 1998). LRP, a multifunctional endocytic receptor, has a role in controlling cellular uptake and subsequent degradation of FVIII and FVIII has been shown to have multiple binding sites for LRP (Lenting *et al* 1998; Saenko *et al* 1999: Schwarz et al 2000). As LRP is particularly expressed in the liver in Kupffer cells and hepatocytes, it is consequently decreased in liver disease (Moestrup et al 1992). Hence a decrease in LRP results in increased plasma FVIII concentration by decreasing cellular intake and subsequent degradation of FVIII.

Dysfunctional coagulation proteins may also be formed in liver disease. FII, VII, IX, and X, and the anticoagulant factors protein C, protein S, and protein Z contain- many glutamic acid residues (GLU). These glutamic residues are modified by the addition of the carboxyl group to the γ-carbon post-translationally by a vitamin-K-dependent carboxylase to form γ-carboxyglutamic acid. This γ-carboxyglutamic acid (GLA) domain provides affinity to negatively charged phospholipid membranes, such as those of activated platelets or endothelial cells, promoting the assembly of functional multiprotein complexes on these surfaces. In liver disease, part of the plasma prothrombin and protein C lack their GLA residues, which is also expected for the other GLA containing proteins (Blanchard *et al* 1981; Yoshikawa *et al* 1988). Abnormal γ-carboxylation might be due to an intrinsic enzymatic defect or to a vitamin K deficiency.

In both chronic and acute liver failure, abnormal fibrinogen molecules are commonly found (Francis and Armstrong 1982). These dysfibrinogens have an excessive content of sialic acid residues, which leads to impaired fibrin polymerisation (Martinez *et al* 1983). The prevalence of dysfibrinogenemia is higher in patients with liver disease (76% to 86%) than in those with obstructive jaundice (8%) (Francis and Armstrong 1982).

Anticoagulant

Antithrombin (AT), Protein C, Protein S, Protein Z, alpha2 macroglobulin, and Heparin Cofactor II and are synthesised primarily in the liver and hence are reduced in liver disease (Tripodi *et al* 2009; Tripody *et al* 2013; Kemkes-Matthes *et al* 1995; Damus and Wallace 1975). Tissue factor pathway inhibitor (TFPI) is produced by endothelial cells and levels are expectedly normal in patients with chronic liver disease (Bajaj *et al* 1987; Bajaj *et al* 1990). TFPI anticoagulant pathway however is functionally impaired by decreased concentration of protein S and hence there is a decreased downregulation of thrombin generation (Potze *et al* 2013). Additionally, functional defects may be produced due to part of Protein C, Protein S, and Protein Z lacking gamma-carboxylation.

Effect of procoagulants and anticoagulants: Low levels of coagulation factors (FII, V, VII, IX, X, and X), decreased fibrinogen, abnormal gamma-carboxylation of Factors

(FII, VII, IX, and X), and dysfibrinogenemia are balanced by elevated levels of FVIII, decreased level of anticoagulants (AT, Protein C, Protein S, Protein Z, alpha2 macroglobulin and Heparin Cofactor II), abnormal gamma-carboxylation of anticoagulants (Protein C, Protein S and Protein Z) and impaired TFPI pathway.

Although the prothrombin time (PT) and APTT suggest defective coagulation, these tests do not represent the balance between the pro- and anticoagulant proteins because the PT and APTT are not sensitive to deficiencies of the anticoagulants. The addition of thrombomodulin to a test system such as thrombin generation tests makes it sensitive to anticoagulants (Tripody *et al* 2005). The median ratio of thrombin generation (with/without thrombomodulin) was higher in patients with liver disease compared to normal patients. These results highlight the resistance to the inhibitory actions of thrombomodulin in the case of liver disease. This resistance points to greater hypercoagulability in liver disease which increases from Child-Pugh Class A to C. Indeed, it has been found that an increase of FVIII and decrease in protein C, FII, FV and AT is progressive with Child-Pugh score (from Child-Pugh Class A to Class C). Increased FVIII and decreased Protein C are a major reason for hypercoagulability leading to thrombosis (Tripody *et al* 2009).

The fibrinolytic system

Except for tissue plasminogen activator (tPA)and plasminogen activator inhibitor type 1 (PAI-1), all proteins involved in fibrinolysis are produced in the liver, hence reduced plasma levels of plasminogen (Stein and Harker LA 1982), α2-antiplasmin (Lisman T *et al* 2001), factor XIII (Biland *et al* 1978) and thrombin-activatable fibrinolysis inhibitor (TAFI) (Lisman *et al* 2001; Colucci *et al* 2003) have been found in patients with liver disease. Plasma tPA levels are often increased in patients with hepatic failure (Leiper *et al* 1994). The decreased plasma tPA levels may be a result of either increased secretion from endothelial cells or decreased clearance by the ailing liver. PAI-1 levels are only slightly increased in plasma from patients with liver disease and do not seem to balance the elevated plasma tPA levels (Hersch SL *et al* 1987, Leebeek *et al* 1991), except in acute hepatic failure, in which plasma PAI-1 levels are dramatically increased (Pernambuco *et al* 1993).

Effect of changes in fibrinolysis

Low levels of α2-antiplasmin, factor XIII, and TAFI and elevated tPA levels are balanced in liver disease with low levels of plasminogen and high PAI.

Physiological stress including infection may be key in tipping the balance towards fibrinolysis by increased release of tPA (Thalheimer *et al* 2005). Another cause may be the presence of ascites in liver disease (Agarwal *et al* 2000).

Hyperfibrinolysis has been reported in liver disease as far back as 1914 (Goodpasture 1914). This has been confirmed multiple times using clot-lysis experiments or global fibrinolytic assay (Ratnoff 1949; Kwaan *et al* 1956; Colucci *et al* 2003; Aytac *et al* 2007; Rijken *et al* 2012)

Although there have been conflicting reports about the role of TAFI in inducing hyperfibrinolysis (Lisman *et al* 2001; Colucci *et al* 2003), the conflicting results may be due to different clot lysis models used by the two researchers particularly with respect to trigger of coagulation i.e. thromboplastin by Lisman *et al* (2001) and thrombin and increased dilution of plasma by Colucci *et al* (2003). However, Colucci *et al* (2003) still got decreased plasma clot lysis time results using a lysis mixture similar to Lisman *et al* (2001). Colucci *et al* (2003) however could not exclude the minor methodologic difference or differences in the clot lysis method and or differences in selection of patients as cause for this discrepancy.

There have also been reports of low-level underlying disseminated intravascular coagulopathy (DIC) in liver disease. This might be due to similarities of test results such as D-dimers and FDP in DIC and cirrhosis. In contrast to DIC, liver disease has relatively stable platelet levels and FVIII (Joist *et al* 1999). It was concluded in a study in 1999 that DIC is not part of the coagulopathy in stable liver cirrhosis without recent complications (Ben-Ari *et al* 1999). The mechanism of fibrinolysis may be due to the formation of a fibrin clot which is more susceptible to fibrinolysis due to increased level of tPA (usually increased by stress like infection) coupled with the inadequate release of PAI. There is also a lack of α2-plasmin inhibitor to stop plasmin activity and there is the maintenance of a high concentration of plasminogen on the clot surface despite lower plasminogen production.

Joist (1999) reviewed studies using highly sensitive tests such as quantification of proteolytic cleavage products of the coagulation reaction and reported increased levels of D-dimer, prothrombin fragment F1+2, fibrinopeptide A, TAT, and plasmin-α_2-antiplasmin complexes and proposed accelerated intravascular coagulation and fibrinolysis (AICF- previously low-grade DIC) as a feature in less than a third of patients with CLD. The presence of AICF correlated with disease severity; it was seen at increased frequency in those with advanced or decompensated CLD and those with additional risk factors, such as sepsis, shock, or malignancy.

The concept of rebalanced haemostasis

A normal individual has an excess of both prohaemostatic and antihaemostatic factors which leads to a stable

equilibrium; even if there is hemostatic alteration the excess of prohaemostatic and antihaemostatic does not allow the equilibrium to tip in favour of thrombosis or bleeding.

In contrast, in liver disease, the relative deficiency and functional defects of both prohaemostatic and antihaemostatic factors result in a rebalancing between prohaemostatic and antihaemostatic factors. This is shown in Figure 1.

This balance is fragile and can easily tip towards bleeding and/or thrombosis depending on triggering risk factors (infection, renal disease, portal hypertension, and blood stasis). Conditions apart from haemostasis factors that can lead to a bleeding tendency in patients with decompensated CLD are (1) hemodynamic alterations owing to portal hypertension, (2) endothelial dysfunction, (3) development of endogenous heparin-like substances owing to bacterial infections, and (4) renal failure (Caldwell et al 2006).

While portal hypertension is recognised as the leading cause of bleeding in patients with cirrhosis, the role played by coagulation defects in the occurrence of bleeding is still not clear (Garcia-Tsao et al 1985). There seems to be a partial association between the severity of bleeding and the degree of coagulation abnormalities, as well as the fact that conventional coagulation tests fail to reflect blood coagulation as it occurs *in vivo*.

The laboratory investigation of haemostasis in liver disease/tests of primary haemostasis

Platelet count

As previously noted, platelets support both primary haemostasis and secondary haemostasis. The primary haemostasis is supported by the adhesion of platelets to the subendothelium and subsequent aggregation, both being mediated by vWF and fibrinogen.

The secondary haemostasis is supported by platelets providing negatively charged phospholipid surfaces for assembly of macromolecular enzymatic (tenase and prothrombinase) complexes needed for thrombin generation and fibrin formation. Platelet counts as low as 56×10^9/l in platelet-rich plasma from patients with cirrhosis are usually sufficient to preserve thrombin generation at a level equivalent to the lower limit of the normal range in healthy subjects. Current guidelines recommend initiating platelet transfusion in an actively bleeding patient when platelet counts are under 50 X 10^9/l (PBM Guidelines Module 4; Szczepiorkowski and Dunbar 2013). The requirements should be individually assessed in cases of functional defects of platelets (Peterson *et al* 2016).

Platelet counts and size are usually performed by automated analysers and the blood smear is used to differentiate pseudo thrombocytopenia from actual thrombocytopenia.

Viscoelastic testing (VET) and platelet count

The correlation between VET and platelet count is elucidated in the section of platelet function.

Bleeding time: This is one of the earliest tests for the evaluation of primary haemostasis. The bleeding time (BT) is increased in cirrhosis and has been used to measure primary haemostasis and maybe also reflects a decrease in vasoconstrictor response (Violi *et al* 1994; Laffi and Marra 1999). Although prolonged BT was associated with a 5-fold increase risk of haemoglobin reduction after liver biopsy (Boberg *et al* 1999), the correction of the bleeding time with 1-desamino-8-d-arginine vasopressin (DDAVP) worsened bleeding in cases of acute variceal haemorrhage in cirrhotic patients (de Franchis *et al* 1993). Another study with DDAVP did not result in decreased transfusion requirements in patients undergoing hepatectomy.

In a prospective study it was found that although bleeding time increased with the degree of liver deficiency, the subsequent statistical model showed that bleeding time was not a predictor of gastrointestinal bleeding. Only severity of the liver failure and variceal size were found to be independent predictors of bleeding (Basili *et al* 1996). This raises questions about the test's practical utility. In a position paper by the College of American Pathologists and the American Society of Clinical Pathologists, it was concluded that the bleeding time test fails as a screening test and was not indicated as a routine preoperative test in the absence of a history of excessive bleeding (Peterson *et al* 1998). This test is not in use in most of the laboratories in developed countries as it is invasive, shows greater variability, and also poor sensitivity.

Platelet function tests

The PFA-100 is a system for analysing the complex process of primary haemostasis in which citrated whole blood is aspirated under vacuum at high shear rates, similar to the physiologic environment, through self-contained disposable cartridges through a capillary and a microscopic aperture within a membrane coated with either collagen and epinephrine (CEPI) or collagen and ADP (CADP). When blood comes into contact with the coated membrane, platelets adhere, activate aggregate, and aggregate to form a plug that occludes the aperture with consecutive cessation of blood flow called the closure time (CT). The PFA-200 is essentially similar but includes an additional cartridge - the INNOVANCE PFA P2Y, that detects platelet P2Y12-receptor blockade in patients on therapy with a P2Y12-receptor antagonist.

An abnormal CT may therefore reflect vWD or platelet dysfunction, but may also reflect a low platelet count, a low haematocrit, or some anti-platelet therapy.

Prolonged CT occurs in patients with end-stage liver disease (Kujovich 2005) and advanced cirrhosis (Escolar *et al* 1999). Prolonged CT in case of advanced cirrhosis may be due to associated anaemia, as the abnormalities correct with an *in vitro* elevation of the haematocrit (Escolar *et al* 1999). Conventional aggregometry tests were able to identify the intrinsic platelet abnormality thus pointing to the fact that platelet function defects were present. As anaemia and thrombocytopenia are usually features of liver disease, it may be difficult to differentiate between increased closure time due to anaemia/thrombocytopenia or platelet function defects. The value of the PFA-100 in predicting bleeding complications in cirrhosis patients has not yet been studied (Lisman *et al* 2006). An international consensus panel has evaluated the literature on this device in terms of its clinical utility and has concluded that this assay should be considered optional in the evaluation of platelet function, based on a paucity of outcome data that has been published with the PFA-100 (Hayward *et al* 2006).

Platelet aggregation test

Platelet aggregation can be evaluated by light transmission aggregometry (LTA) or whole blood aggregometry/ Multiplate (WBA). However, these tests do not take into consideration the influence of blood flow, a key variable increasing the complexity of the aggregation process. The response of various agonists in liver disease has been discussed in the section on the effect of liver disease on platelet function.

Both WBA and LTA may be enhanced with a luminescence channel that employs the luciferin-luciferase reaction to measure and detect platelet-dense granule ATP secretion after *in vitro* platelet activation. These are also useful to evaluate platelet function in thrombocytopenia (Paniccia *et al* 2015). The luminescence channel adds its name to the terms LTA or WBA, yielding the terms light transmittance lumiaggregometry or whole blood lumiaggregometry (LTLA, WBLA).

Light transmission aggregometry: Platelet aggregation testing measures the ability of various agonists to induce *in vitro* platelet activation and platelet-to-platelet activation. Platelet-rich plasma is stirred in a cuvette that is placed between a light source and a photocell. The plasma is cloudy due to the suspension of platelets and allows relatively little light to pass through. Upon the addition of an agonist, platelets aggregate, and the sample becomes clearer, permitting greater light transmission. Transmission of light is detected by the photocell and recorded as a function of time

It has the disadvantage of variable reproducibility, high costs, large sample volume, sample preparation, and is time-consuming.

Impedance aggregometry

Impedance aggregometry is based on the principle that blood platelets are nonthrombogenic in their resting state, but expose receptors on their surface after activation by agonists that allow them to attach to artificial surfaces. The change in resistance is continuously measured by the instrument and is directly proportional to the number of platelets adhering to the electrodes.

Multiplate

The commercial Multiplate® analyser has five separate channels and five sample test cells. Each probe is equipped with two pairs of electrodes to automatically perform each assay in duplicate, offering improved reliability. It allows simultaneous measurement of multiple different whole blood samples or a single whole blood sample using a variety of different agonists.

The disposable reaction cell detects platelet aggregation as an increase in electrical resistance as activated platelets aggregate on the sensor wires.

The proprietary software integrates the paired output aggregation curves and generates an area under the curve (AUC) expression for each assay. Various assays available in Multiplate are ADPtest, ASPItest, and TRAPtest.

In a study (Stegewerth *et al* 2019) regarding early detection of clinically relevant platelet dysfunction in liver disease, the ASPItest and ADPtest AU/min values at T3 were found to be the most reasonable predictor for impaired platelet function in multiplate and, in case of bleeding, the need for haemotherapy. Previously published studies (Toth *et al* 2006; Mutlak *et al* 2015) found the ASPItest and ADPtest to be of overriding sensitivity and specificity for both the characterisation of platelet dysfunction (irrespective of their aetiologies) and monitoring of haemotherapy.

Disadvantages of this test are that it is expensive, uses a large sample volume, requires sample preparation, and is time-consuming. Also, it has a limited haematocrit (HCT) and platelet count range.

Flow cytometry

In recent times flow cytometry has been used to study markers of platelet activation on platelet surface such as membrane p-selectin (basal and post-stimulation), platelet monocyte complexes, platelet leucocyte complexes, GP IIb IIIa (post stimulation). As previously noted, there is still no consensus on platelet activation in liver disease.

The advantages of this test are that it has low sample volume and is a whole blood assay. It is also sensitive and considered the test of choice to define the presence of thrombocytopathy in a thrombocytopenic patient (Kehrel and Brodde2013)

The disadvantages of the test are increased sample preparation and high costs. It also requires expertise and a flow cytometer. In addition, it only tests platelet activation and not aggregation.

Thromboelastography (TEG®) and Thromboelastometry (ROTEM®) provide global information on the dynamics of clot development, stabilisation, and dissolution that reflect in *vivo* haemostasis.

Using whole blood, these tests analyse all components of haemostasis including clot initiation (factor deficiency, inhibitors, severe hypofibrinogenemia), clot kinetics (hypofibrinogenemia, thrombocytopenia, inhibitors), clot strength (thrombocytopenia, inhibitors, hypofibrinogenemia), and clot lysis (tPA release or severe factor XIII deficiency).

TEG®/ROTEM® and secondary haemostasis

Conventional laboratory tests frequently indicate just hypocoagulable state in patients with liver disease, whereas viscoelastic haemostatic assays (VHA) exhibit a spectrum from hypo- to hypercoagulable.

VHAs have a better predictivity for bleeding in hepatic disease and rebleeding than standard lab tests (Fayed *et al* 2015; Tafur *et al* 2016; Lawson PJ *et al* 2017; Pustavoitau *et al* 2017; Chau *et al* 1998).

A retrospective, single-centre study (Dotsch *et al* 2017) comparing standard lab tests and ROTEM® in post-op bleeding in adult liver transplant patients found that APTT and PT were the standard lab tests predictive of bleeding. ROTEM® tests predictive of bleeding were CTEXTEM, CFTINTEM, A10FIBTEM, and MCFFIBTEMROTEM®-FIBTEM® analyses were found to be better predictors of postoperative bleeding than plasma fibrinogen concentrations in liver transplantation.

British Society of Haemostasis guidelines state that a normal VHA trace has a 95% negative predictive value for transfusion requirement and VHA can be used in liver transplant patients to reduce overall transfusion requirement.

