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New Zealand Journal of Medical Laboratory Science

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In this issue

Celiac disease is a heritable chronic inflammatory disease that generally leads to a wide spectrum of clinical symptoms. Yasaman Hajinabi and colleagues from Iran investigated whether the expression of key genes (NF-kB, REL, and TNFAIP3) induces inflammatory meditated NF-kB signalling changes in celiac patients treated with a gluten-free diet. They found that the disrupted gene pattern of the NF-kB pathway affected the optimal immune response control, indicating some interactive inflammatory reactions in celiac disease.

Autoimmune Bullous Dermatoses (AIBD) is a blister forming disease of skin and mucous membrane caused by production of autoantibodies against structural proteins in the skin. Paul Austin and colleagues investigated if any clinical and/or operational benefits accrued from changing the skin autoantibody indirect immunofluorescence methodology type from tissue-based pattern recognition to specific antibody (Dsg1, Dsg3, BP180, BP210) determination. They found that superior NPV and PPV values were obtained for the specific antibody versus the tissue – based method. An improved diagnostic value clinical service with faster reporting times at a reduced cost were the key findings from the study and ultimately were the drivers for dermatologists-endorsed decision to transition from tissue-based pattern to specific skin antibody target reporting at LabPLUS, Auckland City Hospital.

The role of anti-inflammatory cytokines in obesity is unidentified. In a case-control study Moushira Zaki and colleagues from Egypt measured serum IL-10 concentrations and messenger RNA expression in obese women with metabolic syndrome. They showed that obese women have low levels of IL-10, suggesting that this anti-inflammatory cytokine plays a crucial role in metabolic syndrome risk and obesity-related problems. Moreover, low mRNA expression suggests the contribution of genes to insulin-resistant states and metabolic syndrome in obese women.

Alterations in zinc, copper, and magnesium blood levels have been linked to gestational diabetes. Ewen Akpan and colleagues from Nigeria measured these minerals in women with pre-eclampsia and gestational diabetes mellitus to understand their relationship with these pregnancy disorders. They found significant changes in serum zinc in pre-eclampsia suggesting a relationship with pathogenesis of this disorder. No significant difference in copper and magnesium levels were found.

Monogenic involvement has been shown to be among the major determinants in the development of paediatric-onset systemic lupus erythematosus (SLE). In a case-control study Eman Eissa and colleagues from Egypt evaluated the expression pattern of deoxyribonuclease1-Like 3 (DNase1L3) at the mRNA and protein levels and their association with the disease activity and inflammatory markers in juvenile-onset SLE. They found that DNase1l3 expression pattern at the mRNA and protein levels was significantly lower while IL-1β and TNF-α levels were significantly higher. They conclude that DNase1L3 could be involved in the immune regulation of juvenile SLE patients and could be considered as a potential regulator of immune activation in those patients.

Factor XIII is a rare clotting deficiency whereby the formed fibrin clot is unstable and leads to a bleeding tendency. Routine clotting factor assays are usually normal leading to this disorder being missed in the first instance. In this case report a historic case of Factor XIII deficiency in New Zealand is described by Syd Shepherd (formally from Hamilton Hospital Laboratory and now retired) and highlights the classical initial problems with identifying this inherited disorder.

Pseudomonas aeruginosa is an important cause of nosocomial infections worldwide. Many *P. aeruginosa* are multidrug-resistant, and the carbapenem-resistant *P. aeruginosa* have been recognised as a global threat by the World Health Organization. A prevalent international clone of *P. aeruginosa* is ST235, which is associated with high-level antibiotic resistance and poor clinical outcomes. Sean Munroe and colleagues from Hamilton and ESR, Wellington report an isolate of ST235 *P. aeruginosa* harbouring IMP-26 in a patient with no history of overseas travel. They believe it is the first recorded case of IMP -26 producing *P. aeruginosa* in New Zealand.

Shugo Kawamoto from LabPlus, Auckland reports a case of a 31-year-old female with no medical history of liver disease or intake of herbal medicine with elevated liver function tests and marked coagulopathy consistent with a diagnosis of acute liver failure and a liver transplant was considered. Plasma paracetamol levels at 121 µmol/L (therapeutic range up to 100µmol/L) prompted clinical consideration of paracetamol drug overdose, but the patient denied taking paracetamol. The colorimetric principle for the paracetamol quantification gave rise to a falsely elevated paracetamol, confounding the clinical picture to suggest paracetamol overdose. Re-analysis of the patient's samples by ultrafiltration and HPLC demonstrated undetectable paracetamol levels. The colorimetric method is susceptible to spectrophotometric interference from bilirubin, and it is highly recommended that total bilirubin is analysed for all paracetamol analysis by laboratories using the colorimetric method.

Carbapenemase producing organisms have become a global health concern because they are multidrug resistant thus limiting therapeutic options for clinical management of infections. Ephraim Ibadin and colleagues from Nigeria and Canada. determined the prevalence of carbapenemase genes among clinical Gram-negative bacterial isolates in Benin City, Nigeria. They found that the prevalence of carbapenemase producing organisms was 8.9% with 80% being *P. aeruginosa*. The majority of carbapenemase producing organisms were MBL-producing, harboring NDM, VIM and NDM + VIM genes. All carbapenemase producing organisms were multidrug resistant.

Flammable refrigerant remains a commonly used chemical product in household type refrigerators and freezers used in the medical laboratory. Dennis Mok and colleagues from Australia, Armenia, Saudi Arabia, United Arab Emirates, Philippines, United Kingdom, and Vietnam bring to attention the medical laboratory's awareness of requirements relating to provision of relevant exposure control measures for flammable refrigerant in refrigeration systems to laboratory personnel.

The Editor, Rob Siebers from Wellington analysed the Journal using the Scopus database for the top ten most cited articles in the Journal and determined some characteristics of them. Out of a total of 420 articles from 1999-2022, 127 articles were cited at least once (30.2%). Fifty-seven of these articles had been cited one time only, the rest of cited articles (n=70) more than once. The ten top cited articles in the Journal were cited between eight to 29 times, The top cited article was from Nigeria. The author concluded that despite not being covered by the Web of Science or PubMed databases, the Journal has attracted citations to its published articles in the world biomedical literature as evidenced by the top 10 citated articles.

The current Editor, Rob Siebers is standing down in September 2022 and will be succeeded by the Deputy Editor, Lisa Cambridge. In a joint editorial Lisa identifies some of her own guidelines for the road ahead and Rob reflects on his 28 years as Editor. So, its hello from her and goodbye from him.

In a commentary the NZIMLS President, Terry Taylor, outlines the legislation for the health reforms, the Pae Ora Health Futures bill, and the involvement of the NZIMLS.

Rob Siebers
Editor

EDITORIAL

It's hello from her and goodbye from him

Lisa Cambridge and Rob Siebers

LISA

Hello! In this issue I find myself contemplating on those very large shoes I need to fill after Assoc. Prof. Rob Siebers' imminent departure. He has been a principle member of the Journal for many years and his contribution, advocacy and tenacity have been key to this Journal's success and is reflected in the Journal's readership and in the number of citations reported in this issue (1).

I share Rob's enthusiasm to help members achieve that very first publication and it is really what enticed me to this Journal. I am keen to continue this support for those outside the realms of academia and who have few opportunities to publish their work in a scientific peer-reviewed journal, and who contribute their experience and practical knowledge and improve healthcare at its grass-roots.

Writing and editing have been constant throughout my career and the tools I now apply to the Journal come from working in research and diagnostic sciences. I enjoy helping people communicate through writing but with my scientist's brain I always advocate for the delivery of solid evidence, clear research and well supported arguments. It seemed a natural step to move into a role as Editor and in the last 12 months with thanks to Rob, Michael Legge, the editorial team and Sharon Tozer I have learnt a lot and already identified some of my own guidelines for the road ahead:

- Be prepared for manuscripts to arrive in any state of completion, from minor proofreading to large meandering essays.
- Peer reviewers are gold and just as hard to find.
- There is so much out there you don't know that you don't know.
- No one is an expert on everything, ask for help.
- Authors do not read journal instructions.
- Show consistency in style, language, abbreviations, and terminology.
- Check for plagiarism, predatory journals, ethics, and author consent.
- Offer regular and respectful communication with authors and reviewers.

It is exciting times! The decision for its open access some years ago puts the Journal in a position to successfully navigate the constantly changing trends in the way we learn and our appetite for online information that is driving the future direction of scientific publishing.

Numerous technical advances and perspectives in laboratory medicine (2) will also be sure to feature, with more Artificial Intelligence (AI), Point-of-Care testing, automation, green technologies, new methods of testing and detection of disease.

The Medical Laboratory Science profession increased its profile in New Zealand and across the world during the Covid-19 pandemic and will do so again as New Zealand prepares for significant changes in the health and disability system. With the release of this July issue, Health NZ replaces the 20 District Health Boards and take responsibility for the day-to-day running of our health system together with the newly created Māori Health Authority who will work with Health NZ and the Ministry to ensure the reformed health system delivers improved outcomes for Māori (3).

As I look forward to these challenges ahead, I would like to thank you all for the warm welcome I have received and join with me in wishing Rob the very best and in thanking him for the massive contributions he has made to the Journal and to the profession.

ROB

In 1993 an icon of our profession and friend of mine, Jim le Grice, tragically passed away in a mountain climbing accident. While attending his funeral I was approached by the then NZIMLS President, Dennis Reilly, and offered the position of Editor as Marie Gillies, then Editor, had indicated to the NZIMLS Council that she wished to stand down from that position. Apparently, my name was suggested as I had published several papers in the Journal, and it was assumed I would know what editing a journal was all about. This could not have been further from the truth as I quickly became aware of. Over the years we have had to deal with editorial issues such as plagiarism, ghost authorship, author disputes, and attracting suitable reviewers, among others. Just being an established author does not prepare an Editor for such issues.

My first issue as Editor was in May 1994. I was aware that previous Editors had struggled at times to attract papers, and this was also my experience. Despite attending numerous SIG and other scientific NZIMLS meetings urging presenters to submit, it was hard work attracting papers and we would often end up with only a few papers for an issue. This was an issue for the Journal questionnaire, which was started in 2007. The questionnaire, comprising 10 questions, is derived from published articles in each journal issue and provides valuable CPD points for our practitioners.

Nearly 10 years ago I persuaded the NZIMLS Council to make the Journal platinum open access. This is a model where an organisation (NZIMLS) owns the journal but does not charge authors a publishing fee and the authors retain copyright of their published article under a Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original authors and source are credited. This has resulted in an increase in articles submitted to the Journal, many from overseas. I have been criticised by some of our members who believe our Journal should remain focussed on New Zealand, as implied by our Journal's name. However, we believe our Journal's focus is on medical laboratory science and as long as the science is valid and conclusions are based on results, the country of origin is irrelevant. Since making the Journal truly international we have had a satisfactory increase in accepted articles without compromising quality and the Journal has an international reputation (4).

I particularly like case studies for the Journal as they highlight what medical laboratory science is all about and have written on how to write up a case study for the Journal (5). All articles submitted to the Journal undergo single blind peer review (where the identity of the reviewers is anonymous, but identity of the authors is known to the reviewers). To help our reviewers, many of whom are practicing medical laboratory personnel without reviewing experience, we have also published guidelines for reviewers as an aid (6).

A few years ago, we called for expressions of interest to take over as Editor once I relinquished the role. Lisa Cambridge from Dunedin expressed an interest and joined the editorial team as a Deputy Editor from the April 2021 issue onward. Under tutelage and support from myself and Michael Legge (the other Deputy Editor), Lisa quickly mastered all aspects of what the position entailed. She will take over the baton as Editor at the AGM of the NZIMLS early September this year. I have no doubt she will be an excellent Editor and hope the members of our profession will give her all their support, be it as an Editorial Board member, author, or reviewer when requested, and wish her well in her new role.

Finally, my heartfelt thanks go to present and past NZIMLS Councils, Sharon Tozer (and Fran van Til in the past) in the

Executive Office, and the Editorial Board members during the last 28 years. It's a role I have thoroughly enjoyed, and my biggest thrill always has been helping a member of our profession here in New Zealand achieve their very first publication in the Journal. So, it is goodbye from me and a warm hello from her.

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Rob Siebers

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NZIMLS Journal Prize

The NZIMLS Council has approved an annual Journal prize to the value of NZ$300 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 NZIMLS 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 (April, July and November issues) will be considered. The Editor, Deputy Editor 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 2021 Journal prize was awarded to Paul Austin from LabPlus, Auckland for the article **The design and implementation of a novel standardised training and assessment tool at LabPLUS, Auckland Hospital, NZ for anti-nuclear antibody (ANA) pattern reading using IIF methodology in a non-automated digital microscopy setting** published in the April 2021 issue, pages 42-50.

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PERSPECTIVE

Covid-19 journey: A scientist's warning from inside out amid 'mild' labelling of Omicron

Terry Taylor

Terry Taylor, NZIMLS President

I was shocked at the ferocity of my symptoms when I tested positive for Covid-19. We are constantly told Omicron is a mild illness; nothing to worry about, most people get mild symptoms and certainly nothing to get concerned about. For context, I am a fit and healthy 55-year-old man (1.80m tall and 90kg) who exercises most days. I work full time as a medical laboratory scientist in a hospital setting. I am double vaxed, boosted and closely follow public health measures to the nth degree.

I was woken on a Thursday night by an intense sneezing fit that was like nothing I had ever had before. To be safe and sure I thought I had better take a rapid antigen test (RAT) before heading into work at the laboratory on the Friday. The positive test strip appeared within 30 second and confirmed that I was now another positive Covid-19 case. I was shocked, as apart from the sneezing fit, I felt completely fine. I had been for two long runs and trained at the gym during the previous few days and most certainly didn't have an inkling about having Covid-19. This is clearly one very stealthy virus that knows how to spread, prosper, and survive.

The first 24 hours I experienced a variety of minor symptoms: a headache coming and going, more sneezing and the start of a dry hacking cough. I did still have my taste and smell and no drop in appetite. I was thinking 'this isn't all bad, I don't feel too bad, and I have a week at home to catch up on all my overdue tasks'. I had a full day of remote meetings and phone calls on the Friday to keep me busy and was wondering how I was going to fill in my sudden increase in spare time. Just when things looked rosy, the fever hit overnight, I woke to a soaking T-shirt and sheets and a blinding headache that wasn't quite like anything I had had before. I normally don't take any medication for anything but took some anti-inflammatory and antihistamines, both recommended by trusted health professionals.

The next part is harder to explain, random joint and bone pain that came and went. My headache dropped down a level and I tried to write and answer a few e-mails. I found I couldn't concentrate at all, and I was forgetting names, places, and terminology for no good reason.

Figure 1. The RAT which alerted Terry Taylor that he had contracted Covid-19.

Even though it was a warm, calm day I started to shiver and feel very cold, I put more clothes on, but this didn't help so I lit the fire. My neighbours must have wondered whether I had truly gone mad! And the damn headache was back, along with the random bone pain. My dry cough was now constant, and every cough made my head throb more. Luckily, I still had my sense of taste and an appetite, so it was time for dinner and an early night. Unfortunately, it was another night of drenching sweats, but I woke feeling better than the day before. I took more medication and wondered whether I was ever going to improve. The lingering dry cough was very annoying and interrupted just about anything I tried to do, my nose was dry, but I had an ache in the sinus region.

I noticed I was strangely fatigued while just moving around. Standing up felt like I had just done a 200kg squat and simply stepping outside saw me huffing and puffing like I had run up a steep hill. My brain was still not functioning right but at least I could remember my colleagues' names again. The random body pains were becoming more debilitating as the day went on.

By Sunday night I was feeling strangely cold again despite the warm day and evening. And that damn headache wouldn't go away. The muscles in my legs felt tired and I was starting to get random cramps despite eating and drinking normally and not exercising. I take magnesium and zinc supplements after long

runs so took some prophylactic tablets before bed. I felt warm in bed but every time I rolled over, I got that awful leg cramp feeling and ended up drifting off to sleep on my back. I was only up to day four and already it seemed a lingering infection that would be difficult to shake.

Every time I medicated to control my symptoms; I got a short period of relief before another set of different symptoms kicked in. By day six, I was well and truly over it. I felt weak, shaken and had a dry hacking cough like a pack-a-day smoker. I did my obligatory RAT, but alas it was still positive, though it took five minutes to become positive this time not 30 seconds, so I believed I must be healing. Finally, on day eight, the test line on the RAT was barely detectable and the remnants of my hacking cough were all but gone.

Omicron for me was not some mild cold or flu. I seldom get ill with anything and can't remember when I last had more than two days with any illness. My body feels shaken up and I know it will take a while to get through and over this. It felt like my immune response was chasing the virus around my body as it played a real time cat-and-mouse game of mimicry and antibody evasion. It still feels like a mild concussion, without the impact injury.

As a scientist, I think a lot about symptoms translating to medical findings: it seems realistic to hypothesise that Omicron may stimulate reactions that can cause long-term immune activation against our neurologic systems, so I urge other medical professionals to be mindful of this. Having this virus render my usually fit and active body a pile of misery has been disconcerting and the ease with which it promoted immune chaos is scientifically worrying. We clearly need to listen to and support those scientists who are chasing the vital diagnostic markers that may hold the key to long term reactivation of lingering symptoms.

The big thing I worry about is that we're once again going to have an 'ambulance at the bottom of the cliff' approach when it comes to the longer-term impacts of Covid-19 infection on people's health. This has been an experience that I most certainly don't want others to go through and I urge everyone to use every health measure available to mitigate the risk to the most vulnerable members of our communities. I don't wish my experience on anyone.

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Dr Ashley Bloomfield

Past Director-General of Health and Chief Executive

Dr Bloomfield qualified in medicine at the University of Auckland in 1990 and after several years of clinical work specialised in public health medicine. His particular area of professional interest is non-communicable disease prevention and control, and he spent 2011 at the World Health Organization in Geneva working on this topic at a global level.

Dr Bloomfield was Chief Executive at Hutt Valley District Health Board from 2015 to 2018. Prior to that, he held a number of senior leadership roles within the Ministry of Health.

Dr Bloomfield will be giving the Keynote Address at the NZIMLS Annual Scientific Meeting on Wednesday 31 August.

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ORIGINAL ARTICLE

The genes expression of NF-κB inflammation pathway in treated celiac disease patients

Yasaman Hajinabi, Elham Aghamohammadi Khamene, Flora Forouzesh, Fahimeh Sadat Gholam-Mostafaei, Seyed Abedin Hosseini Ahangari, Reza Mahmoudi Lamouki and Mohammad Rostami Nejad

ABSTRACT

Objectives: Celiac disease (CD) is a heritable chronic inflammatory disease that generally leads to a wide spectrum of clinical symptoms. The present research aimed to investigate whether the expression of key genes (NF-kB, REL, and TNFAIP3) induces inflammatory meditated NF-kB signaling changes in patients treated with a gluten-free diet compared to the healthy group.

Methods: Biopsy specimens from the distal duodenum and blood sampling were collected from 50 patients with CD (37.06 ± 7.02 years old) under gluten-free diet for at least 1 year and 50 healthy individuals (34.12 ± 4.90 years old) served as a control group. RNA was extracted from samples, cDNA synthesised and primer pairs were designed for NF-kB, REL, and TNFAIP3 gene expression. Quantitative real-time PCR was used to analyze the relative gene expression.

Results: A total of 50 CD patients (72% men and 23 % women) were included in this study. The results showed that the expression of NF-κB1 and c-Rel in tissue sample and blood samples did not have a significant difference compared to the control group (P > 0.05), whereas the expression of TNFAIP3 was significantly lower than the control group (P< 0.05).

Conclusions: Generally, it seems that the disrupted gene pattern of the NF-kB pathway can affect the optimal immune response control, indicating some interactive inflammatory reactions in CD patients. These results light unknowns in interactive inflammatory reactions in CD patients and explain the common complex immune reaction in CD.

Keywords: Celiac disease, gluten-free diet, NF-kB, c-Rel, TNFAIP3.

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INTRODUCTION

The common autoimmune gluten-sensitive enteropathy is called celiac disease (CD) (1). CD is a heritable chronic inflammatory diseases that generally leads to a wide spectrum of clinical symptoms (2). The anti-tissue transglutaminase antibodies elevations and histopathological damage to the small intestine with villous shortening, chronic inflammation, intra-epithelial lymphocyte infiltration, and activation of lamina propria T cells are generally seen in CD patients (3,4). Nevertheless, when the patients are follow the gluten-free diet, the histopathological signal goes into remission (5). Some evidences indicates that the T-cell mediated immune response to gliadin plays a key role in starting events in the pathogenic cascade of CD (6,7). Also, the pathway of the gluten-induced immune response in CD is not yet completely understood to date (8). However, the recent discovery of candidate genes identified by genome-wide association studies has led to tremendous progress (9,10). Therefore, determining the pattern of these gene expressions may guide to finding reliable pieces of functional pathways that complete the puzzle of the gluten-induced immune response (11). A common paradigm in the pathogenesis of the CD is the genes whose expression is induced in the inflamed mucosa such as NF-kB pathway genes and important mediators such as REL and TNFAIP3 present CD-associated variants (9,12).

NF-kB is a transcription factor and a crucial regulator of lymphocyte activation and adaptive immune response. Additionally, recent studies showed that gliadin effects on enterocytes could be mediated through oxidative stress, followed by NF-kB activation and IL15 up-regulation (13). Some signaling mediators and regulatory mechanisms such as TCR/CD28 colligation can induce NF-kB activation (14). The NF-kB activation involves phosphorylation and degradation of small cytosolic IkB inhibitors, catalyzed by the IkB kinase complex leading to the transcriptional activity of NFkB1/RELA heterodimers (15,16). In this signaling pathway, a MALT1 inhibitor is an important procaspase modulator that can cleave TNFAIP3 leading to the loss of NF-kB inhibition downstream of IL1- and toll-like receptor signaling (17). Thus, the study of the gene expression of the most central functional components in the NFkB route may help to understand the inflammatory reaction in the CD patients. The present research aimed to investigate whether the expression of key genes (NF-kB, REL, and TNFAIP3) induces inflammatory meditated NF-kB signaling changes in the blood and biopsy specimens of celiac disease patients under gluten-free diet compared to the healthy group.

MATERIALS AND METHODS

Patients

Biopsy specimens were obtained from 50 patients with CD and 50 individuals without any history of autoimmune diseases served as the healthy control groups. Subjects were referred to the Research Institute for Gastroenterology and Liver Diseases, Taleghani Hospital,Tehran, Iran during 2018-2019. Four biopsy specimens were obtained, histological examination was performed on three of these, while one sample tissue was immediately frozen in liquid nitrogen beforentesting. Diagnosis of CD was based on positivity for anti-transglutaminase antibody (anti-tTG IgA), villous atrophy, typical mucosal lesions with crypt hyperplasia, and increased number of intraepithelial lymphocytes according to the modified Marsh classification (18). The control group was negative for anti-tTG IgA, and their duodenal histology was normal. Blood samples were collected from all CD patients and healthy controls. Informed consent was received from each individual who has been nominated for blood and tissue screening and gene expression analysis of Nf-κb1, c-Rel, and TNFAIP3. This study was approved by the local ethics committee of the University Islamic Azad, Iran (IR.SBMU.IRGLD.REC.1398.006).

RNA extraction and cDNA synthesis

RNA was extracted from blood buffy coat and biopsy specimens by using a commercial kit (YTA Total RNA Purification Mini Kit, Yektatajhiz Azma, Tehran, Iran) according to the manufacturer's instructions. The quantity of the extracted RNA was determined by a NanoDrop spectrophotometry (NanoDrop Technologies, Wilmington, US) at wavelengths: 260 and 280 nm and the quality of the RNA was analysed by agarose gel electrophoresis. Then, total RNA (1 µg) was reverse transcribed into High Capacity cDNA using a Reverse Transcription Kit (Primer Script TMRT Reagent Kit, Takara Bio, Kusatsu, Shiga, Japan) according to the manufacturer's instructions. cDNA samples were stored at -20°C until used as a template for Q-PCR.

Quantitative real-time PCR

The primers were designed using the GeneRunner software and Primer3 (ver.4) online program (19). The sequence of these genes is shown in Table 1. Quantitative real-time PCR was performed by using a Rotorgene Real-Time PCR System (Hilden, Germany) and SYBR Master Mix (BioFACT™ 2X Real-Time PCR Master Mix including SYBR® Green I (Daejeon, Korea) according to the manufacturer's instructions for the detection. The samples were amplified in 20 µL reaction mixtures containing 10 µL SYBR Green Master Mix, 1 µL of cDNA, and 0.5 µL of each primer and 8 µL water for three

genes, as well as 1μL of each primer and 7μL water for housekeeping gene (β2M). For each primer pair, NTC (no template control) was used, and cDNA sample was replaced by RNase-free water. Instrument settings for all amplification reactions were 95°C for 15 min; 40 cycles of 95°C for 5 sec; 59°C for 40 sec; and 72°C for 40 sec for NFkB1 gene; 95°C for 15 min; 40 cycles of 95°C for 5 sec; and 59°C for 40 sec for c-Rel gene. For the TNFAIP3 gene amplification reactions were 95°C for 15 min; 40 cycles of 95°C for 5 sec; and 59°C for 40 sec. The melting curve shows the absorption peak, and all samples were run in triplicate. The analysis of gene expression was performed based on the relative gene expression by analyzing the melting curve of the ΔΔCt method. The constitutive expression of target genes in the tissues was calculated as a ratio of the $2^{-\triangle Ct}$ target gene and endogenous reference gene, according to the 2 method (20), where the ΔΔCt is the number of PCR cycles at which the signal of fluorescence exceeds the threshold level. The relative gene expression in each group samples was shown as a fold change between the level of gene expression in control and patient samples and was calculated using the $2^{-\triangle\triangle Ct}$ method (20-22).

Data analysis and statistics

The exact ΔΔCt method was used to calculate the relative expression of each gene. SPSS (ver.20) software was used for statistical analysis of clinical symptoms and findings. Graph pad Prism (ver.7) software was also used to assess differences between groups using t-test and ANOVA, and Spearman correlation was used to assess differences between groups (23). Sstatistical significance was defined at P<0.05

RESULTS

Characteristics of patients

The average age of the patients and control groups were 37.06, ± 7.02 and 34.12 ± 4.90 years respectively and the majority of CD patients were male (72%). The mean of BMI in controls (27.11 ± 3.42) was higher than the patients' group (20.23 ± 5.78). The frequency of gastrointestinal symptoms and extra- intestinal symptoms in the CD patients are shown in Table 2. Based on this result diarrhea (60%) and abdominal cramps (58%) were the most common GI symptoms and weight loss (70%) and anaemia (62%) were the most frequent extra-GI symptoms. Based on the results of Fisher's exact test, no statistically significant difference was observed between extra-gastrointestinal symptoms and pathological findings in the CD group (P<0.05).

Gene expression in monocytes

The results of real-time PCR data showed that the mean expression of NFkB1 gene in the PBMC of patients group was 1.039 and the mean expression of NFkB1 gene in the healthy group was 0.987 and this difference was not statistically significant (p=0.472). In addition, the mean expression of c-Rel gene in the group of patients and control group were 1.087 and 0.895 respectively. Accordingly, there was no significant difference in the expression of c-Rel gene in the control group and CD patients (p=0.439). On the other hand, the results showed that the mean expression of TNFAIP3 gene in the group of patients was 0.8032 and 1.6882 in the control group. Therefore, the expression of TNFAIP3 from patients who underwent a gluten-free diet for six months to one year was significantly lower than the control group (P<0.001; Figure1).

Gene expression in tissues

As shown in figure 2, the analysis of gene expression of NF-κB1 and c-Rel in tissue of patients were not significantly different compared to the control group (p=0.254 and p=0.341 respectively). Our results demonstrated that the expression of TNFAIP3 of patients who were on a gluten-free diet for more than six months was significantly lower than the control group (p=0.03).

Table1. Sequences and specifications of primers.

Target gene	Oligonucleotide sequences (5'-3')	Product length (bp)	TM (°c)
NFkB1	AGAAGAAGTGCAGAGGAAACGT CCACCGCCACTACCAAACAT	111	58.39 59.35
c-Rel	TGAACAACCCAGGCAGAGG TGTTCGGTTGTTGTCTGTGC	100	58.83 57.30
TNFAIP3	TCATCCACAAAGCCCTCATC ATT GCCGTCACCGTTCGTTT	119	57.30 57.30
β2M	TGCTGTCTCCATGTTTGATGTATCT TCTCTGCTCCCCACCTCTAAGT	112	59.70 62.12

Table 2. Frequency distribution of people based on gastrointestinal symptoms and extra-intestinal symptoms in CD patients.

Symptoms		(%)
Gastrointestinal symptoms	Bloating	22
	Diarrhea	60
	Nausea and vomiting	32
	Abdominal cramps	58
Extraintestinal symptoms	Weight loss	70
	Anaemia	62
	Bone diseases	34
	Nervous problems	16
	Menstrual problems	12
	Infertility	6
	Abortion	16
	Skin problems and aphthous stomatitis	2

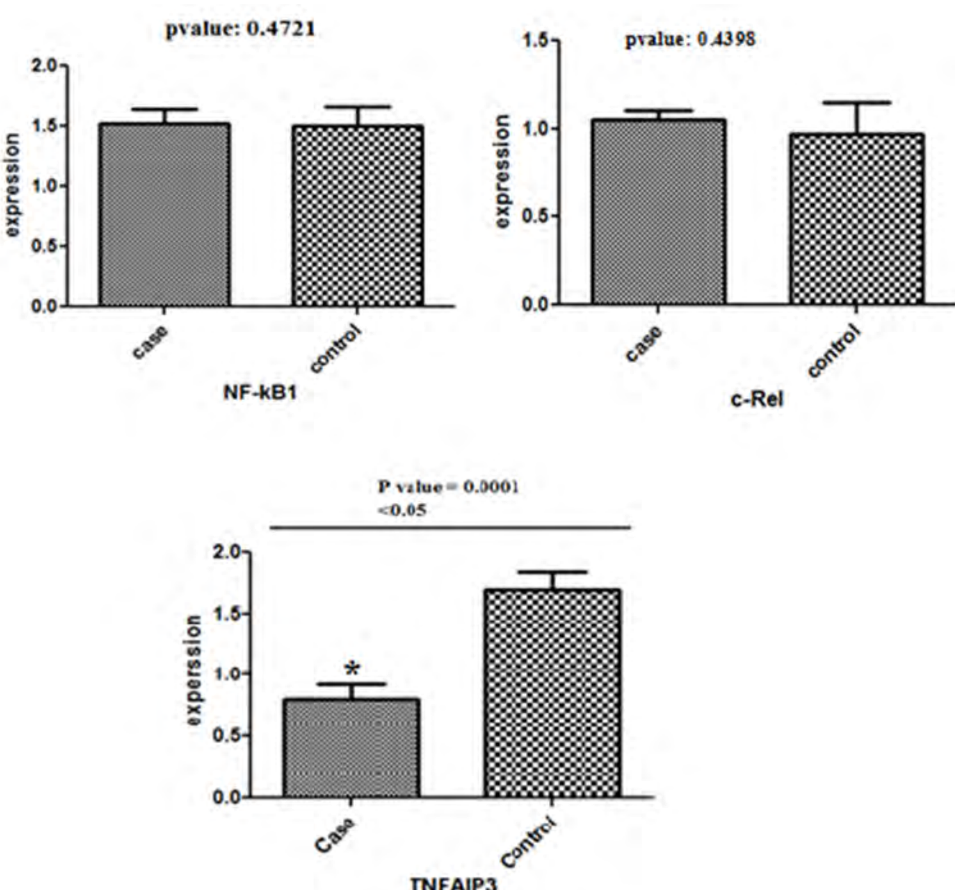

Figure 1. Constitutive expression of NF-kB1, c-Rel, and TNFAIP3 in monocytes from the control group (right bar) and CD patients who received the gluten-free diet for six months to one year (left bar). Gene expression was quantified by RT-qPCR and their expression level was expressed relative to the expression of the β2M gene (n=25). The star indicates significant differences between groups (p<0.05, mean± SD).

