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The *New Zealand Journal of Medical Laboratory Science* (the Journal) is the official publication of the New Zealand Institute of Medical Laboratory Science (NZIMLS). The Journal is peer reviewed and publishes original and review articles, case studies, technical communications, and letters to the Editor on all subjects pertaining to the practice of medical laboratory science. The Journal is open access (www.nzimls.org.nz/nzimls-journal) and is published three times per year in April, August, and November. Hard copies are circulated to all NZIMLS members and universities and research units in New Zealand and overseas. Current circulation is about 2,200 copies per issue. Printing is by Griffin Press, Christchurch on environmentally responsible paper using elemental chlorine free third party certified pulp sourced from well managed and legally harvested forests and manufactured under the strict ISO14001 Environmental Management System. The Journal is indexed by CINAHL, EMBASE, SCOPUS, Informit, Thomson Gale, EBSCO and Biosis Citation Index, and the Journal Editors are members of the World Association of Medical Editors (www.wame.org).

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Expression of interest for reviewers of the Journal

Articles submitted to the New Zealand Journal of Medical Laboratory Science undergo single-blind peer review. The subject of medical laboratory science encompasses many specialities and at times it is difficult for the Editors to find suitably qualified experts to peer review.

We are now setting up a data base of potential reviewers and ask NZIMLS members to express an interest to be in this data base. What we require of you, if you are interested, is to state what your specific experience and interest in your speciality is, together with up to six keywords. Send these details, together with your place of employment and email, to Sharon Tozer at the NZIMLS (sharon@nzimls.org.nz) with 'Reviewer expression of interest' in the subject line.
Further information may be obtained form the Editor, A/Prof. Rob Siebers at rob.siebers@otago.ac.nz

You do not necessarily have to have experience in peer review, as comprehensive instructions will be given as well as the Editorial team guidance. Upon satisfactory completion of a review, the member can claim 10 CPD points.

The Editor and Deputy Editor: Associate Professors Rob Siebers and Michael Legge

EXECUTIVE OFFICE HOURS OVER THE CHRISTMAS PERIOD

The Executive Office will close for the Christmas Break on Wednesday 23 December 2020

We will re-open on Monday 11 January 2021

We would like to wish all our members, advertisers, supporters and their families a very Happy Christmas and hope for a better year in 2021!

In this issue

Rob Siebers, Editor

Fran van Til, the Executive Officer of the New Zealand Institute of Medical Laboratory Science (NZIMLS) will be retiring at the end of 2020. In this issue Tony Barnett, the NZIMLS Secretary/ Treasurer, gives a fitting tribute to Fran's involvement with the NZIMLS over the last 30 years.

Articles submitted to this journal undergo peer review. As many of the New Zealand reviewers are working medical laboratory scientists, rather than academics, the question they often raise when asked to review is 'what am I supposed to look for'. In this issue the Editor and Deputy Editor, Rob Siebers and Michael Legge, provide guidelines for reviewing scientific articles. Not only are these guidelines of use for reviewers, they are also of potential use for submitting authors and readers.

COVID-19, the virus responsible for the current global pandemic, causes severe acute respiratory syndrome. In severe cases it causes multiple organ injuries with a high morbidity and mortality, causing changes in many laboratory parameters. Ikeagwulona and colleagues from Nigeria reviewed studies that reported changes in laboratory parameters in COVID-19 hospitalised patients to identify those parameters that predict disease severity. The authors found 28 articles comprising nearly 3,000 laboratory confirmed cases of COVID-19 patients with 32 different laboratory parameters identified. D-dimer, lactate dehydrogenase, lymphocytes, neutrophils, platelets, fibrinogen, NLR, oxygen index, and blood gases should be considered important in risk stratification to predict severe and fatal COVID-19 outcome in hospitalised patients.

Significant metabolic complications can appear over time with spinal cord injuries with a strong association with the increased incidence of heart disease and type 2 diabetes. Lynnette Jones and Michael Legge from Dunedin present a hypothesis linking dysfunctional skeletal muscle molecular clocks as a primary causative factor for the development of metabolic syndrome in spinal cord injury. They hypothesise that, following spinal cord injuries, the skeletal muscle metabolic clock uncouples and fails to provide the correct signalling cues for normal skeletal muscle metabolism and that the consequence of stopping or slowing the muscle molecular clock results in metabolic chaos.

Early diagnosis and prompt surgical intervention reduces the rate of complications in acute appendicitis. However, accurate diagnosis remains a common surgical problem. Olaogun and colleagues from Nigeria determined the value of preoperative serum bilirubin level and white blood cells count in patients with simple and complicated appendicitis. They found that high total serum bilirubin is a useful adjunct to improve the diagnostic yield in perforated appendicitis.

Anthropometric indices are known markers of obesity and predictors of metabolic syndrome. Adejumo and colleagues from Nigeria assessed the ability of neck circumference to predict metabolic syndrome in undergraduates in Nigeria. They found that neck circumference has a positive correlation with markers of central obesity and can discriminate metabolic syndrome.

Mok and colleagues from Australia, Armenia, and Saudi Arabia provide an update on selected internationally oriented guidance documents and relevant literature at the application level that are associated with the implementation of the process evaluation and improvement stage of ISO 15189:2012. Their study contributes to the medical laboratory's development and improvement of implementations of ISO 15189:2012 in areas of responsibilities by fulfilling management system and technical competence requirements to an acceptable level of conformance.

Invasive breast cancers have two major histological subtypes, invasive ductal carcinoma, the most common subtype, and invasive lobular carcinoma. Hussam Khalil investigated BRCA2 mutations in Syrian familial breast cancer cases. He found that BRCA2 mutations were more likely to be correlated with invasive lobular breast carcinoma and tended to be associated with hormone receptor positive tumours.

Silver nanoparticles have been postulated as the next generation of antimicrobials ascribed to their recognised attraction for the DNA of microbes and production of reactive chemical species containing oxygen. Siddique and colleagues from the United Arab Emirates and Malaysia tested drugs that are clinically used for CNS disorders (phenobarbitone, phenytoin, and levetiracetam) in their available formulation and following conjugation with silver nanoparticles against *Escherichia coli* and methicillin-resistant *Staphylococcus aureus*. Drug-conjugated silver nanoparticles and silver nanoparticles displayed bactericidal effects when compared to the drugs alone. Drug-conjugated silver nanoparticles also inhibited bacterial-mediated host cell death. These findings suggest that drug repurposing is a viable approach and currently available CNS drugs should be tested following conjugation with nanoparticles against CNS pathogens.

Wilson and colleagues looked at the implementation of a targeted next generation sequencing panel (AmpliSeq for Illumina Cancer Hotspot Panel v2 consisting of 207 amplicons representing the covered hotspot regions of 50 oncogenes and tumour suppressor genes) at the Wellington Regional Genetics Laboratory and compared results to those previously reported externally. All detected variants were concordant with previously reported results from other laboratories overseas. Variant allele frequencies in the OncoSpan gDNA reference standard are concordant with variant allele frequencies observed in-house.

Lauren Eddington from Dunedin gauged New Zealand haematology medical laboratory scientist's opinions on a possible Clinical Laboratory Scientist position in New Zealand medical Laboratories. Responsibilities thought to be appropriate for this role included educating laboratory scientists, requesting further laboratory testing, and commenting and consulting on result interpretation for clinicians.

Rapid urine antigen tests are available for the detection of *Legionella pneumophila* serogroup 1 antigens, but not for the detection of *L. longbeachae*. Ros Podmore and Mona Schousboe from Christchurch evaluated a prototype lateral flow urine antigen test for the rapid detection of *L. longbeachae* urinary antigen and found that the assay was an extremely useful tool for the early detection of *L. longbeachae,* despite the low sensitivity achieved.

Shirley Gates and Elizabeth Lombard from Whangarei present a case of cold autoimmune haemolytic anaemia as the presenting feature in a patient with angioimmunoblastic T-cell lymphoma (AITL). Bone marrow investigation demonstrated a small B-cell clone in keeping with a monoclonal B-cell lymphocytosis. No evidence of a concurrent lymphoproliferative process was present, however, radiological studies indicated extensive lymphadenopathy and a lymph node biopsy indicated a diagnosis of AITL. Diagnosis was confirmed by clonal T-cell gene rearrangement on molecular studies.

Professor Birgitta Tomkinson from Uppsala University in Sweden recently visited the BMLSc course at Otago University on a teaching sabbatical. In this issue she presents a comparison between the Otago BMLSc and the equivalent Bachelor of Biomedical Laboratory Science at Uppsala University. While there are many similarities between the two degrees, there are also many differences, especially in the area of organisational control with responsibility for the programme at Uppsala University shared between more staff members.

EDITORIAL

Fran van Til's retirement

Tony Barnett

December 31, 2020 marks a milestone moment in the career of Fran van Til. Fran will be retiring as the Executive Officer of the New Zealand Institute of Medical Laboratory Science (NZIMLS). Fran has dedicated the last 30 years of her working life to the effective and efficient function of the NZIMLS. All members will be aware of Fran's attention to detail, her willingness to accommodate and her kind and considered response to any request. For those reasons alone Fran has been the perfect point of contact for the NZIMLS. However, when Fran started with the organisation there was not really a vacancy to fill or job description in place. The organisation just needed someone… and that person was Fran.

Paul McLeod, the NZIMLS President at the time, provides "some thoughts and memories of those times".

I had been involved with the Council of the NZIMLS for some years before being elected as the President. For those of you with a good memory you will remember Barrie Edwards and the pivotal role that he played as the Secretary to the Institute. In a nutshell, he was the major driver of the Institute. I clearly recall the time when he pulled me to one side just prior to the annual conference being held at Invercargill to say that he was stepping aside immediately to give himself space and time to pursue his career at Canterbury Health. It was not the kind of news that an incoming President really wanted to hear. However, in his usual proficient way he said that he had arranged for someone to be present at our Council meeting to take the minutes. Enter Fran van Til. From the very outset I personally knew that Fran was exactly what we needed. She was professional, confident, and demonstrated all those lovely qualities that were required to fit in and understand our organisation. As the years have passed, Fran has become much more than the 'secretary'. The complexities involved with preparation for the Council meetings, staging the annual meetings, SIG meetings and supporting the ongoing education programmes have all become part of her skills and dedication to the Institute. I also recall Barrie saying to me at the time, 'don't worry, Fran will do a good job'. How right he was! To Fran I say "you made my task as President a real pleasure and a breeze and I have nothing but happy memories of our friendship and time together serving our membership. All the best for your future years".

Paul McLeod

Over the past 30 years Fran has worked closely with a number of different elected Presidents, Vice Presidents, Secretary/Treasurers and Council members and she has always maintained a friendly and cheerful disposition while ensuring a high level of professionalism and enthusiasm for our organisation. Fran is one of only a few individuals who is not member of the scientific community to be made a Life Member of the NZIMLS and this was bestowed on Fran because of her dedication and loyalty to our profession.

Fran is not only extremely knowledgeable about the function of the NZIMLS, including the CPD programme, the Journal, and educational programmes, but also the relationship with the Medical Sciences Council and legislation pertaining to scopes of practice. But for those of us who attend Council meetings, are involved in organising annual scientific meetings or SIGs, been examiners or moderators, have attended any scientific meeting, or been an examination candidate, we can only marvel at Fran's organisational skills to ensure everything goes according to plan. It is this skill and commitment that ensures the NZIMLS is recognised as a quality professional organisation within the New Zealand health network.

From all current and past Council members, we thank you for your tireless work ensuring the services of the organisation are maintained at the highest standard. And on behalf of all past and current members and the profession, we thank you for making our contact with the NZIMLS an easy and friendly experience. We wish you all the best in your retirement and hope you can enjoy some well-earned quality time with your family.

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Tony Barnett, MNZIMLS, Secretary /Treasurer

NZIMLS, Rangiora

Advertisers in this Issue

Guidelines for reviewers for the New Zealand Journal of Medical Laboratory Science

Rob Siebers and Michael Legge

The New Zealand Journal of Medical Laboratory Science (Journal) has a proud history of having been published continuously since 1946. Right from the birth of our Journal, all scientific and review articles have been peer reviewed and this is a major reason for the Journal being abstracted in major data bases, such as Scopus and CINAHL. Normally, submitted articles are reviewed by at least two reviewers who are experts or knowledgeable in the topic of the submitted article. The Journal uses a single-blind review process in which the reviewers' identities are unknown to the authors, but the authors' identities and study locations are known to the reviewers. This is the most common model of peer review worldwide, although some journals use the open peer review model where reviewers' names are known to the authors and published, or the double-blind peer review model where neither authors or reviewers know each other's identities. In the latter model it is often not possible to blind the authors' names or their institutions especially when the authors reference their own publications in the submitted article.

Quality peer review takes significant effort and time and is unpaid. Even the major journals struggle at times to attract reviewers, mainly due to the time required. We are lucky that we have an excellent Editorial Board whose members span all the major disciplines of medical laboratory science. We also rely on the Special Interest Group conveners for suggesting potential reviewers, and both the Editor and Deputy-Editor are academics experienced in international publishing and peer review. However, most of our reviewers are practicing medical laboratory scientists with no or limited publication experience and often their response to a request to review is to say "*I know little or nothing about peer reviewing*". We normally provide a brief list of instructions to peer reviewers highlighting the points to consider. In these guidelines we expand on what to look for in an article review. These guidelines are not aimed at reviewers alone, they are a useful reference for authors as well. Knowing what reviewers and Editors look for in a good scientific article can aid the authors when preparing their articles. It may also be useful in critically evaluating published articles.

Before submitted articles are sent out for review, the Editors first determine whether the topic is medical laboratory science related. The Journal does not publish animal studies. We also determine whether the study is ethical and whether ethical approval for the study has been obtained by an approved authority, if required. Nowadays many submissions are from overseas where English is not the primary language of the authors. Often there are problems with grammar, spelling and punctuations. However, the Editors primarily focus on scientific quality and will aid the authors in correcting grammar and spelling if their article has been accepted for publication. The focus of these guidelines is primarily for scientific articles, not for review articles or case studies. For the latter, an article has previously been published in the Journal on what constitutes a good case study and can be used as a guide in evaluating this type of article (1).

When receiving an article for review, first read through entirely without making notes. This is to get a general feeling for the article. Next look if clear aims or a hypothesis is stated and then look if the methods employed are appropriate to test those aims or hypothesis. The stated methods must be described sufficiently and succinctly for experienced scientists to be able to replicate the study. Statistical methods must be appropriate. The Journal does have statistical experts at hand who often will look at this aspect, thus having no or little statistical ability is not a problem for reviewers for the Journal. Additionally, whether ethical approval is needed and mentioned will already be determined by the Editors during pre-screening.

Next look at the Introduction section. Is this a succinct, but brief, background to the study. This section should only have a few key references and should not be an extensive literature review. Check to make sure these references are appropriate and accurately quoted. State if you think more appropriate published studies should have been quoted. The last paragraph should clearly state what the study's aims were.

It has already been mentioned that the Methods section should briefly describe the methods and materials used. Manufacturers of equipment and reagents should be identified. If described methods are standard ones, an appropriate reference to them will suffice without extensive details. However, any modifications to established methods should be described.

Make sure that results are clearly presented in the Results section without comments. Discussion of the results in this section should be in the Discussion section. Ensure that results in Tables and Figures are not repeated in the text in this section. The Journal uses SI units for expression of laboratory parameters. Please note if results are not in SI units. P values should be actual values and not stated as <0.05. Actual p values give a clearer indication of the degree of significance.

The Discussion section should, in the opening paragraph, state what the main findings of the study were. Note if the authors make any claims which are not supported by the data or published studies. If the authors claim significance if their results reached statistical significance at the p0.05 level, note whether this is of clinical or methodological significance. The authors should then discuss their results in relation to other similar published findings. If their results do not agree with other studies the authors must come up with plausible reasons for their different findings. Next see if the authors have addressed limitations to their study. No study is perfect. It may be that their results may only be applicable to certain populations or age groups for example. Are there any implications for diagnosis or laboratory procedures? Clear conclusions must be stated by the authors for their findings.

Finally look at the Abstract. The Journal's requirements are that the Abstract should be structured under the headings of Background, Aims, Methods, Results, and Conclusions. The Abstract should be brief and succinct and only present key aspects. The Abstract should be no more than 300 words or thereabouts and include up to six key words. Also check that results noted in the Abstract are consistent with what is in the text and are indeed presented in the Results section. Previous studies have demonstrated significant differences in those aspects (2,3).

References used in either the Introduction and Discussion sections should be appropriate and accurately quoted. The reviewer should look at least the Abstract of the quoted reference ensuring that what the authors have stated is accurate (4). Sometimes the reviewer may have difficulty in retrieving a quoted study because the references used by the authors have major inaccuracies rendering retrieval difficult (5). It is not an uncommon finding despite reference management software being available. We find that articles can often be found by just putting the article's title in a Google search. Do not worry about correcting references, this is done in the Editorial Office prior to publication.

A good review takes time, effort, and knowledge of the topic of the submitted article. The main aim is for an expert opinion for the Editors to use in their decision to accept, ask for a resubmission, or to reject. Indicate to the Editor, but not to the authors, if the article should be accepted as is, acceptable with minor or major modifications, or outrightly rejected. These recommendations should be supported with evidence-based reasons.

We hope these guidelines are useful in providing good review which are helpful for the Editors of the Journal and of course for the authors in assuring that thoughtful comments and suggestions made by reviewers help authors in ensuring that their articles, if accepted, are put in front of the reader in the best possible light. Finally:

- Do be courteous to the authors.
- Notify the Editor if you have any possible conflicts of interest, such as having published previously with the authors or the authors being from your direct workplace.
- Notify the Editor if you are unable to provide a review within a reasonable time period (< 4 weeks).
- Do not communicate directly with the authors.
- Do not share the manuscript with anyone without the Editor's permission.

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REFERENCES

1. Siebers R. How to write up a laboratory-based case study for the Journal. *N Z J Med Lab Sci* 2010; 64: 22-23.
2. Siebers R. Data in abstracts of research articles. Are they consistent with those reported in the article? *Br J Biomed Sci* 2002; 59: 67-68.
3. Pitkin RM, Branagan MA, Burmeister LF. Accuracy of data in abstracts of published research articles. *JAMA* 1999; 281: 1110–1111.
4. De Lacey G, Record C, Wade J. How accurate are quotations and references in medical journals? *BMJ (Clin Res Ed)* 1985; 291: 884–886.
5. Siebers R, Holt S. Accuracy of references in five leading medical journals. *Lancet* 2000; 356: 1445.

