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NZIMLS

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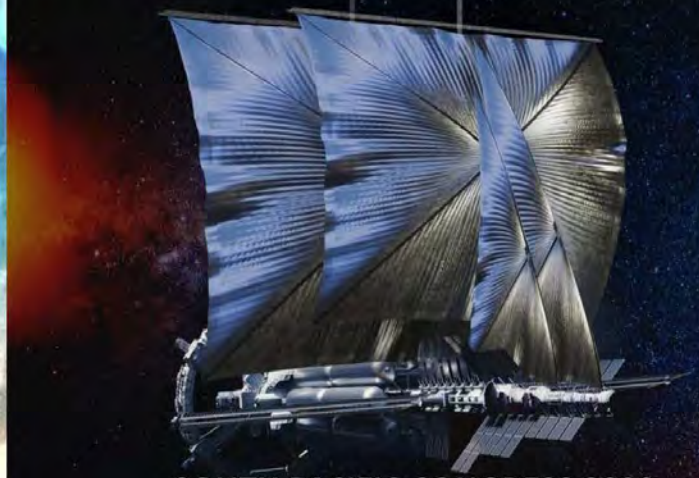

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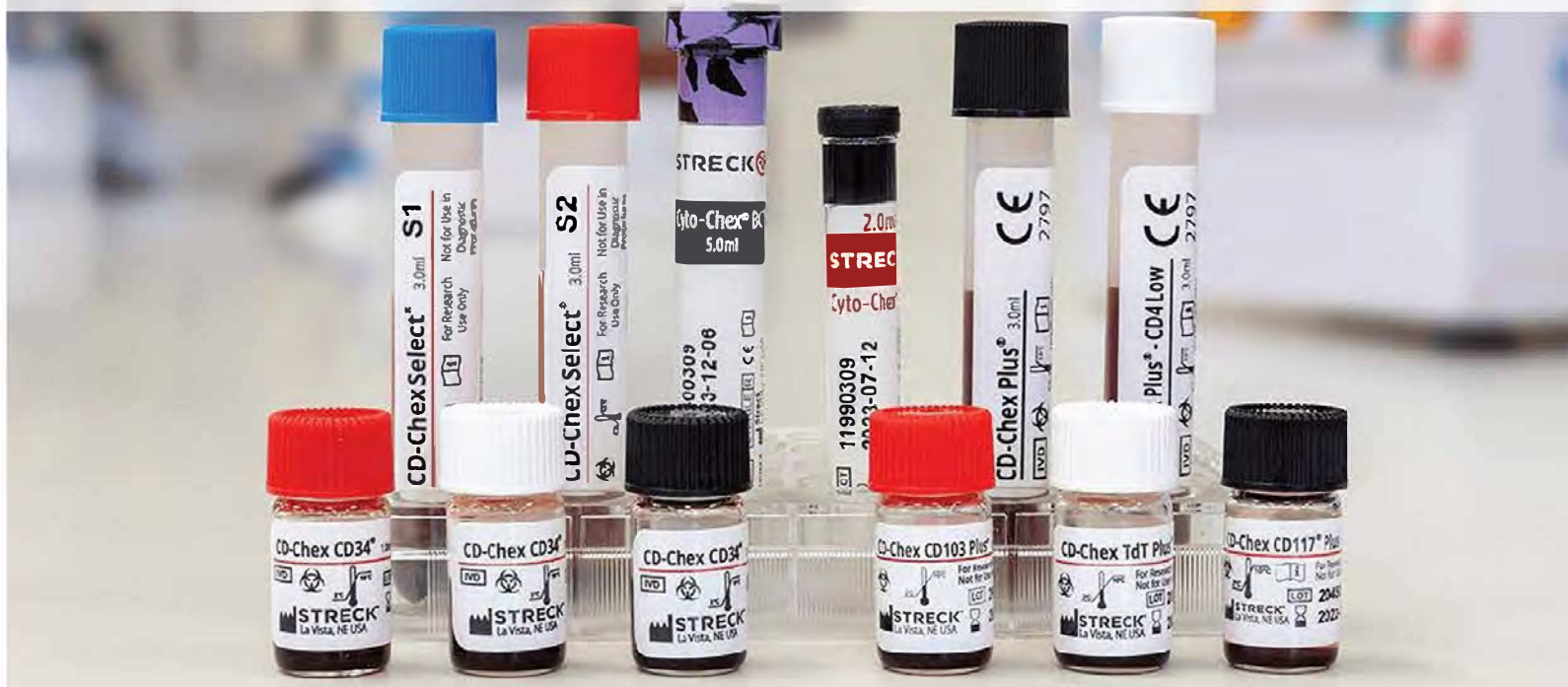

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New Zealand Journal of

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In this issue

President of the NZIMLS, Terry Taylor, shines a light on the premeditated strategy of some managers to employ BMLSc graduates as medical laboratory technicians rather than the scopes that they have trained hard for. This Issue's editorial advocates for solidarity within the profession for these new graduates and for those members who work in a technically advanced role but are frequently unrecognised or unrewarded. These practices are detrimental to the profession and have been highlighted to the top level of the Ministry of Health.

Cervical cancer is one of the leading causes of death among women worldwide including the Pacific Island Countries and Territories (PICT). Sharita Meharry and her colleagues at Auckland University of Technology and Fiji National University discuss the incidence of cervical cancer in the Pacific and how the high mortality rates can be related to the availability of resources and the barriers that exist in current screening programmes. They offer recommendations to local authorities, the Ministry of Health and NGOs to consider for a global elimination strategy, which includes; encouraging education, building regional partnerships to reduce the burden of cervical cancer and improving outcomes for women in the Pacific region.

Water Soluble Vitamins (WSV) play a crucial role in body growth, skin, nerves, red blood cells and heart function. Although deficiency is uncommon, there are no New Zealand specific reference intervals and overseas intervals may not be appropriate. Reza Nemati and colleagues from Canterbury Health Laboratories, University of Otago and Southern Community Laboratories in New Zealand review and summarise analytical and clinical aspects of water-soluble vitamins and advocate for more reliable, accurate and standardised measurements for better diagnosis of these vitamin deficiencies.

D-dimer is a small protein fragment derived from plasmin-mediated degeneration of cross-linked fibrin. The presence of D -dimer is an indication of the activation of intravascular coagulation and subsequent fibrinolysis (enzymatic breakdown of fibrin in blood clots). The D-dimer test is one of the most frequently requested coagulation tests used to exclude venous thromboembolisms, however there are many preanalytical variables that can affect the performance of the assay. Yii Sen Wee from the Haematology Department at Southern Community Laboratories and team conducted a study using INNOVANCE® D-dimer assay to determine if stability time could be extended from 4 to 6 or 8 hours at room temperature to account for late arrivals and added test requests in the clinical laboratories. They concluded that the assay could be reliably tested at 8 hours post-sample collection for both centrifuged and uncentrifuged samples at room temperature.

Holly Perry and colleagues at Auckland University of Technology and University of Otago conducted a study to compare tube and column agglutination technique (CAT) for monitoring quantities of blood group antibody anti-D by titration. It is important to monitor the quantity of anti-D in mothers throughout an immunized pregnancy. This antibody has the capacity to cause severe clinical Haemolytic disease of the fetus and new-born (HDFN), and higher levels often correlate with greater disease severity. All laboratory methods such as titration, continuous flow analysis and flow cytometry suffer from technical variation leading to a lack of reproducibility within and between laboratories. A trigger titre of 32 is used in tube methods but there is no trigger titre established for column methods. Investigation of sensitivity data between CAT and tube titration of anti-D found that the trigger titre for monitoring fetus risk should be reviewed using the more sensitive and reproducible automated CAT method.

Certain serovars of *Salmonella* show a higher predilection for causing bacteraemia in HIV infected persons and differ with geographic location. Individuals with human immunodeficiency virus (HIV) infection are at greater risk of bacteraemia due to their defective immune status. Dora Udoh and colleagues in Nigeria undertook a study to characterise serovars of *Salmonella* to determine the relationship of the infection with the low $CD4^+$ cell counts of patients. The prevalence of *Salmonella* associated bacteraemia was significantly higher in HIV individuals compared to non-HIV subjects, however regardless of HIV/AIDS sero-status, subjects with CD4+ counts below 200 counts/μL had the highest rates of *Salmonella* associated bacteraemia.

Dr Idemudia and medical laboratory science colleagues at the University of Benin in Nigeria report on dyslipidaemia in HIV infections with an increased risk of 1.5 - 2 times higher of cardiovascular abnormalities as a result of chronic inflammation in HIV positive people compared to uninfected people. Selected surrogate markers of inflammation and cardiovascular disease were evaluated in participants compared with controls and the team observed that HAART-naive HIV patients had a higher risk of developing cardiovascular disease among the groups in their study, consistent with other studies findings.

Our Emeritus Editor Rob Siebers writes to the Journal comparing article citations of female verses male authors. Under-citation can have serious consequences to promotion for female scholars. The Scopus database was searched and Rob reports a higher mean number of citations to male-authored articles (3.34) compared that of female authored articles (2.23) in the New Zealand Journal of Medical Laboratory Science and is consistent with a similar study published in *Nature Physics*.

[As Editor, I am inspired by this particular issue where four of the six original and review articles have female 1st author or senior corresponding authors.]

We have our regular features, the Science Digest, Pacific Way and two book reviews from Michael Legge, as well as an exciting competition announcement for our new Journal cover for any aspiring New Zealand artists.

Lisa Cambridge, Editor

EDITORIAL

When is a scientist not a scientist?

Terry Taylor

Our new medical laboratory science graduates have studied for four long years including their clinical placements to gain their BMLSc qualifications. The world is theoretically their oyster and to see the excitement in the graduating practitioners and the immense pride their families show is something I have treasured in my professional leadership role.

What I have not enjoyed is the continual and premeditated contempt that some of our managers have for these enthusiastic but often naïve graduates. It is simply not acceptable for any manager who sees fit to take advantage of the keenness of BMLSc graduates and employ them as medical laboratory technicians in a blatant 'cost cutting' move. Whilst everyone understands that there are dedicated technician positions advertised, it is the view of the NZIMLS (and the then Minister of Health, Chief of Allied Health, and Te Whatu Ora leadership) that this needs to be made clear to the new graduates and is not taken advantage of by employers. While we have such well-documented scientist shortages (1,2,3), it is completely short-sighted not to incorporate the BMLSc graduate workforce into the scopes they are trained for.

Just for context after correspondence and a meeting with the Health Minister, the Hon Andrew Little, the NZIMLS received a letter from the Minister on 16 February 2022 stating the following; "I understand the Ministry of Health has made a commitment that all medical laboratory science graduates are guaranteed employment, however it is important that graduates are employed in a way that they can work at the top of their scope. I understand that the Chief of Allied Health Professions, Dr Martin Chadwick has reached out to the Medical Sciences Council of New Zealand to provide better oversight and monitoring of the graduates to ensure their employment is appropriate. I am pleased that you [Terry Taylor] are also directly involved in this work".

I can assure the profession that this narrative is reciprocated through Te Whatu Ora through our NZIMLS discussions at the leadership level. Whilst I have never seen a policy or directive from any laboratory provider stating that they are changing the makeup of their workforce to a technician-based approach based on purely fiscal grounds, it is up to our professional members to stand up and callout any hint this is happening. This is particularly pertinent when the new graduate is being utilised in a technically advanced role but not appropriately recognised. As we have seen over the period of the pandemic increasing pressure and responsibility above what is reasonably expected has detrimental results at every level of the medical laboratory workforce.

Everyone needs to be aware that over the next two years, our pathology services are going to undergo a generational change in governance, strategy, and priority. It is an expectation that our specialist scientist workforce will have significant role extension opportunities with our expertise spilling across the entire health system. It is up to all members of the NZIMLS to ensure that we maintain the push for professional excellence and not allow degrading or abuse of what we have worked so hard to achieve over the past three years.

He aha mea nui o te ao, he tangata, he tangata, he tangata.
'What is the most important thing in the world? It is people, it is people, it is people'.

AUTHOR INFORMATION

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Rob Siebers Journal Prize

The NZIMLS Council has approved an annual Journal prize to the value of NZ$1,500 for the best peer-reviewed article published by NZIMLS members in the Journal during the calendar year. The article can be a review article, original article, case study, research letter or technical communication. Excluded are Fellowship dissertations.

Many studies are presented at the Annual Scientific Meeting, SIG meetings, and the North and South Island Seminars, yet are rarely submitted to the Journal for wider dissemination to the profession. Consider submitting your presentation to the Journal. If accepted, you are in consideration for the Rob Siebers Journal Prize and will also earn you valuable CPD points.

Please contact the Editor or any Editorial Board Member for advice and help. Contact details are on the NZIMLS web site (www.nzimls.org.nz) as are instructions to authors.

All articles published during the calendar year (March, June and November issues) will be considered. The Editor, Deputy Editors and the President of the NZIMLS, who themselves are ineligible, will judge all eligible articles in December. Their decision will be final and no correspondence will be entered into.

The inaugural winner of the Rob Siebers Journal prize for 2022 is Rebecca Busch for her article *Immunotherapy induced cerebral vasculitis,* published in Vol. 76. No. 3, November 2022.

REVIEW ARTICLE

Cervical cancer screening in the Pacific Island countries: an overview of current management for therapy

Shamal S Chand, Atlesh N Sudhakar and Sharita Meharry

ABSTRACT

Cervical cancer is a common form of cancer in women worldwide including the Pacific Island Countries and Territories (PICT's). Mortality rates with cervical cancer are high in the PICTs and can be related to a number of factors including lack of available resources and cultural acceptance of screening, which contribute to this high rate. Cervical cancer screening methods have evolved from cell morphology observations to the more specialized techniques of molecular testing. High-risk Human Papilloma Viruis (HPV) genotyping and liquid-based cytology are the most common methods and have been "gold standard" tools used in cervical screening for some time now. However, in the Pacific Island Countries and Territories (PICTs), there is great disparity in the use of such methods. These disparities in testing have seen an increase in cervical cancer morbidity and mortality rates.

Here, we consider the factors necessary to develop a low-cost effective and acceptable cervical cancer screening programme made accessible to all women in the Pacific Islands and thereby decrease the mortality rates and improving outcomes for women in the PICTS.

Keywords: Cervical cancer, Pacific Islands, Human papilloma virus (HPV).

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INTRODUCTION

Cervical cancer is one of the leading causes of death among women in the developing countries (2). According to a report from Global Cancer Observatory (GLOBOCAN), in 2018, 311,000 women died from the disease with a global incidence of 570,000 cases. However, more than 80% of the total deaths were seen mainly in low-resource countries with a lack of organised cervical screening programs (3). Cervical malignancies have been strongly linked to persistent infection with high-risk human papillomavirus (HPV). However, certain cofactors, are essential in the progression of the disease, for example smoking, high parity, long-term usage of hormonal contraceptive and immunosuppressants (4, 5).

The incidence and mortality from cervical cancer in the Pacific Island Countries (PICTs) are equally alarming. The PICTs is regarded as a developing country, that is populated by a combination of Micronesians, Melanesians and Polynesians (Figure 1.) (6).

Melanesia is one of the regions with the highest cervical cancer incidence and mortality in the world (Figure 2), with an age-standardised incidence rate of 27.7 cases per 100,000 and a mortality rate of 19 per 100,000 population (7). According to the International Agency for Research on Cancer (IARC), the highest rates of cervical cancer incidence and mortality in PICTs (per 100,000 population) are seen in Papua New (29.1 and 19.8), Fiji (25.1 and 19.7), Solomon Islands (22.6 and 16.0) and Vanuatu (17 and 10.6) respectively. Similarly, Micronesia and Polynesia, except for New Zealand, demonstrate an alarming representation of this disease (Figure 2.) (3).

Whilst HPV vaccination has been used as a primary prevention strategy in the control of cervical cancer, screening programmes associated with cervical cancer, strategies such as cytology based screening, visual inspection with acetic acid (VIA) and HPV deoxyribose nucleic acid (DNA) testing also play a crucial role in reducing cervical cancer incidence and mortality (8). Unfortunately, screening in most of the Pacific countries is not routinely available. Where available, the screening strategy differ due to various contextual factors such as the availability of experienced health personnel, equipment, health facilities and sociocultural issues (9).

It is essential to identify the loopholes in screening efforts and formulate strategies in the fight against cervical cancer in a resource-constrained country such as the PICs. This article will focus on some areas that need evaluation such as effectiveness of the current cervical screening program, barriers and facilitators of the cervical screening program and the current survival rate of women diagnosed with cervical cancer.

Figure 1. Map of ocean based on Geoscheme M49 coding *(Retrieved from United Nations Statistics Division)*

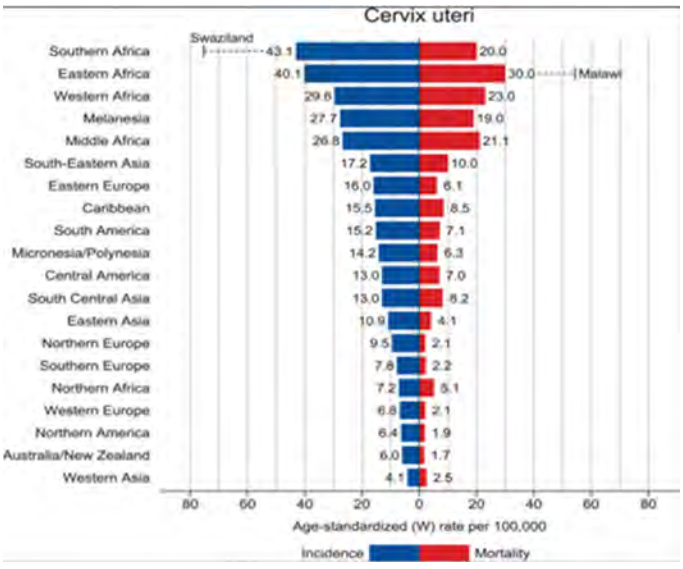

Figure 2. Estimate of incidence and mortality of cervical cancer in 2018 *(Retrieved from GLOBOCAM cancer statistics)*

MATERIALS AND METHODS

This is an overview of current management and therapy of cervical cancer in the Pacific Island countries. No materials or personal interviews formed part of this overview. For the purpose of this work the authors surveyed the current regimes relating to the management of cervical cancer in the Pacific Island countries based on current HPV screening programmes and the literature. The results of the survey are presented in the form of an overview with some suggested recommendations relating to improving outcomes for screening women for HPV in the Pacific.

DISCUSSION

Current Cervical Screening Programme in Pacific Island Countries

Cervical cancer screening, as a secondary prevention tool, helps to identify the pre-cancerous stage of cervical malignancies. Therefore, a well organised cervical cancer screening programme helps to reduce cervical cancer burden. For instance, the National Cervical Screening Program in Australia has halved its cervical cancer mortality, and incidence since its establishment in 1991 (10). Similar results were seen in New Zealand after implementing the population-based screening programs in the 1990s. The population-based screening identifies woman 25 to 70 years who have been sexually active and encourage them to attend screening through invitation (11). However, disparities in access to screening services among different ethnic groups still exist in New Zealand. Nevertheless, the New Zealand Ministry of Health has developed a cancer action strategic plan to reduce equity gaps by introducing self-sampling and HPV DNA testing as a primary screening tool which commenced in the year 2021 (12).

In contrast, other PICT's such as Fiji, Papua New Guinea (PNG), Nauru Federated States of Micronesia (FSM), Kiribati, Marshall, Palau, and Cook Island has an opportunistic cervical screening program based on individuals' decision to be screened which affects the pap smear coverage and cervical cancer incidence (7). This is demonstrated with opportunistic screening in Malaysia (although not in PICT) where the Pap smear coverage dropped from 74.5% in 1996 to 59.7% in 2006, and the mortality rate increased from 0.29% to 0.41% respectively (13).

Similar results were seen in Palau following the implementation of screening services in 1997, where the overall cytology-based Pap smear tests decreased from 1647 in 2004 to 527 in 2013 (14). These results indicates the need for screening strategies in the PICTs to be revised. Additionally, countries with no effective screening programs, such as Solomon Islands, Vanuatu, Tonga, and Samoa, require a collaborative regional approach to address the issue (3). Similarly, with the exception of New Zealand and Australia, none of the PICTs have a well-structured quality assurance program to supervise or monitor the cervical screening process (4).

Primary Screening Methods Used in the PICTs

There are different types of cervical screening methods used in PICTs, such as: cytology-based screening, visual inspection with acetic acid (VIA) and HPV DNA testing (4). The most common form of the primary screening in the PICTs is cytology-based Pap smear screening. However, VIA is also introduced as a co-test to cytology-based screening in countries such as Fiji, FSM, Kiribati, and Marshall, whereas HPV DNA testing is anticipated as an adjunct to cytology in Palau (4, 15).

The Pap smear test involves collecting exfoliated cells from the squamocolumnar junction of the cervix, which are transferred onto the slides, stained, and examined under a microscope for cell changes. However, these techniques require technical expertise for the reporting of the results (16). The Pap smear technique has been very successful in countries such as Australia, New Zealand, and other developed countries. However, due to a limited number of cytologists in the PICTs, the samples are sent elsewhere for reporting, causing delays in the turnaround time (17). For example, in Palau, the samples are sent to Philippines for reporting, which takes several weeks for results to be finalised.

In contrast, VIA technique is a cost-effective and efficient method, also referred to as "see and treat" approach which involves applying acetic acid on the cervix to identify the presence of abnormal lesions (15). The World Health Organisation (WHO) has recommended the use of VIA to prevent cervical cancer in a low resource setting. A study conducted by Fong et al. (2014) in Fiji, demonstrated that VIA and cryotherapy have been effective and acceptable among the Fijian population. However, VIA method has high false-negative results, whereby high-grade lesions can be easily missed (18). A study conducted in PNG compared the VIA with HPV DNA testing demonstrated that only 47% of high-grade diseases were detected using VIA in comparison to 92% by HPV DNA test (19). Whilst VIA technique has higher sensitivity, it is less specific in the detection of low and high-grade lesions when compared with Pap smear technique. Vahedpoor et al. (2019) reported that using VIA along with Pap smear increases the sensitivity up to 97.3% in detecting low- and high-grade lesions. Therefore, countries such as Kiribati, Marshall Islands and FSM have incorporated VIA in conjunction with cytology as the tools for detection(4).

The HPV DNA method detects the presence or absence of the human papilloma virus in the vaginal or cervical smears. While some studies indicate that HPV testing is costly and adds an extra processing step when used as a triage test to cervical cytology, other cost-effective analyses support the implementation of HPV as a primary screening tool. A study conducted in Thailand demonstrated a higher detection rate of CIN 2 lesion (1520 women per 100,000) using the HPV test in comparison to Liquid-based Pap test (1013 women per 100,000).

Another study in Papua New Guinea proved that the HPV DNA testing was cost-effective and demonstrated sufficient accuracy with self-sampling (21). Similar recommendations were made from a study conducted amongst Samoan population and the emphasis on self-sampling technique in this study could potentially remove the cultural barriers associated with pelvic examination currently faced by Pacific women (21). In addition, a clinical control trial in Mexico demonstrated higher acceptability of self-collected testing (98%) in comparison to Pap screening (89%) (22). Therefore, small island nations where cytological tests are not available, or VIA had poor results, HPV DNA testing could be a promising approach with the support from outside donor agencies.

Screening Age and Screening Interval

The screening age and the screening interval vary across different countries in the Pacific region. For example, at the Fiji School of Medicine, the screening age for women who have been sexually active is between 25 to 49 years while in Fiji, women of 25-60 years are screened for cervical cancer. In contrast, Marshall Islands, Palau and Kiribati, perform cervical screening for sexually active female which begins as early as 21 years of age. However, there are no evidence that screening from age 20-24 reduce cervical cancer, thus screening women who are under the age of 25 years, can lead to over diagnosis, mistreatment, and increased risk of infertility (23).

The American Cancer Society has similar recommendation on screening intervals as the PICTs. For example, a primary HPV test is done every five years, whereas the Pap test alone is done every three years (24). When low grade or high-grade abnormality is detected, the tests to be repeated at shorter intervals. For example, in New Zealand, when low-grade abnormality is detected at cytology screening, the women will undergo a reflex (cytology and HPV DNA) testing, and if HPV DNA is negative, the cytology test is repeated in 12 months (11). A similar approach is seen in Fiji, Palau, and FSM, whereby patients with abnormal pap smear must repeat screening in 12 months for effective management (15).

The primary concern for Pacific women is the follow-up rate upon the detection of abnormal results. A study conducted by

Foliaki et al. (2014) in Fiji showed that only two out of 13 women with abnormal Pap smear test, attended follow up health clinics. Again, women in the PICTs face specific challenges to access health care facilities which can be a significant factor contributing to the low follow-up rate. Pacific women also face specific barriers that need to be addressed to improve the engagement of women in the cervical screening process.

Barriers of Cervical Cancer Screening

While barriers to cervical cancer screening are multifaceted, some of the common themes highlighted in the literature include:

Cultural Beliefs and Attitudes

The culture across all PICTs is very similar in terms of how body parts are treated. In general, it is considered that the lower part of the body particularly the genitalia area is a sacred part of the body. Discussions surrounding sex and sex related topics are "taboo". As a result of this, the majority of the Pacific women feel uncomfortable discussing their reproductive health with the health care providers, especially if the health care worker is a male (26). In addition, women undergoing cervical screening in PICTs are viewed as being associated with promiscuous behaviour, and to prevent themselves from discrimination and stigma, these women do not engage in the screening process (9, 27). Similarly, some Pacific women have competing priorities and responsibilities to their families and may not see the need for screening being asymptomatic (26). However, culturally appropriate and an effective cervical screening program should overcome the cultural barriers and attitudes of the Pacific women towards cervical cancer.