Hypercoagulability detection

In a study (Ben-Ari *et al* 1997) in patients suffering from primary biliary cirrhosis, primary sclerosing cholangitis or non-cholestatic cirrhosis, hypercoagulability was found by TEG® in 13 out of 47 (28%) primary biliary cirrhosis and in nine out of 21 (43%) primary sclerosing cholangitis patients independent of cirrhosis, and bilirubin concentration, but in only 2 out of 40 (5%) patients with non-cholestatic cirrhosis. No evidence of hypercoagulability was found in the normal control group. The clinical implications of these findings were unclear at that time, however, emerging evidence suggests that hypercoagulability detected by VHAs puts patients in an 'at risk' group for both venous and arterial thrombotic events (Kashuk JL *et al* 2009; McCrath DJ *et al* 2005).

The disadvantages of VHAs are that they are not indicative of VWF levels because of lack of shear stress which is essential for the activation of VWF and they are insensitive to Protein C.

TEG®/ROTEM® and functional fibrinogen

TEG® and ROTEM® can give a qualitative indication of functional fibrinogen concentration during the management of major bleeding. Clot strength measures (MA in TEG® and MCF in ROTEM®) are highly dependent on fibrinogen, platelet count and function. To differentiate low clot strength due to low platelet function or number from that due to low fibrinogen, the clot strength of the fibrinogen assay (ff – MA; FIBTEM –MCF) should be compared with the standard MA or MCF trace, respectively. A low clot strength in standard MA/MCF with normal clot strength in ff/FIBTEM would suggest a lack of platelets contributing to the clot, whereas a low clot strength in standard MA/MCF with low clot strength in ff/FIBTEM would point to a lack of fibrinogen.

TEG®/ROTEM® and platelet function

TEG®) with platelet mapping: It is based upon the evaluation of the rate of clot formation based on low shear-induced and agonist addition. In the TEG® system, arachidonic acid and ADP have been used as agonists using the PlateletMapping™ assay for testing platelet function, but the test lacks sensitivity to detect moderate or subtle changes in platelet function (Chen and Teruya 2009; Scharbert *et al* 2009).

ROTEM® platelet system: The ROTEM® platelet measures platelet aggregation in whole blood samples using impedance aggregometry. The device is run in conjunction with the ROTEM® delta. Measurements can be performed on the ROTEM® platelet device while running measurements on the ROTEM® delta system simultaneously and can be visualised together. However, so far very sparse documentation exists for the use of the ROTEM® Platelet as regards platelet function testing. Its disadvantages are similar to multiplate.

TEG® and ROTEM® for detecting fibrinolysis

These analysers are only sensitive for profound hyperfibrinolysis. They are insensitive to mild to moderate fibrinolysis and should not be used to rule out fibrinolysis or a reason to withhold tranexamic acid (Raza *et al* 2013). A prospective observational study in 37 liver transplant patients comparing ROTEM® and TEG® showed that tissue factor-triggered ROTEM tests were more sensitive than contact-activated kaolin TEG in identifying hyperfibrinolysis (Abuelkasem *et al* 2016).

Tests to measure secondary haemostasis

Both procoagulant and anticoagulant factors can be measured in plasma by antigen or activity assays. In liver disease, multiple factors are disturbed simultaneously and therefore global assays reflecting the balance of all factors are required to assess secondary haemostasis.

Traditional coagulation tests PT

The test has been successfully used in the diagnosis of congenital or acquired coagulopathies and to regulate vitamin K antagonists (VKA). This test is also a part of MELD algorithm and Child-Pugh Score for liver disease.

There are issues with using PT in liver disease as the test in its present form is insensitive to the effect of natural anticoagulants because these tests are performed without added thrombomodulin hence they do not allow full activation of protein C. The activation of Protein C without thrombomodulin is 20,000 times slower than that attained in the presence of thrombomodulin (Esmon 1989). They are therefore inadequate to explore the physiological mechanisms regulating thrombin formation. There have been efforts to improve the test by making the test sensitive to natural anticoagulants by including thrombomodulin in the test system and finding out the thrombin potential of the system.

The ISI of thromboplastin is calculated for warfarinized patients. The ISI of thromboplastin for liver disease was found to be significantly different and was found to be better in standardising the INR and subsequent improvement in reporting of MELD score.

Clot-based tests like INR also cannot detect hypercoagulability (Krzanicki *et al* 2013) and in liver transplantation, for example, the PT or INR does not predict which patients will or will not bleed excessively (Massicotte *et al* 2014).

APTT is usually not very sensitive to liver disease. Similar to PT it only measures the procoagulant pathway and is insensitive to natural anticoagulants. FVIII increase in liver disease may blunt the sensitivity of APTT to measure the decrease of other factors in liver disease.

A limitation of these conventional laboratory tests is that plasma starts to clot after as little as 5% of the whole thrombin is generated (Mann *et al* 2003) and null thrombin potential cannot be evaluated with clotting-based assays. It also does not measure clot strength and stability and they are insensitive to modest but clinically relevant reductions in factor concentration (Morrow *et al* 2020).

Thrombin generation tests

Thrombin generation tests (TGT) dynamically measure the total amount of thrombin generated during *in vitro* coagulation. In presence of thrombomodulin (TM), which activates Protein C, TGT assesses the balance between procoagulant and anticoagulant factors by evaluating thrombin generation (resulting from the action of the procoagulant driver) and decay (resulting from the action of the anticoagulant driver). TGT is therefore meant to be used to investigate hypo- or hypercoagulability. Tripody *et al* (2005) demonstrated that plasma from patients with stable cirrhosis generates thrombin at a normal or even increased rate despite prolonged PT/INR and APTT values. In a later study, (Zermatten*et al* 2020) the TM-mediated inhibition had an inverse linear relation to the MELD score, which indicated an increasing prothrombotic state with increasing cirrhosis stage. Butenas *et al* (1999) demonstrated that thrombin generation varies up to 40-fold when individual coagulation factors are measured at the extremes of the normal ranges in a synthetic plasma system. Tripody (2005, 2009) demonstrated that thrombin generation is preserved in cirrhosis despite prolonged conventional coagulation assays due to decreased protein C. Also, in a study on liver disease patients (Morrow *et al* 2020) where PT was prolonged, thrombin generation (ST Genesia) was found to be normal. Of particular interest in this study was the fact that fresh frozen plasma transfusion in patients did not affect thrombin generation.

There are three main types of TGTs – the traditional test, chromogenic assays and, fluorogenic assays. The ST Genesia, launched in June 2018, is a novel walkaway automated analyser, based on the fluorescence principles of thrombin generation described by Hemker *et al* (2003).

One of the disadvantages of TGTs is that they lack information on protein-cell interactions.

Fibrinogen assay

Different types of assay done for fibrinogen estimation are the Clauss assay, PT derived fibrinogen assay, and immunological assays.

The Clauss assay is a functional assay based upon the time for fibrin clot formation. A high concentration of thrombin is added to buffer-diluted test plasma and the clotting time is measured. The PT-derived fibrinogen assay is based on

the PT determined by optical density change for a range of plasma dilutions with known fibrinogen levels. The optical change for each different fibrinogen level is plotted as a calibration curve. A PT is performed on the patient's platelet-poor plasma and the fibrinogen derived from the change in optical density compared to the calibration curve.

Immunological assays are either enzyme-linked immunosorbent assays (ELISA), radial immunodiffusion or electrophoresis.

Immunological assays measure protein concentration rather than functional activity. They are of value in the investigation of congenital dysfibrinogenaemia where there is a discrepancy between functional activity and antigen level.

TEG and ROTEM can also detect fibrinogen and have been discussed previously.

The latest guidelines from BSH regarding lab aspects of assays used in hemostasis and thrombosis investigation recommend:

- fibrinogen should be measured by Clauss assay;
- Clauss assay with concomitant fibrinogen antigen assays should be used to distinguish between hypofibrinogenaemia and dysfibrinogenaemia;
- PT-derived fibrinogen assays are not recommended for clinical use.

Fibrinogen sialylation may be increased in liver disease which may prolong the thrombin time but does not alter the Clauss assay (Baker *et al* 2020) so despite the BSH guidelines dysfibrinogenemia in liver disease can only be detected using thrombin time and an immunological assay. The presence of dysfibrinogenemia in liver disease is unlikely to contribute to systemic bleeding (Abshire 2009).

Fibrinolytic factors can be measured in plasma by antigen or activity assays. In liver disease, however multiple factors are disturbed simultaneously and global assays reflecting the balance of all factors are required to assess the fibrinolytic capacity.

Euglobulin clot lysis time (ECLT)] is a test that reflects the overall fibrinolytic activity of plasma. In many cases (perhaps all) it has now been replaced by specific functional and immunological assays.

The turbidimetric plasma clot lysis assay is performed by adding platelet-poor plasma to the wells of a microtiter plate containing a buffer with tissue factor, $CaCl^2$, and phospholipid vesicles to induce clotting and purified t-PA to induce lysis. The optical density is measured at regular time intervals for a couple of hours. The clot lysis time (CLT) is the time from the midpoint of minimum turbidity to maximum turbidity, which represents clot formation, to the midpoint of maximum turbidity to minimum turbidity, which represents clot lysis.

Global fibrinolytic capacity in a whole blood test is an additional test to measure fibrinolysis (Rijken *et al* 2008). In this test blood is collected in a tube containing thrombin and the resulting clot is incubated for 3 hours at 37°C. The serum is then separated and the generated fibrin degradation products are determined by an immunoassay. This test is fully dependent on t-PA (and possibly u-PA) in the blood sample and on the potential effects of blood cells. *In vitro* inhibition of t-PA by PAI-1 before the assay start is minimised because the clot is formed immediately after blood collection in thrombin.

Fibrinolysis can also be measured by VHAs as discussed previously.

Designing tests for fibrinolysis have some issues.

Endogenous t-PA activity in normal plasma is already inhibited by 50% by PAI-1 within 5 minutes (Rijken *et al* 2009). Therefore, blood collected in citrate and stored for some time before analysis starts is acceptable for the coagulation phase, but not for the lytic phase.

To eliminate this problem, either excess t-PA is added to the system (turbidimetric plasma clot lysis assay) or whole blood (global fibrinolytic capacity assay) and needs to be tested immediately. The first approach makes the test insensitive to the effect of endogenous t-PA and the second approach does not allow the test to be done on stored samples. Also, as expected, the turbidimetric plasma clot assay and the global fibrinolytic assay do not correlate very well. In a comparison done by Rijken *et al* (2012) between the two assays, 15% of patients showed hyperfibrinolysis only in plasma clot assay and 19% showed hyperfibrinolysis only in global fibrinolytic assay.

In general, TEG is considerably less sensitive to newer global tests of fibrinolysis. Even when the global fibrinolysis shows evidence of hyperfibrinolysis, there have been no observed direct implications for the clinical practice (Rijken *et al* 2012). In a clinical study, laboratory evidence of hyperfibrinolysis was found in a third of hospitalised cirrhosis patients, but clinically evident in only 6% of patients (Hu *et al* 2001). Although relatively rare, the clinical signs of fibrinolysis characterised by diffuse oozing from mucosal surfaces or bleeding from puncture wounds and delayed post-procedure bleeding must be recognised and correlated with results from global fibrinolytic assays to facilitate appropriate therapy.

Conclusion

The problem with interpreting different individual tests of coagulation in liver disease is complicated by the effects of rebalanced haemostasis. An increase or decrease of an individual component of primary or secondary haemostasis can be balanced by another component. Measuring and interpreting individual components of primary or secondary haemostasis can consequently be misleading and this is the reason why global tests for primary or secondary haemostasis are required. No global haemostasis test at this moment can interpret all aspects of primary or secondary haemostasis.

Current guidelines recommend initiating platelet transfusion in an actively bleeding patient when platelet counts are under $50x10^9$/L. Individual tests of platelet function tests are not routinely recommended due to rebalanced haemostasis and there is no overall consensus regarding the role of tests of primary haemostasis in liver disease. The complexity of platelet function in predicting the progression of liver cirrhosis, including bleeding and thrombosis, is still debatable and needs further investigation. Currently, the use of routine *in vivo* and *ex vivo* tests to explore global platelet function in cirrhosis is not useful for clinicians.

Conventional lab tests such as PT and APTT do not measure bleeding or thrombosis risk in liver disease. These tests only measure the procoagulant pathway without measuring the counterbalancing anticoagulant pathways and hypercoagulation cannot be predicted by these tests.

Platelet count and fibrinogen are currently considered to be useful in predicting bleeding in cirrhosis patients (Intagliata *et al* 2018). Current BSH guidelines recommend measuring fibrinogen by the Clauss assay.

In liver diseases, multiple factors are disturbed simultaneously and global assays reflecting the balance of all factors are required to assess primary haemostasis, secondary haemostasis or fibrinolysis. The PFA100 is the only shear-based test in routine use to measure primary haemostasis. However as both anaemia and thrombocytopenia may increase CT, it might be difficult to differentiate between increased closure time due to anaemia/thrombocytopenia or platelet function defects. An international consensus panel has concluded that this assay should be considered optional in the evaluation of platelet function.

TEG®/ROTEM® are the only global tests measuring all aspects of secondary haemostasis (clot development, stabilisation and dissolution). It can measure both hypo- and hypercoagulability. It is not indicative of vWF levels because of lack of shear stress which is essential for the activation of vWF. It is also insensitive to protein C and mild to moderate fibrinolysis. Although it is only a qualitative indicator for fibrinogen and platelets, Dotsch *et al* (2017) found that ROTEM®-FIBTEM® analyses were better predictors of postoperative bleeding than plasma fibrinogen concentrations in liver transplantation.

The TGT measures the procoagulant and anticoagulant balance in the plasma sample but it lacks information on protein cell interactions. Due to the presence of thrombomodulin, it is sensitive to protein C levels and also very sensitive to factor deficiencies. It is designed to investigate both hypo- and hypercoagulability. This test was considered a research test before the advent of ST Genesia. However, it is still meant to be used in conjunction with conventional coagulation tests like PT and APTT as per the manufacturer's recommendation. The advances in TGT calls for a novel diagnostic strategy based upon thrombin generation tests.

As per recommendation from Proceedings of the 7th International Coagulation in Liver Disease Conference (Intagliata*et al* 2018) "Available laboratory tests of hyperfibrinolysis are of limited value in cirrhosis and clinical diagnosis is often necessary." It did however recommend considering using anti-fibrinolytic therapy in case of delayed and diffuse mucosal bleeding in decompensated cirrhosis (hyperfibrinolysis physiology).

Despite a variety of global tests to assess prohaemostatic and antihaemostatic pathways in blood or its components the design of a "gold standard" assay has remained an elusive goal due to the complexity of the system. A review of current global tests shows that there is a lack of a systematic approach to their use. There is also low-quality published data that has not been linked to important clinical outcomes. Presently the evidence base to guide practice is limited.

Future research may be directed through large prospective multicentre studies with clinically relevant endpoints and prediction models for bleeding or thrombosis.

References

Abshire TC 2009. Bleeding risks with Liver Disease. In: Hillyer CD, Shaz BH, Zimerring JC, Abshire TC eds *Transfusion Medicine and Hemostasis Clinical and Laboratory Aspects.* 1st Edition: Elsevier:565-569.

Abuelkasem E, Lu S, Tanaka K, Planinsic R & Sakai T 2016. Comparison between throm-boelastography and thromboelastometry in hyperfibrinolysis detection during adult liver transplantation. BJA: *British Journal of Anaesthesia* 116: 507–512.

Agarwal S, Joyner KA, Swaim MW 2000. Ascites fluid as a possible origin for hyperfibrinolysis in advanced liver disease. *Am J Gastroenterol* 95: 3218-3224.

Albornoz L, Bandi JC, Otaso JC, Laudanno O, Mastai R 1999. Prolonged bleeding time in experimental cirrhosis: role of nitric oxide. *J Hepatol* 30:456–460.

Alkozai E M, Porte R J, Adelmeijer J, Zanetto A, Simioni P, Senzolo M, & Lisman, T 2015. No evidence for increased platelet activation in patients with hepatitis B- or C-related cirrhosis and hepatocellular carcinoma. *Thrombosis Research* 135(2): 292–297. https://doi.org/10.1016/j.thromres.2014.11.016

Aster RH 1966. Pooling of platelets in the spleen: role in the pathogenesis of "hypersplenic" thrombocytopenia. J Clin Invest 45(5):645–657.

Aytac S, Turkay C, Bavbek N, Kosar A 2007. Hemostasis and global fibrinolytic capacity in chronic liver disease. *Blood Coagul Fibrinolysis* 18: 623–6.

Baker P, Platton S, Gibson C, Gray E, Jennings I, Murphy P, & Laffan M 2020. Guidelines on the laboratory aspects of assays used in haemostasis and thrombosis. *British Journal of Haematology 191*(3): 347–362. https://doi.org/10.1111/bjh.16776

Bajaj MS, Kuppuswamy MN, Saito H, Spitzer SG, Bajaj SP 1990. Cultured normal human hepatocytes do not synthesise lipoprotein-associated coagulation inhibitor: evidence that endothelium is the principal site of its synthesis. *Proc Natl Acad Sci* (USA) 87:8869–8873.

Bajaj MS, Rana SV, Wysolmerski RB, Bajaj SP 1987. Inhibitor of the factor VIIa-tissue factor complex is reduced in patients with disseminated intravascular coagulation but not in patients with severe hepatocellular disease. *J Clin Invest* 79:1874–1878.

Basili S, Ferro D, Leo R, Juliano L, Alessandri C, Cordova C and Violi F 1996. Bleeding time does not predict gastrointestinal bleeding in patients with cirrhosis. *Journal of Hepatology* 24(5): 574-580.

Basili S, Raparelli V, Riggio O, Merli M, Carnevale R, Angelico F, Tellan G, Pignatelli P, Violi F, Group C 2011. NADPH oxidase-mediated platelet isoprostane over-production in cirrhotic patients: Implication for platelet activation. ***Liver Int***. 31: 1533–1540.

Ben-Ari Z, Panagou M., Patch D, Bates S, Osman E, Pasi J and Burroughs A 1997. Hypercoagulability in patients with primary biliary cirrhosis and primary sclerosing cholangitis evaluated by thrombelastography. *Journal of Hepatology*, *26*(3): 554-559.

Ben-Ari Z, Osman E, Hutton RA and Burroughs AK 1999. Disseminated intravascular coagulation in liver cirrhosis: fact or fiction? *The American Journal of Gastroenterology 94*(10): 2977-2982.