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NZIMLS Annual Conference
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REVIEW ARTICLE

Systematic review of laboratory parameters predicting severity and fatality of COVID-19 hospitalised patients

Richard Chinaza Ikeagwulonu, Mark Uchejeso Obeta and Immaculata Ngozi Ugwu

ABSTRACT

Background: We reviewed studies that reported changes in laboratory parameters in COVID-19 hospitalised patients to identify those parameters that predict disease severity with a high degree of sensitivity to aid early clinical decision which is essential for timely triaging of patients.

Methods: An electronic search of four databases comprising PubMed/Medline, Google Scholar, AJOL and JSTOR was performed to identify studies reporting changes in laboratory parameters in COVID-19 patients using key words "laboratory" OR "haematological" OR "cardiac" and "coronavirus 2019" OR ''COVID-19" OR "2019-nCoV" OR "SARS-CoV-2".

Results: A total of twenty-eight articles were reviewed out of 593 identified after duplicates removal. These 28 articles comprise 2,688 laboratory confirmed cases of COVID-19 patients comprising 1,418 male and 1,270 female patients with 32 different laboratory parameters identified. Comparing the mild to severe/critical cases our result showed a significant increase in lactate dehydrogenase (100%), D-dimer (100%), neutrophil to lymphocyte ratio (100%) as well as lymphocytopenia (96.0%), thrombocytopenia (83.3%), leukocytosis (71.4%) and neutrophilia (86.4%). Severe cases of COVID-19 had increased serum levels of myoglobin, cardiac troponin I, creatine kinase, creatine kinase-MB, N-terminal brain natriuretic peptide, urea, creatinine, cystatin C, platelet to lymphocyte ratio, international normalised ratio (NLR), platelet count, fibrin degradation products; and decreased levels of Hb, fibrinogen, uric acid, blood gases (PaO_2 and $PaCO_2$), and oxygenation index.

Conclusions: The observations in this study provide evidence of multiple organ involvement in COVID-19 disease. D-dimer, lactate dehydrogenase, lymphocytes, neutrophils, platelets, fibrinogen, NLR, oxygen index, and blood gases should be considered important in risk stratification to predict severe and fatal COVID-19 outcome in hospitalised patients.

Keywords: haematological markers, cardiac biomarkers, severity, COVID-19, laboratory parameters.

N Z J Med Lab Sci 2020; 74: 165-180.

INTRODUCTION

Wuhan, a city within the Hubei province of China, was thrown into a serious health trap following the discovery of a strange disease of unknown etiology that manifested with severe pneumonia since December 8, 2019. The disease was named severe acute respiratory syndrome coronavirus-2 (SARS-COV-2) following sequencing by the Chinese Center for Disease Control and Prevention in early January 2020 (1,2), but was subsequently renamed coronavirus disease 2019 (COVID-19) by the World Health Organization (WHO) and by 11 March 2020 was declared a global pandemic (3).

SARS-COV-2 is zoonotic in origin, shares similarity with MERS-CoV and SARS-CoV in gene sequences and is the seventh member of the family *Coronaviridae* that infects man (4). Though SARS-COV-2 is known to cause respiratory distress and human to human transmission has been established, its pathogenesis is still not yet fully understood. There has been evidence of multi-organ involvement (5,6) which has been linked to the high morbidity and mortality rate associated with the disease, especially in its severe form, and where there is already an underlying health challenge like hypertension, diabetes, renal disease, and cardiovascular diseases at time of infection. Medical laboratory investigations have identified blood parameters that may predict severity and monitor prognosis of SARS-COV-2 infected persons (6-34). Early clinical knowledge of infected individuals at the risk of developing complications could help reduce mortality and improve prognosis and outcome. One of the best ways of doing this is to identify those blood parameters that have been shown through research to have good predictive value and timely monitor their levels in infected/hospitalised persons. Many studies have identified these blood markers (6-33). Thus the present study was aimed to systematically review these studies and present the findings in such a way as to guide clinical decisions and risk stratification.

METHODS

An electronic search of four databases comprising PubMed/ Medline, Google Scholar, AJOL and JSTOR was performed using the key words "laboratory" OR "haematological" OR "cardiac" and "coronavirus 2019" OR ''COVID-19" OR "2019-nCoV" OR "SARS-CoV-2". Initial search was done from May 18 to 31st May 2020 and another one on 29th June 2020. There was no date of publication restrictions, and only studies reported in English were included. Snowballing was used to identify additional studies. Studies were included if they were primary research articles, assessed the association between laboratory parameters and COVID-19 infection, reported in mean (SD) or median (IQR), and with the full text of the article available online. Expert opinions, case reports, books, newsletters, commentaries, theses, and editorials were excluded. Patients were grouped as mild or severe cases. All patients in intensive care units (ICU) and non-survival or diseased cases were classified as severe cases, whereas patients who were not in ICU and those who were survivors were grouped as mild cases. All publications were retrieved online while data extraction was carried out for each article highlighting the following: name of first author and year of publication, country, study center, laboratory parameters, previous exposure, sample size, age, gender, presence of co-morbidities, and disease severity criteria. The search strategy and results are shown in Figure 1.

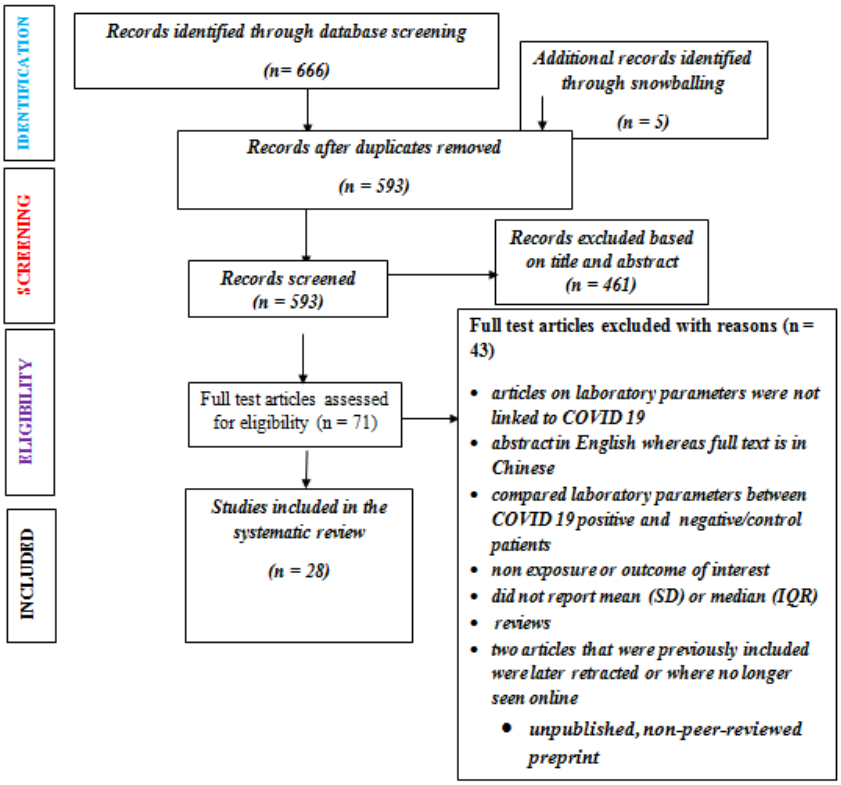

Figure 1. PRISMA flow diagram for the selection of studies based on study inclusion and exclusion criteria.

RESULTS

A total of 671 articles were retrieved following search of the databases and was later reduced to 593 after duplicates were sorted out and removed (Figure 1). These 593 articles were then subjected to the study inclusion and exclusion criteria through a thorough scrutiny of the title, abstract, and full texts to yield a total of 28 articles that were then systematically reviewed (6-33). These 28 studies comprised 2,688 laboratory confirmed cases of COVID-19 comprising 1,418 male and 1,270 female patients. All studies were observational (retrospective) in nature and were predominantly from China, except for four, from Singapore (25,28), Spain (30), Turkey (31), and Greece (32). Some of the infected patients were reported to be exposed to the Huanan seafood market. The general characteristics of the included studies are presented in Table 1.

Medical laboratory parameters

Thirty-two medical laboratory parameters were identified and were classified into six different subgroups: haematological parameters, coagulation profiles, cardiac markers, blood gas parameters, biochemical profiles, and laboratory parameters ratios (Table 2).

White blood cell count (WBC)

Twenty-one studies reported changes in the WBC. Fifteen of these studies showed increased levels of WBC (6-8,10,11,14-16,18,24-29) among the severe cases, eight were significant (10,11,14,16,24,26,27,29) while seven were non-significant (6,7,8,15,18,25,28). Decreased WBC was seen in six studies (9,13,17,19,22,23) of which five was non-significant (13,17,19,22,23).

Neutrophils

Twenty-two studies reported changes in neutrophil levels, 19 of which reported raised neutrophil levels (7-16,18,19,23,24,26-31). Fourteen studies reported that the raised neutrophil levels were significant (7,9,10,11,14,15,16,24,26-31) as against five studies which were reported as non-significant (8,13,18,19,23). Significant decreases in neutrophil levels were seen in three studies (17,22,25).

Lymphocytes

Lymphocyte levels were reported in twenty-five studies. Twenty-four studies reported decreased lymphocyte counts (6-19, 22-31). Decreased lymphocyte counts were reported as significant in 18 studies (9-18,19,24-29,31) while in six studies it was reported as non- significant (6,7,8,22,23,30).

Platelets

Changes in platelets levels were reported in eighteen studies of which 15 studies reported that platelets decreased in severe cases (7-11,14,16-19,22,24,27,28,32). In six studies the decrease in platelets was significant (7,10,16,18,22,28) while non-significant in nine studies (8,9,11,14,17,19,24,27,32). Three studies reported an increase in platelets which were significant (25,30,31).

Haemoglobin (Hb)

Eleven studies investigated changes in Hb levels and were reported to be decreased in nine studies (9-11,16,17,19,24,27,29). However, in six studies this decrease was non-significant (11,16,17,19,14,29) as against three studies that reported a significant decrease (9,10,27). Increased Hb levels were seen in two studies but were non-significant (14,28).

Red blood cell count (RBC)

RBC changes were reported in two studies, both reported to have significantly decreased in severe cases (10,27).

D-dimer

Serum D-dimer levels were reported in 19 studies and were increased in all studies. A significant increase was seen in 11 studies (9,10,13,14,16,20,21,23,26,29,32) while non-significant increases occurred in eight studies (7,8,15,18,22,27,30,31).

Prothrombin time

Seven studies reported prothrombin time changes and six of these showed levels to be raised in severe cases (9,14,16,21,26,27). The increase in prothrombin time was non-significant in five of these studies (9,14,16,26,27).

Activated partial thromboplastin time (APTT)

In six studies APTT was reported to be associated with COVID-19 cases. Interestingly, both increased and decreased APTT were observed at equal frequency. A significant increase in levels were seen in three studies (16,21,22) while decreased levels were found in three other studies (14,16,23), though this decrease was not significant in two of the three studies (16,23).

Fibrinogen
Nine studies investigated changes in fibrinogen levels. Eight of these studies reported that levels of fibrinogen decreased in severe cases, five of which showed this decrease to be significant (9,18,23,29,32) while in three studies it was non-significant (21,27,31).

Thrombin time
Thrombin time was reported in one study where levels were seen to be decreased non-significantly in severe cases (23).

Fibrin degradation products
Fibrin degradation products, as reported in one study, were significantly increased in severe cases of COVID-19 (21).

Lactate dehydrogenase (LDH)
Fifteen studies investigated changes in LDH levels and in all studies LDH serum levels were increased in severe cases. The increase was significant in thirteen of these studies (6,7,9,11,13-17,19,25,27,32).

Myoglobin
Myoglobin levels were reported in four studies and in all levels were significantly increased (13,26,27,33).

Cardiac troponin 1
Five studies investigated changes in troponin I levels. In three of these studies troponin I levels were increased (26,27,31) and the increase was seen to be significant in two of these studies (26,31) while unchanged in two other studies (18,33).

Creatine kinase (CK)
Seven studies reported changes in CK enzyme levels. Six of these studies noted that CK levels increased in severe cases (7,9,13,14,16,17,27) while a significant increase was seen in five of these studies (9,13,14,17,27).

Creatine kinase MB (CK-MB)
The isoenzyme of CK, CK-MB, was reported in two studies where the levels weree non-significantly raised (27,33).

N-terminal brain natriuretic peptide (NT-BNP)
Three studies investigated NT-BNP changes and reported an increase in NT-BNP levels in severe cases (18,26,33). Significant increases were noted in two of these studies (27,33)

Urea
Changes in urea levels were reported in nine studies, and all these studies showed increased levels in severe cases. The increase was seen to be significant in six studies (9,13,14,16,27,31) and non-significant in three studies (17,22,23).

Creatinine
Fourteen studies investigated creatinine levels. In ten studies creatinine levels were increased in severe cases. However, this increase was seen to be non-significant in six studies (7,11,14,16,17,19) while significantly increased in three studies (9,13,27). Creatinine was significantly decreased in three studies (8,15,23).

Uric acid
Two studies reported a non-significant decrease in uric acid levels in severe cases (23,27).

Cystatin C
Cystatin C changes were reported in two studies and was increased in both studies (23,27).

Arterial oxygen tension (P_aO_2)
Five studies investigated changes in P_aO_2 and in four reported a *decrease* in P_aO_2 in severe cases (8,18,26,30). This decrease was significant in two studies (12,21) and non-significant in the other two studies (2,25).

Arterial carbon dioxide tension (P_aCO_2)
Two studies reported changes in P_aCO_2 and in both levels of P_aCO_2 decreased in severe cases (18,26).

pH
One study reported pH levels in COVID-19 infected individuals and was increased in severe cases (18).

Lactate
Lactate was reported in one study and was increased in severe COVID-19 cases (18).

Neutrophil to lymphocyte ratio (NLR)
A total of nine studies reported NLR and in all the studies NLR was seen to be increased in severe cases. The increase was significant in seven studies (10,15,18,24,27,29,32) and non-significant in two studies (19,30).

Platelet to lymphocyte ratio (PLR)
PLR was reported in two studies and was increased (18,27).

Lymphocyte to monocyte ratio (LMR)
One study reported LMR and the ratio was seen to be decreased non-significantly in severe cases (24).

International normalised ratio (INR)
INR was investigated in two studies and was raised in severe cases of COVID-19 (17,27).

Oxygenation index
$P_aO_2{:}F_1O_2$ was reported in three studies and the ratio was decreased significantly in all three studies (16,17,26).

Table 1. General characteristics of reviewed studies.

Reference	Country	COVID-19 +ve	Age (range)	Gender	Previous exposure to Hunan market	History of co- morbidities/ pre-existing disease
6	China	148	50 (36–64)	M: 75 (50.7%) F: 73 (49.3%)	All cases had a history of exposure and most had clinical manifestations including fever or respiratory symptoms.	*NS
7	China	155	54 (42-66)	M: 86 (55.5%) F: 69 (44.5%)	*NS	Hypertension (23.9%), Diabetes (9.7%) and cardiovascular diseases (9.7%).
8	China	78	38 (33-57)	M: 39 (50%) F:39 (50%)	No previous exposure.	Twenty patients (25.6%) with comorbidities, of which hypertension was the most common.
9	China	63	47 (3-85)	M: 37 (58/7%) F: 26 (41.3%)	*NS	29 patients had one or more coexisting diseases, including hypertension (12), diabetes (5), cerebral infarction (2), cardiac arrhythmia (2), prostate cancer (1), bronchial asthma (1), pulmonary tuberculosis (1),

						claustrophobia (1), thyroid disease (1), chronic hepatitis (2), chickenpox (1).
10	China	298	57 (40-69)	M:150 (50.3%) F:148 (46.7%)	*NS	86 cases of hypertension, 32 cases of cerebrovascular diseases, 45 cases of diabetes, 26 cases of coronary heart disease, 23 cases of chronic pulmonary disease, 16 cases of cirrhosis, and 12 cases of anaemia.
11	China	60	42 (35-62)	M:32 (46.4%) F: 37 (53.6%)	*NS	Less than half (25) had underlying comorbidities (36%).
12	China	32	*NS	M:13 (40.6%) F: 19 (59.4%)	*NS	*NS
13	China	298	47 (33-61)	M:145 (48.7%) F:153 (51.3%)	233 (81.5%) had been to Hubei, while 42 (14%) did not have a clear travel history.	A small percentage of patients had pre-existing conditions including diabetes (6.4%), hypertension (12.8%), cardiovascular disease (3.7%), liver disease (2.7%), and malignancies (1.3%).
14	China	28	68.6 (53-82)	M:21 (75%) F:7 (25%)	11 (39.3%) patients had a history of exposure to confirmed COVID-19 patients.	17 (60.7%) patients had coexisting chronic diseases, including hypertension, heart disease, cerebrovascular disease, and chronic respiratory disease.
15	China	299	53.4 ± 6.7	M:160 (53.5%) F:139 (46.5%)	113 patients (37.8%) had been to Wuhan before the illness.	Approximately one - third of the patients had comorbidities. Comorbidities of interest were hypertension, diabetes, coronary heart disease , cerebrovascular disease, chronic obstructive pulmonary disease, and cancer.
16	China	21	56.0 (50-65)	M:17 (81.0%) F:4 (19.0)	Four, including a familial cluster of three confirmed cases, had direct exposure to the Huanan seafood market .	More severe cases had comorbidities.
17	China	645	46.65 ± 13.82	M:328 (50.9%) F:317 (49.1%)	Among patients with abnormal imaging findings, the proportion who had a clear exposure to Wuhan and/ or confirmed patients was 81.0%	Patients with at least one coexisting underlying conditions and patients with hypertension were observed in 28.8% and 16.8% of the 573 patients, respectively, which was significantly higher than non-pneumonia patients ($P < 0.05$).
18	China	127	50.9	M:45 (35.4%) F:82 (64.6%)	*NS	Hypertension (31 [24.42%]) was the most common underlying disease.
19	China	76	44.5	M:42 (55.3%) F:34 (44.7%)	*NS	Diabetes absent in patients. Prevalence of hypertension: 14.58% (7 cases) and 39.29% (11 cases) in groups 1 and 2, respectively.
20	China	19	73 (38-91)	M:11 (57.9%) F:8 (42.1%)	All patients were citizens of Wuhan, China and had no history of contact with COVID-19.	Fifteen (78.9%) patients had underlying diseases, mainly high blood pressure or diabetes. Seven of patients who died had underlying diseases.
21	China	183	54.1 (14.94)	M:98 (53.6%) F:85 (46.4%)	April, 2020	Seventy - five (41.0%) patients with chronic diseases, including cardiovascular, cerebrovascular, respiratory, malignant tumors, chronic liver and kidney disease.