Knowledge and Education

Most women in the PICTs have limited knowledge relating to HPV and cervical cancer in general because of lack of awareness and education. Naidu et al. (2015) reported that 72% of Fijian women in three rural settlements did not know about cervical cancer, while 80% had no idea of the risk factors. However, comprehensive research in the different provinces of the PICTs should be conducted to access the level of knowledge of these women on the topic of cervical cancer. A similar study by DiStefano et al. (2012) revealed that Chamorro and Tongan communities in California had minimal knowledge of HPV. Thus, to increase the knowledge among people in the Pacific community, a culturally tailored and language-specific programs will engage more women in cervical screening.

Fear and Health Care Experience

Some women reveal a fear of possible cancer diagnosis as a barrier to screening, and others complain about the fear of pain and discomfort regarding the procedure (27). Similarly, women with previous bad experiences with health care services are negatively influenced to participate in cervical cancer screening. These experiences include long waiting hours, language barrier and inadequate information communicated to the patients (30). Fitzgerald (2018) further elaborated that the slow turnaround time of results in the PICTs adds to the disinterest for a "follow-up". It is therefore essential to identify the reasons for the low turnout in the Pacific women and to determine whether is it due to a lack of knowledge, negligence or because of poor health care service. Georgina et al. (2019) argued that effective communication is the strong factor in the PICTs that may drive women to take part in screening programs continuously.

Practical Issues

Several studies have discussed a range of "accessible to service" issues such as: transportation cost, taking time off from work, childcare and socioeconomic status, which prevent women from accessing screening services (9, 15, 28). Clarke (2019) suggested that to overcome these problems, services such as: after hour appointments should be made free in both general practitioner surgeries and health clinics, cover transportation costs. Hence, reaching out to communities to provide these services will increase the likelihood of participation. Additionally, women staying on the outer islands in the Pacific must travel long distance with the only mode of transport as a boat, horseback, or walking, which prevent them from attending clinics (15, 18). To overcome these barriers, population-based outreach programs will increase the participation of cervical screening.

Facilitators

Some of the factors that may remove the barriers to screening among Pacific women, addressed in this review include:

Awareness Through Education

Creating awareness of the disease will enhance knowledge among people. Both males and females should be informed of the importance of screening practices. According to Wong and Kawamoto (2010), when the husband has a better understanding of the disease, he will offer better support to his wife. While most of the families in the PICTs carry traditional and strong cultural values strongly, a tailored education supported by church and community leaders can be very beneficial.

In contrast, in communities with strong cultural taboos, culturally tailored programs may not be as effective. As stated by Mishra et al. (2009), Samoan women with culture-specific beliefs showed no increase in self-reported cervical screening. Therefore, this raises the question of whether creating awareness in a church setting is the appropriate place. Nevertheless, other means of creating awareness, such as radio, television and social media advertising could be an essential facilitator of engagement. A 12% increase in the screening coverage among Pacific women living in New Zealand was noted following 12 months of a culturally appropriate media campaign (33).

Health Service Delivery

Anaman-Torgbor et al. (2017) highlighted that if women are given the option to decide on the gender of the service provider, they will ultimately improve their participation. According to Wong and Kawamoto (2010), the likelihood of Pacific women attending the screening was greater when in the presence of a female provider. However, due to limited number of qualified health personnel in PICTs, it would be necessary to train nurses and other health care personnel to enhance the screening activities (15). Other than accessible health care services such as mobile clinics, outreach programs and extended hours on weekends are important facilitators that can be initiated in the PICTs. Nevertheless, encouragement from health care personnel is an essential factor that will assist in the facilitation of screening practices.

Effective Communications

By providing standardised information on tests, women will be better informed. The health care providers communication style carries a significant role in how women engage for the screening programme. A study by Foliaki and Matheson (2015) concluded that the Pacific women who have had positive experience with health care services were more likely to return for screening. Additionally, a reminder system similar to the one used in New Zealand which is lacking in the PICTs will help with engagement for screening (11).

The Survival Rate of Cervical Cancer Patients in the PICTs

The survival rates of cervical cancer patients are dependent on factors such as the stage of the disease, age of the patient, access to treatment facilities, availability of treatment modalities, and socioeconomic status. The clinical stage at presentation is a significant predictor of survival rate. For example, if diagnosed with stage 1 cervical cancer, the chances of a 5-year survival rate are more than 90%, while stages 3 and 4 have less than 20% survival rate (35). Although this may be true, survival rates are just estimates, and may vary in a particular person's situation due to certain factors such as diet and individuals' immunity to combat cancers.

In addition, the survival rate of patients differs across different countries (Table 1.). Some reasons for survival differences may include factors such as socioeconomic status, the viability of health facilities, and failure to follow up (Jayant et al., 2016).

The five-year survival rates in regions such as Korea (77.3%), Japan (71.4%) and China (67.6%) reported the highest, while India (59%), Malaysia (57.1%) and Thailand (53.9%) reported a lower 5-year survival rate (Huang et al (2022). These results reflect the differences in the effectiveness of screening programs and accessibility to high-quality service.

Table 1. Data on cervical cancer survival demonstrated in different studies conducted in developed and developing countries.

Country	Author/ Year	Number (n)	Outcome	Value
New Zealand	Patricia, Lynn (36)	Māori 138 Non-122	2-year survival rate (Age 40-59)	M -68% Non-M (88%)
Saudi Arabia	Nisreen and Khalid (37)	190	5-year survival	53.2%
India	Jayant, Thorat (35)	558	5-year survival	35-60%
Ethiopia	Wassie, Argaw (38)	634	5-year survival	38.62%

In a study conducted in Ethiopia, which has no effective screening programme, the five-year survival rate was as low as 38%. However, the authors in this study did not look at the specific cause of death, which overestimated the cervical cancer-related mortality (38). Similarly, a study in India revealed that the survival rate for five years varies between 35% to 60% in the different rural regions in India. The primary reasons emphasised by the authors in both the studies were lack of access to health clinics, late diagnosis, and lack of treatment facilities.

A similar trend is seen among Pacific women where they present to the hospital with an advanced stage of the disease which impacts their survival rate (28). A retrospective study in Palau demonstrated that the survival rate of women diagnosed with cervical, or breast cancer decreased from 58% to 39 % between the years (2004-2008) and (2009-2013) respectively (14). The result of these studies suggests that an ineffective screening program with the delayed intervention has a major impact on the survival rate of women with breast or cervical cancer.

Furthermore, the lack of current information on cervical cancer survival rate in the Pacific context makes it difficult to compare the finding with other studies. However, the results from various literature provide an insight on the survival rate in the PICTs, based on the similar challenges of limited treatments options, lack of access to health services and socioeconomic barriers faced by the Pacific women. Ethnicity and socioeconomic survival disparities has been discussed in several studies. Patricia et al. (2010) reported that the 2-year survival rate in the non-Māori population (88%) was significantly higher in comparison to the Māori population (68%). Also, Brewer et al. (2012) stated that the 5-year survival rate between Māori and Pacific people is as twice as lower than the non-Māori and non-Pacific people in New Zealand. Similarly, in the United States, the relative survival rates in white and black women are 71% and 58%, respectively (7). The inequalities in the survival rates of women were due to late-stage diagnosis. Therefore, equity issues in the Pacific women should be evaluated if the data on survival rate is made available.

The lack of available data in the Pacific context raises a question on the availability of cancer registries and management of patient's information. Therefore, updated cancer registries and future studies are necessary to investigate the survival rate in cervical cancer patients in PICT's. The local information will determine the effectiveness of cancer management while reflecting on the degree of awareness on screening, so that early diagnosis and treatment can be provided.

Primary Prevention Strategies of Cervical Cancer in the Pacific Island Countries

Because virtually all cases of cervical malignancies are attributable to HPV, vaccinating young females against the HPV could substantially reduce the cervical cancer burden in resource-limited countries. The WHO recommended vaccinating girls aged 9-13 years against HPV and screening women aged 30-49 years as a part of their "Best Buys" intervention in developing countries (40). However, the impact of vaccination depends on several factors that vary across different populations in the Pacific such as; vaccination type, age of vaccination, vaccination coverage, and the prevalence of age-specific HPV infection (41).

There are three types of vaccines available varying on the number of HPV types they target. The bivalent vaccines contain and target types 16, and 18 HPV whereas quadrivalent vaccines provides additional protection against types 6 and 11 that causes 90% of genital warts. The second-generation 9-valent vaccines target similar HPV types as quadrivalent vaccines as well as types 31,33,45,52 and 58 responsible for over 90% of cervical cancer (42). However, the choice of vaccines used in the Pacific region entirely depends on the cost, availability, and support from international organisation such as Global Alliance for Vaccine (GAVI), United Nations Children's Fund (UNICEF), and Centres for Disease Control (CDC). The current cost of full vaccination ranges from US$10 to more than US$100; but additional operational and delivery costs need careful analysis in the decision-making process. While procuring vaccines on their own is highly costly for individual countries, but through the regional approach of collective bargaining, and bulk procurement will ensure some cost reduction similar to what has been achieved in the Caribbean Island territories (43). Therefore, affordability and sustainability of vaccines in the Pacific region call for regionalisation and collaboration between national governments, global partners, and donors (44).

Moreover, the choice of vaccine delivery strategy plays a vital role in the effectiveness, affordability and equitability of the primary prevention of cervical cancer (44). The efficacy trials have demonstrated that vaccine is most cost-effective in the adolescent females between the ages of 9-14 who mount a more robust immune response in comparison to 16-23 years old individuals (45). While school enrolment in the 9-14 age group is high in the PICTs, school-based vaccination programs could be most suitable to increase coverage. However, other approaches such as community outreach vaccination programmes are needed for those girls not in school (e.g., migrants, street children, school dropouts). A National school-based campaign in Fiji initiated by the Global Alliances for Vaccine and Immunisation (GAVI) was successful with regards to community acceptance and vaccine uptake (8). However, the integration of sex education in the school curriculum is required in the Pacific region to raise awareness on other risk factors, such as high parity, smoking, and use of hormonal contraceptives responsible for cervical cancer progression (46).

Subsequently, many clinical trials have been conducted to determine the suitable number of doses of HPV vaccination. In developing countries, three doses of HPV vaccination are a logistical and economic barrier. However, the efficacy and high immunogenicity of vaccines suggest that even if administered less than three doses, the vaccines will retain long-term effectiveness (42). A trial in Costa Rica demonstrated that antibodies to HPV 16 and18 were stable for a four-year trial period after receiving only one or two doses of the vaccine (47). The surprising nature of this finding suggests that total costs will be reduced if only one dose of the vaccine is administered to girls in the resource-constrained setting (48).

National HPV vaccination programs have started in several PICTs (Fiji, Solomon Islands, New Caledonia, Cook Islands, Guam, Federated States of Micronesia, Marshall Islands, Kiribati, Wallis and Futuna Northern Mariana Islands and Palau) through significant donor agency support (49). However, HPV vaccination coverage in the majority of the PICTs is unknown or low with weak monitoring mechanisms and requires a robust system of continuous monitoring and evaluation of vaccine coverage to monitor the effectiveness of the HPV vaccination program (50).

RECOMMENDATIONS

Given that most of the screening is performed opportunistically in the PICTs may explain for the low participation rate of women, it is therefore recommended that population-based screening is implemented in the PICTs to increase coverage and cervical screening participation. Similarly, VIA screening should be introduced in a resource-constrained setting, with a recommendation that PICTs can implement VIA and cytology co-testing to increase sensitivity in detecting low- and high-grade lesions. Above all, HPV testing may revolutionise cervical screening in the developing countries. Local authorities such as the Ministry of Health, cancer societies and other non-government organisations (NGOs) should consider implementing HPV testing in the cervical cancer program at the earliest.

Moreover, policies and standard operating procedures will ensure quality results delivered to the patients in a timely manner. Additionally, training health care providers will enable the cervical screening process to be conducted efficiently and effectively. Similarly, health seminars on cervical cancer should be encouraged through increased publicity. Health practitioners should be encouraged to educate women on cervical health and the advantages for screening. Comparatively, health promotion activities should be culturally appropriate and delivered in collaboration with Pacific communities.

The screening age in the PICTs should be reviewed and standardised to 25 years as recommended by the National Cervical Screening Program Australia. The data on the survival rate of the patients could be a potential indicator of the effectiveness of current cervical cancer prevention strategies; therefore, policies on reporting cervical cancer surveillance data should be made compulsories for all PICTs.

Most importantly, an improved cancer surveillance system is recommended with an ongoing registration of records that include every woman's vaccination history, clinical records, appointments scheduled, and treatment administered. Leaders in the PICTs must continue to seek capacity building through regional partnership and prioritise their local cancer control agenda in line with the global elimination strategy of vaccinating 90% of girls by age 15, 70% screening coverage by the ages 35 to 45, treating 90% of precancerous lesions and managing 90% of invasive cervical cancer (51, 52).

CONCLUSION

The increasing burden of cervical cancer in the PICTs warrant a regional initiative in collaboration with local health authorities. This collaboration will assist for capacity building among local health care providers to improve the outcome of women in the PICTs with the disease. Additionally, there is a need for strengthening research capabilities in the Pacific region so that comprehensive data on cervical cancer epidemiology can be attained for developing effective strategies to improve the outcome of cervical cancer in women in the Pacific region.

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REVIEW ARTICLE

Water soluble vitamins: mechanism and metabolism. A narrative review

Reza Nemati, Christopher McEntyre, James Yeo, Ian Phillips, Bobby Li, Christine Leaver and Christiaan Sies

ABSTRACT

Vitamins are essential nutrients that are classified into two groups, fat soluble vitamins (FSVs) and water-soluble vitamins (WSV). WSVs include thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxal phosphate (B6), biotin (B7), folate (or folic acid) (B9), cobalamin (B12), and ascorbic acid (C). Although deficiency of WSVs is uncommon, it can still be seen among individuals with different disorders such as short bowel syndrome, chronic alcoholism, and malnourished or post bariatric surgery patients. Despite the use of advanced technologies such as chromatography and mass spectrometry to measure vitamins, there is a need for more consistent standardisation and reference intervals. Covariates such as requirement for sample collection, fasting and avoiding supplementation, require more work to assess their effects in the interpretation of vitamin results. The aim of this review is to highlight pre-analytical factors such as fasting and cessation of supplementation before sampling. There are no comprehensive guidelines found in the literature describing how long vitamin supplementation should be ceased before sampling, and further studies are warranted.

Keywords: water soluble vitamins, reference range, B vitamins, vitamin C, vitamin supplement, fasting and non-fasting vitamins.

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OVERVIEW

Vitamins play a crucial role in many biochemical mechanisms in the human body. Water soluble vitamins (WSVs) are found in a variety of foods such as fruits, vegetables and meat and play vital roles in body growth, skin, nerve, red blood cells (RBCs) and heart function (1). Although deficiency of WSVs is uncommon in New Zealand, it can be seen among malnourished people, such as: chronic alcohol abuse; strict veganism; and malabsorption syndromes. Unlike fat soluble vitamins (FSVs) where excess amounts of vitamins can be stored in the human body (2), WSVs are readily excreted in the urine and most do not have large stores. The structures of the biologically active forms of WSVs are shown in Figure 1.

WSVs are measured by immunoassay, high performance liquid chromatography (HPLC) and liquid chromatography tandem mass spectrometry (LC-MS/MS). HPLC and LC-MS/MS assays have been reported for vitamins B1, B2, and B6 (3, 4). Vitamins B9 and B12 are routinely measured on auto-analysers, while vitamins B3, B5 and B7 are not routinely measured in most clinical laboratories. This may be due to both analytical challenges and lack of clinical demand. Little information is available about the bio-accessibility and bioavailability of many WSV's. Currently, there are no New Zealand specific reference intervals for WSVs, and overseas reference intervals may not be appropriate.

In this review we provide an overview of WSVs and challenges for measurement and interpretation of results.

Vitamin B1 (Thiamine)

Introduction

Thiamine was described more than 4000 years ago in Egypt (5) and was the first member of the B complex to be discovered.

Chemistry and measurement

The main active form of thiamine is thiamine pyrophosphate (TPP) (6) (Figure 1). Thiamine is often measured using erythrocyte thiamine transketolase activity (ETKA) (6). However, due to the limited sensitivity and specificity of ETKA, HPLC and LC-MS assays are considered more reliable assays (3). TPP is usually measured in whole blood because thiamine is more concentrated in RBCs.

Indications for measurement

Thiamine testing can be considered in patients with suspected beriberi, particularly in the context of poor diet. Measurement of thiamine can help confirm deficiency in patients with poor intake. Patients with suspected thiamine deficiency should be treated with thiamine to prevent the development of Wernicke encephalopathy and Korsakoff psychosis. Thiamine testing can also be considered in patients with behavioural changes, ocular disorders, delirium, and encephalopathy (7), and in patients who undergo insulin therapy, due to thiamine deficiency lowering insulin synthesis and secretion (8).

Limitations of measurement

Systemic inflammation, variations in testing methods between laboratories, and the presence of hypoalbuminemia may interfere with the measurement of thiamine (9). Thiamine can degrade if samples are not light protected. Non-fasting and thiamine supplementation may falsely increase thiamine results.

Sources

High levels of thiamine are found in foods such as yeast, legumes, pork and brown rice. However, high temperature and modified pH can destroy thiamine (6).

Metabolism and action

Thiamine is mainly absorbed in the small intestine (6). Thiamine is dephosphorylated to facilitate entering the blood through an adenosine triphosphate (ATP) dependent pump (*i.e.*, active transport), then phosphorylated intracellularly to the active form. Thiamine is most commonly passed via active transport into to most cells and by passive diffusion to RBCs (6).

Thiamine has a 10–20-day half-life in the body. Therefore, it is necessary to maintain thiamine levels with continuous intake. Thiamine is mostly excreted in the urine and partially in bile (6). TPP is a crucial cofactor for enzymes involved in carbohydrate and branched-chain amino acid metabolism. In the Krebs cycle, TPP works as a catalyst to convert pyruvate to acetyl CoA.(6).

Deficiency

Thiamine deficiency may cause a reduction in carbohydrate metabolism, which can affect amino acid homeostasis leading to decreased formation of acetylcholine needed for neural function (10). Thiamine deficiency is mostly reported in people who consume polished rice, or milled white cereals, due to the refining process (6). Thiamine deficiency leads to beriberi, a neurological disorder which is related to the synthesis of glutamate and γ-aminobutyric acid as well as myelin sheath maintenance (6). Thiamine deficiency occurs in both children and adults. Infantile beriberi usually happens in babies who were born to a mother with thiamine deficiency with features such as cardiomegaly, tachycardia, and pulmonary hypertension (6). In older babies thiamine deficiency can be present as meningitis with no abnormalities on cerebrospinal fluid analysis (6). The main reason for thiamine deficiency in babies and new-borns is inadequate parenteral nutrition (6). In adults there are two types of beriberi. Wet beriberi which is characterised by signs of cardiac disorders such as cardiomegaly and heart failure. Dry beriberi is recognised by development of a symmetrical peripheral neuropathy classified through sensory and motor impairments (6).

Another neuropathic complication of thiamine deficiency is Wernicke-Korsakoff syndrome (WE). WE is often recognised by short-term impaired memory, confabulation (honest lying), nystagmus, ophthalmoplegia, ataxia, and confusion.

Toxicity
There are no reports of thiamine toxicity, due to the rapid clearance of excess thiamine from the kidneys (6).
Therapeutic application
The role of thiamine deficiency in diabetic nephropathy was proposed a long time ago, but is still controversial (11).

Vitamin B2 (Riboflavin)
Introduction
Vitamin B2 is a member of the flavin family with a critical role in a myriad of biochemical reactions.
Chemistry and measurement
Riboflavin is mostly present in the form of flavin-adenine dinucleotide (FAD) (6) (Figure 1). Flavins in food are derivatives of FAD, flavin mononucleotide (FMN) or free flavins. After ingestion and absorption of dietary riboflavin, FAD and FMN are hydrolysed into free riboflavin through gastric acid, proteolytic enzymes and bile salts (6). Free riboflavin binds to albumin and immunoglobulins (6) and is absorbed in the proximal small intestine. When Free riboflavin reaches cells of the liver, heart and kidney it is metabolised into FMN and FAD (6). Riboflavin is first phosphorylated to form FMN. FMN can be either further phosphorylated into FAD or become part of coenzyme flavin complex. Both reactions are ATP dependent.

Plasma and urine riboflavin measurement reflect recent dietary intake. Urinary riboflavin is primarily used as a test to determine dietary intake in a population for riboflavin deficiency (6). FMN has been introduced as a biomarker to predict outcomes after kidney transplantation (12).

Vitamin B2 is usually measured as the active form, FAD. FAD can be measured in serum or whole blood by HPLC-fluorescence directly without the need for derivatisation (13).
Indications for measurement
Riboflavin deficiency can be seen in people who consume few dairy products (*i.e.*, lactose intolerant people) (14). In addition, malabsorption syndromes, HIV, malignancy, and some inborn genetic defects (*e.g.*, acyl-coenzyme A (CoA) dehydrogenases) have been linked to riboflavin deficiency (15, 16). Medications such as tricyclic phenothiazine, barbiturates and chlorpromazine also affect measurement.

Non-fasting or taking a dietary supplement can falsely increase the blood level of vitamin B2.
Sources
Riboflavin is present in different foods such as milk, eggs, meats and green vegetables (17).
Metabolism and action
FAD is often combined with proteins to form flavoproteins. Flavoprotein is the most common form of riboflavin storage in the human body (6). Riboflavin is an essential cofactor in energy producing respiratory pathways such as Krebs cycle, beta-oxidation of fatty acids, as a catalyst in mitochondrial oxidative and reductive reactions and electron transporters (6).

The absorption of riboflavin is regulated by bile salt in two ways. Firstly, bile salts induce solubility and permeability of riboflavin in the small intestine. Secondly, bile salts assist gastric emptying and increase the dephosphorylation time of FMN and FAD to riboflavin in the small intestine; then, riboflavin is stored in the liver as FAD (16).
Deficiency
A deficiency of vitamin B2 results in a disorder called ariboflavinosis. Symptoms include sore throat, hyperaemia of pharyngeal mucous membranes, oedema of mucous membranes, cheilitis, stomatitis, glossitis, normocytic-normochromic anaemia, and seborrheic dermatitis (6). Whether these symptoms are due to riboflavin deficiency is sometimes unclear, and the condition can be confused with other WSV deficiencies (6).

The other condition that can lead to riboflavin deficiency is multiple acyl-CoA dehydrogenation deficiency (MADD), a genetic disorder that is usually manageable with riboflavin supplementation (18).
Toxicity
There are no adverse effects reported after high doses of riboflavin. This may be due to limited absorption in the intestine (6).
Therapeutic application
Riboflavin can be used to prevent migraines. Riboflavin has shown some promise in preventing Parkinson's disease, though more research is required to confirm these findings (19).

Vitamin B3 (Niacin)
Introduction
Vitamin B3 (nicotinic acid or niacin) (Fig. 1) is essential in the synthesis and metabolism of carbohydrates, fatty acids, and proteins. A deficiency of this vitamin results in a disease called pellagra. This condition can progress to both physical and mental deterioration if left untreated.
Chemistry and measurement
Niacin is rapidly taken up by a passive process in liver, kidney and RBCs. During metabolism of niacin dietary nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) are converted to nicotinamide and nicotinic acid for intestinal absorption by hydrolysis and microbial mediation and released through passive and facilitated diffusion. Then, they are returned to NAD and NADP form to be used in cellular functions (20). Many enzymatic redox reactions need NAD and NADP as a cofactor.

High levels of niacin metabolites, such as N-methyl nicotinamide are the indicators of sufficient concentrations of intracellular niacin (21). Niacin can be measured by urinary N-methyl nicotinamide or by measuring the erythrocyte NAD: NADP (ratio). Nevertheless, this test is not offered in many laboratories (6). Niacin can be measured in serum by HPLC using derivatisation and fluorometric detection (22), or using LC -MS methods (23).
Indications for measurement
High consumption of corn or corn meal can lead to niacin deficiency due to inadequate tryptophan, a precursor of niacin synthesis, in maize. For instance, niacin deficiency has been seen in resource-rich countries where people consume a lot of corn (24). Niacin measurement should also be considered for chronic alcoholics, people undergoing bariatric surgery, anorexia or malabsorption disorders (6).
Limitations of measurement
Non-fasting and niacin supplement can provide falsely elevate niacin results. Some medications for tuberculosis (e.g., isoniazid and pyrazinamide) can falsely reduce plasma levels (24).
Sources
High levels of niacin are found in plant and animal foods such as yeast, meats (especially liver), legumes and grains (6).
Metabolism and action
NAD and NADP are necessary for enzymes involved in redox reactions. For instance, NAD dependent enzymes work in oxidation of fatty acids and producing of NADH via glycolysis and Krebs cycle (6).
Deficiency
Deficiency of niacin is called pellagra or "raw skin" which is characterised by a photosensitive pigmented dermatitis, diarrhoea, and dementia, and eventually death (if not treated) (6).
Toxicity
Although toxicity of niacin is not normally problematic, high doses of niacin may lead to nausea, vomiting, pruritus, hives and liver dysfunction with elevation of serum aminotransferases (6).
Therapeutic application
Niacin is well-known as an antihyperlipidemic agent by reducing very low density lipoprotein (VLDL) as a result lower low density lipoprotein (LDL), thus increasing the level of high density lipoprotein (HDL) (6).