Biland L, Duckert F, Prisender S, Nyman D 1978. Quantitative estimation of coagulation factors in liver disease. The diagnostic and prognostic value of factor XIII, factor V and plasminogen. *Thromb Haemost* 39: 646–656.

Blanchard RA, Furie BC, Jorgensen M, Kruger SF, Furie B 1981. Acquired vitamin K-dependent carboxylation deficiency in liver disease. *N Engl J Med* 305: 242–248.

Boberg KM, Brosstad F, Egeland T, Egge T, Schrumpf E 1999. Is a prolonged bleeding time associated with an increased risk of hemorrhage after liver biopsy? *Thromb Haemost* 81:378-381.

Butenas S, Van't Veer C. & Mann KG 1999. "Normal" thrombin generation. *Blood* 94: 2169– 2178.

Caldwell SH, Hoffman M, Lisman T, Macik BG, Northup PG, Reddy KR, Tripodi A, Sanyal AJ and Coagulation in Liver Disease Group, 2006. Coagulation disorders and hemostasis in liver disease: pathophysiology and critical assessment of current management. *Hepatology 44*(4): 1039-1046.

Chau TN, Chan YW, Patch D, Tokunaga S, Greenslade L & Burroughs AK 1998. Thromboelastographic changes and early rebleeding in cirrhotic patients with variceal bleeding. *Gut* 43: 267–271.

Chen A, Teruya J 2009. Global hemostasis testing thromboelastography: old technology, new applications. *Clin Lab Med* 29:391–407

Child CG, Turcotte JG 1964. Surgery and portal hypertension. In Child CG (ed.). *The liver and portal hypertension*. Philadelphia: Saunders: 50–64.

Cholongitas, E., Papatheodoridis, G.V., Vangeli, M., Terreni, N., Patch, D. and Burroughs, A.K., 2005. Systematic review: the model for end-stage liver disease–should it replace Child-Pugh's classification for assessing prognosis in cirrhosis? *Alimentary Pharmacology & Therapeutics*, *22*(11-12): 1079-1089.

Clauss A 1957. Rapid physiological coagulation method in determination of Fibrinogen. *Acta Haematol.* 17: 237–46.

Colucci M, Binetti BM, Branca MG, Clerici C, Morelli A, Semeraro N, Gresele P 2003. Deficiency of thrombin activatable fibrinolysis inhibitor in cirrhosis is associated with increased plasma fibrinolysis. *Hepatology* 38: 230–7.

Damus PS, Wallace GA 1975. Immunologic measurement of antithrombin III-heparin cofactor and alpha2 macroglobulin in disseminated intravascular coagulation and hepatic failure coagulopathy. *Thromb Res* 6: 27–38.

Davì G, Ferro D, Basili S, Iuliano L, Camastra C, Giammarresi C, Santarone S, Rocca B, Landolfi R, Ciabattoni G and Cordova C 1998. Increased thromboxane metabolites excretion in liver cirrhosis. *Thrombosis and Haemostasis 79*(4): 747-751.

Dotsch TM, Dirkmann D, Bezinover D, Hartmann M, Treckmann JW, Paul A & Saner FH 2017. Assessment of standard laboratory tests and rotational thromboelastometry for the prediction of postoperative bleeding in liver transplantation. BJA: *British Journal of Anaesthesia* 119: 402–410

de Franchis R, Arcidiacono PG, Carpinelli L, Andreoni B, Cestari L, Brunati S, Zambelli A, Battaglia G and Mannucci PM 1993.

Randomised controlled trial of desmopressin plus terlipressin vs. terlipressin alone for the treatment of acute variceal hemorrhage in cirrhotic patients: A multicenter, double-blind study. *Hepatology 18*(5): 1102-1107.

Egan K, Dillon A, Dunne E, Kevane B, Galvin Z, Maguire P, Kenny D, Stewart S and Ainle FN 2017. Increased soluble GPVI levels in cirrhosis: evidence for early in vivo platelet activation. *Journal of Thrombosis and Thrombolysis 43*(1): 54-59.

Escolar G, Cases A, Vinas M, Pino M, Calls J, Cirera I and Ordinas 1999. Evaluation of acquired platelet dysfunctions in uremic and cirrhotic patients using the platelet function analyser (PFA-100): influence of hematocrit elevation. *Haematologica 84*(7): 614-619.

Esmon CT 1989. The roles of protein C and thrombomodulin in the regulation of blood coagulation. *J Biol Chem* 264: 4743-4746.

Eyraud D, Suner L, Dupont A, Bachelot-Loza C, Smadja DM, Helley D, Bertil S, Gostian O, Szymezak J, Loncar Y and Puybasset L 2018. Evolution of platelet functions in cirrhotic patients undergoing liver transplantation: A prospective exploration over a month. *PloS one*, *13*(8), p.e0200364.

Fayed N, Mourad W, Yassen K & Gorlinger K 2015. Preoperative thromboelastometry as a predictor of transfusion requirements during adult living donor liver transplantation. *Transfusion Medicine and Hemotherapy* 42: 99–108.

Ferro D, Quintarelli C, Lattuada A, Leo R, Alessandroni M, Mannucci PM and Violi F 1996. High plasma levels of von Willebrand factor as a marker of endothelial perturbation in cirrhosis: relationship to endotoxemia. *Hepatology 23*(6): 1377-1383.

Feys HB, Canciani MT, Peyvandi F, Deckmyn H, Vanhoorelbeke K, Mannucci PM 2007. ADAMTS13 activity to antigen ratio in physiological and pathological conditions associated with an increased risk of thrombosis. *Br J Haematol* 138: 534-40.

Francis JL, Armstrong DJ 1982. Acquired dysfibrinogenaemia in liver disease. *J Clin Pathol* 35:667–672.

Garcia-Tsao G, Groszmann RJ, Fisher RL, Conn HO, Atterbury CE, Glickman M 1985. Portal pressure, presence of gastroesophageal varices and variceal bleeding. *Hepatology* 5: 419–424.

Giannini EG, Savarino V 2008. Thrombocytopenia in liver disease. *Curr Opin Hematol* 15:473-80.

Goodpasture E 1914. Fibrinolysis in chronic hepatic insufficiency. *Bull Johns Hopkins Hosp* 25(285):330–336.

Guarner C, Soriano G, Such J, Teixidó M, Ramis I, Bulbena O, Roselló J, Guarner F, Gelpi E, Balanzó J and Vilardell F 1992. Systemic prostacyclin in cirrhotic patients: relationship with portal hypertension and changes after intestinal decontamination. *Gastroenterology 102*(1): 303-309.

Hayward CPM, Harrison P, Cattaneo M, Ortel TL, Rao AK and Platelet Physiology Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis, 2006. Platelet function analyser (PFA)-100® closure time in the evaluation of platelet disorders and platelet function. *Journal of Thrombosis and Haemostasis 4*(2): 312-319.

Hemker HC, Giesen P, Al Dieri R, Regnault V, de Smedt E, Wagenvoord R, Lecompte T & Beguin S 2003. Calibrated automated thrombin generation measurement in clotting plasma. *Pathophysiology of Haemostasis and Thrombosis* 33: 4–15.

Hersch SL, Kunelis T, Francis Jr RB 1987. The pathogenesis of accelerated fibrinolysis in liver cirrhosis: a critical role for tissue plasminogen activator inhibitor. *Blood* 69:1315–1319.

Hollestelle MJ, Geertzen HG, Straatsburg, IH, van Gulik TM and van Mourik JA 2004. Factor VIII expression in liver disease. *Thrombosis and Haemostasis 91*(2): 267-275.

Hu KQ, Yu AS, Tiyyagura L, Redeker AG, Reynolds TB 2001. Hyperfibrinolytic activity in hospitalised cirrhotic patients in a referral liver unit. *Am J Gastroenterol* 96(05):1581–1586.

Ingeberg S, Jacobsen P, Fischer E, Bentsen KD 1985. Platelet aggregation and release of atp in patients with hepatic cirrhosis. *Scand J Gastroenterol* 20: 285–8.

Intagliata NM, Argo CK, Stine JG, Lisman T, Caldwell SH and Violi F 2018. Concepts and Controversies in Haemostasis and Thrombosis Associated with Liver Disease: Proceedings of the 7th International Coagulation in Liver Disease Conference: October 6th and 7th, 2017 Rome, Italy. *Thrombosis and Haemostasis* 118(8): 1491-1506.

Joist JH 1999. AICF and DIC in liver cirrhosis: expressions of a hypercoagulable state. *Am J Gastroenterol* 94:2801-2803.

Jüttner B, Brock J, Weißig A, Becker T, Studzinski A, Osthaus WA, Bornscheuer A and Scheinichen D 2009. Dependence of platelet function on underlying liver disease in orthotopic liver transplantation. *Thrombosis Research 124*(4): 433-438.

Kamath, P.S., Wiesner, R.H., Malinchoc, M., Kremers, W., Therneau, T.M., Kosberg, C.L., D'Amico, G., Dickson, E.R. and Kim, W.R., 2001. A model to predict survival in patients with end-stage liver disease. *Hepatology*, *33*(2): 464-470.

Kamath, P.S. and Kim, W.R., 2007. The model for end-stage liver disease (MELD). *Hepatology*, *45*(3): 797-805.

Kashuk JL, Moore EE, Sabel A, Barnett C, Haenel J, Le T, Pezold M, Lawrence J, Biffl WL, Cothren CC and Johnson JL 2009. Rapid thrombelastography (r-TEG) identifies hypercoagulability and predicts thromboembolic events in surgical patients. *Surgery 146*(4): 764-774.

Kehrel BE, Brodde MF 2013. State of the art in platelet function testing. *Transfus Med Hemother*. 40:73–86.

Kemkes-Matthes B, Matthes KJ 1995. Protein Z a new

haemostatic factor, in liver diseases. *Haemostasis* 25:312–316.

Koike Y, Yoneyama A, Shirai J, Ishida T, Shoda E, Miyazaki K, Sunaga S, Horie R, Aoki K, Koike K and Ogata I 1998. Evaluation of thrombopoiesis in thrombocytopenic disorders by simultaneous measurement of reticulated platelets of whole blood and serum thrombopoietin concentrations. *Thrombosis and Haemostasis 79*(06): 1106-1110.

Krzanicki D, Sugavanam A and Mallett S 2013. Intraoperative hypercoagulability during liver transplantation as demonstrated by thromboelastography. *Liver Transplantation 19*(8): 852-861.

Kujovich JL 2005. Hemostatic defects in end stage liver disease. *Crit Care Clin* 21:563–587.

Kwaan HC, McFadzean AJ, Cook J 1956. Plasma fibrinolytic activity in cirrhosis of the liver. *Lancet* 270(6908):132–136

Laffi G, Cominelli F, Ruggiero M, Fedi S, Chiarugi V and Gentilini P 1987. Molecular mechanism underlying impaired platelet responsiveness in liver cirrhosis. *FEBS letters* 220(1): 217-219.

Laffi G, Cominelli F, Ruggiero M, Fedi S, Chiarugi VP, La Villa G, Pinzani M and Gentilini P 1988. Altered platelet function in cirrhosis of the liver: impairment of inositol lipid and arachidonic acid metabolism in response to agonists. *Hepatology* 8(6): 1620-1626.

Laffi G, Marra F, Gresele P, Romagnoli P, Palermo A, Bartolini O, Simoni A, Orlandi L, Selli ML, Nenci GG and Gentilini P 1992. Evidence for a storage pool defect in platelets from cirrhotic patients with defective aggregation. *Gastroenterology* 103(2): 641-646.

Laffi G, Marra F, Failli P, Ruggiero M, Carloni V, Giotti A and Gentilini P 1993. Defective signal transduction in platelets from cirrhotics is associated with increased cyclic nucleotides. *Gastroenterology* 105(1): 148-156.

Laffi G, Cinotti S, Filimberti E, Ciabattoni G, Caporale R, Marra F, Melani L, Grossi A, Carloni V and Gentilini P 1996. Defective aggregation in cirrhosis is independent of in vivo platelet activation. *Journal of Hepatology* 24(4):436-443.

Laffi G, Marra F 1999. Complications of cirrhosis: Is the endothelium guilty? *Journal of hepatology* 30: 532-535.

Lawson PJ, Moore HB, Moore EE, Stettler GR, Pshak TJ, Kam I, Silliman CC and Nydam TL 2017. Preoperative thrombelastography maximum amplitude predicts massive transfusion in liver transplantation. *Journal of Surgical Research 220*: 171-175.

Leebeek FWG, Kluft C, Knot EAR, de Maat MPM, Wilson JHP 1991. A shift in balance between profibrinolytic and antifibrinolytic factors causes enhanced fibrinolysis in cirrhosis. *Gastroenterology* 101:1382–1390.

Leiper K, Croll A, Booth NA, Moore NR, Sinclair T, Bennett B 1994. Tissue plasminogen activator, plasminogen activator inhibitors, and activator-inhibitor complex in liver disease. *J Clin Pathol* 47: 214–217.

Lenting PJ, van Mourik JA, Mertens K 1998. The life cycle of coagulation factor VIII in view of its structure and function. *Blood* 92:3983-96.

Lisman T, Leebeek FW, Mosnier LO, Bouma BN, Meijers JC, Janssen HL, Nieuwenhuis HK and De Groot PG 2001. Thrombin-activatable fibrinolysis inhibitor deficiency in cirrhosis is not associated with increased plasma fibrinolysis. *Gastroenterology 121*(1):131-139.

Lisman T, Bongers TN, Adelmeijer J, Janssen HL, de Maat MP, de Groot PG and Leebeek FW, 2006. Elevated levels of von Willebrand Factor in cirrhosis support platelet adhesion despite reduced functional capacity. *Hepatology 44*(1): 53-61.

Lisman T, Caldwell SH, Porte RJ, Leebeek FW 2006. Consequences of abnormal hemostasis tests for clinical practice. *J Thromb Haemost* 4: 2062–2063.

Lisman T, Adelmeijer J, de Groot PG, Janssen HL, Leebeek FW 2006. No evidence for an intrinsic platelet defect in patients with liver cirrhosis—studies under flow conditions. *J Thromb Haemost* 4: 2070- 2072.

Malinchoc, M., Kamath, P.S., Gordon, F.D., Peine, C.J., Rank, J. and Ter Borg, P.C., 2000. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. *Hepatology*, *31*(4): 864-871.

Mann KG, Brummel K, Butenas S 2003. What is all that thrombin for? *J Thromb Haemost* 1:1504-1514.

Martinez J, MacDonald KA, Palascak JE 1983. The role of sialic acid in the dysfibrinogenemia associated with liver disease: distribution of sialic acid on the constituent chains. *Blood* 61:1196–1202.

Massicotte L, Denault AY, Thibeault L, Hevesi Z, Nozza A & Roy A 2014. Relationship between conventional coagulation tests and bleeding for 600 consecutive liver transplantations. *Transplantation* 98: e13–e15.

McCrath DJ, Cerboni E, Frumento RJ, Hirsh AL, Bennett-Guerrero E 2005. Thromboelastography maximum amplitude predicts postoperative thrombotic complications including myocardial infarction. *Anesth Analg* 100: 1576– 83.

Mitchell O, Feldman DM, Diakow M and Sigal SH 2016. The pathophysiology of thrombocytopenia in chronic liver disease. *Hepatic medicine: evidence and research 8*: 39-50.

Michelson AD, Barnard MR 1990. Plasmin-induced redistribution of platelet glycoprotein Ib. *Blood* 76:2005–2010.

Moestrup SK, Gliemann J, Pallesen G 1992. Distribution of the alpha 2-macroglobulin receptor/low density lipoprotein receptor-related protein in human tissues. *Cell Tissue Res* 269:

375-82

Morrow GB, Beavis J, Harper S, Baker P, Desborough MJ, Curry N, Stanworth SJ and Laffan MA 2020. Coagulation status of critically ill patients with and without liver disease assessed using a novel thrombin generation analyser. *Journal of Thrombosis and Haemostasis 18*(7): 1576-1585.

Mutlak H, Reyher C, Meybohm P, Papadopoulos N, Hanke AA, Zacharowski K and Weber CF 2015. Multiple electrode aggregometry for the assessment of acquired platelet dysfunctions during extracorporeal circulation. *The Thoracic and cardiovascular surgeon 63*(01): 021-027.

Ogasawara F, Fusegawa H, Haruki Y, Shiraishi K, Watanabe N, Matsuzaki S 2005. Platelet activation in patients with alcoholic liver disease. *Tokai J Exp Clin Med 30*: 41- 48.

Ordinas A, Escolar G, Cirera I, Vinas M, Cobo F, Bosch J, Teres J and Rodés J 1996. Existence of a platelet-adhesion defect in patients with cirrhosis independent of hematocrit: studies under flow conditions. *Hepatology 24*(5):1137-1142.

Owen JS, Hutton RA, Day RC, Bruckdorfer KR, McIntyre N 1981. Platelet lipid composition and platelet aggregation in human liver disease. *JLipid Res* 22:423–430

Panasiuk A, Zak J, Kasprzycka E, Janicka K, Prokopowicz D 2005. Blood platelet and monocyte activations and relation to stages of liver cirrhosis. *World J Gastroenterol* 11:2754-2758.

Paniccia R, Priora R, Liotta AA and Abbate R 2015. Platelet function tests: a comparative review. *Vascular health and risk management 11*: 133. https://doi.org/10.2147/VHRM.S44469

Pantaleo P, Marra F, Vizzutti F, Spadoni S, Ciabattoni G, Galli C, VILLA GL, Gentilini P and Laffi G 2004. Effects of dietary supplementation with arachidonic acid on platelet and renal function in patients with cirrhosis. *Clinical Science 106*(1): 27-34.

Pasche B, Ouimet H, Francis S, Loscalzo J 1994. Structural changes in platelet glycoprotein IIb/IIIa by plasmin: determinants and functional consequences. *Blood* 83:404–414.

Pernambuco JR, Langley PG, Hughes RD, Izumi S, Williams R 1993. Activation of the fibrinolytic system in patients with fulminant liver failure. *Hepatology* 18:1350–1356.

Peterson P, Hayes TE, Arkin CF, Bovill EG, Fairweather RB, Rock Jr WA, Triplett DA and Brandt JT 1998. The preoperative bleeding time test lacks clinical benefit: College of American Pathologists' and American Society of Clinical Pathologists' position article. *Archives of surgery 133*(2): 134-139.

Peterson TJ, Webb AMB and Vipler BS 2016. Use of thromboelastography in the management of liver cirrhosis and accelerated intravascular coagulation and fibrinolysis (AICF). BMJ *Case Reports*, *2016*, https://doi.org/10.1136/bcr-2016-218294.