22	China	34	66 (58-76)	M:23 (67.6%) F:11 (32.4%)	None had direct exposure to the Huanan seafood market.	24 (70.6%) patients had chronic diseases, including hypertension (64.7%), diabetes (23.5%), cardiovascular (11.8%), chronic obstructive pulmonary disease (5.9%), chronic liver disease (11.8%), and chronic kidney disease (5.9%).
23	China	43	43.74 ± 12.12	M:26 (60.5%) F:17 (39.5%)	*NS	*NS
24	China	72	49 (37-64)	M:33 (45.8%) F:39 (54.2%)	37 cases (51.4%) had recently been to Wuhan City and 23 (31.9%) had contact with COVID-19 patients.	Most common comorbidities included hypertension (9) , cardiovascular disease (6), and diabetes (5).
25	Singapore	69	42 (35-54)	M: 37 (55.2) F: 30 (44.8)	*NS	*NS
26	China	179	57.6 ±13.7 (18–87)	M:97 (54.2%) F:82 (45.8%)	*NS	*NS
27	China	45	39 (16-62)	M:23 (51.1%) F:22 (48.9%)	26 patients had been to Wuhan within 2 weeks before hospitalisation, Three had visited the South China seafood market.	Four patients with hypertension-based diseases, three of whom were severe.
28	Singapore	76	46 (19-71)	M:44 (57.9%) F:32 (42 1%)	*NS	Severe cases were older and had a higher incidence of comorbidities.
29	China	75	46.6 ± 14 (22–77)	M:45 (60%) F:30 (40%)	41 patients (54.7%) had a history of Hubei travel or residency.	Thirteen patients had underlying chronic diseases, including hypertension (7), chronic respiratory disease (4), diabetes (4), and malignant tumors (1).
30	Spain	60 pregnant women	34 (22-43)	F:60 (100%)	*NS	*NS
31	Turkey	681	56.9 ± 15.7	M:347 (51%) F:334 (49%)	*NS	*NS
32	Greece	64	57.11 ± 16.3	M:31 (47.7%) F:33 (52.3%)	*NS	*NS
33	China	273	*NS	M:97 (35.5%) F:176 (64.5%)	*NS	*NS

*NS: not specified; M: male; F: female. Data are presented as numbers, median (interquartile range), or mean ±sd.

Table 2. Major laboratory findings in mild and severe/critical cases.

Reference	Disease severity criteria	Disease/case severity classification	Laboratory parameters	Levels in mild cases	Levels in severe/ critical cases	P value	COVID-19 detection
6	Clinical criteria of diagnosis and discharge as per standards for "Diagnosis and Treatment Scheme of New Coronavirus Infected Pneumonia" (tria l version 6).	Severe cases: 55 (37.2%) had abnormal liver function. Mild cases: 93 (4.3%) of patients with normal liver function.	WBC Lymphocytes LDH	4.35 (3.55-5.6) 1.2 (0.785-1.5) 207 (186–243)	5.04 (3.89–5.72) 1.05 (0.69–1.57) 257 (227–369)	0.1033 0.3208 < 0001	Real-time RT-PCR

7	Pneumonia diagnosis based on clinical characteristics and chest imaging.	Severe cases: 85 Mild cases: 70	WBC Neutrophil count Lymphocyte count Platelet count Creatinine LDH CK D-dimer	4.16 (3.33-5.18) 2.72 (1.88-3.53) 0.97 (0.79-1.28) 179 (146-219) 65 (58-78) 241 (198-338) 100 (60-146) 178 (100-289)	4.65 (3.14-6.84) 3.28 (1.99-5.08) 0.80 (0.56-1.04) 159 (119-202) 79 (65-96) 293 (193-434) 89 (60-140) 213 (126-447)	0.059 0.017 0.105 0.049 0.158 0.017 0.560 0.288	Real-time RT-PCR
9	Guidelines for diagnosis and management of COVID-19 (4th edition, in Chinese) by the National Health Commission of China	Mild cases:8 Moderate cases:36 Severe cases:10 Critical cases: 9	WBC Neutrophil count Lymphocyte count Platelet count Hb Creatinine Urea LDH	5.92 ±1.29 3.25 ±0.82 2.00 ±0.64 204.38 ±49.1 144.88 ±12.63 77.13 ±21.33 4.33 ±0.67 231.75 ±122.92	Moderate cases: 4.56 ±1.21 Severe cases: 5.01 ± 1.76 Critical cases: 6.84 ± 3.57 Moderate cases: 2.70 ±1.01 Severe cases: 3.76 ± 1.85 Critical cases: 5.54 ± 3.70 Moderate cases: 1.42 ± 0.56 Severe cases: 0.90 ± 0.47 Critical cases: 0.83 ± 0.50 Moderate cases: 180.31 ± 56.84 Severe cases: 183.40 ± 71.87 Critical cases: 193.56 ± 75.8 Moderate cases: 137.67 ± 14.07 Severe cases: 134.5 ±16.79 Critical cases: 119.33 ± 30.8 Moderate cases: 77.3 ± 12.92 Severe cases: 74.80 ± 10.99 Critical cases: 103.11 ± 48 Moderate cases: 3.99 ± 1.21 Severe cases: 4.49 ± 1.05 Critical cases: 9.41 ± 8.69 Moderate cases: 217.47 ± 51.12 Severe cases: 279.70 ± 84.36 Critical cases: 376.89 ± 161.55	0.007 0.001 0.001 0.759 0.017 0.021 0.017 0.001	Real-time RT-PCR

			CK	80.75 ± 28.9	Moderate cases: 101.55 ±124.69 Severe cases: 132.57 ±111.91 Critical cases: 137.0 ±166.5	0.000	
			Prothrombin time	12.01 ± 1.13	Moderate cases: 14.0 ±0.81 Severe cases: 12.57 ±2.19 Critical cases 21.30 ±8.0	0.826	
			Fibrinogen	2.04 ± 0.39	Moderate cases: 3.14 ±1.00 Severe cases: 3.69 ±2.04 Critical cases: 3.62 ±1.52	0.045	
			D-dimer	0.16 ± 0.06	Moderate cases: 0.35 ±0.25 Severe cases: 3.15 ±3.31	0.000	
10	Patients diagnosed according to WHO interim guidance for COVID-19	Severe cases (those that died): 84 Mild cases (those that recovered): 214	WBC	5.19 (3.98-6.48)	8.58 (5.26-12.70)	0.001	Real-time PCR.
			Neutrophil count	3.20 (2.53-4.56)	6.92 (4.33-10.79)	0.001	
			Lymphocyte count	1.04 (0.83-1.50)	0.83 (0.63-1.09)	0.000	
			Platelet count	205 (151-252)	154 (111-213)	0.000	
			Hb	128.0 ± 17.2	125.5 ± 20.0	0.280	
			RBC	4.20 ± 0.61	3.99 ± 0.71	0.013	
			Neutrophil-lymphocyte ratio	2.96 (2.13-4.61)	8.17 (6.15-10.90)	0.000	
			D-dimer	0.50 (0.29-1.10)	4.59 (0.95-17.14)	0.000	
11	All patients were diagnosed and admitted in accordance with guidelines of the National Health Commission of China	Mild cases (SpO_2 ≥ 90%): 55 Severe cases (SpO_2 ≥ 90%): 14	WBC	3.57 (2.96-4.93)	6.52 (4.3-7.73)	0.006	Real-time PCR
			Neutrophil count	2.16 (1.60-2.70)	5.24 (2.9-6.44)	<0.001	
			Lymphocyte count	1.19 (0.95-1.46)	0.61 (0.37-1.00)	0.002	
			Platelet Count	172.0 (138.0-206.0)	167.0 (144.0-215.0)	0.829	
			Hb	131.0 (121.0-141.0)	128.0 (117.0-136.0)	0.507	
			Creatine	65.3 (58.0-78.5)	71.5 (52.5-80.4)	0.623	
			LDH	207.0 (181.1-274.0)	517.5 (267.0-549.0)	0.001	
12	Diagnosis in line with WHO diagnostic criteria and inclusion criteria for confirmed cases	Critical cases: 11 Severe cases:10 Mild cases:11	Lymphocyte count	1.04 ± 0.32	Severe cases: 0.76 ± 0.49 Critical cases: 0.85 ± 0.40	0.044	Real-time PCR

13	Diagnosis based on WHO interim guidance and diagnostic criteria based on recommendations by National Centers for Disease Control and Prevention of China	Mild cases: 240 (80.5%) Severe cases: 58 (19.5%)	WBC Neutrophil count Lymphocyte count D-dimer Creatinine Urea LDH CK Myoglobin	4.525 (3.57-5.585) 6.65 (5.3-8.7) 1.345 (1.025-1.855) 0.36 (0.24-0.51) 61 (50-74) 3.84 (3.12-4.75) 216 (170-349) 64.5 (46-93) 33.52 (26.13-42.7)	4.5 (3.7-6.2) 7.35 (5.4-9.6) 0.985 (0.7-1.24) 0.56 (0.39 - 0.96) 72 (55-98) 5.195 (3.84-6.39) 387 (224-593) 87 (51.1-170) 67.9 (43.21-110.16)	0.442 0.158 <.0.001 <.0.001 <.0.001 <.0.001 <.0.001 0.006 <0.001	Real-time PCR.
14	Diagnosis according to New Coronavirus Pneumonia Prevention and Control Program (5th edition), National Health Commission of China	Mild cases (patients in isolation):14 Severe cases (patients in ICU):14	WBC Neutrophil count Lymphocyte count Platelet count Hb Prothrombin time APTT Creatinine Urea	5.9 ± 2.0 4.6 ± 2.1 0.82 (0.63-1.09) 186.9 ± 79.3 130.0 (127.5- 140.8) 14.1 (13.7- 14.6) 40.7 ± 7.5 74.0 (56.0- 89.3) 5.0 (3.0- 5.5)	13.1 ± 6.6 12.0 ± 6.6 0.47 (0.31- 0.77) 174.8 ± 90.97 135.5 (119.8- 148.3) 14.5 (13.5- 17.5) 39.0 ± 4.6 85.5 (62.3- 96.8) 9.2 (7.2- 11.3)	0.0007 0.001 0.0345 0.7112 0.5494 0.3818 0.4698 0.2905 0.0007	Real-time PCR.
15	Diagnostic criteria for COVID-19 by WHO	Mild cases (survivors): 283 Severe cases (non survivors): 16	WBC Neutrophil count Lymphocyte count Neutrophil-lymphocyte ratio Creatinine LDH D-dimmer Fibrinogen	4.8 ± 2.1 3.2 ± 2 112 (39.6) 3.3 ± 4.3 74.9 ± 48 229.1 ± 122.5 1.3 ± 3.9 3.2 ± 1	7.0 ± 5.2 5.6 ± 3.4 15 (93.8) 13.3 ± 14.3 70.2 ± 14.8 531.1 ± 487.3 6 ± 16.1 3.2 ± 1.3	0.111 0.012 <0.001 0.013 0.326 0.026 0.266 0.98	Real-time PCR
16	Guidelines for diagnosis and management of COVID-19 (6th edition, in Chinese) by the National Health Commission of China		WBC Neutrophil count Lymphocyte count Platelet count	4.5 (3.9–5.5) 2.7 (2.1–3.7 1.1 (1.0–1.2) 175.6 (148.3–194.0)	8.3 (6.2–10.4) 6.9 (4.9–9.1) 0.7 (0.5–0.9) 157.0 (134.0–184.5)	0.003 0.002 0.049 0.88	Real-time RT-PCR

			Hb	139.5 (132.8–146.0)	136.0 (125.5–144.5)	0.78	
			Prothrombin time	13.4 (12.8–13.7)	14.3 (13.6–14.6)	0.15	
			APTT	44.0 (42.6–47.6)	33.7 (32.1–38.4)	0.002	
			D-dimer	0.3 (0.3–0.4)	2.6 (0.6–18.7)	0.029	
			Creatinine	76.5 (63.3–81.0)	82.0 (67.5–91.5)	0.21	
			Urea	4.0 (3.4–4.8)	6.1 (5.2–9.1)	0.015	
			LDH	224.0 (200.3–251.8	537.0 (433.5–707.5)	0.001	
			CK	64.0 (57.5–83.5)	214.0 (90.0–329.0)	0.16	
			PaO_2	0 (0)	11 (100.0)	0.000	
			PaO_2 / F_1O_2	371.7 (350.0-422.7)	104.8 (94.6-119.0)	0.001	
17	Diagnosis of novel COVID-19 based on WHO interim guidance (WHO, 2020) Subtype definition of COVID-19 according to diagnosis and treatment scheme for SARS-CoV-2 of China (5th edn.) National Administration of Traditional Chinese Medicine, 2020	Severe cases: 573 Mild cases: 72	WBC	5.42 ± 2.00	5.01 ± 1.87	0.079	Real-time RT-PCR
			Neutrophil count	3.48 ± 1.88	3.29 ± 1.68	0.397	
			Lymphocyte count	1.39 ± 0.61	1.23 ± 0.52	0.021	
			Platelet count	198.44 ± 58.44	185.22 ± 62.42	0.088	
			Hb	141.04 ± 5.62	138.09 ± 16.47	0.15	
			Creatinine	65.54 ± 13.16	69.17 ± 24.52	0.053	
			Urea	3.90 ± 1.13	4.04 ± 1.69	0.505	
			LDH	174.5 (148 ± 235.5)	213.0 (173.0 ± 268.0)	<0.001	
			CK	62.5 (47 ± 83.75)	73.0 (48.0 ± 111.0)	0.01	
			International normalided ratio	1030.09	1040.10	0.384	
			Oxygenation index (PaO_2/FiO_2)	478.79 (468.9-478.79)	381.46 (354.73-419.05)	<0.001	
18	Patients diagnosed according to guidelines for diagnosis and treatment for COVID-19 (Trial Version 6)	Mild cases: 111 (87.40) Severe cases: 16 (12.60%)	WBC	5.00 (4.30-6.50)	5.35 (4.13-7.53)	0.568	Real-time RT-PCR
			Neutrophil count	3.29 (2.54-4.40)	3.89 (2.25-6.57)	0.309	
			Lymphocyte count	24.30 (18.70-31.40)	17.45 (8.23-22.25)	0.012	
			Platelet count	205.0 (165.0-246.0)	155.0 (125.75-206.0)	0.01	
			Neutrophil-lymphocyte ratio	2.75 (1.90-3.95)	4.24 (3.00-10.87)	0.015	
			Platelet-lymphocyte ratio	160.0 (129.64-215.0)	210.46 (116.33-300.88)	0.299	

			Fibrinogen	423.0 (363.0-550.0)	574.3 (405.3-668.0)	0.018	
			pH	7.45 (7.40-7.48)	7.47 (7.42-7.49)	0.234	
			PO_2	102.0 (77.0-123.0)	63.0 (57.75-74.5)	<0.001	
			PCO_2	40.0 (35.0-43.0)	35.90 (33.33-37.45)	0.015	
			Lactate	1.50 (1.0-2.70)	2.30 (1.23-2.70)	0.215	
			D-dimer	100.0 (82.0-158.0)	160.95 (73.75-282.75)	0.195	
			Cardiac troponin 1	<0.03 (<0.03-0.04)	<0.03 (<0.03-<0.03)	0.082	
			NT-BNP	118.0 (78.38-218.75)	196.45 (75.82-405.10)	0.25	
19	Diagnostic criteria for COVID-19 severity based on CDCP (China) Diagnosis and Treatment of COVID-19	48 mild cases (C-reactive protein: < 20.44 g/L) 28 severe cases (C-reactive protein: ≥ 20.44 g/L)	WBC	4.57 ± 1.68	4.25 ± 1.10	0.377	Real-time RT-PCR
			Neutrophil count	2.90 ± 1.40	2.98 ± 0.82	0.806	
			Neutrophil-lumphocyte ratio	1.24 ± 0.55	0.94 ± 0.38	0.011	
			Hb	2.95 ± 3.05	3.77 ± 1.89	0.202	
			Platelet count	177.81 ± 68.38	171.64 ± 55.16	0.686	
			Creatinine	69.50 ± 15.02	73.32 ± 18.55	0.330	
			LDH	203.21 ± 65.28	266.14 ± 83.33	<0.001	
20	*NS	Mild cases (survivors): 11 Severe cases (Non-survisors): 8	D-dimer	0.48 (0.42-0.97)	2.15 (1.4-9.2)	<0.05	*NS
21	Diagnosis according to WHO interim guidance and according to the International Society on Thrombosis and Haemostasis (ISTH) diagnostic criteria for disseminated intravascular coagulation	Mild cases (survivors): 162 Severe cases (non survivors): 21	Prothrombin time	13.6 (13.0-14.3)	15.5 (14.4-16.3)	<.001	*NS
			APTT	41.2 (36.9-44.0)	44.8 (40.2 - 51.0)	.096	
			Fibrinogen	4.51 (3.65-5.09)	5.16 (3.74-5.69)	.149	
			D-dimer	0.61 (0.35-1.29)	2.12 (0.77-5.27)	<.001	
			FDP	4.0 (4.0-4.3)	7.6 (4.0-23.4)	<.001	
			AT	91 (84-97)	84 (78-90)	0.096	
22	Diagnosis according to guidance of National Health Commission, the People's Republic of China	Mild cases (Noninvasive ventilation): 19 Severe cases (requiring invasive mechanical ventilation): 15	WBC	9.3 (6.6-16.0)	6.2 (4.4-13.5)	0.160	Real-time RT-PCR
			Neutrophil count	8.0 (5.5-14.8)	5.7 (3.6-12.8)	0.223	
			Lymphocyte count	0.6 (0.5-0.9)	0.5 (0.4-0.8)	0.539	
			Platelet count	191 (172-201)	156 (111-176)	0.012	
			D-dimer	467 (370-1073)	675 (433-1613)	0.405	