Vitamin B5 (Pantothenic acid)

Introduction

Pantothenic acid was discovered less than a century ago (25) and plays a crucial role to the metabolism of CoA and acyl-carrier-protein (26).

Chemistry and measurement

Pantothenic acid got its name from a Greek word meaning 'everywhere' because it is found in nearly all foodstuffs (25). Due to the wide availability of pantothenic acid, deficiencies are uncommon and there is low interest in measuring it clinically.

Different methods that have been used to quantify pantothenic acid include: microbiological assays; radioimmunoassay; and HPLC (27). In addition, measurement of pantothenic acid in different sample matrices can produce different results (6). The ideal sample for pantothenic acid measurement is urine because urinary pantothenic acid is well-correlated to dietary intake (6). Measurement of pantothenic acid with LC-MS/MS is considered the "gold standard" (28).

Indications for measurement

It is generally unnecessary to measure pantothenic acid in a clinical setting (29). However, measurement might be considered in malnourished people (30).

Limitations of measurement

Literature regarding the interferences of pantothenic acid not readily available.

Sources

Pantothenic acid is found in many dietary sources and is also partially produced via gut bacteria (6). Pantothenic acid levels are high in egg yolk, chicken, animal organs such as liver and kidney, and vegetables such as broccoli and potatoes (6).

Metabolism and action

Pantothenic acid is a coenzyme involved in mitochondrial carbohydrate and lipid biosynthesis, the Krebs cycle, and in the biosynthesis of haemoglobin and cytochromes (26). CoA is hydrolysed in the small intestine by gut microbiota to form pantothenic acid. It is absorbed in the jejunum and secreted into the blood circulation through a sodium-dependent transport system. In the cell, pantothenic acid undergoes phosphorylation and re-conversion to CoA. Any excess of pantothenic acid is hydrolysed and excreted as cysteamine and pantothenate through the renal system (6).

Deficiency

Deficiency of pantothenic acid is characterised by paresthesia and dysesthesias or 'burning feet syndrome (6). Although pantothenic acid deficiency is a rare disorder in humans it can still be seen where chronic starvation occurs (6).

Toxicity

No toxicity has been reported as pantothenic acid is readily excreted by the kidneys.

Therapeutic application

Pantothenic acid is used in skin care, in particular for dry skin and acne (31).

Vitamin B6 (Pyridoxine)

Introduction

Vitamin B6 consists of pyridoxine, pyridoxamine, pyridoxal, and the phosphorylated derivatives of each of these compounds. Vitamin B6 plays a vital role in the metabolism of fats, carbohydrates and protein.

Chemistry and measurement

Vitamin B6 has seven forms: pyridoxine (PN), pyridoxal (PL), pyridoxamine (PM), pyridoxine 5′-phosphate (PNP), pyridoxal 5′-phosphate (PLP), pyridoxamine 5′-phosphate, and pyridoxine -5-β-D-glucoside (PNG). However, the biologically active form PLP is the form most commonly measured. (Figure 1.)

Methods available for measuring vitamin B6 include erythrocyte transaminase activity, urinary 4-pyridoxic acid and xanthurenic acid detection (6). However, HPLC and LC-MS are the most reliable methods to measure vitamin B6 as PLP. PLP can be measured by HPLC with fluorescence detection using either serum or whole blood (32). Derivatisation is required to increase assay sensitivity (33, 34).

Indications for measurement

Early markers of vitamin B6 deficiency include decreased plasma PLP and urinary 4-pyridoxic acid. Other signs of deficiency include reduced circulating lymphocytes and normocytic, microcytic, or sideroblastic anaemia (10). Plasma PLP is the best single indicator to reflect storage of vitamin B6 in tissues (10).

Limitations of measurement

Pre-analytical factors such as: not protecting samples from light; non-fasting samples; and multivitamin supplements may affect the result. As this vitamin is photosensitive, a non-light protected specimen may report a falsely-lowered measurement. Non-fasting samples falsely increased the vitamin B6 level. Vitamin B6 can be reduced in alcoholics, and those with coeliac disease.

Sources

Pyridoxine and pyridoxamine are high in plant foods while pyridoxal is most predominantly in animal-based foods. However, cooking, food processing and canning may reduce the availability of vitamin B6 by up to 50% (6).

Metabolism and action

PLP is the active form found in circulation. It acts as a coenzyme in more than 100 known enzymatic reactions (35). It is also involved in the synthesis of NAD from tryptophan and homocysteine metabolism. Pyridoxine is converted to PLP in the liver and catabolised to 4-pyridoxic acid and excreted in the urine.

Vitamin B6 is a crucial cofactor in transamination of amino acids, gluconeogenesis, decarboxylation of amino acids (*e.g.* tryptophan to niacin), haem synthesis, sphingolipid biosynthesis, neurotransmitter synthesis, immune function and transsulfuration of homocysteine to cystathionine and to cysteine; Vitamin B6 insufficiency can result in elevation of plasma homocysteine concentration and this can indirectly lead to atherosclerosis and vascular thromboembolism (6).

Deficiency

Although vitamin B6 deficiency is rare, marginal deficiency is relatively common. Vitamin B6 deficiency may lead to a variety of clinical abnormalities such as heart disease, lung cancer, diabetes mellitus, gastrointestinal and ocular disorders, and depression (17). Vitamin B6 deficiency usually occurs with other B-complex deficiencies. For instance, hypovitaminosis B6 may occur with riboflavin deficiency because riboflavin is needed to make PLP. Non-specific manifestations include dermatitis, glossitis, microcytic anaemia, irritability, confusion, depression, and possibly peripheral neuropathy and seizures (5, 6). Lack of vitamin B6 is reported in diseases such as asthma, diabetes, alcoholism, heart disease, pregnancy, breast cancer, Hodgkin lymphoma, and sickle-cell anaemia, cardiovascular diseases, colon and lung cancers, diabetes mellitus complications, digestive system disorders (5, 6). Moreover, medication such as isoniazid, penicillamine, hydralazine, and levodopa are associated with deficiency of vitamin B6 (5, 6). Infants are the most susceptible to vitamin B6 insufficiency, which can lead to epileptiform convulsions, dermatitis with cheilosis and glossitis (5).

Toxicity

Vitamin B6 toxicity is common and may present with peripheral neuropathy, dermatoses, photosensitivity, dizziness, and nausea.

Therapeutic application

Vitamin B6 is used for several complications such as reducing the severity of nausea in the first trimester, and to reduce the risk of dental decay in pregnant women (36, 37). Vitamin B6 is also prescribed for pre-menstrual syndrome (due to its same effect as serotonin and dopamine), carpal tunnel syndrome (due to its effect on the rising pain threshold through analgesic property) and other neurologic disorders (10).

Vitamin B7 (Biotin)

Introduction

Early in the 20th century, growth factors found in yeasts and called "Bios" were identified as *myo*-inositol, pantothenate, and biotin (6). Biotin was introduced as a vitamin when its deficiency

caused a clinical syndrome mediated by carboxylase enzymes (6). Biotin or vitamin B7, also called vitamin H, coenzyme R, factor S, factor W, vitamin B, and protective factor X due to its protective property of biotin to avoid dermatosis and hair loss in animals (38).

Chemistry and measurement

D-Biotin is the active form of biotin (Figure 1.); biocytin (biotin bound with lysine) is the end product of biotin-containing enzyme degradation and is convertible to biotin through the action of biotinidase (6). Biotin is mostly absorbed in the proximal small intestine and to a lesser degree in the caecum. It is either ingested as the biotin form or produced by gut bacteria. Unabsorbed biotin is excreted either in faeces or urine (6). Measurement of biotin is done by immunoassay and LC-MS/MS (39).

Indications for measurement

Biotin may interfere with several immunoassays including thyroid function tests and may give a pattern that mimics Graves' disease (6). In addition, high dose of biotin may interfere with troponin, ferritin, testosterone, B-type natriuretic peptide, digoxin, and progesterone (6). Avoidance of biotin supplement for 2 to 5 days before testing can eliminate the potential interference (39).

Limitations of measurement

Metabolism of biotin is increased in smokers, which may affect biotin measurement because smoking increases the catabolism of the vitamin (40)**.**

Sources

High levels of biotin are found in different plants (e.g., peanuts and soybeans), liver and egg yolk (6). However, egg yolk is the best source of biotin.

Metabolism and action

Biocytin (biotin bound with lysine) is the end product of biotin-containing enzyme degradation and is convertible to biotin through the action of biotinidase (6). It is either ingested as the biotin form or produced by gut bacteria. Unabsorbed biotin is excreted either in the faeces or urine (6). Biotin is necessary for a range of carboxylase enzymes such as acetyl coenzyme A (CoA) carboxylase (ACC), pyruvate carboxylase (PC), propionyl CoA (PCC) and methylcrotonyl CoA carboxylase (MCC) in mammals. The vitamin acts as a CO_2 carrier on the surface of biotin carboxylase, and it is essential in the metabolism of carbohydrates, protein and lipids. PC and PCC are involved in Krebs for the metabolism of lipid (odd-chain fatty acids) and proteins (3-carbon non-carbohydrate precursors for gluconeogenesis) and MCC is needed for the metabolism of leucine (41).

Deficiency

Biotin deficiency may result in dermatitis around the eyes, nose, mouth, and cause conjunctivitis, alopecia, and changes in mental status (42). Most of these clinical manifestations are from malfunctioning of the biotin dependent enzymes mentioned above (6). Deficiency of biotin was firstly detected among patients on parenteral nutrition (6). Biotin deficiency reports are very rare, apart from lack of biotinidase (6). Deficiency of biotin can also result from inherited disorders such as multiple carboxylase deficiency which is usually diagnosed in the first weeks of life, and manifests as lethargy, poor muscle tone and vomiting and biotinidase deficiency. Biotin deficiency occurring later in life leads to loss of biotin in urine and organic aciduria is characterised by ataxia, ketoacidosis, dermatitis, seizures, myoclonus, and nystagmus (43).

Toxicity

No toxicity has been reported for biotin.

Therapeutic application

It has been shown that biotin may have a potential to prevent hair loss and build nail, but these benefits are yet to be proven (44).

Vitamins B9 (Folate) and B12 (Cobalamin) Introduction

As vitamin B9 and B12 share similarities and are measured simultaneously, both are reviewed together in this section. Vitamin B9 is necessary for the normal function of RBCs and white blood cells and vitamin B12 is required to convert inactive folate to the active form, maintenance of myelin, and methionine synthesis.

Chemistry and measurement

Folate is necessary during early pregnancy for neural tube formation and folic acid supplementation is required to prevent or reduce the risk of neural tube defects.

The diagnosis of vitamin B12 deficiency is complicated due to poor sensitivity and specificity of conventional tests (45). There are many different forms of vitamin B12. Cyanocobalamin (Fig.1) is commonly found in vitamin supplements. However, the cyanide group can be replaced by an adenosyl, methyl, or hydroxyl groups. Holotranscobalamin (or the active protein bound fraction of vitamin B12) was introduced to improve the detection of vitamin B12 (45, 46).

Indications for measurement

One of the biggest concerns about folate and vitamin B12 deficiency is that most people with deficiency are asymptomatic. As a result, the detection of the insufficiency is usually diagnosed by laboratory analysis or gradual development of symptoms. In some situations, such as pregnancy and symptomatic anaemia (or neurologic or neuropsychiatric findings) the treatment with folate and vitamin B12 should be the first priority. For symptomatic patients the first line of treatment is parenteral administration, and after symptoms have resolved, oral therapy. However, in those who do not have the ability to absorb oral therapy, parenteral administration should be considered (6).

Due to the link between vitamin B12 deficiency and anaemia, screening tests such as a complete blood count (CBC) with a peripheral smear (morphology studies) along with measurement of serum B12 and folate can be performed. Folic acid deficiency may also lead to macrocytic anaemia and can be confused with vitamin B12 deficiency. Therefore, testing for serum vitamin B12 and folate at the same time can help to differentiate between them. However, if the case is still unclear the measurement of methyl malonic acid (MMA), homocysteine, and active B12 is useful. Total B12 has a large borderline interval, and testing for MMA, homocysteine, and active B12 can help in these cases. For instance, active vitamin B12 usually makes up 10% to 30% of the entire B12 in the body, thus someone whose B12's range is normal might be actually B12 deficient (49). Homocysteine is elevated in either folate or B12 deficiency, whereas MMA is elevated in B12 deficiency but not folate deficiency.

Limitations of measurement

There is no standardised or harmonised assay available for the measurement of folate, B12 and active B12. Vitamin B12 deficiency is seen among people with small intestine disorders, neonates from B12 deficient mothers, people who are exposed to nitrous oxide (N_2O) and aging (47).

Sources

Vitamins B9 and B12 are derived from plants (e.g., green vegetables) and animal products (e.g., red meat), respectively. Foods such as breads and cereals in NZ are fortified with a range of vitamins including B9 and B12.

Metabolism and action

Folate is needed for metabolism of purines and pyrimidines, which are precursors for DNA synthesis. Vitamin B12 has other roles such as being a cofactor for methionine synthesis. In the absence of vitamin B12, homocysteine cannot be converted to methionine, thus homocysteine accumulates. As a result, DNA synthesis is decreased, and this results in megaloblastic anaemia. Malfunctioning DNA synthesis results in polymorphonuclear leukocytes. Altogether, vitamin B12 deficiency leads to the formation of hyper-segmented neutrophils (a classical indicator of megaloblastic anaemia).

The other role of vitamin B12 is converting methylmalonyl-CoA to succinyl-CoA via methylmalonyl-CoA mutase. Patients with vitamin B12 deficiency have accumulated MMA because MMA cannot be converted to succinyl-CoA. It has been shown that an increased level of MMA and homocysteine is associated with neurologic deficits and myelin damage. The latter affects various parts of spinal cord and results in ataxia, peripheral neuropathy, and dementia (47).

Folate is absorbed through the intestinal tract and a decreased level of folate indicates deficient dietary absorption. Folate can be also found in the tissues and RBCs (48).

Deficiency

Folate deficiency is uncommon. A decreased level of folate in RBCs means either there is tissue folate deficiency or the patient has primary vitamin B12 deficiency resulting in an inability of cells to uptake folate. The former needs folate therapy while the latter requires vitamin B12 therapy (47). However, it can be seen in some conditions such as inflammatory bowel disease (IBD) severe malnourishment, alcoholism, diets low in green leafy vegetables, chronic haemolytic anaemia, and disorders with high cellular turnover.

Vitamin B12 deficiency usually occurs by three mechanisms: 1.) autoimmune; 2.) malabsorption, and 3.) dietary insufficiency. Vitamin B12 deficiency is usually indicated by clinical manifestations such as gastrointestinal, neurologic findings plus fatigue and pallor manifestations along with laboratory findings such as macrocytic anaemia, haemolysis, and jaundice. There are other nonspecific findings such as peripheral neuropathy, glossitis, diarrhoea, headaches, and neuropsychiatric disturbances. A history of coeliac or Crohn's disease, bowel resection, surgical history of gastrectomy and strict vegan diet increase suspicion for vitamin B12 deficiency.

Toxicity

There is an association between high folic acid consumption and increased risk of cancer (e.g. cutaneous melanoma, a type of skin cancer) and developmental delay in new-borns (47).Toxicity for vitamin B12 has not been reported.

Therapeutic application

Restricted vegans and vegetarians often need to take vitamin B12 supplements (49). In addition, vitamin B9 is prescribed to women who are planning a pregnancy to prevent neural tube defects (50). Folic acid deficiency is not very common in NZ as many foods are now fortified with folic acid (51).

Vitamin C (Ascorbic Acid)

Introduction

Vitamin C (or ascorbic acid) is an antioxidant, and a crucial cofactor for collagen, carnitine, catecholamine and iron metabolism. The human body is unable to produce vitamin C, therefore we need to obtain vitamin C through the regular dietary intake of fruit and vegetables.

Chemistry and measurement

Vitamin C is an alpha-ketolactone (Figure. 1). Although the measurement of vitamin C in leukocytes was previously shown to be a better indicator of vitamin C in body stores (52), this method is not widely utilised now.

Vitamin C (ascorbic acid) can be measured in plasma using HPLC with either UV or electrochemical detection (53). HPLC is often performed using a reversed phase column with an ion-pairing agent in the mobile phase(54).

Indications for measurement

Vitamin C should be measured in patients with disorders such as alcoholism, anorexia, cancer, asthma, glaucoma, and collagen disorders (55). It is worth noting that people with autism may also require to be monitored for vitamin C due to the possibility of an inadequate diet (56).

Limitations of measurement

Samples for vitamin C need to be stored in light protected tubes. The levels of vitamin C increase significantly among people who take a vitamin C supplement before sample collection. Measurement of vitamin C is difficult. For instance, pre-analytical sample treatment is important due to the limited stability of vitamin C, which is readily oxidised to dehydroascorbic acid (DHA). Metal ions present in blood increase the oxidation of vitamin C; the choice of chelating reagent is important. It is recommended that samples are collected in lithium heparin tubes and kept cold until analysis. DHA can be reduced back to ascorbic acid using strong reducing agents such as tris (2-carboxyethyl) phosphine (TCEP). This is often added to convert all DHA to ascorbic acid, so that a total vitamin C is measured. If the samples have been stored at room temperature for extended periods, then DHA can oxidise to diketogulonic acid, which cannot be converted back to ascorbic acid (57), but frozen (*i.e.* -80℃) samples seem to be more stable (58).

Sources

Vitamin C is found in citrus fruits, berries, tomatoes, potatoes, and green leafy vegetables (6). It is an essential nutrient for humans, and other living creatures.

Metabolism and action

Vitamin C, a biological electron donor or reducing agent, plays a crucial role in the maintenance of enzymes involved in iron and copper metabolism, and stabilisation of vitamin E and folic acid (6). Other functions of vitamin C include fatty acid transport (6), a crucial cofactor in wound healing (6), neurotransmitter synthesis for instance, synthesis of norepinephrine (6), prostaglandin metabolism, an anti-inflammatory (6) and a potent vasodilator (i.e. nitric oxide synthesis). It is absorbed in the distal small intestine via an energy-dependent active transport pathway (6) and regulated by kidney excretion. Vitamin C is necessary for the integrity of the skin, mucous membranes, blood vessels, and bone via its nature in collagen synthesis (59).

Deficiency

Deficiency of vitamin C is called scurvy. It is a historical disease, which is manifested in haemorrhage, hyperkeratosis, and haematological abnormalities bruising, gingivitis, arthralgia, and impaired wound healing (6). Vitamin C deficiency is mainly diagnosed by clinical symptoms based on a history of insufficient vitamin C intake. Early onset of the condition usually occurs by follicular hyperkeratosis and perifollicular haemorrhage, with petechiae and coiled hairs, ecchymoses, gingivitis, anaemia and impaired wound healing (6).

Although vitamin C deficiency has generalised systemic symptoms such as weakness, malaise, depression etc., (6), it can have life-threatening symptoms such as dyspnoea, hypotension and sudden death (6). It occurs mainly among people with severe malnourishment, chronic drug and alcohol abuse, children with autism, iron overload such as sickle cell anaemia or thalassaemia, and bone marrow transplantation because ferric deposits increase the catabolism of vitamin C (6).

Toxicity

Large doses of vitamin C can be toxic and associated with diarrhoea, and abdominal bloating, and oxalate kidney stones in males. Higher doses of vitamin C interfere with guaiac results (give a false negative result) but not immunological faecal haemoglobin tests (6, 60)

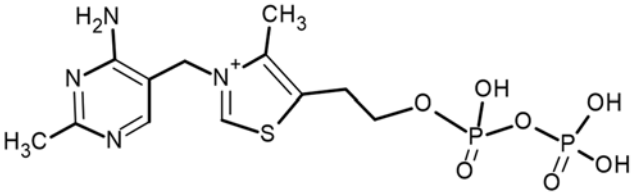
Therapeutic application

Vitamin C has several therapeutically and prophylactic roles such as cardiovascular diseases and cancer prevention (61), but does not prevent common colds except in people doing extreme physical exertion (62-64).

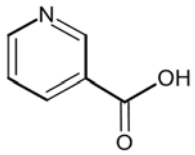
CONCLUSIONS

Requests for vitamin testing are increasing. However, there is no standardised or harmonised procedure for pre-analytical sample treatment, measurement, and interpretation of vitamin results. There are different references used for recommended ranges, reference range and interval range that can be very overwhelming and confusing, not only for health professionals but also for patients. Establishing a universally adopted recommended interval is not a simple task. In order to establish a normal range, a large number of healthy subjects with tight inclusion and exclusion criteria (e.g., not fasting and not taking supplementation) would need to be analysed for WSVs in the New Zealand population. Most samples measured in clinical laboratories are not taken from healthy people, and often, little consideration is given as to whether they are taking a supplement and what effect that has on the interpretation of the results, especially when testing for a vitamin deficiency or how long it will take to excrete from the body. An effective vitamin working group with input from Pathologists is useful in order to widely agree and adopt a standardised approach to vitamin testing.

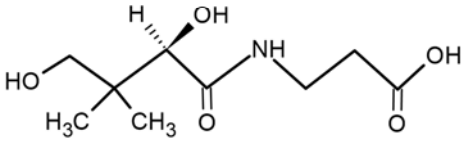
There is further work to do to for better agreement on best practices to be achieved in New Zealand (and Australian) laboratories. Deficiency of some WSVs is a serious clinical issue that can lead to severe morbidity and mortality. Therefore, reliable, accurate and standardised measurement is needed to assist in better diagnosis of vitamin deficiencies.



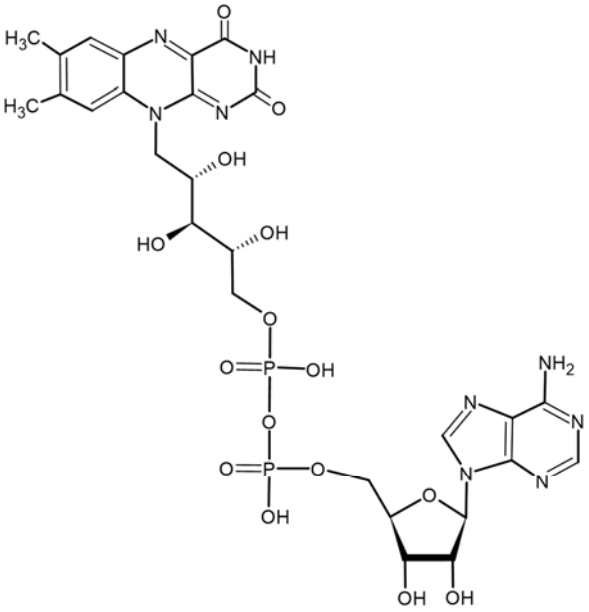
A. Thiamine pyrophosphate (B1)



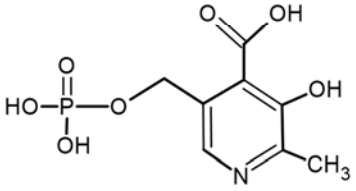
C. Nicotinic acid (B3)



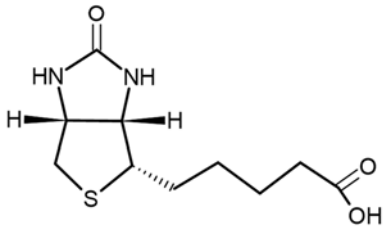
D. Pantothenic acid (B5)



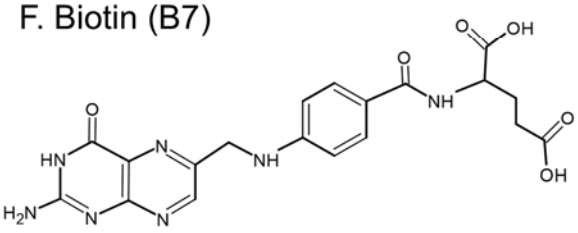
B. Flavin adenine dinucleotide (B2)



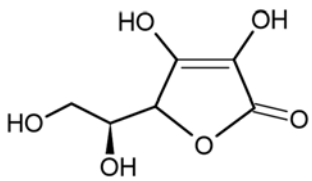
E. Pyridoxal 5'-phosphate (B6)



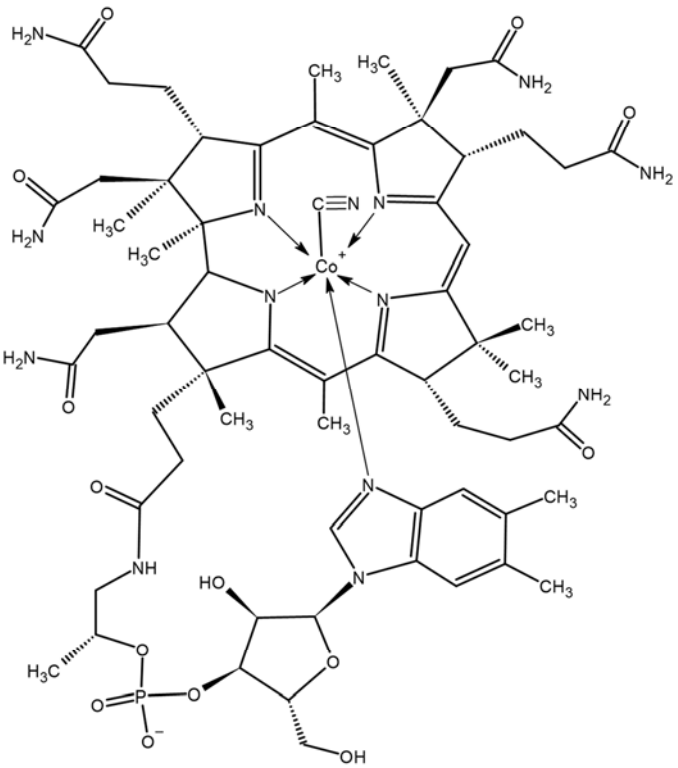
F. Biotin (B7)



G. Folic acid (B9)



I. Ascorbic acid (C)



H. Cyanocobalamin (B12)

Figure 1. Structures of biologically active forms of WSVs: A.) thiamine pyrophosphate (vitamin B1); B) Flavin adenine dinucleotide (B2); C) nicotinic acid (B3); D) pantothenic acid (B5); E) pyridoxal-L-phosphate (B6); F) biotin (B7);G) folic acid (B9); H) cyanocobalamin (B12); I) ascorbic acid (vitamin C).