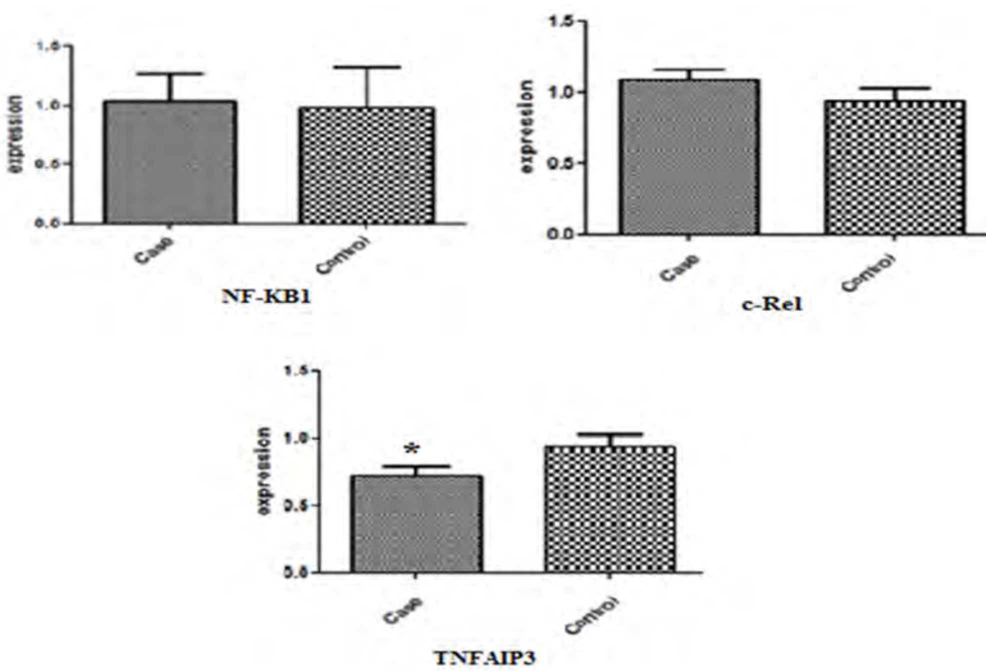

Figure 2. Constitutive expression of NF-kB1, c-Rel, and TNFAIP3 in the tissue of control group (right bar) and tissues of CD patients who received the gluten-free diet for six months to one year (left bar). Gene expression was quantified by RT-qPCR and their expression level was expressed relative to the expression of the β2M gene (n=25). The star indicates significant differences between groups P<0.05; mean ± SD.

DISCUSSION

Evidence show that the NF-kB pathway is constitutively upregulated in CD (12). Previous studies confirmed that NF-kB is a key regulator of inducible gene expression in both innate and adaptive immune responses (24). Besides, the NF-kB family transcription factors can control the development and maintenance of the cells and organs that comprise the immune system at multiple stages (25,26). Since the characteristics and functions of NF-kB and key NF-kB-mediators, such as REL and TNFAIP3, have been associated with susceptibility to CD (9), this pathway is an interesting candidate to have a prominent effective role in the development of the CD.

According to our results, the expression of NF-kB and Rel genes in monocytes and intestinal tissue of CD patients was not significantly different from the control group but the expression of the TNFAIP3 gene in both targets were significantly decreased in CD patients. The tumor necrosis factor-alpha-induced protein 3 (TNFAIP3) gene is a TNF-inducible gene, which is a negative feedback inhibitor of TNF signaling (27). Recent genome-wide association studies have revealed associations between TNFAIP3 and CD (9). The expression of TNFAIP3 in inflammatory responses can modulate the ubiquitination status of central components in NF-κB, IRF3, and apoptosis signaling cascades; therefore, it can restrict and terminate inflammatory responses (28).

Cielo *et al.* showed that the expression of NF-kB pathway genes, such as NF-kB and TNFAIP3, were upregulated in untreated CD patients and consequently the inflammatory processes genes were up-regulated, whereas the genes involved in the cell adhesion/integrity of the intestinal barrier were down-regulated (29). Thus, our study showed that strict gluten-free diet for six months to one year may modulate NF-kB pathway genes and cause downregulation of TNFAIP3 genes. It is hypothesised that, the abnormal response to dietary antigens may not be related to the regulation of molecular pathways but abnormalities of gene structure. Besides, disrupting the regulatory equilibrium of the NF-kB pathway and the effect of gene expression may be related to alteration in the methylation of NF-kB-related gene promoter (13). These hypotheses require further research.

A small sample size, the absence of CD patients who are not on a gluten-free diet (new cases), as well as the lack of protein base methods for validation of the results were the limitations of this study.

In conclusion, our study has revealed the different expression of the candidate genes in the NF-kB pathway between CD patients who received a gluten-free diet for six months to one year and the control group in monocytes and intestinal tissues. We also observed downregulation of the TNFAIP3 gene in CD. The disrupted gene pattern of the NF-kB pathway can affect different gene levels of this pathway and provoke the disruption of the tight regulatory equilibrium that ensures its optimal immune response control. These results highlight unknowns in interactive inflammatory reactions in CD patients and explain the common complex immune reaction in CD.

ACKNOWLEDGMENTS

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Science Snippet

A brief view of pandemics.

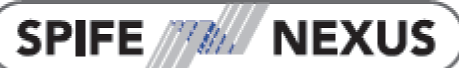
While the COVID-19 pandemic is a significant concern it is important to remember that humans have always been subject to the outbreaks of pandemics throughout history. Infectious agents have either directly or indirectly caused significant numbers of deaths in human recorded history. *Yersinia pestis* is considered to have killed approximately 100 million people in the Justine plague (541BCE) and the "Black" Death (1340s). Tuberculosis became a pandemic disease in Europe in the 1500s, killing in the high millions. Yellow fever killed approximately 25000 American colonial settlers from 1793. In the 20th century "Spanish" influenza is estimate to have caused approximately 50 million deaths and HIV/AIDs an estimated 37 million deaths. Influenza-like epidemics were regularly observed in Europe notably 1173 to 1187 and 1458 to 1551. Although it is taken for granted that in the 20th and 21st centuries the infectious agents can be identified and hopefully treated, it is a sobering thought that the agent causing "Spanish" influenza pandemic in 1918 was unknown at the time and was identified as *Bacillus influenza* (subsequently shown to be *Haemophilus influenzae* in 1930) from work with the Russian influenza outbreak in the 19th century. Laboratory based research with "Spanish" influenza during the pandemic using tissue extracts from the infected dead clearly demonstrated that the infectious agent passed through Chamberlin candle microbial filters and the filtrate retained infectious properties giving rise to speculation of a virus. However, it was not until the 1930s that the virus was identified and its highly infectious properties demonstrated by a virus-infected ferret sneezing on a laboratory worker who subsequently developed flu. The origins of "Spanish" influenza are still not fully understood but the troop movements and accommodation conditions at the time in both the USA and Europe certainly helped to cause the pandemic as well as the failure to recognize the highly infectious nature of the disease at the time. A sharp contrast to the international response to COVID-19.

Dr Joanne Hewitt

ESR Ltd

Dr Joanne Hewitt leads the enteric, food and environmental virology work at the Institute of Environmental Science and Research (ESR), Porirua, where she has worked for 25 years. Joanne and her team work on the detection, characterisation and surveillance of enteric viruses including norovirus, rotavirus and hepatitis A virus. Her research work centres on the detection and persistence of human viruses in the environment/waters, food and shellfish. In the past 2 years, Joanne has also been working on the detection and quantitation of SARS-CoV-2 RNA in wastewater. Dr. Hewitt has published over 50 peer-reviewed publications and is an IANZ technical expert.

Dr Hewitt will be speaking at Plenary Session 3 of the NZIMLS Annual Scientific Meeting on Thursday 1 September.



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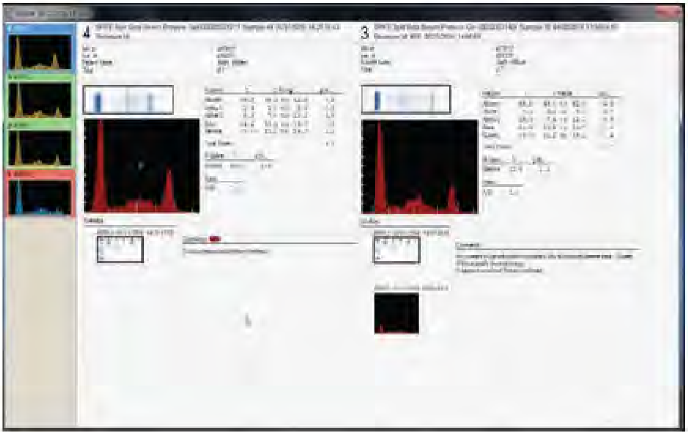

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ORIGINAL ARTICLE

Clinical and technical assessment of the EUROIMMUN Dermatology Mosaic 7 BIOCHIP IIF assay: evidence in favour of change from traditional tissue based indirect immunofluorescence methodology

Paul M Austin, Yulia J Hwang, Caroline L Allan, Helena T Thompson-Faiva and Rong Zhou

ABSTRACT

Objective: To determine if any clinical and/or operational benefits accrued from changing the skin autoantibody Indirect Immunofluorescence (IIF) methodology type from tissue-based pattern (ICS / BMZ) recognition to specific antibody (Dsg1, Dsg3, BP180, BP210) determination.

Methods: 168 unselected retained patient sera from routine skin autoantibody testing were tested in a randomised format using the Euroimmun Dermatology Mosaic 7 BIOCHIP IIF assay. Results were correlated against those patients (N=89) that had skin biopsy results available.

Results: Superior NPV and PPV values were obtained for the specific antibody versus the tissue – based method (NPV: 89.5% vs. 84.8%, PPV: 86.4% vs. 69.6%). At LabPLUS, transition of methodology would allow reduced reporting times for antibody positive patients (35% - 95% improvement) as well as delivering reduced operational consumable costs (NZD $7,000 – NZD$8,000 p.a.).

Conclusions: An improved diagnostic value clinical service with faster reporting times at a reduced cost were the key findings from the study and ultimately were the drivers for dermatologists endorsed decision to transition from tissue-based pattern to specific skin antibody target reporting at LabPLUS, Auckland City Hospital, New Zealand.

Key words: Euroimmun Dermatology mosaic 7 IIF biochip [EIIF], PV disease, BP disease, Skin biopsy, Tissue-based Indirect Immunofluorescence [TBIF]

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INTRODUCTION

Autoimmune Bullous Dermatoses (AIBD) is a blister forming disease of skin and mucous membrane caused by production of autoantibodies against structural proteins in the skin. The two main structural proteins that are targeted by autoantibodies in AIBD are desmosome and hemi-desmosome, where desmosomes are responsible for cell-to-cell adhesion between keratinocytes within epidermis and hemi-desmosomes serve as an anchorage for basal keratinocytes to the basal membrane zone allowing adhesion between epidermis to dermis. The loss of adhesion between keratinocytes or between basal keratinocytes and underlying epidermal basal membranes cause lack of resilience of epidermises, resulting in intra-epidermal blistering or sub-epidermal blistering (1).

Based on the specific antigenic targets of the autoantibodies and the site of blistering AIBD can be classified into 2 main groups namely (a) pemphigus diseases which includes pemphigus vulgaris, pemphigus foliaceus and paraneoplastic pemphigus and (b) pemphigoid diseases which includes bullous pemphigoid, mucous membrane pemphigoid, epidermolysis bullosa acquisita and dermatitis herpetiformis (2).

Pemphigus vulgaris [PV] is the commonest of the pemphigus diseases [PD] accounting for 70% of all cases in an age range of 40 to 60 years (3). In PV, antibodies target desmosomal proteins Desmoglein 1 [Dsg1] and Desmoglein 3 [Dsg3] in which Dsg1 is typically expressed on epidermal surface whereas Dsg3 is in deeper epidermal layers and mucous membranes (4, 5). Dsg3 is the major autoantigen in PV however 50 to 60% of patients are known to have additional antibodies to Dsg1. Depending upon the combination of Dsg1 and Dsg3 antibodies, phenotypic PV clinical variants are seen (4-6).

Bullous pemphigoid [BP] is the most common type of AIBD which primarily affects older patients [>70 years] and is seen more frequently in female subjects (1-2). In BP antibodies target the hemi-desmosomal proteins namely BP180, a transmembrane-based glycoprotein and BP230 which is a cytoplasmic-based glycoprotein (7- 8). BP180 is known as the major immunogenic antigen in BP where 85-90% of BP patients are positive for anti-BP180. Although the seroprevalence of anti -BP230 in patients with BP approximates 50%, anti-BP230 is an important additional marker for the diagnosis of BP since a proportion of anti-BP180 negative BP patients are known to have positive anti-BP230 antibodies (9). Autoimmune reaction triggered by antibodies against BP180 and BP230 cause sub-epidermal loss of adhesion which results in tense and thick roofed blisters mostly impacting skin whereas mucous membrane involvements are rare (7).

Laboratory diagnosis of AIBD is mediated via (a) direct examination of lesional skin or mucosa (Direct Immunofluorescence [DIF] and/or histopathological evaluation) and (b) indirect serological detection of circulating autoantibodies using indirect immunofluorescence [IIF] or Enzyme-linked immunosorbent assays [ELISA]. DIF is widely regarded as the gold standard method for diagnosis of AIBD. However, serology is advantageous in terms of its minimal invasiveness of the procedure and in those cases where obtaining a biopsy specimen is either not possible or is problematic (10). Further, in two recent large patient cohort studies where there were clinically characterised PV and BP patients as well as disease controls, the monkey oesophagus tissue based IIF method was shown to have high levels of specificity [PV:93%, BP:96.5%] and moderate levels of sensitivity [PV:87.1%, BP:73.5%] (11-12).

At LabPLUS, the serological detection of skin autoantibodies is delivered via IIF methodology using monkey oesophagus substrate. This allows differentiation of two characteristic patterns namely (a) linear staining of basal membrane zone [BMZ] which is associated with pemphigoid diseases and (b) “chicken wire” like fluorescence staining of intercellular cement substance [ICS] which is associated with PD (13).

In 2018, an audit conducted at LabPLUS indicated that a high frequency 17/28 [61%] of reported low titre [<160] positive ICS antibodies were identified in patients who were biopsy negative for both BP and PD. Conversely in the same audit, 25/30 patients [83%] with reported BMZ patterns had biopsy findings consistent with BP and, high end point titres were typically found [mode end point titre: 1280]. All of the above were the principal drivers for the evaluation of Euroimmun Dermatology Mosaic 7 BIOCHIP IIF assay where transfected cells allow detection of the specific skin autoantibody targets Dsg1, Dsg3, BP180 and BP230.

The purposes of this study were to (a) confirm the frequency of the previously identified non-specific ICS patterns by tissue IIF in a new clinically characterised population (b) determine the clinical utility of the specific antibody testing in that same

population (c) determine if fiscal and / or productivity benefits accrued by use of the specific antibody testing system over that of the tissue-based IIF and (d) comment on any operational limitations that were identified using the specific-antibody testing system.

MATERIALS AND METHODS

Historical 2018/2019 tissue IIF data extract

A data extract for the 16-month period from January 2018 through to April 2019 was obtained from routine clinical requests for skin autoantibody testing. This extract was used to determine tissue based IIF utility compared to biopsy findings. In total, there were 452 patients tested, 92 of whom had positive IIF results. Of the 92-tissue based IIF reactive patients, 58 had biopsies performed. The extract was additionally used to determine (a) tissue-based IIF result turn-around time (TAT) [patient registration to patient reporting (days) and (b) determine a comparative assessment of cost efficiency between the tissue-based IIF method and the Euroimmun Dermatology Mosaic 7 BIOCHIP IIF method under the testing regimen at LabPLUS, using the results that were reported.

Serum evaluation panel: 2019/2020

Over the six-month period from October 2019 through to March 2020, 168 sequential sera referred for skin autoantibody testing by tissue-based IIF were retained after analysis at -80°C for testing using the Euroimmun Dermatology Mosaic 7 BIOCHIP IIF method.

Seventy-three patients were excluded from data analysis due to either (a) absence of biopsy [N=63] or (b) the biopsy result was inconclusive [N=4] or (c) sera demonstrated strong non-specific cell-associated reactivity [N=6]. From the original test group of 168 patients, 89 [52.9%] had dual IIF methodology results as well as definitive biopsy results. Clinical performance assessments across both IIF methodologies were determined using this 89-patient cohort group.

Tissue-based IIF (standard methodology)

The remainder of this manuscript will refer to this technique by the abbreviation TBIF.

INOVA monkey oesophagus was used as the tissue substrate. Patient sera were screened at dilutions of 1:10 and 1:40 in PBS in accordance with manufacturer instructions. A primate absorbed anti-human IgG-FITC conjugate was used to exclude potential false positive cross-species reactions. To exclude potential interference due to autologous autoantibodies directed against RBC A and B antigens, presumptive ICS antibody positive sera were absorbed with commercially sourced human A1 and B red cells (BIORAD) and then re-tested in parallel (absorbed / un-absorbed on separate slides) in an extended 5-well dilution sequence (1:10, 1:40, 1:160, 1:640, 1:1280) to determine comparative end-point titres. All presumptive BMZ antibody positive sera were re-tested on a single slide using the same extended dilution sequence as defined for presumptive ICS positive sera undergoing absorption. Slides were viewed using Zeiss LED-based fluorescence microscopy (excitation filter – 470/40nm; emission filter – 515nm) at a magnification of x200 for both ICS and BMZ patterns [Figure 1 (a) and 1 (b).

Specific antibody IIF (EUROIMMUN Dermatology Mosaic 7 BIOCHIP)

The remainder of this manuscript will refer to this technique by the abbreviation EIIF.

Each testing field contains a mosaic of 6 BIOCHIPs which are: primate oesophagus tissue, primate salt-split skin tissue, Dsg1 transfected cells, Dsg3 transfected cells, BP230 transfected cells and recombinant purified BP180 NC16A domain. Dsg1, Dsg3 and BP230 transfected cells allow specific detection of respective autoantibodies by fine granular fluorescent staining of cytoplasm. In BP180-NC16A BIOCHIP, purified recombinant tetramer of the NC16A domain of BP180 is applied in diamond shape which allows specific detection of anti -BP180 by bright green fluorescent staining of the antigen [Figure 2 (a) – 2(d)]. Refer to Results / Technical overview section for the exclusion of monkey oesophagus and salt-split-skin tissue BIOCHIP analysis.

Patient sera were tested at dilutions of 1:20 and 1:40 in PBS. Kit-based anti-human IgG-FITC conjugate was used. Patient samples were randomly assigned onto the slides to remove any potential observer bias in the blinded reading of the immunofluorescence. Observation was performed independently by three senior medical laboratory scientists and an immunopathology registrar using Zeiss LED-based fluorescence microscopy as described previously. All technical steps were performed in accordance with the manufacturer's instructions.

Data analysis

Readings from the four practitioners were reviewed for concordance. Non-agreements were resolved by consensus discussion and re-testing. After practitioners' reading concordance had been established, IIF results of both tissue-based and transfected cell-based methods were matched against biopsy findings. Using 2x2 contingency tables assay sensitivity, specificity, PPV and NPV values were calculated.

Final diagnoses were determined by clinical chart review of medical records. Patient identities were not disclosed, and data was used anonymously. As this was a retrospective study with no modification on clinical decision making or individual follow up, patient consent was not required.

RESULTS

Technical assessment of the EIIF assay

In general, transfected reactive patterns were relatively easy to identify for an experienced IIF user as demonstrated by the 100% concordance across the four pattern observers. However, it is recognised that a degree of familiarisation is required for effective evaluation of the BP180-NC16A 4-X BIOCHIP due to the unusual grid-based preparation of the coating antigen. Biochips containing monkey oesophagus tissue and salt-split-skin tissue were excluded from the evaluation due to high associated background staining and compressed anatomical presentation respectively. Our comments were fed back to EUROIMMUN at the time of the validation study.

Of the originally tested group of 168 patient sera, 6 [3.6%] exhibited non-specific cell-based reactivity (Figure 3) which precluded antibody determination and were therefore excluded from analysis. Where the non-specific reactivity occurred, it was exclusively associated with the Dsg antigens and typically was seen in both Dsg 1 and Dsg 3 BIOCHIPs [5/6, 83%] at the manufacturer's recommended serum screening dilution of 1:10. Further titration of such sera demonstrated that the non-specific reactivity was fully extinguished at serum dilutions ranging between 1:40 to 1:80. In clinical practice, the affected targets of such sera would be reported as undetermined at the manufacturer's recommended screening dilution of 1:10 due to non-specific binding. Serial dilutions of high titre (≥2560) ICS and BMZ reactive sera demonstrated that at the manufacturer's recommended serum screening dilution of 1:10, prozone was not evident.

Historical 2018/2019 Tissue-based IIF Data Extract Tissue-based IIF [TBIF] positive results versus biopsy findings

A review of biopsy findings was restricted to those patients that had ICS [[N=47] and BMZ [N=45] reported patterns. For the ICS pattern group, 28 patients had tissue biopsy and 6 [21%] were reactive. For the BMZ pattern group 30 patients had tissue biopsy and 25 [83%] were reactive (Table 1). With respect to reported end point titres, 77% of patients with a reported ICS pattern and were biopsy negative had titres <160. Conversely, 21 / 25 [84%] of patients with a reported BMZ pattern that were biopsy positive had titres ≥160 (Table 1). Within this group the mode end point titre was 1280.

Efficiency / Consumable Cost

From the 2018 / 2019 audit, based upon the results that were reported we calculated that 666 tissue IIF slides would have been used to process the 452 patient specimens. Using a configuration of three patients per slide and two dilutions per

patient for the EIIF assay, 166 slides would have been required. The lower slide demand of the EIIF assay was due to (a) no requirement for RBC absorption for presumptive ICS patterns and (b) no end point titration requirement. With the lower slide usage and the discontinuation of the consumable purchase of commercial RBC's we estimated that at LabPLUS an on-going consumable saving in the order of NZD $600 to NZD $650 per month (NZD $7,200 - $7,800 p.a.) would accrue by transitioning from TBIF methodology to the EIIF method.

Reporting Turn-Around Time [TAT]

On average, reporting TAT in an antibody negative setting took just over a single 5-day working week. That TAT was significantly extended if the pattern was either BMZ [+34%] or most notably ICS [+93%] (Figure 4). For the same reasons stated in the 'Efficiency/ Consumable' section we believe that a transition from TBIF methodology to the EIIF method would result in a reporting TAT of 3 to 5 days for all patient specimens under test irrespective of reported result.

Serum evaluation panel (2019/2020)

Tissue-based IIF [TBIF]

While having adequate levels of specificity and NPV [88.9% and 84.8% respectively] the method was challenged by poor analytical sensitivity and PPV [61.5% and 69.6% respectively]. The principal cause of the poor sensitivity was 10 false negative results, 80% being from patients with BP disease (Table 2a). In addition to the poor sensitivity, there were 7/89 false positive results [[7.9%] (Table 2a). The majority of the false positive sera had ICS patterns [6/7, 86%], all of which had reported end point titres of ≤160.

Specific antibody IIF [EIIF]

The assay demonstrated high levels of sensitivity and specificity, superior to that of the conventional TBIF resulting in comparatively improved NPV and PPV values (Table 2b). The improvement was most obvious for PPV seeing an increase of 16.8% for this parameter (Tables 2a, 2b). The PPV shift in favour of the EIIF assay was principally due to the assay being able to exclude 5 /6 [83%] of the TBIF ICS pattern false positive results. Critically, the assay allowed the serological identification of patients with autoimmune skin diseases that were seronegative using conventional tissue based IIF methodology. This enhanced detection capacity was seen for 7% (potentially increasing to 9%) of the 89-patient cohort (Figure 5). The specific antibodies directed against Dsg1, Dsg 3 and BP180 all played a role, but the most notable effect was seen for independent reactivity against the BP180 target (Figure 5).

In the 89 patients who had tissue biopsies performed, the antibodies associated with pemphigus diseases [Dsg1, Dsg3; N=7] and BP [BP180, BP230; N=15] demonstrated very high specificity levels [Dsg1, Dsg3: 96.8%; BP180, BP230: 98.4%]. Sensitivity levels were lower for both diseases [Dsg1, Dsg3: 83.3%; BP180, BP230: 70.0%], most notably in the BP disease group where there were six false negative results (Table 3).

Table 1: Clinical value of reported tissue – based IIF patterns from an audit of 452 unselected patients routinely tested for skin antibodies at LabPLUS over the period January 2018 – April 2019.

IIF pattern	N	Biopsy Performed	Biopsy Positive	Pattern True Positive (%)	Pattern False Positive (%)
ICS	47 (10.4%)	28	6	21%	79% (17 / 22 [77%] patients had end point titres <160)
BMZ	45 (10.0%)	30	25	83% (21 / 25 [84%] patients had end point titres >160)	17%
Negative	360 (79.6%)	Evaluation not performed			

Table 2(a): Analytical performance of tissue based IFF method verses biopsy outcome (n=89)

		Tissue Biopsy		
		Positive	**Negative**	**Total**
Tissue based IIF (BMZ and ICS patterns)	**Positive**	16	7	23
	Negative	10	56	66
	Total	26	63	89
Sensitivity: 16/26 = 61.5%, Specificity: 56/63 = 88.9%, PPV: 16/23 = 69.6%, NPV: 56/66 = 84.8%				

Table 2(b): Analytical performance of the Euroimmun Dermatology Mosaic 7 BIOCHIP IIF method versus biopsy outcome (N=89)

		Tissue Biopsy		
		Positive	**Negative**	**Total**
Euroimmun BIOCHIP Mosaic 7 IIF (Dsg1, Dsg3, BP180, BP230)	**Positive**	19	3	22
	Negative	7	60	67
	Total	26	63	89
Sensitivity: 19/26 = 73.1%, Specificity: 60/63 = 95.2%, PPV: 19/22 = 86.4%, NPV: 60/67 = 89.5%				

Table 3. Specific antibody performance in biopsy confirmed or excluded cases of either pemphigus disease (PD) or bullous pemphigoid disease (BP)

Group	True Positives	False Positives	False Negatives	True Negatives	Sensitivity/Specificity (%)
PD (Dsg1, Dsg3 antibodies)	5	2	1	60	Sensitivity: 83.3% Specificity: 96.9%
BP (BP180, BP230 antibodies)	14	1	6	60	Sensitivity: 70.0% Specificity: 98.4%

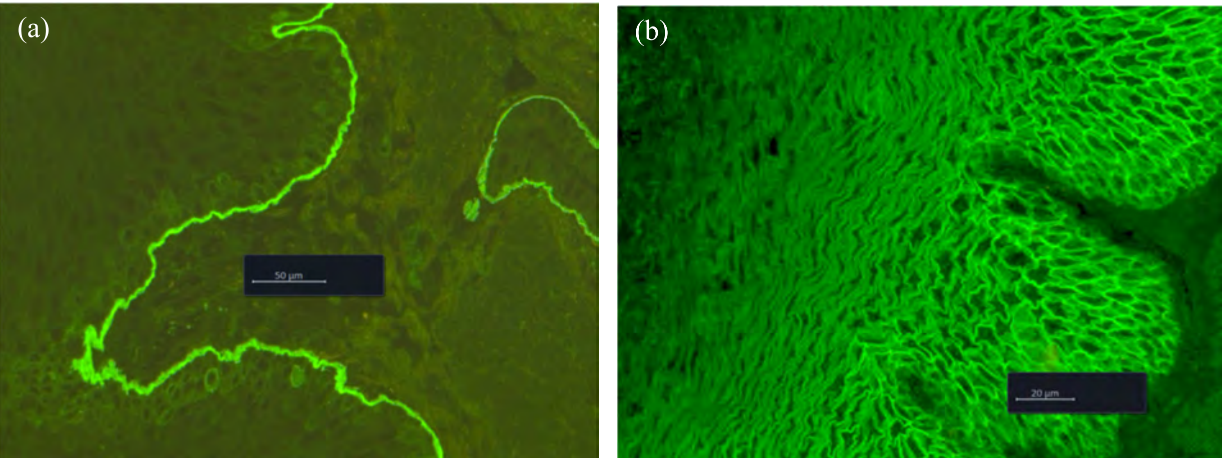

Figure 1: (a) BMZ and (b) ICS immunofluorescence patterns on INOVA monkey oesophagus tissue.

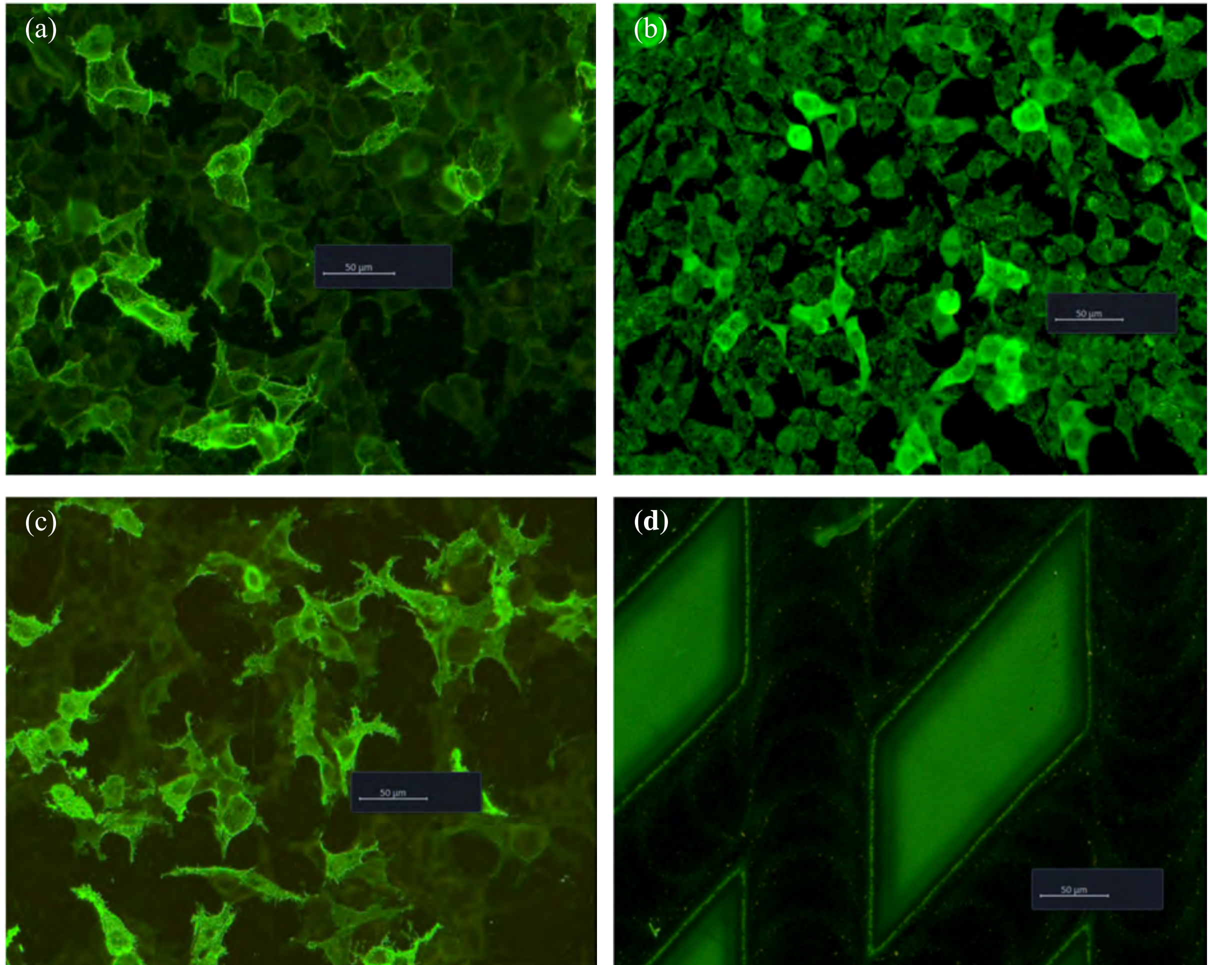

Figure 2: (a) Dsg1 transfected cells, (b) BP230 transfected cells, (c) Dsg3 transfected cells and (d) recombinant BP180 NC16A on EUROIMMUN Dermatology mosaic 7 IIF.