			Urea	6.6 (5.0-8.1)	7.5 (6.0-14.2)	0.111	
			Creatinine	84 (67-99)	84 (67-106)	0.445	
23	Diagnoses according to WHO interim guidance for COVID-19	Mild cases: 28 Severe cases: 15	WBC	4.96 ± 1.85	4.26 ± 1.64	0.220	Flourescent reverse transcription-PCR
			Lymphocyte count	1.07 ± 0.40	1.20 ± 0.42	0.309	
			Neutrophil count	3.43 ± 1.63	2.65 ± 1.49	0.127	
			Thrombin time	14.5 ± 1.71	15.87 ± 2.11	0.025	
			Fibrinogen	3.11 ± 0.83	3.84 ± 1.00	0.014	
			APTT	30.41 ± 0.83	27.29 ± 6.09	0.089	
			Prothrombin time	12.03 ± 1.21	11.26 ± 1.42	0.068	
			D- dimmer	0.21 (0.19-0.27)	0.49 (0.29-0.91)	0007	
			Urea	4.09 ± 1.29	4.51 ± 1.76	0.367	
			Creatinine	66.96 ± 13.38	65.33 ± 15.55	0.696	
			Uric acid	256.54 ± 85.86	201.60 ± 90.59	.056	
			Cystatin C	0.820 ± 0.130	0.862 ± 0.21	.452	
24	Diagnosis based on program (6th trial edition) issued by the National Health Commission of the People's Republic of China	Mild cases: 57 (79.2%) Severe cases: 15 (20.8%)	WBC	4.0 (3.4-4.4)	6.3 (3.7-7.6)	0.019	Sequencing or real-time RT-PCR
			Neutrophil count	2.2 (1.8-2.8)	4.2 (2.2-5.8)	0.004	
			Lymphocyte count	1.1 (0.8-1.4)	0.8 (0.6-1.0)	0.038	
			Neutrophil - lymphocyte ratio	1.9 (1.3-2.9)	3.6 (2.4-9.6)	0.002	
			Lymphocyte-to-monocyte ratio	4.3 (2.9-6.0)	3.2 (2.0-4.7)	0.102	
			Hb	140 (130-145)	139 (133-150)	0.51	
			Platelet Count	180 (149-227)	160 (134-216)	0.515	
25	NA	Mild cases (non-ICU patients): 58 Severe Cases (ICU patients): 9	WBC	4.7 (4.0-5.8)	5.1 (3.5-8.2)	0.87	Real time PCR
			Hb	14.2 (12.9-15.2)	13.2 (12.5-14.0)	0.07	
			Lymphocyte count	1.3 (0.9-1.7)	0.5 (0.48-0.8)	0.0002	
			Neutrophil count	2.6 (2.1-3.8)	4.2 (2.1-6.9)	0.17	
			Platelet count	201 (157-263)	217 (154-301)	0.81	
			LDH	401 (352-513)	1684 (1053-2051)	0.003	
26	Probable and definite diagnosis of COVID-19 pneumonia established according to case definition established by WHO interim guidance	Mild cases (survivors):158 Severe cases (deceased):21	WBC	5.1 (3.8-7.3)	8.9 (4.8–13.1)	0.003	*NS
			Neutrophil count	3.9 (2.6-6.1)	7.7 (3.0–11.5)	0.007	
			Lymphocyte count	0.8 (0.6-1.1)	0.7 (0.5–0.8)	0.046	
			D-dimmer	0.5 (0.2-1.2)	1.1 (0.4–10.5)	0.044	
			Prothombin time	13.7 (12.4-15.2)	13.9 (12.3-16.3)	0.758	
			APTT	35.3 (30.9-39.1)	37.8 (30.8-41.5)	0.383	

			Cardiac troponin I	0.00 (0.00-0.00)	0.1 (0.0-0.8)	<0.001	*NS
			Myoglobin	32.3 (15.5-60.3)	162.0 (48.5-342.8)	<0.001	
			NT-BNP	390 (58-1118.5)	970 (620.5–3531)	0.004	
			PaO_2	74.5 (59.0-92.0)	56.0 (49.0 –71.0)	0.001	
			$PaCO_2$	37.0 (34.0-41.0)	34.0 (29.0–41.0)	0.068	
			PaO_2:FiO_2	261.5 ± 108.2	185.5 ± 64.8	0.002	
27	Diagnosed and classified according to the Diagnosis and Treatment Guidelines (Trial Sixth Edition)	Mild (moderate) cases: 35 Severe cases: 10	WBC	8.59 ± 4.01	11.46 ± 5.32	0.001	Nucleic acid detection kit (fluorescent PCR method)
			Neutrophil count	6.69 ± 3.83	10.51 ± 5.10	0.000	
			Lymphocyte count	1.34 ± 0.78	0.50 ± 0.39	0.000	
			Neutrophil-lymphocyte ratio	7.93 ± 8.36	29.9 ± 18.7	0.000	
			Platelet-lymphocyte ratio	238.8 ± 196.0	655.6 ± 457.4	0.000	
			RBC	4.36 ± 0.46	3.89 ± 0.88	0.007	
			Hb	134.5 ± 12.1	122.3 ± 27.3	0.023	
			Platelet count	222.7 ± 73.01	210.6 ± 60.43	0.403	
			Creatinine	65.78 ± 15.12	240.55 ± 290.66	0.001	
			Urea	4.77 ± 1.94	11.43 ± 10.55	0.000	
			Uric acid	254.84 ± 81.14	245.44 ± 143.57	0.707	
			Cystatin C	0.81 ± 0.26	2.33 ± 2.5	0.002	
			CK	85.43 ± 60.34	181.21 ± 184.0	0.015	
			CK-MB	15.96 ± 12.76	19.47 ± 6.89	0.182	
			Myoglobin	29.44 ± 29.19	150.51 ± 208.21	0.049	
			Cardiac troponin I	0.001 ± 0.001	0.04 ± 0.04	0.061	
			LDH	203.11 ± 70.31	280.25 ± 37.42	0.001	
			Prothrombin time	12.22 ± 1.56	13.08 ± 3.38	0.225	
			APTT	31.14 ± 4.89	33.75 ± 13.58	0.299	
			Fibrinogen	4.94 ± 1.47	5.81 ± 1.21	0.102	
			D-dimer	108.9 ± 86.48	409.14 ± 376.11	0.080	
			International normalised ratio	1.16 ± 0.15	1.24 ± 0.32	0.222	
28	NA	Mild cases: 56 Severe/critical cases: 20	WBC	5.16 (2.6-18.55)	6.11 (3.48-16.39)	0.1533	Real-time PCR
			Hb	14.4 (0.98-16.8)	15.05 (8.7-15.5)	0.1758	
			Neutrophil count	3.15 (0.98-16.76)	4.56 (1.79-14.75)	0.0054	
			Platelet count	221 (140-400)	173 5 (64–299)	0.0003	
			Lymphocyte count	1.34 (0.54-3.88)	1 015 (0 43–2 36)	0.0089	
			LDH	398 (135.849)	403 (290–6374)	0.05	
			Creatinine	71 (41-134)	89 (70-502)	>0.05	

29	Diagnosis and classification of Covid-19 based on the trial version 1–5 guidelines on the novel coronavirus-infected pneumonia diagnosis and treatment (National Health Commission of China)	Mild/moderate cases: 59 Severe cases: 16	WBC Neutrophil count Lymphocyte count Neutrophil-lymphocyte ratio Hb Figrinogen D-dimer	4.87 ± 1.70 2.92 ± 1.21 1.42 ± 0.66 2.33 ± 1.22 138.6 ± 16.0 0.94 ± 0.12 190 (70-1180)	7.14 ± 3.61 5.63 ± 3.50 0.97 ± 0.33 6.29 ± 3.72 135.9 ± 16.5 1.57 ± 0.39 315 (70-1220)	0.026 0.008 0.009 0.001 0.548 0.010 0.001	Real-time PCR
30	Severity of Pneumonia classified following radiography-based score in which each of five lung lobes were assessed for degree of involvement and classified as normal	Moderate cases (moderate pneumonia): 15 Severe cases (severe pneumonia): 3	Neutrophil count Neutrophil-lymphocyte ratio Platelet count D-dimer LDH PaO_2	3.9 (2.4-6.9) 3.7 (1.3-10.2) 196 (164-288) 0.9 (0.5-2.2) 180 (154-437) 97(91-100)	Moderate cases: 5.6 (1.4-10.1) Severe cases: 6.2 (2.4-10.3) Moderate cases: 5.2 (1.7-14.4) Severe cases: 6.8 (2.5-13.9) Moderate cases: 233 (100-466) Severe cases: 240 (132-321) Moderate cases: 0.9 (0.4-1.8) Severe cases: 1.9 (0.4-7.8) Moderate cases: 210.3 (154-379) Severe cases: 225.5 (161-297) Moderate cases: 96 (85-99) Severe cases: 95 (87-99)	0.024 0.056 0.507 0.841 0.256 0.063	Real-time PCR
31	COVID-19 diagnosis and treatment protocol issued by Turkish Ministry of Health	Mild cases: (alive: 626) Severe cases (deceased: 55)	Lymphocyte count Platelet count Neutrophil-lymphocyte ratio Fibrinogen D-dimer LDH	1511 ± 938 249.3 ± 84.2 3.09 ± 2.38 402 ± 83 481 ± 338 211 ± 55	2677 ± 9438 242.3 ± 105 3.98 ± 2.9 556 ± 151 1498 ± 1613 323 ± 134	0.809 0.619 0.001 0.004 0.024 0.019	Real-time PCR
32	*NS	Mile cases (group 2) Severe cases (group 1)	Lymphocyte count Platelet count Neutrophil-lymphocyte ratio Fibrinogen D-dimer LDH	1511 ± 938 249.3 ± 84.2 3.09 ± 2.38 402 ± 83 481 ± 338 211 ± 55	2677 ± 9438 242.3 ± 105 3.98 ± 2.9 556 ± 151 1498 ± 1613 323 ± 134	0.809 0.619 0.001 0.004 0.024 0.019	Real-time PCR

33	Patients diagnosed as having SARS - CoV - 2 infection according to WHO interim guidance	Mild cases: 198 Severe cases: 60 Critical cases: 15	CK-MB	0.91 (0.61-1.41)	Severe cases: 1.10 (0.76 - 2.12) Critical cases: 0.97 (0.32 - 2.37)	>0.05	Real PCR
			Myoglobin	34.66 (26.46-54.54)	Severe cases: 57.73 (37.43 - 100.71) Critical cases: 75.34 (23.24 - 112.47)	>0.05	
			Troponon I	0.01 (0.01-0.01)	Severe cases: 0.01 (0.01 - 0.04) Critical cases: 0.01 (0.01 - 0.03)	>0.05	
			N-terminal pro-brain natriuretic peptide	113.65 (45.92-274.23)	Severe cases: 290.85(106.13-958.08) Critical cases:	>0.05	

Data presented as numbers, median (interquartile range) or mean ±sd. PaO_2 : arterial oxygen tension; $PaCO_2$: arterial carbon dioxide tension are in percentages; FiO_2 : inspiratory oxygen fraction, APTT: activated partial thromboplastin time; AT: antithrombin activity; FDP: fibrin degradation product; WBC: white blood cell count; Hb: haemoglobin.
*NS: not stated. *P* values for differences between mild and severe/critical groups, $P<0.05$ statistically significant.

DISCUSSION

COVID-19 is known to cause severe acute respiratory syndrome which in its severe form causes multiple organ injuries with high morbidity and mortality rate when compared to other forms of coronavirus diseases. The multi organ involvement in pathogenesis of COVID-19 could be due to its affinity for angiotensin-converting enzyme 2 (ACE-2) which is a functional receptor expressed in some organs, including lungs, heart, and kidney (5,35). As in most other infections, organ injury is associated with changes in blood parameters and the degree of change depends on the level of involvement of the target organ and the severity of the disease. In our study COVID-19 severity was associated with an increase in neutrophils (86.4%), WBC (71.4%), and decrease in lymphocytes (96.0%) and platelets (83.3%). Hb and RBC were decreased. This finding is similar to previous reviews (36,37). Velavan *et al.* explained that lymphopenia was associated with disease severity (36). Henry *et al*. carried out a review of laboratory abnormalities in infected children and reported decreased neutrophils as the most common haematologic aberration as well as decreased HB in mild cases (38). Leukocytosis and neutropenia are also observed in influenza (39). The ability of the SARS-COV-2 virus to directly attack T-lymphocytes has been suggested as one mechanism for the lymphopenia observed and it has also been hypothesised to be associated with an increased risk of in-hospital mortality (40). However, the increased WBC in this study could be due to elevated neutrophils. In-house mortality has also been linked to thrombocytopaenia (41) and though IL.6 was not included in this study, it has been suggested that markers for potential progression to critical illness include a combination of platelet count, lymphocyte count, and IL-6 (37).

Severe COVID-19 appear to be associated with elevated serum levels of cardiac markers as the findings from our study showed increased levels of all the cardiac markers studied. A 100% increase was seen in LDH, myoglobin, CK-MB, and NT-BNP. Increases in CK (85.7%) and CTI (60%) were also observed. Though the number of studies is small in some instances, the findings are supported by previous studies where increased cardiac markers were also reported (36-38). CK-MB enzyme is solely secreted by cardiac muscles and the finding of elevated levels is evidence that suggest either viral infiltration of cardiac tissue or cardiac ischemia (38). Troponin, which is more sensitive and specific, can also serve as a likely indicator of ongoing or imminent cardiac injury and should be closely monitored (38).

High-sensitivity cardiac troponin has been associated with higher mortality (42). Interestingly, severe acute respiratory syndrome and Middle East respiratory syndrome have also been associated with increased LDH (43,44) and was an independent risk factor for severe acute respiratory syndrome (43). Chronic cardiovascular disease has been known as one of the outcomes of viral pathogenicity and may partly be explained either by the imbalance between infections-induced increased metabolic demand and reduced cardiac reserve (35), or due to atherosclerotic plaque instability and rupture as they were directly linked to severe systemic inflammation (5), one of the common features of coronaviruses. SARS-COV-2 is known to have affinity for ACE-2 receptor present in vascular epithelium and myocardium and there is a significant increased risk of death in patients with cardiovascular disease infected with COVID-19 (45).

Abnormalities in coagulation profiles were associated with COVID-19 severity as a 100% increase was seen in D-dimer levels (Table 2). Other findings include 85.7% and 88.7% increase and decrease in prothrombin time and fibrinogen, respectively. These results showed that hypercoagulability is associated with disease severity which could be related to a sustained inflammatory response. These findings have also been reported by others (36-38). Prothrombin and fibrinogen are synthesised in the liver, therefore a change in blood levels is an indication of liver dysfunction. D-dimer is a product of fibrinolysis, which increases in acute sepsis. Prothrombin time, fibrinogen, and D-dimer have all been reported as predictors of disease severity as test abnormalities connotes the onset of disseminated intravascular coagulopathy (5). D-dimer has equally been reported to be the most single independent predictor of mortality in COVID-19 (46).

The 100% and 71.4% increase in serum urea and creatinine levels, respectively, observed is a pointer that the kidney/renal system is not equally spared as the pathogenesis of COVID-19 spread (46). Though this may be due to underlying medical conditions before infection, nevertheless studies have linked renal failure to COVID-19 disease. The increase in creatinine and urea levels seen in our study have been reported by others (37). Serum urea levels can reflect an intricate relationship between renal situation, protein metabolism, and nutritional status of patients, and the increase in serum levels in that study (37) may be caused by high catabolism, which also corroborates the report of the possibility of catabolism syndrome in COVID-19 patients with prolonged mechanical ventilation (24).

As creatinine is predominantly excreted through the renal system, an increase in serum levels suggest renal dysfunction.

Arterial/venous blood gases measures the clinical status of patients and has generally been used to determine infected individuals in need of admittance to ICU. In our study a decrease in PaO_2 and $PaCO_2$ levels were observed among the severe/critical cases just as blood pH and lactate levels were seen to be elevated. The use of arterial/venous blood gas parameters (pH, PaO_2, $PaCO_2$, FiO_2, and lactate) to monitor the patients' respiratory function and to inform clinical decision-making has been reported (5) while lactate levels of ≥ 2 mmol/L has been linked with septic shock in adults (47).

Studies have used the neutrophil to lymphocyte ratio, the platelet to lymphocyte ratio, the international normalized ratio, the lymphocyte to monocyte ratio, and the oxygenation index to predict severity and/or mortality from COVID-19, and an increase in the neutrophil to lymphocyte ratio, the platelet to lymphocyte ratio, and the neutrophil to lymphocyte ratio levels have been observed (47). However, the lymphocyte to monocyte ratio and oxygenation index were decreased. Patients with oxygen saturation (resting state) ≤93% or PaO_2/FiO_2≤300mmHg have been classified as being severe/critical and required intensive care management and mechanical ventilation (5,24,35).Significance of the oxygenation index in COVID-19 was also shown in another study (48).The uniqueness of the neutrophil to lymphocyte ratio lies in the characteristic changes in lymphocyte counts and have been used in evaluating progression and prognosis in tumors and infections (26,49). The significance of using the neutrophil to lymphocyte ratio as an independent predictor of COVID-19 severity and mortality has previously been reported (50,51).

Our study is limited by the fact that most of the included studies are observational in nature, with some reporting incomplete patient information. However, unlike other systematic reviews on laboratory changes in COVID-19, we did not include case reports and case series thus increasing the level of confidence in the generated estimates. Our study gives up to date information on the laboratory abnormalities seen with COVID-19 and includes studies from countries outside of China. In addition, the sample population size in our study is higher than those reported in previous reviews. Nevertheless, our study did not separate findings according to age and sex and did not attempt to demonstrate the causality of the observed association between COVID-19 and abnormal laboratory parameters.