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ORIGINAL ARTICLE

Investigation of D-dimer stability with INNOVANCE D-dimer assay

Yan Fu, Yii Sen Wee and Rhonda Lucas

ABSTRACT

Objectives: The present study aims to examine whether the D-dimer stability (using INNOVANCE® D-dimer assay) time can be extended from 4 hours to 6 or 8 hours at room temperature to account for late arrival samples and test add requests received after 4 hours.

Methods: Three citrate blood tubes were collected from each of the 25 patients. For each patient, a baseline tube was centrifuged and tested for D-dimer upon arrival (within 4 hours). This baseline tube was then repeatedly tested at 6 hours and 8 hours post-sample collection. The other two tubes were stored horizontally at room temperature until 6 hours and 8 hours post-sample collection, at which they were centrifuged and tested, respectively. All tests were analysed with the INNOVANCE® D-dimer assay.

Results: The D-dimer levels at baseline ranged from less than 190 µg/L FEU to 10512 µg/L FEU. The mean percentage changes at 6 hours and 8 hours were all below the clinically acceptable cut-off of 10% compared with the baseline. Specifically, for centrifuged samples, a decrease of 2.4% and 2.2% were found after 6 and 8 hours, respectively. For uncentrifuged samples, a decrease of 2.8% and 1.9% were observed at 6 and 8 hours, respectively. Results from each tested condition all met the allowed limit of performance in EFLM (European Federation of Clinical Chemistry and Laboratory Medicine). Further analysis using Passing-Bablock regression showed excellent agreement between D-dimer levels at baseline and D-dimer levels after 6- and 8-hours storage.

Conclusion: Our study demonstrated that the stability of D-dimer using INNOVANCE® D-dimer assay at room temperature can be reliably extended to 8 hours for both centrifuged and uncentrifuged whole blood samples.

Keywords: D-dimer, stability, INNOVANCE® D-dimer assay

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INTRODUCTION

D-dimer is a small protein fragment derived from plasmin-mediated degradation of cross-linked fibrin (1). The generation of D-dimer requires the activity of thrombin, activated factor XIII, and plasmin. First, thrombin generated by the coagulation system converts soluble fibrinogen to fibrin monomers. Then, the fibrin monomers are covalently crosslinked into insoluble stable fibrin polymers by factor XIIIa. This stabilising crosslink occurs between the D domains of adjacent fibrin monomers and the E domain of a third fibrin monomer. The adjacent dimeric D domains are referred to as D-dimers. Lastly, plasmin digests fibrin clots and results in the D-dimer molecules and other fibrin degradation products (FDPs). Therefore, the presence of D-dimer is an indication of the activation of intravascular coagulation and subsequent fibrinolysis (1). The measurement of D-dimer is mainly using immunoassays with monoclonal antibodies specific for plasmin-degraded fibrin (2).

D-dimer test is one of the most frequently requested coagulation tests, which is mainly employed for the exclusion of venous thromboembolism (3) such as deep venous thrombosis (DVT), pulmonary embolism (PE), disseminated intravascular coagulation (DIC) and stroke (4). A negative D-dimer result, along with a low clinical pre-test probability, can be used to exclude venous thromboembolism in suspected patients (5). However, a positive D-dimer value is not conclusive of thrombosis, given that various diseases and conditions can increase D-dimer levels.

Many pre-analytical variables can affect the performance of a specific D-dimer assay (6,7), such as sample collection device (butterfly devices, needle bore size), sample transportation, processing method, storage time, storage temperature, and the status of the specimens (whole blood or separated platelet poor plasma). In Dunedin, Southern Community Laboratory (SCL), the blood is collected into a 0.109M buffered sodium citrate tube with 21G BD vacutainer flashback needles to the required level. The sample is immediately carefully mixed, the tourniquet released, stored and transported at room temperature (15–25 °C). The whole blood must arrive in the laboratory within 3 hours and testing performed in <4 hours. This short time frame can be challenging when samples are collected in satellite centres outside of the major testing centre.

In addition, clinicians often request that D-dimer testing be performed on samples already drawn for other coagulation tests after the 4-hour window. While many studies have examined the stability of centrifuged and refrigerated D-dimer, very few studies have investigated room-temperature D-dimer stability. Therefore, it would be useful to explore whether the stability of the D-dimer assay can be extended to a wider time window at room temperature for whole blood samples.

The current study aims to examine: first, whether the D-dimer stability time on whole, uncentrifuged sodium citrate blood can be extended from 4 hours to 6 or 8 hours. This accounts for late arrival samples. Secondly, whether the D-dimer stability time on whole centrifuged samples can be used after 4 to 6 or 8 hours, given that add-on test requests are frequently received on tested samples after 4 hours.

MATERIAL AND METHODS

Blood sample collection and processing

Twenty-five sets of patient laboratory samples participated in this study. Three whole blood samples were collected from each patient in 0.109M buffered sodium citrated tubes by venepuncture staff during routine blood tests. Consent forms or consent stickers were obtained from each patient before sample collection.

After samples arrived, one tube was randomly assigned as the baseline tube, which was centrifuged (10 min, 2000 g) and tested for D-dimer immediately, within 4 hours. Any sample that has HIL level ≥2 (equivalent to 40 mg/dl free haemoglobin in the plasma) is deemed as haemolysed and therefore unsuitable for this study. After analysis, the baseline sample tube was stored on a rack at room temperature. Repeated tests were conducted on the tested centrifuged tube at 6 hours and 8 hours post-sample collection. The other two uncentrifuged whole blood sodium citrate tubes were stored in a bag horizontally after arrival at RT; they were centrifuged and tested respectively at 6 and 8 hours post-sample collection.

Of all the 25 samples, four patients' data were incomplete due to sampling collection error (3 patients only had two sample collected) or testing error (1 patient missed a repeated test at 6 hr and one patient missed a repeated test at 8 hr).

Principle of the INNOVANCE® D-dimer assay

INNOVANCE® D-dimer assay is a particle-enhanced immunoturbidimetric assay (8) for quantitatively determining cross-linked fibrin degradation products (D-dimer) in plasma. The assay relies on the 8D3 monoclonal antibody, which is covalently coupled to polystyrene particles that aggregate when mixed with samples containing D-dimer. The degree of agglutination was directly proportional to the concentration of D-dimer in the sample and detected turbidimetrically via the increase in turbidity. Results were expressed in µg/L fibrinogen equivalent units (FEU) with report limits between 190 µg /L FEU to 35200 µg /L FEU and clinical cut-off at 500 µg /L FEU.

In this study, D-dimer testing was performed using the Sysmex CS2500 automated coagulation analyser with the INNOVANCE® D-Dimer. INNOVANCE® D-Dimer is a particle-enhanced, immunoturbidimetric assay for the quantitative determination of cross-linked fibrin degradation products (D-Dimers). Daily QCs were performed on the analyser, and good diagnostic capability was maintained.

Statistical analysis

To assess stability, the percentage changes were calculated to compare with the clinically relevant difference, which was defined as a percentage change greater than 10% (9). This was done by taking the result obtained from 6 hr, and the 8 hr sample minus the baseline result and then divide that result by the baseline result and multipled by 100. If the percentage change is greater than 10% at 6 hr but corrected back to values less than 10% at 8 hr, it was assumed that deviation was not entirely caused by the storage-dependent change of D-dimer levels (10). Results were further assessed against the Allowed Limit of Performance (ALP) guideline set by EFLM (European Federation of Clinical Chemistry and Laboratory Medicine), which has the Imprecision% at 11.6%, Bias % at 8.8 % and Total Error % at 28 % (11).

Bland-Altman plots (12) were used to display the relative difference of D-dimer levels between baseline and different storage conditions (see Figure 2 and 3) by Analyse-it add-in of Microsoft Excel. Statistical analysis were conducted using Passing-Bablok regression (13) to estimate the agreement between the baseline test and each storage condition test, which is a robust, non-parametric statistical analysis that is not sensitive to the distribution of errors and data outliers. For this analysis, a slope between 0.9 and 1.1, an intercept lower than 50µg/L FEU and a Pearson correlation coefficient higher than 0.99 were considered to indicate substantial agreement (10).

RESULTS

The D-dimer level at baseline ranged from <190µg/L FEU to >4000µg/L FEU (Figure 1). The line charts for each patient showed an overall stable pattern at 6 hr and 8 hr post-sample collection for both centrifuged and uncentrifuged whole blood samples (Figure 2). The relative difference between D-dimer levels at baseline and D-dimer levels at different storage conditions were plotted in Bland-Altman plots for centrifuged (Figure 3.) and uncentrifuged whole blood samples (Figure 4). The mean percentage changes were all below the clinically acceptable cut-off of 10% (11) at 6 hours and 8 hours for both centrifuged and uncentrifuged samples. For centrifuged samples, a median decrease of 2.4% and 2.2% were found after 6 and 8 hours, respectively. For uncentrifuged samples, a median decrease of 2.8% and 1.9% were observed at 6 and 8 hours, respectively. Samples with results below the measuring range of 190µg/L FEU were excluded from the line charts and absolute number for percentage change calculations were not calculated.

The Bias%, Imprecision%, and Total Error% for each storage condition were calculated and compared against the allowed limit of performance in EFLM. Results from each conditions all met the guideline (Table 1). Passing-Bablock regression analysis showed an excellent agreement between D-dimer levels at baseline and D-dimer levels in different storage conditions by showing regression slopes between 0.9–1.10, intercepts <50µg/L and r > 0.99 (Table 2 & Figure 5), which suggested that the D-Dimer levels were stable after 6 hours and 8 hours for both centrifuged and uncentrifuged sample. Therefore, the stability time for D-dimer can be confidently extended to 8 hours.

DISCUSSION

The present study aimed to examine whether the D-dimer stability time on centrifuged and uncentrifuged whole blood can be extended from the current 4 hours to 6 or 8 hours to account for the test add requests and late samples received after 4 hours. Twenty-five patients with a wide range of D-dimer levels were tested with INNOVANCE® D-Dimer Assay. For each patient, three 0.109M buffered citrated tubes were collected. One baseline tube was centrifuged and tested after arrival and repeatedly tested at 6- and 8-hours post-sample collection. The other two tubes were centrifuged and tested at 6 and 8 hours post-sample collection, respectively. The results showed that the mean percentages change was all less than the clinically acceptable age related cut-off of 10% (17) after 6 hours and 8 hours storage for both centrifuged and uncentrifuged samples. In addition, all results met the criteria set in EFLM guideline and excellent agreements were shown between tests at baseline and tests at 6 and 8 hours.

Of all 25 patients, a decrease of percentage change over 10% occurred only in two patients. For one patient, the baseline D-dimer value was at 2716µg/L FEU and the deviation over 10% only occurred at 6 hours (-20.8% and -21.5% for centrifuged and uncentrifuged), However, the percentage changes were lower than 10% at the 8 hours measurement (3.1% and -3.2% for centrifuged and uncentrifuged). Therefore, the latter correction suggested that the storage-dependent change of D-dimer levels did not fully cause the deviation. So 8 hours stability is still acceptable in this case. For the second patient, the baseline D-dimer level was around the borderline (605 µg/L FEU, with age adjusted cut-off of 600µg/L), the percentage changes were above 10% for the centrifuged sample at 6 hours (-27.3%) but not for uncentrifuged samples at 6 hours (-9.09%). They were also above 10% at 8 hours for both centrifuged (-18.7%) and uncentrifuged samples (-16.7%). Therefore, this result suggests that it is potentially possible to have false negative results when measuring the D-dimer at 8 hours, especially when the patient's D-dimer level is around the borderline. However, these two outliers do not significantly change the overall statistical results. Future studies with larger sample sizes can be useful to further clarify this problem.

According to the guideline from the Clinical and Laboratory Standards Institute (CLSI), D-dimer is a stable measurand and may tolerate storage times of up to 24 hours at room temperature or in a refrigerator (14). However, the manufacturer's package insert for INNOVANCE® D-Dimer assay suggests that the stability time for D-Dimer is 24 hours at 2-8°C and only 4 hours at room temperature (15). Therefore, it is necessary to do local validations on INNOVANCE® D-Dimer assay to extend the stability time.

Previous studies using the same INNOVANCE® D-Dimer assay have demonstrated supportive evidence for 24 hours D-dimer stability at room temperature. For example, Böhm-Weigert et al.(10) used a different sample collection tube (0.106 mol/L trisodium citrate), a different analyser system (BCS system), and different sample processing methods (separated plasma) with a larger sample size (n=147) showed the D-dimer were stable after 8 and 24h storage, which suggested that the acceptable storage time can be extended to 24 hours. Same findings were also reported in another study (16), using the Sysmex CA7000 system and fasting samples (n=80). The current study further confirmed the D-dimer stability at 8 hours, which extended the acceptability age of D-dimer samples from the previous 4 hours to 8 hours. This allowed the late arrival samples and test add requests to be processed in a wider time range and saved time and money for rebleeding patients.

There are some limitations in this study. First, compared with the Böhm-Weigert et al (10) and Zhao Y, Lv G. (16) studies, the

sample size in the present study is relatively small. Secondly, D -dimer levels over 500µg/L FEU are routinely checked for clots before reporting the result as per Dunedin SCL Protocol - Routine Coagulation manual. Clotted samples can have falsely elevated D dimer levels. However, the clot check was not conducted on all patients in this study. This might affect the accuracy of D dimer results. Lastly, some patients (n=3) only have two tubes collected, so the 8-hour test cannot be undertaken. Despite these limitations, the overall result was not significantly affected.

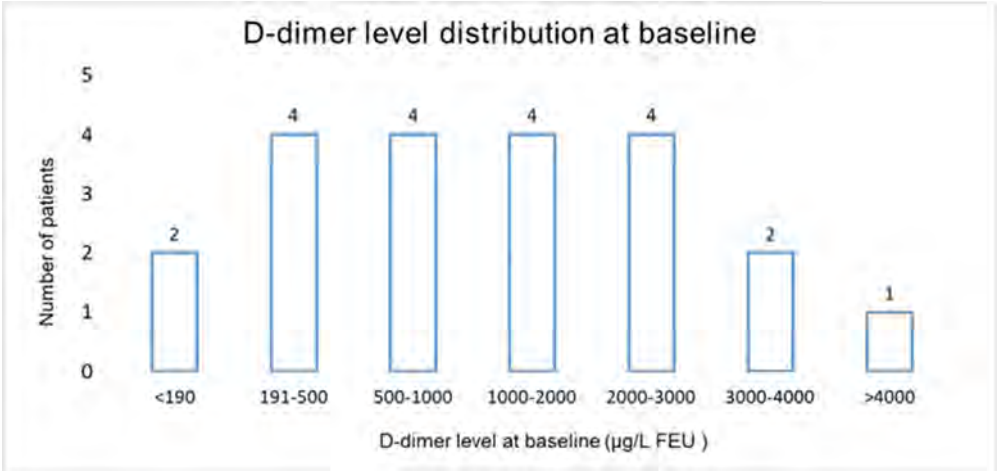

Figure 1. 21 D-dimer level distribution at baseline test.

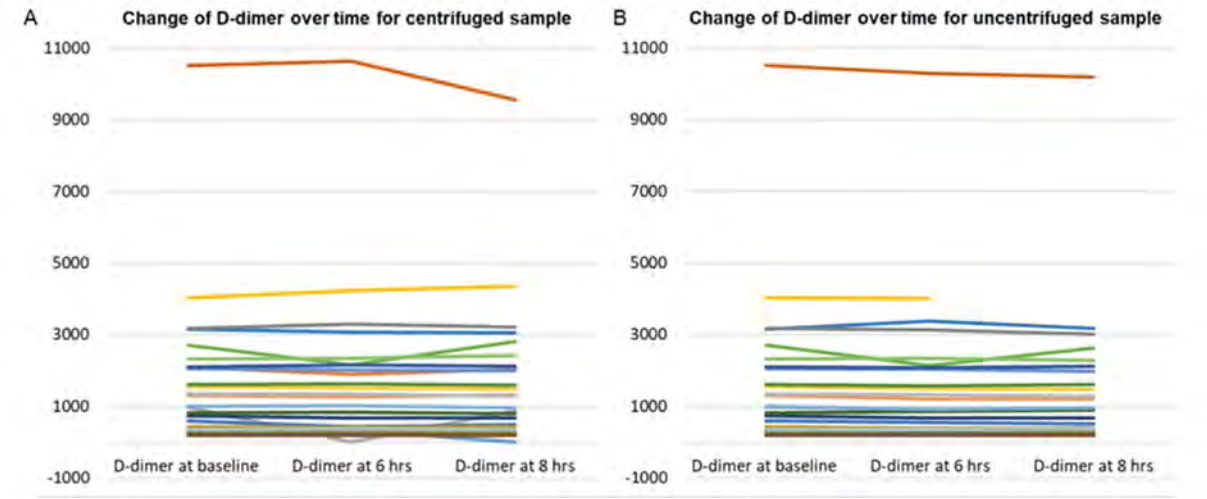

Figure 2. Line charts of D-dimer variation over time for 24 centrifuged samples (2A) and 25 uncentrifuged whole blood samples (2B). The median change for centrifuged sample is -2.4% for 6hr and -2.2% for 8hr. The median change for uncentrifuged sample is -2.8% in 6hr and -1.9 in 8hr.

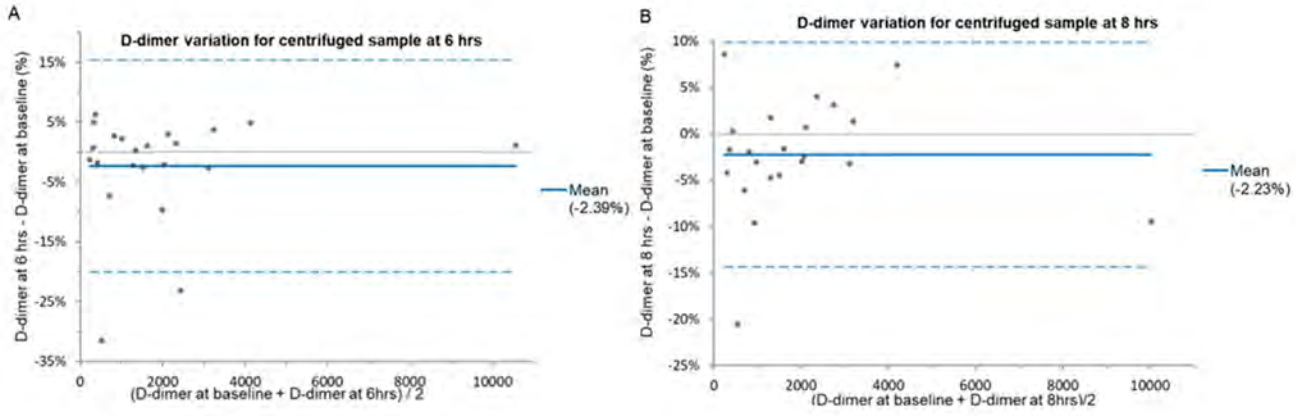

Figure 3. Bland-Altman plots of D-dimer variation for all individuals between baseline and after 6hrs (3A) and 8 hrs (3B) for 24 centrifuged samples.

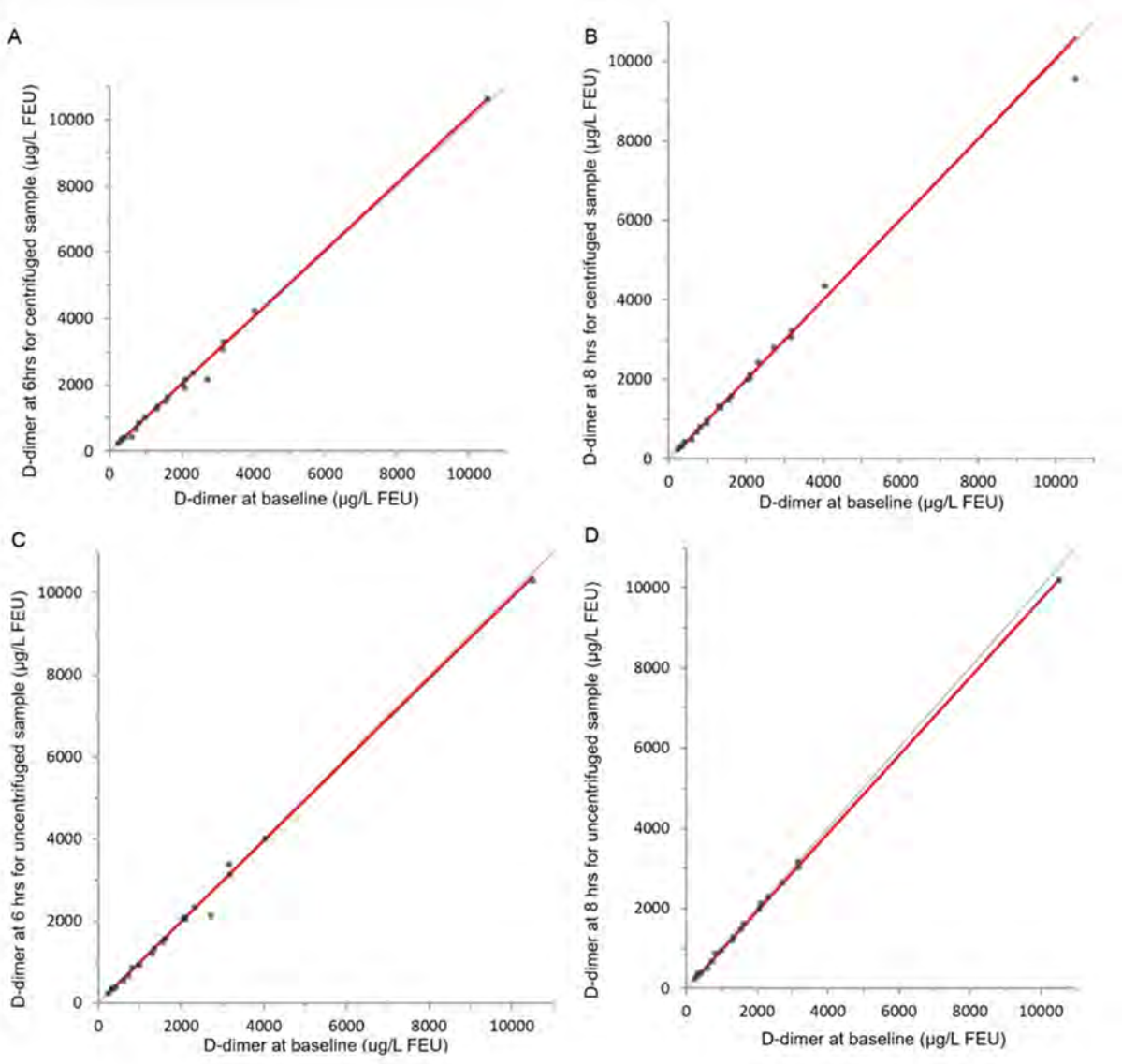

Figure 5. Comparison of D-dimer values at baseline versus D-dimer values at 6 hours (5A) and 8 hours (5B) for 24 centrifuged samples and 22 uncentrifuged whole blood samples (5C and 5D). The results of the regression analysis are given in Table 2.

Table 1. Summary of tested samples compared against the allowed limit of performance

Storage Condition	Sample Tested	% change versus baseline				Test Pass/Fail
		Mean (B%)	SD	CV (I%)	TE%	
6 hours centrifuged	24	-2.39	9.03	-3.78	12.51	PASS
8 hours centrifuged	24	-2.23	6.17	-2.77	7.95	PASS
6 hours uncentrifuged	25	-2.83	6.69	-2.36	8.21	PASS
8 hours uncentrifuged	22	-1.93	6.36	-3.29	8.56	PASS

Table 2. Passing-Bablock regression result for different storage conditions

Storage condition	Slope	Intercept	Pearson's r
6 hours centrifuged	1.011	-3.488	0.998
8 hours centrifuged	1.010	-32.16	0.997
6 hours uncentrifuged	0.988	-0.896	0.998
8 hours uncentrifuged	0.970	-5.596	1

CONCLUSIONS

In summary, our study demonstrates that INNOVANCE® D-dimer assay can be reliably tested at 8 hours post-sample collection and the acceptability age of D-dimer samples can be extended to 8 hours for both centrifuged and uncentrifuged samples at room temperature.