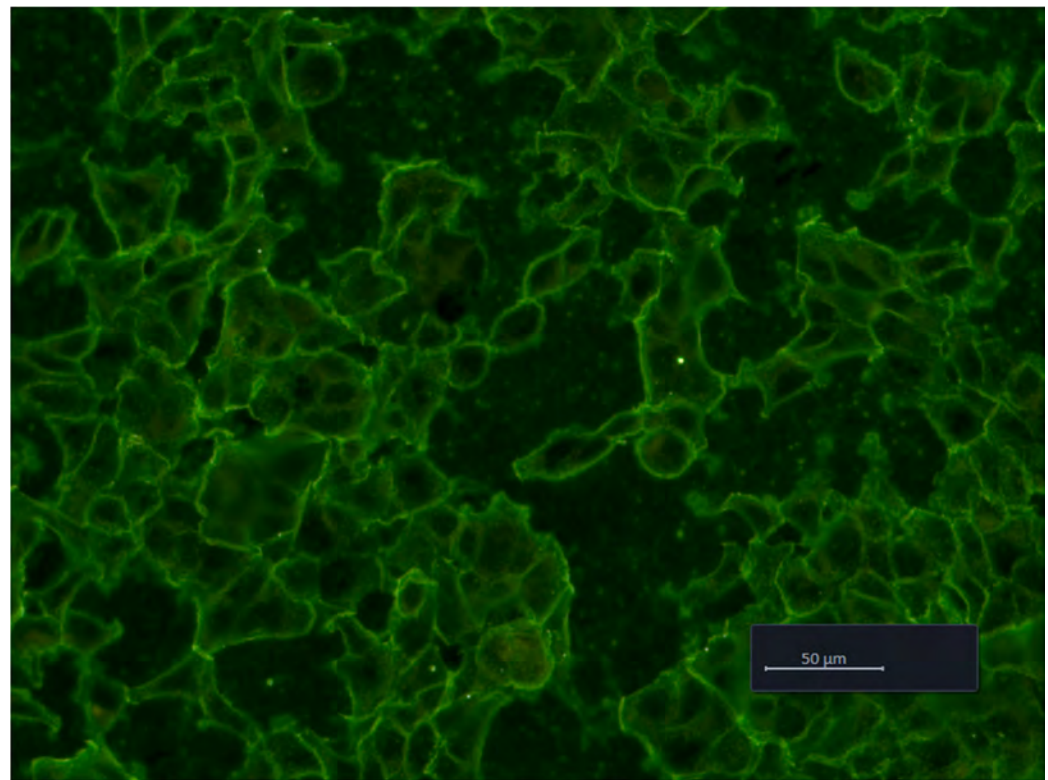

Figure 3: An example of non-specific staining seen in a Dsg transfected cell on the EUROIMMUN Dermatology 7 BIOCHIP

Figure 4: Patient reporting turn-around times (TAT) for skin antibody testing by tissue IIF in antibody negative (N=360) and reactive settings (ICS: N= 48; BMZ: N=44).

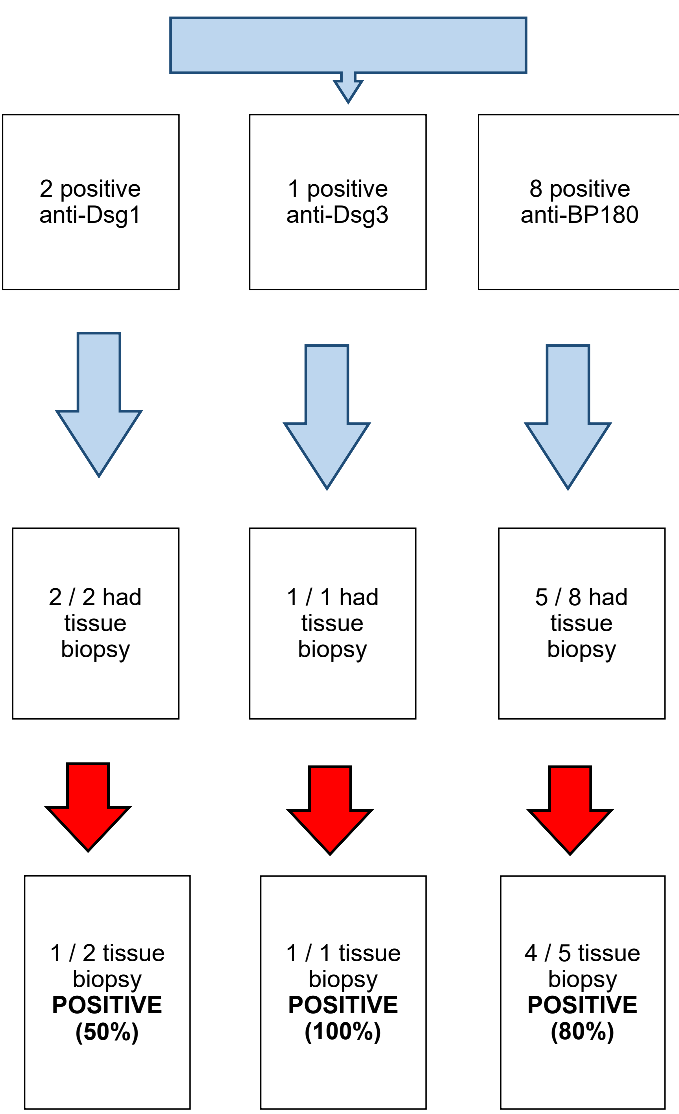

Figure 5: Enhanced diagnostic sensitivity of Euroimmun BIOCHIP Dermatology Mosaic 7 IIF over conventional tissue based IIF.

DISCUSSION

Over two independent cohorts of unselected patient sera that were referred for skin autoantibody testing at LabPLUS spanning a 3-year time frame we were able to confirm that the TBIF methodology persistently produced low (<160) titre antibody patterns at a frequency in the order of 10%. Despite all presumptive ICS pattern sera undergoing absorption with human AB type RBCs, as per the method of Lee et al (14) to correct for non-specific reactivity, a high proportion of the low titre ICS results were proven to be false positives. Our experience has been that we infrequently identify extinction of ICS pattern fluorescence in absorbed sera. Typically, we find that end point titres are identical between absorbed and unabsorbed sera using either preserved RBC's or soluble AB antigens, implying a different, as yet unidentified interfering serum-based factor is involved in producing an ICS-like pattern. In the evaluation panel testing, 5/6 [83%] of the non-specific low titre ICS pattern sera were excluded by specific antibody testing. A single serum was independently reactive for Dsg1 antibody.

This retrospective study clearly demonstrated that the overall assay performance characteristics of the EIIF method were superior to that of the TBIF procedure. Although the reader was blinded to biopsy results at the time of evaluation thereby eliminating reader bias, a limitation of this study design was the delivery of relatively low numbers of patients with biopsy proven PD [N=6] or BP disease [N=20]. A larger study conducted by Yang et al in 2020 using the EIIF assay found similar levels of sensitivity for both PD [N=31] and BP [N=38] disease groups (15). However, assay specificity levels were significantly lower [20-25% range] compared to our findings. The most likely reason for this difference is that in the study by Yang et al the inclusion of relatively high numbers of disease controls [N=63] and healthy volunteers [N=39] would have impacted on assay specificity performance characteristics (15).

Apart from the significant reduction in false positive results for PD patients we additionally saw that the EIIF method detected biopsy positive cases for patients with both PD [N=2] and BP [N=4] disease that the TBIF method gave negative results for. For the PD patients, one had isolated reactivity to Dsg1, the other having isolated reactivity to Dsg3. For the BP disease patients, all had isolated reactivity to the BP180 target. There are numerous publications in the literature identifying that

antibodies against BP180 are found in healthy patients at a frequency of 2%-7% as well as in patients with neuronal-based diseases (stroke and Alzheimer's) at a frequency of between 10%-20% (16-18). These results may be due entirely or in part to a combination of the methodology used (ELISA) and the manufacturer's recommended assay cut-offs. We were unable to find any published studies in non-dermatological settings that used the EIIF method. In apparent contrast to the enhanced sensitivity of the BP180 target we had to account for the seven false negative results that we saw in the evaluation panel testing. Six of the seven patients had BP, one having PD, and all were under treatment with steroids. In a publication by Ghohestani et al in 1996 it was identified that the TBIF BMZ pattern was largely triggered by antibodies directed at BP230 and as such did not correlate with disease activity in BP patients (19). A later publication by Schmidt et al in 2000 where 15 patients with BP under treatment were followed longitudinally clearly demonstrated that BP180 antibody levels became undetectable over time and matched clinical disease activity whereas TBIF assessments did not (20). It follows, that care must be taken in the interpretation of the results from the specific targets, in particular correlation with treatment and disease expression being mandatory.

To summarise the clinical utility of the EIIF method versus traditional TBIF methodology the results of this study support, in favour of the EIIF method (a) a superior diagnostic capacity for both PD and BP patients and (b) an improved reporting of serological results for both PD and BP patients under treatment that matches clinical disease activity. Likely follow-on beneficial outcomes for patients as a consequence of transitioning away from the TBIF diagnostic testing will be (a) reductions in the number of required biopsy procedures (b) shorter waiting times for dermatological assessments and (c) potentially improved targeted application of steroid medications for patients under treatment

A unique feature of this publication was to assess the potential impact of methodology change on operational aspects of the diagnostic laboratory. Although every diagnostic laboratory will have its own unique processing environment, at LabPLUS a transition to the EIIF method would accrue significant benefits for both (a) reporting TAT and (b) consumable costs. The improvement for both measurable parameters would be mediated by immediate qualitative reporting as opposed to secondary titration and / or RBC absorption

Although this study demonstrated that a methodology transition at LabPLUS would deliver a faster, more clinically appropriate service at a reduced cost, operationally, laboratories considering methodology change need to have an awareness that (a) this study did not assess the value of reporting semi-quantitated results for any of the specific targets (b) a degree of patients (5-10%) will demonstrate low titre non-specific reactivity, principally for the Dsg targets (c) it was our observation and contention that, as seen across all slides the compressed anatomical presentation of the biochips containing both the monkey oesophagus and salt-split skin tissue were too challenging to allow confident determination of reactive ICS and/or BMZ patterns. Additionally, and most notably for the monkey oesophagus tissue, there was high associated background staining. It was for these reasons that these two fields were excluded from the evaluation process and (d) expertise in IIF methodology, preferably with experience in reading and recording results from multiple fields for individual patients would be desirable.

In conclusion, when the results from this study were presented to a group of regional dermatologists, the proposed transition from TBIF testing to the EIIF method using qualitative reporting for the four specific targets was fully endorsed.

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ORIGINAL ARTICLE

Effect of obesity on serum IL-10 concentrations and messenger RNA expression in women with metabolic syndrome

Moushira Zaki, Hala T El-Bassyouni, Eman R Youness, Walaa A Basha, Maha Abdelhadi Ali, Wagdy KB Khalil, Sara M Abdo and Walaa Yousef

ABSTRACT

Objectives: In humans, the probable role of anti-inflammatory cytokines in obesity is unidentified. The objective of this work was to investigate serum IL-10 concentrations and the messenger RNA expression (mRNA) in peripheral blood of 30 obese women with metabolic syndrome (MS) and 20 lean healthy controls matching sex and age.

Methods:In this cross-sectional study, 50 Egyptian women (age <45 years) were included. Blood pressure, anthropometric measurements, lipid parameters, obesity indices and HOMA-IR were measured. The expression of IL-10 in blood was assessed by extraction of RNA followed by real time PCR analysis. The IL-10 level in serum was assessed using enzyme linked immunosorbent assay (ELISA).

Results: Women with MS had lower values of IL-10 and significant higher levels of blood pressure, lipid parameters, obesity measures and HOMA-IR compared to control women (all P <0.01). Similarly, mRNA expressions were down regulated in MS women compared to the control group (P = 0.003).

Conclusion: Our study demontstrated that obese women have low levels of IL-10, suggesting that this anti-inflammatory cytokine plays a crucial role in MS risk and obesity-related problems. Moreover, low mRNA expression suggests the contribution of genes to insulin-resistant states and the metabolic syndrome in obese women.

Keywords: Interleukin 10; metabolic syndrome; obesity; mRNA expression; cytokines.

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INTRODUCTION

Interleukin 10 (IL-10) has a crucial role in limiting inflammation and regulating the immune response. IL-10 represses inflammation via numerous mechanisms comprising supression of the of pro-inflammatory cytokines synthesis (1). Growing evidence has been found linking metabolic syndrome (MS) to IL-10, cardiovascular diseases, and obesity (2). It has been found that low circulating IL-10 is related to obesity in adults (3). Also IL-10 plays a protective part against insulin resistance development.

In young healthy adults, the concentration of IL-10 in plasma correlated positively with insulin sensitivity (4,5). IL-10 is secreted by T-cells, B-cells, macrophages and monocytes, this is under powerful genetic control, with heritability assessment as high as 75% (6). Genetic and environmental factors can share in the metabilic hazard. Genome wide association studies have found significant relations among MS and single nucleotide polymorphisms (SNP), predominantly located in genes encoding apolipoprotein synthesis and proteins that control the central nervous system hunger-satiety control (7). Gene variants of IL-10 have been related with type 2 diabetes mellitus risk (8); however, the association between gene expression of IL10 and its serum levels in Egyptian obese women with MS have not been previously investigated. The present study aimed to investigate serum IL-10 levels and the mRNA expression of IL-10 and the risk of MS in as sample of Egyptian obese women.

SUBJECTS AND METHODS

Study population

Thirty obese women between the ages of 25 and 35 years were recruited from the obesity clinic, National Research Centre. Exclusion criteria of the study group were pregnancy, systemic diseases (kidney, liver, heart, or any systemic diseases). The control group was twenty women with associated other endocrine disorder between the ages of 25 and 45 years. The study was approved by the Ethical Committee of the National Research Centre, Egypt (number = 16361), in accordance with the World Medical Association's Declaration of Helsinki. Written informed consent was obtained from all participants.

Anthropometric and clinical measures

Patients and controls were clinically examined and a full medical history was obtained. Anthropometric parameters were taken three times on the left side of the body and the mean value was used. Body weight, height, waist circumference (WC) and hip circumference (HC) were measured were measured as previously described (9,10). Body mass index (BMI) and waist hip ratio (WHR) were calculated. The anthropometric measurements and instruments followed the International Biological Program (10). Systolic and diastolic blood pressures (SBP and DBP) were measured three times with a standard mercury sphygmomanometer and appropriately sized adult cuffs on the right arm of each subject after a 10-minute rest in the sitting position, and the mean values were used for analysis. Metabolic syndrome (MS) and its individual components were assessed according to the National Cholesterol Education Program definition(11).

Measurement of glucose and lipids

Serum lipids (total cholesterol, high-density lipoprotein cholesterol (HDL-C) triglycerides (TG) and fasting plasma glucose were assessed using colorimetric enzymatic methods using a Hitachi auto-analyzer 704 (Roche Diagnostics, Switzerland). Low density lipoprotein cholesterol (LDL-C) was calculated according to a specific equation (LDL-C= Total cholesterol – Triglycerides/5+HDL-C). Serum insulin concentration was evaluated by a chemiluminescent immunoassay (Immulite 2000, Siemens, Germany. The insulin resistance was determined via the Homeostasis Model Insulin Resistance (HOMA-IR), calculated as the product of the fasting plasma insulin level (IU/mL) and the fasting plasma glucose level (mmol/L), divided by 22.5 (12).

Measurement of IL-10

The total concentrations of IL-10 in plasma samples were evaluated using an ELISA kit (R&D System, Inc., Minneapolis, MN), in accordance to the manufacturer's instructions.

Expression analysis of IL-10 RNA

RNA isolation and reverse transcription reaction

Total RNA from blood samples of control healthy persons (n=20) and from obese women with metabolic syndrome (n=30) was isolated by the standard TRIzol® Reagent extraction method (Invitrogen, Germany). After completion of the isolation procedures, RNA pellets were stored in DEPC treated water. To digest the potential DNA residues the pellet of isolated RNA was treated with RNAse-free DNAse kit (Invitrogen, Germany).

RNA aliquots were stored at -20°C or utilized immediately for reverse transcription.

First Strand cDNA Synthesis Kit (RevertAidTM, MBI Fermentas) was used to synthesize the cDNA copy from human samples via reverse transcription reaction (RT). A RT reaction programme of 25°C for 10 min, then one hour at 42°C then 5 min at 95°C was used to obtain the cDNA copy of human genome. Finally, tubes of reaction containing cDNA copy were collected on ice up to use for cDNA amplification.

Quantitative Real Time-PCR

SYBR® Premix Ex TaqTM kit (TaKaRa, Biotech. Co. Ltd.) was used to perform the qRT-PCR analyses using the synthesised cDNA copies from human samples. For each reaction a melting curve profile was conducted.

Statistical analysis

All obtained data were statistically analysed using SPSS16.0 software (SPSS Inc). To verify that the data of the present study was normally distributed the Kolmogorov–Smirnov test of normality followed by a Gaussian pattern was utilised. Normally distributed data in this study were exhibited as means ± SD. To examine the significant differences between the two studied groups, the data were analysed by Mann–Whitney U test or unpaired "t-test" as appropriate. All used tests were two-sided and considered statistically significant when $p < 0.05$.

RESULTS

The quantitative values of the target genes were normalised on the expression of the housekeeping gene (β-actin). The $2^{-\Delta\Delta CT}$ method was used to determine the quantitative values of the specific RNA to the b-actin gene (Table 1). The clinical characteristics of the studied population are summarised in Table 2. MS women showed significant higher obesity meaures, including BMI, WC, WHR and SBP as well as higher blood pressure levels compared with control healthy women (Table 2).

Table 3 shows the biochemical characteristics of the two groups. The results showed that MS women had significant decreased value of IL-10 levels and higher levels of serum lipids and HOMA-IR than the control group. Morover, MS women showed significant higher levels of TC, TG and LDL-C compared to control women.

The expression values of IL-10 gene ($2^{-\Delta Ct}$) were significantly decreased in MS patients (0.818) compared to controls (2.293), P=0.003. The relative expression levels of IL-10 RNA are demonstated in Figure 1, which exhibited that the expression levels in healthy control samples were significantly higher that those of MS patients.

Table 1. Primers sequence used for RT-qPCR

Gene	Primer sequences used for RT-qPCR	NCBI reference sequence
IL-10	F: GTT CTT TGG GGA GCC AAC AG	NM_000572.3
	R: GCT CCC TGG TTT CTC TTC CT	
β-actin	F: AGA GCT ATG AGC TGC CTG AC	Jing Z, *et al.* (13)
	R: AAT TGA ATG TAG TTT CAT GGA TG	

Table 2. Clinical characteristics.

Variables	Group	Mean±SD	*P*-value
Age (years)	Controls	33.90 ± 3.46	0.61
	MS	35.90 ± 4.45	
BMI (kg/m^2)	Controls	21.89 ± 2.08	0.01
	MS	33.28 ± 5.89	
WC (cm)	Controls	74.91 ± 9.039	0.05
	MS	100.07 ± 12.540	
WHR	Controls	0.76 ± 0.09	0.03
	MS	0.8463 ± 0.06	
SBP (mmHg)	Controls	93.89 ± 11.95	0.02
	MS	108.68 ± 15.64	
DBP (mmHg)	Controls	63.06 ± 5.18	0.02
	MS	71.76 ± 9.82	

BMI: body mass index; WC: waist circumference; WHR: waist to hip ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure.

Table 3: The biochemical characteristics of the studied women.

Variables	Group	Mean±SD	*P*-value
IL-10 (pg/mL)	Controls	13.29 ± 3.61	0.01
	MS	10.82 ± 2.95	
Total cholesterol (mmol/L)	Controls	4.194 ± 1.68	0.01
	MS	6.21 ± 1.18	
Triglycerides (mmol/L)	Controls	0.80 ± 0.32	0.02
	MS	1.19 ± 0.47	
HDLc (mmol/L)	Controls	1.31 ± 0.33	0.04
	MS	1.13 ± 0.26	
LDLc (mmol/L)	Controls	2.56 ± 0.92	0.03
	MS	4.39 ± 1.13	
HOMA-IR	Controls	2.29 ± 1.09	0.01
	MS	7.19 ± 2.19	

HDLc = high density lipoprotein cholesterol, LDLc = low density lipoprotein cholesterol, HOMA-IR: homeostasis model assessment-insulin resistance.

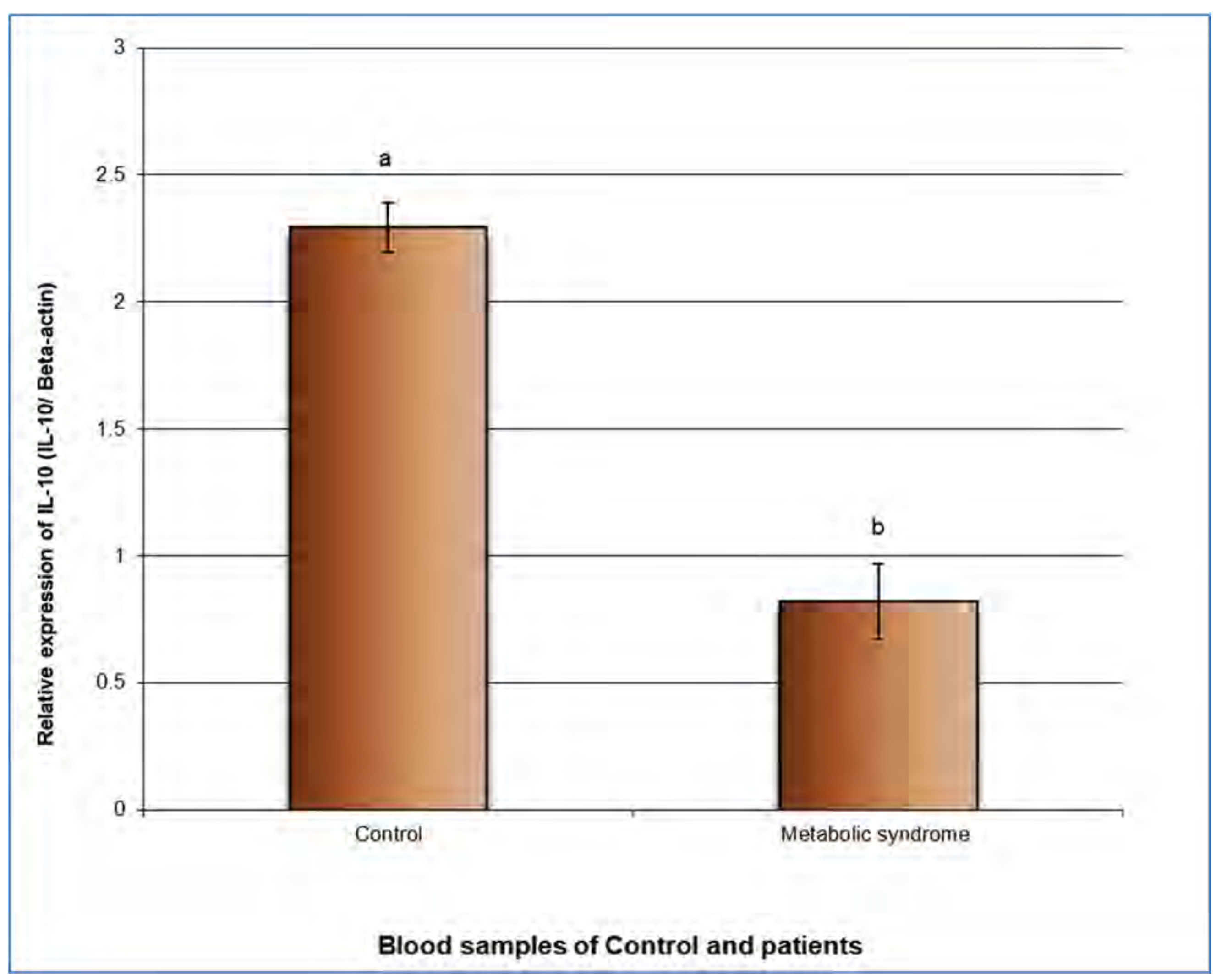

Figure 1. Expression alterations of IL-10 RNA in the control group (n=20) and the metabolic syndrome group (n=30). Data are presented as mean ± SD.

DISCUSSION

Various studies have shown increased inflammation in adipose tissue of obese subjects and disclosed its propable role in the progress of insulin resistance. The decreased production capacity of IL-10 (i.e., a pro-inflammatory response) is in association to type 2 diabetes and metabolic syndrome (6,14). MS is distinguished by related risk factors such as low high-density lipoprotein (HDL) levels, hypertriglyceridemia, impaired glucose tolerance, obesity (particularly visceral adiposity) and raised blood pressure. MS is an established clinical condition and a public health concern highly associated with the incidence of obesity, excessive caloric intake and sedentary lifestyle (15). Moreover , it has been shown that obesity and MS are accompanied with chronic low-grade systemic inflammation, particularly higher in women (16). Pro-inflammatory cytokines have been related to the development of type 2 diabetes and metabolic syndrome. The total cholesterol, triglycerides, LDL-C and HOMA-IR were significantly higher in the obese women in comparison to control. While HDL-C was diminished in the obese women compared to the control. This is interrelated with lipids accumulation in adipose tissue, secretion of lipoproteins, and increase in hepatic synthesis. Insulin resistance has been suggested as the leading cause for this cardiovascular and metabolic syndrome, despite that its molecular basis is not yet clear (17). Experimental studies in animals and humans have revealed that treatment with pro-inflammatory cytokines stimulates insulin resistance and hypertriglyceridemia (18-20). The eventuality of a significant correlation between HOMA and the studied variables was predominantly lower in male than female subjects. Such is the case for triglycerides, total cholesterol, HDL-cholesterol, interleukin-6, hs-CRP, adiponectin. and TNF-α (21,22).

Obesity is a multifactorial disease with epigenetic alterations (23). IL-10 is an anti-inflammatory cytokine with an imperative role in the immunological system regulation via inactivation of pro-inflammatory cytokines through the repression of macrophage function, with a consequentual crucial role in immunity. Gene expression of IL-10 in our study was deficient in the obese women compared to the control group. A previous study by Viesti *et al.* (24) did not delineate a difference in IL-10 expression among their groups. Other studies have reported an association between obesity and dysregulated expression of miRs in inflammatory adipocytes (25,26). On the basis of the present data, the low levels of IL-10 can be used as a treatment to ameliorate the obesity-related problems. It may be concluded that the correlation between the gene expression of IL-10 in individuals with obesity and MS in the Egyptian population extends the current understanding of the biological passages of obesity.

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Dr Dianne Webster
Clinical Scientist, LabPLUS, Auckland District Health Board

Dr Dianne Webster, with responsibilities for newborn metabolic screening, has won an international award for excellence in global leadership in standards development. She has directed the national newborn metabolic screening programme (NMSP) for more than 25 years.

She has held several panel and leadership roles with the Clinical and Laboratory Standards Institute (USA). She chairs the newborn screening subcommittee of the Human Genetics Society of Australasia and has supported screening initiatives in many other countries.

Dr Webster has been active for many years in the establishment of both written and physical standards and initiated the International Society for Neonatal Screening (ISNS) lexicon and minimum data set some 20 years ago. She is a past chair of ISNS Standard Committee for Quality Assurance, and was involved in the development of the ISNS Dried Blood Spot Reference Preparation.

More recently she was appointed co-chair of a new ISNS Committee on Guidelines and Quality Assurance and co-chair of the CLSI-ISNS working group on terminology in newborn screening to undertake a project that will lead to a concise list of terms that should be used in newborn screening publications.

Dr Dianne Webster was awarded the Queen's Service Order in 2007, and in 2020 was further recognised to become a Companion of the NZ Order of Merit for services to health, particularly paediatrics.

Dr Webster will be giving the TH Pullar address at the NZIMLS Annual Scientific Meeting on Wednesday 31 August.

ORIGINAL ARTICLE

Evaluation of serum copper, zinc and magnesium in pre-eclampsia and gestational diabetes in Calabar, Cross River State, Nigeria

Idongesit KokoAbasi P. Isong, Ntongha Nelson Ofem, Uwem Okon Akpan, Euphoria C Akwiwu, Bassey Edward Icha and Kingsley Emmanuel John

ABSTRACT

Objectives: Alterations in zinc (Zn), copper (Cu) and magnesium (Mg) levels in blood have been linked to gestational diabetes mellitus (GDM) and pre-eclampsia. This study aimed at evaluating the levels of some serum minerals in women with pre-eclampsia and gestational diabetes mellitus in order to understand their relationship with these pregnancy disorders.

Methods: Ninety (90) pregnant women (18-45 years) attending ante-natal clinic at General Hospital, Calabar were recruited into this case control cross sectional study. Thirty (30) were clinically diagnosed with gestational diabetes mellitus, 30 clinically diagnosed with pre-eclampsia and 30 were apparently healthy women. Serum Zn, Cu and Mg were determined using Atomic Absorption Spectrometry. Random blood glucose (RBG) was estimated using a glucometer. Urine protein estimation was done turbidimetrically with trichloroacetic acid. Data was analysed using SPSS version 22.0 with ANOVA and Pearson's correlation. P <0.05 was considered statistically significant.