Pitney WR & Dacie JV 1953. A simple method of studying the generation of thrombin in recalcified plasma; application in the investigation of haemophilia. *Journal of Clinical Pathology*, 6: 9–14.

Potze W, Arshad F, Adelmeijer J, Blokzijl H, van den Berg AP, Meijers JC, Porte RJ and Lisman T 2013. Decreased tissue factor pathway inhibitor (TFPI)-dependent anticoagulant capacity in patients with cirrhosis who have decreased protein S but normal TFPI plasma levels. *British journal of haematology 162*(6):819-826.

Potze W, Siddiqui MS, Boyett SL, Adelmeijer J, Daita K, Sanyal AJ and Lisman T 2016. Preserved hemostatic status in patients with non-alcoholic fatty liver disease. *Journal of hepatology 65*(5):980-987.

Pugh, R.N.H., Murray-Lyon, I.M., Dawson, J.L., Pietroni, M.C. and Williams, R., 1973. Transection of the oesophagus for bleeding oesophageal varices. *Journal of British Surgery*, *60*(8): 646-649.

Pustavoitau A, Lesley M, Ariyo P, Latif A, Villamayor AJ, Frank SM, Rizkalla N, Merritt W, Cameron A, Dagher N, Philosophe B, Gurakar A & Gottschalk A 2017. Predictive modelling of massive transfusion requirements during liver transplantation and its potential to reduce utilisation of blood bank resources. *Anesthesia and Analgesia* 124: 1644–1652.

Qamar AA, Grace ND, Groszmann RJ, Garcia–Tsao G, Bosch J, Burroughs AK, Ripoll C, Maurer R, Planas R, Escorsell A and Garcia–Pagan JC 2009. Incidence, prevalence, and clinical significance of abnormal hematologic indices in compensated cirrhosis. *Clinical Gastroenterology and Hepatology 7*(6): 689-695.

Queck A, Thomas D, Jansen C, Schreiber Y, Rüschenbaum S, Praktiknjo M, Schwarzkopf KM, Mücke MM, Schierwagen R, Uschner FE and Meyer C 2019. Pathophysiological role of prostanoids in coagulation of the portal venous system in liver cirrhosis. *Plos one 14*(10): p.e0222840.

Quick AJ 1935. The prothrombin in hemophilia and in obstructive jaundice. *J Biol Chem* 109:73–74

Raparelli V, Basili S, Carnevale R, Napoleone L, Del Ben M, Nocella C, Bartimoccia S, Lucidi C, Talerico G, Riggio O and Violi F 2017. Low-grade endotoxemia and platelet activation in cirrhosis. *Hepatology 65*(2): 571-581.

Ratnoff OD 1949. Studies on a proteolytic enzyme in human plasma; the rate of lysis of plasma clots in normal and diseased individuals, with particular reference to hepatic diseases. *Bulletin of the Johns Hopkins Hospital 84*(1): 29-42.

Raza I, Davenport R, Rourke C, Platton S, Manson J, Spoors C, Khan S, De'ath HD, Allard S, Hart DP, Pasi KJ, Hunt BJ, Stanworth S, MacCallum PK & Brohi K 2013. The incidence and magnitude of fibrinolytic activation in trauma patients. *Journal of Thrombosis and Haemostasis* 11: 307–314

Rijken DC, Hoegee-de Nobel E, Jie AF, Atsma DE, Schalij MJ, Nieuwenhuizen W 2008. Development of a new test for the global fibrinolytic capacity in whole blood. *J Thromb Haemost* 6(1):151–157

Rijken DC, Lijnen HR 2009. New insights into the molecular mechanisms of the fibrinolytic system. *J Thromb Haemost* 7(1):4–13

Rijken DC, Kock EL, Guimarães AH, Talens S, Murad SD, Janssen HL and Leebeek FW 2012. Evidence for an enhanced fibrinolytic capacity in cirrhosis as measured with two different global fibrinolysis tests. *Journal of Thrombosis and Haemostasis 10*(10): 2116-2122.

Rubin MH, Weston MJ, Langley PG, White Y, Williams R 1979. Platelet function in chronic liver disease: relationship to disease severity. *Dig Dis Sci* 24: 197–202.

Saenko EL, Yakhyaev AV, Mikhailenko I, Strickland DK, Sarafanov AG1999. Role of the low-density lipoprotein-related protein receptor in mediation of factor VIII catabolism. *J Biol Chem* 274:37685- 92.

Sa´nchez-Roig MJ, Rivera J, Moraleda JM, Garcı´a VV 1994. Quantitative defect of glycoprotein Ib in severe cirrhotic patients. *Am J Hematol* 45: 10– 5.

Sayed D, Amin NF, Galal GM 2010. Monocyte-platelet aggregates and platelet micro-particles in patients with post-hepatitic liver cirrhosis. *Thromb Res* 125: e228- e233.

Scharbert G, Auer A, Kozek-Langenecker S 2009. Evaluation of the platelet mapping assay on rotational thromboelastometry ROTEM. *Platelets* 20:125–130

Schwarz HP, Lenting PJ, Binder B, Mihaly J, Denis C, Dorner F and Turecek PL 2000. Involvement of low-density lipoprotein receptor-related protein (LRP) in the clearance of factor VIII in von Willebrand factor–deficient mice: Presented in part at the 41st annual meeting of the American Society of Hematology, New Orleans, LA, December 1999. *Blood, The Journal of the American Society of Hematology 95*(5): 1703-1708.

Stegewerth K, Weber CF, Moehlmann M, Adam EH, Zacharowski K, Zeuzem S, & Weiler N 2019. Fast interpretation of thromboelastometry and aggregometry in patients suffering from chronic liver disease. *Clinical Laboratory 65*(8): 1513–1521. https://doi.org/10.7754/Clin.Lab.2019.190505

Stein SF, Harker LA 1982. Kinetic and functional studies of platelets, Fibrinogen, and plasminogen in patients with hepatic cirrhosis. *J Lab Clin Med* 99:217–230.

Stoy S, Patel VC, Sturgeon JP, Manakkat Vijay GK, Lisman, T, Bernal W, Shawcross DL 2018. Platelet-leucocyte aggregation is augmented in cirrhosis and further increased by platelet transfusion. *Aliment. Pharm*. 47: 1375–1386.

Szczepiorkowski ZM and Dunbar NM 2013. Transfusion guidelines: when to transfuse. *Hematology 2013*(1): 638-644.

Tafur LA, Taura P, Blasi A, Beltran J, Martinez-Palli G, Balust J & Garcia-Valdecasas JC 2016. Rotation thromboelastometry velocity curve predicts blood loss during liver transplantation. *British Journal of Anaesthesia* 117: 741– 748.

Thalheimer U, Triantos CK, Samonakis DN, Patch D, Burroughs AK 2005. Infection, coagulation and variceal bleeding in cirrhosis. *Gut* 54: 556-563.

Thorsen LI, Brosstad F, Gogstad G, Sletten K and Solum NO 1986. Competitions between Fibrinogen with its degradation products for interactions with the platelet-fibrinogen receptor. *Thrombosis research 44*(5): 611-623.

Toth O, Calatzis A, Penz S, Losonczy H, Siess W 2006. Multiple electrode aggregometry: a new device to measure platelet aggregation in whole blood. *Thromb Haemost* 96:781-8

Tripodi A, Salerno F, Chantarangkul V, Clerici M, Cazzaniga M, Primignani M and Mannuccio Mannucci P 2005. Evidence of normal thrombin generation in cirrhosis despite abnormal conventional coagulation tests. *Hepatology 41*(3): 553-558.

Tripodi A, Primignani M, Chantarangkul V, Dell'Era A, Clerici M, De Franchis R, Colombo M and Mannucci PM 2009. An imbalance of pro-vs anti-coagulation factors in plasma from patients with cirrhosis. *Gastroenterology 137*(6): 2105-2111.

Tripodi A, Primignani M, Lemma L, Chantarangkul V and Mannucci PM 2013. Evidence that low protein C contributes to the procoagulant imbalance in cirrhosis. *Journal of hepatology 59*(2): 265-270.

Turitto VT, Baumgartner HR 1975. Platelet interaction with sub endothelium in a perfusion system: physical role of red blood cells. *Microvasc Res*9:335–344.

Uemura M, Fujimura Y, Matsumoto M, Ishizashi H, Kato S, Matsuyama T, Isonishi A, Ishikawa M, Yagita M, Morioka C and Yoshiji H 2008. Comprehensive analysis of ADAMTS13 in patients with liver cirrhosis. *Thrombosis and haemostasis 99*(06): 1019-1029.

Vinholt PJ, Hvas AM, Nielsen C, Söderström AC, Sprogøe U, Fialla AD and Nybo M 2018. Reduced platelet activation and platelet aggregation in patients with alcoholic liver cirrhosis. *Platelets 29*(5): 520-527.

Violi F, Leo R, Vezza E, Basili S, Cordova C, Balsano F and CALC group, 1994. Bleeding time in patients with cirrhosis: relation with degree of liver failure and clotting abnormalities. *Journal of hepatology 20*(4): 531-536.

Yoshikawa Y, Sakata Y, Toda G, Oka H 1988. The acquired vitamin K- dependent gamma-carboxylation deficiency in hepatocellular carcinoma involves not only prothrombin, but also protein C. *Hepatology* 8:524–530.

Zermatten MG, Fraga M, Calderara DB, Aliotta A, Moradpour D and Alberio L 2020. Biomarkers of liver dysfunction correlate with a prothrombotic and not with a prohaemorrhagic profile in patients with cirrhosis. *JHEP Reports 2*(4): 100120.

AIMS NSM 2021 Abstracts

The risk of Ivig therapy

G Mann

Cabrini Pathology, Melbourne, VIC, Australia

E-mail: gmann@cabrini.com.au

Introduction

What is it, why do we give it and how does it work?

How is it made and can this be important?

What can be in this product that may cause adverse events?

Case Study

A 58-year-old male was diagnosed with Hemophagocytic lymphohistiocytosis (HLH).

Over 8 weeks he was transfused with red cells and large doses of IVIG and then deceased.

Initially the DAT was negative, but later became positive and he developed haemolysis with brown plasma. Anti-A1 was eluted off the cells. Was this due to the large amounts of IVIG and/or possibly he was a Group A2 and developed an anti-A1 from transfused red cells?

Discussion

Risk factors for haemolysis from IVIG:

1. Administration of high-dose IVIG

2. Non-O blood group recipients and non-secretor status

3. Titre of the isoagglutinin in the product?

4. An underlying inflammatory or autoimmune disorder

5. Method of preparation:

- Chromatographic – gives higher yields and purity, higher anti-A and anti-B titres, but lower levels of IgA and prekallikrein activator

- Cohns cold ethanol fractionation – lower levels isoagglutinins but has proenzymes and lipoproteins

6. Additive in IVIG eg glycine

7. Liquid preparation more implicated than lyophilized product

8. Genetic predisposition eg Fc receptor or complement polymorphisms on our macrophages, red cell antigen density, cells lacking Cromer CD55 or CD59 are more prone to haemolysis eg also PNH as they are lacking complement mediators

9. Patients who are IgA deficient can form anti-IgA (IgG or IgE forms) and have anaphylaxis

Conclusion

IVIG can cause adverse events and complicate testing.

A DAT result can be very important.

Look at what products the patient has received.

Put the eluate up against not only Group O cells with various antigens, but against A and B cells also.

Ibrutinib, but not zanubrutinib, induces platelet receptor shedding of GPIb-IX complex and integrin αIIbβ3 from the platelet surface-Role of ADAM17 (TACE).

DE Jackson[1], G Dobie[1], FA Kuriri[1], M Omar[1], F Alanazi[1], A Gazwani[1], C Tang[2], D M Sze[1], S Handunnetti[2], C Tam[2]

[1]RMIT University, Bundoora. Victoria. 3083.

[2]Peter MacCallum Cancer Centre, Parkville and St. Vincent's Hospital, Melbourne, Victoria

Introduction

The Bruton's tyrosine kinase (BTK) inhibitor, ibrutinib, has proven to be efficacious in the treatment of B-CLL and related diseases. However, a major adverse side effect of ibrutinib is bleeding including major haemorrhages. The bleeding associated with ibrutinib use is thought to be due to a combination of on-target irreversible Btk inhibition as well as off targeted inhibition of other kinases including EGFR, ITK, JAK3 and Tec kinase. In this study, we investigated the effects of ibrutinib versus zanubrutinib (a more selective Btk inhibitor) on platelet activation, glycoprotein expression and thrombus formation.

Methods

Platelet glycoprotein expression was measured with respective fluorescently labelled antibodies by flow cytometry. *Ex vivo* thrombus formation was determined platelet adhesion under flow on immobilised type I collagen at arterial shear flow rate. *In vivo* thrombus formation was determined using ferric chloride induced vascular injury of mesenteric arterioles and intravital microscopy over time. Soluble GPIbα and αIIb levels were determined using ELISA based assays.

Results

Ibrutinib, but not zanubrutinib, induced a time and dose dependent shedding of GPIb-IX complex and integrin $\alpha_{IIb}\beta_3$ but not GPVI and GPV from the platelet surface. The shedding of GPIbα and GPIX was blocked by GM6001 and

TAPI-2, an ADAM17 inhibitor but not ADAM10 inhibitor. Ibrutinib but not zanubrutinib treatment of human platelets increased ADAM17 activation. Pretreatment of C57BL/6 mice with ibrutinib (10 mg/kg) but not zanubrutinib (10 mg/kg) inhibited *ex vivo* and *in vivo* thrombus growth over time. Platelets from ibrutinib-treated CLL patients showed reduced GPIb-IX complex and integrin $\alpha_{IIb}\beta_3$ surface expression and reduced *ex vivo* thrombus formation under arterial flow, which was not observed in zanubrutinib-treated patients. Ibrutinib but not zanubrutinib treated mice led to increased soluble GPIbα and soluble α_{IIb} levels in plasma.

Conclusions

These data demonstrate that ibrutinib induces shedding of GPIbα and GPIX by an ADAM17-dependent mechanism and integrin $\alpha_{IIb}\beta_3$ by an unknown sheddase, and this process occurs *in vivo* to regulate thrombus formation.

Measles breakthrough infection (reinfection/waning immunity) – The Victorian Experience.

S Nicholson[1], K Gibney[2,3], J Strachan[2], G Cross[4,5], L Attwood[6], J Healy[2], V Stambos[1], M Catton[1].

[1]Victorian Infectious Diseases Reference Laboratory (VIDRL) The Doherty Institute, Melbourne, Australia

[2]Health Protection Branch, Victorian Department of Health & Human Services, Melbourne, Australia

[3]The Doherty Institute, the University of Melbourne and the Royal Melbourne Hospital

[4]Department of Medicine, Yong Loo Lin School of Medicine, National University of Singapore

[5]Department of Infectious Diseases, National University Hospital, Singapore

[6]Department of Infectious Diseases, Alfred Hospital, Melbourne, Australia

Introduction

After prolonged periods of high vaccine coverage Australia was declared free of endemic measles in March 2014. However, returning travellers from measles endemic countries may transmit measles to non-immune individuals in Australia. In Victoria between 2014 and 2016, there were a number of measles cases reported in individuals with prior measles immunity. Some of these had IgG avidity testing performed, and were found to have high avidity antibody, consistent with past measles immunologic responses, and referred to as 'measles breakthrough infection cases. Our objective was to determine if there is an increase in measles diagnoses among Victorians with serologic evidence of prior measles immunity, and to explore evidence of an attenuated measles illness in this group.

Methods

Review measles cases notified to the Public Health Surveillance System (PHESS) of the Victorian Department of Health and Human Services between January 2008 and December 2017. Cases were included in the analysis if they were Measles PCR positive and IgM and IgG tested at the time of diagnosis.

Results

There were 297 measles cases notified in Victoria between 2008 and 2017 and 190 of these subjects fulfilled the study inclusion criteria. Thirteen (7%) were IgM-/IgG+, 26 (14%) were IgM+/IgG+ and 151 (80%) were IgG- at diagnosis. There was an increase in the number of cases reported with PCR positive IgM– and IgG+ results, from none reported between 2008-2013 to 13/103 (13%) between 2014-2017.

Conclusions

It is not clear if these results represent an emerging clinical scenario in Victoria reflecting increasing partial immunity among measles cases (IgM-/IgG+) at diagnosis, or more complete diagnosis of measles re-infection. Cases tended to have modified clinical illness however transmission potential was shown. This highlights the need for on-going measles surveillance in monitoring the status of population immunity even in an elimination setting.

Investigating The Link Between Insulin Dysregulation And Breast Cancer Prognosis Via Immunohistochemistry: A Systematic Review And Meta-Analysis

SS Chinni, K Wilson, D Pouniotis

Discipline of Laboratory Medicine School of Health and Biomedical Sciences, Royal Melbourne Institute of Technology, Bundoora, Victoria, 3083, Australia

E-mail: chinnishivali98@gmail.com

Introduction

Dysregulation of insulin and its downstream insulin-like-growth-factor-1 (IGF-1) expression caused by obesity has been observed in breast cancer (BC), influencing a pro-inflammatory state of tumour microenvironment. Tumour microenvironment plays a pivotal role in tumour development and progression. Dysregulation and overexpression of insulin has been observed in BC via IGF-1 receptor expression and known to be correlated with poor BC prognosis. This systematic review aimed to investigate

the link between insulin dysregulation and the prognosis of breast cancer via insulin-like-growth-factor-1 receptor (IGF-1R) IHC marker.

Methods

Literature search was performed using PubMed and ProQuest to identify 13 studies that observed IGF-1R expression via immunohistochemistry in human BC samples and measured the associated prognostic outcomes. All included studies were assessed for quality using CASP cohort-studies checklist. Meta-analysis was performed on hazard ratios associated with outcomes such as overall, disease-free, and breast-cancer-specific survival (OS, DFS and BCSS).

Results

Strong overexpression (SOE) of IGF-1R was observed in hormone-receptor positive subtypes (Estrogen-receptor (ER) and Progesterone-receptor (PR) positive) subtypes and weak expression was observed in human-epidermal-growth-factor-2 (HER2) and triple-negative (TNBC) subtypes. No significant correlation was observed between IGF-1R expression and prognostic outcomes like OS, DFS and BCSS. SOE of IGF-1R was observed with Transzumab-resistant BC patients as well.