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ORIGINAL ARTICLE

Comparison of tube and column agglutination technique for the quantification of blood group antibody anti-D by titration

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ABSTRACT

Objectives: Laboratory titration of blood group antibody anti-D is used to monitor immunized pregnancies. A titre of 32 is commonly used to trigger clinical procedures to investigate whether a fetus is suffering complications of haemolytic disease of the fetus and new-born. This so called "trigger titre" is based on historical tube methods. Column agglutination technology (CAT) has largely superseded tube for routine blood group investigations and is used by some laboratories for antibody titration. However, sensitivity differences exist between the two testing platforms and no trigger titre for clinical intervention has been established for column. Furthermore, titres suffer from a lack of reproducibility, produced by many factors including variation in equipment, consumables and techniques of different laboratory scientists. As a consequence, results of studies reporting sensitivity differences between tube and column titres vary widely. In this study, variations were minimised by having one scientist perform titration on the same samples at the same time, in tube and column. The work aims to add to the body of data available on sensitivity differences between CAT and tube for titration of anti-D, with a view to informing a trigger titre for CAT.

Methods: Twenty plasmapheresis donations containing anti-D were titrated under standardised conditions by one individual by indirect antiglobulin technique in tubes and columns. Reproducibility was assessed by titrating a reagent anti-D with each batch of plasmapheresis donations tested.

Results: Column titres of anti-D in plasmapheresis donations were on average 0.8 dilutions higher than tube, showing that column is more sensitive than tube. Reproducibility was high for both techniques, with titre varying ±1 dilution from the mode titre in both tube and column.

Conclusions: Consideration should be given to raising the trigger titre by one dilution when performed by CAT for titration of anti-D. However, it is key that laboratories communicate with clinicians to further evaluate whether the laboratory platform and the trigger titre are appropriate in their local setting.

Keywords: Anti-D, titration, column agglutination technology.

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INTRODUCTION

Haemolytic disease of the fetus and new-born (HDFN) is the destruction of red blood cells (RBC) of a fetus or neonate by antibodies produced by the mother (1). These antibodies are maternal immunoglobulin G (IgG) directed against fetal red cell antigens and include anti-D. It is important to monitor the quantity of anti-D in mothers throughout an immunized pregnancy, as this antibody has the capacity to cause severe clinical HDFN, and higher levels often correlate with greater disease severity (2,3).

There are different laboratory methods available to estimate the quantity of anti-D in a mother's plasma including titration, continuous-flow analysis and flow cytometry. All methods suffer from some degree of technical variation leading to a lack of reproducibility both within and between laboratories (4-6), but titration remains the most used method worldwide.

Titration is performed by preparing a series of dilutions of mother's plasma, with dilutions tested against RBC which express the target antigen, in a haemagglutination technique capable of detecting IgG. The end point is the highest dilution (representing the lowest concentration of antibody) which can agglutinate RBC expressing the antigen. A titre of 32 is commonly regarded as the "trigger titre"; the level at or above which clinical investigations should be performed to ascertain the health of the fetus (7,8). The traditional method for titration is the manual tube technique. Dilutions of patient plasma are incubated in tubes with a suspension of antigen positive RBC, then the RBC are washed to remove unbound immunoglobulins naturally present in the patient plasma. Anti-human globulin (AHG) is added to allow agglutination of IgG-coated RBC. The tube is briefly centrifuged and examined and graded for haemagglutination.

In contrast, column agglutination technology (CAT) is performed in a microcolumn filled with either dextran-acrylamide gel or glass bead matrix (9,10). AHG is already in the column and no washing is required because the unbound immunoglobulins remain in the supernatant and do not contact the AHG. In a positive reaction, red cells will agglutinate and are trapped in the matrix during centrifugation, whilst non-agglutinated cells form a pellet of free cells at the bottom of the column.

Lack of reproducibility is an accuracy limiting factor in manual titration methods (5) and can be largely attributed to technique variability between personnel, with the practice of doubling dilutions magnifying any inaccuracy in the volume delivered across the titre series (1). CAT titres show improved reproducibility compared to tube (5) and CAT is easily automated, which further improves reproducibility (11,12) and removes the subjectivity associated with reading tubes.

Although surveys show some labs now use CAT in preference to tube for antibody titration (7), CAT has not been universally adopted due to sensitivity differences between tube and CAT. CAT is more sensitive than tube for detection of some antibodies, notably Rh antibodies (13-17). Due to this sensitivity difference, and a lack of large prospective studies comparing CAT titre results with clinical outcomes, no universally accepted trigger titre has been established for CAT.

This study was an opportunity to compare 20 plasmas in real time using standardised reagents in techniques performed by one individual, thus providing optimum conditions for reproducibility. Reproducibility was assessed by titrating a reagent anti-D with each batch of testing. The aim was to assess if titres were consistently higher, lower or the same when conducted in tube and gel-based CAT, with a view to adding to the data to establish a CAT trigger titre for anti-D levels in pregnant women.

MATERIALS AND METHODS

The cohort consisted of 20 plasmapheresis donations from New Zealand blood donors, supplied by New Zealand Blood Service (NZBS) with permission (NZBS 2020/04). Donations were all from Rh(D) negative individuals with anti-D. The mechanism for immunisation in these donors is unknown. Plasmapheresis bags (average volume 565mL) were received frozen and stored at minus 20 degrees Celsius for up to two years. Bags were

thawed at 4°C. Once thawed, the bags were emptied into a glass beaker, mixed and clotted with 5mL of thrombin (Siemens). After addition of the thrombin, the bags were mixed by inversion and left at 4°C overnight. After mixing again, sieving through muslin was performed to remove clotted material. The filtered serum was then aliquoted into 20mL vials with one vial used immediately for this study. These clotted plasmapheresis donations containing anti-D were tested in batches of two, together with a reagent antiserum (Epiclone IgG + IgM anti-D) as a control to assess reproducibility between batches.

All testing was performed by one operator, using the same electronic pipette (Rainin E250) and the same reagents by the same method (18). The same set of master dilutions was used to titrate both tube and CAT methods. Master serial two-fold dilutions were prepared for each sample using standard protocols in total volumes of 250μL, using phosphate buffered saline (PBS) as diluent. For each plasmapheresis donation, a dilution series was prepared up to a dilution factor of 2048.

For the tube method, 100μL of each master dilution was mixed with 50μL of 3% red cells (R_2R_2 DiaCell II cells (Biorad) and incubated for 1 hour at 37°C. Cells were washed four times with PBS before two drops of poly-specific antihuman globulin (AHG) reagent (Epiclone Anti-IgG-C3d polyspecific green) were added to the dry red cell button. Tubes were centrifuged and examined for agglutination from highest to lowest dilution and graded using the standard 0-4 grading scale (18). Washing adequacy was checked with Coombs Control cells (Biorad, Coombs-Control 4%).

For the CAT method, a 0.8% solution of cells was prepared by adding 2.67mL of the 3% DiaCell II R_2R_2 (BioRad) to 7.33mL Cellstab (BioRad). Fifty microlitres of 0.8% red cells and 25μL of each master dilution were added to columns of Biorad Liss/ Coombs gel cards. Cards were incubated for 15 minutes at 37°C and then centrifuged before reading for agglutination and graded using 0-4 grading scale (18). For both tube and CAT methods, the titre was determined as the reciprocal of the highest dilution at which grade 1 agglutination was observed (18).

The number of additional dilutions for CAT compared to tube was calculated for each plasmapheresis donation. For example, where a titre was 8 by tube, and 16 by CAT, this was calculated as plus 1. Where a titre was 8 by tube and 4 by CAT, this was calculated as minus 1. Where the titre was the same by tube and CAT, the calculated additional dilutions equalled zero. This data was used to calculate the average difference in dilutions between CAT and tube.

In addition, a paired t-test was calculated to assess if the titre pairs were significantly different by either method, with raw titre results converted to base 2 logarithm to reduce the large numerical difference between one raw titre value and the next (19).

To assess reproducibility of titres of the control Epiclone anti-D antisera, the mode titre was established, and the number of dilutions falling either side of the mode was counted.

RESULTS

The end point titres of the plasmapheresis donations performed by tube and CAT agglutination are presented in Table 1 and Figure 1. Titres ranged from 1 to 512 by tube, and 2 to 1024 by CAT. Of the 20 samples tested, five (25%) had the same titre by CAT and tube. Eleven (55%) of the samples showed a one dilution higher result and three (15%) showed a two dilution higher result in CAT compared to tube. One sample (5%) showed a one dilution higher result in tube. On average, CAT was 0.8 dilutions higher than tube. When comparing differences between individual pairs of titre results on the same sample the difference is statistically significant (p = 0.0002). The results of the control anti-D used to assess batch to batch reproducibility are shown in Table 2. Titres for both tube and CAT varied by a maximum of ±1 dilution of the mode, which was 512 for both tube and CAT.

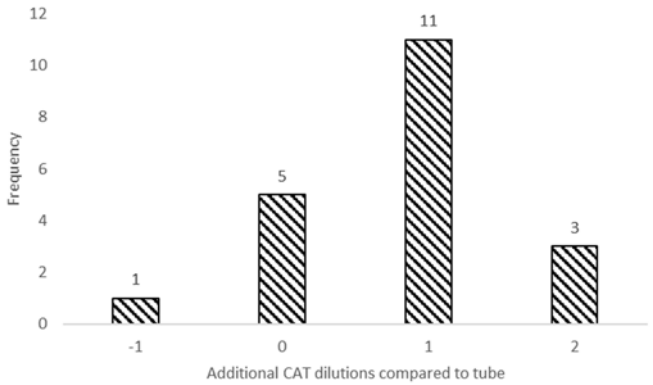

Figure 1. Additional CAT dilutions compared to tube dilutions. The number of additional dilutions for CAT compared to tube was calculated for each plasmapheresis donation. For example, where a titre was 8 by tube, and 16 by CAT, this was calculated as plus 1. Where a titre was 8 by tube and 4 by CAT, this was calculated as minus 1. Where the titre was the same by tube and CAT, the calculated additional dilutions equalled zero.

Table 1. Comparison of titres of anti-D in 20 plasmapheresis donations by manual methods of tube and CAT. Numbers represent the number of samples per titre.

		CAT titre											
Tube titre		1*	2	4	8	16	32	64	128	256	512	1024	2048
	Log base 2	0	1	2	3	4	5	6	7	8	9	10	11
1*	0	-	-	1	-	-	-	-	-	-	-	-	-
2	1	-	1	2	-	-	-	-	-	-	-	-	-
4	2	-	-	1	-	-	-	-	-	-	-	-	-
8	3	-	-	1	-	1	-	-	-	-	-	-	-
16	4	-	-	-	-	-	-	-	-	-	-	-	-
32	5	-	-	-	-	-	-	-	-	-	-	-	-
64	6	-	-	-	-	-	-	-	4	1	-	-	-
128	7	-	-	-	-	-	-	-	-	1	-	-	-
256	8	-	-	-	-	-	-	-	-	2	1	1	-
512	9	-	-	-	-	-	-	-	-	-	1	2	-
1024	10	-	-	-	-	-	-	-	-	-	-	-	-
2048	11	-	-	-	-	-	-	-	-	-	-	-	-

1 = undiluted sample; highlighted cells show when tube and CAT titres are identical*

Table 2. Results of titres of control (Epiclone anti-D) tested on 10 occasions by tube and CAT.

Titre	Tube	CAT
256 (log 8)	4	3
512 (log 9)	5	6
1024 (log 10)	1	1

DISCUSSION

This study confirms that in most cases CAT yields higher titres of anti-D than tube does. This finding emphasizes the need for communication between clinicians and laboratory scientists, to discuss and further investigate the impact of this finding on clinical outcomes for pregnancies in mothers immunized with anti-D.

Column has largely overtaken tube as the platform of choice for methods using haemagglutination as the end point for standard blood grouping methods. Compared to tube, column technology has several advantages; stable and reproducible reactions, ease of automation, and no requirement for washing of cells in the antiglobulin technique. However, many laboratories have not adopted column for titration methods of antibody quantification, due to a lack of internationally established trigger titre for column.

For the clinical application of antenatal monitoring, maternal Rh antibody titres which reach and exceed 32, and titres which increase throughout the pregnancy are used as triggers to inform clinicians that the baby may be at risk of HDFN (7,8). The trigger value of 32 is historical, based on correlations established in manual tube technology, and has not been reviewed or updated to reflect the use of manual or automated CAT. It is of concern that despite this lack of review some laboratories do use CAT as their routine titration platform (7, 17, 20, 21).

In cases where a titre triggers clinical investigation, fetal middle cerebral artery peak systolic velocity measurements (MCA PSV) are performed and depending on the result of this non-invasive screening test, more invasive measures may be needed to ascertain the presence and degree of fetal anaemia (1). MCA PSV can yield false positive results (22), leading to invasive measures which are not without risk to the fetus (1). It is therefore important that the trigger titre used for referral for clinical investigations correlates well with clinical outcome.

It is well established that CAT is more sensitive than tube for detection of Rh antibodies (13-17). Several studies have found CAT titres of anti-D to be higher than tube, with some between one and two dilutions higher on average across a study cohort (13,14,20,23), and in some cases between three and five dilutions higher by CAT (11,21). One study found a 0.5 average dilution higher in CAT than tube (24). Inter-laboratory study variables in these studies included Rh(D) phenotype, cell diluents, incubation times, cell suspension strengths and number of tube washes.

This work had access to twenty plasmapheresis donations which could be tested in one study in a standardised environment, and aimed to assess if titres of red cell antibody anti-D were consistently higher, lower or the same when tested by manual techniques in tube and CAT haemagglutination. Results were highly reproducible (±1 dilution of the mode value). For the 20 plasmapheresis donations, CAT titres were on average 0.8 dilutions higher than tube.

The results of this study showed a lower difference in additional dilutions in CAT compared to tube (average of 0.8 dilutions higher in CAT than tube) than other studies (11,13,14,20,21,23), where the mean across studies was 2.7 dilutions higher in CAT than tube (17). One reason for this could be that in this study all agglutination reactions were read by a second experienced scientist immediately after being read by the person conducting the tests. Sometimes, weak reactions in tube can be misinterpreted as negative (25), particularly if the scientist is not reading tube reactions very often, or lacks experience with tube reading (26). In this case tube titres can be reported falsely low.

Based on correlation with clinical HDFN in 17 cases of anti-D immunized pregnancies and laboratory results, Steiner et al suggested that a titre of 64 could be used as the trigger for gel-based CAT (13). This was supported by work of Thakur et al (14). This study further supports that 64 could be used as the gel CAT trigger, as CAT titres were on average 0.8 dilutions higher than tube. The figure of 0.8 is close to 1, and it is impossible to raise a titre trigger by 0.8, therefore it seems reasonable to round this to the nearest whole number of 1. However, it should be noted that one sample had a one dilution higher result in tube rather than CAT, and further work to correlate CAT and tube titres with clinical outcome is essential (11,14,17,27). A lack of communication between clinical and laboratory teams has hampered progress in this arena, and efforts must be made in further studies to overcome this communication barrier, to ensure involvement from both scientists and obstetric clinicians.

In routine practice, titres are considered acceptably reproducible if results of the same sample are within one (±1) dilution (27), as was the case in this study. This study demonstrated high (but not absolute) reproducibility because one scientist did all the manual pipetting. In clinical practice it is not practical for one person to do all the work across the laboratory, and therefore it is strongly recommended that if CAT titres become the norm, that they be performed on an automated platform, where an accurately calibrated robot performs the pipetting steps. One reason for lack of absolute reproducibility in this study could be that the indicator cells, whilst always of the same Rh type (R_2R_2), were not from the same individual, and individuals of the same Rh type can have differing numbers of RhD antigens (28).

CONCLUSION

The trigger titre for monitoring a fetus at risk of HDFN due to anti-D should be reviewed in laboratories using CAT as their routine method.

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ORIGINAL ARTICLE

Salmonella serovars associated with bacteraemia infection in persons infected with human immunodeficiency virus with low CD4$^+$ cell counts in Akwa Ibom State, Nigeria

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ABSTRACT

Background: Certain serovars of *Salmonella* show a much higher predilection for causing bacteraemia as opportunistic infections in human immunodeficiency virus (HIV) infected persons.

Objectives: The study was undertaken to characterize serovars of *Salmonella* isolated from bacteraemia infections in HIV/AIDS patients in Akwa Ibom State and to determine the relationship of the infection with the low CD4$^+$ cell counts of the subjects.

Materials and Methods: A cross-sectional descriptive epidemiological study was conducted among 300 HIV/AIDS patients and 105 non-HIV subjects. Blood culture samples were collected and cultured on *Salmonella-Shigella* agar (SSA) and Deoxycholate citrate agar (DCA) for recovery of *Salmonellae.* Isolates were identified using conventional tests and Microgen^{TM} GN ID System (Microgen Bioproducts). Serotyping was done using *Salmonella* antisera (Statens Serum Institut, Denmark). Estimation of CD4+ cell counts was by Partec flow cytometry.

Results: *Salmonella species* and *Salmonella enterica arizonae* were identified. Seven serovars were obtained; *S. typhi, S. paratyphi* A, *S. paratyphi* B*, S. paratyphi* C, *S. typhimurium, S. cholerasius* and *S. enteritidis*. The prevalence of *Salmonella* associated bactereamia was significantly higher ($p<0.05$) in HIV persons with 13.7% in contrast to 3.8% non-HIV subjects. Regardless of the HIV/AIDS sero-status, subjects with CD4$^+$ counts below 200µL had the highest rates of *Salmonella* associated bacteraemia.

Conclusion: *Salmonella typhi, S. paratyphi* A, *S. paratyphi* B*, S. paratyphi* C, *S. typhimurium, S. cholerasius* and *S. enteritidis* are identified as serovars of *Salmonella* which are endemic in the study area and associated with bacteraemia infection with significantly higher occurrence in subjects with low CD4$^+$ counts compared to HIV seronegative subjects.

Keywords: *Salmonella*, Bacteraemia, HIV infection, CD4$^+$ counts.

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INTRODUCTION

Bacteraemia is defined as the presence of bacteria in the bloodstream (1). Bacteria can enter the bloodstream through severe complications of infections, dental procedures, catheterization, other foreign bodies entering the arteries and veins and during surgery involving the mucous membranes (2). Individuals with human immunodeficiency virus (HIV) infections are at greater risk of bacteraemia because of their defective immune status (3-5).

The rates of *Salmonella* invasive infections and mortality are higher in infants, elderly and people living with HIV and hemoglobinopathies (6). *Salmonella* species have emerged as a major public health problem in developing countries, particularly sub-Saharan Africa, including Nigeria (7,8). The increasing prevalence of people living with HIV/AIDS has increased the occurrence of both the typhoidal and non-typhoidal salmonellae in bacteraemia in sub-Saharan Africa (9, 10).

There are two species of *Salmonella* complex namely *Salmonella bongori* and *Salmonella enterica* (11). The species of *Salmonella enterica* has six subspecies with more than 2579 serovars currently identified (12). Certain serovars of *Salmonella* show a much higher predilection for causing bacteraemia and these serovars differ with geographic location (13, 14).

Infection with HIV is associated with a progressive decrease of CD4$^+$ T-Cells in the immune system, resulting in an increase in viral load. The normal CD4$^+$ T-Cell count for an adult is over 1,000 cells/µl (15). In HIV pathogenesis, the primary target is the cells bearing CD4$^+$ markers on their surfaces (16-18). Thus, CD4$^+$ cell count determination is the best validated predictor of the likelihood of developing series of opportunistic infections as well as an important parameter to initiate prophylaxis that helps to reduce the risk and severity which would have resulted in death of the patients (19, 20). Moreover, screening for opportunistic infections is an aspect of care and support to these immunosuppressed persons (18).

Therefore, this study aimed at screening and identifying *Salmonella* serovars causing bacteraemia in HIV/AIDS patients in the study area and to determine the relationship between CD4$^+$cell counts and the distribution of *Salmonella* infections in HIV-infected persons.

MATERIALS AND METHODS

Study Design

This research work is a cross-sectional descriptive study undertaken to isolate and characterize *Salmonella* serovars associated with bacteraemia infection in persons infected with HIV/AIDS patients with CD4$^+$ cell counts of less than 1,000 cells /µL (15), in Akwa Ibom State, Nigeria.

Ethical approval

Approval for the work was obtained from the Ethical Committee of the University of Uyo Teaching Hospital (UUTH), Akwa Ibom State, Nigeria. Consents were also sought from the subjects who were willing to volunteer. Consents for minors (below 18 years) were obtained from the parents and guardians.

Collection of samples

Fresh blood samples for culture were collected from a total of 300 confirmed HIV/AIDS infected persons and 105 HIV negative people (control subjects) control who consented to be enrolled into the study.

Inoculation of Samples

Five millilitres (5mL) of blood sample was collected intravenously from each patient and 1mL of the blood was dispensed into blood cultured bottles containing 5mL tryptone soy broth medium and was incubated at 37^{o}C for 48 hours in a standard incubator (DNP-9052 Laboratory incubator, TCA, England). Subculture was made on *Salmonella-Shigella* agar (SSA) plates, while the remaining 4mL was dispensed into blood sample bottles containing ethylene diamine tetra-acetic acid (EDTA) as anticoagulant for HIV test and for CD4$^+$ cell estimation.

Identification of isolates

All purified colonies were identified using the different biochemical tests (15,21,22) and Microgen^{TM} GN ID System (Microgen Bioproducts, USA), an identification system for all currently recognized Enterobactereriaceae were used to confirm the species of *Salmonella* isolates obtained in the study.

Serotyping of *Salmonella*

Serotyping of *Salmonella* isolates was carried out using *Salmonella* antisera and monoclonal antibodies for serological confirmation. Polyvalent “H” (poly A –E + Vi) antisera and “O” (O:2, O:4, O:7, O:9) antisera (Statens Serum Institut, Denmark) were used.

Estimation of $CD4^+$ cell counts

Estimation of $CD4^+$ cell counts was conducted using Partec flow cytometry method. The analysis involves the use of cytoflow beads with a Partec flow cytometry counter machine. Flow cytometry uses laser to excite fluorescent antibody, probes specifically for $CD4^+$ and other cell surface markers, to distinguish one type of lymphocyte from another.

The method involves measuring 20µL of fresh whole EDTA blood (0.02mL) and this quantity of blood was added to 20µL of $CD4^+$/MAb (0.02mL of counting beads) in a test tube and incubated in the dark at room temperature of 37°C for 15 minutes after which, eight hundred microlitres (800µL/0.8mL) of phosphate buffered saline was added to the blood sample and run on the cytometry machine. Thereafter, the machine automatically generated the report of the quantity of the red blood cells present and the number of $CD4^+$ in the cells.

Statistical analysis

Percentages were employed to express the prevalence and the frequency of occurrences of the infection. Analysis of Variance (ANOVA) was used to determine the significant level of *Salmonella* associated infections in HIV sero-positive subjects and to determine the role between the infection and $CD4^+$cell counts of the subjects studied.

RESULTS

Prevalence of *Salmonella b*acteraemia among HIV/AIDS and the controls

Prevalence of *Salmonella b*acteraemia among HIV/AIDS and the control subjects is presented on Figure 1. The results revealed that of the 300 HIV/AIDS patients studied, a total of 41 (13.7%) had *Salmonella* associated bacteraemia while 4 (3.8%) of the blood samples of the 105 control subjects yielded *Salmonella* species. The prevalence in the test group differed significantly from that in the controls ($p<0.05$). The results of biochemical characterizations and Microgen™ GN ID System identification of the isolates are presented on Table 1. The isolates were identified as *Salmonella enterica* species and *Salmonella enterica Arizonae* respectively. The biochemical reaction results obtained from the biochemical tests carried out on the test isolates are shown in Table 2.

Table 3 shows serotyping results of *Salmonella* isolates obtained in the study. *Salmonella* isolates agglutinated the polyvalent H antisera used as genus specific antisera. Further, Results from agglutination of O2, O4, O7, O9 and Vi antisera further identified 7 different serovars including *S. typhi, S. paratyphi* A, *S. paratyphi* B, *S.* p*aratyphi* C, *S. typhimurium, S. choleraesius,* and *S. enteritidis*.

The recovery rates of different serovars of *Salmonella* isolated from blood cultures of HIV/AIDS subjects and the control subjects are presented on Table 4. A total of 58 *Salmonella* isolates agglutinated the polyvalent H antisera used as genus specific antisera. These isolates were obtained from 41 HIV /AIDS patients and 4 control subjects. Some subjects had more than one serovar of *Salmonella. Salmonella typhi* was the most commonly observed *Salmonella* serovars identified from the samples in our study, making up 32% (17/53) of the *Salmonella* isolates in HIV/AIDS patients studied, and 40% (2/5) from the control subjects.

It was observed that irrespective of the HIV/AIDS status, subjects with low $CD4^+$ cell counts of ≤ 200µL had higher percentages *Salmonella* associated bacteremia (Table 5). Data analysis showed a significant relationship ($p< 0.05$) between $CD4^+$ cell counts of HIV/AIDS subjects and the prevalence of *Salmonella* bacteraemia.