Results: The zinc levels were significantly lower in gestational diabetes mellitus and pre-eclamptic group compared to the control group (p<0.001). No significant difference was found in mean serum copper and magnesium levels. A positive correlation (r=0.422, p=0.020) was observed between magnesium and random blood glucose in the gestational diabetes mellitus group. A negative correlation was observed between zinc and systolic blood pressure (r=-0.471, p=0.001), diastolic blood pressure (r=-0.485, p=0.001) and urine protein (r=-0.399, p=0.001) in all study participants.

Conclusion: Significant changes in serum zinc was demonstrated in pre-eclampsia suggesting a relationship with pathogenesis of this disorder. There was however no significant difference in levels of copper and magnesium, thus no relationship between them and gestational diabetes mellitus and pre-eclampsia.

Keywords: Pre-eclampsia, gestational diabetes Mellitus, Copper, Zinc, Magnesium.

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INTRODUCTION

Pregnancy initiates many metabolic and physiological changes over a period of 40 weeks, and may have complications such as hyperemesis gravidarum, gestational diabetes (GDM), intrauterine growth restriction (IUGR) and pre-eclampsia (1). Nigeria's approximate 40 million women of child-bearing age (between 15-49 years) suffer a disproportionately high level of health issues contributing 10% of global deaths for pregnant mothers (2). Gestational diabetes mellitus (GDM) and pre-eclampsia are both serious complications of pregnancy threatening both mother and foetus's life (3).

Gestational diabetes is defined as glucose intolerance resulting in hyperglycaemia of variable severity with onset or first recognition during pregnancy. According to the most recent International Diabetes Federation (IDF) estimate GDM affects approximately 14% of pregnancies worldwide, representing approximately 18 million births annually (4). Pre-eclampsia is a pregnancy specific disorder characterised by hypertension, significant proteinuria with or without oedema. It complicates 2-7% of all pregnancies. It remains one of the most common medical complications of pregnancy and is a major cause of maternal and perinatal mortality worldwide (5).

The exact pathogenic mechanism of both pre-eclampsia and GDM is uncertain however disturbances in mineral status have been suggested to play essential or contributory roles in the pathogenesis and progression of these disorders (6). The important role of zinc and copper in robust antioxidant capacity and magnesium's vital role in blood pressure regulation and signal transduction of insulin make them important in understanding suggested roles of serum minerals in the pathogenesis of both disorders. Previous studies have established significant alterations of serum zinc, copper and magnesium levels in pre-eclampsia and GDM (6, 7) suggesting they may contribute to these disorders. However, reports describing their associations vary greatly (7, 8).

This research was therefore designed to determine whether abnormal levels of these trace elements in pregnant women exist in these disorders.

MATERIALS AND METHODS

Study design/subject selection

A total of 90 women attending ante-natal clinic of General Hospital, Calabar were recruited into this case control cross sectional study. They were aged between 18-45 years. Out of the 90 pregnant women, 30 were clinically diagnosed with gestational diabetes Mellitus (GDM), 30 clinically diagnosed with pre-eclampsia and 30 were apparently healthy women (controls). Ethical clearance was obtained from the Cross River State Ministry of Health. A well-structured questionnaire was administered to obtain demographic data and their informed consent to participate in the study was obtained. The study was conducted between June 2021 and August 2021.

Inclusion criteria

Apparently healthy multiparous pregnant women in their second and third trimesters were recruited as controls and pregnant women diagnosed with pre-eclampsia and GDM as test subjects. They were within the age range of 18 and 45 years.

Exclusion criteria

Those excluded from the study were those whose informed consent were not obtained, those who did not fall within the specified age range (18-45 years), Pregnant women in their first trimester. Pregnant women diagnosed with other conditions, non-pregnant females and those on iron supplements.

Sample size

A sample size of 18 was obtained using the formula described by Naing *et al* (9) allowing an expected proportion of occurrence of 1.2% from data of the study by Koofreh *et al* (10) with 95% confidence limit. However, the sample size was increased to 30 for better representation of the study population and reduction in error margin.

Sample collection

Using aseptic technique, 5mL of blood was collected via venepuncture into a clean dry plain sample container and kept from sunlight. The sample was allowed to clot, dislodged and then spun in a centrifuge at 3000rpm for 5 minutes to obtain serum. The serum was transferred into a 5mL plain container, correctly labelled and stored frozen at -20°C until time of analysis. On the spot urine was also collected into a sterile universal container, correctly labelled and stored frozen (-20°C) until time of analysis.

Measurement of blood pressure
Blood pressure was measured in a well-seated and relaxed position after resting about 10 minutes, using a digital blood pressure monitor from OMRON HEALTHCARE LTD, United Kingdom. Two readings were taken from each subject and the systolic and diastolic blood pressures were recorded after computing the average of the two readings.

Measurement of weight and height
The measurement of weight was achieved using a bathroom weighing scale. The participants were made to stand erect bare -footed and facing front. The values were read to the nearest 0.1kg. A stadiometer was used in the measurement of height. Each participant was instructed to stand erect, without any shoes and cap against a wall. Their heights were read in metres.

Determination of body mass index
Body Mass Index (BMI) was obtained by finding the ratio of weight (kilograms) to the square of height (metres^2). This is expressed in kg/m^2.

Laboratory assays
Serum copper, zinc and magnesium were estimated for both the test and control samples using Atomic Absorption Spectrometry (AAS) with instrument from Agilent Technologies. Santa Clara, USA. Random blood glucose was estimated with the aid of a glucometer (Fine Test by Osang Healthcare). Urine protein analysis was by the turbidimetric technique using trichloroacetic acid (11). Qualitative analysis of protein and glucose in urine was done using combi-2 test strip (Medi-test by Macherey-Nagel Inc, Allentown, USA).

Statistical analysis
Data collected was entered into Microsoft excel spreadsheet and analysed using Statistical Packages for Social Science (SPSS) version 20.0 for determination of mean, standard deviation and comparison of variables was done using analysis of variance (ANOVA) and correlation analysis with Pearson's correlation, with a p<0.05 considered statistically significant.

RESULTS

A comparison of the age, BMI, blood pressure and biochemical parameters among the three groups studied showed no statistically significant variation in the mean values of age (p=0.208), magnesium (p=0.344) and Copper (p=0.253). However, there were statistically significant variations in the mean values of zinc, body mass index (BMI), random blood glucose (RBG), urine protein, systolic and diastolic pressures (p<0.05) among the groups. The mean zinc values of the pre-eclamptic and GDM groups were significantly lower than those of the controls. However, the mean zinc values of the pre-eclamptic group were significantly lower than the GDM group.

The Pre-eclamptic group also had significantly higher BMI, systolic and diastolic pressures and urine protein compared to other groups. GDM subjects had significantly higher RBG compared to other groups (Table 1). The blood pressure and urine protein were significantly higher in the pre-eclamptic group compared to the controls while Zn was significantly lower. The GDM subjects had significantly higher Zn and RBG levels, and significantly lower BMI, Blood pressures and urine protein levels compared to the pre-eclamptic subjects (Table 2). A significant positive correlation (r=0.422, p=0.020) was observed between magnesium and random blood sugar in the GDM group (Table 3).

Table 1: Comparison of mean physical and biochemical parameters of normotensive, gestational diabetes mellitus and pre-eclamptic women.

Parameter	Control n=30	Pre-eclampsia n=30	Gestational Diabetes n=30	F-cal	p-value
Age (years)	30.0 ± 5.39	28.70 ± 5.27	31.1 ± 4.94	1.600	0.208
Magnesium (mmol/L)	0.93 ± 0.39	0.81 ± 0.17	0.91 ± 0.35	113.41	0.344
Zinc (ug/L)	106.1 ± 16.6	80.0 ± 14.2	97.9 ± 16.9	23.026	<0.001*
Copper (ug/L)	116.9 ± 19.4	117.8 ± 18.2	124.3 ± 17.9	32.178	0.253
BMI (kg/m^2)	30.33 ± 3.61	33.39 ± 6.57	30.32 ± 4.19	14.937	0.027*
Systolic BP (mmHg)	116.6 ± 4.84	138.7 ± 11.4	115.3 ± 5.71	0.312	<0.001*
Diastolic BP (mmHg)	73.10 ± 4.70	93.00 ± 8.37	75.33 ± 5.71	1.363	<0.001*
Urine Protein (g/L)	0.037 ± 0.024	0.161 ± 0.071	0.03 ± 0.012	134.0	<0.001*
RBG (mmol/L)	5.6 ± 0.68	5.3 ± 0.69	9.1 ± 0.95	80.54	<0.001*

*Significant at p<0.05

Table 2: Comparison of mean physical and biochemical parameters of normotensive, gestational diabetes mellitus and pre-eclamptic women using LSD post-hoc analysis.

Parameter	Groups		Mean Difference	p-value
	Control	Pre-eclampsia		
Zinc (ug/L)	106.1 ± 16.6	80 ± 14.2	26.05	<0.001*
BMI (kg/m^2)	30.33 ± 3.61	33.4 ± 6.6	-3.057	0.021*
Systolic BP (mmHg)	116.6 ± 4.84	139 ± 11.4	-22.11	<0.001*
Diastolic BP (mmHg)	73.10 ± 4.70	93 ± 8.37	-19.89	<0.001*
Urine Protein(g/l)	0.04 ± 0.02	0.16 ± 0.07	-0.124	<0.001*
	Control	Gestational Diabetes		
RBG(mmol/l)	5.6 ± 0.68	9.1 ± 0.95	-3.55	<0.001*
	Gestational Diabetes	Pre-eclampsia		
Zinc (ug/L)	97.9 ± 16.9	80 ± 14.2	17.90	<0.001*
BMI (kg/m^2)	30.32 ± 4.19	33 ± 6.6	-3.07	0.019*
Systolic BP (mmHg)	115.3 ± 5.71	139 ± 11	-23.3	<0.001*
Diastolic BP (mmHg)	75.33 ± 5.71	93 ± 8.37	-17.67	<0.001*
RBG(mmol/L)	9.1 ± 0.95	5.3 ± 0.69	3.780	<0.001*
Urine Protein(g/L)	0.03 ± 0.012	0.16 ± 0.07	-0.131	<0.001*

*Significant at p<0.05

Table 3: Correlation of serum magnesium with systolic blood pressure, diastolic blood pressure, random blood sugar and urine protein across the groups.

Parameters	Control n=30 r (p-value)	Preeclampsia n=30 r (p-value)	GDM n=30 r (p-value)
SBP	0.088(0.650)	0.051(0.788)	0.226(0.230)
DBP	0.345(0.067)	-0.225(0.231)	0.085(0.657)
RBG	-0.015(0.939)	0.007(0.971)	0.422(0.020)*
Urine Protein	-0.059(0.760)	-0.137(0.472)	0.227(0.228)

*Significant at p<0.05

DISCUSSION

The reduced levels of zinc observed in the GDM compared to the controls, is similar to previous report of Mishu *et al* (12) but in contrast to previous report of Hamdan *et al* (13) which did not show any significant difference in mean zinc levels between pregnant women with GDM and controls. The serum zinc levels were also significantly lower in pre-eclampsia which agrees with previous study by Farzin and Sajadi, (14). Contrary to the present study, Mahomed *et al* (15) reported significantly high serum zinc levels as compared to controls. Studies suggested that lower serum zinc levels in gestational diabetes and pre-eclampsia was related to low oestrogen and Zn-binding protein levels caused by oxidative stress (16).

The disproportional elevation of plasma volume, increased zinc requirement, the reduced dietary bioavailability and the ultra-high dietary iron or copper content competing with zinc for the absorption sites were also suggested to cause reduced serum zinc levels (17). The increased urine protein observed in the pre-eclamptic subjects compared to other groups may be due, in part, to impaired integrity of the glomerular filtration barrier and altered tubular handling of filtered proteins (hypofiltration) leading to increased non-selective protein excretion. This observation agrees with the findings of previous studies who reported elevated urine protein levels in pregnant women with pre-eclampsia (18, 19). As expected, the BMI was increased in the pre-eclamptic pregnant women compared to the controls as the amniotic fluid and foetal body weights

increased. A positive correlation was observed between magnesium and random blood sugar in the GDM group which agrees with the study of Ertberg *et al* (20).

It has been established that Mg participates directly in glucose metabolism disorders in humans and has also been shown to function in hyperglycaemia, hyperinsulinemia and insulin resistance, although its exact role has not been elucidated (21).

CONCLUSION

It is concluded that low serum zinc levels may play a significant role in the pathogenesis of pre-eclampsia. Therefore, dietary intake or supplementation of zinc in pre-eclamptic patients may be a potential area for further studies.

LIMITATION OF STUDY

A relatively small sample size was used in this study.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare

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ORIGINAL ARTICLE

Evaluation of deoxyribonuclease 1-Like 3 as a potential regulator for immune activation in juvenile-onset systemic lupus erythematosus patients: a case-control study

Eman Eissa, Basma M Medhat, Botros Morcos, Dalia Dorgham, Rania Kandil, Nehal El-Ghobashy and Naglaa M. Kholoussi

ABSTRACT

Objectives: This study aimed to evaluate the expression pattern of Deoxyribonuclease1-Like 3 (DNase1L3), an endonuclease that catalyses the degradation of chromatin within apoptotic or necrotic cells, at the mRNA and protein levels and their association with the disease activity and inflammatory markers in Juvenile-onset systemic lupus erythematosus (jSLE) patients.
Methods: 36 Juvenile-onset systemic lupus erythematosus patients and 25 healthy controls were included in the study. DNase1L3 and Interleukin (IL)-1β expression in peripheral blood were determined using qRT-PCR, while ELISA was used to determine plasma levels of DNase1L3 and tumour necrosis factor (TNF)-*α*.
Results: DNase1L3 expression pattern at the mRNA and protein levels was significantly lower while IL-1β and TNF-a levels were significantly higher in Juvenile-onset systemic lupus erythematosus patients than healthy controls. There were significant negative associations between DNase1L3 expression at mRNA and protein levels with SLEDAI-2K of patients. Also, DNase1L3 plasma levels of patients were inversely correlated with IL-1β levels in a significant manner.
Conclusion: DNase1L3 could be involved in the immune regulation of jSLE patients and could be considered as a potential regulator of immune activation in those patients.

Keywords: DNase1L3, Juvenile-onset systemic lupus erythematosus (jSLE), inflammatory cytokines, IL-1b, TNF-*α*.

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INTRODUCTION

Monogenic involvement (1) rather than heterogenous polygenic affection (2), has been shown to be among the major determinants in the development of paediatric-onset SLE, a peculiar cohort with distinct clinical and immunologic features (3,4). The French Genetic and Immunologic Abnormalities in SLE (GENIAL/LUMUGENE) study is a longitudinal cohort (64 patients) describing several laboratory and genetic aspects of juvenile-onset SLE (jSLE) patients (5). The investigators divide the cohort into three groups being a) syndromic SLE (10 patients), with those patients being characterized by distinct clinical characteristics including growth failure, intracranial calcifications, b) familial SLE (12 patients), whereby patients have either familial consanguinity or first-degree SLE relatives, and c) all other early-onset SLE (42 patients).

Hence, differences in the pathophysiologic pathways of monogenic involvement could lead to various presentations. Monogenic affection includes complement deficiencies, interferonopathies, and abnormalities in deoxyribonucleic acid (DNA) damage, clearance, and repair; with the latter being regulated by a group of nucleases including deoxyribonucleases (DNases) (6), which are a group of enzymes that catalyse the degradation of DNA molecules and thus prevent self-DNA recognition and subsequent damage (7). Four different DNases have been identified, including DNase I (8), DNase1L3 (DNase γ) (9), DNase II (10), and TREX1 (DNase III) (11-13). Similarly, distinct clinical phenotypes vary among different DNases, with clinical (9) and experimental (7) studies demonstrating that DNase1L3 deficiency is associated with an earlier SLE-onset, renal involvement, hypocomplementemia, and high anti-double stranded deoxyribonucleic acid antibodies titres (anti-ds DNA).

It is of note that a complex interplay between DNase1L3 and other aspects of the innate immunity has been demonstrated in several studies, including inflammasome activation and subsequent interleukin (IL)-1β elevated levels (14), a proinflammatory cytokine that has been linked to lupus nephritis murine models (15). Moreover, several endonucleases (16) including DNase1L3 (17), have been linked to clearance of nuclear extra-cellular traps (NETs) that are strongly implicated in the pathogenesis of SLE (18). Impaired NETosis leads to a downstream of abnormal cytokine milieu including elevated tumour necrosis factor-α (TNF-α) (19), a cytokine that has been shown to be associated with several aspects of SLE (20).

In this case-control study, we aimed at determining DNase1L3 expression at mRNA and protein levels, IL-1β and TNF-a levels, and their potential association with several aspects of the disease in a cohort of jSLE patients.

PATIENTS AND METHODS

Ethics

This study was approved by the ethics committee of National Research Centre, Giza, Egypt with an approval number 16/109 and has therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All samples were obtained with the written informed consents of the subjects.

Study subjects

This study included 25 healthy subjects and 36 juvenile-onset SLE patients (age of onset ≤16 years) recruited from Rheumatology and Rehabilitation outpatient clinic, Kasr Al Ainy Hospital, Cairo University from March to July 2019. All patients fulfilled the 2012 Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE (21). Demographic and cumulative clinical manifestations were recorded, and disease activity at the last visit was assessed through the Systemic Lupus Erythematosus Disease Activity Index-2K (SLEDAI-2K) (22).

RNA extraction and quantitative real-time PCR

Total RNA was extracted and isolated from fresh blood of all subjects of the study populations using QIAamp RNA Blood Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. For reverse transcription, RNA was reverse-transcribed to cDNA using HiSenScrip™ RH(-) cDNA Synthesis Kit (iNtRON Biotechnology, Korea) according to the manufacturer's instructions. Reverse transcription was performed under the following conditions: 5 min at 25°C, 45 min at 45°C and followed by 10 min at 85°C and the resulting cDNA was kept at −80°C until use.

A quantitative real-time PCR (qRT-PCR) was carried out to quantify the expression levels in triplicate of DNase1L3 and IL-1b using TaqMan® DNase1L3 and IL-1b Assay kits and TaqMan® Universal Master Mix (Applied Biosystems) using 7500 fast real-time PCR system according to the manufacturer's instructions. GAPDH was used as endogenous control to normalize the expression levels of DNase1L3 and IL-1β. Relative quantification (Rq) was calculated using the $2^{-\Delta\Delta CT}$

threshold cycle method. ΔCt was determined by subtracting the Ct values for GAPDH from the Ct values for the gene of interest. qRT-PCR was performed under the following conditions: 2 min at 50°C, 10 min at 95°C, followed by 45 cycles at 95°C for 15s and at 60°C for 1 min (23, 24).

Enzyme-linked immunosorbent assay (ELISA)

Plasma DNase1L3 and TNF-a levels of all study subjects were determined using Human DNase1L3 ELISA kit (Sunlong Biotech Co. Ltd, China) and TNF-a ELISA kit (Elabscience, Elabscience Biotechnology Co., Ltd), respectively according to the manufacturer's protocol.

Statistical analysis

Data were statistically analysed using SPSS version 19.0 software (SPSS Inc., Chicago, Illinois, USA). Non-parametric Mann-Whitney U test was used to compare the expression pattern of DNase1L3 and the inflammatory cytokine levels between groups. The correlation of DNase1L3 expression with the clinical data, SLEDAI score and the inflammatory cytokine levels of jSLE patients was done using Spearman correlation analysis. Data were presented as median and mean ± SD. A *P* value of less than 0.05 was considered statistically significant.

RESULTS

Baseline characteristics

The study included 36 jSLE patients, of whom 35 were females (97%). The mean age of patients at onset and at time of sampling was 12.66 ± 3 and 24.5 ± 7 years, respectively, whereas the median disease duration (interquartile ratio) was 138 (115) months. Furthermore, 25 normal controls, 22 females (88%) and 3 males (12%), were involved with a mean age 26 ± 6.4 years. It is of note that 4/36 (11%) patients had a family history of SLE, and 4/36 (11%) had a family history of other autoimmune diseases. Cumulative clinical and serologic patients' characteristics, and the medications received at the time of sampling are shown in Table 1.

Expression pattern of DNase1L3 at mRNA and protein levels

DNase1L3 mRNA expression pattern was significantly reduced in our patients compared to the control group (Rq median = 0.37, p = 0.001), with a 2.7-fold decrease (Figure. 1a). Moreover, plasma levels of DNase1L3 were significantly lower among our patients compared to their controls (p = 0.019) (Figure. 1b).

Association of DNase1L3 with disease characteristics and serological data

Among the patients' recorded characteristics, there was no association of DNase1L3 expression and plasma levels with any of the different clinical and serological features (Data not shown). Interestingly, there was a significant negative correlation between DNase1L3 expression at mRNA (r = - 0.531, p = 0.001) and protein levels (r = - 0.430, p = 0.012) with disease activity (SLEDAI-2K). On the other hand, there was no association between DNase1L3 mRNA expression (r = - 0.222, p = 0.214) and protein levels (r = - 0.111, p = 0.537) with disease damage (SDI).

Expression levels of Interleukin (IL)-1b and Tumour necrosis factor (TNF)-α

Interleukin-1β expression pattern was significantly higher among our patients in comparison to the controls (Rq median = 43.8, p = 0.001) (Figure. 2a). Moreover, patients' TNF-α plasma levels were significantly higher than those detected among the controls (p = 0.015) (Figure. 2b).

Association of DNase1L3 levels with IL-1β and TNF-α Apart from a weak negative correlation between patients' DNase1L3 protein and IL-1β levels (r = 0.3; p =0.04), there was no association between DNase1L3 mRNA expression and IL-1β levels (r = - 0.1; p = 0.3); and moreover, there was no association between DNase1L3 mRNA expression (r= -0.13; p=0.4) and protein (r=-0.2; p=0.09) levels with TNF-α levels.

Table 1. Baseline patients' characteristics*

	N = 36 (%)
Cumulative clinical manifestations	
Constitutional	28 (78)
Mucocutaneous	10 (28)
Neuropsychiatric	9 (25)
Nephritis	29 (80.5)
Arthritis	24 (67)
Serositis	10 (28)
Secondary antiphospholipid syndrome	16 (44.4)
Cumulative immune profile	
ANA	32(89)
Anti-ds DNA	29(80.5)
Hypocomplementemia	17 (47)
aPL	16(44.4)
Disease activity at sampling visit	
SLEDAI-2K (Median (IQR))	9 (7)
Disease damage at sampling visit	
SDI (Median (IQR))	1 (2)
Medications received at sampling visit	
Glucocorticoids	36(100)
Hydroxychloroquine	25(69.4)
Azathioprine	12(33.3)
Mycophenolate mofetil	6(16.7)
Cyclophosphamide	5(14)

*Unless indicated, data are presented in number (N) and percentage. Abbreviations: IQR: interquartile ratio; ANA: anti-nuclear antibodies; Anti-ds DNA: anti-double stranded deoxyribonucleic acid antibodies; aPL: antiphospholipid antibodies; SLEDAI-2K: Systemic Lupus Erythematosus Disease Activity Index-2K; SDI: Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index

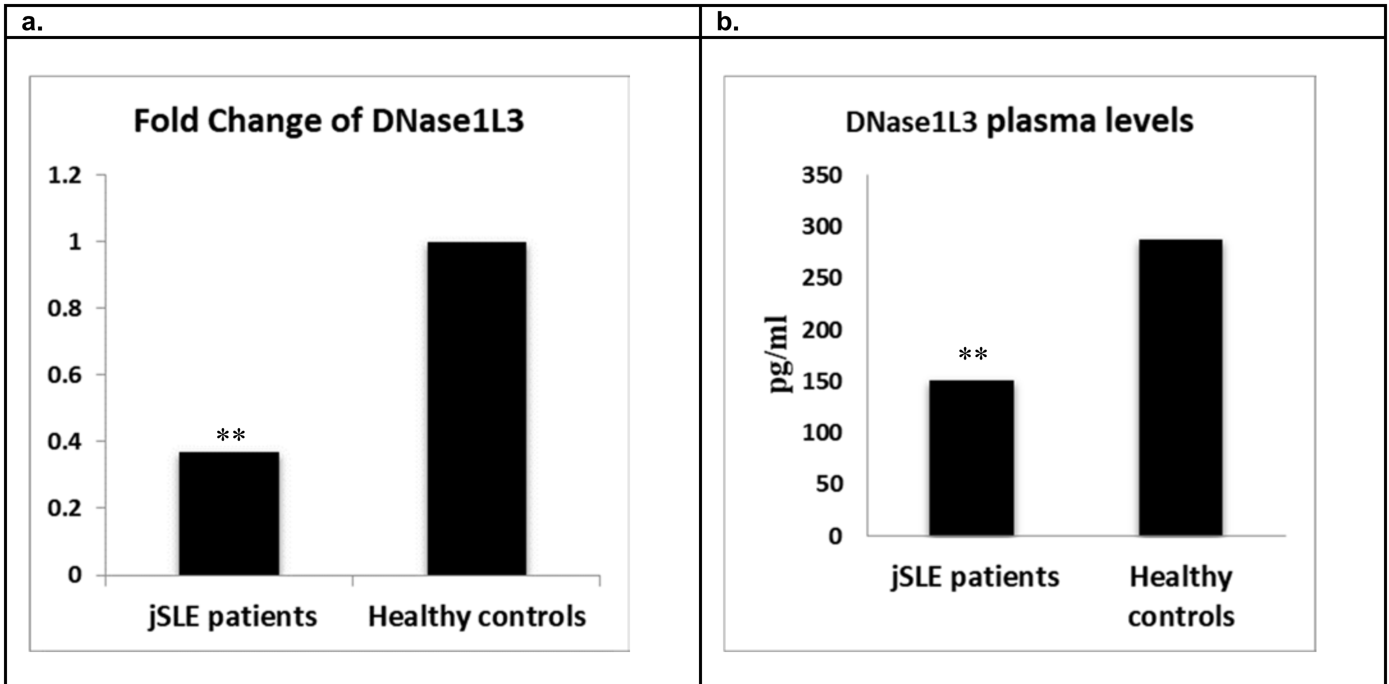

Figure. 1 a: Fold Change of DNase1L3 in jSLE patients relative to healthy controls. Data were presented as Rq median (p = 0.001, by Mann Whitney U Test).
b: Plasma levels of DNase1L3 in jSLE patients compared with healthy controls. Data were presented as median (p = 0.019, by Mann Whitney U Test).

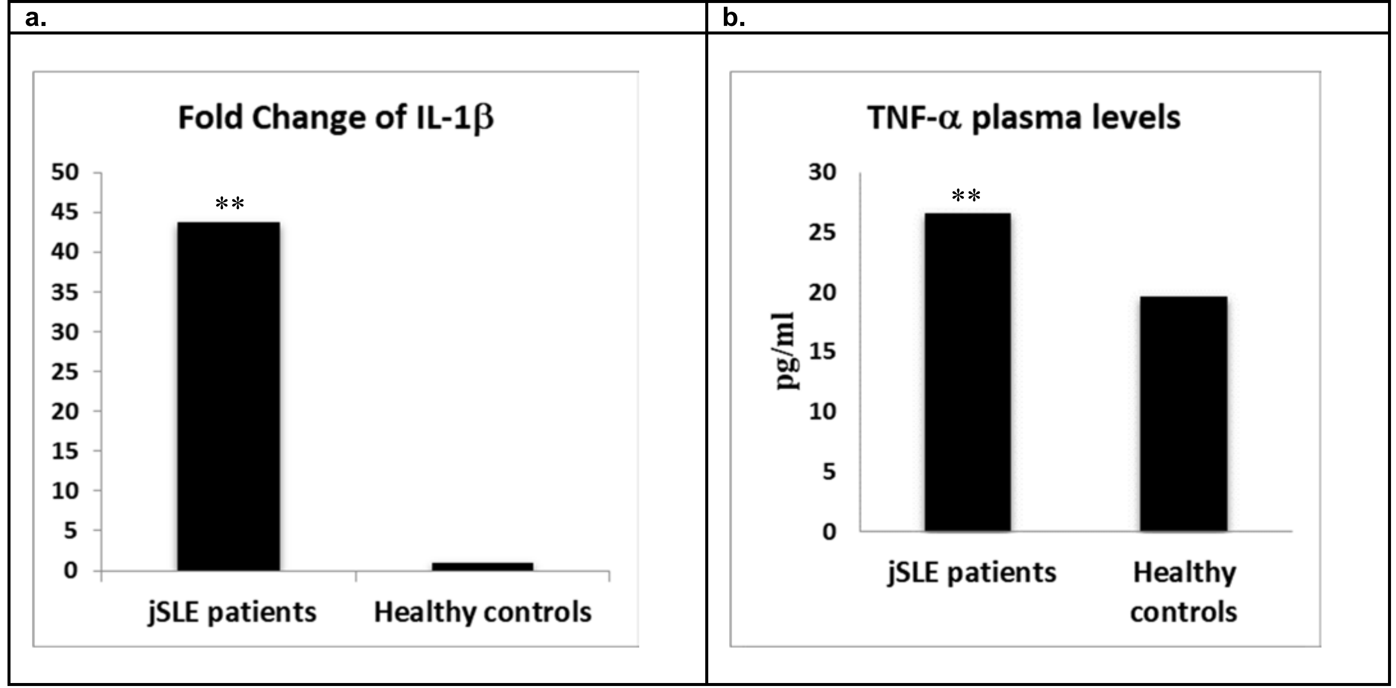

Figure. 2 a: Fold Change of IL-1β in jSLE patients relative to healthy controls. Data were presented as Rq median (p = 0.001, by Mann Whitney U Test).
b: TNF-α plasma levels in jSLE patients in comparison with healthy controls. Data were presented as median (p = 0.015, by Mann Whitney U Test)

DISCUSSION

DNase1L3 is one of the endonucleases that when aberrant, could lead to impaired DNA elimination and subsequent innate immunity activation and a downstream of altered cytokine milieu (14). Herein, we analyse the potential association of jSLE with DNase1L3 and the possible inter-play between DNase1L3 with IL-1β and TNF-α.

In our study, DNase1L3 pattern at both mRNA expression (2.7-fold) and plasma levels were significantly reduced in patients compared with the control group, hence resembling previous clinical study (25) and experimental studies (7). DNase1L3 LacZ/LacZ knockout (KO) murine models (7) that developed progressive anti-ds DNA levels increase at five weeks and was further followed by renal immune complex deposition and glomerulonephritis.

Interestingly, we found a significant negative correlation between DNase1L3 expression at mRNA and protein levels with disease activity (SLEDAI-2K). This is consistent with Zhao et al. (2017) who demonstrated a significant decrease in DNase1L3 level in the patients with active SLE versus those with inactive disease as well as in the patients with positive anti-ds DNA compared to those with negative anti-ds DNA (25).

We have investigated IL-1β and TNF-α as potential markers associated with SLE and linked to DNase1L3. Although both cytokines' expression pattern was higher among our cases rather than the controls, TNF-α showed no association with DNase1L3 mRNA expression or protein level, whereas, apart from a weak negative correlation between patients' DNase1L3 protein and IL-1β levels, there were no other significant associations between them. It is of note that several investigators demonstrated that DNase1L3 activity loss resulting in DNA accumulation could lead to subsequent toll like receptors (TLRs) activation (7,26) and hence, NF-kB dependent IL-1β gene encoding transcription, a pro-IL-1β protein (27,28). These insights into the pathophysiology entailing DNase deficiency give rise to potential therapeutic implications. Exogenous administration of recombinant DNase1L3 protein could prevent immunogenic DNA-dependent TLR signalling (29). Other proposed therapeutic implications include NET remnant elimination by DNase1 and 1L3 (17). In interest of SLE per se, recombinant DNase 1 improved laboratory and renal markers in a lupus prone murine model (30), but these findings were not replicated when survival was set as a primary endpoint (31). Interestingly, DNase1L3, being an extracellular nuclease rather than an intracellular one, could lead to engineering and modification of DNase1L3 protein to enhance its nuclease activity and increase its half-life in circulation (7,29,32).