Conclusions

SOE of IGF-1R was observed in hormone-receptor positive subtypes due to the possible interaction with IGF-1R and ER/PR signalling pathways promoting BC and its invasiveness. Further studies need to be performed to optimise IGF-1R IHC protocols and quantification methods to decrease data heterogeneity of IGF-1R expression between studies. These findings can also potentially help develop therapies for ER/PR positive tumour subtypes & improve BC prognosis if the correlation of IGF-1R and BC are further studied and proven with larger cohort.

Parathyroid Hormone Related Peptide

Dr Myron Lee

NSW Health Pathology, Sydney, Australia

Parathyroid hormone related peptide (PTH-RP) is a protein with a wide range of functions in multiple organ systems, but is most well-known for causing humoral hypercalcaemia of malignancy. This is a common cause of hypercalcaemia in hospitalised patients, and the most common cause of PTH independent hypercalcaemia. The mechanism is due to the common N-terminus that PTH-RP shares with PTH which interacts with the PTH receptor. In contrast, the C-terminal end differs widely from PTH and many fragments of variable length exist. This poses a challenge to PTH-RP assays in terms of both sensitivity and specificity. The clinical utility of such assays in helping determine the cause of PTH independent hypercalcaemia rests on managing this challenge.

Research and teaching insights of Australian Medical Laboratory Science Academics

R Donkin, K Broome, and L Swanepoel

University of the Sunshine Coast, Sippy Downs, Queensland, Australia

Email: rdonkin@usc.edu.au

Introduction

While teaching remains a primary responsibility of many medical laboratory science (MLS) academics, research is also a major role. Research contributes to the evidence base of the MLS profession and contributes to academic career advancement.

With the loss of experienced academics and scientists retiring from the baby boomer era, the problem arises on how universities recruit and retain faculty to teach MLS programs who are adequately qualified and maintain a MLS research agenda.

The aim of the study was to examine MLS academic characteristics (qualification, level of appointment, research and teaching interests) to provide insight into how these characteristics relate across MLS programs and universities.

Methods

In December, 2019 a bibliographic analysis of MLS university websites and corresponding Scopus citation database profiles was conducted. Data was collected on research output and themes of teaching and research, along with academic appointment level.

Results

From 13 universities, 124 academic positions in undergraduate MLS programs were identified. Academics at the level of lecturer or higher typically held a doctorate which strongly influenced the number of publications. However, institution (metropolitan versus regional) and research interest appeared to influence publication number and level of appointment. The majority of academics did not have alignment of their major research and teaching areas.

Conclusions

A shortage of MLS academics with clinical and research experience could impact the overall quality of university programs and their MLS research agenda. This may ultimately impact the quality of MLS graduates and the MLS industry. Further investigation into how academic research influences MLS student learning and graduate outcomes is still required.

Molecular Testing in Thalassaemia

W M Hutchison, J.N. Wells

THMRL, Monash Health Pathology, Level 4 E-Block Monash Medical Centre, 246 Clayton Road Clayton, Victoria 3168, Australia

Email: wendy.hutchison@monashhealth.org

Introduction

This talk will provide an overview of genetic testing for thalassaemia in the Thalassaemia and Haemophilia Molecular Reference Laboratory (THMRL) at Monash Health Pathology.

Methods

I will discuss methods currently used in the laboratory and potential future methods.

Results

Australia's cultural melting pot raises some challenges for the testing and diagnosis of thalassaemia, particularly in the context of reproductive risk assessment. We see, and therefore must test for, a wide variety of variants, and frequently observe reproductive risk combinations which have not previously been reported. I will discuss this and some interesting cases which challenged the laboratory.

Conclusions

Rapid molecular assay for the detection of COVID-19 coupled on a dipstick: potential for use at point-of-care

Comparison of bead-based multiplex assay and enzyme-linked immunosorbent assay for detection of serum interleukin 6.

P Amoretty-Matthews, L. Forster, D. Westerman, S. Fox

Peter MacCallum Cancer Centre, Melbourne, Victoria, 3000, Australia

E-mail: Philip.Amoretty-Matthews@petermac.org

Introduction

Circulating interleukin (IL)-6, a major inflammatory cytokine involved in malignant disease and other chronic disease conditions associated with inflammation, has classically been measured using enzyme-linked immunosorbent assay (ELISA). However, researchers are increasingly using bead-based multiplex assays driven by Luminex xMAP (Multi-Analyte Profiling) technology.

Despite this trend, only limited studies have directly compared Luminex xMAP Technology to ELISA for their ability to detect serum IL-6. Here we tested the prediction that a bead-based multiplex assays using the Luminex MAGPIX® System would detect concentrations of serum IL-6 in a manner comparable to ELISA.

Methods

Twenty patients tested for routine diagnostic serum IL-6 quantification were run on both the Triturus® ELISA system (Human IL-6 Quantikine ELISA Kit, R&D Systems) and the Luminex MAGPIX® System (Human Cytokine Magnetic Bead Panel, MILLIPLEX®). Samples were performed in duplicate for both assays. Correlations between data sets were evaluated using Pearson's correlation coefficient (r).

Results

Values for the Triturus® ELISA ranged from 3.5-119.0 with a median of 19.6 while the Luminex MAGPIX® System ranged from 2.8-70.4 with a median of 8.2. There was strong correlation between the MAGPIX concentrations and those determined by ELISA across all samples ($r=0.9603$, $p<0.00001$). The Luminex MAGPIX® System values were 1.8 fold (SD = 0.8, CV (%) = 44) lower compared with the Triturus® ELISA system.

Conclusion

The Luminex MAGPIX® System has distinct advantages when compared to ELISA. The ability to multiplex up to 50 cytokines at one time brings extensive cost and time savings. We observed lower absolute concentrations with this technology when compared to ELISA, however as a strong correlation was observed, this demonstrates the ability to use this technology in experiments that follow standard baseline and multi-cycle/dose methodologies in a clinical trial setting.

Broadened education and collaboration opportunities supporting certification: an outcome of the pandemic

RM French, L Bielby

Blood Matters, Melbourne, Victoria, 3016, Australia
rfrench@redcrossblood.org.au

Introduction

The Blood Matters Scientist role includes providing education/resources to support best practice and compliance in the areas of blood management for scientists. With the pandemic came changed work practices, and face to face education was converted to virtual platforms, providing increased access for professional development. Providing educational opportunities to transfusion scientists addressing current issues and topics supports professional growth and also assist in the early stages of the newly implemented voluntary certification scheme for the scientific workforce.

Methods

Several virtual platforms were tested and evaluated for ease of use (presenters/participants), accessibility (across firewalls) and capability for interaction. Webex platforms provided the most suitable option.

To reduce possible education duplication, discussion and collaboration occurred with other relevant parties.

Results

Fifteen education sessions have been conducted virtually with outstanding attendance, not only local (rural and regional), but also interstate colleagues across borders. While target audiences were scientists, attendance was multidisciplinary. Sessions are recorded and shared with registered participants to allow education to reach an even broader audience.

Evaluation feedback has been extremely positive with suggested topics included in our current program, including collaborative sessions with peak bodies.

There is resounding support for ongoing virtual education even when COVID-19 restrictions have eased.

Conclusions

The pandemic has led to increased opportunities to provide and engage virtually with colleagues who are motivated and eager for professional development. The change of education delivery coincided with the launch of the voluntary national certification scheme for the medical scientific workforce, which Blood Matters is committed to supporting.

Transition to virtual education has allowed education to be more accessible and inclusive, even extending beyond our borders. While restrictions have now eased, the use of virtual platforms to provide opportunities to collaborate and communicate for best patient care will continue.

A unique model for delivery of an innovative AIMS accredited medical laboratory science course

N Roman, W Kalle

Charles Sturt University, Wagga Wagga, NSW, 2650 Australia

E-mail: nroman@csu.edu.au

During a course review in 2020, the only AIMS accredited undergraduate course in NSW underwent a major restructure. The new Medical Laboratory Science (Pathology) course is taught across six microsessions that are eight weeks in length. This has many benefits for students from content focus, content integration; including theory application and workplace readiness.

The new course model has a distinct feature making it the first of its kind for an AIMS accredited course, where the practical classes are no longer embedded into a theory class and are stand alone in their own session. By setting the practicals in the middle of the year it allows students to focus on skills and competencies required by AIMS and preferred by industry and allows us to combine relevant practicals, allowing us to include practicals that show students clear clinical applications. Standalone practicals allow the university greater flexibility in providing students that are currently working in industry the ability to apply for credit, where evidence of meeting the requirements and skills can be provided. This supports industry by reducing the amount of time that staff are not at work and allows staff to use study leave for study prior to exams or assessments. Students will now only have to attend campus once a year which potentially reduces the financial, social and psychological impact of being away from home. This is particularly true for our indigenous students who are required to leave country to attend these practical sessions.

These changes have not only transformed the way content is delivered, but assessments have also been modified to support the delivery methods and content integration. With these changes, we have managed to maintain the high-quality content that the course is known to deliver. At the recent AIMS accreditation site visit, the course was accredited for two years.

Would a national antibody register contribute to improving patient outcomes?

Z Powell, N Jiang, R Shrestha, D E Jackson

School of Health and Biomedical Sciences, RMIT University, Bundoora, Victoria, Australia

E-mail: zjpowell@gmail.com

Introduction

Despite stringent testing protocols, there always remains a chance of a delayed haemolytic transfusion reaction

(DHTR) occurring as a result of an undetected or unknown antibody. In this systemic review and meta-analysis, we aimed to investigate improvements to patient outcomes that could be achieved through the implementation of a national antibody registry.

Methods

A series of searches through PubMed and SCOPUS identified a collection of articles with relevant information, restricted to full text, English language articles available through the RMIT Library service. 25 articles were considered for the review, four of these found to have relevant, extractable data for use in the meta-analysis.

Results

Alloantibody evanescence rates were analysed for the potential for reducing DHTRs associated with transfusion services, returning significant results indicating antibody evanescence rates of up to 68.4% in one study, with p-values less than 0.001. Due to the small number of included studies however, the interference values were quite high for these analyses at greater than 90% for each. Additional, beneficial side-effects of such a system, including staffing and efficiency improvements were also considered, along with reductions in DHTRs.

Conclusions

In conclusion it was determined that a National antibody registry would contribute to improving patient outcomes, however further studies would need to be performed to determine a stronger correlation, and the exact levels of improvement that could be achieved.

Discrepancy between genotype and phenotype for factor V Leiden mutation in recipients of liver transplantation (oltx) and stem cell transplantation (sctx)

J Rigano

Alfred Health, Melbourne, Victoria, 3004, Australia

E-mail: j.rigano@alfred.org.au

Introduction

Activated protein C resistance (APCR) first described in 1993 (Dahlbäck *et el.*) is the most common hereditary risk factor for venous thromboembolism (VTE) in Caucasian population. Approximately 90–95% of cases, APCR results from factor V Leiden (FVL) mutation (R506Q). FVL mutation causes activated FV resistance to cleavage by APC resulting in a hypercoagulable state. Thrombophilia testing for APCR of liver FV and FVL mutation in DNA of peripheral blood leukocytes can lead to genotype and phenotype discrepancies in OLTX and SCTX recipients. This report describes 2 cases; acquired APCR without FVL mutation and inherited APCR without FVL mutation both associated with DVT.

Methods

First case a 68-year-old male with DVT nine months post OLTX for liver cirrhosis. Second case a 42-year-old female with CRT four months post SCTX for AML. Both patients reported no history of thrombosis. Thrombophilia assays were performed using ACL TOP CTS 500, IL AcuStar and Qiagen Rotor-Gene.

Results

For both patients; protein C, protein S and antithrombin levels were normal and anti-phospholipid antibodies assays (lupus anti-coagulant, anti-cardiolipin and anti-β_2-glycoprotein I antibodies) were negative. FVL and prothrombin gene (G20210A) mutations not detected. APCR detected in both patients with ratios of 1.75 and 1.64 for case one and two respectively (normal APCR ratio 2.2-3.3). In case one, APCR was detected in donor liver and the recipient's peripheral leucocyte DNA lacked FVL mutation. In case two, APCR was detected in recipient's liver and peripheral leucocyte DNA of donor lacked FVL mutation. OLTX and SCTX recipients are at risk of VTE, therefore consideration should be given to thrombophilia testing of OLTX and SCTX donors and recipients which may indicate anticoagulation to prevent additional morbidity.

Conclusion

Here reported were two cases of genotype and phenotype discrepancies for FVL mutation in recipients of OLTX and SCTX both associated with DVT.

Inherited Protection Against Malaria; A Case of Co-Existing Sickle Cell Disease, Alpha Thalassaemia, G6PD Deficiency and Duffy Null Phenotype

J Rigano

Alfred Health, Melbourne, Victoria, 3004, Australia

E-mail: j.rigano@alfred.org.au

Introduction

The high mortality and widespread impact of malaria have resulted in genetic resistance to this disease and strongest evolutionary selective force in human history. There is extensive overlap of historical geographical distribution of malaria and human genetic variants that confer malaria resistance. The strong selective pressure for malaria resistance has resulted in high frequency detrimental

genetic diseases, such as sickle cell anaemia, thalassemia, G6PD deficiency and ovalocytosis. This report describes four co-inherited mutations in a pregnant woman that confers protection against malaria.

Methods

29-year-old women 18 weeks pregnant for antenatal testing. Routine haematology, biochemistry and haemoglobin electrophoresis analysis was performed. Follow-up testing was performed at 34 weeks with signs of pre-eclampsia treated with magnesium sulphate and RBC transfusion. The woman delivered at 39 weeks without complications.

Results

Initial FBE and blood film revealed mild microcytic hypochromic anaemia with elliptocytes, target cells and normal ferritin. Haemoglobin electrophoresis detected Hb S (69.9%), Hb F (28.1%) and HbA_2 (2.0%). Molecular analysis confirmed sickle cell disease and alpha thalassaemia. Follow-up testing revealed moderate microcytic hypochromic anaemia with sickle cells and schistocytes. LD was elevated with proteinuria and G6PD deficiency detected. RBC phenotyping and genotyping detected Duffy Null. The genetic mutations detected have protection mechanisms against malaria. Hb S impairs *P. falciparum* RBC invasion and growth under low oxygen conditions, enhances removal of parasite-infected RBCs, reduces pathogenicity and improves acquisition of malaria immunity. Alpha thalassaemia protects against malaria-induced anaemia and reduces pathogenicity. G6PD deficient RBCs are fragile to malaria-induced oxidative damage reducing parasite growth rate and increasing phagocytosis of infected RBCs. Duffy antigen is used by *P. vivax* and *P. knowlesi* as a receptor to mediate its entry into the RBC and its absence prevents parasite entry.

Conclusions

Here reported was a pregnant woman who co-inherited four different mutations that confers protection against malaria.

Familial Multiple Coagulation Factor Deficiencies (FMCFDs); A Rare Case of Combined Deficiency of Factor V and Factor VIII (F5F8D)

J Rigano

Alfred Health, Melbourne, Victoria, 3004, Australia

E-mail: j.rigano@alfred.org.au

Introduction

FMCFDs are characterised by the presence of more than one coagulation factor deficiency arising from a genetic defect or defects and transmissible as a familial trait. F5F8D is one of the most common FMCFDs caused by mutations in either the *LMAN1* or *MCFD2* genes responsible for the transportation of FV and FVIII from the ER to the Golgi for post-translational modification and secretion into the circulation. This case describes a child with F5F8D that was not initially diagnosed at presentation of bleeding.

Methods

A 3-year-old girl requiring treatment for post-operative infection and debridement presented with intermittent bleeding. She recently required sutures from lower lip bleed caused by a fall. She previously had upper lip injury at 18-months-old which bled for three days. The initial PT and APTT were markedly prolonged and post-operative haemoglobin 79 g/L. IV vitamin K were administered with slight improvement in APTT only. She received Prothrombinex® with slight improvement in PT and normalised APTT. NovoSeven® was given to cease bleeding prior to discharge. Two weeks later the PT and APTT were repeated and consistently markedly prolonged. Factor assays were then requested.

Results

Initial PT and APTT suggested common pathway factor deficiency. PT and APTT mixing studies corrected immediately and after 2 hours incubation at 37°C excluding an inhibitor. Factor V and Factor VIII levels were deficient explaining ineffective vitamin K and Prothrombinex® and confirmed on repeat analysis. F5F8D is a rare autosomal recessive congenital bleeding disorder common in consanguineous families from middle eastern countries. The girl's family originated from Dubai. The majority of patients present with prolonged bleeding following trauma or surgery. Bleeding episodes are treated on demand with DDAVP, FVIII concentrates and FFP.

Conclusions

Here reported was the detection of F5F8D in a child who presented with bleeding following trauma and prolonged PT and APTT.

Fibrinogen Austin (aα17gly→cys and aα381ser→phe); identification of a novel fibrinogen mutation causing dysfibrinogenaemia

J Rigano

Alfred Health, Melbourne, Victoria, 3004, Australia
E-mail: j.rigano@alfred.org.au

Introduction

Fibrinogen encoded by the *FGA*, *FGB* and *FGG* genes on chromosome 4q is a 340 kDa glycoprotein organised as two identical heterotrimers Aa, Bβ and γ chains. Fibrinogen abnormalities can be caused by acquired or congenital disorders. Mutations causing hypofibrinogenaemia and/or dysfibrinogenaemia phenotypically give rise to haemorrhage and/or thrombosis. This report describes two novel fibrinogen mutations in a woman causing dysfibrinogenaemia with a bleeding phenotype.

Methods

The proband a 51-year-old female of Chinese ancestry presented for investigation of menorrhagia with history of mild bleeding from uterine fibroids, haemorrhoidectomy and three vaginal births. Ten family members were also investigated. Coagulation assays were performed on ACL TOP CTS 500. Fibrinopeptide release assays, HPLC fibrinogen, SDS-PAGE mass spectrometry and DNA sequencing were performed.

Results

Coagulation investigations showed low Fibrinogen-Clauss with prolonged Thrombin and Reptilase times indicating dysfibrinogenaemia. Fibrinopeptide release assays showed delayed polymer formation and decreased fibrinopeptide A release. Control fibrinogen formed rigid clots after 2 minutes while proband's fibrin clots remained sloppy after 20 minutes. Released peptides were in a A:B ratio of 0.53 compared to control ratio of 1:1. SDS-PAGE, mass spectrometry revealed abnormal masses of Aα chains. DNA sequencing showed proband was heterozygous for two novel mutations Aα17Gly→Cys and Aα381Ser→Phe. Thrombin cleaves AaArg^{16}-Gly^{17} bond releasing Fibrinopeptide A, this bond was not cleaved in 50% of the Aa chains in the proband implicating Aα17Gly→Cys the cause of dysfibrinogenaemia. The connector region AaC domain contributing to fibrin polymer formation showed delayed polymer formation in the proband implicating Aα381Ser→Phe which occurs in this region. DNA analysis of ten family members confirmed the two mutations were co-inherited on the *FGA* allele. This novel double mutation variant was named fibrinogen Austin.