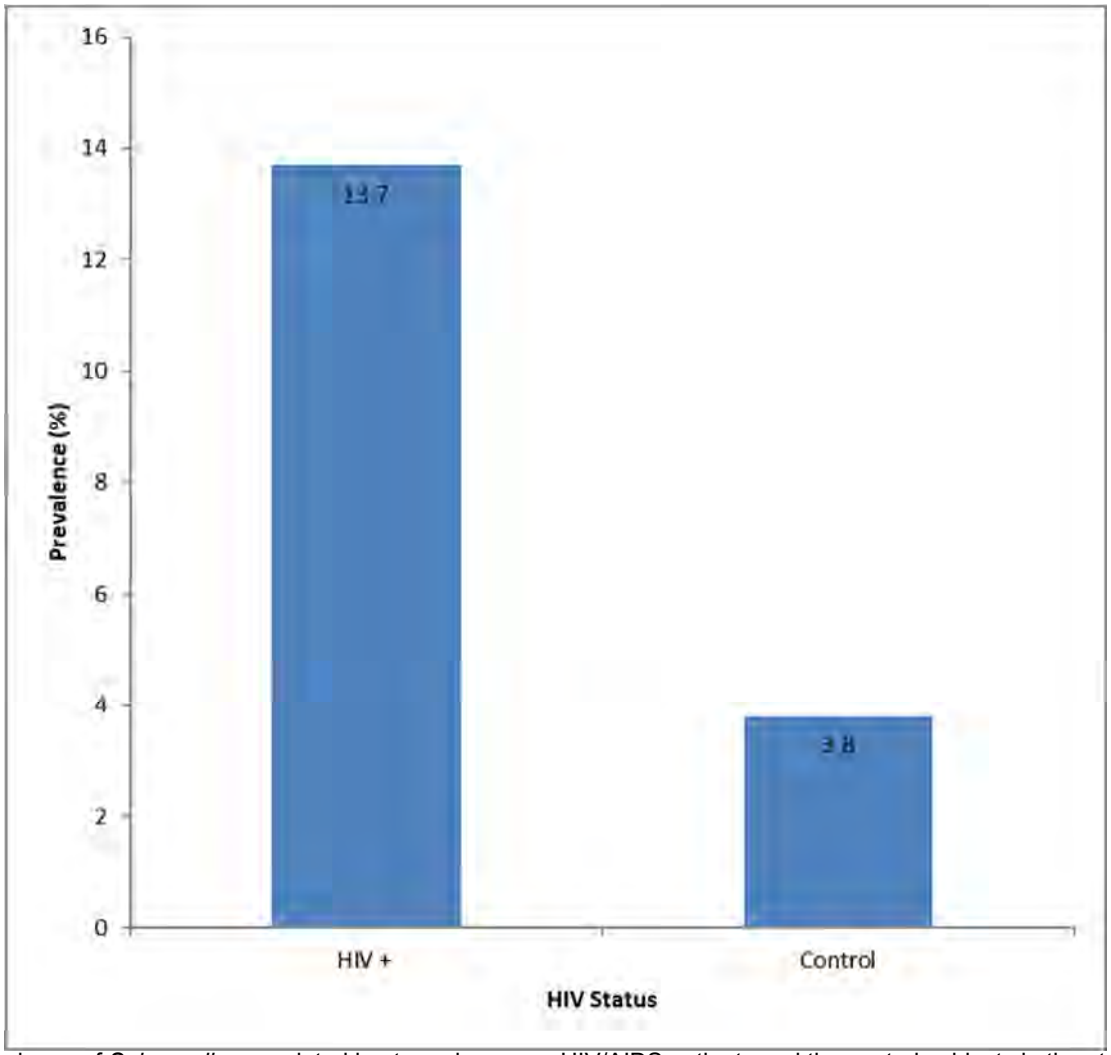

Figure 1: Prevalence of *Salmonella* associated bacteremia among HIV/AIDS patients and the control subjects in the study

Table 1. Biochemical characterizations and Microgen identification of the isolates obtained in the study

Isolates	Biochemical Tests														
	TDA	Cit	Mot	Oxi	Glu	Lys	Orni	Man	H2S	Xyl	ONPG	IT	UT	VP	Nit
S. spp	+	+	+	-	+	+	-	+	±	-	-	±	-	-	+
S. ariz	±	±	+	-	+	±	±	+	±	+	±	±	-	-	+

***S spp :** *Salmonella enterica* species, **S ariz:** *Salmonella enterica Arizonae*, **TDA:** Tryptophan deaminase test, **Cit:** Citrate Utilization test, **Mot**: Motility test, **Oxi**: Oxidase test, **Glu:** Glucose fermentation test, **Lys:** Lysine decarboxylase test, **Orth**: Orthinine decarboxylase test, **Man:** mannitol fermentation test, **H2S**: Hydrogen sulphide production test, **Xyl**: Xylose oxidation test, **ONPG:** beta -galactosidase test, **IT:** Indole production test, **UT:** Urease test **VP**: Voges Prokauer Test, **NT**: Nitrate reduction test. + = positive reaction, - = negative reaction, ± = variable.

Table 2. Biochemical reactions obtained from the serovars of *Salmonella* isolates in the study.

Salmonella serovars	Biochemical Tests and Their Reactions								
	KIA	Cit	Lys	Lac	Orth	ONPG	IT	H2S	Mot
S. typhi	A	-	+	-	-	-	-	+	+
S. paratyphi A	AG	-	-	-	-	+	-	-	+
S. paratyphi B	AG	+	-	-	+	+	-	+	+
S. paratyphi C	AG	+	+	-	-	-	+	+	+
S. typhimurium	AG	+	+	-	+	+	+	+	+
S. cholerasius	AG	+	+	-	-	+	+	-	+
S. enteritidis	A	+	-	-	-	+	-	+	+

***KIA=** Kligler iron agar test, **Cit:** Citrate Utilization test, **Lys:** Lysine decarboxylase test, **Lac**: Lactose fermentation, **Orth**: Orthinine decarboxylase test, **ONPG:** betagalactosidase, **IT:** Indole production test, **H2S**: Hydrogen sulphide production test, **Mot**: Motility test, **+ :** Positive reaction, **- :** Negative reaction.

Table 3. Serotyping results of Salmonella isolates obtained from blood cultures

Antisera used	Serotypes of *Salmonella* species	'O' & Vi antigens possessed by the isolates	'H' antigens possessed by the isolates
O2	*S. paratyphi* A	1, 2, 12	a
O4	*S. paratyphi* B	1, 4, 5, 12	b
	S. typhimurium	1, 4, 5, 12	i
O7	*S. paratyphi* C	6, 7 (Vi)	c
	S. choleraesius	6, 7	y
O9	*S. typhi*	9, 12(Vi)	d
	S. enteritidis	4, 9, 12	g

Table 4: Recovery rate of *Salmonella* serovars from blood of HIV/AIDS subjects and the control subjects

Salmonella species	No. (%) of isolates from HIV/AIDS (n =53)	No. (%) of isolates from control subjects (n = 5)
S. typhi	17 (32.1)	2 (40.0)
S. paratyphi A	7 (13.2)	0 (0.00)
S. paratyphi B	9 (17.0)	1 (20.0)
S. paratyphi C	5 (9.4)	1 (20.0)
S. typhimurium	4 (7.6)	0 (0.00)
S. cholaerasius	6 (11.3)	0 (0.00)
S. enteritidis	5 (9.4)	1 (20.0)

Table 5: Relationship between $CD4^+$ cell counts and the distribution of *Salmonella* bacteraemia in HIV/AIDS patients.

$CD4^+$ Counts/µL	(%) of HIV/AIDS subjects with *Salmonella* bacteraemia (n=41)	(%) of controls subjects with *Salmonella* bacteraemia (n=4)
≤ 200	21 (51.2)	2 (50.0)
201-300	11 (26.8)	1 (25.0)
301-400	3 (7.32)	1 (25. 0)
401-500	2 (4.88)	0 (0.00)
501-600	3 (7.32)	0 (0.00)
601- 700	1 (2.44)	0 (0.00)
≥ 701	0 (0.00)	0 (0.00)

DISCUSSION

In this study, the 13.7% prevalence of *Salmonella* isolation from blood of HIV/AIDS subjects is of public health concern. Immunocompromised persons are more susceptible to *Salmonella* infection especially from its life-threatening bacteraemia (23). According to Keddy *et al.*, (2009) and Sharma *et al., (*2010), *Salmonella* organisms especially *S. typhi,* infection in people living with HIV/AIDS may result in complications like septic shock, meningitis, and local abscess formation. The isolation of *S. typhi* and other serovars including the non- typhoidal ones in this study, corroborates the work of (25, 26) Taramasso et al., (2016), Adisa *et al.,* (2017). *Salmonella* is known to cause serious infections ranging from febrile illness, typhoid fever, gastroenteritis and could lead to death (27). Previous studies have reported more than 2.16 million episodes of typhoid occurred worldwide, resulting in 216,000 deaths, and is described as a common systemic infection in tropical regions (28).

People living with HIV/AIDS are predisposed to bacteremia and their systemic spread is uninhibited due to their defective adaptive immune system (29).

The isolation of serovars *S. typhimurium* and *S. enteritidis* agrees with the works of Brent, *et al*., (2006) and Gordon et *al.,* (2010) who reported these organisms as the leading cause of community-acquired bloodstream and focal infection in Sub-Saharan Africa. Their study further highlighted that non-typhoidal *Salmonella (NTS)* serotypes were predominant among the HIV/AIDS infected persons and children with HIV, malaria, and malnutrition (30).

In this study, the majority of HIV/AIDS infected persons had low CD4+ cell counts with a higher recovery rate of *Salmonella* serovars. Other studies have reported that in HIV infection, the primary targets by the virus are the cells bearing $CD4^+$ markers on their surfaces, hence the gradual decrease in $CD4^+$ marker receptors (16-18). Fisk *et al*., (2005) noted that the dysfunction observed with HIV/AIDS infected patients is usually as a result of opportunistic infections and the vulnerability to *Salmonella* infections which correlates inversely with the $CD4^+$ lymphocyte count.

CONCLUSION

This study isolated two species of *Salmonella* and seven serovars from 13.7% of the HIV/AIDS infected persons in the study area, and their distribution largely depends on the level of $CD4^+$ cell counts of the subjects. Therefore, emphasis should not be laid only on retroviral drugs for the HIV/AIDS infected persons, but also regular screening of the subjects for bacteraemia as well as advising the subjects to consume a balanced diet including fruits to boost their immune systems, maintain personal hygiene and sanitation coupled with provision of available *Salmonella* vaccines by Government to HIV/AIDS infected individuals so to eliminate *Salmonella* associated infections among this group.

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ORIGINAL ARTICLE

Correlation of selected inflammatory markers with cardiovascular diseases markers among HIV patients in Benin City, Nigeria

Godwin A. Aikpitanyi-Iduitua, Nosakhare L. Idemudia, Rosemary O. Aikpitanyi-Iduitua and Richard Omoregie

ABSTRACT

Objectives: Dyslipidaemia has been reported in HIV infections which often results in cardiovascular disease (CVD). Given that HIV is associated with inflammation with resultant adverse clinical outcomes, this study seeks to assess the correlation between selected markers of inflammation and markers of cardiovascular diseases among HIV patients in Benin City, Nigeria.
Methods: Selected inflammatory markers (such as Albumin, CD4, neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), and systemic immune-inflammatory index (SII)) and markers of CVD (such as atherogenic index of plasma (AIP), Castelli's risk index(CRI), triglyceride-high density lipoprotein cholesterol ratio (TG/HDL-c) and triglyceride glucose index (TyG index)) were evaluated in 173 participants comprising 81 HIV patients on Highly Active Antiretroviral Therapy (HAART), 45 HAART-naïve HIV patients and 48 non-HIV individuals (Controls) attending out-patients clinics in the University of Benin Teaching Hospital, Benin City. Using blood samples obtained from each of the participants, albumin, CD4 count, Full blood count (FBC) and lipid profile test were determined using standard methods from where other markers were calculated.
Results: CD4 count and albumin were lower in HAART-naïve HIV patients than in both those on HAART and non-HIV ($P<0.001$) whereas PLR were higher. AIP and TG/HDL-c were significantly higher in HAART-naïve HIV patients than in those on-HAART and non-HIV subjects. In HAART-naïve patients, albumin with AIP, TyG index and TG/HDL-c, and CD4 with CRI and TG/HDL-c correlated negatively. This was the same for albumin with TyG index amongst HIV patients on HAART. In non-HIV patients, CRI had a significant positive correlation with NLR, PLR and SII, while CD4 with TG/HDL-c, AIP and TyG index was the reverse relationship. Using AIP and TG/HDL-c cut-off values, HAART naïve HIV patients had a higher risk of developing cardiovascular disease than non-HIV patients, followed by HIV patients on HAART.
Conclusion: This study showed that HIV is linked to lipid abnormalities as a result of chronic inflammation. Therefore, HIV patients should be monitored for inflammation and CVD.
Keywords: Albumin, Antiretroviral therapy, cardiovascular disease, HIV, inflammation

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INTRODUCTION

Human immunodeficiency virus (HIV) patients on antiretroviral therapy (ART), particularly those on protease inhibitor-based regimens, have been found to have a variety of lipid abnormalities (1). Reports have shown that adults with HIV who have lipid abnormalities may be at risk for vascular permeability, arterial stiffness and cardiovascular disease (2-4). In HIV-positive people, the risk of cardiovascular abnormalities (especially coronary artery disease) is 1.5 – 2 times greater than in uninfected people. Although HIV infection and the antiretroviral therapy have been reported to be among the known conventional risk factors, persistent immunological activation and systemic inflammation that are crucial in HIV pathogenesis also increase the risk of coronary artery disease in HIV patients who are receiving ART as well as in those who have never received the drug (1,5).

Due to a variety of methodological issues, including differences in study designs, choices of inflammatory biomarker panels, and selection of clinically relevant outcomes, evaluating inflammatory biomarkers in the context of CVD risk in HIV-infected people is difficult especially in resource limited settings (6). However, some novel biomarkers such as atherogenic index of plasma (AIP), triglyceride-high density lipoprotein cholesterol (TG/HDL-c) ratio, triglyceride glucose index (TyG index) and Castelli's Risk Index (CRI) have been utilised as strong and reliable predictor of dyslipidaemia and related disorders such as cardiovascular diseases (7,8).

Recently, we had observed a correlation between some surrogate markers of inflammation and non-invasive markers of liver fibrosis (unpublished observation). Some of these earlier studied surrogate markers of inflammation such as platelet-to-lymphocyte ratio (PLR), monocyte-to-lymphocyte ratio (MLR), and neutrophil-to-lymphocyte ratio (NLR) have all been discovered as markers of inflammation in a variety of illnesses, including malignancies and cardiovascular disease (9-11).

Therefore, this study aims to assess the correlation of some surrogate inflammatory markers with some markers of cardiovascular disorders among HIV patients attending clinic in the University of Benin Teaching Hospital in Benin City, Nigeria.

MATERIALS AND METHODS

Study location and population

A total of 125 HIV patients were recruited for the study at the University of Benin Teaching Hospital in Benin City, Edo State, Nigeria. Of the 125 HIV patients, 81 (20 males and 61 females) were on Highly Active Antiretroviral Therapy (HAART), whereas the remaining 44 (18 males and 26 females) were HAART-naive. The study also included 48 (28 males and 20 females) HIV seronegative individuals who served as controls. All participants were asymptomatic. The ages of participants (mean ± standard deviation) across the groups were 40.64 ± 9.63years for HAART-naïve, 41.19± 11.57years for those on HAART and 30.36± 10.07years for controls. All participants were instructed to be on 10 – 12 hours fasting prior to specimen collection. Informed consent was obtained from all participants prior to specimen collection. The Ethical Committee of Edo State Ministry of Health, Benin City, Nigeria, approved the protocol for this study.

Inclusion and exclusion criteria

Clinical stage I HIV patients and apparently healthy seronegative HIV subjects were recruited for this study. Subjects that were pregnant, diabetic, hypertensive, smokers, hepatitis B and C seropositive, as well as on lipid and glucose medications were excluded from this study.

Sample collection and processing

Ten millilitres of blood was collected from each subject and dispensed in 5m/L amounts in ethylene diamine tetra acetic acid (EDTA), fluoride-oxalate and plain containers. The EDTA

blood was used for complete blood count and CD4 count, fluoride-oxalate specimen for glucose estimation and sera samples obtained were used to analyse some biochemical parameters (albumin, and lipid profiles) of all participants.

Full Blood Count Analysis

Complete blood count (CBC) was determined with the EDTA blood sample using haematology auto-analyser (Sysmex K2IN, Sysmex Corporation, Kobe, Japan) by following the manufacturer's instructions.

CD4 Cell Count Estimation

Flow cytometry was used for the CD4 cell count estimation using the Partec Cyflow Counter II® and the Sysmex CD4 Easy Count Kit® following the manufacturers' instruction.

Biochemical Analysis

Blood from fluoride-oxalate containers and sera samples were used to determine the following biochemical parameters – fasting blood glucose, total cholesterol (TC), high density lipoprotein cholesterol (HDL-c), triglyceride (TG) and albumin using Selectra ProS chemistry auto analyser (Vital Scientific Inc., Germany) following the manufacturer's instruction. The low -density lipoprotein cholesterol (LDL-c) was calculated using the Friedewald's equation as previously described (12).

Inflammatory Markers

Some inflammatory markers such as neutrophil/lymphocyte ratio (NLR), platelet/lymphocyte ratio (PLR), and systemic immune-inflammatory index (SII) were calculated from the obtained parameters of the CBC (11). Briefly, NLR was calculated as the ratio of the neutrophils count to lymphocyte counts and PLR was calculated as the ratio of the platelet count to lymphocyte counts. The SII was defined as: SII = neutrophil x platelet/lymphocyte.

Markers of Cardiovascular Diseases

The markers of cardiovascular diseases such as atherogenic index of plasma (AIP), Castelli's Risk Index (CRI), TG/HDL-c ratio and triglyceride glucose index (TyG index) were calculated from the obtained biochemical parameters as follows:

AIP was calculated as $\log_{10}$ (TG/HDL-c) and, according to previous studies, classified into three groups: low risk (<0.11), intermediate risk (0.11–0.21), and increased risk (>0.21) (13).

Castelli's Risk Index (CRI) is calculated as the ratio of TC to HDL-c. A value of ≥ 5 was associated with cardiovascular disease (14).

The TG/HDL-c ratio was calculated by dividing the serum concentration of TG by HDL-c measured in mg/dL and a cut-off for TG/HDL-c ≥ 3.5 has been reported to be associated with CVD (15). TyG index was calculated based on the formula: Ln (TG (mg/dL) x FG (mg/dL)/2) (16).

Statistical Analysis

The data obtained were analysed using the statistical software INSTAT® version 3.10 for Windows 7 (Graph Pad Inc., La Jolla, California, USA). The parametric data were analysed with ANOVA and correlation while the non-parametric data were analysed with chi-square (χ^2) test. Statistical significance was set at $p<0.05$.

RESULTS

In this study, comparing the levels of selected surrogate markers of inflammation with the various study groups shows that PLR was significantly higher in HAART-naive HIV patients than non-HIV patients and HIV patients on HAART at p<0.001. CD4 count and albumin levels of HAART naïve HIV patients were significantly lower than those of both HIV patients on HAART and non-HIV Patients (p<0.001). NLR and SII were also significantly higher in HAART-naïve patients compared with those on HAART (p<0.05) while NLR of HAART-naïve HIV patients was also significantly higher than that of non-HIV patients (p<0.05) (Table 1). The comparison of the markers of cardiovascular diseases among the study group reveals that AIP was significantly higher in HAART-naïve HIV patients than those on-HAART at P<0.01 and non-HIV patients at P<0.001. Although there was no statistically significant difference in the levels of TyG index and CRI amongst the study groups, TG/HDL-c ratio was significantly higher in HAART-naïve HIV patients than non-HIV patients (p<0.05) (Table 2).

The correlation of markers of cardiovascular disease with some surrogate markers of inflammation reveals that albumin significantly correlates negatively with AIP, TyG index and TG/HDL-c in HAART-naïve patients while CD4 also has the same relationship with CRI and TG/HDL-c in the same group whereas only albumin had a significant negative correlation with TyG index amongst HIV patients on HAART. CRI had a significant positive correlation with NLR, PLR and SII among non-HIV patients; also, CD4 count had a significant negative correlation with TG/HDL-c, AIP and TyG index in the same group of participants (Table 3).

Although, TyG index was not used in the determination of the risk of cardiovascular disease because there are no known cut-off values, the study however showed that there was no statistically significant difference in the use of CRI, AIP and TG/HDL-c in the determination of the risk of cardiovascular disease across the group (p = 0.8973) (Table 4). Using AIP, HIV patients on HAART had higher risk of developing cardiovascular disease than the other group as 52.27% of HAART-naïve HIV patients had high risk of cardiovascular disease compared to HIV patients on HAART (12.35%) and non - HIV patients (25.00%) (p<0.001) whereas non-HIV patients had lower risk of cardiovascular disease than the other groups (p<0.0001) and HIV patients on HAART had moderate risk of cardiovascular disease than the other groups (p=0.0081). (Table 4).

Table 1. Comparison of Some Surrogate Markers of Inflammation among the Study Groups.

Parameters	HAART-naïve (n=44)	On HAART (n=81)	Non-HIV (n=48)	p values
Albumin	4.09± 0.09*,†	4.59± 0.04*	4.62± 0.04†	0.0001
Neutrophil-to-Lymphocyte ratio (NLR)	2.22 ± 0.74^{a,b}	1.07 ± 0.07^{a}	0.89 ± 0.11^{b}	0.0232
Platelet-to-Lymphocyte ratio (PLR)	203.72 ± 40.92*,†	103.33 ± 5.35*	93.13 ± 7.63†	0.0002
Systemic Immune-inflammatory Index (SII)	699.70 ± 295.80^{a}	250.85 ± 23.35^{a}	210.35 ± 28.56	0.0313
CD4 count (cells/µL)	261.27 ± 29.02*,†	520.16 ± 29.40*	613.07 ± 38.53†	0.0001

Values are mean ± SEM; *,† = p<0.001; a,b = P<0.05

Table 2. Comparison of markers of cardiovascular disease among the study groups.

Parameters	HAART-naïve (n=44)	On HAART (n=81)	Non-HIV (n=48)	P values
Atherogenic Index of Plasma (AIP)	0.23 ± 0.04*, †	0.09 ± 0.02*	0.05 ± 0.04†	0.0006
Triglyceride-HDL-c Ratio (TG/HDL-c)	1.98 ± 0.19#	1.44 ± 0.16	1.28 ± 0.11#	0.0185
TyG Index	8.42 ± 0.09	8.31 ± 0.07	8.17 ± 0.08	0.1375
Castelli's Risk Index (CRI)	2.96 ± 0.12	2.85 ± 0.08	3.05 ± 0.25	0.6041

Values are mean ± SEM; * = p<0.01, † = p<0.001; # = p<0.05

Table 3: Correlation of Markers of Cardiovascular Disease with some surrogate markers of inflammation

Inflammatory markers	CRI	TG/HDL-c	AIP	TyG Index
HAART-naïve				
Albumin	-0.1855	-0.5864*	-0.5866*	-0.4750*
CD4	-0.3446*	-0.3217*	-0.2748	-0.0671
NLR	0.1126	0.2727	0.2707	0.2255
PLR	0.0831	0.2053	0.2254	0.2244
SII	0.0862	0.2429	0.2477	0.2181
On HAART				
Albumin	0.0953	0.0271	0.0137	0.3132*
CD4	-0.0739	0.0144	-0.0995	-0.1664
NLR	-0.0187	-0.0695	0.0321	0.0736
PLR	-0.0348	-0.0535	-0.0102	0.0043
SII	-0.0551	-0.0719	0.0244	0.1068
Non-HIV				
Albumin	-0.0526	0.0411	0.0647	-0.0251
CD4	-0.2208	-0.3259*	-0.4007*	-0.3793*
NLR	0.6562*	0.2501	0.1812	0.1772
PLR	0.3385*	0.0594	0.0409	0.0974
SII	0.6685*	0.2050	0.1357	0.1065

*Significant correlation (p<0.05)

Table 4. Prevalence of Risk of Cardiovascular Disease among study population

	HAART-naïve (n=44)	On HAART (n=81)	Non-HIV (n=48)	P value
CRI	0 (0.00)	2(2.47)	1(2.08)	0.5864
TG/HDL-c	4 (9.09)	3 (3.70)	2 (4.17)	0.4020
AIP				
Low Risk <0.11	4 (9.09)	41 (50.62)	27 (56.25)	<0.0001
Moderate Risk 0.11- 0.21	7 (15.91)	30 (37.04)	8 (16.67)	0.0081
High Risk >0.21	23 (52.27)	10 (12.35)	12 (25.00)	<0.0001

Values are number (Percentage %)

DISCUSSION

Infection with the human immunodeficiency virus (HIV) and subsequent antiretroviral therapy (ART) are frequently associated with lipid profile changes. Furthermore, despite the fact that antiretroviral therapy (ART) suppresses viral replication, prolonged inflammation is expected to cause changes in lipid composition and function, increasing the risk of cardiovascular disease (CVD) (17). Therefore, this study which seeks to examine the correlation of some surrogate markers of inflammation with some markers of cardiovascular disease has some interesting findings.

Hypoalbuminemia has been established as the result of the combined effects of inflammation and inadequate protein and caloric intake in patients with chronic diseases such as chronic renal failure (18). This agrees with the findings in our study, where albumin levels in HAART naïve HIV patients were significantly lower than its level in HIV patients on HAART and non-HIV patients because HAART naïve HIV patients have more inflammation than the other groups. PLR have been shown to be markers of systemic inflammation and predict mortality in the general population, whether or not they are infected with HIV (19). As a result of viral suppression following HAART commencement, there was substantially higher PLR values in HAART naive HIV patients than in HIV patients on HAART. As a result, the significantly higher PLR levels and lower CD4 counts in HAART naive HIV patients compared to the other groups in our study reflects the severity of the underlying systemic inflammatory and coagulation disturbances that lead to increased mortality in HIV positive people without HAART (20).