The main limitation of our study resides in the limited number of patients. However, our study strengths include investigate DNase1L3 in an Egyptian cohort, which is to the best of our knowledge was yet to be investigated, and we have studied its potential association with IL-1β and TNF-α.

To conclude, our jSLE cohort demonstrated that DNase1L3 expression pattern at both mRNA and protein levels were significantly lower than healthy controls. Moreover, DNase1L3 expression at mRNA and protein levels inversely correlated with disease activity. On the other hand, IL-1β and TNF-α levels were significantly elevated in jSLE patients in comparison to the control group; yet their association with DNase1L3 was unremarkable.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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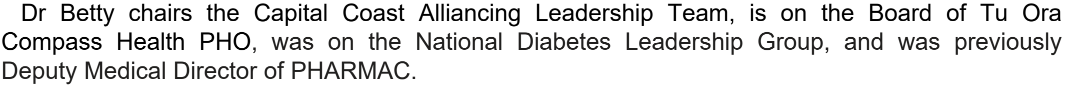
Dr Bryan Betty

Medical Director of The Royal New Zealand College of General Practitioners

Dr Bryan Betty is a Wellington-based GP who works at Porirua Union and Community Health Services in Cannons Creek, East Porirua, a low-cost access practice. The practice has 7000 registered patients, is 90% high needs with 25% Māori, 50% Pacific the remainder mainly refugee - Syrian, Burmese, Columbian. A strong motivator throughout his career has been issues of equity and access to quality healthcare for everyone. Dr Betty has always been a strong advocate for general practice and the role of general practitioners within the primary healthcare system.

Dr Betty became the College's Medical Director in August 2019. He provides clinical advice and guidance on policy and medicolegal issues. He is also a media spokesperson for the College and speaks regularly on issues affecting the GP workforce and patient care in order to advocate for change. His opinion editorials appear in New Zealand Doctor, *Stuff* and the *New Zealand Herald.* In his role as Medical Director, Dr Betty represents the College and its 5,500 members on various health sector committees.



Dr Betty chairs the Capital Coast Alliancing Leadership Team, is on the Board of Tu Ora Compass Health PHO, was on the National Diabetes Leadership Group, and was previously Deputy Medical Director of PHARMAC.

Dr Betty will be speaking at Plenary Session 3 of the NZIMLS Annual Scientific Meeting on Thursday 1 September.

CASE STUDY

A case of Factor XIII deficiency in New Zealand

C Sydney Shepherd

ABSTRACT

Factor XIII is a rare clotting deficiency whereby the formed fibrin clot is unstable and leads to a bleeding tendency. Routine clotting factor assays are usually normal leading to this disorder being missed in the first instance. In this case report a historic case of Factor XIII deficiency in New Zealand is provided and highlights the classical initial problems with identifying this inherited disorder.
Key words: Factor XIII deficiency, missed diagnosis, fibrin stability.

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INTRODUCTION

Formerly known as fibrin stabilising factor or Laki-Lorand factor, Factor XIII is a pro-transglutaminase for a plasma transglutaminase and is converted to the active form (XIIIa) by the action of thrombin in the presence of calcium ions. In the terminal clotting stage, Factor XIIIa acts by cross-linking the formed fibrin clots to stabilise and protect them from fibrinolysis (1).

First formally described by Duckert *et al.* (2,3) in a young boy with a cut to his head associated with a prolonged bleeding time, which was subsequently identified as Factor XIII deficiency. In Factor XIII deficiency, clots form as normal after bleeding or tissue damage but tend to break down if not stabilised by Factor XIIIa leading to a delay in the healing process. Factor XIII deficiency (with less than 1 per cent of the normal Factor XIII) is inherited as an autosomal recessive trait that affects about one in three million people (1). However, there are a number of missense and nonsense point mutations in the Factor XIII gene which will produce a range of bleeding tendencies, the majority of which may be familial and are considered to be autosomal recessive in origin (4).

CASE STUDY

MP presented to our laboratory for investigation of bruising episodes especially around his legs when about two years old, routine blood screening showed his haematology results to be normal including platelet count and coagulation screening tests (PCT, APPT, TCT, fibrinogen) were all normal.

The patient again presented for investigation some two and a half years later, this time with the added information that the bruising on his arms and legs appeared to occur in fairly regular cycles of about six-week intervals. Again, all the coagulation screening tests were within normal range. However, careful observation of the clots formed during these tests noted that the clots gradually broke down and showed dissolution.

A study of the literature indicated the possibility of Factor XIII deficiency and samples were submitted to Professor Duckert's reference laboratory in Geneva where confirmation showed that MP had a Factor XIII level of between 10 to 15% of normal range. Family studies showed that MP's two siblings both had normal levels of Factor XIII, but interestingly both parents showed subnormal levels (40 to 50% of normal). Unfortunately, we were unable to obtain samples from any other extended family members due to privacy reasons.

Treatment was begun with transfusion of cryoprecipitate with dosage appropriate for age and body size at six weeks intervals and the bruising episodes subsided. Sadly, the patient was killed in an accident before long-term survey of his condition could be monitored.

In conclusion, Factor XIII deficiency should always be considered and either confirmed or excluded in investigation of bleeding, bruising disorders when all clotting screening test show normal results.

ACKNOWLEDGEMENTS

Dr Symman's family for encouragement to proceed with the publication. MP's mother for supplying information and being supportive while preparing this manuscript.

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Science Snippet

Be careful how you test your theories.

Alexander Bogdanov was a significant figure in the development of blood transfusion in the 1920s. However, he firmly believed that blood transfusions especially from young people could provide the answer to eternal youth. Over two years he transfused himself with 11 litres of blood taken from students (presumably with the same ABO group.) Despite his claim for perpetual youthfulness his final (and terminal) transfusion was from a student who had tuberculosis and severe malaria and Bogdanov died.

CASE STUDY

An isolate of ST235 Pseudomonas aeruginosa harbouring IMP-26 in New Zealand

Sean Munroe, Hermes Pérez Cardona , Kristin Dyet and Julia Howard

ABSTRACT

Pseudomonas aeruginosa is an important cause of nosocomial infections worldwide. Worryingly, many *P. aeruginosa* are multidrug -resistant, and the carbapenem-resistant *P. aeruginosa* have been recognised as a global threat by the World Health Organisation (WHO).A prevalent international clone of *P. aeruginosa* is ST235, which is associated with high-level antibiotic resistance and poor clinical outcomes. We report an isolate of ST235 *P. aeruginosa* harbouring IMP-26, which to the best of our knowledge is the first case of in New Zealand, in a patient with no history of overseas travel.

Keywords: *Pseudomonas aeruginosa*, Carbapenemase, IMP-26.

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INTRODUCTION

Pseudomonas aeruginosa is an important cause of nosocomial infections worldwide (1). These infections can be difficult to treat due to isolates of *P. aeruginosa* possessing intrinsic resistance, and the ability of the organism to acquire further resistance to multiple classes of antibiotic agents (2).The most prevalent and widespread clone of *P. aeruginosa* worldwide is ST235, and this clone has the potential to become readily resistant to aminoglycosides, beta-lactams and carbapenems (3).Carbapenem-resistant *P. aeruginosa* is recognised as a global threat by the World Health Organisation (WHO). It is listed as one of three bacteria with critical priority for antibiotic research therapy due to the increased spread of these strains (4).

CASE REPORT

In September 2020 in Hamilton, NZ, we isolated a *P. aeruginosa*, identified by the Vitek MALDI-TOF (BioMérieux), from the abdominal drain fluid of an inpatient who had no history of overseas travel. Disc (Oxoid) susceptibility testing showed the isolate was resistant to ceftazidime, cefepime, piperacillin-tazobactam, meropenem, gentamicin, tobramycin and ciprofloxacin but susceptible to aztreonam and amikacin (Table 1.) using European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints. The isolate was resistant to ceftazidime-avibactam and ceftolozane-tazobactam (Liofilchem MIC Strip) and susceptible to colistin (broth microdilution) (Table 1.) Phenotypic screening for carbapenemase activity was performed by CARBA PAcE (MAST Group) and was positive within 10 minutes. Based on this a GeneXpert Carba R (Cepheid) was performed, which revealed the isolate possessed the bla_{IMP} gene.

Genomic DNA was extracted using the Roche High Pure PCR template preparation kit, the DNA library was created using the Nextera XT DNA preparation kit (Illumina), and sequencing was performed using Illumina technology. Whole Genome Sequencing (WGS) data was analysed using an in-house developed pipeline linking together open-source established packages and in-house scripts. Open-source packages used included the Nullarbor (https://github.com/tseemann/nullarbor): 'Reads to report' for public health and clinical microbiology pipeline. WGS data was analysed using an in-house developed pipeline linking together open-source established packages and in-house scripts. Open-source packages used included the Nullarbor2: 'Reads to report' for public health and clinical microbiology pipeline SKESA v.2.3.0 (https://github.com/ncbi/SKESA), MLST (https://github.com/tseemann/mlst) and ABRicate (https://github.com/tseemann/abricate) using ResFinder and PlasmidFinder databases. The isolate was sequenced with a depth of 93x. Analysis of the *de novo* assembly of the WGS reads identified the *bla*IMP-26 gene and showed that the isolate was ST235.

Table 1. Susceptibility profile.

Antibiotic	Interpretation (Zone size/MIC)
Amikacin	Susceptible (19mm)
Aztreonam	Susceptible (20mm)
Cefepime	Resistant (6mm)
Ceftazidime	Resistant (6mm)
Ceftazidime-avibactam	Resistant (>256 mg/L)
Ceftolozane-tazobactam	Resistant (>256 mg/L)
Ciprofloxacin	Resistant (6mm)
Colistin	Susceptible (1.0 mg/L)
Gentamicin	Resistant (6mm)
Meropenem	Resistant (>32 mg/L)
Piperacillin-tazobactam	Resistant (19mm)
Tobramycin	Resistant (6mm)

DISCUSSION

IMP-26 producing *P. aeruginosa* was first described in Singapore in 2009, (5) and then subsequently described from retrospective analysis of clinical samples from Malaysia and the Philippines (6,7), as well as in Vietnam, where isolates had spread in a medical setting (8). It has also been found in clinical samples from Uganda (9). IMP-26 has also been found in other Gram-negative bacteria, such as in *Enterobacter cloacae* in China (10).

Carbapenemase producing *P.aeruginosa* are infrequently isolated in New Zealand. The first *P. aeruginosa* harbouring a carbapenemase gene in New Zealand was isolated in 2009. Between 2009 and December 2021 39 isolates of *P. aeruginosa* harbouring acquired carbapenemase genes have been submitted to ESR. Metallo-beta lactamases have been the most common gene type identified, with VIM the most predominant, at the time of writing (ESR data).

This is the first known case of the IMP-26-producing *P. aeruginosa* in New Zealand. To date, most isolates with acquired carbapenemase genes in New Zealand are thought to have been acquired overseas, so it is of concern that this isolate with IMP-26 was found in a patient with no history of overseas travel and no history of travel from family in the same household. Only six other *P. aeruginosa* isolates possessing

IMP beta-lactamase genes have been reported in New Zealand (unpublished data). Travel history was reported for five of the cases, indicating they were likely to have been acquired in an overseas hospital (China, Thailand (three cases) and Peru).

Patient screening for carbapenemase-producing *P. aeruginosa* can be challenging for laboratories, as highlighted by the WHO Guidelines for the prevention and control of carbapenem-resistant Enterobacteriaceae, *Acinetobacter baumannii* and *P. aeruginosa* in health care facilities (11). These guidelines stopped short of recommending active surveillance for carbapenem-resistant *P. aeruginosa* as whilst it may be beneficial, the potential benefit is dependent on clinical setting, epidemiological stage and body sites sampled. Unlike carbapenemase-producing Enterobacterales (CPE) where faecal material or rectal swabs were considered the best methods for surveillance, detection of carbapenem-resistant *P. aeruginosa* carriage may be improved by the addition of urine and pharyngeal swabs as well (12).

Interestingly, our patient had a rectal screening swab collected during his hospital stay and P. aeruginosa was not isolated, but subsequent abdominal drains continued to grow the IMP-producing *P. aeruginosa*.

In conclusion, to the best of our knowledge this the first case of IMP-26 producing *P. aeruginosa* in New Zealand, from a patient with no overseas travel history. Active surveillance and outbreak screening for organisms such as these may prove difficult for laboratories in the absence of official recommendations of appropriate sites and methods.

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Dr Joep de Ligt PhD
Senior Science Lead Bioinformatics & Genomics
Institute of Environmental Science and Research Limited (ESR)

"Dr. Joep de Ligt is the Senior Science Lead bioinformatics and genomics at ESR New Zealand, where the teams work covers topics ranging from Human genetics to Infectious disease genomics.

The pathogen genomics team supports the Ministries of Health and Primary industries in the surveillance of notifiable diseases.

Genome sequencing is becoming an increasingly important tool in monitoring incursions, investigating outbreaks and helping understand and track drug resistance.

The team was able to respond quickly to the emergence of SARS-CoV-2 and demonstrated the capability and value of rapid whole genome sequencing in New Zealand's COVID-19 response.

The research done within the team ranges from understanding virulence of certain species to applied research geared at delivering on the health impact potential of sequencing-based technologies."

Dr de Ligt will be speaking at Plenary Session 3 of the NZIMLS Annual Scientific Meeting on Thursday 1 September.

CASE STUDY

False positive paracetamol results due to interference in a colorimetric assay. A case study

Shugo Kawamoto

ABSTRACT

A 31-year-old female with no medical history of liver disease or intake of herbal medicine was admitted to hospital for abdominal pain, decreased appetite, malaise, tea coloured urine and confusion. Routine laboratory testing revealed elevated liver function tests (LFTs) and marked coagulopathy consistent with a diagnosis of acute liver failure (ALF). Plasma paracetamol level at 121 µmol/L prompted clinical consideration of paracetamol drug overdose; however, the patient denied taking paracetamol. Over the course of her hospitalisation general conditions and liver functions improved; however, plasma paracetamol concentration remained greater than 100 µmol/L . This article discusses the case where the colorimetric principle for paracetamol quantification gave rise to a falsely elevated paracetamol, confounding the clinical picture to suggest paracetamol overdose and recommendations for laboratories using the colorimetric principle to help identify this interference.

Key words: acute liver failure, paracetamol, acetaminophen, coagulopathy, hepatic necrosis, colorimetric.

N Z J Med Lab Sci 2022; 76(2): 86-89.

CASE

A 31-year-old female, with no medical history of liver disease or intake of herbal medicine, was admitted to hospital for abdominal pain, decreased appetite, malaise, and confusion. Routine laboratory testing of blood sample shown in Table 1 revealed elevated LFTs and marked coagulopathy consistent with a diagnosis of ALF. Plasma paracetamol levels at 121 µmol/L (therapeutic range up to 100 µmol/L) prompted clinical consideration of paracetamol drug overdose, but the patient denied taking paracetamol. Over the course of her hospitalisation general conditions and liver functions improved, however plasma paracetamol concentration remained greater than 100 µmol/L. Failure of the liver to metabolise the drug was suspected and liver transplantation was considered for treatment (1).

Acute liver failure

ALF is defined as a rapid loss of liver function, which in turn results in impaired protein synthesis, and is evidenced by coagulopathy. It can ultimately result in necrosis of hepatic cells and death of the patient if left untreated. Common causes of ALF, but not limited to this list, include:

- Toxic injury (e.g., paracetamol, natural products, isoniazids)
- Idiosyncratic reaction to medical treatment; e.g., antibiotics, NSADs, statins (2)
- Alcoholic and autoimmune hepatitis
- Viral hepatitis: Hepatitis B and C most common in New Zealand (3)
- Wilson's Disease, a rare genetic disorder of copper accumulation in liver and brain
- Idiopathic, cryptogenic, or indeterminate (without obvious cause)

Liver transplantation is a significant clinical decision and candidates must have an irreversible disease for which medical and surgical therapies have been exhausted. Despite being a last resort in critical situations, paracetamol aetiology is the fourth leading cause of liver transplants, as summarised in Figure 1 (4). Routine laboratory tests can aid in the differential diagnosis of ALF, and therefore it is important for laboratories to provide rapid and accurate laboratory test results to help determine the cause and ascertain effective treatment without relying on a liver transplant.

Pathophysiology of paracetamol

Although paracetamol is widely used for pain relief, the mechanism of action is a topic of debate. It is known that paracetamol confers its analgesic effects by activating the descending serotonergic pathways, and Anderson (5) proposes two mechanisms: inhibition of prostaglandin (PG) synthesis or influencing cannabinoids receptors. In the inhibition pathway, prostaglandin H2 synthetase (PGHS) is responsible for arachidonic acid metabolism to the unstable PGH2. There are two active sites for PGH2: a cyclooxygenase (COX) and a peroxidase (POX) site. Paracetamol acts on the POX site and reduces the amount of the oxidised form of the COX site, thereby inhibiting the synthesis of PG. Alternatively, the synthesis can be inhibited by an active metabolite of paracetamol (p-aminophenol) and acts on the cannabinoid receptor, which exerts analgesic effects such as relaxation and tranquillity. At therapeutic doses, more than 90% of the paracetamol is metabolised by the liver and excreted in urine as nontoxic glucuronide and sulphate conjugates.

As schematically represented in Figure 2, less than 5% of paracetamol is metabolised by cytochrome P450 1AE and 2E1 to N-acetyl-p-benzoquinoeimine (NAPQI), which is a highly toxic intermediate that causes free radical damage. Moreover, the cytochromes can be induced to increase activity and thereby produce more NAPQI under cases of paracetamol overdose, alcohol consumption, or induction by isoniazid and phenobarbitones (6). This toxic intermediate is usually reduced to nontoxic mercapturic acid and cysteine conjugates, but in the case of paracetamol overdose, where sulfation and the glucordination pathway becomes saturated, glutathione becomes deficient and excessive NAPQI nonspecifically binds to intracellular proteins causing cell apoptosis and ultimately death.

A timely measurement paracetamol concentration allows treatment with N-acetylcysteine (NAC) that is known to minimise or prevent hepatic damage (7). The mechanism for NAC to be the antidote is not fully understood. It appears NAC is able to work as a sulfhydryl donor and replenish the hepatic glutathione storage and sulphate conjugation (8,9). However, the effectiveness of the drug rapidly diminishes 12 to 24 hours after exposure to paracetamol. This reinforces the importance of a rapid and accurate measurement of paracetamol to establish whether NAC treatment is beneficial. Therefore, plasma paracetamol concentrations should be measured as soon as possible for all cases of suspected drug overdose. Falsely positive paracetamol results can lead to inappropriate use of NAC. This can trigger potential serious adverse effects, where gastrointestinal effects are the most common. Most importantly, anaphylactoid reactions occur in around 60% of patients. In addition, clinicians may make false assumptions about the cause of the ALF and take no further investigation for other possible causes. The true cause may never be found, and patients may miss the needed treatment.

Methods of paracetamol measurement

Many methods have been described for the assay of paracetamol. The gold standard is Gas Liquid Chromatography (GLC) and High Pressure Liquid Chromatography (HPLC) as it

has proven to produce accurate quantification of paracetamol with minimal interference (1). Notwithstanding, these tests are time consuming, require specialised laboratory equipment, and highly skilled staff to operate the instrument making it unsuitable for routine laboratory measurement. For rapid, automated measurement of paracetamol colorimetric enzymatic methods are more commonly used. Another automated method is the competition immunoassay that is widely available but more expensive. In 2019, 117 on the 322 laboratories enrolled in the Royal College of Pathologists of Australasia's Quality Assurance Program (RCPAQAP) used methods with a colorimetric measurement. Incidentally, 69 of the laboratories used immunoassay. In our laboratory at LabPLUS, paracetamol is measured with Cobas ACET2 which is based on competition immunoassay (10).

Interference with paracetamol measurement

Colorimetric principles, whilst economical, are more susceptible to spectrophotometric interferences that immunoassays are less likely to experience. This includes haemolytic and icteric separated plasma that create a strong background interference in the absorbance of the ultraviolet and visible regions of the electromagnetic spectrum. In particular, icteric samples can raise the background absorbance at 600 nm that contributes to a false elevation in paracetamol. According to the package insert for an enzymatic principle (11) interference occurs at unconjugated bilirubin concentrations of >86 µmol/L. Moreover, enzymatic principles may also have a false positive in the presence of therapeutic concentrations of NAC (8). Unlike enzymatic based principles, immunoassays are less likely to have interference from bilirubin, up to 510µmol/L of unconjugated bilirubin, and unaffected by presence of NAC.

DISCUSSION

Because paracetamol levels were consistently elevated throughout the subsequent samples taken during hospitalisation, interference with the assay was considered to be an issue. Particularly, bilirubin interference is known to cause aberrant results in colourimetry. To reduce this interference, plasma ultrafiltration was applied to remove the interfering substances before measuring paracetamol with the enzymatic method. An ultrafiltrate has the same concentrations of analytes but is virtually free of proteins, including protein-bound bilirubin, haemoglobin, and lipoproteins. Using Centrifree micropartition devices from Amicon Bioseparations/Milipore, 1 mL of patient plasma and positive control in the reservoir of the device was centrifuged at 1000-2000g for 10 min. The ultrafiltrate was collected, assayed, and quantified for paracetamol and bilirubin with the Vitros system.

The results summarised in Table 2 indicated that the patient had not ingested paracetamol, as the analyte was undetectable in the patient's plasma by the enzymatic method assay post-treatment. These findings were supported by the HPLC method that consistently produced negative results on the patient's plasma samples. Interpreted together, the results strongly indicate bilirubin interference and false positive paracetamol concentrations when measured by the enzymatic method. An alternative method for identifying bilirubin interference for laboratories that do not have the ultrafiltration equipment is dilutions of the sample to confirm the bilirubin effect.

From previous research, the colorimetric method on the Vitros system was the only method that expressed positively proportional linear relationship between the false positive value of paracetamol and bilirubin concentration (12). Among other colorimetric methods, the Vitros system is the only one using ferricyanide as chromogen activator. It is possible that ferricyanide reacts with bilirubin or unknown substances in the sample to produce product that absorbs at or around the wavelength λ=670 nm. This concept was supported when normal human serum with added bilirubin was tested. Since conjugated and unconjugated bilirubin have an absorption peak between 390 and 460 nm it is therefore likely that bilirubin by-products are causing interference in the assay.

The patient's liver functions improved during hospitalisation, and she made an uneventful full recovery after three weeks. The exact pathophysiology of acute hepatic derangement remains obscure, as Fong *et al.* conclude (1). Possible explanations of over-the-counter health supplements containing hepatotoxic ingredients, such as alternative herbal remedies or a rare viral hepatitis caused by the Epstein-Barr virus were suggested, but not rigorously excluded in this case.

CONCLUSIONS

Persistently increased levels of paracetamol were seen in a patient with acute liver failure which was initially suspected due to the inability to metabolise the drug, despite patient describing they had not taken the drug. Timely laboratory results of paracetamol measurement are critical in the management of overdose, as prompt treatment using glutathione precursor NAC can minimise hepatic damage, but its effectiveness diminishes rapidly by 12-24 hours after exposure. Many methods of paracetamol analysis have been described and the colorimetric enzymatic assays are commonly used for its rapid and economic automated protocol. Despite these benefits, the colorimetric principle is more susceptible to spectrophotometric interference from bilirubin and is used in more than a third of the laboratories enrolled in RCPAQAP in Australasia. Particularly in the case of acute liver failure, high total bilirubin can give rise to false positive paracetamol levels, confounding the clinical picture. It is highly recommended that total bilirubin is analysed for all paracetamol analysis, particularly if the laboratory is using a colorimetric method.

Table 1. Initial presentation of laboratory results.

Test	Results	Units	Reference range
Total Bilirubin	1210	µmol/L	<25
AST	5080	U/L	0-45
ALT	6170	U/L	0-45
ALP	150	U/L	40-110
Ammonia	171	µmol/L	<70
LDH	6830	U/L	120-250
Paracetamol	121	µmol/L	<100
PT	39.7	seconds	9-18
INR	3.3	-	2-3
Hepatitis A Serology	Negative	-	-
Hepatitis B Serology	Negative	-	-

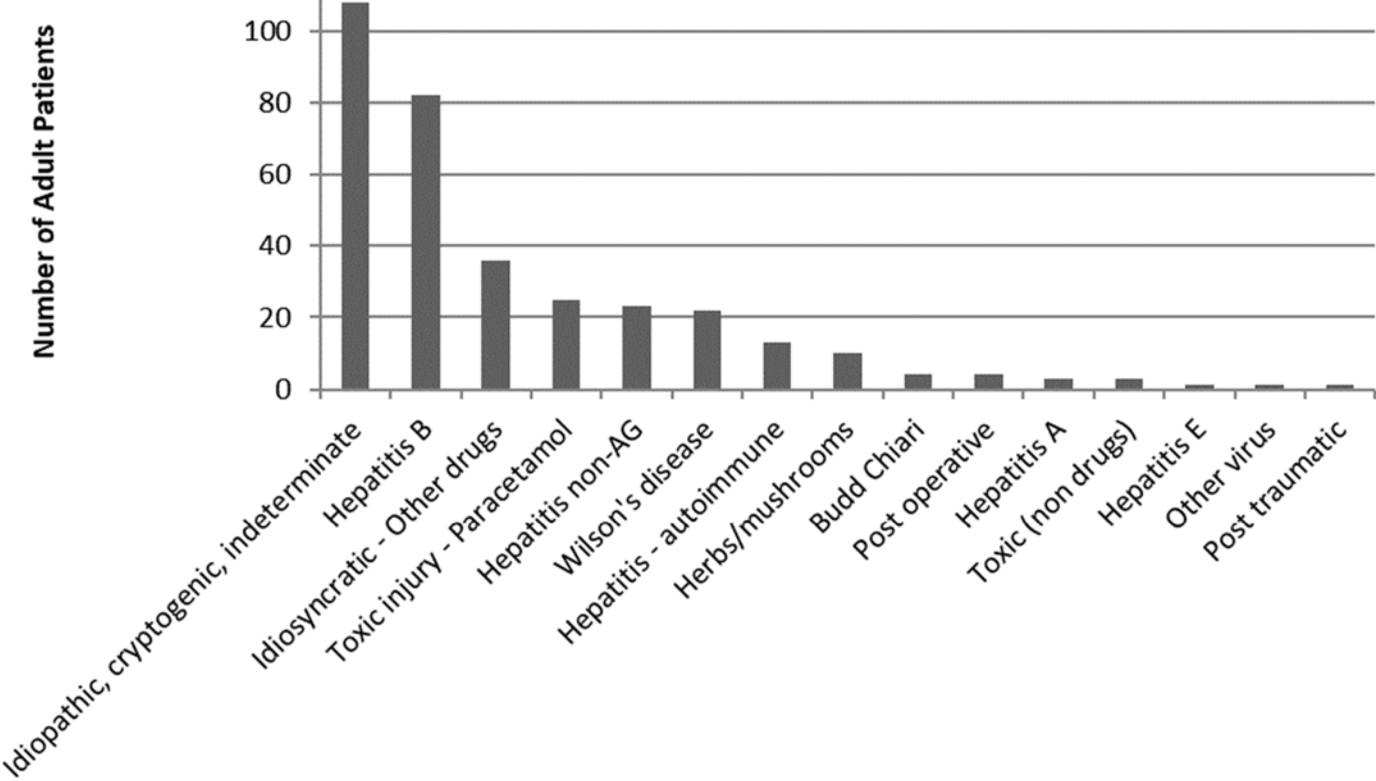

Figure 1. Data of adult acute liver transplant cases in Australia and New Zealand to 31/12/2019. Adapted from the Australia and New Zealand Liver and Intestinal Transplant Registry 31st Annual Report 2019.

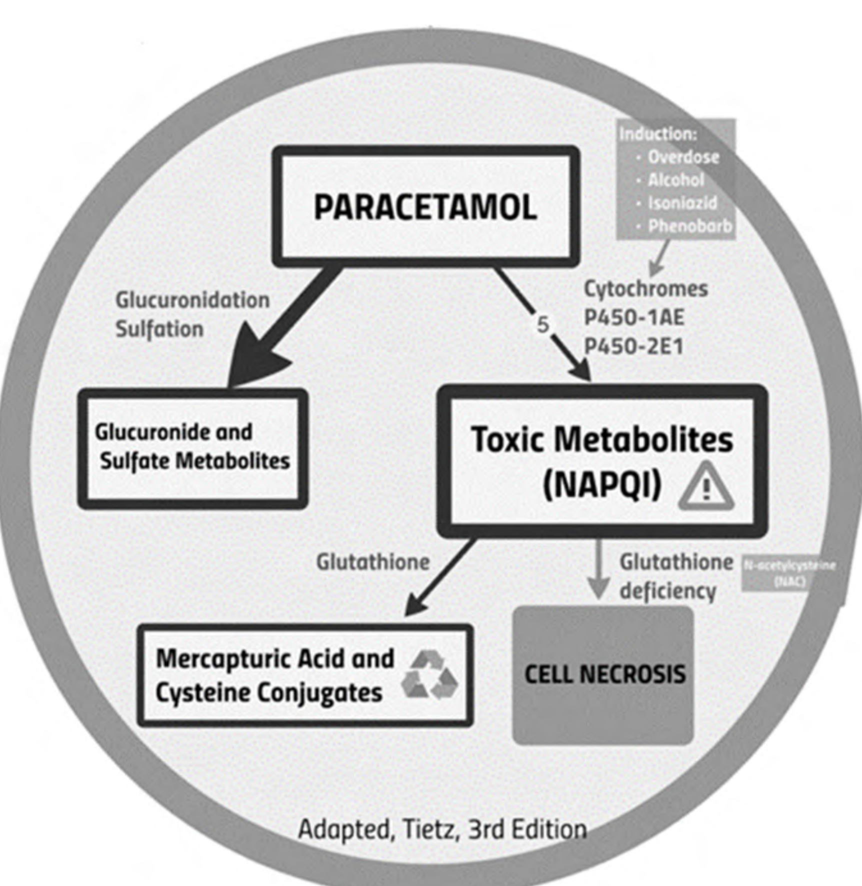

Figure 2. Paracetamol pathophysiology (adapted from Tietz, 3rd Edition, 1999).

Table 2. Paracetamol and bilirubin concentrations for patient samples day 1-3.