Conclusions

Here reported was the identification of two novel fibrinogen mutations co-inherited on the same *FGA* allele in a woman causing dysfibrinogenaemia.

A case of erythropoietic protoporphyria treated with an allogeneic stem cell transplantation

J Rigano

Alfred Health, Melbourne, Victoria, 3004, Australia

E-mail: j.rigano@alfred.org.au

Introduction

Porphyrias are a group of metabolic disorders caused by enzyme defects in the haem biosynthetic pathway. Biochemical hallmark of porphyrias is overproduction and overexcretion of porphyrin precursor compounds. Erythropoietic protoporphyria (EPP) is caused by deficiency in mitochondrial enzyme ferrochelatase (FECH). FECH inserts ferrous iron into protoporphyrin forming haem. Major site of protoporphyrin overproduction is BM. Protoporphyrin is removed by hepatic excretion and accumulation has propensity for pigmentary liver cirrhosis. Liver transplantation (OLTX) alleviates symptoms of chronic liver cirrhosis while stem cell transplantation (SCTX) corrects underlying defect. This case describes a woman diagnosed with EPP who developed liver cirrhosis and treated with allogeneic SCTX.

Methods

May 2011, a 21-year-old female with EPP and liver cirrhosis presented with chest and abdominal pain, fever with haemoptysis, thrombocytopenia and worsening liver enzymes. She was diagnosed at 18-months-old, protected from sunlight all her life and asymptomatic until December 2010. She suffered refractory RUQ pain, jaundice, splenomegaly, nausea, vomiting and anxiety. She received RBC transfusions, plasmapheresis and haem arginate to manage symptoms. She urgently required OLTX prior to consideration of SCTX.

Results

November 2011, she received a OLTX then fortnightly exchange transfusions and plasmapheresis for six years. Regular liver biopsies showed EPP recurrence and progression, cholestasis, cholangitis, biliary injury, cirrhosis and stage 4 haemosiderosis. March 2017, she received a second OLTX and splenectomy. May 2017, BMAT revealed dyserythropoiesis and 18% ring sideroblasts. August 2017, she received a SCTX however poor chimerism resulted in graft loss. February 2018, she received a second SCTX from the same donor. February 2019 she was in complete haematological remission receiving no exchange transfusion or plasmapheresis in last 12 months. Protoporphyrin, bilirubin and liver enzymes were normal and she has had no restrictions to sun exposure.

Conclusions

Here reported was a woman with EPP successfully treated with a OLTX and SCTX

Troublesome travel companions

J A Faull

Pathology New South Wales Coffs Harbour Laboratory Microbiology Department, NSW, Australia

Email: james.faull@nsw.health.gov.au

A 47-year-old lady was admitted into hospital via ambulance, drowsy and barely responsive. She was a recent traveler from India, where she suffered an upper GI bleed and had spent time in ICU post-surgery with subsequent complications including pneumonia, pneumothorax and pressure sores.

Initial indications in the emergency department were accidental paracetamol overdose. Microbiological samples were taken including urine cultures and a deep wound culture of a sacral pressure ulcer. ICU admission occurred where MRO screening swabs were collected soon after.

The urine samples grew a CPE *Klebsiella pneumoniae*, and ESBL *Escherichia coli*.

The wound swab also contained the *K. pneumoniae* and *E. coli,* however it also had a third gram negative organism, *Acinetobacter Baumanii*. This was also pan resistant (MRAB), though it did not possess an MBL mechanism.

E coli, K pneumoniae, and a VRE *E. faecium* was also detected from the patients MRO rectal swab.

The patient outcome was poor due to the overwhelming organ failure, and she passed at day 4 of admission. Surprisingly not one of the multiple sets of blood cultures collected showed bacteremia with any of the gram-negative organisms.

Massive Transfusion Competency

N K Whiting

Monash Health Pathology, Dandenong, Victoria, 3175, Australia

Email: narelle.whiting@monashhealth.org

Introduction

A scenario was devised to assess competency in the area of Massive Transfusion Protocol (MTP). The Aim was to bring the execution of MTP to the forefront and enable scientists to assess their competency to perform the task and to identify areas for improvement.

Methods

Scenario started with a 'phone call' request for 8 units of O Negative STAT. The scientist must ask the appropriate questions then prepare suitable products.

MTP: prepare 4 Red cells, 2 Fresh Frozen Plasma (FFP) and 1 platelet (4/2/1) and maintain availability until the MTP ceased.

The scenario was set up in real time. Apart from thawing products, all the usual protocols and preparation was performed as if it were a real patient, i.e. entering in computer, labelling of units, printing of reports, issuing of products to runner, 'ordering of extra products', 'phoning' the haematologist.

Times were noted for preparation of Red cells, FFP and Platelets.

At the end of the scenario each scientist was given individual feedback on their performance and ways to improve workflow discussed.

Results

Average time to availability of red cells. 7 min, AB FFP 14min and platelets 15min-a target 5 min showed room for improvement. Each scientist was given their times with aim to improve those times when the competency is repeated. A number of issues were identified for individual scientists to work on, as well as issues common to more than one scientist. These included the handling of the initial phone call, thinking beyond the initial order and usage of thawed FFP.

Conclusion

Overall an interesting exercise that showed room for improvement in some areas and enabled the scientists to understand what was required of them in MTP. An updated exercise with our new MTP protocol is currently underway with aim to show improvement in competency.

Global coagulation assays and their role in thrombosis

Lim HY

Northern Health, Epping VIC 3076 Australia

Email: huiyin.lim@nh.org.au

Global coagulation assays such as thromboelastography have been widely used in trauma and bleeding disorder

settings to assess bleeding risk and guide blood product replacement. However, the role of these assays in thrombosis is less clear and predicting the risk of cardiovascular and thrombotic complications still remains an important unmet need.

Various studies have explored these assays separately in a variety of small cardiovascular and thrombotic studies, and have demonstrated the role of these assays in predicting thrombotic risk. Interestingly, some studies have demonstrated contradictory results, with reduced thrombin generation levels seen in patients with cardiovascular disease. Similarly, we have previously reported that global coagulation assays such as thromboelastography and thrombin generation can show subtle difference in age, gender and race, which is not seen on standard coagulation assays. Hence, these global coagulation assays, particularly when used in combination, may provide a better assessment of cardiovascular and thrombosis risk.

We provide a review of the literature of the role of these assays in thrombosis, with a specific focus on data we have collected in normal controls, cardiovascular disease, COVID-19, plasma cell dyscrasia and transwomen on hormonal therapy. Specifically, the role of thromboelastography, fibrin and thrombin generation have been evaluated in each of these conditions, with unique differences and contradictions seen with each assay, which may help further our understanding of the complexities of the coagulation system.

Serological interference of anti-cd47 immunotherapy in Pre-transfusion testing

R Kumar, W. Magee

Peter MacCallum Cancer Centre, Melbourne, Victoria, 3000, Australia

Email: Kumar.Ranjeef@petermac.org

Introduction

CD47, a glycoprotein expressed on all human cells, binds to signal-regulatory protein α on macrophages triggering the inhibition of phagocytosis.[1] Magrolimab (Anti-CD47) is a human monoclonal Immunoglobulin G (IgG) subclass 4 antibody that blocks CD47 thereby targeting cells for destruction via macrophage phagocytosis. Plasma from patients receiving anti-CD47 immunotherapy have been shown to interfere with all pre-transfusion testing phases.

We report anti-CD47 interference in pretransfusion testing in one patient and evaluate mitigation strategies.

Methods

Samples from a patient on Magrolimab were tested for blood grouping, direct antiglobulin test (DAT), antibody identification with and without papainised cells, titration and carryover studies using column agglutination testing (CAT).

Indirect antiglobulin testing (IAT) with Rapid Antibody Medium (RAM) and without enhancement media (classic) were subsequently performed using tube techniques. Different AHG reagents (polyspecific anti-IgG-C3d, monoclonal anti-IgG with or without anti-IgG4) were used at IAT phase. Eluates were made using rapid acid elution.

Results

Panagglutination was observed in antibody screen and identification. There were no interferences when performing ABO forward typing. The patient was determined to be group O Positive. In addition, no carryover was noted, in spite of the anti-CD47 in the sample showing a very high titer (1: 8192 dilution).

Tube IAT results (with and without enhancement) and using 3 AHG reagents will be presented.

The eluate reacted 3+ to 4+ with 11-cell panel by tube IAT using polyspecific anti-IgG-C3d and monoclonal anti-IgG. IAT was negative with a monoclonal anti-IgG not detecting IgG4.

Conclusions

Magrolimab immunotherapy reported here interfered with CAT. However, ABO forward typing using CAT was unaffected. In addition, no carryover was seen when using automated CAT.

As expected from previous reports, interference with IAT was dependent on the anti-IgG used. Monoclonal reagents without anti-IgG4 activity were helpful in mitigating the interference at AHG.

References

Velliquette RM, *et al*. Monoclonal anti-CD47 interference in red cell and platelet testing. *Transfusion* 2019; 59; 730-773

Tuberculosis of the appendix

C Aburrow

RMIT University, Bundoora, VIC, 3083 Australia

Email: aburrowcailin@gmail.com

Introduction

Cabrini Health received a specimen from a 25-year-old female from Papua New Guinea presenting with RIF pain. No previous clinical history is known. Operative findings illustrated the presence of necrosis and fibrosis occuring at the base of the appendix.

Methods

Routine H & E staining showed necrosis and inflammatory cells in the tissue. The special stains Ziehl Nielson and PAS were conducted to observe the necrosis in the specimen to determine if the infection was caused by the prevalence of mycobacterium or a fungus in the appendix.

Results

The Ziehl Nielson staining highlighted the acid-fast bacilli in the tissue, this correlates with the provisional diagnosis of *mycobacterium tuberculosis.*

Conclusions

The most common diagnosis of RIF pain is acute appendicitis, however due to the limited access to patient information it is indeterminate if the appendicitis is primary or secondary. Although, due to the necrosis at the base of the appendix it is most likely a secondary tuberculosis infection.

The source of the TB may be due to the consumption of unpasteurised milk, especially as this is common in developing countries.

Liquid biopsy in multiple myeloma

S Mithraprabhu

Alfred Hospital – Monash University, Melbourne, Victoria 3004, Australia

Email: durga.mithraprabhu@monash.edu

Multiple myeloma (MM) is an incurable plasma cell malignancy that manifests at multiple-sites within the bone marrow (BM). Genomic heterogeneity in MM contributes to disease relapse. The methodology to characterise the tumour genome relies on single-site BM biopsies that fail to capture the recognised spatial and genetic heterogeneity of this multi-focal disease. An alternative to BM biopsy is liquid biopsy - the interrogation of circulating tumour DNA (ctDNA) released into the blood stream by MM tumour cells. We investigated the utility of ctDNA for mutational characterisation and therapeutic monitoring in MM.

We performed analysis of paired ctDNA and BM MM cell DNA from 76 patients for *KRAS*, *NRAS*, *BRAF* and *TP53* mutations using the OnTarget™ Mutation Detection (OMD) platform. Patients with ctDNA-specific *TP53* (a DNA-repair gene) mutations had shorter OS compared to patients with no ctDNA-*TP53* mutations (p=0.003). We validated this utilising a targeted amplicon sequencing (TAS) panel of 23-MM specific genes with an independent cohort of 36 paired BM MM and ctDNA to reveal that higher levels of DNA-repair gene mutations were present in ctDNA compared to *RAS-RAF* mutations (p=0.0095).

OMD analyses also revealed that patients with >2 mutations or >1% tumour burden in the PL had shorter OS (p=0.04 and p=0.0006, respectively). This was validated in an independent cohort of uniformly treated MM patients (n=24), in whom patients with a higher number of mutations or tumour burden in PL demonstrated a shorter OS (p=0.005 and p=0.018, respectively). We performed droplet digital PCR analyses of specific mutations at entry and at day 5 of treatment and observed that a decrease in ctDNA levels at day 5 correlated with superior progression-free survival (PFS) (p=0.017).

In conclusion, our analyses has confirmed that ctDNA analyses offers a comprehensive and robust methodology for mutational characterisation and therapeutic monitoring in MM.

It's a no-brainer: the impetus to remove neural material from brain heart infusion media

P Traynor, M Summerford, A Catalano,

Thermo Fisher Scientific, Oxoid Australia Pty Limited, Thebarton, South Australia, 5031, Australia

Email: peter.traynor@thermofisher.com

Introduction

For over a century, brain material has been used in microbiological media. Since the emergence of bovine spongiform encephalopathy (BSE) in the late 1980s, the risk of brain and brain stem materials has been widely recognised. In culture media manufacture, some changes in sourcing of brain materials has occurred, but it remains in use.

Brain materials continued use in media is from a presumption that it provides extra, essential nutritive qualities. However, our recent review of the literature into the original uses of brain infusion highlighted that those reasons for its inclusion are totally unrelated to the actual performance of the media today. Those historical rationales, and the fact it is present only in homeopathic quantities, suggests that its removal will not adversely affect performance.

Methods

Performance testing of heart infusion media, compared with media also containing brain infusion, was undertaken.

Results

Our testing confirmed that, with the range of organisms tested, the minute quantities of brain materials did not provide any advantage regarding media performance.

Conclusions

Brain infusion in microbiological culture media provides significant biosecurity risks but no performance advantages.

The historical inclusion of brain materials in media were for fallacious reasons, unrelated to any current usage of the media.

The inclusion of brain and brain materials in microbiological media has no justified place in the 21st century.

Morphology – making a difference

C. Durkin

Peter MacCallum Cancer Centre, Melbourne 3000, Victoria, Australia

E-mail: catherine.durkin@petermacallum.org

Over recent decades the role of the morphologist has changed dramatically; from examining mostly normal blood films to examining mostly abnormal films. The sophistication of today's haematology analysers means that we now have access to a vast amount of information about a patient's sample before we review their blood film. As good as these analysers are though, they do not always detect the presence of abnormal cells, they are often unable to identify the nature of abnormal cells, they cannot give a diagnostic interpretation of abnormal samples and may not alert operators to spurious results. These are areas where the skilled morphologist can really make a difference to patient care.

The role of morphology trainer is hugely rewarding. There is great satisfaction in seeing a trainee make sense of a challenging blood film. It is the trainer's job to ensure that a trainee has the requisite theoretical knowledge, shows attention to detail, and most importantly, has a clear focus on, and commitment to, the patient. The trainee needs to be shown how to piece together key features on a blood film with patient history and other pathology results to determine if a diagnosis can be made. The trainee also needs to learn that while a university education is essential, there is no substitute for time spent examining blood films.

Blood film morphology is an exciting field that provides medical scientists with an opportunity to use their theoretical knowledge and expertise to play a vital role in the diagnosis and management of many diseases. Morphologists can thereby directly improve patient outcomes. From the diagnosis of iron deficiency and thalassaemia/haemoglobinopathies to haematological malignancies and potentially life-threatening disorders, the role of the morphologist can be demanding, but is ultimately very satisfying.

Copeptin- Biochemistry and its role in Clinical Diagnosis

N J Perera[1,2]

[1]Royal Prince Alfred Hospital, Camperdown, NSW, 2050, Australia

[2]Faculty of Medicine, University of New South Wales, NSW, Australia

Introduction

Antidiuretic Hormone (ADH) also called Arginine Vasopressin (AVP) is a key hormone secreted from the posterior pituitary. It has important physiological functions that include homeostasis of fluid balance, vascular tonus and regulation of endocrine stress response. AVP is elevated in response to high serum osmolality, low effective circulating volume and physiological stress. However, measurement of mature AVP is difficult and subject to pre-analytical errors. Due to this AVP or ADH testing have not been routinely useful.

Copeptin (aka C- Terminus Pro-Arginine Vasopressin, CTproAVP), is the C-terminal part of the AVP precursor. It has been found to be a stable and sensitive marker of AVP release. It is secreted in equimolar amounts to AVP. It has the advantage of having a much longer plasma half-life than ADH, greater stability at room temperature and therefore is less prone to pre-analytical errors. For these reasons Copeptin has replaced ADH testing and is valid surrogate marker of AVP.

Copeptin is now identified as a useful tool in the investigation of polyuria –polydipsia syndromes. It is now used as an adjunct to the water deprivation test which is the diagnostic "gold standard" for the assessment of AVP activity. An Immuno- luminometric proAVP or copeptin assay is now available in our laboratory.

For the investigation of diabetes insipidus (DI) and primary polydipsia (PP) in patients with confirmed polyuria (>40 mL/kg/d), [without concurrent diabetes mellitus, hypercalcemia, pregnancy, uncorrected thyroid or adrenal

insufficiency, heart failure and **sodium levels >14mmol/L following water deprivation]

A baseline Copeptin level >21.4 pmol/L (100% sensitive and specific for nephrogenic diabetes insipidus)

A baseline Copeptin level < 2.6 pmol/L with prior fluid deprivation (> 8hrs) will indicate complete central DI likely

A stimulated Copeptin** > 4.9 pmol/L PP likely and < 4.9 pmol/L partial central DI likely (94.0% specificity and 94.4% sensitivity)

References

Fenske W, Quinkler M, Lorenz D, et al. Copeptin in the differential diagnosis of the polydipsia-polyuria syndrome - revisiting the direct and indirect water deprivation tests. *J Clin Endocrinol Metab.* 2011; 96(5): 1506-1515.

The Reference Interval for Non-water Deprived and Non-Fasting Adults was determined from an in-house RPAH Endocrinology Laboratory Study.

Reference Interval for fasting and water deprived adults (> 8hours) was adopted from the Mayo Clinic in house study, www.mayocliniclabs.com

A curly blood culture isolate

T Abajo

Alfred Health, Melbourne, Victoria, 3004 Australia

Email: t.abajo@alfred.org.au

Introduction

A 27-year-old MSM presented with fevers and diarrhoea. Several sets of blood cultures were collected and four aerobic bottles flagged positive after 96 hours. Long spiral-shaped Gram-negative rods were seen. They were sluggishly motile.

Methods

The blood was subcultured onto chocolate and horse blood agars and incubated in CO^2, anaerobic and microaerophilic atmospheres. Cultures were examined daily for 5 days. There was no growth at all. At this point it was decided to send one of the positive bottles to a reference laboratory for 16s rRNA.