Our study also compared the markers of cardiovascular disease among the various study groups; the findings of AIP and TG/HDL-c being significantly higher in HAART-naïve compared to non-HIV patients is consistent with several previous studies (21-24). However, contrary to some of these studies that have identified higher prevalence of dyslipidaemia in HIV patients undergoing treatment with HAART, our study has shown that the level of some markers of cardiovascular disease were higher in HAART naïve HIV patients compared to those on HAART. This may be due to the fact that most of these studies used the levels of lipid profile parameters for assessment, unlike our study where we used novel biomarkers of cardiovascular disorders and may also be due to the fact that all HIV patients in our study were not known to have progressed beyond clinical stage one. Furthermore, studies have revealed that AIP may serve as a diagnostic alternative in situations where other atherogenic risk parameters such as TG appear normal and that it has a role in predicting CVD risk and treatment success. In the same vein, high ratio of TG/HDL-c has been strongly indicated to correlate with coronary disease than lipid variables (25-27). Our finding further buttresses the fact that HIV as an inflammatory disease induces dyslipidaemia in a lot of people living with it to the extent that cardiovascular disease (CVD) is currently the second most frequent cause of death (after cancer) among HIV-positive subjects (28,29).

However, the observed non statistically significant difference in the levels of TyG index and CRI among the different study group in our study agrees with the report of Kamoru and colleagues being a study done in a similar setting as ours (23). This therefore implies that these markers may not be good markers for assessment of cardiovascular diseases in our locality. However, the claim that before initiation of antiretroviral medication, an HIV-positive person's lipid metabolism is severely disrupted and that ART resets the balance, sometimes for the better, sometimes for the worse justifies our findings in this research (30).

Our study also revealed that a significant relationship exists between some markers of cardiovascular disease with some surrogate markers of inflammation in HAART-naïve HIV patients especially albumin and CD4 counts. Although there is paucity of data on the relationship between the surrogate markers of inflammation used in this study and markers of cardiovascular disease, some studies have identified that there is a relationship with inflammatory markers and cardiovascular diseases as inflammation arising from HIV infection modulates lipid particle and lipid transport (18). The mechanism by which this happens is that inflammation impairs reverse cholesterol transfer (RCT), in which HDL-c transports atherogenic lipid molecules from atherosclerotic plaques for elimination by the liver (30). Also, a variety of ATP-binding cassette transporters (ABC transporters) in the arterial wall like macrophages ferry atherogenic lipid molecules onto HDL-c or ApoA1 for reverse cholesterol efflux, commencing RCT and leading to cholesterol excretion (30). Therefore, our findings in this study suggests that these surrogate inflammatory markers can also be assessed as a predictor of developing cardiovascular disorders in HIV patients.

Finally, we also observed that the HAART-naïve HIV patients had higher risk of developing cardiovascular disease among the study groups in our study. This finding is consistent with other studies that have associated HAART-naïve HIV patients with higher risk of cardiovascular diseases (3-5,7). This finding establishes that the link between HIV infection and dyslipidaemia is inflammation as HAART-naïve HIV patients had more inflammation on higher risk of cardiovascular disease.

This study has some limitations like not including demographic and anthropometric data in the analysis as well as not inquiring about the risk factors for cardiovascular diseases among study participants, it is however, believed that this can become a follow-up study.

In conclusion, our study has observed that HIV is associated with lipid disturbances which is a result of persistent inflammation, hence the need to monitor inflammation and CVD markers in HIV.

ACKNOWLEDGEMENTS

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Conflict of Interest

The authors report no conflict of interest in the course of this research.

AUTHOR CONTRIBUTIONS

GAA and RO conceptualised the study. GAA, NLI, ROA and RO carried out sample collection and analysis. NLI and RO analysed data and drafted the manuscript. GAA, ROA and RO contributed to reviewing and finalising the manuscript. All Authors approved the final version of the manuscript.

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Did you know?

The SJR indicator measures the scientific influence of the average article in the journal. It expresses how central to the global scientific discussion an average article of the journal is.

Cites per doc (2y) measures the scientific impact of an average article published in the journal. It is computed using the same formula as that of the journal Impact Factor ™ (Thomson Reuters).

RESEARCH LETTER

Are articles authored by women under-cited compared to articles authored by men?

Rob Siebers

A recent article in *Nature Physics* described that articles authored by women in general physics journals were significantly under-cited compared to articles authored by men (1). This has also been demonstrated previously in high impact medical, epidemiology and social journals (2-4). To our knowledge no such studies have been reported in medical laboratory science journals. Therefore, the present study aimed at determining whether there is under-citation in articles authored by women in the *New Zealand Journal of Medical Laboratory Science* (the Journal).

The Scopus™ database was searched for the Journal and the number of citations each published article received from August 2000 to July 2022. Articles were categorised in whether women or men were the 1st or senior corresponding author. Comparison between citations in the two categories was by Mann-Whitney u test (2-tailed) and statistical significance set at the p 0.05 level.

Of the 435 articles published in the Journal (August 2000 to July 2022), 128 were cited at least once. Table 1 shows the mean, standard deviation, and range of citations in the two categories (female, male). The Mann-Whitney u test returned a z-score of 1.438 resulting in a p value of 0.150 and a 95% confidence interval of the differences was -0.103 to 2.323.

Table 1. Citations to articles authored by women and men.

	Female authors	Male authors
Mean	2.23	3.34
Standard deviation	1.79	4.16
Range of citations	1-9	1-30
Number	52	76

Our study, although showing a higher mean number of citations to male authored articles compared to female authored articles, did not demonstrate a statistically significant difference. This contrasts with previously published articles on this topic (2-5). However, compared to those studies, the present study had a much smaller number of articles and citations, which is a limitation of the study.

The under-citation of articles by women in international journals (2-4) has been ascribed to the "Matthew effect" or the Matilda effect". The "Matthew effect" is where research by men is deemed to be the most important and central in any field while the "Matilda effect" is where research by women is deemed less important than that by men or their ideas are attributed to male scholars.

Citations to published peer-reviewed articles are important in the academic environment for promotion and research grant funding. Therefore, potential under-citing to articles published by women could have serious consequences for them in the academic environment. However, in New Zealand most medical laboratory science personnel are not academics and therefore potential under-citation is not of importance in their work environment. However, under-citation would be important to them personally as female authors are under-represented in the Journal (6).

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RECENT REVIEWS

A PILOT

The editorial team would like to try and provide regular updates on recent reviews appearing in the scientific literature. There are often excellent review articles being published and the intention is to provide the references to such reviews as information for the membership. Unfortunately, due to copyright requirement these cannot be reprinted by the NZIMLS, but the Abstracts are usually available on-line.

Comments on this pilot would be welcomed.
Please email: editor@nzimls.org.nz

1. Ahmadi e, Ali A, Sharma AK et al. Molecular approaches to cancer. *Clin Chim Acta* 2022; 537: 60-73.
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BOOK REVIEW

The wine-dark sea within; a turbulent history of blood

Author: Dhun Sethna, Basic Books, June 2022
ISBN: 9781541600669

It is difficult for us to conceive the idea that the heart has no function in the circulation of blood, but this concept lasted for nearly 1500 years, based on the ideas of the Greek Physician and Philosopher Aelius Claudius Galenus Galen (c. 130-220 CE) who believed the heart septum had minute pores to allow blood to flow between chambers of the heart and was the source of the "body fire". This became the dogma until the discovery of the circulation of blood by William Harvey, published in 1628. However, prior to this major discovery it was widely believed, based on the dogma of Galen, that the heart was a 'furnace' to fuel/power the body and the venous circulation came from the liver. This was responsible for 'fuelling' the heart and the body, and the arteries contained the 'pneuma' (Ancient Greek) for air and in religious terms 'spirit' or 'soul'. This not only became embedded in the early medical literature and therapies but also in the early Christian beliefs. Prominent philosophers embraced this or modifications of the concept over time and it became dangerous for others to propose alternative ideas to the "Galen philosophy" relating to blood even to the point of being burned at the stake for heresy.

The book under review provides a fascinating history from the Ancient Greeks through the ages to the final discovery that blood actually circulates around the body, which was strongly resisted despite William Harvey's discovery as it went against the 'wisdom' of 1500 years. This took 25 years to be accepted in Britain and longer in Europe where data and experiments were fabricated to try and disprove the circulation of blood. Such was the rigor of Harvey's experiments and proof that he could defend his circulation theory against the detractors. The pulmonary circulation and the transfer of gases still remined an enigma until the discovery of atmospheric gases. It is appropriate therefore, that the final chapters of the book move to the then vexed question of respiration and the subsequent discovery of oxygen in the early 1800s as well as other gases and the evidence that oxygen was necessary to support life, but the mechanism of oxygen transport i.e., discovery of haemoglobin had to wait until the late 1800s.

The author is a senior clinical and academic cardiologist who has worked at the Cleveland Clinic and the Cedars Sinai Medical Centre and has produced an extremely interesting and well written book discussing the many and varied theories of blood and functions of the heart from the Ancient Greeks to the proof of what we now acknowledge as the circulation of the blood. It is extensively researched and provides insights into early personalities of the major natural philosophers (now scientists) of their day. The title is taken from Homer's concept of a "wine dark sea" followed by Alchmaeon's ebb-and flow of idea that the circulation was analogous to an irrigation system proposed by Aristotle.

Reviewed by: *Michael Legge, Deputy Editor, NZIMLS*

BOOK REVIEW

Planting clues: how plants solve crimes

Author: David J Gibson, Oxford University Press, Oct 2022
ISBN: 9780198868606

The author, David Gibson, a Professor of Plant Biology at Southern Illinois University Carbondale has written extensively on plant biology. In this current book, he explores the use of plants in both solving and facilitating crimes. In the seven chapters he uses cases to provide the evidence and explains how understanding plants and their biology has led to successful prosecutions where plant forensic evidence provided the vital clues. In the first chapter he discusses how understanding wood patterns successfully led to the conviction in the 1935 Lindberg kidnapping case and the development of the use of 'expert evidence' in court. Evidence based on plant materials from the scenes of crime are presented in the second chapter whereby plant material found on two perpetrators linked two separate murders with the crime scenes. This chapter has several successful convictions for murder linked to plant-based evidence. There is also a discussion on Edmond Locard, who was probably the first real forensic scientist and coined the phrase; "…when two objects come into contact, each takes something from the other or leaves something behind". Moving on, the use of pollens and fungal material from crime scenes is presented in solving several murders. The use of DNA is introduced in chapter 5 and the author succinctly describes the basis for the use of DNA in murders and the establishment of DNA databases for serious crimes. Again, he links the use of plant molecular evidence to kidnappings, murders and successful prosecutions, as well as the use of DNA in major marijuana trading and the illegal timber trade. There is a change of direction for the penultimate chapter which describes the use of plants as poisons starting with the death of Socrates (399 BCE) with hemlock tea. Here the author outlines the narrow margins between survival and death with an example of Renaissance women using belladonna (from deadly nightshade) eye drops which was believed to enhance their beauty by dilating their pupils as well as several other historic plant poisonings. The author identifies famous plant poisoning cases and describes the use of plant poisons including cyanotoxins and the use of 'traditional' medicines. The use of strychnine, ricin pellets and mushroom poisonings makes interesting reading in this chapter. The final chapter describes rare and endangered plants and timber smuggling. Here satellite imaging of endangered forests is used to detect logging as well as sophisticated tracking systems to follow the timber. New instrumental analytical technologies and DNA analysis are being developed to "fingerprint" rare plants and timber. Cases using these techniques are discussed. Although this is not a biomedically-related book it is a fascinating journey of the development and evolution of forensic science to show how plants will be used to commit and solve crimes.

Reviewed by*: Michael Legge, Deputy Editor, NZIMLS*

THE Pacific WAY

Welcome to the PPTC's new Financial Controller, Karl Romijn

Karl is a qualified Chartered Accountant who gained his Bachelor of Commerce and Administration from Victoria University, Wellington in 2010. His professional roles have included senior business advisor, commercial account manager, and chief financial officer for several organisations in Wellington and apart from having an absolute love for numbers, spreadsheets and financial data, he is an avid and keen sportsman who continues to play and coach a variety of sports including football, tennis, and cricket.

Farewell to John Farrell

John is a qualified Chartered Accountant who provided accounting services to a number of not-for-profit organisations including the PPTC. John was Chief Accountant for 14 years with Lotto NZ where he managed a team of accounting professionals. John has also provided accounting services to the National Bank of NZ as well as Child, Youth and Family.

John worked for not-for-profit organisations for many years, an area in which he developed broad knowledge and skills in both their accounting and business needs.

The PPTC thanks John for his fine efforts as Financial Controller of the PPTC for over 9 years and his dedication and commitment to the Pacific Region in assisting PPTC Consultants in meeting the health needs within Pacific communities. The PPTC wishes John the very best for his approaching retirement and hopes he can now take the long-awaited rest that he is deserving of.

WHO COLLABORATING CENTRE FORUM: 28th and 29th November 2022 Cambodia

The World Health Organization (WHO) Regional Office for the Western Pacific presented the Fourth Regional Forum of WHO Collaborating Centres in the Western Pacific from 28 to 29 November 2022 in Siem Reap, Cambodia and Phil Wakem (CEO) and John Elliot (PPTC Chair) were invited to attend as representatives of the PPTC. The PPTC has carried the title of WHO Collaborating Centre for External Quality Assessment since 1990.

The Forum enabled WHO and its Collaborating Centres to reflect on how they have worked together since the previous Forum, and using the lessons identified from the last 4 years, participants were able to identify opportunities to maximize the contribution of WHO Collaborating Centres in countries to respond to current and future health challenges.

Dr Tedros Adhanom Ghebreyesus, WHO's Director-General, addressing participants via video message.

Can you help?

If any New Zealand medical laboratories have items of diagnostic instrumentation that have been recently upgraded or continue to be stored in the laboratory but are actually surplus to requirements, the PPTC would be most grateful if such items could be donated through its Centre to Pacific Island laboratories where there is an exceptional need. Pacific laboratories have very restricted budgets and often cannot afford to replace troublesome instrumentation that continues to breakdown and which is often discontinued because it is so outdated.

The PPTC would also welcome teaching resources in terms of wall charts, Haematology case studies (stained blood films), projector slides, textbooks and journals (within 10 yrs of publication) etc , for teaching purposes in the Pacific, if you no longer have a use for them. Any contribution is so valuable to us.

Please contact:
Phil Wakem
Chief Executive Officer
Pacific Pathology Training Centre
Wellington
New Zealand
Email: pptc @ pptc.org.nz or phil @ pptc.org.nz

SCIENCE DIGEST

Michael Legge

Occupational health issues experience by embryologists

Health data from the UK indicated that the most common work-related ill health issues were stress, depression, anxiety and musculoskeletal problems. In addition health and social work professions had significantly higher rates of work-related illness than many other industries. In response to these data, the UK Association of Reproductive and Clinical Scientists (ARCS) undertook a workforce survey to determine what occupational health issues were being experienced by the profession (1). The survey only included those employed as embryologists employed in 80 different licenced fertility treatment centres plus six from overseas centres. Over half of the 62.2% respondents had 10 or more years experience. In summary, 43.5% had musculoskeletal problems relating to static positions and repetitive actions. Stress and mental health problems were identified in 27.8% of respondents frequently associated with high work demands and low work autonomy. Ocular problems in 12.2% were associated with microscopy work in clean rooms and auditory problems (3.6%) were associated with workplace noise. Needlestick injuries (3.1%) were associated with mental health problems presumably related to the transmission of infectious diseases and liquid nitrogen related injuries were reported in 3.1% of respondents. Overall the survey concluded that embryologists experienced a number of occupational health problems of which musculoskeletal and mental health and work stress issues were the dominate issues emerging from the survey which appeared to be linked to lack of control of workflow and irregular breaks as well as workplace stress.

Proof of innocence by next generation sequencing?

Sometimes what appears to be the obvious is not proof of effect or guilt. In 2003 an Australian woman was convicted of killing her four children aged between 19 days to 18 months over a 10 -year period. All four died in their sleep and the woman was sentenced to 30 years imprisonment. No clear motive for all the murders was established and there was no history of child abuse. Importantly, at the times of the children's deaths there was no evidence of asphyxiation, at least two died from cardiac arrest and the conviction was based on circumstantial evidence. At the time of the trail reference was made to "Meadow's Law" despite it being discredited as having no basis in fact i.e. the paediatrician made it up! In 2020 a judicial enquiry was launched to consider the quality of the medical evidence provided at the original trial and as part of the enquiry next generation sequencing (NGS) was undertaken on the mothers and children's stored DNA (the father refused to give a sample). The investigators initially looked at genes known to be associated with sudden infant death but expanded the search to genes associated with sudden unexpected death (SUD). The mother was identified as having a rare (often lethal) variant in the calmodulin 2 gene associated with myocardial malfunction, which has been associated with SUD and is known to have incomplete penetrance. The pathogenic variant was identified in her two daughters and could account for a natural death. The two sons were identified as compound heterozygotes for a rare missense mutations associated with lethal epilepsy in mice but unreported in humans, and one of the sons was an epileptic and blind. The authors of the publication (2) who collaborated internationally to investigate this case, conclude that NGS and WGS are necessary and justified to identify cases of sudden unexplained deaths in infants. Currently the mother is still serving the sentence and a petition signed by 90 scientists, medical practitioners and related health professionals indicating the mutations could have caused the infant deaths has been presented to the review.

N95 masks and cardiorespiratory function

The advent of the SARS-Cov-2 (COVID-19) pandemic brought about the almost universal wearing of face masks with an ensuing debate of which mask was best. Generally the N95 mask was acknowledged as the most likely face mask to provide optimal protection. In a recently published report, researchers from China have investigated the effects of surgical and N95 masks may have when exercising (3). Three groups of volunteers with normal BMI were divided into no masks, surgical masks and N95 masks. The volunteers then underwent cardiopulmonary exercise testing (CPET). The outcome of this research was that both masks were associated with shortness of breath with the N95 masks having a more negative impact on ability to perform exercise. N95 masks in particular were associated with carbon dioxide retention, a reduction in exercise tolerance, lower breathing frequency and a lower ventilation efficiency. Although surgical masks demonstrated a similar trend it was not as significant as the N95 masks. The authors conclude that both mask types have a negative impact on cardiopulmonary function but the effect was greater with N95 masks and caution about wearing either mask when exercising.

Is it just *BRCA1* and *BRCA2* we should be concerned about in breast cancer?

It is well established that both *BRCA1* and *BRCA2* are important in hereditary breast cancer and it is now well established that approximately 10% of patients with breast cancer carry a predisposing germ-line mutation. Although *BRCA1* and *BRCA2* are both the first described and best known breast cancer genes, and are linked to the most hereditary risk for breast cancer, there are other genes that are linked to breast cancer. Until now these have been difficult to describe but with the ability to sequence multiple genes in a single assay decisions will have to be made on which genes should be identified as being responsible for susceptibility to breast cancer. Recently two high quality major studies (4,5) have identified a number of genes that are statistically associated with breast cancer risk. The Breast Cancer Association Consortium (BCAC) involved 60,466 breast cancer patients and 53,461 control patients from 44 international sites and sequenced 34 genes known or suspected to be causing breast cancer. The second study, Cancer Risk Estimates Relating to Susceptibility Consortium (CARRIERS), sequenced 37 cancer related genes. Detailed statistical analysis of the resulting data was undertaken in both studies. The major findings were that nine genes were statistically associated with breast cancer and across both studies *ATM, BRCA1, BRCA2, PALB2* and *CHEK2* had the highest penetrance for causing breast cancer and possibly *TP53,* although the latter was just below the significant cut-off. Discussion concerned the position of laboratories when testing for breast cancer susceptibility genes and whether a single pan-cancer panel was the better option rather than testing for single genes. An additional factor related to some of the genes had variable penetrance for breast cancer but may be significant for ovarian cancer raising the question of how well the functions of the identified 'risk' genes are understood.

A new method for malaria testing

Malaria is a life-threatening disease and is at times difficult to diagnose. The use of the thick blood film and microscopic examination is still the most common method for malarial parasite identification. However, despite the relative ease of establishing this technique, it requires significant skill especially in identifying the different malarial parasites, particularly when a mixed infection may occur. Newer techniques such as immunochromatography may be affected by malarial parasite density and suffer from lack of high sensitivity.

More recently the use of PCR techniques have emerged and are showing potential especially for low malarial parasite numbers. In a recent publication from China (6) the researchers undertook the use of digital PCR (dPCR) as a new technique for the detection of malaria using all four main *Plasmodium* species: *P. vivax, P. falciparum, P. ovale* and *P. malariae.* Using blood from 29 patients with known malaria infections, 20 with fever (but not malaria), 20 healthy controls and six patients with other parasitic infections. Of the malaria patients 22 had *P. falciparum*, 6 *P. ovale,* 4 *P. vivax* and 4 *P. malariae.* Using dPCR the researchers were able to distinguish all four types of malaria species simulttaneously. Using the two sets of control data it was established that the dPCR technique had a sensitivity of 98% and a specificity of 100% and was able to distinguish mixed infections. The authors conclude that the new technique is both rapid and accurate for the diagnosis of malaria and can be used on stored blood samples.

Genomic autopsies and pregnancy loss and perinatal death

Despite the advances in maternal and fetal care in developed countries eight fetal and neonatal deaths occur in Australia every day. Frequently the cause or uncertainty about these deaths creates significant issue for future pregnancies. While many of these deaths are investigated less than 50% have an autopsy performed. While congenital abnormalities can account for approximately one-third of the deaths the underlying cause of many of such abnormalities is not determined, which limits the ability for counselling for future pregnancies. Currently approximately 70% of congenital abnormalities related deaths are unexplained. In the present publication (7) an international collaboration by researchers from Australia, Turkey, Spain, and the USA offered genomic autopsy using exome sequencing or genome sequencing. Using samples obtained from the parents and those retained at routine autopsy from the child and the placenta all samples underwent microarray analysis. A total of 200 consecutively referred families were included in the current publication. Genomic sequencing was undertaken and sequence mapping, variant calling was undertaken. Analysis and interpretation was in two stages, to identify mendelian related disease variants and the second to identify potential disease variants in novel genes. RNA analysis was also undertaken to identify any splice variants. A total of 105 families were able to receive a definitive or candidate genetic diagnosis. These were either an autosomal recessive (30.8%), X-linked recessive (3.8%) or autosomal dominant (7.7%) with a risk of recurrence. These data informed some families to use preimplantation or prenatal diagnosis for future pregnancies. The authors propose that the use of genomic autopsies improves the standard of care and enhances families options where fetal or neonatal death occurs.

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4. Darling L, Canalho S, Allen J, Gonzalex-Neira A et al. Breast Cancer Association Consortium. Breast cancer risk genes-association analysis in more than 113,000 women. *N Eng J Med* 2021;384: 428-439.
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7. Bryne AB, Arts P, Ha TT, Kassahn KS et al. Genomic autopsy to identify underlying causes of pregnancy loss and perinatal death. *Nat Med* 2023; 29(1): 180-189.

COMPETITION CALL!

We invite Medical Laboratory Professionals and practitioners, university students, design students, youth/rangatahi and the general public to completely redesign the cover of our NZ Journal of Medical Laboratory Science.

The Journal is looking for a contemporary Maori/Pacifica design with Molecular overtones and a focus to the future of our new NZ health system. The Journal cover will run for approximately two years (6 issues).

Prize for winning design is $1,000.00

Design must be submitted to the NZIMLS Executive Officer sharon@nzimls.org.nz no later than 01 June 2023

Prepress Specifications:

- Include 3mm bleed on all edges
- Send as single page pdf's (don't impose)
- Black text to be 100% black
- Final size A4
- If sending Indesign working files, please supply ALL fonts and links

Judges will be the NZIMLS Council and Journal Editors. The Judges' decision will be final. Winning designer will hold the copyright to their design.

JOURNAL QUESTIONNAIRE

Read the articles carefully as most questions require more than one answer. Answers are to be submitted through the NZIMLS website. Make sure you supply your correct email address and membership number. It is recommended that you write your answers in a word document and then cut and paste your answers on the web site.

You are reminded that to claim valid CPD points for successfully completing the journal questionnaire you must submit an individual entry. It must not be part of a consultative or group process. In addition, members who have successfully completed the journal questionnaire cannot then claim additional CPD points for reading the articles from which the questions were derived.

The site will remain open until **Friday 16th June 2023**. You must get a minimum of eight questions right per questionnaire to obtain five CPD points. The Editors set the questions and the CPD Co-Ordinator, Jillian Broadbent, marks the answers.
Direct any queries to her at cpd@nzimls.org.nz.

MARCH 2023 QUESTIONNAIRE

1. In addition to preventative HPV vaccination, what screening strategies are associated with cervical cancer? How do screening strategies differ in many pacific countries?

2. List the common barriers to screening in Pacific countries and provide one example of each.

3. Water soluble vitamin deficiency is uncommon in New Zealand, however in what groups is deficiency seen? And how does storage of water soluble vitamins differ from that of fat-soluble vitamins?

4. Provide one example of a limitation of measurement for each of the following water soluble vitamins; B1 (Thiamine), B6 (Pyridoxine) and B7 (Biotin).