Method (Day 1 sample)	Test	Patient (µmol/L)	Positive control (µmol/L)
Plasma, enzymatic	Paracetamol	121	610
	Bilirubin	1210	12
Ultrafiltrate, enzymatic	Paracetamol	<30	609
	Bilirubin	<2	<2
Plasma, HPLC	Paracetamol	<1	612

Method (Day 2 sample)	Test	Patient (µmol/L)	Positive control (µmol/L)
Plasma, enzymatic	Paracetamol	115	611
	Bilirubin	1170	12
Ultrafiltrate, enzymatic	Paracetamol	<30	610
	Bilirubin	<2	<2
Plasma, HPLC	Paracetamol	<1	613

Method (Day 3 sample)	Test	Patient (µmol/L)	Positive control (µmol/L)
Plasma, enzymatic	Paracetamol	104	609
	Bilirubin	960	13
Ultrafiltrate, enzymatic	Paracetamol	<30	607
	Bilirubin	<2	<2
Plasma, HPLC	Paracetamol	<1	612

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SHORT COMMUNICATION

High prevalence of NDM genes among Carbapenemase-producing clinical Gram-negative bacilli in Benin City, Nigeria: Pseudomonas aeruginosa - a leading culprit

Ephraim Ehidiamen Ibadin, Helen Oroboghae Ogefere, Gisele Peirano and Johann Pitout

Carbapenemase producing organisms (CPOs) have become a global health concern because they are multidrug resistant thus limiting therapeutic options for clinical management of infections (1). Carbapenemases are capable of inactivating the carbapenems, cephalosporins, monobactams and penicillins and their genetic determinants are mostly spread by horizontal transmission on mobile genetic elements such as transposons and/or conjugative plasmids (1,2). This facilitates their spread between Enterobacteriales and non-fermenters, a worrying fact for regions with fragile healthcare systems characterised by poor infection prevention and control practices, absence of policies on antibiotic stewardship, and absence of surveillance on antimicrobial resistance (3). Such favorable conditions for the spread of CPOs are rife in most African countries.

In Nigeria literature is emerging on the role of carbapenemase genes such as NDM, $OXA_{48\text{-like}}$, $OXA\text{-}_{181}$, IMP, KPC, GES, VIM and CPOs across various regions with varying prevalence rates (2,4,5). However, owing to paucity of data in the South-South and our locality (Benin City), this study was carried out to determine the prevalence of carbapenemase genes among clinical Gram-negative bacterial isolates in Benin City, Edo state, Nigeria.

The study was cross-sectional and was conducted at the University of Benin Teaching Hospital. Gram-negative rods recovered from consecutive non-repetitive routine specimens between February 24th 2020 and July 21st 2020 were identified using the Microbact 24E identification system. Antimicrobial susceptibility tests were conducted according to CLSI (2018) (6), using the following antibiotics as appropriate: Meropenem (10µg), Imipenem (10µg), Ceftazidime (30µg), Cefuroxime (30µg), Cefoxitin (30µg) Levofloxacin (5µg), Tazobactam-piperacillin (110µg), Amoxicillin-clavulanate (30µg) and Amikacin (30µg). Bacterial strains that were multidrug resistant (resistant to ≥ 3 classes of antibacterial drugs and/or resistant to the carbapenems) were screened using the simplified carbapenemase inactivation method (sCIM) (7). Isolates that were carbapenemase producing were further screened for carbapenemase genes by PCR.

PCR was used to identify genes encoding various known carbapenemases. DNA extracts were obtained by boiling method and multiplex PCR amplification for the simultaneous detection of bla_{KPC}, bla_{NDM}, $bla_{OXA\text{-}48\text{-like}}$, and bla_{VIM}, β-lactamase genes was carried out on a Veriti 96-well thermal cycler instrument (Applied Biosystems,Life Technologies, Foster City, CA) with the AmpliTaq Gold PCR master mix (Applied Biosystems, Life Technologies, Hammonton, NJ) as previously described (8). The PCR products were analysed by electrophoresis with 1.5% agarose gels in 0.5X Tris-Borate-EDTA (TBE) buffer. The data obtained was analysed with appropriate statistical tool (chi square) using the statistical software INSTAT®.

A total of 451 Gram-negative bacilli were recovered from clinical specimens comprising 329 (72.9%) of Enterobacteriales and 122 (27.1%) of non-fermenters (Table 1). A total of 189 (41.9%) of these isolates were MDR and 39 (20.6%) MDR strains were carbapenemase producing using phenotypic method. Of this number, 37 (92.5%) were confirmed by PCR (Figure 1), with two (7.5%) isolates being negative. *Pseudomonas aeruginosa* was more likely to be carbapenemase producing in comparison with any other isolate ($p<0.0001$). The most detected carbapenemase gene was NDM (67.5%), with *P. aeruginosa* showing the highest prevalence of the gene (71.9%). Verona integron metallo-β-lactamase (VIM) gene ranked next (17.5%). Three isolates harbored two carbapenemase genes with one of these combinations NDM + VIM and NDM + $OXA_{48\text{-like}}$). Wounds had the highest prevalence of CPOs (35.9%) while cerebrospinal fluid and stool samples had the least prevalence (2.6%).

In this study the most detected carbapenemase gene was NDM (67.5%), with *P. aeruginosa* showing the highest prevalence of the gene while KPC gene was strikingly absent among CPOs, including *Klebsiella* spp. This finding is in contrast to the prevailing narrative in Europe and North America where KPC and its variants are the dominant carbapenemase genes found mostly in Enterobacterales (4). It also differs from few studies in Nigeria where KPC and GES were the dominant genes (2,4). Our finding is, however, similar to findings on the Indian subcontinent where an increased prevalence of metallo-beta-lactamase MBL-producing CPOs has been observed, and a recent study in Northwestern Nigeria where NDM gene dominated among CPO (1,4,9). New Delhi Metallo-beta-lactamase-1 was first detected in a *K. pneumoniae* isolate from a Swedish patient of Indian origin in 2008. Since then, it has been detected in bacteria from patients in India, Pakistan, the United Kingdom, Canada, the United States, and in several Asian and African nations (1,10). Although previous studies have highlighted indiscriminate use of antibiotics (especially beta-lactamases) in Nigeria as contributing to selective pressure leading to the survival and proliferation of beta-lactamase producing organisms (2), the cost of the carbapenems is prohibitive and its abuse not as rampant. A significant number of Nigerian citizens of Edo extraction visit Europe and Asian countries, including the Indian subcontinent, for economic reasons and medical tourism. They therefore, inadvertently, may have played key roles in the spread and distribution of CPOs harboring MBL genes. Further research exploring this facet would give clarity.

Strikingly, VIM type MBL-producing *Pseudomonas* spp were incriminated in infections in this study, as 17.5% of bacterial strains harboured the gene. Although previous studies in Southwestern and Northern Nigeria had reported VIM gene from Enterobacterales and *P. aeruginosa* (3,4,9), we for the first time report VIM-producing isolates from Edo state, South-South Nigeria.

Summarily, the prevalence of CPO was 8.9% with 80% being *P. aeruginosa*. Majority of CPOs were MBL-producing, harboring NDM, VIM and NDM + VIM genes; all CPOs were multidrug resistant. There is an urgent need to deeply entrench infection prevention and control practices as well as enact institutional and national policies on antibiotic stewardship.

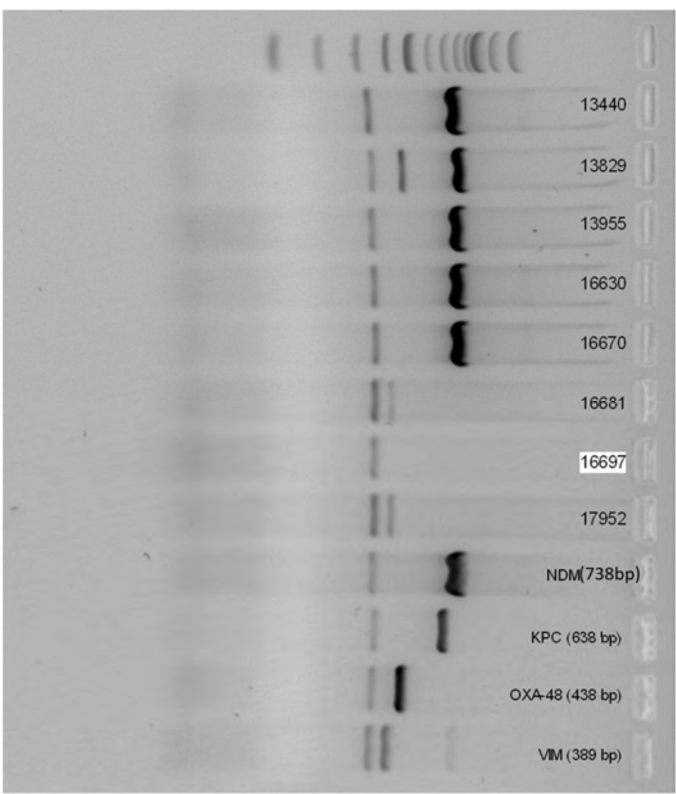

Figure 1. Agarose gel of PCR products (amplified carbapenemase genes): Isolates number 13440, 13955, 16630 and 16670 are NDM positive, while 13829 is NDM + OXA-48-like positive. Isolate 16681 and 17952 are VIM positive while 16697 is negative for all genes. Positive control genes are seen in the last four wells (NDM, KPC, OXA-48-like and VIM).

Table 1. Distribution of Gram-negative bacterial isolates in relation to carbapenemase genes and clinical specimens.

Organism	No. of isolates	Phenotypic detection (CPO)	Molecular detection				Clinical specimens					
			NDM	VIM	NDM ++VIM	NDM + OXA_{48}	Aspirate	Catheter tip	CSF	Stool	Wound	Urine
E. cloacae	27	1 (3.7)	0	1 (100)	0	0	0	0	0	0	1 (100)	0
K. oxytoca	26	1 (3.8)	0	0	0	1 (100)	0	0	0	0	1 (100)	0
K. pneumoniae	47	4 (8.5)	3 (75)	0	0	1 (25)	0	1 (25)	0	0	2 (50)	1 (25)
Providencia retgerri	4	1 (25)	1 (100)	0	0	0	0	0	0	1 (100)	0	0
P. aeruginosa	81	31 (38.3)	23 (71.9)	5 (15.6)	1 (3.1)	0	4 (12.9)	6 (19.4)	1 (3.1)	0	10 (32.3)	8 (25.8)
P. putida	4	1 (25)	0	1 (100)	0	0	0	0	0	0	0	1 (100)
Total	189	39 (20.6)	27 (67.5)	7 (17.5)	1 (2.5)	2 (5)	4 (10.3)	7 (17.9)	1 (2.6)	1 (2.6)	14 (35.9)	10 (25.6)

Number of isolates vs CPO: $p < 0.0001$, CPO- carbapenemase producing organism, NDM- New Dehli metallo-β-lactamase, VIM- Verona integron metallo-β-lactamase, OXA- Oxacillinase-like carbapenemase, CSF-cerebrospinal fluid.

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Acknowledgement of the Barrie Edwards and Rod Kennedy Scholarship in both the presentation and any subsequent publication is a required condition of this award. The NZIMLS Council reserves the right to request the full conference presentation.

The 2022 recipient of the Barrie Edwards and Rod Kennedy Scholarship is Associate Professor Rob Siebers. Rob will be attending The International Congress on Peer Review and Scientific Publication. Chicago - September 2022.

SCIENTIFIC LETTER

Measures to control exposure to flammable refrigerant in household type refrigerators and freezers in the medical laboratory

Dennis Mok, Naria Eloyan, Sharfuddin Chowdhury, Rana Nabulsi, Geraldine Budomo Dayrit, Arisina Chung Yee Ma and Dung Thi Cong Nguyen

Flammable refrigerant remains a commonly used chemical product in household type refrigerators and freezers used in the medical laboratory (1,pp.17.1-17.13). The medical laboratory must provide relevant exposure control measures to laboratory personnel when flammable refrigerant is used in the refrigeration system for storage of reagents, consumables and specimens. The main objective of this paper is to enhance the medical laboratory's awareness of requirements relating to provision of relevant exposure control measures for flammable refrigerant in refrigeration systems to laboratory personnel. Selected organisations were identified to provide relevant information to support communication of hazard information to laboratory personnel: the American Society of Heating, Refrigerating and Air-Conditioning-Engineers (ASHRAE) (2,pp.742743), the International Electrotechnical Commission (IEC) (3,p.1741;4,p.1178), the International of Electrical and Electronics Engineers (3,p.1481), the International Organization for Standardization (ISO) (3,p.1896;4,p.881), and the United Nations Economic Commission for Europe (5,p.2391).

Definitions

The following definitions should be noted by the medical laboratory:

Refrigerant

Defined by the ISO as 'fluid used for heat transfer in a refrigerating system, which absorbs heat at a low temperature and a low pressure of the fluid and rejects it at a higher temperature and a higher pressure of the fluid usually involving changes of the phase of the fluid' (Item 3.1.36 of ISO 817:2014).

Coolant

Defined by the ISO as 'heat absorbing medium or process' (Item 3.1.2 of ISO 224491:2020).

Secondary coolant

Defined by the ASHRAE as 'any liquid used for transmission of heat, without a change in state. Examples of secondary coolants include glycol and brine' (1,p.50.10).

Exposure and control measures

The exposure control measures relating to hazard information communication should include conveying relevant information by: marking, defined by the ISO and the IEC as 'symbols, pictograms, warnings, logos, or inscriptions on the consumer product, label or packaging to identify its type, which can also include short textual messages' (Item 3.12 of ISO/IEC Guide 14:2018); the safety data sheet, defined by the ISO as 'technical bulletin providing detailed hazard and precautionary information' (Item 3.25 of ISO 15190:2020); and the instructions for use, defined by the ISO and the IEC as 'information provided by the supplier of a product to the user, containing all the necessary provisions to convey the actions to be performed for the safe and efficient use of the product' (Item 3.9 of ISO/IEC Guide 14:2018).

Table 1. An action list for the medical laboratory to ensure the relevant exposure control measures for flammable refrigerant in refrigeration systems are identified and displayed for hazard communication.

Areas	Action list	References
Marking	The medical laboratory to ensure flammable refrigerants in refrigerators and freezers are identified and properly labelled.	Subclause 8.2.1 b) of ISO 15190:2020
	The medical laboratory to ensure the symbol ISO 7010-W021 (2011-05) is used by the manufacturer to indicate the flammability hazard.	Clause 5 of ISO 7010:2019
	The medical laboratory to ensure compressiontype appliances that use flammable refrigerants are marked with the symbol ISO 7010-W021 (2011-05).	Clause 7 of IEC 603352-24:2020
	The symbol ISO 7010-W021 (2011-05):	Clause 5 of ISO 7010:2019
Safety data sheet	The medical laboratory to ensure the pictogram GHS02 used by the manufacturer to indicate the flammability hazard in the safety data sheet is noted.	Annex A.3 of ISO 11014:2009
	The pictogram GHS02:	(6, p. 375)
Instructions for use	The medical laboratory to ensure relevant safetyrelated information provided in the equipment instructions for use is noted.	Subclause 7.11.2 of IEC/ IEEE 820791:2019
	The medical laboratory to ensure the instructions for use and safety notifications are reviewed to ensure correct usage.	Subclause 13.1 of ISO 15190:2020
Communication requirements	The medical laboratory to ensure the symbol ISO 7010-W021 (2011-05) is displayed at perpendicular height of ≥ 15 mm.	Subclause 5.2 of IEC 610102011:2019

Marking
The medical laboratory is to ensure that: hazardous products in use, including flammable refrigerants in household type refrigerators and freezers, are identified and properly labelled [Subclause 8.2.1 b) of ISO 15190:2020]. The symbol ISO 7010 -W021 (2011-05) should be used as the marking by the manufacturer to indicate the flammability hazard when household type refrigerators and freezers contain flammable refrigerant. In addition, the pictogram GHS02 should be used by the manufacturer to display the flammability hazard in the relevant section of the safety data sheet (6,p.375).

Instructions for use
Relevant safety related-information must be provided in the equipment instructions for use supplied by the manufacturer to support hazard communication (Subclause 7.11.2 of IEC/IEEE 82079-1:2019). The medical laboratory must ensure that the equipment instructions for use are read thoroughly by laboratory personnel.

Communication requirements
The medical laboratory must ensure the symbol ISO 7010-W021 (2011-05) is displayed at a reasonable location to assist in alerting laboratory personnel to the specific hazard; together with the provision of information in standardised supplementary safety information panel by the manufacturers, if provided (Subclause 6.3 of ISO 3864-2:2016), and standardised information on symbols in the globally harmonised system of classification suitable for use in the safety data sheet, if provided (Annex A.3 of ISO 11014:2009).

The medical laboratory must do what is reasonably practicable to ensure the relevant exposure control measures for flammable refrigerant in refrigeration systems are identified and displayed unambiguously for hazard communication to laboratory personnel.

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Dr Richard Charlewood
Transfusion Medicine Specialist at New Zealand Blood Service

Dr Richard Charlewood is a Transfusion Medicine Specialist at the New Zealand Blood Service, based in Auckland. He trained in South Africa and the United Kingdom, qualifying as a haematologist. He has also studied computer science in South Africa. He has served on the councils of the South African Society of Haematology, the Australian and New Zealand Society for Blood Transfusion and the Biotherapeutics Association of Australasia. He has over two dozen published citations, including a book chapter. His clinical interests are wide-ranging and include clinical transfusion practice improvement, transfusion information systems and infectious disease testing.

Dr Charlewood will be speaking at Plenary Session 3 of the NZIMLS Annual Scientific Meeting on Thursday 1 September.

SCIENTIFIC LETTER

Characteristics of top citations to articles from the New Zealand Journal of Medical Laboratory Science

Rob Siebers

Citations to published articles are a measure of those articles' worth in the biomedical literature. The more citations to an article, the more it is deemed of worth and importance (unless controversial). It is estimated that the average citation rate to published articles is 1.75 citations per article and that articles with ≥10 citations are in the top 24% of most cited research worldwide. About 12% of articles are never cited and citations to articles within a given journal are skewed. Weale *et al.* estimated that in immunology and surgical journals one-sixth of articles gather about a half of all citations and that the degree of non-citation varies between journals and the subject fields (1). Additionally, the number of citations is increased if the journal is listed in the Web of Science and PubMed data bases. The *New Zealand Journal of Medical Laboratory Science* (the Journal) is not covered by those two databases but is covered by Scopus, second only to PubMed. The objective of this study was to list the top ten most cited articles in the Journal and determine some characteristics of them. The Scopus database was searched for total number of articles published therein from 1999-2022 and how many times they had been cited in the world literature. The top ten cited articles were then examined for several characteristics (country of origin and subject matter).

A total of 420 articles (1999-2022) from the Journal were in the Scopus database, of which 127 articles were cited at least once (30.2%). Fifty-seven of these articles had been cited one time only, the rest of cited articles (n=70) more than once. The ten top cited articles in the Journal were cited between eight to 29 times (medium: 10 citations). The top cited article was by Omoregie *et. al.* from Nigeria which has been cited 27 times to date (2). The first New Zealand article in the top ten came in at number five with 10 citations (3). In total, six of the top 10 cited articles came from overseas, the rest (n=4) from New Zealand. Publications from overseas came from Nigeria, Thailand, United Kingdom, United Arab States, Singapore, Italy, Australia, India, and Bahrain (some of these overseas articles had authors from different countries). Five of the top 10 cited articles were in the field of microbiology (n=3) and haematology (n=2).

Citations to published articles are deemed a good indicator of the article's significance and a useful measure of its scholarly impact. An article attracting ≥10 citations is deemed of significant importance. Six of the Journal's top cited articles each had ≥10 citations. Despite the Journal not indexed in the Web of Science or PubMed databases, a number of articles have attracted significant numbers of citations in the biomedical literature worldwide, as evidenced by the top 10 cited articles. Some of the citations to some of the top 10 cited articles in the Journal were self-citations, i.e., where a Journal article cites another Journal article. This could be a limitation of the study. However, this was not determined as one would have to see whether this journal self-citation was justified or not.

In conclusion, despite not being covered by the Web of Science or PubMed databases, the Journal has attracted citations to its published articles in the world biomedical literature as evidenced by the top 10 citated articles.

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Dr Martin Chadwick
Chief Allied Health Professions Officer

Martin is the Chief Allied Health Professions Officer within the Ministry of Health, working in partnership with the Chief Medical Officer and Chief Nursing Office in providing transdisciplinary clinical leadership and advice. The role works at a systems level as well as providing support to clinicians, programmes and projects across the Ministry. He is aligning a work programme around what he describes as the five challenges facing allied health. This includes the challenges of demonstrating the value add of the allied health professions to population health outcomes; and working towards the concept of transdisciplinary working in the provision of healthcare services. Martin was the 2019-20 New Zealand Harkness Fellow in Health Care Policy and Practice. He has completed his doctoral degree in 2019 examining health workforce change. He is passionate about the untapped potential that allied health professions can bring in improving the quality of care provided to our populations, and in turn better meeting equitable population health needs.

Dr Chadwick will be speaking at Plenary Session 3 of the NZIMLS Annual Scientific Meeting on Thursday 1 September.

Commentary

The Pae Ora Healthy Futures Bill and health reforms

Terry Taylor

In 2018, the then Minister of Health, Hon Dr David Clark announced that he was setting up a comprehensive independent review of the current Health and Disability System. The expert review panel was chaired by Heather Simpson and included six other experts from a variety of backgrounds and stakeholders within the health system. The aim of the review was to identify opportunities to improve the performance, structure, and sustainability of the system with a goal of achieving equity of outcomes, and contributing to wellness for all, particularly Māori and Pacific peoples.

After a meeting with the Hon Dr David Clark in 2018 the NZIMLS Executive was encouraged to provide professional input to the review panel. The NZIMLS Executive had a meeting with Professor Peter Crampton from the panel to submit background on the profession of medical laboratory science, and the challenges that the profession faces operating within the current health system structure.

The reporting timeframes were initially set but due to the COVID-19 pandemic the final report was not released until June 2020 (1). The report provided a comprehensive overview and recommendations that few working in the health sector would have disagreed with. From the time the report was released the Department of the Prime Minister and Cabinet (DPMC), under the leadership of Stephen McKernan, began working on the white paper summary. On April 21st 2021 the Health Minister, the Hon Andrew Little publicly released the white paper containing the Cabinet signed off plan for reforming the Health and Disability System (2). The outcome of this was a new national operational entity to be called Health NZ alongside a Māori Health Authority (MHA) and the formation of a national Public Health Agency (PHA).

That was the start of the consultation and engagement phase within the newly formed Health Transition Unit (HTU) within the DPMC. Prominent health stakeholders provided detailed assessments and were able to meet and provide position papers and recommendations for the merger of publicly funded health services into Health NZ governance. The NZIMLS Executive on behalf of the NZIMLS provided a submission, and position document with professional recommendations and has had meetings and correspondence with the leadership of the HTU.

At this time the NZIMLS Executive on behalf of the NZIMLS, was involved in providing submissions on the future structure of the PHA which will be a national agency that will provides significant public health research and diagnostic testing input into Health NZ and the MHA. Within this proposed structure there is a strong hint of an overarching governance position for an appropriate expert specialist medical laboratory scientist. The NZIMLS Executive has been lobbying hard for a Chief Medical Scientist Officer position for several years and it finally appears like that will end up within the PHA.

The legislation for the health reforms, the Pae Ora Health Futures bill, was released to Parliament on 20th October 2021 and underwent the first reading on the 27th October 2021 (3).

The bill was referred to the Pae Ora Legislation Select Committee and submissions were called for with a closing date of 9th December 2021. The NZIMLS Executive on behalf of the NZIMLS provided a submission to this Bill (4) and appeared by Zoom in front of the Select Committee on January 28th 2022. Unfortunately we were due to appear in person but the emergence of Omicron in the community put paid to that. The second Parliamentary reading of the legislation was voted in on May 5th 2022 and is currently awaiting the third and final reading before being incorporated into law as an Act before July 1st 2022. Following Royal Assent by the Governor General, all DHB's will cease to exist and all health and disability system governance and functions merge into Health NZ with oversight from the MHA and PHA. The Ministry of Health (MOH) will take on a purely policy implementation role and pass on all operational functions to the new health entities.

The Health NZ and MHA Boards have all been appointed and the Board Chairs are Rob Campbell and Co-chairs Sharon Shea and Tipa Mahuta respectively. The Chief Executive of Health NZ is Margie Apa and the Chief Executive of the MHA is Riana Manuel. There have been no leadership appointments to the PHA as at 16th May 2022.

Over the period of the health reforms the NZIMLS Executive has been involved with hui and discussion documents with other medical professional colleagues. The NZIMLS Executive on behalf of the NZIMLS has provided integral input into collaborative publications and position reports throughout 2021. These include Creating Solutions, A roadmap to health equity 2040 (5), Role of laboratory science in the future of public health (2021 MOH in draft), and the NZIMLS position statements on merging into Health NZ, as well as wider Allied Health discussions on merging into Health NZ.

Through this continued involvement the NZIMLS Executive is optimistic that the health reforms will provide a positive effect on the diagnostic laboratory industry. To have been recognised as a key contributor at every step has been a credit to the NZIMLS proactive approach and newly found political and professional clout.

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Science snippet

A mythical property of blood.
Whilst blood transfusions and blood products use are widely acknowledged in modern medicine as 'life-savers' …… Historically blood has been seen as a 'life-giving force'. In ancient Rome a wide spread practice was to drink the blood of the slain gladiators after they died in combat. This was considered a potent cure for epilepsy as well as other serious conditions and was a practice that persisted through Europe for nearly 2000 years with epileptics taking bowls and cups to public executions to collect the blood.

Validating the method for inhibited specimens for the analysis of faecal pathogens

Richard Chen[1], Jenny Grant[2], Kaye McGeorge[2] and Fernalynn Tiongko[2]
[1]University of Otago, Dunedin and [2]Southern Community Laboratories, Dunedin

Objectives: The Molecular Pathology Department at SCL, Dunedin is planning to implement extraction free faecal PCR from AusDiagnostics Faecal Buffer specimens. In order to have a backup method in the event of sample inhibition, we aimed to validate the performance of faecal PCR assay with extraction of Faecal Buffer by AusDiagnostics MT-Prep, a nucleic acid extraction instrument.
Methods: Faecal samples (44) were inoculated in Faecal Buffer tubes and tested in this experiment. At least 2 samples positive for each faecal pathogen target in the AusDiagnostics Faecal Pathogens assay were included. For each specimen, PCR was performed on both extraction-free supernatant and nucleic acid extract from MT-Prep, by AusDiagnostics High-Plex instrument. The take-off values for all target pathogens and corresponding spikes were measured. Eleven of the 44 included faecal samples were also inoculated in STAR buffer and had PCR performed after MT-Prep extraction.
Results: All the faecal samples inoculated into the faecal buffer and then extracted on the MT prep had later target and spike take off values than those from extraction-free faecal buffer samples. There were also 4 extracted samples with spike inhibition. The outcome of MT-Prep extracted specimens has shown poor agreement with extraction-free ones. Regarding the extracted STAR buffer samples, none were inhibited, and their target and spike take-off values were closer to results from their corresponding extraction-free specimens.
Conclusion: This study suggested that it is unsuitable to apply MT-Prep extraction to Faecal Buffer specimens as the back-up method for extraction-free faecal PCR. Limited testing of extracted STAR buffer samples showed better performance. Therefore, we may consider specimens in STAR buffer as the alternative plan after future studies.

EvaGreen-based digital droplet PCR for copy number variation analysis: A systematic review of the literature

Jasmine Chew[1] and Natasha Henden[2]
[1]University of Otago, Dunedin and [2]LabPLUS, Auckland

Objectives: Digital droplet polymerase chain reaction (ddPCR) is purported to be a robust platform for quantitating copy number (CN). This literature review aimed to assess the accuracy and assay optimization of EvaGreen-based ddPCR for CNV analysis. Findings from this review will help guide an in-house validation project for adopting EvaGreenbased ddPCR to orthogonally confirm CNVs detected by a targeted next-generation sequencing (NGS) panel at LabPLUS, Auckland.
Methods: PubMed and Embase (Ovid) databases were searched for relevant studies. Studies were included which reported adopting EvaGreen-based ddPCR for CNV analysis using any sources of DNA template and/or assay optimization of EvaGreen-based ddPCR. All studies were written and published in English. Relevant information on assay protocols, accuracy for quantitating CN, assay optimization, and multiplexing strategies of EvaGreen-based ddPCR was extracted.
Results: Seven studies were eligible for data extraction. The assay protocols reported by all studies emphasized the significance of primer design, DNA preparation, droplet generation, and thermal cycling conditions on the final assay performance. The accuracy for quantitating CN was high, with an average calculated percentage error of 4.86% across five studies. Assay annealing temperature, primer concentration, and the amount of input DNA added per reaction were highlighted as parameters critical in assay optimisation. Multiplexing could be achieved through manipulation of amplicon length or the final primer concentration.
Conclusion: EvaGreen-based ddPCR is an accurate platform for CNV analysis, which is relatively easy to optimize and multiplex. Although EvaGreen-based ddPCR could be potentially employed as an orthogonal method for confirmation of CNVs detected via NGS, further validation and studies are required to fully evaluate its diagnostic performance.

Investigation of an alternative method for managing patients with EDTA mediated platelet clumping

Ella Edwards[1], Yii Sen Wee[2] and Katherine Christie[2]
[1]University of Otago, Dunedin and [2]Southern Community Laboratories, Dunedin

Objectives: In the diagnostic haematology laboratory, platelet clumping is a cause of pseudothrombocytopenia, which can lead to potential interpretation errors. Platelet clumping is typically due to platelet activation during venepuncture or the action of an EDTA mediated antibody. It is critically important to obtain accurate platelet counts, as treatment is often transfusion dependent, which is a dangerous procedure if the platelet count is incorrect. The efficacy of ThromboExact and gentamicin in preventing platelet clumping is currently unknown and poorly characterised in the literature. This study aimed to assess the validity of ThromboExact and gentamicin for overcoming platelet clumping and to compare their effectiveness in rectifying EDTA-mediated platelet clumping.
Methods: A full blood count was performed on thirty normal controls and three known platelet clumping patients. Normal controls had one EDTA and one ThromboExact bled, whilst the clumping population had two EDTA, one ThromboExact and one sodium citrate bled. The normal control samples were tested upon lab arrival, and after five hours, while the known platelet clumping samples were tested hourly over six hours. This was to assess the tube stability in reducing platelet clumping. Analysis for both groups was carried out on the same Sysmex XN-20 Automated Cell Counter.
Results: Preliminary results indicated EDTA was more stable in normal controls than in patients with a known history platelet clumping. Gentamicin and ThromboExact were deemed the preferential method in known platelet clumping patients, due to the smaller decrease in platelet number over six hours.
Conclusion: This study suggests the use of gentamicin as the preferred method for rectifying EDTA mediated platelet clumping, due to statistical and cost analysis and overall performance.

Comparison between BD BACTEC™ blood culturing and standard culturing for suspected prosthetic joint infections

Angharad Greening Christian
University of Otago, Dunedin

Objectives: The New Zealand Health Quality and Safety Commission's (HQSC) prosthetic joint infection (PJI) sampling and culture guide states that any broth culturing performed must be in addition to solid media culturing. Multiple studies have shown improved detection of PJI pathogens with the use of blood culturing broth systems. This study was performed to determine whether a testing protocol using the BD BACTEC™ FX40 blood culture instrument could replace the Canterbury

Southern Community Laboratories' (CSCL) current solid media and thioglycollate broth method.

Methods: Tissue samples (44) from 16 patients were homogenised and cultured using the current CSCL and study blood culture methods. MedCalc Software was used to analyse data.