We returned to the bottles to reattempt culture of this organism. This time the cultures were left undisturbed for 5 days. Thin, spreading colonies were visible, with better growth anaerobically than in microaerophilic atmosphere. We attempted to identify the organism by MALDI-TOF on the Vitek MS with no result, and then on the Bruker BioTyper, which identified the organism as *Helicobacter cinaedi*. This identification was questioned as our isolate initially tested negative for oxidase.

Results

The organism was confirmed as *Helicobacter cinaed.*

Conclusion

This is an extremely rare isolate for our laboratory, and emphasises the need to combine old methodologies with newer technologies in order to identify unusual organisms.

Identification of a *vibrio parahaemolyticus* cluster by bacterial culture of faecal specimens

T Mahony, G McCluskey, G Robertson, C Perera, L Prendergast, L Waring

Melbourne Pathology, Collingwood, Vic 3066, Australia

Email: Tess.Mahony@mps.com.au

Vibrio parahaemolyticus is an occasional cause of seafood associated foodborne diarrhoea. Most laboratories utilise selective media (eg TCBS) where a clinical suspicion exists based on consumption of seafood. Recently, many laboratories have moved to multiplex PCR assays for bacterial faecal pathogens, which do not include *V. parahaemolyticus,* while we continue to culture. This is advantageous, as further testing may be performed. From 01/2/2021 - 02/05/2021, we had 10 cases of *V. parahaemolyticus*, isolated from community patients with histories of diarrhoea, but no history given of recent seafood ingestion. There was no apparent geographic clustering of the cases. In Victoria, *V. parahaemolyticus*, is not notifiable, unless there is suspicion of an outbreak. Because this was more prevalent than usual, we notified the DHHS (health department) and referred the isolates to the reference laboratory for strain typing. After Multi-Locus Sequence Typing (MLST), 9 of the 10 were found to be genetically related, suggesting a point source outbreak.

Conclusion: Culture is a broader method of testing for gastro-intestinal pathogens than restricted panel Faecal Multiplex PCR, and isolates are available for further testing if required. We recommend the inclusion of *V. parahaemolyticus* as a notifiable disease due to the risk of missing an outbreak.

Melanin pigment…get the peroxide!

M Boyer

Monash Health
Monash Medical Centre, Clayton, Victoria, 3168
Email: Maria.chavez@monashhealth.org

Melanin is a structurally complex brown-black pigment found in living organisms across multiple biological kingdoms inclusive of plants, animals and microorganisms. Melanin pigment is produced by melanocytes, within melanocytes the pigment is synthesized in membrane bound organelles (melanosomes) and is visible when melanocytes reach stage 3 maturation. There are two chemically distinct types of melanin, the brown black eumelanin and the yellow red pheomelanin. Melanogenesis is a complex process involving multiple regulatory enzymes and pathways, of which the enzyme tyrosinase is critical and regulates the quality and quantity of melanin produced. Melanin is tightly bound to the melanosome by structural proteins, making the pigment insoluble in organic solvents.

Whilst eumelanin has numerous beneficial functions such as pigmentation, radical scavenging, radiation protection and thermal regulation, it can cause significant implications in the histopathological and molecular diagnosis of heavily pigmented melanocytic lesions. Dense melanin pigment obscures cellular morphology and physically masks antigen-antibody interactions.

Bleaching methods using strong oxidants potassium permanganate/oxalic acid and hydrogen peroxide have been successful in removing the masking effects of melanin pigment, however both have their limitations. Review of the literature indicates that 10% hydrogen peroxide warmed to 60^{o}C is a superior method of bleaching that preserves antigenic sites suitable for immunohistochemical detection. This bleaching method was applied to the Masson Fontana silver stain.

The Masson Fontana silver stain for the detection of melanin pigment incorporates a control slide that is bleached to confirm the presence of melanin in an unbleached test section. This presentation will discuss the success and limitations of using hydrogen peroxide as an alternative bleaching method to the traditional potassium permanganate/oxalic acid method.

Rapid detection of viral and bacterial meningitis in a paediatric population

S Burgess,[1] G Gonis,[2] C Lowe[3]

[1,2,3]Royal Children's Hospital, Melbourne, Victoria, 3052, Australia

Email: stephen.burgess@rch.org.au

The bioMerieux BioFire is an instrument based on filmarray technology, which allows for rapid detection of multiple nucleic acid targets in clinical specimens. The ME (Meningitis/Encephalitis) panel is used for the early detection of both bacterial and viral meningitis in cerebrospinal fluid (CSF). Over the course of seven months, the bacteriology department at the Royal Children's Hospital (RCH) ran over two hundred assays on CSF, using the BioFire ME panel. Specific criteria were established so that only the most urgent and at-risk patients were tested, so as to preserve resources. Only CSF from patients less than three months old, or patients from the emergency department were tested. Twenty-three of these assays were positive, detecting targets such as *E.coli*, *H.influenzae*, *S.agalactiae*, *Herpes simplex virus* and *Enterovirus.* Interestingly many of the positive CSF results did not have a classic presentation such as raised leukocytes counts, positive gram stains or bacterial culture growth. Additionally, almost half of the positive viral cases were from patients where either viral studies were not requested, or a different virus was detected. Despite some limitations, the BioFire has integrated seamlessly into the RCH bacteriology department and has become an important tool for the rapid detection of meningitis.

ICSH Morphology standardization project

L Palmer

Middlemore Hospital, Auckland 1640, New Zealand

E-mail: Lynn.Palmer@middlemore.co.nz

The identification, classification and grading of peripheral blood cell morphology can be a challenging and subjective exercise. In 2010, the International Council for Standardization in Haematology (ICSH), began an international project to try and standardise the nomenclature and reporting of peripheral blood cell morphology. This led to the development of consensus guidelines, published in 2015 in the International Journal of Laboratory Haematology, on how to reliably and consistently report abnormal red cell, white cell and platelet morphology using manual microscopy.

This presentation will provide a brief background to the project, outline some of the challenges we faced and cover key recommendations from the guideline.

Chimeric antigen receptor (CAR) T-cell therapy in all: the RCH experience

K Clerici

Royal Children's Hospital, Parkville, Victoria, 3052, Australia

Chimeric Antigen Receptor (CAR) T cell therapy is a new form of cellular immune therapy, using T cells engineered to express a hybrid receptor that is specific for a chosen antigen. In the case of B cell Acute Lymphoblastic Leukaemia

(B ALL) the antigen is CD19. These CD19-CAR T cells attack normal and malignant B cells, providing a durable anti-cancer treatment as long as the CAR T cells persist.

The first paediatric patient to receive CAR T cells for B ALL therapy was in 2012 at the Children's Hospital of Philadelphia. The US Food and Drug Administration (FDA) approved this treatment in 2017 as the first ever approved gene therapy in the US. This followed with approval by the Australian Therapeutic Goods Administration (TGA) in December 2018. The Royal Children's Hospital (RCH) first infused a patient with CAR T cells in 2016 on a clinical trial and has since treated 20 patients with this therapy.

The delivery of this treatment involves multiple departments at the RCH. This presentation will highlight the role played by the Medical Scientists in the Cell Therapy Laboratory at the RCH in delivering this exciting, and ground breaking, new treatment for B ALL.

Exploring the microbiome of probiotic-treated preterm infants

J Westaway[1,2], Dr R Huerliman[2,3,4], Dr Y Kandasamy[5,6], Dr K Staunton[1,7], Dr R Norton[8], C M Miller[1,7], Dr D Watson[9], S I Vilamil[2,4], Dr D Rudd[5]

[1] College of Public Health, Medical and Veterinary Science, James Cook University, 1/14-88 McGregor Road, Smithfield, QLD 4878, Australia.

[2] Centre for Tropical Bioinformatics and Molecular Biology, James Cook University, 1 James Cook Dr, Douglas, QLD 4811 Australia.

[3] Marine Climate Change Unit, Okinawa Institute of Science and Technology (OIST). 1919-1 Tancha, Onna-son, Okinawa, 904-0495 Japan.

[4] College of Science and Engineering, James Cook University, 1 James Cook Dr, Douglas QLD, 4811 Australia.

[5] College of Public Health, Medical and Veterinary Science, James Cook University, 1 James Cook Dr, Douglas QLD, 4811 Australia.

[6] Neonatology, Townsville University Hospital, 100 Angus Smith Dr, Douglas QLD, 4814 Australia.

[7] Australian Institute for Tropical Health and Medicine, James Cook University, 1/14-88 McGregor Road, Smithfield QLD 4878, Australia.

[8] Microbiology, Pathology Queensland, 100 Angus Smith Dr, Douglas QLD, 4814 Australia.

[9] Maternal-Fetal Medicine, Townsville University Hospital, 100 Angus Smith Dr, Douglas QLD, 4814 Australia

The role of prematurity in several diseases may be linked to the disruption of gut microbiome development. Probiotics may correct this microbial disruption, mitigating disease risk. We used both amplicon and shotgun metagenomic sequencing to characterise the gut microbiome of probiotic-treated preterm infants. We aimed to identify and understand variation in bacterial flora between admission to discharge and in association with clinical variables. We also assessed the current probiotic treatment criteria at the Townsville University Hospital (TUH) by comparing against an un-treated preterm infant group who fall outside the criteria for treatment.

127 preterm infants were recruited, and 182 stool samples collected. Significant differences in the microbiome of probiotic-treated infants were observed between admission and discharge. Modelling showed significantly lower alpha diversity in infants diagnosed with either sepsis or retinopathy of prematurity (ROP), and those formula-fed. Chorioamnionitis, preeclampsia, sepsis, necrotizing enterocolitis and ROP were associated with differential abundance of taxa. Relative to probiotic-treated infants, untreated infants had significantly different microbial communities, characterised by lower alpha diversity and differential abundance of taxa. Shotgun metagenomics showed clustering by probiotic-treatment, and colonisation by probiotic-species. However, this shotgun approach was limited by sample size.

Lower diversity in sick or formula-fed infants, as well as the differing abundances of several taxa across variables, highlights the role of the microbiome in disease and supports the need for promoting healthy microbiome development. The differences observed between probiotic treatment groups suggests older preterm infants may benefit from probiotics, but further research is needed.

Non-invasive prenatal assessment (NIPA)

E Black

Australian Red Cross Lifeblood, Brisbane, QLD, 4059, Australia

Email: eblack@redcrossblood.org.au

Introduction

Non-Invasive Prenatal Analysis (NIPA) for fetal RHD is a molecular blood group genotyping assay used to predict the RhD status of the fetus in pregnancies where the mother is RhD negative and the fetus is at risk of being affected

by Haemolytic Disease of the Fetus and Newborn (HDFN) due to anti-D.

Methods

NIPA utilises a maternal peripheral whole blood sample for the extraction of cell-free fetal DNA (cffDNA), which is analysed for the presence of the RHD gene. After extracting cffDNA from the maternal plasma, a quantitative Polymerase Chain Reaction (qPCR) assay is used to amplify exons 4, 5 and 10 of the RHD gene in quadruplicate. The combination of these exons ensures that fetuses with RhD variant genes are not missed and called RhD negative. The assay also amplifies the male-associated SRY gene, and a chemokine receptor gene, CCR5, which serve as internal controls for the presence of fetal DNA, and a measure of sample integrity. In the event that the RHD gene is not detected from the sample, the SRY gene (a Y-chromosome specific gene) is useful in male fetuses to ensure our samples contain cffDNA. If the SRY gene is not detected a supplemental qPCR assay for hypermethylated RASSF1A is used to confirm the presence of fetal DNA sequences in the plasma DNA sample.

Results

Case study 1 predicted a RhD positive fetus. Case study 2 predicted a RhD negative fetus, confirmed via qPCR assay for hypermethylated RASSF1A. Case study 3 was indeterminate due to the mother having a RhD variant.

Conclusion

NIPA improves clinical practice in transfusion medicine by giving clinicians an additional diagnostic marker to guide them in determining the appropriate management of pregnancy.

APACE an overview

M Jacobsen

AIMS APACE Committee, Australia

The presentation provides an overview of Australasian Professional Acknowledgement of Continuing Education (APACE) as a Continuing Professional Development (CPD) program, for all members of the medical laboratory workforce. This will include the reason for setting up the program, the roles of AIMS, the APACE committee and the participants. Describe how the program operates, the recent changes to the program, time frame, points required and division of activities into categories and the rationale behind these changes. The presentation will conclude with some tips for participants to enable easy submissions for APACE certification for participation.

For additional information about APACE please contact programs@aims.org.au

APACE Certification is achievable by all.

The value of a public health ethics approach to reproductive genetic carrier screening

A J Newson, L. Dive

Sydney Health Ethics, Sydney School of Public Health, Faculty of Medicine and Health, University of Sydney, Sydney, New South Wales, 2006, Australia

Email: ainsley.newson@sydney.edu.au

Introduction

Reproductive genetic carrier screening (RCS) tests people for a range of autosomal or x-linked conditions to determine the chance they will have a baby with a serious genetic condition. Determining the chance, a person or couple will have an affected baby has typically taken place in two contexts: (i) clinical carrier testing, for those with a relevant family history; or (ii) population screening, for groups with higher prevalence of certain conditions. Traditionally, these paradigms have been underscored by different values.

RCS is now increasingly available to whole populations, by way of publicly funded testing or via commercial providers. In Australia, the Mackenzie's Mission trial is undertaking RCS in around 8,500 couples, to inform subsequent implementation. This project is building on well-established infrastructure, including clinical, counselling and laboratory resources.

However, expanded RCS also gives rise to a possible convergence between clinical and public health approaches to testing, and their respective ethical paradigms. Which is more appropriate for RCS?

Methods

Clinical ethics and public health ethics approaches will be described and critically assessed as they apply to RCS. The clinical paradigm emphasises reproductive autonomy, while the public health paradigm focuses more on prevention.

Results

RCS meets the definition of a screening intervention and can therefore be considered as a form of public health. However, existing public health ethics approaches are inappropriate for RCS. Instead, an ethical approach that brings together elements from both public health and clinical ethics is favourable.

Conclusion

Determining the goals of RCS and their implementation requires a commitment to plural values, drawn from both clinical and public health ethics. This includes supporting reproductive autonomy as well as paying heed to social factors that can undermine or limit reproductive choices. Such an approach will ensure that the needs of both individuals and populations in RCS can be considered and addressed.

National Blood Authority Update

J Cameron

National Blood Authority

Email: Jo.cameron@blood.gov.au

The National Blood Authority is a statutory authority that represents the interests of the Australian and state and territory governments, and sits within the Australian Government's Health portfolio. Our work would not be complete without our valued staff, suppliers, committee members, health professionals and other key stakeholders that support our strategies, programs and systems.

The National Blood Agreement's primary policy objectives are:

- to provide an adequate, safe, secure and affordable supply of blood products, blood-related products and blood-related services in Australia; and
- to promote safe, high-quality management and use of blood products, blood-related products and blood-related services in Australia.

A supporting principle is that blood and blood-related products can be accessed by patients at no direct cost, provided such use is in accordance with clinical need and appropriate clinical practice.

The financial year 2020-21 was a busy year for the National Blood Authority, with a number of achievements in the areas of ensuring blood supply, release and maintenance of guidelines, improvements to the Blood Sector Systems and Immunoglobulin use and management.

R.O.S.E.: Advantages and disadvantages in multi-disciplinary pathology testing

K L Jones

Anatomical Pathology Department, St Vincent's Hospital Melbourne, Victoria

Email: Kerryn.jones@svha.org.au

Introduction

ROSE is an acronym for Rapid, On Site, Evaluation. ROSE is a routine service that most cytology laboratories provide to clinician's and surgeons at the time of a Fine Needle Aspirate (FNA) procedure. The attendance of a Scientist or Pathologists at the time of aspiration collection, allows the specimen to be assessed for adequacy in real time.

Due to the COVID 19 Pandemic, attendance at FNA procedures was identified as a risk to staff safety. This was due to the small procedure rooms that did not allow social distancing, and inadequate ventilation for the aerosols generated during FNA collection and preparation.

At St Vincent's Hospital in Melbourne, it was deemed an unnecessary risk to attend FNA procedures. We opted to provide clinicians with a Liquid based collection (LBC) medium, CytoLyt™, for collection of aspirated specimens. Preparation of the FNA samples was then performed in the safety of the laboratory.

This study evaluates data from 2014 to 2020 for FNA specimens received in our laboratory with and without ROSE. The aim was to establish whether or not ROSE is truly of benefit in ensuring the collection of an adequate specimen, that meets the needs of all morphological and ancillary pathology testing.

Methods

Data from 2014 to 2020 for FNA cytology specimens was evaluated.

The data was for all FNA specimens that had been collected with or without ROSE.

The most significant data was that for 2020, where no routine ROSE was performed from February due to the COVID 19 pandemic.

Results

Adequacy rates were shown not to differ greatly by the collection of the specimen into CytoLyt™, without ROSE.

The collection of FNA specimens into CytoLyt™, demonstrated greater specimen utility for ancillary testing.

LBC of FNA specimens also allowed for more efficient allocation of scientific resources.

Conclusion

Consideration should be given to whether ROSE for all FNA's is essential.

LBC collection of FNA specimens provides greater specimen utility to further meet pathology testing requirements, instrumental in guiding patient care and management.

Human Colours - The Rainbow Garden of Pathology

A Laslowski

Monash Health, Clayton, Australia

Human tissue presents a range of colours from white to black and a myriad of colours in between. But why is it that we see fat as yellow and muscle as red? Cells as singular units are colourless but, in the millions, demonstrate the predominant cellular contents for the cell type. This gives tissues and organs their unique colours and as scientists a better understanding of them can help us to better identify normal from abnormal pathology. The presentation looks at how the natural environment and individual cellular elements influence the rainbow of colours we see in the pathologic tissue.

The diagnosis of blood-borne parasites- a review of current methods and preview of future diagnostic applications

F Azzato

Victorian Infectious Disease Reference Laboratory, Doherty Institute, Melbourne, Victoria, 3000, Australia.