5. What pre-analytical variables affect the performance of a D-dimer assay? And what time-frame must testing be performed in?

6. What stability time for the D-dimer assay did this small study confirm? And what would be the suggested benefits of this stability time?

7. What is HDFN? What antibody has the capacity to cause severe clinical HDFN? And what methods are used to estimate the antibody's quantity in plasma?

8. Column agglutination technology (CAT) has overtaken manual tube methods for blood grouping methods. What are the advantages cited in using CAT compared to tube and what is a limitation of CAT seen by many laboratories?

9. What is Bacteraemia? Provide one example of how it can be introduced. How are individuals with HIV at greater risk of these infections?

10. Patients of protease inhibitor based antiretroviral therapy ART for HIV have found to have a variety of what? And how do these put adults with HIV at risk?

NOVEMBER 2022 QUESTIONNAIRE ANSWERS

1. What factors can be used to predict recurrence of hepatitis after liver transplantation?
   age; viral load; the degree of immunosuppression and surgical complications occurring during the first months' post -transplantation, mainly biliary complications

2. Where is the Adropin hormone expressed? In what diseases is it shown to be diminished?
   Brain and Liver, coronary atherosclerosis, diabetic nephropathies, type 2 diabetes, polycystic ovary disease and hypertension

3. In Acute Kidney Injuries (AKI), what protein is found in urine or blood earlier than serum creatine elevation? And what do levels correlate to?
   Elevated levels of Neutrophil gelatinase-associated lipocalin (NGAL) in serum blood correlate with AKI severity

4. What is Familial Mediterranean Fever (FMF), what does the mutation in pyrin inflammasome increase the production of and what does this initiate in the disease?
   Familial Mediterranean fever (FMF) is a monogenic autosomal recessive autoinflammatory disease, (or, FMF is the most common monogenic autoinflammatory disease) characterised by self- inflammatory attacks of recurrent fever and painful serositis mediated by inflammatory cytokines leading to an increased in the production of Interleukin-1B, which initiates the progress of inflammation and influences the disease pathogenesis.

5. What health problems can the most common daily laboratory activities such as pipetting, microscopy and long periods of standing, create for biomedical scientists?
   Musculoskeletal disorders (MSDs) can affect organs such as muscles, joints, tendons, nerves, ligaments, cartilage, and spinal discs.

6. What is Cyclophilin A (CypA) production stimulated by, where is it synthesised and what event will increase CypA concentration?
   CypA production is stimulated by oxidative stress and inflammation, synthesized by a normal kidney and concentration will increase with any damage to the kidney.

7. Immune checkpoint inhibitor (ICI) immunotherapeutic agents such as Pembrolizumab have become the standard of care for treating patients with advanced metastatic melanoma. What is one of the major disadvantages in the use of immunotherapy highlighted in this case study? What are two examples of underlying conditions that should be excluded through further work up?
   The Major disadvantage in the use of Immunotherapy is the appearance of inflammatory manifestations – immune-related adverse events (irAE). Examples of immune-related adverse events (irAE) are vasculitis and neurological irAE reactions. Underlying conditions such as infections, tumour progression, tumour cell spread to the CSF, vascular complications such as ischemia or bleeding, brain parenchyma, metabolic alterations should be considered for further work up and exclusion.

8. What is the intestinal fatty acid-binding protein 2 (FABP2) gene involved with in the small intestine? What conditions have shown an increase in FABP2 serum levels?
   FABP2 is involved in transport and metabolism of long-chain fatty acids. Increased FABP2 serum levels are seen in conditions such as celiac disease, acute intestinal ischemia, necrotizing enterocolitis, sepsis, and Crohn's disease.

9. What causes 3q29 microdeletion Syndrome? What are some of the clinical manifestations seen by this deletion? *(Please list 5)*
   3q29 microdeletion syndrome is caused by a 1.6Mb deletion in chromosome 3q29. Clinical manifestations of 3q29 syndrome include; developmental milestone delay, gait abnormality, musculoskeletal abnormalities, urinary voiding dysfunction, heart defects, gastro-oesophageal reflux disorder, feeding problems, chronic constipation, anxiety disorder, autism, depression, schizophrenia, facial dysmorphology, chest-wall deformity, and long and tapering fingers.

10. Where is transferrin receptor 1 (CD71) expressed? How does CD71 by flow cytometry help distinguish indolent from aggressive B cell lymphomas?
    CD71 is expressed by replicating cells of all haematopoietic lineages but not by mature resting lymphocytes, monocytes, granulocytes and erythrocytes. There was a correlation between expression of CD71 by flow cytometry with more aggressive lymphomas, differentiating them from indolent lymphomas.

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SCIENTIFIC LETTERS

COMMENTARY

BOOK REVIEWS

OBITUARY

AUTHOR INDEX

2023 NZIMLS CALENDAR

Dates may be subject to change
Visit https://www.nzimls.org.nz/calendar for updates

2023		**NZIMLS Office Opens 9 January 2023**	
January	01	Membership and CPD enrolment due for renewal	cpd@nzimls.org.nz
	10	Material for the March issue of the Journal must be with the Editor	sharon@nzimls.org.nz
	31	Last day for 2022 CPD points to be entered	cpd@nzimls.org.nz
February	01	QMLT applications open	admin@nzimls.org.nz
March	02-03	Council Meeting, Christchurch	sharon@nzimls.org.nz
April	20	Closing date for QMLT applications	admin@nzimls.org.nz
	15	Material for the July Journal must be with the Editor	sharon@nzimls.org.nz
June	9-11	NICE Weekend, Christchurch	raewyn.cameron@pathlab.co.nz
August	01	Annual Reports and Balance Sheet to be with the Membership (14 days prior to AGM)	sharon@nzimls.org.nz
	15	Material for the November Journal must be with the Editor	sharon@nzimls.org.ng
	16-18	**SOUTH PACIFIC CONGRESS VIADUCT EVENTS CENTRE, AUCKLAND And NZIMLS AGM**	**sharon@nzimls.org.nz ailsa.bunker@middlemore.co.nz**
	17	**NZIMLS Annual General Meeting, Viaduct Events Centre, Auckland**	**sharon@nzimls.org.nz**
October	07	QMLT Examinations	sharon@nzimls.org.nz
		Special Interest Group Meetings (dates to be confirmed)	
May		South Island Seminar	willem.vandyk@medlabsouth.co.nz
May		North Island Seminar	TBC
June	9-11	NICE Weekend, Christchurch	Raewyn.cameron@pathlab.co.nz
September		Biochemistry	leoluk@adhb.govt.nz
October		Anatomical Pathology	tanyaF@adhb.govt.nz
October		Haematology	shona.brougham@medlabsouth.co.nz
October		Microbiology	tracy.camp@middlemore.co.nz
October		Molecular Diagnostics	robertom@adhb.govt.nz
October		Preanalytical	Ajesh.joseph@waikatodhb.health.nz
November		Immunology	sarah.burge@wellingtonscl.co.nz
November		Mortuary	rod.smith@cdhb.health.nz

More events (and dates) will be added as they are confirmed.

All NZIMLS events will be held in conjunction with current NZ Government COVID-19 regulations.

Mask wearing is not a requirement, however is encouraged.

IMMUNOLOGY SIG REPORT 2022

The Immunology SIG was held at the Marion Davis Hall at Te Toku Tumai on the 11th November 2022. There was a wonderful turn out of over 50 medical laboratory professionals, suppliers and pathologists. The sponsors for the event were Abacus dx and TG diagnostics. A range of topics were covered from the complement system to ocular syphilis.

The day started off with an interesting presentation from Dr Edward Lea on the complement system describing both the classic and alternate pathway as well as the lectin pathway who's clinical use is somewhat controversial. Pathologies of the complement testing were also discussed and methods for testing. An interesting case was presented of a patient who presented with a rash characteristic of meningococcal septicaemia. He was treated with antibiotics and referred to immunology where clinical suspicion of terminal complement deficiency was confirmed by absence of complement pathway activity. Ed also summarised a request audit which indicated that a significant number of complement pathway requests for adults were for inappropriate indications.

Dr Richard Steele then took to the floor to talk about determining assay performance and optimal cut offs. He highlighted the importance of considering a tissue bank and the challenges of assessing assay performance for rare antibodies where presentations may be varied and manifestations may occur at different times. In the absence of tissue banks, retrospective reviews could provide insight as was suggested by a review of inflammatory myopathy.

Paul Austin was again at his best presenting on a rare case of AMPA limbic encephalitis with engaging flair. He gave a brief overview of the various components of the limbic system and their functions emphasising the complexity and sophistication of the central nervous system. He presented on the importance of the relatively new demand managed neuroimmunology diagnostic service and an interesting case presentation on a 79-year-old patient diagnosed with a rare case of AMPA limbic encephalitis.

Andrew Soepnal gave an engaging presentation on considerations given when building one's fantasy integrated immunology laboratory and the challenges that come with new technologies. Of interest was Nova view which may assist with more efficient reporting of ANA slides and presents the possibility of working from home. Andrew however highlighted the challenges associated with reviewing results from the thumbnail view only stressing the possibility of missing weaker positives.

Dr Russell Barker presented on learnings from the European society of immunodeficiency conference. He talked about the sheer amount of information discussed at these types of conferences and how in order to bring these improvements and new technologies/assays back, more attendance is required from the scientist cohort in order to allow group digestion and dissemination of all the information and content.

Dr Campbell Heron presented on The role of the laboratory in IgG4-related disease providing an overview of IgG4 related disease, its pathophysiology, epidemiology and clinical manifestations. He interestingly highlighted irregularities seen in serum protein electrophoresis which demonstrated an association with IgG4 related disease but was not sufficiently sensitive/specific to be diagnostic. Several interesting case studies indicated electrophoresis and immunofixation can be helpful and that IgG4 concentration is useful but normal levels do not exclude IgG4 related disease.

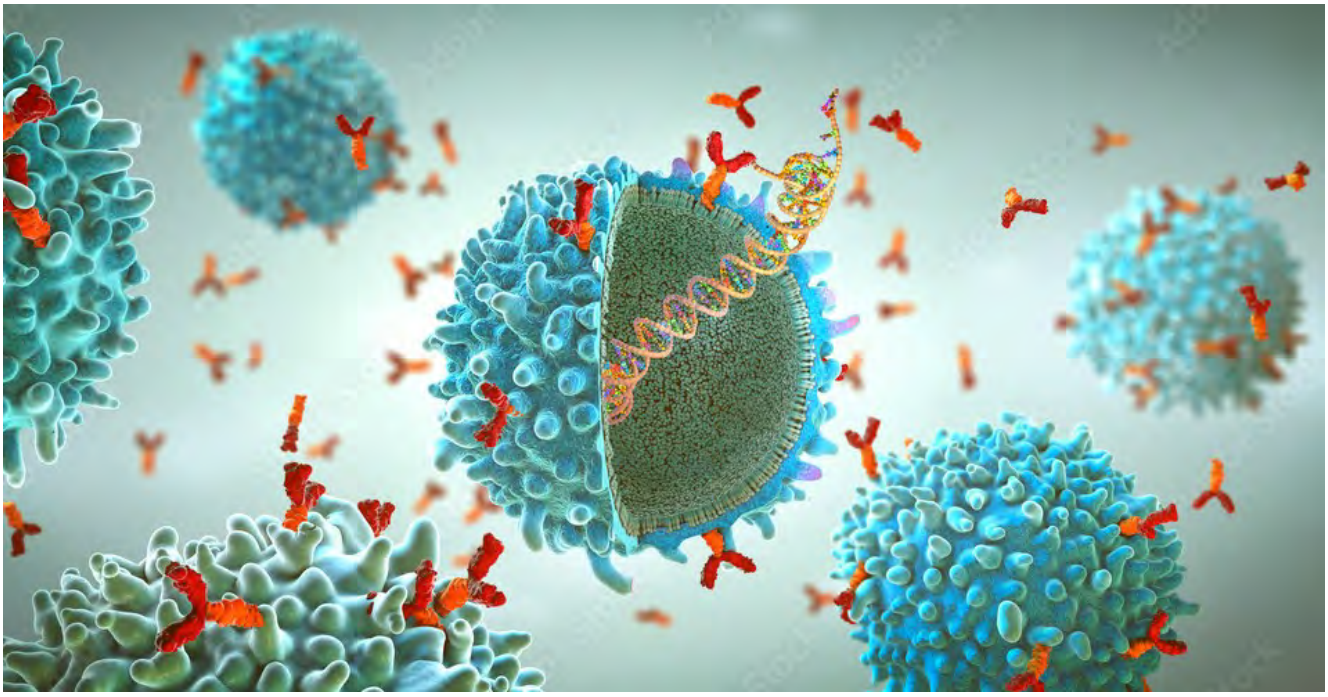
Puka Thakkar delivered an interesting presentation on Occular syphilis. She presented on a 69-year-old patient with rapid vision loss who was referred to neuro-opthamology and diagnosed with syphilitic chorioretinitis. The neurosyphilis was treated successfully indicating the importance of the laboratory in diagnosis of more complex cases.

To wrap up the day Shaleen Shah presented on a patient with type 2 cryoglobulinaemia and IgM paraproteinemia who despite being cured of hepatitis C after ribavirin treatment had persistent cryoglobulinaemia. Significant comorbidities paired with the thermolabile nature of cryoglobulins made this case a challenge for the clinical and laboratory staff.

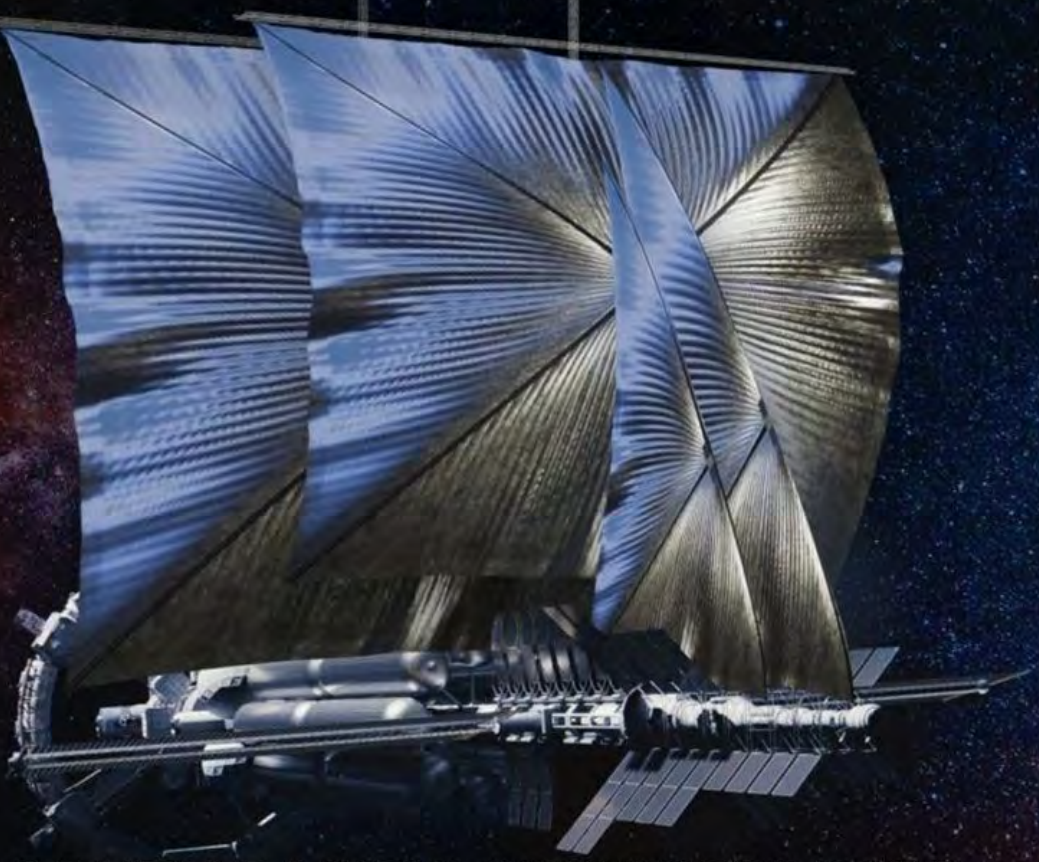
At the end of the day the two judges Sarah Burge and Helena Thompson-Faiva concluded that Paul Austin would win the prize for best presentation with an engaging and interactive presentation on a rare case of limbic encephalitis and Shaleen Shah would win the prize for best runner up presentation with a presentation on A "Tricky Picture" of type 2 cryoglobulinaemia and IgM paraprotein.

All acknowledged that it was a great privilege to be able to gather together again face to face to enjoy learning from one another, catching up with old acquaintances and building new connections.

Shaleen Shah
Immunology SIG Organiser 2022



Sailing Into the Future.....



SOUTH PACIFIC CONGRESS 2023

A full scientific programme
and great networking events await.

Join your colleagues from Australia, New Zealand and the Pacific.

Bloodisloe Cup to be contested!

Convenor: Ailsa Bunker:
Ailsa.Bunker@middlemore.co.nz

Registration opens in April at www.nzimls.org.nz

Proffered Papers & Posters Guidelines for Presentation at the South Pacific Congress 2023.

ABSTRACT GUIDELINES

For all presentations an abstract must be submitted for consideration by the deadline of **31 May 2023** and must adhere to the following guidelines. Abstracts will be printed in the conference booklet.

All abstracts must be:

- Submitted as a *Microsoft Word* .doc file ONLY.
- Use Times New Roman font, size 12, single line spacing, right justified.
- A maximum of 300 words in length, excluding references.
- Specify all abbreviations in full at first use, followed by the abbreviation in parentheses. Thereafter only abbreviations should be used.
- Checked thoroughly for spelling and grammar.
- References should be limited to a maximum of five. They should be numbered consecutively in the order they appear in the text and follow the Vancouver style.
- Structured as follows:

1. *Original* research:
   Title: in bold
   Authors: The principal author should appear first. Underline the name of the author who will be presenting the paper/poster (may be different to principal author). Use forename, initials and surname and omit degrees and titles. Include affiliations for each author. Use superscript numbering after the authors name to indicate affiliations.
   Objective: The purpose of the study; hypothesis tested.
   Methods: Brief description of materials, subjects and methods used.
   Results: The main findings of the study. Do not include tables, graphs or diagrams.
   Conclusion: The main outcomes and implications of the study.
2. *Case Studies*:
   These should follow the same guidelines as for original research but with the following headings in the body of the abstract:
   Clinical presentation: Relevant presenting clinical/radiological findings
   Discussion: Consideration of differential diagnoses and important points illustrated by the case.

Disclosure of interest statement:
NZIMLS and AIMS recognise the need for transparency of disclosure of potential conflicts of interest by acknowledging these relationships in publications and presentations. If your abstract is accepted, any financial support or sponsorship relevant to your presentation must be stated in your presentation or poster.

Selection criteria
Abstracts will be favourably reviewed if they are novel and incorporate original data of high quality that extends existing knowledge in the discipline of Medical Laboratory Science.
In balancing the programme, the organising committee may request authors to present their work in an alternate format e.g. poster rather than platform presentation.

Abstract submission
Abstracts must be submitted prior to the closing date by e-mail ailsa.bunker@middlemore.co.nz

Closing date for abstract submissions is 31 May 2023

By submitting an abstract all authors agree to the NZIMLS publishing the abstract in the conference booklet and in so doing certify that the abstract is original work. If the abstract does not conform to the guidelines detailed above, it will be returned to the submitting author to revise.

PRESENTATION GUIDELINES

ORAL PRESENTATIONS

- Audio visual material must be in a digital format suitable for data projection.
- Presentations should be in the latest version of Microsoft PowerPoint. If you intend using any alternative application to display your presentation you must confirm the feasibility of this with the conference organising committee via the NZIMLS.
- Bring your Power Point presentation on a USB storage device.

Tips for oral presenters

The following suggestions may be helpful when preparing your presentation:

- Check the information to be presented fits the time frame
- A coloured background works better than black and white. Good contrast between text and background is necessary to ensure visibility from the back of lecture theatres.
- Provide a brief (one slide) “Background” to start
- Limit the number of slides (Leave out text/images/tables you do not plan to discuss)
- Do not overcrowd each slide
- Identify up to five key points for each slide
- Avoid large amounts of text
- Use minimal text on a slide. Use several slides to cover a detailed topic that cannot logically be included on one slide.
- Ensure the font size is sufficiently large to be viewed from the back of the auditorium.
- Avoid fancy fonts – use fonts that are easy to read e.g., Times New Roman or Arial
- Use of different colours should be kept to a minimum.
- Where possible, utilise relevant graphs and diagrams to convey a message.
- Images should be of good quality and selected to highlight relevant features.
- Do not rely on computer screen colours, have the presentation projected prior to the conference
- If video clips or downloaded images are used, these should be checked well ahead of the presentation, preferably on a different computer if that is possible.
- Try not to read what is already on the slides – use them as a prompt to provide more details relating to the subject.
- Briefly state any anticipated outcome from the presentation
- Rehearse the presentation by projecting the presentation and presenting out loud to ensure it does not exceed the allotted time. Screen reading is faster than spoken words

NOTE: Do not attempt to download internet images or video clips during the presentation, this takes up the presentation time and often does not work.

Prior to the Session

Report to the speaker/rehearsal room at least one hour before the scheduled time of your presentation.
Notify the AV operator of any special instructions regarding your presentation.
Take advantage of the facilities provided to review your slides one last time.
Acquaint yourself with the operation of the podium, remote control and location of equipment.
Speak clearly in accordance with your slide sequence and use a pointer sparingly to guide the audience.

After your Presentation

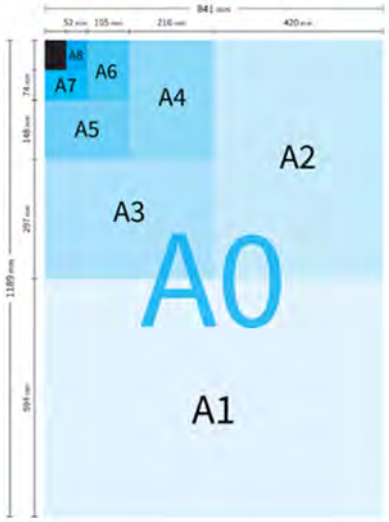
Collect your USB drive from the speaker rehearsal room/registration desk.

POSTER PRESENTATIONS

- The posters will be displayed throughout the conference in the main exhibition area.
- Poster boards will be available. Please bring your own Velcro to affix the poster to the display boards.
- On arrival at the conference please advise the registration desk that you have a poster, and you will be directed to the board for your presentation.
- The NZIMLS Hugh Bloore Memorial Poster Prize of $1,000.00 is awarded to the best poster provided by a NZIMLS member.

Tips for poster presenters

The purpose of a poster is to provide a visual summary of a study that will stimulate interest and discussion. The information needs to be displayed in such a manner that facilitates easy assimilation by the reader. Therefore, the poster should be easy to read and free from clutter.



The following guidelines are offered to assist you in the preparation of your poster:

- The poster **must either be no bigger than A1 in size**, refer to the paper size diagram on the right. Poster orientation (landscape or portrait) and layout is optional.
- At the top of the poster include the title, authors, affiliations and an abstract. The abstract on the top left-hand side of your poster is preferable but not compulsory and has to be 300 or less words.

Include the following subheadings:

- ◊ Introduction
- ◊ Methods
- ◊ Results / Conclusion
- ◊ Discussion / Summary
- ◊ References

- Try to indicate the order of the material, either by numbering or by using arrows if you are not using subheadings making it easy to follow when reading.
- There is no word limit, try to communicate the content clearly and precisely to the point with minimal description as it is a poster and not a journal article. Keep it simple and basic.
- Concentrate on the essential findings and cut out the rest, be selective with the information you are trying to convey. Make it interesting but keep it on topic. Your discussion/summary should leave the observer focused on the topic on hand and wanting more information from you.
- Avoid complex sentences; sentence fragments may be easier to comprehend. An alternative is to break text into chunks surrounded by plenty of ‘white space’ to facilitate easy reading. Bulleted lists are effective
- Graphs and diagrams are easier to scan than columns and data in a table. Legends should be brief
- Photographs should be carefully selected. Ensure they have sufficient sharpness and contrast to highlight the features discussed. The magnification of the image (at least the objective power use and stain type should be noted on relevant photographs).
- There is no set font or size, however it must be clear and adequately sized to be read from a distance of two meters. (e.g. 24 pt for the body text). The subheadings must be in bold and must be slightly larger than the main text.
- **Avoid having background that is a photo or a colour that will be conflicting with the text**, as this will make it harder for the observer to read. Dark print on a light background is easier to read. Minimise the range of colours used
- Do not write or paint on your poster. Laminating of the poster is optional.

Most importantly have fun designing your posters.

Prior to the Session

Report to the registration desk with your poster and you will be advised which display board to use.

At the Time of your Presentation

You must be present during the Poster Session when delegates are viewing your poster to answer any questions that may arise. Your presence may also facilitate a discussion that will encourage a two-way exchange of information.

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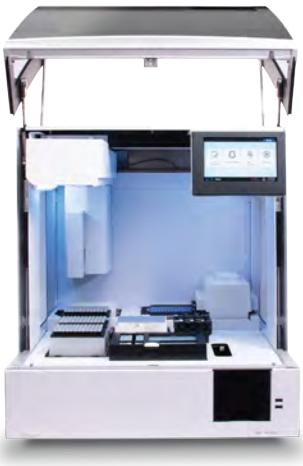

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