Results: The 16 true positive samples were all *Cutibacterium acnes* shoulder infections. The current method identified 16 true positives (100% sensitivity; 82.14% specificity; 76.19% positive predictive value; 100% negative predictive value) with an average true positive identification time of 8.06 days. The study method identified 14 true positives (87.5% sensitivity; 85.71% specificity; 77.78% positive predictive value; 92.31% negative predictive value) with an average of 10.38 days.

Conclusion: This study suggests that the blood culturing method cannot replace the current CSCL PJI method. The current standard method detected all true positives and, on average, did so faster by two days. Only *Cutibacterium acnes* was isolated in true PJI patients in this study. In another study, this microorganism was shown to be poorly recovered specifically in PJI blood culturing compared to standard culturing. Further investigation could assess, especially if other PJI organisms are frequently isolated, if blood culturing (without negative vial terminal subculturing as per the HQSC PJI guidelines) may replace thioglycollate broth culturing.

Acknowledgement: The author thanks staff at Canterbury Southern Community Laboratories, Christchurch for supervision of this project.

Verification study of the Abbott® i-STAT total βhCG Point-of-Care test for rural hospital use

Amanda Hamilton-White[1], Sue Melvin[2] and Tracey Hollings[3]

[1]University of Otago, Dunedin, [2]Southern Community Laboratories Dunstan and [3]Southern Community Laboratories Otago/Southland

Objectives: A need for a point-of-care (POC) testing method to detect βhCG was identified for the Dunstan Hospital, to assist with the differential diagnosis of abdominal pain in women of childbearing age presenting to the hospital if miscarriage or ectopic pregnancy were suspected. This study was designed to assess both the accuracy and precision of the i-STAT βHCG quantification POC test, to verify this method for use in clinical decision making in a rural setting.

Methods: Measurements (40) were made, on two available i-STAT devices, for samples with a HCG quantification test request or known second trimester pregnancy. These were measured against the reference method (Roche® 2010/ E170 assay) for correlation analyses. Individual samples (38) were analysed. Samples at each end of the measurement range were used for precision calculations: co-efficient of variation (CV) and uncertainty of measurement (UoM). Quality control (QC) was also performed on all days the patient samples were measured to calculate CV and UoM.

Results: The I-stat method showed high correlation with the reference method, adjusted R^2=1.0. Bland-Altman analysis showed a mean difference of 1.88% between the methods. CV values obtained from patient precision were 5.6 and 7.2, which was consistent with CV values calculated from daily precision runs which had CV values for each level of 6.6, 3.7, and 6.0. Sensitivity and specificity were 100% for the I-Stat method.

Conclusion: The i-STAT β-hCG POC test provided hCG values that were consistent with the reference method and sufficiently precise for the purpose of emergency clinical decision making where pregnancy may be suspected.

Verification of the Hologic Panther Fusion system for diagnostic testing of parainfluenza, adenovirus, human metapneumovirus and rhinovirus

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[1]University of Otago Dunedin and [2]Wellington Southern Community Laboratories, Wellington

Objectives: Respiratory viruses are seen globally, producing similar symptoms while requiring different treatments and clinical management. This makes testing and diagnosis important. Wellington Southern Community Laboratories use the (verified) AusDiagnostic Respiratory 24 assay for such testing. This method is time-consuming and laborious unlike the Hologic Panther Fusion, already used to test some respiratory viruses in the laboratory.

The aim of this study was to verify the Hologic Panther Fusion for diagnostic testing of parainfluenza 1/2/3/4, adenovirus, human metapneumovirus and rhinovirus in comparison to the AusDiagnostic.

Methods: The Southern Hemisphere Influenza and Vaccine Effectiveness Research and Surveillance (SHIVERS) study-five criteria was used to determine the sample population. All samples were run on the Hologic Panther Fusion for these respiratory targets and their cycle thresholds recorded. Five positive samples (covering upper and lower limits of detection) were selected for each target. These samples were then tested on the AusDiagnostic, which produces a take-off value. These values are two different measurements making direct comparison difficult.

Results: Each sample's position within both measurements' limits of detection were approximately the same, indicating the Hologic Panther Fusion detected results similar to the AusDiagnostic. A linear regression graph indicated the two measurements had a strong concordance as discordant results were only found in the lower limits of detection, which have increased chance of sampling bias due to low viral loads and less clinical significance. There were no positive parainfluenza 1/2 samples in the study population so these could not be verified.

Conclusion: This report indicated the Hologic Panther Fusion is verified for diagnostic testing of parainfluenza 3/4, adenovirus, human metapneumovirus and rhinovirus at Wellington Southern Community Laboratories.

Verification of total prolactin and post-PEG monomeric prolactin reference intervals validation of phosphate-buffered saline polyethylene glycol method

Katja Holgate[1], Joanne Webb[2] and Max Reed[2]

[1]University of Otago, Dunedin and [2]Southern Community Laboratories, Wellington

Objectives: Macroprolactin complexes positively interfere with the Roche prolactin assay. Precipitation of macroprolactin by 25% polyethylene glycol provides an estimate of monomeric prolactin. Wellington SCL is reviewing its methodology by comparing the performance of phosphate-buffered saline polyethylene glycol (PBS-PEG) to de-ionised water polyethylene glycol (DI-PEG) solution, as monomeric prolactin recovery may be more consistent with PBS-PEG.

Methods: Forty samples with normal prolactin concentrations, 25 with elevated concentrations, and 20 normal-health male samples had serum frozen for testing. After warming to room temperature and removing particulate matter, 200µL was combined with 200µL DI-PEG or 200µL PBS-PEG and vortexed, incubated, then centrifuged and tested on the Cobas e602. Monomeric prolactin recovery rates and percentage difference between the methods were calculated. Normal serum (4mL) and elevated-prolactin serum (4mL) were repeatedly tested to determine CV%.

Results: Monomeric prolactin recovery was 2.3% less using PBS-PEG for the normal group, and 5.6% less in the elevated group. Method correlation was strong, R^2 of 0.95 for the normal group and 0.99 for the elevated group. Recovery reduction was more pronounced with higher total prolactin concentrations. The 20 normal-health male total prolactin concentrations ranged from 98.5mU/L to 380.6mU/L. CV% using DI-PEG was 3.8% and 2.0% for normal and elevated groups, whereas PBS-PEG was 2.1% and 1.9% respectively. Most method differences fell within the RCPA prolactin analytical performance specification.
Conclusion: Wellington SCL's current total prolactin and post-PEG monomeric prolactin reference intervals were verified for the testing population, as well as the 40% cut-off for macroprolactinaemia. PBS-PEG had better reproducibility, supporting a change from DI-PEG.

Phoenix 100 identification and antimicrobial sensitivity validation

Shane Hutchinson[1] and Tony Barnett[2]
[1]University of Otago, Dunedin and [2]Medlab South Nelson Hospital, Nelson

Objectives: The Nelson hospital laboratory microbiology department has a BD Phoenix 100 bacterial identification and antimicrobial susceptibility analyser. The primary goal of this project is to validate the Phoenix 100 for diagnostic use by verifying that the Phoenix 100 produces the same results as would be produced by the laboratory with their current methods. The current methods include MALDI-TOF MS as the gold standard for identification and phenotypic bacterial profiles as the gold standard for antimicrobial susceptibility and as another identification method.
Method: A comparison of 24-gram positive organisms and 21-gram negative organisms was constructed. Their identification was compared on genus and species levels and antimicrobial susceptibility results were compared with the reference methods for any variation.
Results: All 45 organism identifications correlated with the reference methods on the genus level and 38 on the species level. There were 21-gram positives and 17-gram negatives with the same species identifications as the reference methods. Not all organisms had antimicrobial sensitivity panels performed but of the 36 organisms that did, there was only one discrepancy which was the determination of the source of an AmpC. The percentage of corroborating identifications were comparatively low considering results from other studies, but this was due to the way the project was executed and organisms were selected. Organisms which would routinely have confirmatory testing with the Phoenix 100 were appropriately identified.
Conclusion: The Phoenix 100 analyser should be appropriate to include in routine confirmatory testing as it produces the same results as methods already in use. This can help improve patient outcomes by reducing turnaround times and providing confirmatory diagnostic information.

Platelet function analysis on PFA-200 and the introduction of preanalytical variation by pneumatic tube transportation

Carne Lincoln[1] and Sunny Jamati[2]
[1]University of Otago, Dunedin and [2]Waikato District Health Board, Hamilton

Objectives: Determine the preanalytical variation and corresponding clinical significance of introduced to samples undergoing pneumatic tube system transportation prior to Platelet Function Analysis testing.
Methods: Paired samples were collected from participating donors through venepuncture with a 21-gauge needle. As per the standard operating procedure of Waikato District Health Board, one sample was walked to the laboratory, the paired samples were allocated a randomly assigned Lamson station located throughout Waikato Hospital for pneumatic tube transportation to the laboratory. Upon reception, paired samples were tested in parallel on the Siemens INNOVANCE® PFA-200 System measuring closure time of specimens in PFA Collagen/ Epinephrine Test Cartridges.
Results: Removal of outliers following a Shapiro-Wilk Test, normally distributed data plotted on a Bland-Altman plot expressed agreement between the two methodologies. The obtained bias of -2.9 seconds, p-value of 0.5, R-squared value of 0.89 and difference of interquartile ranges of 0.5 seconds identified a statistically insignificance between the difference in closure times of walked and Lamson transported samples. The null hypothesis stating no difference exists between the two methodologies failed to be rejected (p-value >0.05).
Conclusion: A statistically insignificant difference was identified between the closure times of walked and pneumatic tube transported samples for Platelet Function Analysis. Further continuation of this study is recommended to finalise an overall conclusion and to establish the appropriate clinical and statistical significance of the preanalytical variation introduced by Waikato District Health Board Lamson system.

Evaluating the performance of visual observation to detect haemolysed, icteric and lipaemic samples: Are our eyes good enough?

Holly McMahon[1] and Louise Nutbean[2]
[1]University of Otago, Dunedin and [2]Southern Community Laboratories, Oamaru

Objectives: Common preanalytical interferences such as haemolysis, icterus and lipaemia can significantly affect test results and compromise patient safety. The current method for haemolysis, icterus and lipaemia detection in routine coagulation samples is visual observation. The aim of this study was to evaluate the accuracy and agreement between visual observation and automated serum indices methods.
Methods: This study was performed on 40 routine coagulation samples. Visual observation was performed by five trained laboratory staff members, according to standard operating procedures. Automated haemolysis, icterus and lipaemia detection was completed using the Cobas c501 serum indices Generation 2 assay. The accuracy and agreement between visual observation and automated methods was determined.
Results: Accuracy of 100% was achieved for the correct handling of samples with haemolysis values over 100mg/dL. This agreement was reflected in the k value of 1. Observer error occurred most frequently for samples with haemolysis around 50mg/dL. No clinically relevant levels of lipaemia or icterus were present in the sample population, which likely contributed to the high level of accuracy and agreement achieved.
Conclusion: Very low frequencies of clinically relevant levels of haemolysis, icterus and lipaemia were detected in the sample population. This is representative of the quality of samples received in the Oamaru, Southern Community Laboratory. Routine coagulation specimens with observed interference should have automated serum indices results obtained, particularly when observed haemolysis is around 50mg/dL. This is to safeguard the accuracy of routine coagulation results. A more extensive study to assess the performance of visual observation for varying degrees of haemolysis, icterus and lipaemia would be useful to reveal further errors of sample categorisation.

Evaluation of an established urine total porphyrin screening protocol for implementation in Southern Community Laboratories, Dunedin.

Zara Mullally[1], Christian Christian[2] and Sian Horan[2]
[1]University of Otago, Dunedin and [2]Southern Community Laboratories, Dunedin

Objectives: Porphyria is a group of disorders affecting haem metabolism, resulting in the accumulation of toxic haem precursors, porphyrins. Porphobilinogen is a porphyrin and can be detected in a screening test for acute intermittent porphyria. A positive porphobilinogen result indicates the requirement for more specific testing, such as total urine porphyrin testing. Currently, total urine porphyrin testing is conducted by Canterbury Health Laboratories. Our laboratory intends to bring this test in-house by evaluating an established protocol utilised by Southern Community Laboratories, Wellington.
Methods: Twelve Royal College of Pathologists Australasian (RCPA) samples of known median concentration and two ClinChek quality control samples were utilised to evaluate the respective accuracy and precision of this protocol. The samples were acidified using hydrochloric acid to dissociate porphyrin chelation and scanned between 300nm and 600nm on the Thermo Fisher Evolution 220 spectrophotometer. The peak absorbance was determined, quantified, and reported in umol/L for comparison to the expected value.
Results: A strong correlation was observed between the median value of RCPA samples and the calculated results. A calculated bias of 4.5% from the median was acceptable and within the RCPA analytical performance specification of 10%. A Student t-test was performed (p=0.296), demonstrating statistically insignificant differences. Additionally, this protocol displayed high precision with a CV of 0-1.16% when repeatedly analysing the quality control samples.
Conclusion: This study showed agreement between the expected and calculated results. The differences between these results were not statistically or clinically significant. This evaluation has validated the reliability of this protocol to determine the concentration of total porphyrins in fresh urine and should be adopted by Southern Community Laboratories Dunedin.

A comparison between GMS (manual) and PASF (automated and manual) for the diagnosis of fungal infection

Mark Paca[1] and Daniel Smith[2]
[1]University of Otago, Dunedin and
[2]Waikato DHB Laboratories, Hamilton

Objectives: The effective management of fungal infection heavily depends on the early diagnosis and timely initiation of treatment. Periodic Acid Schiff (PAS) and Gomori methenamine silver (GMS) are the two most common stains for fungal diagnosis. The aim of this research project was to compare the quality and sensitivity of both stains using automated and manual methods.
Methods: PAS and GMS were performed on 20 specimens that were previously positive for fungal infection. Sixty slides were evaluated based on a 5-grading scale. Statistical tests were also performed to compare the different staining methods.
Results: There were 15 out of the 20 specimens that were positive for one or both stains for fungi. The mean score of the stain quality for both manual PAS and GMS was evaluated using a paired sample t-test (p=0.21) which is statistically insignificant. The automated PAS and manual PAS also failed to show a significant difference with a p-value of 0.58. Furthermore, a chi-squared test was performed to assess the difference in sensitivity of both stains (p-value<0.05) which was statistically significant and both stains are strongly associated.
Conclusion: PAS and GMS stains were slightly different in quality and positivity rate. However, both stains are still considered optimal for fungal detection as they failed to show significant difference and they were both strongly associated. Overall, PAS was easier to perform whereas GMS was easier to read.

Evaluation of the Roche Ca15-3 assay on the Cobas 8000

Jessica Quinn[1], Sian Horan[2] and Christian Christian[2]
[1]University of Otago, Dunedin and [2]Southern Community Laboratories, Dunedin

Objectives: Ca15-3 is a tumour marker used for monitoring breast cancer treatment or progression. Southern Community Laboratories (SCL), Dunedin, currently sends serum samples requiring Ca15-3 analysis to Canterbury Health Laboratories (CHL) but will soon bring the assay in-house. The aim of this evaluation was to compare the Ca15-3 results obtained by CHL using the Beckman-Coulter DXL800 analyser to the results obtained on the Roche Cobas 8000.
Methods: Both analysers employ a chemiluminescent microparticle immunoassay method but differ in their monoclonal antibody specificity. Breast cancer patient samples (n=17) were analysed in parallel and their results compared using statistical analysis. Two levels of Roche PreciControl Tumour Marker (TM) were analysed daily on the Cobas 8000 over a two-week period to assess assay precision.
Results: A mean difference of 53.02 U/mL was identified between the two assays. The Bland-Altman plot had limits of agreement (95%) from -102.676 to 208.724 U/mL and the Passing-Bablok generated a slope with the equation y= 0.6033 + 1.56x. The t-test derived a p-value of 0.0142. The CV% for PreciControl TM1 (n=16) and PreciControl TM2 (n=17) was 3.54% and 3.64%, respectively.
Conclusion: The results obtained by the Cobas 8000 have an upwards bias of 56% compared to the Beckman-Coulter DXL800. This is statistically significant as deemed by the p-value. It is clinically significant as it is higher than the analytical performance specification for Ca15-3 set by the Royal College of Pathologists of Australasia. This indicates that while the Cobas 8000 has good precision, the two assays are not interchangeable. During the period of assay changeover at SCL, patient results should not be directly compared to their older results obtained from CHL.

A comparison of the growth of *Streptococcus pneumoniae* in aerobic and anaerobic conditions

Melissa Reeve and Esther Lau[2]
[1]University of Otago, Dunedin and [2]Canterbury Health Laboratories, Christchurch

Objectives: *Streptococcus pneumoniae* is a Gram-positive diplococcus capable of causing infections such as pneumonia. Scientists at Canterbury Health Laboratories (CHL) currently culture respiratory samples using aerobic incubation in 5% carbon dioxide (CO_2) to identify *S. pneumoniae* colonies. The aim of this study was to compare the growth of *S. pneumoniae* in aerobic and anaerobic conditions in order to determine if the permanent addition of anaerobic incubation for all respiratory samples would facilitate more efficient *S. pneumoniae* identification.
Methods: Twenty-two *S. pneumoniae* clinical isolates were each inoculated onto three 5% blood agar plates. Each plate was incubated aerobically in 5% CO_2, anaerobically or held for 3 hours in a flow jar in 5% CO_2 at room temperature prior to anaerobic incubation. Colony size and appearance was recorded after 24 hours and 48 hours of incubation. Microsoft Excel was used for data analysis.

Results: Average colony size after 48 hours of incubation aerobically in 5% CO_2, anaerobically and in the flow jar was 0.91mm, 2.32mm and 2.12mm, respectively. ANOVA and post hoc tests indicated a significant difference between aerobic incubation and both anaerobic incubation ($P < 0.001$) and the flow jar ($P < 0.001$). Colony appearance after aerobic incubation resembled draughtsman and were grey and moist. Anaerobic and flow jar colonies were mucoid, white, and convex.
Conclusion: Colony size was found to be consistently larger after both anaerobic and flow jar incubation along with more efficient identification of colonies due to a greater ease of recognition. Despite confirmation of anaerobic incubation increasing the efficacy of *S. pneumoniae* detection, the low numbers of samples positive for *S. pneumoniae* received by CHL and the increase in costs associated with a change in incubation methods has encouraged CHL to maintain their current protocols.

Assessment of a new serological kit for detecting SARS-CoV-2 neutralising antibodies

Hany Sheta[1] and Lynda Hill[2]
[1]University of Otago, Dunedin and [2]Canterbury Health Laboratories (CHL), Christchurch

Objectives: To assess a new serological kit for detecting SARS -CoV-2 neutralising antibodies (NAbs) and compare results to other serological and molecular assays.
Methods: Sixty-six frozen serum or plasma samples were selected to cover a wide range of previously performed tests' results. Two serological assays were previously performed, enzyme-linked immunosorbent assay (ELISA) for anti-SARS-CoV-2 Spike Protein IgG antibodies (S-IgG), and ELISA for anti -SARS-CoV-2 Nucleocapsid Protein antibodies (NCP-IgG). Additionally, nasopharyngeal swabs from all 66 patients were previously tested by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) to detect SARS-CoV-2 RNA. The new kit is a surrogate virus neutralisation test (sVNT), which does not require live virus, hence does not need biosafety level 3 equipment. sVNT was standardised under CHL conditions, then performed on the selected samples. Results were compared to those obtained from assays previously performed.
Results: All positive and negative controls were within the manufacturer's defined range, showing validity of results under CHL conditions. Comparing sVNT results with previous tests, showed agreement of 65.2%, 86.4%, and 74.3% with qRT-PCR, S-IgG, and NCP-IgG assays, respectively. The discrepancies between sVNT and the other assays can be explained by one or more of several reasons: qRT-PCR only detects viral RNA in current infections, while sVNT detects NAbs from current or cleared infections; not all infected individuals seroconvert; some samples were from vaccinated individuals; antibodies are not produced at early infections; and the experiment's small sample size due to the limited kit supply.
Conclusion: sVNT was performed successfully at CHL. Commercial kit may need to be introduced in the future for the detection of NAbs. Standardisation and full comparison are required with the commercial kit with a bigger sample size.

CD56 immunohistochemistry optimisation

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Objectives: An RCPA evaluation of the CD56 immunohistochemistry protocol used in the Department of Histology, Taranaki Pathology Services indicated weak staining with high background pigmentation. The purpose of this study was to review the current protocol and determine if alterations to the methodology could produce higher quality staining.
Methods: Three tissue specimens were selected based on previous positive staining under the current CD56 protocol, and as such were known to have CD56 positive tissue regions for analysis. Each tissue was stained using multiple different protocols with heightened or lowered antigen retrieval or antibody incubation times as well as a repeat of the current protocol.
Results: The staining of these tissues demonstrated that the current protocol produced intense, selective staining in all three specimens, and did not display the background or weak staining observed in the RCPA tissue. The alterations to the antigen retrieval stage of the protocol were observed not to affect staining significantly unless the time was severely reduced, which resulted in pale, sub-optimal staining. Increasing the antibody incubation time was observed to increase the staining intensity and background staining of the section, while decreasing the antibody incubation was observed to result in paler staining. While the reduced antibody incubation still resulted in acceptable staining of strongly CD56 positive tissue, weakly positive tissue was unacceptably pale.
Conclusions: It was determined that the current protocol is capable of consistently producing acceptable staining of CD56 positive tissue, and that other factors besides the antigen retrieval time and antibody incubation times must have interfered with the staining of the RCPA assessment tissue, such as tissue fixation or processing.

The comparison of three commercial rodent tissue indirect immunofluorescence assays for detection of anti-liver kidney microsomal type 1 autoantibodies and other tissue autoantibodies

Tyla Stockdill[1] and Helen van der Loo[2]
[1]University of Otago, Dunedin and [2]Southern Community Laboratories, Dunedin

Objectives: Rodent tissue indirect immunofluorescence assays (IIFAs) are used for detection of tissue autoantibodies to aid in the diagnosis of autoimmune diseases. The aim of this study was to compare the MeDiCa mouse stomach/kidney IIFA, currently used at Southern Community Laboratories (SCL) Dunedin, with the NOVA Lite and ImmuGloTM rodent stomach/ kidney/liver IIFAs, for the detection of anti-liver kidney microsomal type 1 (anti-LKM 1) autoantibodies. The secondary aim was to compare the overall performance of the three IIFAs for detection of other tissue autoantibodies routinely screened for.
Methods: Fifteen tissue autoantibody positive serum samples were tested according to manufactures instructions, with the MeDiCa, NOVA lite, and ImmuGloTM IIFAs. No anti-LKM1 positive samples were able to be obtained, a commercial anti-LKM 1 human serum positive control was used as substitute. The slides were viewed under fluorescence microscope and blind read by five of the immunology staff at SCL Dunedin.
Results: The anti-LKM1 positive control showed distinct staining and was easily identified by the readers with all three IIFAs. The detection of anti-mitochondrial and anti-gastric parietal cell autoantibodies was consistent across all three IIFAs and with the original reported result. Two discrepancies were seen with the MeDiCa IIFA and four were seen with the NOVA lite IIFA in low titre (1:40) anti-smooth muscle antibody positive samples.
Conclusion: All three IIFAs showed good detection of anti-LKM1 autoantibodies, and it was concluded that the MeDiCa IIFA currently used is adequate to identify these autoantibodies. Overall, the MeDiCa IIFA produced comparable results to the NOVA lite, and ImmuGloTM IIFAs, and should continue to be used routinely.

A comparison of the precision and stability of pancreatic amylase and lipase

Esperanza Stuart[1] and Jackie McCullough[2]
University of Otago, Dunedin[1] and Wellington Southern Community Laboratories, Wairarapa[2]

Objectives: Serum enzymes specific to the pancreas are an important metric in the diagnosis of acute pancreatitis. Traditionally, amylase has been the analyte of choice due to the inexpensive and rapid nature of its test. However, with the increased availability of lipase testing kits, both enzymes are frequently requested to diagnose pancreatitis. The aim of this study was to compare the stability and precision of amylase to that of lipase to determine the redundancy of amylase.
Methods: Twenty-eight samples were selected at random. For stability testing, 15 samples were aliquoted into two sets and kept at different temperatures. Lipase and amylase levels were measured in samples from both sets every two days. Precision was measured by repeating each test three times on 13 samples. Statistical values were calculated, and data was graphed and compared.
Results: Both enzymes remained within the acceptable limit of the original value, despite some minor drift. The largest drift was observed in the serum amylase in the samples kept at room temperature, and the least in the serum lipase in the samples from the same set. All repeats fell within the allowable limits of the precision test. Amylase was found to be slightly more precise than lipase, and the increase in lipase concentration did not correlate to increased imprecision.
Conclusion: The stability of serum pancreatic amylase and lipase were comparable, with any drift being clinically insignificant. The precision of lipase and amylase assays were comparable. Therefore, the decision to use the serum pancreatic amylase or the serum lipase assay should not depend on these factors. Further considerations investigators should take into account are sensitivity, specificity, price and availability.

Validation of the Roche lipase assay across the Wellington SCL Laboratories

Annie Tu[1] and Bernice Smith[2]
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Objectives: To review the current literature on serum pancreatic amylase vs serum lipase in the diagnosis of acute pancreatitis. Validate the Roche Lipase assay across the WSCL laboratories to replace the pancreatic amylase assay currently reported.
Methods: The validation of the Roche lipase assay was undertaken across the four WSCL laboratories on three different Roche Cobas platforms: Wellington (c8000) Hutt and Masterton (c501) and Kenepuru (c311). Patient samples within a range of 5.1 – 3281 IU/L were collated at the Wellington Site and distributed to the other three sites for testing on the same day. Each laboratory performed lipase calibrations and quality control before patient samples were tested. Inter-laboratory precision was reviewed using the RCPAQAP analytical performance specifications.
Results: Results from each site were statistically analysed using Analyse-it® for site specific reporting on: Co-efficient of Variation%, Passing – Bablock regression and Bland Altman. Inter laboratory precision was reviewed using the RCPAQAP analytical performance specifications.
Conclusion: The literature review showed the specificity of lipase is slightly superior to pancreatic amylase in patients with acute pancreatitis. This in combination with the fact that serum amylase is elevated earlier and persists longer in acute pancreatitis clinically justifies the replacement of pancreatic amylase with lipase testing. Validation of the Roche lipase assay across the WSCL laboratories showed good correlation of performance between testing platforms. Assay performance was acceptable and within RCPA specifications for lipase. The lipase assay was implemented across the WSCL Laboratories and replaced pancreatic amylase in the diagnosis of acute pancreatitis.

Evaluation of a real-time rt-PCR method on the BD Max™ platform for detecting Mumps orthorubulavirus (MuV) in clinical samples

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Objectives: This project aims to evaluate the performance of a real-time reverse-transcriptase Polymerase Chain Reaction (PCR) method for *Mumps orthorubulavirus* (MuV) detection in clinical specimens using the BD MAX™ automated RT-PCR platform, with comparisons to a method for the ABI 7500 RT-PCR platform.
Methods: A ten-fold dilution series of PRIORIX MMR vaccine containing vaccine-strain MuV was tested via a newly developed in-house "BD MAX™ method", and Canterbury Health Laboratories' current "ABI method". Twenty positive and 22 negative patient samples tested previously with the ABI method for MuV were tested in parallel via the BD MAX™ method; any samples that gave conflicting results were retested via the ABI method in duplicate.
Results: The BD MAX™ method consistently detected MuV in dilutions down to 10^{-5} times the original concentration, whereas the ABI method could only detect down to the 10^{-4} dilution step. The BD MAX™ method appears to have greater analytical sensitivity. Specificity of the BD MAX™ method appears similar to the ABI, as none of the previously negative patient samples tested positive for MuV on the BD MAX™. Unexpectedly, one of the previously positive samples tested negative for MuV on the BD MAX™; this sample later tested positive for MuV twice on the ABI.
Conclusion: This in-house method for the BD MAX™ to detect MuV in samples is at least as specific as the method on the ABI 7500 and has the potential to be more sensitive. Considering the analyser's automated nature and the method's room for future optimisation, the BD MAX™ is well suited for MuV testing.

Evaluation of the clot waveform analysis of the non-anticoagulated population in Christchurch

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Objectives: The clot waveform is a reaction profile that can be generated from the prothrombin time (PT) and activated partial thromboplastin time (APTT) measured by the Sysmex CS-2500 automated coagulation analyser. The clot waveform analysis (CWA) allows for the evaluation of both qualitative and quantitative data derived from the clot waveform, which is known to show different patterns in certain clinical conditions compared with normal. This project was carried out to assess the PT and APTT CWA data of non-anticoagulated patients in Christchurch.
Methods: CWA of 52 non-anticoagulated patients were generated on the Sysmex CS-2500 automated coagulation analyser using Dade Innovin and Dade Actin FS reagents for PT and APTT, respectively. Normal distribution of data was checked by constructing a QQ plot for each parameter.
Results: As the QQ plots supported normal distribution, hypothetical reference ranges (mean ± 2SD) were calculated for each quantitative parameter (with one exception). 12 parameters (PT:6, APTT:6) were generated for every sample tested, each representing a different aspect of the clot waveform; maximum velocity, time taken to reach maximum

velocity, maximum acceleration, time taken to reach maximum acceleration, maximum deceleration, and time taken to reach maximum deceleration. A range for the time taken for maximum acceleration of PT could not be calculated as all results were identical. Due to lack of time, the initial plan of comparing the non-anticoagulated CWA results to the results of patients undergoing warfarin therapy, could not be executed.
Conclusion: By using normal CWA results as a baseline, various conditions and anticoagulant usage may be detected at a higher sensitivity and detail than just clotting time.

Monocyte distribution width: extension of sample age up to 7 hours

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[1]University of Otago, Dunedin and [2]Pathlab, Rotorua

Objectives: Monocyte distribution width was previously demonstrated to be stable for a period of 4 hours. The aim of this study was to evaluate whether monocyte distribution width remained stable in samples aged between 4 and 7 hours.
Methods: Rotorua Hospital Emergency Department and inpatient samples collected in ethylene diamine tetra acetic acid vacutainer tubes were selected between the hours of 4 am and 10 am. Monocyte distribution width was tested at 0, 4, 5, and 6 hours in 28 samples based on the collection time or received time. For 26 samples, monocyte distribution width was tested at 7 hours. Testing was performed on board the UniCel DxH 900 analyser (Beckman Coulter, Incorporated, Brea, California).
Results:
The average coefficient of variation at 7 hours was 5.54%. The average mean increased from 18.04 U at 0 hours post collection, to 19.31 U at 7 hours post collection. By separating the data into 3 groups; monocyte distribution width below 20 U, monocyte distribution width which rose above 20 U after the initial 0 hour test, and monocyte distribution greater than 20 U at 0 hours, it was identified that the two latter groups had a similar trend, while the former had a dissimilar trend between 4 and 7 hours. Monocyte distribution width did not decrease below 20 U in the group which had monocyte distribution width greater than 20 U at 0 hours.
Conclusions:
The results of this study found monocyte distribution width to be stable up to 7 hours, with the greatest amount of deterioration occurring during the first 4 hours. Further studies with larger sample sizes are required to confirm results.