Email: rancesca.azzato@mh.org.au

Blood-borne parasites, often occur in poor socioeconomic areas where hygiene is poor and access to adequate health support is limited. The major parasitic diseases that are categorised as blood-borne include: African Trypanosomiasis, Babesiosis, Chagas disease (American Trypanosomiasis), Leishmaniasis, Malaria, Filariasis and Toxoplasmosis. These are all a cause of significant morbidity worldwide, often leading to chronic illness, malnutrition and in some instances mortality. Transmission to humans can vary, the most common mode of infection is through insect bites and less common modes include ingestion of contaminated food, through blood product transfusions and organ transplantation. Traditionally, the diagnosis of blood borne parasites has largely relied on microscopy, which is still primarily adopted by many laboratories worldwide. However, microscopy is time consuming, laborious and requires a trained microscopist to correctly identify the infecting species. Recent developments in serology based testing and molecular based approaches have provided laboratories with new avenues for improving the diagnosis of these infections. New serology-based tests such as rapid antigen detection assays (RDT's) provide improved sensitivity and a rapid turnaround time, molecular based assay such as PCR also provide improved sensitivity when compared to microscopy and a rapid turnaround time, but in addition they have the ability to test for a greater range of targets. Genomic approaches such as next generation sequencing (NGS) which are in their infancy, are expected to provide future assay development opportunities for the diagnosis and management of these infections.

Benefits of a networked digital morphology system (CellaVision) in a geographically dispersed pathology service

A Giffiths

SA Pathology, Adelaide, Australia

In September 2017, SA Pathology moved to its new flagship laboratory on the campus of the newly completed Royal Adelaide Hospital (RAH). The core laboratory had a new state of the art fully automated track system, including four Sysmex XN analysers and two SP10 slide maker stainer instruments along with a Di60 digital microscope (CellaVision) integrated into the haematology line. In early 2019 digital morphology essentially replaced conventional microscopy for blood film review at the RAH. In April 2020 the system was connected to its own ICT network in preparation for the future rollout of scaled digital solutions at each of its metropolitan and regional sites.

The concept of a fully networked digital morphology system being beneficial to a geographically dispersed pathology service was proven during the height of the COVID 19 pandemic. A digital microscope (DC-1 CellaVision) was placed at a busy regional laboratory that was also required to service a dedicated "pop up" SA Pathology COVID 19 collection centre. This enabled remote blood film review to be performed by experienced blood film morphologists at the RAH, significantly relieving the pressures on this regional laboratory.

Since its inception digital morphology has revolutionised the way SA Pathology performs blood film review, provide morphology training and assess staff competency across the organisation.

Ageing of the plasma proteome

V Ignjatovic

Murdoch Children's Research Institute and the University of Melbourne, Parkville, VIC 3052, Australia

Email: Vera.ignjatovic@mcri.edu.au

Human blood plasma is a complex biological fluid that interacts with a wide array of bodily systems. It is therefore not surprising that changes in specific plasma protein concentration are used regularly in the clinical setting to diagnose disease. Proteomics is an approach that studies hundreds, sometimes thousands of proteins at once, producing a holistic, clinically relevant insights of the

internal physiological environment. Whilst a large body of literature details the plasma proteome changes in the context of disease, there has been minimal focus on the age-specific changes in the plasma proteome, especially in the healthy population. This knowledge is critical in our ability to diagnose disease, identify blood markers associated with clinical outcomes, and the larger opportunity for exploration and design of novel and innovative tools for the ultimate benefit of children who are unwell.

Our team has established the concept of Developmental Proteomics and has made a significant, strategic and focused investment in understanding the age-specific changes in the plasma proteome across the age spectrum, from neonates to adults. In this presentation I will outline the main aspects of the work that we have performed in the Developmental Proteomics space to date, and the ways in which this knowledge is benefiting subsequent clinical proteomics studies which we are currently undertaking and which we have planned for the near future.

A multicentre laboratory assessment of a new automated chemiluminescent assay for adamts13 activity

E Favaloro[1,2,3], S Mohammed[1,2], K Chapman[2,4], P Swanepoel[2,4], D Zebeljan[2,5], O Sefhore[2,5], E Malan[6], J Clifford[6], A Yuen[6], D Donikian[2,7], M Kondo[2,7], E Duncan[8], S Abraham[8], J Beggs[9], R Chatrapati[9], J Perel[9], R Coleman[10], N Klose[10], D Hsu[2,5], P Motum[2,5], C W Tan[8,11], T Brighton[2,7], L Pasalic[1,2,3]

E-mail: joanne.clifford@monashhealth.org

Introduction

Thrombotic thrombocytopenia purpura (TTP) is a rare but potentially fatal disorder caused by ADAMTS13 (a disintegrin and metalloproteinase with thrombospondin type 1 motif, member 13) deficiency. Prompt identification/exclusion of TTP can thus be facilitated by rapid ADAMTS13 testing. The most commonly utilized (enzyme-linked immunosorbent assay [ELISA]-based) assay takes several hours to perform and so does not generally permit rapid testing. We evaluated the utility of a new automated test for ADAMTS13 activity, the HemosIL AcuStar ADAMTS13 Activity assay, based on chemiluminescence and able to be performed on an ACL AcuStar instrument within 33 minutes.

Method

This multicentre (n = 8) assessment included testing of more than 700 test samples, with similar numbers of prospective (n = 348) and retrospective (n = 385) samples. The main comparator was the Technozym ADAMTS13 Activity ELISA. We also assessed comparative performance for detection of ADAMTS13 inhibitors using a Bethesda assay.

Results

Overall, the chemiluminecent assay yielded similar results to the comparator ELISA, albeit with slight negative bias. ADAMTS13 inhibitor detection was also comparable, albeit with slight positive bias with the AcuStar assay. Assay precision was similar with both assays, and we also verified assay normal reference ranges.

Conclusions

The HemosIL AcuStar ADAMTS13 Activity assay provided results rapidly, which were largely comparable with the Technozym ADAMTS13 Activity ELISA assay, albeit lower on average. Conversely, inhibitor levels tended to be identified at a higher level on average. Thus, the HemosIL AcuStar ADAMTS13 Activity assay provides a fast and accurate means to quantitate plasma levels of ADAMTS13 for TTP/ADAMTS13 identification/exclusion, and potentially also for other applications.

Student CPD Grant Final Report

Kevin He

University of South Australia, Adelaide, South Australia

The AIMS National Scientific Meeting was held from 30 August to 1 September and it was my honour to receive a student CPD grant to attend. I will share my positive experience in this report

The scientific programme was comprehensive and I had the great opportunity to learn from leading experts about CAR T-cell therapy, flow cytometry, COVID-19 among other important topics. I would like to now highlight the talks that I found most interesting.

First, my favourite talk was from A/Prof Tracy Heng on mesenchymal stem cell (MSC) therapy. Using various techniques and murine models. She shows that after IV infusion, MSCs undergo apoptosis in the lungs and are taken up by alveolar macrophages, which in turn, exert an anti-inflammatory effect by secreting IL-10 and inhibiting T cell proliferation. These studies provided mechanistic insights into MSCs' once-elusive immunomodulatory effects, which is very intriguing. After listening to her talk, I explored her track record of research, and in particular her previous work on the Immunological Genome Project which was brilliant. Further, her distinguished career has inspired me to consider undertaking a research degree.

Secondly, Prof Vera Ignjatovic and Vicky Karlaftis presented their HAPPI Kids study, which aims to establish paediatric reference intervals (RIs) for common and specialised laboratory tests. This is vital for accurate diagnosis and treatment of diseases in neonates and children, who are not mini-adults. The impact has been impressive, with haemostasis RIs distributed to many laboratories worldwide. I will continue to follow their work, especially on immunology tests and platelet flow cytometry because these tests are often specialised, and validated RIs will play a critical role in patient care. Further, given the importance of testing standardisation, their insights will also apply to my placement.

Thirdly, Dr Sharon Choo covered severe combined immunodeficiency (SCID) and haemophagocytic lymphohistiocytosis (HLH). Two SCID cases with opposite outcomes (death vs survival) were presented to illustrate that "early diagnosis is crucial". Next, she described the low lymphocyte count action plan in her hospital and new NPAAC requirements regarding high-risk pathology result reporting. This makes me think about how medical laboratory scientists (MLS) contribute to improved patient outcomes. As for HLH, the pathophysiology and genetic basis, together with relevant assays were discussed. This offered me an excellent glimpse into the complex and sophisticated testing inside a diagnostic immunology laboratory, which is fascinating.

Taken together, these presentations expanded my understanding of immunology, an exciting discipline that I am strongly passionate about. Moreover, these speakers exemplify the scientific curiosity that is required of a MLS, because basic science has progressed rapidly and advances are translated into clinical practice. As such, I will work hard and challenge myself to become a scientist of such a high calibre one day.

Overall, the conference has, as suggested by the theme, enabled me to "revision for the future". Therefore, I am extremely grateful to AIMS for the grant, and I would also like to take this opportunity to thank my mentors Dr Sheree Bailey and Thi Hoang for their encouragement throughout.

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3 APACE credits per set of questions will be awarded if at least 8 out of 10 questions are answered correctly.
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Journal-based CPD No. 80
Page 1 of 1

Questions relating to the article *'A comparison of column agglutination and solid phase red cell adherence technologies for red cell antibody detection '* at page 115 of this issue.

1.	Generally, clinically significant antibodies are those that react at 30°C, are IgG in nature, and are able to mediate destruction of transfused red cells.	True/False
2.	Since 1954, based upon the American Association of Blood Banks (AABB) recommendation, crossmatches could be omitted provided the antibody screen was negative.	True/False
3.	The tube agglutination technique has its limitations including time taken to perform, and unstable end points making grading and interpretation difficult.	True/False
4.	The following antibodies were detected in samples with positive results: anti-D, anti-C, anti-E, anti-e, anti- c, anti- K, anti-k, anti-Ch, anti-Fya, anti-Jka, anti-Jkb, anti-Lea, anti-Leb, anti-M and anti-Rg.	True/False
5.	The Ortho-Clinical Diagnostics Autovue Innova is not a fully automated system used as the routine analyser at Royal Perth Hospital and Fiona Stanley Hospital.	True/False
6.	The principle of the test is column agglutination technology with the use of glass beads.	True/False
7.	The Immucor Echo SPCRA test consists of a monolayer of red cell membranes bound to the surface of a microtitre plate.	True/False
8.	The principle of the Bio-Rad system is column agglutination technology using six column cards containing a gel matrix with anti-human globulin or other antisera depending on the test requirements.	True/False
9.	The Grifols system, also using the principle of column agglutination technology, is comprised of a gel matrix with added anti-human globulin contained in an eight column card.	True/False
10.	Tetrachoric correlation co-efficient and other metrics such as sensitivity and specificity were calculated using Microsoft Excel software for Windows 10.	True/False

Name: _____

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Journal-based CPD No. 81
Page 1 of 1

Questions relating to the article *'The effectiveness of ultrasound guided fine needle aspiration cytology in detecting head and neck mass pathology'* at page 121 of this issue.

1.	The ultrasound guided fine needle aspiration cytology (USFNAC) is an important test for diagnosis of benign and malignant tumours in head and neck.	True/False
2.	Head and neck masses represent a wide differential diagnosis from inflammation and reactive hyperplasia to benign and malignant neoplasia.	True/False
3.	Primary malignant lesions including lymphoma, adenoid cystic carcinoma, synovial sarcoma, spindle cell carcinoma and mucoepidermoid carcinoma have been reported in up to 14% of cases.	True/False
4.	Fine needle aspiration cytology (FNAC) is a simple test, associated with minimal trauma and complications and results can be rapidly available.	True/False
5.	Ultrasound is a non-invasive procedure and can be very useful in the diagnosis of head and neck masses.	True/False
6.	The nature, echogenicity, edge, presence of calcification and number of head and neck masses can be assessed with ultrasound.	True/False
7.	Other findings suggestive of malignancy includes irregular margin, posterior shadowing and heterogeneous internal echogenicity.	True/False
8.	Recommendations vary regarding the use of ultrasound in improving the accuracy of fine needle aspiration.	True/False
9.	The proportion of benign head and neck masses detected by conventional FNAC and USFNAC was 78.3% and 70.0%, respectively.	True/False
10.	Most of the head and neck masses were from the thyroid gland, followed by the neck nodes and salivary glands.	True/False

Name: _____

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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

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

However, with limited opportunities for CPD due to COVID-19 restrictions, the time frame has been extended to three years. Also, the limits for on-line learning, webinars, structured and journal reading have been removed.

These changes will remain in place until CPD activities return to normal across the country.

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

BOOKS FOR REVIEW

Following is a list of books available for review by resource consultants and members of the Institute with particular expertise in the field. The reviewer is invited to retain the complimentary copy of the book once the review is received.

As per our agreement with the book publishing companies, complimentary books are submitted to the Institute provided that all reviews are published in the Australian Journal of Medical Science. These reviews must be of a high quality as buying decisions and the reputation of the book and author are important considerations.

Books not requested will be allocated at discretion of the Editors for the Australian Journal of Medical Science. Reviews should be 300 to 700 words depending on the volume of the book. Time limit for return of review is **six** weeks.

Please send your request to: Australian Institute of Medical Scientists PO Box 1911 Milton Qld 4064
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1. **Bifidobacteria: Genomics & Molecular Aspects** edited by B. Mayo, & D. Van Sinderen. Caister Academic Press. xii + 260 pages.

2. **Medicine and Sport Science Volume 55: Cytokines, Growth Mediators & Physical Activity in Children during Puberty** edited by J. Jurimae, A.P. Hills & T. Jurimae. Karger. viii+178 pages.

3. **Digestive Diseases The Keys to IBD 2010: Treatment, Diagnosis & Pathophysiology.** Edited by G. Rogler & W. Sandborn. Karger. 188 pages.

4. **Else Kröner-Fresenuis Symposia Volume 1: Molecular Mechanisms of Adult Stem Cell Aging** edited by K.L. Rudolph. Karger. xii+108 pages**.**

5. **Endocrine Development Volume 24: Hormone Resistance and Hypersensitivity** edited by M. Maghnie, S. Loche, M. Cappa, L. Ghizzoni & R. Lorini. Karger. viii + 160 pages.

6. **Frontiers of Hormone Research Volume 41: Endocrine Tumor Syndromes and Their Genetics** edited by C.A Stratakis. Karger. xii + 187 pages.

7. **Frontiers of Hormone Research Volume 39: Kallmann Syndrome & Hypogonadotropic Hypogonadism** edited by R. Quinton. Karger. x+174 pages.

8. **Generic: The Unbranding of Modern Medicine** by Jeremy A. Greene. John Hopkins University Press. 368 pages.

9. **Human Pathogenic Fungi: Molecular Biology and Pathogenic Mechanisms** edited by Derek J. Sullivan & Gary P. Moran, Caister Academic Press. x + 342 pages.

10. **Internal Medicine: A Doctor's Stories** by Terrence Holt. Black Inc. 273 pages.

11. **Intolerant Bodies: A Short History of Autoimmunity** by Warwick Anderson and & Ian R. Mackay. John Hopkins University Press. 250 pages

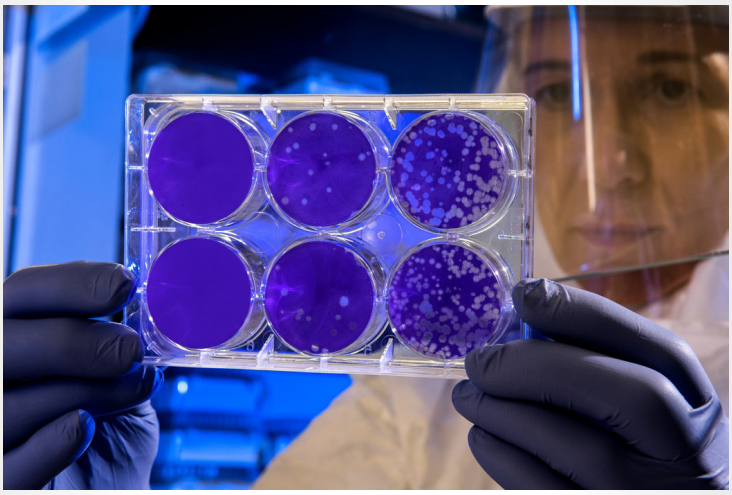
12. **Lyme disease and relapsing fever spirochetes** edited by Justin D. Radolf and D. Scott Samuels. Caiser Academic Press. 760 pages

13. **More Than Hot: A Short History of Fever** by Christopher Hamlin. John Hopkins University Press. 400 pages.

14. **Pediatric and Adolescent Medicine Volume 19: Metabolic Syndrome and Obsesity in Childhood and Adolescence** edited by W. Kiess, M. Wabitsch, C. Maffeis, A.M. Sharma. Karger. x + 202 pages.

15. **Phage Therapy - Current Research and Applications** edited by Jan Borysowski, Ryszard Miedzybrodzki & Andrzej Gorski. Caister Academic Press. 368 pages.

16. **Shigella: Molecular and Cellular Biology** edited by William D. Picking & Wendy L. Picking. Caister Academic Press. 280 pages.



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AUSTRALIAN JOURNAL OF MEDICAL SCIENCE

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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.

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

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- Legends for illustrations.

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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.

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- 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.

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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.

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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.

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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.

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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) …

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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.

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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.

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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:

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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.

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

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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.]

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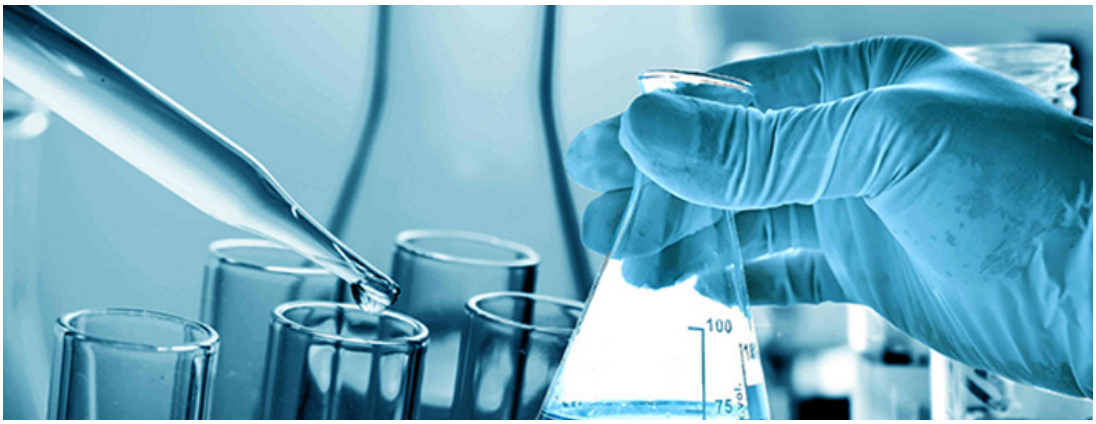

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