CONCLUSIONS

The observations from our study provides evidence of multiple organ involvement in COVID-19 disease. The changes in cardiac markers, coagulation profile, biochemical parameters, blood gases, and laboratory ratios demonstrates clear association between COVID-19 and cardiac, liver, renal, and respiratory/lung dysfunction in these patients. Importantly, lymphocytopenia (96.0%), thrombocytopenia (83.3%), leukocytosis (71.4%), and neutrophilia (86.4%) are common haematological abnormalities in COVID-19 patients as well as elevated serum levels of D-dimer, LDH, urea, creatinine, and the neutrophil to lymphocyte ratio, which were all associated with disease severity. There is a need to include these tests in treatment centers across the world as they are of importance in disease prognosis and monitoring and could aid in reducing the complications and mortality in patients with COVID-19.

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REFERENCES

1. Lu R, Zhao X, Li J, Niu P, Yang B, et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* 2020; 395: 565–574
2. Gorbalenya AE Baker SC, Baric RS, et al. The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. *Nat Microbiol* 2020; 5: 536–544.
3. WHO Director-General's opening remarks at the media briefing on COVID-19 - 11 March 2020. https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-oncovid-19---11-march-2020.
4. Zhu N, Zhang D, Wang W, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* 2020; 382:727–33.
5. O'Shea PM, Lee GR, Griffin TP, et al.COVID-19 in adults: test menu for hospital blood science laboratories. *Ir J Med Sci* 2020 18: 1–6.
6. Fan Z, Chen L, Li J, Cheng X, et al. Clinical Features of COVID-19-Related Liver Functional Abnormality. *Clin Gastroenterol Hepatol* 2020; 18: 1561–1566.
7. Mo P, Xing Y, Xiao Y, et al. Clinical characteristics of refractory COVID-19 pneumonia in Wuhan, China. *Clin Infect Dis* 2020; ciaa270.
8. Liu W, Tao ZW, Wang L, et al. Analysis of factors associated with disease outcomes in hospitalized patients with 2019 novel coronavirus disease. *Chin Med J (Engl)* 2020; 133: 1032-1038.
9. Sun Y, Dong Y, Wang L, et al. Characteristics and prognostic factors of disease severity in patients with COVID-19: The Beijing experience. *J Autoimmun* 2020; 112: 102473.
10. Ding M, Zhang Q, Li Q, et al. Correlation analysis of the severity and clinical prognosis of 32 cases of patients with COVID-19. *Respir Med* 2020; 167: 105981.
11. Wang Z, Yang B, Li Q, et al. Clinical features of 69 cases with coronavirus disease 2019 in Wuhan, China. *Clin Infect Dis* 2020; 71: 769-777.
12. Ding M, Zhang Q, Li Q, et al. Correlation analysis of the severity and clinical prognosis of 32 cases of patients with COVID-19. *Respir Med* 2020; 167: 105981.
13. Cai Q, Huang D, Ou P, et al. COVID - 19 in a designated infectious diseases hospital outside Hubei Province, China. *Allergy* 2020; 75: 1742-1752.
14. Wang F, Yang Y, Dong K, et al Clinical characteristics of 28 patients with diabetes and COVID-19 in Wuhan, China. *Endocr Pract* 2020; 26: 668-674.
15. Huang J, Cheng A, Kumar R, et al. Hypoalbuminemia predicts the outcome of COVID - 19 independent of age and co - morbidity. *J Med Virol* 2020; doi: 10.1002/jmv.26003. Online ahead of print.
16. Chen G, Wu D, Guo W, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. *J Clin Invest* 2020; 130: 2620–2629.
17. Zhang X, Cai H, Hu J, et al. Epidemiological, clinical characteristics of cases of SARS-CoV-2 infection with abnormal imaging findings. *Int J Infect Dis* 2020; 94: 81–87.

18. Zhua Z, Caib T, Fanb L, et al. Clinical value of immune-inflammatory parameters to assess the severity of coronavirus disease 2019. *Int J Infect Dis* 2020; 95: 332–339.
19. Chen W, Zheng KI, Liu S, et al. Plasma CRP level is positively associated with the severity of COVID-19. *Ann Clin Microbiol Antimicrob* 2020; 19: 18.
20. Zhang J, Liu P, Wang M, et al. The clinical data from 19 critically ill patients with coronavirus disease 2019: a single-centered, retrospective, observational study. *Z Gesunh Wiss* 2020; 1-4.
21. Tang N, Li D, Wang X, Sun Z. Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. *J Thromb Haemost* 2020; 18: 844–847.
22. Zheng Y, Sun LJ, Xu M, et al. Clinical characteristics of 34 COVID-19 patients admitted to intensive care unit in Hangzhou, China. *J Zhejiang Univ Sci B* 2020; 21: 378–387.
23. Gao Y, Li T, Han M, et al. Diagnostic utility of clinical laboratory data determinations for patients with the severe COVID - 19. *J Med Virol* 2020; 92: 791-796.
24. Ding X, Yu Y, Lu B, et al. Dynamic profile and clinical implications of hematological parameters in hospitalized patients with coronavirus disease 2019. *Clin Chem Lab Med* 2020; 58: 1365-1371.
25. Fan BE, Chong VCL, Chan SSW, et al. Hematologic parameters in patients with COVID - 19 infection. *Am J Hematol* 2020; 95: E131-E134
26. Du RH, Liang LR, Yang CQ, et al. Predictors of mortality for patients with COVID-19 pneumonia caused by SARS-CoV-2: a prospective cohort study. *Eur Respir J* 2020; 55: 2000524.
27. Wang C, Deng R, Gou L, et al. Preliminary study to identify severe from moderate cases of COVID-19 using combined hematology parameters. *Ann Transl Med* 2020; 8: 593.
28. Yip CYC, Yap ES, De Mel S, et al. Temporal changes in immune blood cell parameters in COVID - 19 infection and recovery from severe infection. *Br J Haematol* 2020; 190: 33-36..
29. Fu J, Kong J, Wang W, et al The clinical implication of dynamic neutrophil to lymphocyte ratio and D-dimer in COVID-19: A retrospective study in Suzhou China. *Thromb Res* 2020; 192: 3–8.
30. Pereira A, Cruz-Melguizo S, Adrien M, et al. Clinical course of Coronavirus Disease-2019 in pregnancy. *Acta Obstet Gynecol Scand* 2020; 99: 839-847.
31. Satici C, Demirkol MA, Altunok ES, et al. Performance of pneumonia Severity index and CURB-65 in predicting 30-day mortality in patients with COVID-19, *Int J Infect Dis* 2020; 98: 84-89.
32. Lagadinou M, Salomou EE, Zareifopoulos N, et al. Prognosis of COVID-19: Changes in laboratory parameters. *Infez Med* 2020; 28(suppl 1): 89-95.
33. Han H, Xie L, Liu R, et al. Analysis of heart injury laboratory parameters in 273 COVID - 19 patients in one hospital in Wuhan, China. *J Med Virol* 2020; 92: 819–823.
34. Ikeagwulonu RC, Etukudoh NS, Obeta MU, Mgbecheta CU. Does Vitamin D serum levels affect the risk of Covid 19 and its clinical outcomes? A review of literature. *E A S J Med Surg* 2020; 2: 146-151.
35. Hamming I, Timens W, Bulthuis MLC, et al. Tissue distribution of ACE_2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. *J* Pathol 2004; 203: 631–637.
36. Velavan TP, Meyer CG. Mild versus severe COVID-19: Laboratory markers. *Int J Infect Dis* 2020; 95: 304–307.
37. Henry B, de Olivera MHS, Benoit S, et al. Hematologic, biochemical, and immune biomarker abnormalities associated with severe illness and mortality in coronavirus disease 2019 (COVID-19): a meta-analysis. *Clin Chem Lab Med* 2020; 58: 1021-1028.
38. Henry BM, Benoit SW, de Oliveira MHS, et al. Laboratory abnormalities in children with mild and severe coronavirus disease 2019 (COVID-19): A pooled analysis and review. *Cline Biochem* 2020; 81: 1–8.
39. Acar M, Sütçü M, Aktürk H, et al. Clinical differences of influenza subspecies among hospitalized children. *Turk Pediatri Ars* 2017; 52: 15–22.
40. Henry BM, Vikse J, Benoit S, et al. Hyperinflammation and derangement of renin-angiotensin-aldosterone system in COVID-19: A novel hypothesis for clinically suspected hypercoagulopathy and microvascular immunothrombosis. *Clin Chim Acta* 2020; 507: 167–173.
41. Yang X, Yang Q, Wang Y, et al. Thrombocytopenia and its association with mortality in patients with COVID-19. *J Thromb Haemost* 2020; 18: 1469-1472.
42. Shi S, Qin M, Shen B, et al. Association of cardiac injury with mortality in hospitalized patients with COVID-19 in Wuhan, China. *JAMA Cardiol* 2020; 5: 802-810.
43. Lee N, Hui D, Wu A, et al. A major outbreak of severe acute respiratory syndrome in Hong Kong. *N Engl J Med* 2003; 348: 1986–1994.
44. Assiri A, Al-Tawfiq JA, Al-Rabeeah AA, et al. Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. *Lancet Infect Dis* 2013; 13: 752–761.
45. Ruan Q, Yang K, Wang W, et al. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Med* 2020; 46: 846-848. Erratum in: *Intensive Care Med* 2020; 46: 1294-1297.
46. Zhou F., Yu T., Du R. Clinical course and risk factors for mortality of adult in patients with COVID-19 in Wuhan. China: a retrospective cohort study. *Lancet* 2020; 359: 1054-1062.
47. Alhazzani W, Møller M.H, Arabi Y.M et al. Surviving sepsis campaign: guidelines on the management of critically ill adults with coronavirus Disease 2019 (COVID-19). *Crit Care Med.* 2020; 48: e440-e469.
48. Cecconi M, Piovani D, Brunetta E, et al. Early predictors of clinical deterioration in a cohort of 239 patients hospitalized for Covid-19 infection in Lombardy, Italy. *J Clin Med* 2020; 9: 1548.
49. Guthrie GJK, Charles KA, Campbell SD, et al. The systemic inflammation-based neutrophil-lymphocyte ratio: experience in patients with cancer. *Crit Rev Oncol Hematol* 2013; 88: 218–30.
50. Yang AP, Liu JP, Tao WQ, Li HM. The diagnostic and predictive role of NLR, d-NLR and PLR in COVID-19 patients. *Int Immunopharmacol* 2020; 84: 106504.
51. Liu Y, Du X, Chen J, et al. Neutrophil-to-lymphocyte ratio as an independent risk factor for mortality in hospitalized patients with COVID-19. *J Infect* 2020; 81: e6-e12.

VIEWPOINT

Is disruption of skeletal muscle molecular clocks the fundamental basis for the development of metabolic syndrome in spinal cord injured individuals?

Lynnette M Jones and Michael Legge

ABSTRACT

Here we consider the inter-connectiveness between the clinical presentation, metabolic changes associated with spinal cord injury and the relationship to skeletal molecular clocks. There is incontrovertible evidence that significant metabolic complications can appear over time with spinal cord injuries, which are associated with significant pathologies, and in many cases, reduced life expectancy. The current biophysical and metabolic data only confirms the presence of pathology but fails to identify the underlying cause at the cellular level. Based on current published experimental evidence on muscle molecular clock function, we propose a hypothesis that the underlying cellular mechanism for the metabolic disruption is that following spinal cord injuries, the skeletal muscle metabolic clock uncouples and fails to provide the correct signaling cues for normal skeletal muscle metabolism. The consequence of stopping or slowing the muscle molecular clock results in the metabolic chaos and comorbidities seen in people with spinal cord injury.

Key words: Spinal cord injury, disrupted metabolism, skeletal metabolic clocks.

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INTRODUCTION

Spinal cord injury resulting in paralysis below the lesion is associated with extensive changes in body composition (1-3). In addition to the primary injury there is a strong association with the increased incidence of heart disease and type 2 diabetes (4-6) which results in the progressive loss of muscle mass and increased adiposity below the lesion (2,7,8). These changes are strongly associated with significant metabolic disruption, thereby increasing the risk factors for metabolic syndrome, such as insulin resistance and dyslipidaemia (5). Previous research has identified alterations in the desaturases and elongase in spinal cord injury that are linked with the development of type 2 diabetes (9). More recently it has been reported that both plasma glycerol and free fatty acids were significantly higher in spinal cord injury than in comparable controls, providing additional evidence for a mechanism to develop insulin resistance (10). We present a hypothesis linking dysfunctional skeletal muscle molecular clocks as a primary causative factor for the development of metabolic syndrome in spinal cord injury.

Muscle metabolic changes consequential to spinal cord injury

The transition from lean tissue muscle mass to adipose tissue is associated with progressive denervation leading to a decrease in the capillary circulation and progressive loss of mitochondria (11,12). These changes result in a shift of normal metabolic response away from muscle to adipose tissue. However, while such metabolic changes occur, other tissue and metabolic changes may precede these and provide an underpinning cellular mechanism for the metabolic changes.

Although there are diverse reasons for general muscle wasting, spinal cord injury and the subsequent muscle wasting below the lesion represents a unique physiological and biochemical situation. Following spinal cord injury there is a progressive loss of the balance between anabolic and catabolic states (1,5) with the injury resulting in changes in muscle fibre and micro-vasculature (12). As disuse atrophy progresses there is a shift from type I (slow) fibre type, to type II (fast) fibre type, with a significant shift to type II by 4.7 months post-injury (11,12). Additionally, there is a progressive increase in sarcoplasm lipid content and denervation (12). The former being considered a function (in part) of decreased mitochondrial function (12,13).

Of note, with the shift from type 1 to type II fibres, an increase in myostatin has been observed (14,15). Previous research has demonstrated increased myostatin production from myotubes from both obese and type 2 diabetes, both of whom had increased type II fibres, which correlated with impaired glucose metabolism and poor fitness (14,16). An imbalance of muscle metabolic homeostasis will result in increased proteolysis and muscle wasting. In addition, increases in myostatin synthesis are compounded in spinal cord injury with both loss of activity below the lesion and the loss of neuronal signaling, which communicates between muscle and bone. The change in muscle-bone interactions leads to the decalcification commonly observed in spinal cord injury (17,18). This interaction between muscle, bone and whole-body metabolism is compromised in spinal cord injury; in particular glucose metabolism where normally up to 80% of post-prandial glucose is utilized by skeletal muscle (19), as well as the utilisation of free fatty acids as an alternative energy source. Therefore, maintaining muscle mass is a key factor in maintaining metabolic homeostasis.

Investigations into progressive loss of muscle mass in humans is limited; however, the major proteolytic pathway to cause loss of muscle mass is the ubiquitin proteasome pathway and is considered to be the major non-lysosomal pathway for intracellular protein degradation (20,21). Whilst a number of activators of the ubiquitin proteasome pathway have been described (22) evidence indicates that in muscle the ubiquitin proteasome pathway can be activated by increased myostatin (23). As indicated earlier in this paper there is a strong association with the transition to type II fibres and the increase in myostatin in spinal cord injury. It is interesting to speculate that alongside this physiological transition, the associated myostatin increase acts as an activating factor (at least in part) to initiate the ubiquitin proteasome pathway and the muscle wasting sequalae. In their review of skeletal muscle atrophy, Jackman and Kandarian indicated that myostatin receptor activin IIB (ACTIIB) is up regulated during muscle atrophy (24). In addition, myostatin inhibits GLUT 4 mRNA and protein expression in-vitro (25), providing a potential mechanism for insulin resistance in muscle following spinal cord injury.

Finding the 'key' to muscle wasting.

The question now arises whether other metabolic mechanisms are disrupted following spinal cord injury and whether there is a disrupted "controller"? Is there a key to the biochemical transitions leading to the development of insulin resistance, type 2 diabetes, and the pathogenesis of the metabolic syndrome? Results from *Bmal1* knock-out mice provides evidence that the molecular clock is directly involved in the transition to type II muscle fibres and disruption of glucose homeostasis (26). More recently Perrin *et al.* (27) confirmed the findings of Harfmann using in-vitro cultures of human myotubes and suppression of BMAL1. Additionally, Perrin *et al.* disrupted lipid diurnal oscillations confirming earlier work of Loizides-Mangold *et al.* (28) who identified that both in-vitro cultured human myotubes and muscle biopsies demonstrated lipid oscillations, which were disrupted when siRNA meditated clock disruption was introduced to the myotubes.

Is it all about loss of 'timing'?

The circadian clock is a well-defined gene regulatory network that controls the expression of transcriptional-translational regulatory networks. These exist in all cells of the body, including muscle, and are expressed in a cyclical manner over an approximate period of 24 hours to synchronize and 'fine tune' local tissue metabolism. Skeletal muscle is entrained by the suprachiasmatic nucleus, which is cued by environmental signals such as light and in turn can modulate muscle response via a number of signaling systems such as neurohormonal, temperature and nutrition control (29). Whilst the mode of suprachiasmatic nucleus signaling is not completely understood, muscle molecular clocks respond in a prescribed manner using the well-established molecular clock regulating system thereby facilitating synchrony with other body organs and tissues (30). This autonomous molecular clock regulates muscle insulin sensitivity via a diurnal rhythm as well as a diurnal rhythm in mitochondrial oxidative capacity (31). In addition, skeletal muscle lipid and amino acid metabolism has been similarly linked to regulation by muscle molecular clocks (32). The mechanism for the cellular metabolic synchronization is linked via the BMAL1:CLOCK transcriptional activation. When the BMAL1:CLOCK heterodimer form a complex in the nucleus they can activate a regulatory loop involving Rev-erb-a, which is highly expressed in oxidative skeletal muscle (33,34) Inactivation or deficiency of Rev-erb-a has been demonstrated to reduce mitochondrial content and the consequential decrease in oxidative function, as well as upregulating autophagy (33,34). Similarly MYOD1, which is involved in skeletal muscle lineage, has been shown to be under the control of the BMAL1:CLOCK complex and it is considered that this control is necessary for maintaining muscle phenotypes by acting as a muscle molecular clock amplifier for down-stream genes (35,36). Taken collectively, disruption of signaling either to or from the suprachiasmatic nucleus will disrupt the integrity of the muscle molecular clock, which in turn will modify the response of the BMAL1:CLOCK complex. Failure or downregulation of BMAL1 activation and the consequential down regulation of Rev-erb-a will disrupt the ability of skeletal muscle to respond to insulin via the GLUT4 receptor and consequently disrupt glucose homeostasis (30). This has been further confirmed using *Bmal1* knock-out mice that demonstrated impaired skeletal muscle glucose uptake and metabolism (37). In addition, disruption of CLOCK in human skeletal muscle biopsies in tissue culture also had a similar effect on disrupting skeletal diurnal lipid response as well as a glucose response as identified with BMAL1 disruption (27).