Comparison of Hemocue haemoglobin and HGB-O in XN-20 for lipaemic samples

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Objectives: Haemoglobin is one of the basic parameters tested in the haematology department. The method used in LabPLUS, called SLS-Hemoglobin, produces falsely high results for lipaemic samples. LabPLUS is using a new parameter HGB-O in XN-20 to substitute the haemoglobin value. Hemocue, a point -of-care analyser is also known to be unaffected by lipaemia. The main aim of this research was to evaluate the comparability between Hemocue haemoglobin and HGB-O.
Methods: Forty-five patient samples were picked for this research according to the lipaemic level, L value (L=50-149: 31, L=150-350: 10, L>350: 4). All the samples were tested by both XN-20 and Hemocue analyser. In XN-20, SLS-Hemoglobin and HGB-O were done. QCs for XN-20 were performed as routine and Hemocue analyser did its QC automatically.
Results: The paired t-test (mean difference 0.42 g/L; 95% confidence interval -0.87-1.71 g/L; p=0.51) and Bland-Altman plot (mean difference 0.42 g/L; Lower LOA -8.00; Upper LOA 8.85) indicated a statistically insignificant difference between the Hemocue haemoglobin and HGB-O results. Passing-Bablok fit for all samples (y = 1.0494x - 5.9965, R2 0.97) showed a positive bias (approximately 5%) for HGB-O compared to Hemocue haemoglobin, which is clinically insignificant according to RCPA Haematology Participant Handbook 2014. However, for samples with L values greater than 150, the Passing-Bablok fit (y = 1.1856x - 23.005, R^2 = 0.9737) showed proportional bias of 18.5%, which is not acceptable.
Conclusion: HGB-O and Hemocue haemoglobin both statistically and clinically agreed with each other according to the statistical tests for all 45 samples. However, Passing-Bablok fit showed they might not be comparable to each other in samples with greater L values. Further investigations with more highly lipaemic samples are necessary.

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Michael Legge

Ketoacidosis at autopsy

Metabolic acidosis is a common finding resulting from diabetes, starvation, and alcohol abuse with an increase in b-oxidative ketone bodies, (primarily β-hydroxybutyrate). In causes of unexplained deaths, elevated ketone bodies may be detected in the blood, vitreous fluid or other body fluids e.g., CSF. A recent systematic review on ketoacidosis at autopsy from the UK indicated that ketoacidosis may be an important factor in unexplained deaths (1). The authors cite a USA study indicating that that of the 93 deaths due to ketoacidosis in Baltimore, USA over a six-year period, 32 had no known cause of diabetes and they overview the cause of ketoacidosis. During the UK study period, 816 cases, 97 of these had β-hydroxybutyrate performed. Where vitreous fluid was unavailable, raised blood acetone was an indicator for ketoacidosis. A raised vitreous β-hydroxybutyrate of >960mmol/L was taken as being associated with significant mortality and was used to select cases. All data was extracted from autopsy reports and medical histories, and included route biochemistry profiles, vitreous fluid chemistry and blood and vitreous fluid ethanol. In total 42 patients clinical, pathological and biochemical findings were obtained. Of the 42 patients over a two-year period, 50% died of alcoholic ketoacidosis, 19% of diabetic ketoacidosis and 12% of both.

The authors also indicate that the differing concentrations of glucose and β-hydroxybutyrate can be obtained at differing sample sites at autopsy. It was emphasised that other causes of ketoacidosis may contribute to death such as starvation, infection, trauma and hypothermia, and that ketoacidosis should be excluded when there is no clear cause of death.

Paracetamol and sperm function

In recent years there have been numerous publications relating to decreasing sperm counts leading to increasing concern of male reproductive health. To-date no single cause for the decreased sperm counts has been identified although some evidence points to environmental endocrine disruptors. These environmental chemicals have been shown to disrupt the CatSper [Ca2+] channel which regulates important aspects of sperm function including motility. Previous research has indicated that men with high urinary levels of paracetamol (acetaminophen) had impaired sperm motility, increased sperm fragmentation and an increased time-to-pregnancy. In the present research from Denmark, the authors investigated the effect of paracetamol and its active metabolites on sperm function both in-vivo and in-vitro (2). Using healthy male volunteers, blood, semen and urine samples were collected prior to and during when paracetamol and its metabolites were administered. Following preparation, the samples were analysed LC/MS, UHHPLC-ESI-HRMS for metabolite identification as well as changes in [Ca2+] and fatty acid amide hydrolase activity (FAAH). All participants demonstrated significantly increased paracetamol exposure by day 3 mirroring concentrations in all three samples. One metabolite (N-arachidonoyl phenolamine, AM404) demonstrated a dose dependent increase in sperm intracellular calcium via activation of the CatSper channel. This metabolite can be conjugated to arachidonic acid by FAAH to form N-arachidoyl phenolamine which also induced calcium influx in sperm. Sperm motility was also decreased via CatSper.

The authors conclude that paracetamol and its metabolites are transferred into seminal fluid have a direct effect on CatSper-mediated calcium influx and that sperm themselves can metabolise paracetamol metabolites which will interfere with sperm [Ca2+] signalling. This is turn will influence sperm motility and delays in achieving a pregnancy.

Stability of SARS-COV-2 nasopharyngeal swabs

Collection, storage and stability of swabs for viral investigations is an important aspect for detection and monitoring of infections. Setting aside the pre-analytical requirements for the swabs, knowing that swabs for viral analysis can provide a reliable result if they have to be transported and possibly stored prior to analysis is vital for diagnosis. A short report from the USA has investigated the storage and stability of nasopharyngeal swabs for the detection of SARS-COV-2 using three different RT-PCR platforms (3). The authors discuss previous work which provided evidence for viral investigation swabs for a range of viral related disorders such as influenza, enterovirus, adenovirus and herpes simplex virus, indicating their stability for viral detection by RT-PCR for up to seven days. In the present work, the authors pooled 30 remnant nasopharyngeal swabs from known SARS-COV-2 positive patients and aliquoted them into 126 samples. These were split and stored for 21 days at both 18 to 25°C and 2 to 8°C and sampled at daily over 21 days. Three different automated RT-PCR platforms were used, Luminex ARIES, Panther Fusion and Abbott m2000. The ‘stability samples’ were analysed along with the routine clinical samples and the single cycle threshold was recorded, providing 244 data points. In addition, the authors tested seven SARS-COV-2 patient samples that had been stored at 4°C for 35 days. The qualitative detection of the virus was 100% for all three systems and storage at the two temperatures demonstrated little difference over time. All three instrument platforms performed extremely well and the seven patient samples tested positive after 35 days at 4°C. The authors concluded that the storage of nasopharyngeal swabs for SARS-CoV-2 detection could be reliably used for the detection of SARS-COV-2 infections.

Cell-free RNA and pregnancy outcome

Although in the Western world pregnancy is considered comparatively safe, there are still unexpected complications which may develop during the pregnancy. Typically, gestational diabetes and hypertension of pregnancy still can remain serious issues in pregnancy management. Of the two, hypertensions in pregnancy and pre-eclampsia still remain life-threatening disorders to both the woman and the fetus, with 14% of maternal deaths being related to these disorders, only below maternal haemorrhage. Hypertension and pre-eclampsia typically develop after 20 weeks' gestation, although pre-eclampsia may develop at any stage of pregnancy. Two recent publications have investigated the use of maternal blood cell-free RNA (cfRNA) to predict the outcomes of pregnancy (4,5). The first (4), a multicentre international collaboration, used transcriptome data analysis from 1,840 racially diverse pregnancies, retrospective analysis of 2,539 banked pregnancy plasma samples and a range of gestational ages. The presence of cfRNA in the maternal blood correlated with ultrasound gestational age assessment. The authors demonstrated that the maternal plasma and placental cfRNA correlated with fetal gene sets. Of these three genes identified the probability of pre-eclampsia with a positive predictive value of 32.3% with maternal BMI or race having no effect. The second publication from the USA (5) published a week later, identified a panel of 18 genes that indicate pre-eclampsia pathology at between 5 to 18 weeks of gestation using 404 plasma samples from 199 pregnancies associated with pre-eclampsia. Both sets of authors conclude that the samples and data indicate evidence of early abnormal placentation and endothelial dysfunction and that non-invasive (to the fetus) monitoring cfRNA could indicate the advent of pre-eclampsia and the signature for organ dysfunction. In conclusion they propose that clinical validation would be required to determine the pathogenesis of pre-eclampsia.

Point of care glucose meter standardisation

Point of Care (POCT) is rapidly becoming the 'norm' for a number of analytes such as glucose. The POCT may be performed in a number of settings such as an individual's home, pharmacies and various POCT stations around a hospital. As new diagnostic technologies emerge, POCT is estimated to grow by 12 to 16% per year. Despite the overall reliability of the instruments themselves, issues arise with the individual's use of the testing system, quality control, result flags, maintenance etc. In a recent publication from the USA a multi-disciplinary group investigated POCT glucose standardisation over a single year (6). This involved approximately 2.4million POCT glucose assays undertaken by 17,000 operators using more than 700 meters. All glucose meters in the survey were anonymised depending on the manufacturer and their distribution and use was spread over all potential users in a hospital setting. The performance of four glucose meters was assessed and validated in a single certified laboratory using the same instruments. A 16-point spreadsheet of operational criteria using a Likert scoring scale was used to compare the performance of each selected instrument. Blood samples included capillary, arterial and venous samples. The validated reference method was the hexokinase colorimetric method on either ABL or Roche c502 analysers. Overall, the analytical performance of all the meters was comparable for both laboratory and clinical evaluation. One meter however, ranked the highest for usability, implementation and streamlined interface connectivity. The authors conclude that standardisation was an integrated system-wide organisational structure requiring a centralised POCT oversight. They also commented that certain instruments were not validated for use with both the critically ill or neonatal venous blood samples. In addition, they commented on the poor connectivity of some system with LIS including patient identification and operators interrupting power on/off sync-sequences. From this work the authors are now investigating the standardisation of other diagnostic based analyser systems. **NOTE:** The authors did not identify the meters in the publication.

Are all SARS-COV-2 methods created equal?

The advent of the world-wide SARS-COV-2 outbreak and declaration of a pandemic by the WHO on March 2020 required the rapid development of an accurate test both for diagnosis and epidemiological tracking of the infection. The identification of the virus sequence resulted in the development and publication of PCR assays, subsequently resulting in establishment of numerous commercial and laboratory-based SARS-COV-2 tests and procedures. Subsequent to the initial outbreak, molecular testing identified a number of SARS-COV-2 variants, which raised the question whether all the PCR diagnostic tests were sufficiently reliable to detect SARS-COV-2 variants. A recent international survey performed on behalf of the International Federation of Clinical Chemistry and Laboratory Medicine (Molecular Diagnostics Committee) investigated the global overview of test methods, laboratory procedures and quality assessment of methods used to detect SARS-COV-2 (7). Using an anonymised online survey, they addressed laboratory demographics, techniques, and virus detection and variant sequencing. A total of 273 laboratories from 49 countries were surveyed. Of these 92.2% used RT-PCR testing, however, the majority of these did not test for SARS-COV-2 variants. In addition, 33.2% of laboratories did not participate in an external quality assurance programme. The testing numbers varied considerably with 17.8% of laboratories performing 0 to 100 tests/week and the majority (31.1%) performing 100-1770 tests/week; 26.6% of laboratories were performing 1400 to 7000 tests/week. Although the majority of participants used RT-PCR there was wide variability on the actual methods used. Reporting was as cycle threshold in 51.1% and Positive/Negative in 57.7% of positives. 4.5% reported copies/mL. For the variants of concern, 25% of laboratories genotyped positive cases in their own laboratory, the remainder did not and 51.9% did not have their positive samples sequenced at all. As a result of the survey the Committee identified the lack of standardisation across different laboratories and countries, as well as sample collection processing and result reporting and concluded that there was insufficient quality assurance being performed by diagnostic testing laboratories for SARS-COV-2 testing. They also commented on comparability of antigen-based methods and molecular methods in the context of analytical and clinical performance including issues relating to quality assurance.

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Science Snippet

Concept of the first face-mask.

In 1870 John Tyndall (a natural philosopher/scientist) identified that "invisible floating matter" was present in the air and could be removed by heat. Knowing of Pasteur's work and his germ theory, Tyndall through a series of experiments determined that the "invisible floating matter' contained germs that could cause disease and decay. In addition, he clearly demonstrated that the "floating matter" could be removed by filtering through cotton wool. This challenged the long-held medical belief of spontaneous generation and the concept of maismas (bad air). Despite the opposition by the medical profession John Tyndall was the first person to demonstrate the complex concept of contagious disease and sepsis under a single germ theory. Additionally, he introduced the concept of filtering germs and the basis of the face mask. However, it was not until the1920s that the medical profession accepted the value and use of the face mask.

OBITUARY

Gilbert Robert Rose

1927 - 2022

Aged 94

I first met Gilbert at the beginning of 1966, when as a callow youth, I and five other raw recruits started as trainee medical laboratory technologists at the Pathology Department, Christchurch Hospital. This first year was all a bit overwhelming and daunting as we learned to understand what was expected of us but our rotation through the Microbiology laboratory quickly became an enjoyable and popular place to be, helped in no small part by the Charge Technologist, Gilbert Rose's amiable and systematic approach to the teaching and development of his acolytes; and which had me specialising and working in this discipline for the next 45 years.

Gilbert commenced as a trainee at the Pathology Dept in 1947 and had a less than auspicious start to his career. Mortuary work was part of the rotational training of the time and it would be fair to say that Health and Safety procedures back then were not as robust as they are today as he contracted tuberculosis when assisting at an autopsy. As this was just prior the introduction of Streptomycin and Isoniazid, the management of Tb at the time was thoracoplasty, a surgical procedure where the affected lung is collapsed and ventilation is impaired, thereby reducing oxygen levels and inhibiting the growth of the oxygen-dependent tubercle bacillus. This was followed by total bed rest for more than a year in the Sanatorium on Cashmere Hill. This now outmoded treatment was, however, successful and another happy outcome of this enforced confinement was where he met Beth, his wife to be.

He went on to qualify with the Certificate of Proficiency in Hospital Laboratory Practice in 1954 and progressed to become Charge Technologist in Microbiology. At a time when rapid diagnostics, minaturisation and automation were starting to be actively developed, Gilbert was awarded the 1976 Wellcome Trust Fund Travel Award which allowed him to travel to Cambridge, England to attend the 2nd International Conference on Rapid Methods and Automation and visit various UK laboratories. He was particularly interested in laboratory computerisation and automated techniques and returned with a vision and direction that was to stand the laboratory in excellent stead for many years to come.

During his tenure he was also co-opted with other responsibilities including the development of new virological and Public Health Laboratory Services, the planning of the new Pathology Laboratories, was Chair of Pathology Services Laboratory Committee, Chair of Allied Health Professions, part time tutor and hospital liasion officer at Christchurch Polytechnic, examiner and moderator for Medical Laboratory Practice Microbiology examinations, an assessor for TELARC (forerunner of IANZ for medical laboratories), was involved in the assessment of and recommendations for re-organisation of small laboratories in the South Island, was on the organising committees for various laboratory conferences and served on the Hospital Service Welfare Society for 15 years. He was also one time Editor for the Institute Journal (when it was then called *Journal of the NZ Association of Bacteriologists*) and was a Life Member of the NZIMLS.

After serving 38years at the Christchurch Hospital Pathology Dept he retired in 1985 but was not about to spend the rest of his days gardening (which, incidentally, he only did under sufferance!) or playing bowls. Instead he signed up for three years at the Tari Research Unit, PNG where he established a microbiology laboratory for acute respiratory infections (ARI) and assisted with routine work at Tari Hospital. Between 1989 and 1991 he was Tutor Co-ordinator at the Pacific Paramedical Training Centre (now Pacific Pathology Training Centre) in Wellington and up until 1995 undertook short term consultancies for ARI laboratory methods, QC procedures or basic laboratory techniques, visiting Samoa, Fiji, Vanuatu, Cook Islands, Tonga, Lao and Port Moresby and Goroka, PNG.

He then joined Volunteer Service Abroad for two assignments; one was for 15 months as Charge Technologist to a hospital laboratory in Kokopo (East New Britain Province, PNG) the other in Quy Nhon, Viet Nam where, under the auspices of the NZ-Viet Nam Health Trust he spent six months preparing their laboratory for an ISO accreditation which back in 2002 was very much in its infancy.

Gilbert was also on the board of the Pacific Leprosy Foundation for five years.

Away from the laboratory his interests were listening to music, particularly classical and jazz, he was an avid reader of most genre and dabbled eclectically with pursuits such as fishing, photography, spinning and dyeing and caligraphy. He loved travel which, apart from his working trips, included Japan, Europe, Australia on many occasions and India; and he enjoyed a good syrah or aromatic white, single malts and ethnic foods.

Gilbert was an outstanding mentor and it was a privilege to have known and worked with him for twenty years. He was a very unassuming person - avoiding any limelight. Always calm, pragmatic and unflappable, he was diplomatic, non judgemental, highly respected by all throughout the NZ medical laboratory scene and his door was always open to anyone seeking advice or direction. An excellent Chair at meetings, he was very good at negotiating pathways and outcomes, often dealing with some very difficult to please people. Working in PNG, the Pacific Islands and SE Asia gave him particular satisfaction as he enjoyed the teaching of "real microbiology", the unrushed life styles and immersing himself in the cultures of his students.

Gilbert was widowed in 1982 and is survived by his daughters, Mary, Alison and Jill, son Graeme, six grandchildren and eight great-grandchildren.

Compiled by Graeme Paltridge

Sources: Mary and Jill, Peter Skidmore and Marilyn Eales.

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 14 October 2022**. 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 Jillian at cpd@nzimls.org.nz.

JULY QUESTIONNAIRE

1. What role does NF-kB have in the immune response?
2. What two main structural proteins are targeted by autoantibodies in Autoimmune Bullous Dermatoses (AIBD) disease? What are these proteins responsible for and how does their loss present on the skin?
3. What crucial roles does Interleukin 10 (IL10) play in regulating the immune response? What conditions have been linked to low IL10 serum levels and IL10 gene variants?
4. What normal roles do the serum minerals zinc, copper and magnesium have in the body? From this study what if any significant changes in these trace elements were found that would suggest a relationship with the pathogenesis and progression of preeclampsia and gestational diabetes mellites?
5. What are DNases? What is DNase1L3 deficiency associated with in systemic lupus erythematosus (SLE) disease?
6. What is Factor XIII deficiency?
7. The carbapenem-resistant P. aeruginosa clone ST235 is associated with high level antibiotic resistance and poor clinical outcomes. What is the significance of this case study to New Zealand? What detection methods are recommended?
8. What caused interference in routine blood test results showing elevated paracetamol levels when the patient insisted that they had not taken the drug? How was it resolved?
9. Why are Carbapenemase producing organisms (CPOs) recognised as a global health concern and what facilitates their spread in African countries?
10. What and how must laboratories display in their laboratory to alert personnel to a fire hazard?

ANSWERS MARCH 2022 JOURNAL QUESTIONNAIRE

1. In invasive aspergillosis fungal infection detection, what is believed to contaminate and cross-react with the widely used EIA biomarker galactomannan assay?
Other fungi, e.g., penicillin, fusarium and paecilomyces spp can cross-react and contaminate GM in antibiotics and food stuffs and cause false positive results.
2. From the LFA assay evaluation what do the researchers recommend the OD cut-off be to define a positive GM using IMMY LFA? And what have the manufacturers informed the researchers?
OD=1.0, Reword their Information for users to; "Indexes between 0.5-1.0 have lower predictive values than specimens with results of >1.0. Indexes between 0.5-1.0 should be interpreted carefully with consideration of other clinical, radiological or laboratory evidence of IA".
3. What role does the endothelium play in regulating and maintaining vascular function? And how does it do this?
Endothelial cells protect the system from the onset of vascular degeneration, serve as an interface between circuiting blood and vascular smooth muscle cells creating a physical barrier.
4. What endothelial cell markers were significantly raised in uncontrolled diabetic subjects compared with that of controlled diabetic subjects?
Soluble thrombomodulin and plasminogen activator inhibitor-1.
5. What is a Gut resistome? And what can happen at this site?
A Gut Resistome is a large potential source of antimicrobial resistance and is a site where resistance genes can be transferred from commensal flora to virulent microorganisms.
6. What antibiotics are the most widely used in animal feed, globally?
Penicillins, tetracyclines and quinolones.
7. What are the critical functions of brain derived neurotrophic factor (BDNF) protein found in the brain and what do researchers recommend supplementing ASD patients with?
Regulation of glycogenesis, synaptogenesis, neurogenesis and neuroprotection and control of long and short duration synaptic interactions in the brain that influence memory mechanisms and cognition. Researchers recommend supplementing with Vitamin D and antioxidants.
8. Measurement of some fat-soluble vitamins can be performed using immunoassay or high-performance liquid chromatography (HPLC) methods. What are the different benefits of using each method?
Immunoassay methods are faster, cheaper and easier to troubleshoot, HPLC methods are more reliable, with greater precision and accuracy.
9. What are examples of some of the symptoms of vitamins A, D, E and K deficiency, respectively?
Vitamin A: Night blindness, Vitamin E: Increased oxidative cell stress, Vitamin D: osteomalacia and Vitamin K: Haemorrhage.
10. When and why were beak masks introduced?
Introduced in the Middle Ages, during the bubonic plague. They were filled with herbs and spices to prevent miasma (which is bad air believed to be the source of infections.)

Welcome to the Board of Trustees

Nicky Beamish, NZCS, DipMLS, PGCertPH

Nicky Beamish, an experienced medical laboratory scientist is currently employed by Wellington SCL as Point of Care Testing Co-ordinator, a diverse role involving education and training of staff, quality and project management, installing and troubleshooting analysers, and managing and troubleshooting IT issues.
Nicky has been involved with the PPTC since 2011 as Microbiology Consultant, writing and co-ordinating the Microbiology QAP for the Pacific and South-East Asian region, and assisting with tutorials for short courses.
Over the past two years Nicky has project managed the installation and validation of molecular instrumentation including the Gene Xpert, ID NOW and COBAS Liat. for rapid detection of SARS-CoV-2 plus provided staff training.
In her role as Team Leader Microbiology, she carried the role of project lead for the implementation of the AusDiagnostics multiplexed tandem PCR and in-house RT-PCR to replace bacterial culture, antigen detection, and microscopy for faecal pathogens. This involved workflow planning, validation of methodology, cross-over studies, procedure writing, and training of staff.
Nicky has had significant experience working in the Pacific, two years as Rural Laboratory Supervisor in Papua New Guinea, and a year as Laboratory Trainer and Adviser in Palau. She has an excellent understanding of the challenges that laboratories in developing countries face with limited budgets, poor infrastructure, technical staff with knowledge gaps, and outdated or poorly planned systems and technology.
The Board of Trustees are delighted to have Nicky as an addition to their team.

Welcome to the PPTCs consultancy staff

Angela Lewis, BSc, GradDipSci.

Angela the PPTC's newly appointed Microbiology Specialist and Programme Support Medical Laboratory Scientist commenced employment with the PPTC on the 26th April 2022.
Since February 2018, Angela has been employed as a Medical Laboratory Scientist in Microbiology at Canterbury Health Laboratories. Prior to this, she was employed as a Medical Laboratory Technician in Serology/Virology. With the various roles she has had in laboratory diagnostics, Angela has gained extensive experience in a number of areas, including general Bacteriology, Mycobacteriology, Virology/Serology and through secondment into the Covid-19 response team. This has allowed her to build proficiency in a range of diagnostic techniques, including molecular analytical platforms such as 7500 RT-PCR, BD Max, Genexpert, Abbott M2000 and thermocyclers. She is also proficient in culture plate reading, antimicrobial susceptibility and automated and manual serological testing. The PPTC is delighted to have Angela join the Consultancy team.

PPTC consultancy staff 2022

Philip Wakem
NZCS, DipMLS, MMLSc (Otago, NZ), MNZIMLS
Chief Executive Officer and Haematology Specialist

Navin Karan
BMLSc (Otago,NZ) PGDipPH (Massey,NZ) MNZIMLS

- Microbiology/Molecular Diagnostics Specialist
- Programme Manager For COVID 19 and Infectious Disease
- Contract Management
- Centre based Courses
- Molecular Diagnostics
- COVID 19 EQA

Filipo Faiga
BSc, DipMLS, MNZIMLS

- Biochemistry Specialist
- Programme Manager
- For PPTC External Quality Assessment
- Health And Safety
- Biochemistry Contracts

Russell Cole
NZCS, DipMLS, MNZIMLS

- Microbiology Specialist
- Programme Manager for Laboratory Quality Management
- Diploma in Medical Laboratory Science
- Microbiology Contracts

Angela Lewis
BSc, GradDipSci, MNZIMLS

- Medical Laboratory Scientist
- Microbiology Specialist and Programme Support.
- Assistance in Molecular diagnostic platforms and Contacts

New PPTC consultants who have joined the PPTC EQA programme

Donna Mitchell, NZCS, DipMLS, DipMktg

Donna has recently taken over the role of Serology Quality Assurance Consultant for the PPTC's Serology REQA Programme.

Donna has been employed as a senior scientist (part time) in Serology/Virology by Canterbury Health Laboratories since Oct 2011. Prior to this Donna was employed by Southern Community Laboratories for 15 years. During this time, she was supervisor of the SCL Molecular Laboratory (from 2004 - 2007) and contributed, as an author, to an in-house informative/marketing publication called "Bug Byte" which was distributed to GP's.
During the introduction of Chlamydia nucleic acid amplification testing in 2000 as a routine test in her Christchurch laboratory, Donna was also involved with the Marketing Department in a Strategic Planning Group for the new product launch.
While at the Princess Margaret Hospital for Canterbury Health Laboratories and later at SCL from 1991, Donna was involved in a variety of work including routine clinical work, research, and evaluation and implementation of Ligase Chain Reaction assays for Chlamydia and M. tuberculosis, and latterly the introduction of the ProbeTec Strand Displacement Assay for both Chlamydia and M. tuberculosis. As a result of the LCR work Donna was asked by Abbott Diagnostics Ltd to introduce the technology to laboratories in both Sydney and Melbourne. The PPTC is delighted to have Donna as a valued member of its consultancy team.

Sharleen Price, BMLSc, MMLSc (Massey)

Sharleen has recently taken over the role as the Microbiology Quality Assurance Consultant for the PPTC's Microbiology REQA Programme.
Sharleen is a Medical Laboratory Scientist with more than 10 years of medical laboratory experience in both hospital and community Microbiology and Molecular settings. Sharleen graduated in 2007 with a BMLSc and in the years to follow completed a Masters in Medical Laboratory Science in 2013. Originally Sharleen was solely involved in Microbiology but over the years has transitioned to mostly Molecular work. Sharleen has a busy family life but manages to work part time at WSCL in Molecular Haematology, and Infectious Molecular and still continues to be involved in general Microbiology

Sharleen is grateful to be working with the PPTC family and being able to contribute to the Pacific communities achieving positive health outcomes. The PPTC is delighted to have Sharleen as a valued member of its consultancy team.

"Give a Little" fundraising towards the devastation in Tonga

As you would be aware, the powerful underwater volcano eruption in Tonga on 15th January 2022, caused widespread devastation to homes and services in Tonga, affecting 84% of the resident population.

The PPTC set up a "Give a Little" page requesting donations that would be paid directly to each of the medical laboratory staff members employed by the Tongan Ministry of Health in Tonga to support themselves and their families.
Navin Karan, (the PPTC's Programme Manager for Microbiology and Molecular Diagnostics) on behalf of the PPTC, spent many hours arranging and administering the "Give a Little Fundraising Programme" and is to be congratulated for his fine efforts in raising $NZD 11,400.00 which was divided and distributed to each of 38 staff members. The laboratory community expressed their sincere gratitude to the PPTC and all those who generously donated.

Appreciation letter received at the PPTC

From, All Medical Laboratory Staff, Tonga

To, PPTC Staff and Board Members,
Laboratory Suppliers (Fort Richard ,EBOS ,Medica and Roche) and product Managers, NZ laboratory and Medical colleagues, NZIMLS members, New Zealand Medical Laboratory (APHG group-Wellington/Dunedin SCL and Labtest), Friends and Families,

We would like to take this opportunity to express our sincere and heartfelt appreciation for the generous monetary donations and support Tonga's Medical Laboratory staff had just received. We are blessed indeed! Thank you for walking alongside with us during this unprecedented time and the crisis of this natural disaster and COVID-19 . Thank you for showing care and support, enabling us to weather the burden of this crisis, knowing that we were not alone. Your donations had greatly helped with our family's essential needs. This will always be remembered and appreciated.
MALO 'AUPITO E 'OFA (THANK YOU SO MUCH FOR THE LOVE!),'OKU HOUNGA MO'ONI (WE ARE SINCERELY GRATEFUL).
May God continue to bless you all in all the ways. Malo 'aupito once again! 'OFAATU

All Medical Laboratory Staff ,Tonga Islands (Nuku'alofa, Vava'u, Ha'apai and 'Eua)

Tongan Laboratory staff accepting the donations given.

Overseas travel

Cook Islands: (30th May- 4th June)

Both Navin Karan and Angela Lewis are scheduled to visit the Rarotonga National Laboratory to carry out an assessment of Molecular Diagnostics, Laboratory Quality Management and the laboratory's progress towards accreditation alignment to the ISO15189: 2012 Standard

The EQAsia project (14-15th June 2022.)

The overall aim of the EQAsia project is to improve the Quality of Bacteriology Diagnostics for Antimicrobial susceptibility testing in the Asian region. The EQAsia project is supported by the Fleming Fund (UK Aid Programme). EQAsia comprehends a multidisciplinary consortium of experts gathered to deliver the outputs of the Fleming Regional Grant – DTU (Technical University of Denmark), IVI (International Vaccine Institute), NIH (National Institute of Health, Thailand) and CU (Faculty of Veterinary Science, Chulalongkorn University.

Navin who has been elected to the Scientific Advisory Board representing the PPTC has been invited to attend the EQAsia 2nd Interim Meeting to be held in Bangkok, from the 14-15th June 2022.

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
E-mail: pptc@pptc.org.nz or phil@pptc.org.nz

31 August - 2 September 2022

Museum of New Zealand
Te Papa Tongarewa

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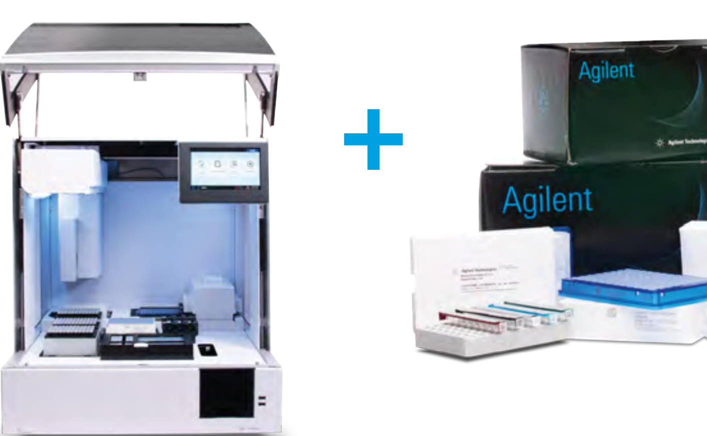

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