In conclusion, we consider that following a spinal cord injury the fundamental mechanism for mediating the metabolic consequences and development of metabolic syndrome is linked to the disruption of the molecular clock in skeletal muscle. The potential uncoupling of the muscle molecular clock not only provides evidence for the development of insulin resistance, but also provides the basis for the transition of muscle fibres from type I to type II and the disruption of muscle myotube function via the down-regulation of MYOD1 function and the consequential loss of signaling to other down-stream genes and gene products.

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REFERENCES

1. Gorgey AS, Dulbow DR, Dolbow JD, et al. Effects of spinal cord injury on body composition and metabolic profile – Part 1. *J Spinal Cord Med* 2014; 37: 693-702.
2. Jones LM, Legge M, Goulding A. Healthy body mass index (BMI) values often underestimates body fat in spinal cord injured males. *Arch Phys Med Rehabil* 2003; 84: 1068-1071
3. O'Shea TM, Burda JE, Safroniew MV. Cell biology of spinal cord injury and repair. *J Clin Invest* 2017; 127: 3259-3270.
4. Bauman WA, Spungen AM. Carbohydrate and lipid metabolism in chronic spinal cord injury. *J Spinal Cord Med* 2001; 24: 266-277.
5. Jones LM, Legge, M Goulding A. Factor analysis of the metabolic syndrome in spinal cord-injured men. *Metabolism* 2004; 53: 1372-1377.
6. Nightingale TE, Walhin J-P, Thompson D, Bilzon JLJ. Biomarkers of cardiometabolic health are associated with body composition characteristics but not physical activity on persons with spinal cord injury. *J Spinal Cord Med* 2017; 42: 328-337.
7. Elder CP, Apple DF, Bickel CS, et al. Intramuscular fat and glucose after spinal cord injury – a cross-sectional study. *Spinal Cord* 2004; 42: 711-716.
8. Sweiss R, Biller J. Systematic complications of spinal cord injury. *Cur Neurol Neurosci Rep* 2017; 17: 8.
9. Jones LM, Legge M. Plasma fatty acids as markers for desaturase and elongase activities in spinal cord injured males. *J Spinal Cord Med* 2019; 42: 163-170.
10. Jones LM, Legge M. Plasma fasting glycerol and fatty acids in spinal cord injured males. *N Z J Med Lab Sci* 2019; 73: 46-48.
11. Burnham R, Martin T, Bell G, et al. Skeletal muscle fiber type transformation following spinal cord injury. *Spinal Cord* 1997; 35: 86-91.
12. Scelsi R. Skeletal muscle pathology after spinal cord injury: Our 20-year experience and results on skeletal muscle changes in paraplegics, related to functional rehabilitation. *Basic Appl Myol* 2001;11: 75-85.
13. Scholpa NE, Schnellmann RG. Mitochondrial-based therapeutics for the treatment of spinal cord injury: Mitochondrial biogenesis as a potential pharmacological target. *J Pharmacol Exp Ther* 2017; 363: 303-313.
14. Brandt C, Nielsen AR, Fischer CP, et al. Plasma and muscle myostatin in relation to type 2 diabetes. *PLoS One* 2012; 7: e37236.
15. Pedersen BK. Muscle as a secretory organ. *Compr Physiol* 2013; 3: 1337-1362.
16. Hittel DS, Axelson M, Sarna N, et al. Myostatin decreases with aerobic exercise and associates with insulin resistance. *Med Sci Sports Exerc* 2010; 42: 2023-2039.
17. Jiang S-D, Dai L-Y, Jiang L-S. Osteoporosis after spinal injury. *Osteoporos Int* 2006; 17: 180-192.

18. Tan CO, Battaglino RA, Morse LR. Spinal cord injury osteoporosis: Causes, mechanisms, and rehabilitation strategies. *Int J Phys Med Rehabil* 2013; 1:1 27.
19. Ferranini E, Simonson DC, Katz LD, et al. The disposal of an oral glucose load in patients with non-insulin dependent diabetes. *Metabolism* 1988; 37: 79-85.
20. Reid MB. Response of the ubiquitin-proteasome pathway to changes in muscle activity. *Am J Physiol Regul Integr Comp Physiol* 2005; 288: R1423-R1431.
21. Bodine SC. Disuse-induced muscle wasting. *Int J Biochem Cell Biol* 2013; 45: 2200-2208.
22. Stadtmueller BM, Hill CP. Proteasome activators. *Mol Cell* 2011; 41: 8-19.
23. Wang D-T, Yang Y-J, Huang R-H, et al. Myostatin activates the ubiquitin-proteasome pathway and autophagy-lysosome systems contributing to muscle wasting activates the ubiquitin-proteasome in chronic kidney disease. *Oxid Med Cell Longev* 2015; 2015: e684965.
24. Jackman RW, Kandarian SC. The molecular basis of skeletal muscle atrophy. *Am J Cell Physiol* 2004; 287: C834-C843.
25. Liu XH, Bauman WA Cardozo CP. Myostatin inhibits glucose uptake via suppression of insulin-dependent and -independent signaling pathways in myoblasts. *Physiol Rep* 2018; 6: e13837.
26. Harfmann BD, Schroder EA, Kachman MT, et al. Muscle specific loss of Bmal1 leads to disrupted tissue glucose metabolism and systematic glucose homeostasis. *Skelet Muscle* 2016; 6: 12.
27. Perrin L, Loizides-Mangold U, Chanon S, et al. Transcriptomic analyses reveal rhythmic and CLOCK-driven pathways in human skeletal muscle. *Elife.* 2018; 7: 34114.
28. Loizides-Mangold U, Perrin L, Vandereycken B, et al. Lipidomics reveals diurnal oscillations in human skeletal muscle persisting in cellular myotubes cultures in vitro. *Proc Natl Acad Sci U S A* 2017; 114: E8565-E8574.
29. Dibner C, Schibler U, Albrecht U. The mammalian circadian timing system: Organization and coordination of central and peripheral clocks. *Annu Rev Physiol* 2010; 72: 517-549.
30. Harfmann BD, Schroder EA, Esser KA. Circadian rhythms, the molecular clock and skeletal muscle. *J Biol Rhythms* 2015; 30: 84-94.
31. Dyar KA, Ciciliot S, Wright LE, et al. Muscle insulin sensitivity and glucose metabolism are controlled by the intrinsic muscle clock. *Mol Metab* 2013; 3:29-41. Erratum in: *Mol Metab* 2014; 3: 857.
32. Dyar KA, Hubert MJ, Mir AA, et al. Transcriptional programming of lipid and amino acid metabolism by the skeletal muscle circadian clock. *PLoS Biol* 2018; 16: e2005886.
33. Woldt E, Sebti Y, Solt LA, et al. Rev-erb-α modulates skeletal muscle oxidative capacity by regulating mitochondrial biogenesis and autophagy. *Nat Med* 2013 19: 1039-1046
34. Mayeuf-Louchart A, Thorel Q, Delhaye S, et al. Rev-erb-a regulates atrophy-related genes to control muscle mass. *Sci Rep* 2017; 7: 14383.
35. Andrews JL, Zhang X, McCarthy JL, et al. CLOCK and BAML1 regulate MyoD and are necessary for maintenance of skeletal muscle phenotype and function. *Proc Natl Acad Sci U S A* 2010; 107:19090-19095.
36. Hodge BA, Zhang X, Gutierrez-Monreal MZ, et al. MYOD1 functions as a clock amplifier as well as a critical co-factor for downstream circadian gene expression in muscle. *eLife.* 2019; 8: e43017.
37. Schiaffino S, Blaauw B, Dyar KA. The functional significance of the skeletal muscle clock: lessons from Bmal1 knockout models. *Skelet Muscle* 2016; 6: 33.

ORIGINAL ARTICLE

The predictive value of preoperative serum bilirubin and white blood cell count in simple and complicated acute appendicitis

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ABSTRACT

Objective: Early diagnosis and prompt surgical intervention can reduce the rate of complications in acute appendicitis. However, accurate diagnosis remains a common surgical problem. We aimed to determine the value of preoperative serum bilirubin level and white blood cells count (WBC) in patients with simple and complicated appendicitis.

Methods: This was a prospective observational study carried out from January 2017 to December 2018 at Ekiti State University Teaching Hospital, Ado-Ekiti, Ekiti State, Southwestern Nigeria. Preoperative samples for total serum bilirubin (TSB), direct bilirubin (DB) and WBC were taken for all patients diagnosed of appendicitis. Data were analyzed using SPSS version 21. A p-value <0.05 was considered statistically significant.

Results: Eighty-one patients were seen during the study period. Their ages ranged between 8 and 77 years (mean: 28.6±13.6). There were 48 (59.3%) males and 33 (40.7%) females (M:F=1.5:1). Sixty-two (76.5%) patients had simple appendicitis while 19 (23.5%) had perforated appendicitis. The mean TSB were 0.8±0.5 and 1.4±0.4 mmol/L (P=0.01), mean DB were 0.37±0.23 and 0.64 ±0.38 mmol/L (P=0.01) while the mean WBC were 9.7±4.3 and 11.4±3.0 $x10^9$/L (P=0.12), for simple and complicated appendicitis respectively. TSB had a sensitivity of 84%, specificity 81%, positive predictive value (PPV) 57% and negative predictive value (NPV) of 94%. Total WBC had a sensitivity of 68%, specificity 60%, PPV 34% and NPV of 86%.

Conclusion: High TSB is a useful adjunct to improving the diagnostic yield in perforated appendicitis. Hyperbilirubinemia and clinical features consistent with complicated appendicitis should warrant early surgery.

Key words: Acute appendicitis, complicated appendicitis, serum bilirubin, white blood cells, predictive value.

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INTRODUCTION

Acute appendicitis is the most common cause of acute abdomen and also the most frequent indication for abdominal surgical emergencies worldwide (1). It accounts for about 40% of all surgical emergencies in the western world. The incidence is increasing in Nigeria like other developing countries, where acute appendicitis was initially considered to be rare, and this probably has been adduced to increasing adoption of western diet (2,3).

The importance of early diagnosis and prompt surgical intervention cannot be overemphasised in the successful treatment of acute appendicitis. Delay in diagnosis and treatment usually results in increased rate of complications, longer hospital stays and high mortality (4). The diagnosis of acute appendicitis is mainly clinical. However, in atypical presentations accurate diagnosis could be a major challenge. Several haematological and biochemical parameters, including white blood cell count (WBC), C-reactive protein, interleukin-6, and procalcitonin have been employed to further improve the clinical diagnosis (5). Imaging studies like ultrasonography, computerised tomography, and magnetic resonance imaging as well as scintigraphy and laparoscopy have all been used for more accurate diagnosis where these facilities are available (6-9). Apart from the ultrasonography, all these other investigations are not available in our hospital, and most patients cannot afford the cost where the facilities could be accessed. In spite of all these diagnostic aids for acute appendicitis, no single test has been found to reduce the rate of negative appendicectomy to zero (10).

The occurrence of jaundice in sepsis is a common phenomenon and is well recognised. This has been found to be associated with a variety of causative bacteria of which Gram-negative bacteria are the most commonly implicated organisms (11). Studies have shown that jaundice can be associated with acute appendicitis and recently hyperbilirubinemia has been found to have a correlation with appendiceal perforation suggesting that it could be a useful predictor of complicated appendicitis (12-14). It was on this background that this study was carried out to determine the diagnostic value of preoperative serum bilirubin levels and WBC in selected patients with simple and complicated appendicitis, and also to determine their predictive values in complicated cases.

MATERIALS AND METHODS

Study design

The study was carried out in Ekiti State University Teaching Hospital, Ado-Ekiti, Ekiti State, Southwestern Nigeria. This is a tertiary health institution that serves as a referral center for the primary and secondary health facilities in Ekiti State and neighbouring states. This was a prospective observational study carried out over two years from January 2017 to December 2018. Approval for the study was obtained from the Ethics and Research Committee of the Ekiti State University Teaching Hospital.

Study population and sampling technique

All patients with features of acute appendicitis, including those complicated by abscess, gangrene, or perforation, that required emergency operation were included in the study. Patients with appendiceal mass and those with atypical presentations that did not require operation were excluded. Other patients excluded were those with haemoglobinopathies, haemolytic anaemia, glucose-6-phosphate dehydrogenase (G6PD) deficiency, recent

history of jaundice, hepatitis, or other liver diseases. Patients who were not willing to participate and those whose blood samples were not completed were also excluded.

Data collection

The following data were collected using a proforma designed for the study: patient's biodata, symptoms and signs of appendicitis, investigation results which included ultrasound scan, full blood count, and preoperative serum bilirubin estimation (total and direct). The blood samples were collected while securing intravenous access and before administration of antibiotics. Samples for serum bilirubin (total and direct) were collected inside lithium heparin specimen bottle which was covered immediately with a black polythene bag to prevent exposure to visible light. The samples were analysed within one hour of collection.

Both total and direct bilirubin were analysed using commercial kits (Randox, United Kingdom). White blood cell count (WBC) was analysed on a 3-part haematology analyser (Swelab, Sweden). Hyperbilirubinaemia was defined as a total serum bilirubin (TSB) level greater than 1.0 mmol/L and normal reference range for total WBC was 2.5-10.0 $x10^9$/L.

Data on the intraoperative findings were documented and histopathology of the resected appendix specimen was considered as the final diagnosis. Thereafter, patients were categorised into those with simple appendicitis in one group and those with complications like abscesses, gangrene, and perforation in another group.

Statistical analysis

Data was analysed using the statistical package for social sciences (SPSS), version 21. Independent sample t-tests were used to compare means across the two groups of patients (simple acute appendicitis and complicated appendicitis). The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and likelihood ratios for TSB and WBC were calculated to determine the validity of the tests as a diagnostic tool in preoperative identification of complicated appendicitis. A *p*-value <0.05 was considered statistically significant.

RESULTS

A total number of 81 patients were seen during the study period. Their ages ranged between 8 and 77 years and the median age was 26 years (mean:28.6±13.6). There were 48 (59.3%) males and 33 (40.7%) females with a male to female ratio of approximately 1.5:1. The age distribution of patients are shown in Table 1. About two-thirds of patients were seen in the second and third decades.

All patients presented with abdominal pain with the duration of pain ranging from 2 hours to 9 days (mean: 59.8±48.0 hours). Thirty (37.0%) patients presented within 24 hours, 48 (59.3%) in 48 hours, while the rest came after 72 hours. Anorexia was present in 75 (92.6%), nausea and vomiting in 66 (81.5%), fever in 55 (67.9%), and dysuria in 6 (7.4%). Intra-operatively, the locations of the appendix were retrocaecal in 67 (82.7%), pelvic 9 (11.1%), paracaecal 3 (3.7%), subhepatic 1 (1.2%), and subcaecal 1 (1.2%).

Histopathology revealed that 62 (76.5%) patients had simple appendicitis while 19 (23.5%) had complicated appendicitis. One patient, who had a normal uninflamed appendix, was not included in the analysis.

The mean values for WBC, TSB, and direct bilirubin (DB) are shown in Table 2. The mean WBC, TSB and direct bilirubin in complicated appendicitis were greater than that of simple appendicitis, but only those of TSB and DB were statistically significant (p-value= 0.01). Table 3 shows test results and their validity.

Table 1. Age distribution of patients .

Age	Frequency
1-10 years	2 (2.5%)
11-20 years	25 (30.9%
21-30 years	27 (33.3%
31-40 years	14 (17.3%)
41-50 years	7 (8.6%)
>50 years	6 (7.4%)
Total	81 (100%)

Table 2. Mean (±SD) white blood cells, total serum bilirubin and direct bilirubin for simple and complicated appendicitis.

Parameter	Simple appendicitis	Complicated appendicitis	p-value with pathology
WBC ($x10^9$/L)	9.7 ± 4.3	11.4 ± 3.0	0.12
TSB (mmol/L)	0.8 ± 0.5	1.4 ± 0.4	0.01
DB (mmol/L)	0.37 ±0.23	0.64 ±0.38	0.01

Table 3. Test results with validity.

Tests	Total bilirubin vs. histology	WBC vs. histology
True positive	16	13
True negative	50	37
False positive	12	25
False negative	3	6
Sensitivity	0.84	0.68
Specificity	0.81	0.60
Positive predictive value	0.57	0.34
Negative predictive value	0.94	0.86
Positive likelihood ratio	4.42	1.72
Negative likelihood ratio	0.20	0.52
Inference	Sometimes useful test	Rarely useful test

DISCUSSION

Most patients in our study were seen in their second and third decades of life with a mean age of 28 years. Nineteen (23.5%) patients had perforations. Complications are still common as a result of delayed presentation and/or delayed diagnosis. In this study, 33 (40.7%) patients presented after 72hrs with the longest duration being 9 days. Studies have shown that perforation usually occurs after 48 hours of onset of symptoms (15).

Accurate diagnosis in patients with acute appendicitis is still a common problem for the clinician (16). The diagnostic uncertainty either leads to negative appendectomies in some cases or at the other extreme an increase in complications like

abscess formation, gangrene, perforation, and generalized peritonitis, with attendant high morbidity and mortality. Despite the fact that the diagnosis of appendicitis is mainly clinical, laboratory investigations, imaging techniques, and other diagnostic armamentaria can aid accurate diagnosis and help strike a balance between the two extremes of removing an uninflamed appendix and increased complications.

In our study the mean TSB was statistically significantly higher in complicated appendicitis than in simple appendicitis. This finding is similar to those reported in previous studies (13,14,17). Therefore, a normal plasma bilirubin level combined with clinical features of acute appendicitis largely supports the presence of an uncomplicated appendicitis but does not rule out perforation in few cases.

Different mechanisms have been adduced to hyperbilirubinaemia in systemic infections. Sepsis in the portal system can disrupt the excretion of bilirubin into biliary canaliculi while pro-inflammatory cytokines and nitric oxide can also trigger intrahepatic cholestasis (11,13,18). The more the severity of infection, the higher the level of serum bilirubin (18,19). Also, the mean value of WBC was higher in complicated appendicitis although this was not statistically significant like those reported by other authors (14,15,20). This disparity may be due to the sample size in our study. Furthermore, our study did not consider the antibiotics use prior to presentation which may affect the haematological features of an infected appendix.

In our study, the sensitivity, specificity, PPV and NPV of TSB in perforated appendicitis were 84%, 81%, 57%, and 94% respectively. Ahmed *et al*. (20) and McGowan *et al*. (14) reported a lower sensitivity of 60% and 63% respectively, but the specificity of 80% and 88% in both were comparable to our findings. The positive likelihood ratio and negative likelihood ratio were 4.42 and 0.20 respectively in our study. A positive likelihood ratio of >5 or a negative likelihood ratio <0.2 is indicative of strong diagnostic evidence (21). With our values very close to this standard, we can infer that serum bilirubin could be a very useful diagnostic marker of complicated acute appendicitis among our patients.

The sensitivity, specificity, PPV and NPV obtained for WBC in perforated appendicitis were 68%, 60%, 34% and 86% respectively. These results were generally lower than those obtained for TSB. However, Wu *et al.* (22) and Xharra *et al.* (23) reported higher sensitivities of 80% and 85% and specificities of 71% and 68% respectively.

While it might be true that the WBC also has a place in diagnosis, TSB could be a more useful test in accurately diagnosing a complicated appendicitis in our setting. However, McGowan *et al*. had reported WBC as a biochemical marker of perforation in acute appendicitis (14). The initial inflammatory response in acute appendicitis is more of an increase in neutrophil count without necessarily affecting the total WBC, but the latter number increases with time as inflammation progresses (24). The WBC can also be elevated in many inflammatory conditions thereby making it a non-specific test in the diagnosis of acute appendicitis (25, 26).

A combination of full clinical evaluation, laboratory tests, and radiological investigations are still needed to improve the diagnostic yield in appendicitis and plan for appropriate management. Even with the recent advances in the diagnostic field, there is no single clinical or laboratory test that can accurately predict acute appendicitis or perforated appendicitis (27).

In conclusion, hyperbilirubinaemia could serve as a biochemical marker for complicated appendicitis. In resource-constrained settings where CT or other imaging techniques are not readily available or too expensive to afford by patients, TSB and DB could be a useful adjunct to improving the diagnostic accuracy in complicated appendicitis and should be included in the assessment of patients. Elevated TSB in a patient with suspected acute appendicitis should warrant urgent surgical intervention.

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REFERENCES

1. Saidi HS, Adwok JA. Acute appendicitis: an overview. *East Afr Med J* 2000; 77: 152-156.
2. Ajao OG. Appendicitis in a tropical African population. *J Natl Med. Assoc* 1979; 7: 997 –999.
3. Adekunle OO, Funmilayo JA. Acute appendicitis in Nigeria. *J R Coll Surg Edinb* 1986; 31: 102-105.
4. Rogers AD, Hampton MI, Bunting M, Atherstone AK. Audit of appendicectomies at Frere Hospital, Eastern Cape. *S Afr J Surg* 2008; 46: 74-77.
5. Blab E, Kohlhuber U, Tillawi S, et al. Advancements in the diagnosis of acute appendicitis. *Eur J Pediatr Surg* 2004; 14: 404-409.
6. Rettenbacher T, Hollerweger A, Gritzmann N, et al. Appendicitis: should diagnostic imaging be performed if the clinical presentation is highly suggestive of the disease? *Gastroenterology* 2002; 123: 992–998.
7. Incesu L, Coskun A, Selcuk MB, et al. Acute appendicitis: MR imaging and sonographic correlation. *AJR Am J Roentgenol* 1997; 168: 669-674.
8. Colak T, Güngör F, Ozuğur S, et al. The value of 99mTc-HMPAO labelled white blood cell scintigraphy in acute appendicitis patients with an equivocal clinical presentation. *Eur J Nucl Med* 2001; 28: 575–580.
9. Larsson PG, Henriksson G, Olsson M, et al. Laparoscopy reduces unnecessary appendicectomies and improves diagnosis in fertile women. A randomized study. *Surg Endosc* 2001; 15: 200–202.
10. Kelley SR, Engel AM, Hatton EK, et al. Do basic laboratory tests add value in predicting the severity of appendicitis in an adult patient population and does it make a difference in how severity is defined? *Am Surg* 2012; 78: E175–E177.

11. Chand N, Sanyal AJ. Sepsis-induced cholestasis. *Hepatology* 2007; 45: 230-241.
12. Chaudhary P, Kumar A, Saxena N, Biswal UC. Hyperbilirubinemia as a predictor of gangrenous/ perforated appendicitis: a prospective study. *Ann Gastroenterol* 2013; 26: 325-331.
13. Burcharth J, Pommergaard HC, Rosenberg J, Gögenur I. Hyperbilirubinemia as a predictor for appendiceal perforation: a systematic review. *Scand J Surg* 2013; 102: 55-60.
14. McGowan DR, Sims HM, Zia K, et al. The value of biochemical markers in predicting a perforation in acute appendicitis. *ANZ J Surg* 2013; 83: 79-83.
15. Prystowsky JB, Pugh CM, Nagle AP. Current problems in surgery: appendicitis. *Curr Probl Surg* 2005; 42: 688-742.
16. Petroianu A. Diagnosis of acute appendicitis. *Int J Surg* 2012; 10: 115-119.
17. Atahan K, Üreyen O, Aslan E, et al. Preoperative diagnostic role of hyperbilirubinaemia as a marker of appendix perforation. *J Int Med Res* 2011; 39: 609-618.
18. Estrada JJ, Petrosyan M, Barnhart J, et al. Hyperbilirubinemia in appendicitis: a new predictor of perforation. *J Gastrointest Surg* 2007; 11: 714-718.
19. Hong YR, Chung C-W, Kim JW, et al. Hyperbilirubinemia is a significant indicator for the severity of acute appendicitis. *J Korean Soc Coloproctol* 2012; 28: 247-252.
20. Elshoura AA, Abo-Ryia MH, Elsadany A, et al. Prospective Assessment of Elevated Serum Bilirubin Level in Diagnosis of Acute Appendicitis. *Adv Surg Sci* 2018 ;6: 27-30.
21. Jaeschke R, Guyatt GH, Sackett DL. Users' guides to the medical literature. III. How to use an article about a diagnostic test. B. What are the results and will they help me in caring for my patients? The Evidence-Based Medicine Working Group. *JAMA* 1994; 271: 703-707.
22. Wu HP, Chen CY, Kuo IT, et al. Diagnostic values of a single serum biomarker at different time points compared with Alvarado score and imaging examinations in pediatric appendicitis. *J Surg Res* 2012; 174: 272–277.
23. Xharra S, Gashi-Luci L, Xharra K, et al. Correlation of serum C-reactive protein, white blood count and neutrophil percentage with histopathology findings in acute appendicitis. *World J Emerg Surg* 2012; 7:27.
24. Nauta RJ, Magnant C. Observation versus operation for abdominal pain in the right lower quadrant. Roles of the clinical examination and the leukocyte count. *Am J Surg* 1986; 151: 746-748.
25. Al-Gaithy ZK. Clinical value of total white blood cells and neutrophil counts in patients with suspected appendicitis: retrospective study. *World J Emerg Surg* 2012; 7: 32.
26. Jamaluddin M, Hussain SMA, Ahmad H. Acute appendicitis with normal total leukocyte Count. *J Surg Acad* 2013; 3: 2-6.
27. D'Souza N, Karim D, Sunthareswaran R. Bilirubin; a diagnostic marker for appendicitis. *Int J Surg* 2013; 11: 1114-1117.

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ORIGINAL ARTICLE

Neck circumference as a useful marker of metabolic syndrome among undergraduates in Southwest Nigeria

Esther Ngozi Adejumo, Faith Eniola Owoloja and Adedeji Olusola Adejumo

ABSTRACT

Background: The neck circumference is an easy, simple and time saving surrogate for central obesity. We assessed its usefulness as a marker of metabolic syndrome among undergraduates of a private University in Southwest Nigeria.

Methods: Descriptive cross-sectional study design. Newly admitted students were consecutively recruited during a pre-admission medical examination. Anthropometric measurements, such as weight, height, neck circumference, waist circumference, waist-to-hip ratio, waist-to-height ratio, and blood pressure, were taken. Fasting venous blood was collected for plasma glucose and lipid profile. The National Cholesterol Education Program criteria were used to identify participants with metabolic syndrome. The Receiver Operative Characteristic curve was used to assess the ability of the neck circumference to discriminate metabolic syndrome.

Results: One hundred and twenty undergraduates participated in the study. The prevalence of metabolic syndrome was 19.2%. The mean values of waist circumference, body mass index (BMI), waist-to-hip ratio, neck circumference and waist-to-height ratio was higher among participants with metabolic syndrome. Neck circumference was positively correlated with waist circumference, waist-to-hip ratio, BMI, and waist-to-height ratio in male and female participants. Among the males, waist-to-height ratio had the largest area under the curve (AUC) followed by waist circumference and neck circumference, while among the females, neck circumference had the largest AUC.

Conclusion: Neck circumference has a positive correlation with markers of central obesity and can discriminate metabolic syndrome.

Keywords: Neck circumference, Anthropometric indices, Metabolic syndrome, Nigeria.

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INTRODUCTION

Metabolic syndrome is a combination of cardio-metabolic risk factors including dyslipidaemia, hypertension, impaired glucose metabolism, insulin resistance and obesity (1). Studies have shown that central obesity or fat distribution is strongly linked with cardiometabolic risk (2.3). Over the years, anthropometric indices such as waist circumference, body mass index (BMI), waist-to-height ratio, and waist-to-hip ratio are known markers of obesity and predictors of metabolic syndrome (4,5).

These anthropometric indices have their drawbacks. Except for waist circumference, others are derived from more than one body measurements on light clothing, which could be discomforting in cold weather (6). BMI may not be a reliable estimate for obesity, depending on body build of individuals and racial variations (7,8). Also, daily variation of the abdominal cavity, variation in measurements, and cultural acceptability may limit the use of waist circumference in certain populations (9-11).

Neck circumference has been shown to correlate with age, weight, waist circumference, and waist-to-height ratio (12-13). Population-based studies from China and the Middle East have demonstrated that neck circumference discriminate participants with metabolic syndrome (14,15). In Nigeria, few studies have demonstrated neck circumference as a measure of obesity (16,17). Our study assessed the ability of neck circumference to discriminate metabolic syndrome in undergraduates of a private University in Southwest Nigeria.

METHODS

Study design, background, and participants

A descriptive cross-sectional study was designed. This study was conducted in a private University in Southwest Nigeria. As part of the admission requirement,s undergraduates are required to undergo a medical examination at the out-patient department of the University Teaching Hospital. Undergraduates who met the selection criteria and consented were recruited into the study.

Selection criteria

Inclusion criterion: Healthy undergraduates. Exclusion criteria: Participants who were known hypertensives or on anti-hypertensives, and known diabetics were excluded. Undergraduates on insulin, lipid-lowering drugs or contraceptives were also excluded. Consenting participants were recruited consecutively into the selection study.

Study procedure

A proforma was used to collect personal details and anthropometric measurements of participants. Participants' anthropometric measurements (weight, height, waist circumference, neck circumference, waist-hip ratio, waist-to-height ratio, and blood pressure were measured. Thereafter, a blood sample (6 ml) was collected for plasma glucose and lipid profile estimation, after an overnight fast.

Anthropometric indices

Waist circumference, height and weight were measured using standard procedures, and BMI calculated (2). The ratio of waist circumference to height was recorded as the waist-to-height ratio. To measure neck circumference, the participant's head was put in a Frankfort horizontal plane and measurement was taken just below the prominence of the larynx perpendicular to the long axis of the neck (18). A standardized digital sphygmomanometer (Omron) was used to measure the blood pressure of participants after 5 minutes rest. High density lipoprotein cholesterol (HDL-c), triglyceride and fasting plasma glucose were estimated by enzymatic methods (Randox Laboratories Ltd., United Kingdom). Participants having three or more abnormalities, as defined by the National Cholesterol Education Program (NCEP) recommendations, were classified as having metabolic syndrome (19).

Data analysis

The Kolmogorov-Smirnov test for normality was used to assess normality before data analysis. Descriptive statistics using measures of central tendencies (means) and dispersion (standard deviation) was done. The means of the anthropometric indices, fasting plasma glucose and lipid profile of the two independent groups were compared by Student t test. Pearson's correlation coefficient was used to assess the correlation between neck circumference and components of metabolic syndrome. The ability of anthropometric indices to discriminate metabolic syndrome was assessed using Receiver Operative Characteristic (ROC) curve. IBM SPSS Statistics was used for data analysis.

Ethics

Ethical approval was obtained from the Institution Review Board of Babcock University, Ilishan-Remo, Ogun State, Nigeria. All participants gave written informed consent.

RESULTS

One hundred and twenty undergraduates participated in the study. The mean age was 20.2 ±4.5 years while males constituted 51.7% of participants. The proportion of participants that smoked cigarettes, took alcohol, and were physically active was 10%, 30.8% and 78.3% respectively (Table 1). A total of 23 (19.2%) participants had metabolic syndrome. Mean values of BMI, waist circumference, neck circumference, waist-to-hip ratio, and waist-to-height ratio were higher in participants with metabolic syndrome, as shown in Table 2.

There was no difference in mean fasting plasma glucose levels of participants with and without metabolic syndrome ($p = 0.115$). Participants with metabolic syndrome had higher mean serum LDL-c and triglyceride levels ($p<0.05$) while serum HDL-c was higher in participants without metabolic syndrome ($p<0.001$) (Table 3).

Table 4 shows correlations of anthropometric indices with metabolic syndrome components in both genders. Correlations of neck circumference, waist circumference, waist-to-hip ratio, BMI, and waist-to-height ratio with systolic and diastolic blood pressure in males were positive. Correlations of neck circumference with waist circumference, waist-to-hip ratio, BMI, and waist-to-height ratio in males and females was positive (Table 5). Among males, the waist-to-height ratio had the largest area under the curve (AUC) followed by waist circumference and neck circumference; while among females, neck circumference had the largest AUC, followed by waist circumference (Table 6).

Table 1. Socio demographic details of participants.

Variable	Frequency (n = 120)	%
Age (years)		
68	68	56.7
≤18	52	43.3
Mean ± SD	20.2 ± 4.5	
Gender		
Male	62	51.7
Female	58	48.3
History of smoking		
Yes	12	10.0
No	108	90.0
History of drinking alcohol		
Yes	37	30.8
No	83	69.2
History of physical activity		
Yes	94	78.3
No	26	21.7

Table 2. Anthropometric indices of participants with and without metabolic syndrome

Variable	MS Mean ±SD n = 97	No MS Mean ± SD n = 23	t	p
BMI (kg/m^2)	27.96 ±6.33	32.52 ±4.05	3.290	0.001
Waist circumference (cm)	88.36 ±13.49	104.26 ±11.83	5.197	<0.001
Neck circumference (cm)	37.13 ±2.82	40.7 0±2.20	5.658	<0.001
Waist-to-hip ratio	0.81 ±0.073	0.87 ±0.062	3.702	<0.001
Waist-to-height ratio	0.52 ±0.087	0.60 ±0.070	4.246	<0.001

The prevalence of metabolic syndrome was 19.2% (23/120).
MS: metabolic syndrome

Table 3. Mean fasting lipid and fasting plasma glucose levels of participants with and without metabolic syndrome.

Variable	Non metabolic syndrome		Metabolic syndrome	
	Mean ±SD n = 97	Mean ± SD n = 23	t	p
FPG (mg/dL)	82.6 ±11.5	88.7 ±16.8	1.628	0.115
HDL-c (mg/dL)	51.9 ±12.4	38.2 ±8.6	4.985	<0.001
LDL-c (mg/dL)	75.3 ±8.5	95.3 ±11.9	2.310	0.023
Total cholesterol (mg/dL)	142.7 ±16.5	158.1 ±18.2	1.885	0.062
Triglyceride (mg/dL)	77.8 ±13.9	122.5 ±17.7	4.546	<0.001

FPG = fasting plasma glucose; HDL-c = high density lipoprotein cholesterol; LDL-c = low density lipoprotein cholesterol.

Table 4. Correlation of anthropometric indices with components of metabolic syndrome.

	NC	WC	WHR	BMI	WHR
Males					
SBP (mm/Hg)	0.478[a]	0.475 [a]	0.308[a]	0.551[a]	0.434[a]
DBP (mm/Hg)	0.393[a]	0.57 [a]	0.445[a]	0.522[a]	0.543[a]
FPG (mg/dL)	0.064[b]	-0.060[b]	-0.054 [b]	-0.106[b]	-0.040[b]
HDL-c (mg/dL)	-0.115[b]	-0.106[b]	-0.156[b]	-0.045[b]	-0.043[b]
Triglyceride (mg/dL)	0.152[b]	0.097[b]	0.131[b]	0.183[b]	0.063[b]
Females					
SBP (mm/Hg)	0.207[b]	0.077[b]	0.096[b]	0.124[b]	0.033[b]
DBP (mm/Hg)	0.268[a]	0.057[a]	0.002[b]	0.112[b]	0.057[b]
FPG (mg/dL)	0.537[a]	0.358[a]	-0.016[b]	0.448[a]	0.336[a]
HDL-c (mg/dL)	-0.267[b]	-0.181[b]	-0.169[b]	-0.234[b]	-0.164[b]
Triglyceride (mg/dL)	0.525[a]	0.310[a]	0.092[b]	0.365[a]	0.344[a]

[a]Significant. [b]Not significant.
NC = neck circumference; WC = waist circumference, WHR = waist-to-hip ratio; BMI = body mass index; WHR = waist-to-height ratio; SBP = systolic blood pressure; DBP = diastolic blood pressure; FPG = fasting plasma glucose; HDL-c = high density lipoprotein cholesterol.

Table 5. Correlation of neck circumference with other anthropometric indices.

	Pearson's correlation coefficient	p
Males		
Weight (kg)	0.744	<0.001
Waist-to-hip ratio	6.616	<0.001
Waist-to-height ratio	0.751	<0.001
Body mass index (kg/m^2)	0.726	<0.001
Waist circumference (cm)	0.751	<0.001
Females		
Weight (kg)	0.688	<0.001
Waist-to-hip ratio	0.342	0.009
Waist-to-height ratio	0.741	<0.001
Body mass index (kg/m^2)	0.750	<0.001
Waist circumference (kg/m^2)	0.742	<0.001

Table 6: Area under the ROC curve of anthropometric indices which best discriminates metabolic syndrome in males and females

	Pearson's correlation coefficient	p
Males		
Neck circumference (cm)	0.860 (0.756 – 0.965)	<0.001
Waist circumference (cm)	0.873 (0.786– 0.959)	<0.001
Waist-to-height ratio	0.890 (0.809 – 0.971)	<0.001
Waist-to-hip ratio	0.819 (0.708 – 0.930)	<0.001
Body mass index (kg/m^2)	0.762 (0.645 – 0.880)	0.002
Females		
Neck circumference (cm)	0.817 (0.636 – 0.998)	0.011
Waist circumference (cm)	0.753 (0.570 – 0.936)	0.044
Waist-to-height ratio	0.716 (0.491 – 0.942)	0.085
Waist-to-hip ratio	0.657 (0.369 – 0.945)	0.211
Body mass index (kg/m^2)	0.731 (0.563 – 0.898)	0.066

DISCUSSION

This study assessed the usefulness of neck circumference in discriminating metabolic syndrome among undergraduate students from a private university. We found that neck circumference was significantly higher among participants with metabolic syndrome. Neck circumference was positively correlated with other anthropometric indices, such as waist circumference, BMI, waist-to-hip ratio, waist-to-height ratio, and components of metabolic syndrome. Lastly, neck circumference had the largest AUC among females, and comparable AUC to waist circumference and waist-to-height ratio among males.

Evaluation of the ability of neck circumference and other anthropometric indices to discriminate metabolic syndrome showed that neck circumference had a large AUC (males: 0.860, females: 0.817), which is comparable to the AUC of other anthropometric indices in our study. Our finding is similar with other studies from China, Saudi Arabia, and Turkey (7,15,20.21). However, other studies have demonstrated that BMI, waist circumference and waist-to-hip ratio discriminates metabolic syndrome better than neck circumference (14,22,23). Despite this, some authors have opined neck circumference to be a more reliable measure of central obesity and effective in predicting metabolic syndrome, because it is not affected by physical conditions, such as hunger, respiratory movement, or wearing of clothes, which are shortcomings of waist circumference and waist-to-hip ratio (15).

Our study shows that there was no consistent pattern in the correlation between neck circumference and other anthropometric indices with components of metabolic syndrome in males and females. This is similar to a Nigerian study, which showed that neck circumference was not correlated with fasting plasma glucose or systolic and diastolic blood pressure (17). Findings from the Framingham Heart Study indicated that neck circumference is correlated with high blood pressure, decreased HDL-c, increased triglyceride, and increased fasting blood glucose. Also, neck circumference was associated with Type 2 diabetes, after adjusting for BMI and waist circumference (24). Our study shows that neck circumference is correlated with blood pressure, consistent with the association of excessive fat accumulation in the neck with sleep apnea and hypertension (25).

Central obesity has been linked with the pathogenesis of the metabolic syndrome. Anthropometric indices, such as waist circumference, waist-to-hip, and waist-to-height ratio are markers of central obesity and are more correlated to visceral fat than BMI. Some studies have also demonstrated these stronger associations with cardio-metabolic risks than BMI (26,27). Our study shows a positive correlation of neck circumference with waist circumference, BMI, waist-to-hip ratio, and waist-to-height ratio, further stressing its importance as a useful marker of metabolic syndrome.

Our study is limited in some ways. First is the study design, as causality of association is limited in cross-sectional studies. Also, our small sample size and study population (undergraduates of a private University) may restrict application of our findings. There is a need for further longitudinal population-based studies to demonstrate neck circumference as a marker for metabolic syndrome. In conclusion, neck circumference has positive correlations with markers of central obesity and can discriminate metabolic syndrome.

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REFERENCES

1. Alberti KGMM, Zimmet P, Shaw J. Metabolic syndrome–a new world-wide definition. A consensus statement from the International Diabetes Federation. *Diabet Med* 2006; 23: 469–480.
2. Beydoun MA, Kuczmarski MT, Wang Y, et al. Receiver-operating characteristics of adiposity for metabolic syndrome: the Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study. *Publ Health Nutr* 2011; 14: 77-92.
3. Cornier MA, Després JP, Davis N, et al. Assessing adiposity: a scientific statement from the American Heart Association. *Circulation* 2011; 124: 1996–2019.
4. Park SH, Choi SJ, Lee KS, Park HY. Waist circumference and waist-to-height ratio as predictors of cardiovascular disease risk in Korean adults. *Circ J* 2009; 73: 1643-1650.
5. Gharipour M, Sadeghi M, Dianatkhah M, et al. The cut-off values of anthropometric indices for identifying subjects at risk for metabolic syndrome in Iranian elderly men. *J Obes* 2014; 2014: 907149.
6. WHO: Waist circumference and waist-hip ratio: Report of a WHO expert consultation, Geneva. http://apps.who.int/iris/bitstream/10665/44583/1/9789241501491_eng.pdf, 2011.
7. Kim MK, Han K, Kwon HS, et al. Normal weight obesity in Korean adults. *Clin Endocrinol (Oxf)* 2014; 80: 214-220.
8. Pasco JA, Holloway KL, Dobbins AG, et al. Body mass index and measures of body fat for defining obesity and underweight: a cross-sectional, population-based study. *BMC Obes* 2014; 1: 9.
9. Lin S, Hu L, Li P, et al. Utility of neck circumference for identifying metabolic syndrome by different definitions in Chinese subjects over 50 years old: a community-based study. *J Diabetes Res* 2018; 2018: 3708939.
10. Dunkley AJ, Stone MA, Patel N, et al Waist circumference measurement: knowledge, attitudes and barriers in patients and practitioners in a multi-ethnic population. *Fam Pract* 2009; 26: 365-371.
11. Brown RE, Randhawa AK, Canning KL, et al. Waist circumference at five common measurement sites in normal weight and overweight adults: which site is most optimal? *Clin Obes* 2018; 8: 21-29.
12. Ferretti Rde L, Cintra Ide P, Passos MA. Elevated neck circumference and associated factors in adolescents. *BMC Public Health* 2015; 15: 208.
13. Fantin F, Comellato G, Rossi AP, et al. Relationship between neck circumference, insulin resistance and arterial stiffness in overweight and obese subjects. *Eur J Prev Cardiol* 2017; 24: 1532–1540.
14. Zhou JY, Ge H, Zhu MF, et al. Neck circumference as an independent predictive contributor to cardio-metabolic syndrome. *Cardiovasc Diabetol* 2013, 12: 76.
15. Alzeidan R, Fayed A, Hersi AS, Elmorshedy H. Performance of neck circumference to predict obesity and metabolic syndrome among adult Saudis: a cross-sectional study. *BMC Obes* 2019; 6: 1.

16. Ukoha U, Jervas E, Clarice UC, et al. Study of neck circumference as a measure of obesity in South-Eastern Nigerian population. *Anthropol Open J* 2018; 3: 11-17.
17. Raimi TH, Dada SA, Solanke A. Positive association of neck circumference and cardiometabolic risk factors in Ekiti, Nigeria. *J Surg Med 2018*; 2: 218-222.
18. Ben-Noun L, Laor A. Relationship of neck circumference to cardiovascular risk factors. *Obes Res* 2003; 11: 226-231.
19. National Cholesterol Education Program (NCEP) Expert Panel on Detection and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. *Circulation* 2002; 106: 3143-3421.
20. Onat A, Hergenç G, Yüksel H, et al. Neck circumference as a measure of central obesity: associations with metabolic syndrome and obstructive sleep apnea syndrome beyond waist circumference. *Clin Nutr 2009*; 28: 46–51.
21. Yanq L, Samarasinqhe YP, Kane P, et al. Visceral adiposity is closely correlated with neck circumference and represents a significant indicator if insulin resistance in WHO grade III obesity. *Clin Endocrinol (Oxf*)2010; 73: 197–200.
22. Preis SR, Pencina MJ, D'Aqostino RB Sr, et al. Neck circumference and the development of cardiovascular disease risk factors in the Framingham heart study. *Diabetes Care* 2013, 36: e 3.
23. Stabe C, Vasques AC, Lima MM, et al. Neck circumference as a simple tool for identifying the metabolic syndrome and insulin resistance: results from the Brazilian Metabolic Syndrome Study. *Clin Endocrinol (Oxf*) 2013; 78: 874–881.
24. Preis SR, Pencina MJ, D'Aqostino RB Sr, et al. Neck circumference and the development of cardiovascular disease risk factors in the Framingham heart study. *Diabetes Care* 2013, 36: e3.
25. Simonenko VB, Goriutskii VN, Dulin PA. [The role of insulin resistance in the pathogenesis of arterial hypertension]. *Klin Med (Mosk)* 2014; 92: 27-33 [Article in Russian].
26. Vasheqhani-Farahani A, Majidzadeh-A K, Masoudkabir F, et al. Sagittal abdominal diameter to triceps skinfold thickness ratio: A novel anthropometric index to predict premature coronary atherosclerosis. *Atherosclerosis* 2013, 227:329–333.
27. Ashwell M, Gunn P, Gibson S. Waist-to-height ratio is a better screening tool than waist circumference and BMI for adult cardiometabolic risk factors: systematic review and meta-analysis. *Obes Rev* 2012, 13: 275–286.

2020 NZIMLS CALENDAR
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27-28 November	Council Meeting, Rangiora	fran@nzimls.org.nz
DATE	**SEMINARS**	**CONTACT**
5-6 November	Immunology Special Interest Group Virtual Seminar	Andrew.soepnel@waikatodhb.health.nz
20 November	Molecular Diagnostics Special Interest Group Virtual Seminar	elsa.parker@cdhb.health.nz
NZIMLS ANNUAL SCIENTIFIC MEETING AND ANNUAL GENERAL MEETING		
24-27 August 2021	Waipuna Hotel and Conference Centre, Auckland	tbathgate@adhb.govt.nz sharon@nzimls.org.nz
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January	Membership and CPD enrolment due for renewal	sharon@nzimls.org.nz
31 January	CPD points for 2020 to be entered before 31 January	cpd@nzimls.org.nz
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15 June	Material for the August Journal must be with the Editor	rob.siebers@otago.ac.nz
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8 July	Nominations close for election of officers (40 days prior to AGM)	sharon@nzimls.org.nz
26 July	Ballot papers to be with the membership (21 days prior to AGM)	sharon@nzimls.org.nz
01 August	Annual Reports and Balance Sheet to be with the membership (14 days prior to AGM)	sharon@nzimls.org.nz
09 August	Ballot papers and proxies to be with the Executive Officer (7 days prior to AGM)	sharon@nzimls.org.nz
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07 November	QMLT Examinations	membership@nzimls.org.nz

ORIGINAL ARTICLE

The process evaluation and improvement stage of ISO 15189:2012 management system standard: an implementation update

Dennis Mok, Naira Eloyan and Sharfuddin Chowdhury

ABSTRACT

Objectives: The primary aim of this paper is to provide an update on selected internationally oriented guidance documents and relevant literature at the application level that are associated with the implementation of the process evaluation and improvement stage of ISO 15189:2012.

Methods: Additional relevant internationally oriented guidance documents were identified from international organisations (Type A to Type F), segmented by the Union of International Associations, and supplemented with additional literature.

Results: Selected international organisations (*n* = 6) were found to provide relevant guidance documents (n = 19) in support of the implementation of the process evaluation and improvement stage of ISO 15189:2012. An updated list of literature (*n* = 101) has been provided for further reference.

Conclusions: The present study contributes to the medical laboratory's development and improvement of implementations of ISO 15189:2012 in areas of responsibilities by fulfilling management system and technical competence requirements to an acceptable level of conformance by using reasonably practical means that are within the medical laboratory's managerial and technical specifications.

Key words: clinical competence, compliance, conformity, ISO 15189:2012, quality improvement, total quality management.

Supplementary material for this article is available on-line at www.nzimls,org.nz/journal

N Z J Med Lab Sci 2020; 74: 194-197.

INTRODUCTION

The primary task of the International Organization for Standardization (1,p.1886) is to set global standards. The International Organization for Standardization promotes collective action among other international organisations by collaboration, such as the International Electrotechnical Commission (1,p.1729), to promote sustainable development. More specifically, the International Organization for Standardization produced International Standard ISO 15189:2012 (2), classified as a Type A management system standard (3,p.118), for supporting medical laboratories. ISO 15189:2012 is intended to be used for the purposes of accreditation (not certification), with the associated benefit that medical laboratories deliver technically competent results (4,5). Another advantage of this accreditation is the associated internationally recognised mutual recognition arrangement scheme, administered by the International Laboratory Accreditation Cooperation (1,p.1830), to promote acceptance and confidence of results.

According to the ISO 15189:2012 process-based quality management system model (6,p.85), one of the major processes is the process evaluation and improvement (PEI) stage of ISO 15189:2012 which comprises six subclauses from Clause 4 (Management requirements) of ISO 15189:2012 (2,pp.6-19) and two subclauses from Clause 5 (Technical requirements) of ISO 15189:2012 (2,pp.19-39). It has been determined that the PEI stage contains 21/119 (17 %) administrative requirements (7) and 252/1515 (17 %) conformance requirements (CRs) (8). The PEI stage contains CRs that provide direct input as referred subclauses of Subclause 4.15.2 (Review input) of ISO 15189:2012 (2,p.18). More specifically, Subclauses 4.15.2 a) to 4.15.2 i), 4.15.2 k) and 4.15.2 l) of ISO 15189:2012 (2,p.18) containing 17/25 (68 %) CRs are directly correlated with the PEI stage (9). The PEI stage fulfills an essential role in providing feedback to the strategic management stage of ISO 15189:2012 for crafting and executing strategic managerial processes (10,pp.3-37).

The main challenge of the PEI stage implementation is that the medical laboratory needs to have an acceptable level of awareness of its internal environment, especially relating to capabilities and resources (11,pp.45-84;12,pp.78-114). Understanding the complexity of such processes can ensure that the outputs are within specifications by the medical laboratory and the level of resources allocated provides evaluation and improvement actions that are both effective and efficient. Competent implementation of the PEI stage can provide relevant support to medical laboratories claiming compliance to ISO 15189:2012.

This article provides an update on internationally oriented guidance documents associated with the PEI stage at the application level in the areas of interest where the medical laboratory should make reasonably practicable effort to fulfil the relevant CRs of ISO 15189:2012. This update should be used in consultation with previously published papers in the *New Zealand Journal of Medical Laboratory Science:* 'ISO 15189:2012 implementation: an update of related international guidance documents for medical laboratory quality management' (13), 'The strategic management stage of ISO 15189:2012 management system standard: an implementation update' (14) and 'The process control, design and planning stage of ISO 15189:2012 management system standard: an implementation update' (15).

MATERIALS AND METHODS

Selection of organisations for inclusion as international organisations

The Union of International Associations classifies international organisations into 15 types (1,pp.xiii-xxi), but only organisations classified as either Type A, Type B, Type C, Type D, Type E or Type F, and published in 'Yearbook of international organizations 2019 - 2020: guide to global civil society networks' (1) were selected for inclusion. Internationally oriented national organisations, Type G (1,p.xix), were excluded from the selection.

Selection of recommended guidance documents associated with the process evaluation and improvement stage of ISO 15189:2012

This update focused on the PEI stage. The subclauses of interest included eight subclauses: Subclauses 4.8 (Resolution of complaints) (2,p.13), 4.9 (Identification and control of nonconformities) (2,pp.13-14), 4.10 (Corrective action) (2,p.14), 4.11 (Preventive action) (2,p.14), 4.12 (Continual improvement) (2,pp.14-15), 4.14 (Evaluation and audits) (2,pp.16-18), 5.6.3 (Interlaboratory comparisons) (2,pp.34-35) and 5.6.4 (Comparability of examination results) (2,p.35) of ISO 15189:2012. Internationally oriented guidance documents that could provide reasonable support for subclauses related to the PEI stage were selected for inclusion.

Selection of relevant literature associated with the process evaluation and improvement stage of ISO 15189:2012

Additional resources associated with the PEI stage were selected for inclusion. Literature presented in the previous update (13) is omitted from in this update.

RESULTS

Selected international organisations providing relevant guidance documents

This update includes additional international organisations (*n* = 4) that provide relevant guidance documents for the implementation of the PEI stage (Table 1). The full list of international organisations (*n* = 6), including the additional ones (*n* = 4), is presented in the supplementary section (Table S1).

Recommended guidance documents associated with the process evaluation and improvement stage of ISO 15189:2012

This update has identified internationally oriented guidance documents (*n* = 19) that provide relevant information for the implementation of the PEI stage (Table 2). Recommended guidance documents were identified and classified in relation to relevant ISO 15189:2012 subclauses (Table S2).

Relevant literature associated with the process evaluation and improvement stage of ISO 15189:2012

Additional references (*n* = 101) that were found to provide further relevant information for the implementation of the PEI stage were identified (Table S3).

Table 1. Additional international organisations providing relevant guidance documents in support of the process evaluation and improvement stage of ISO 15189:2012.

Organisations (*n* = 4)	**Classification** (Type A to Type F)
Cooperative on International Traceability in Analytical Chemistry	F
Eurachem	F
European Committee for Electrotechnical Standardization	D
International Union of Pure and Applied Chemistry	B

Descriptions (1, pp. xiii-xxi)**:**

Type B: universal membership organisations: from either at least 60 countries or at least 30 countries in at least two continents and with a well-balanced geographical distribution; management and policy-making organs reflect a well-balanced geographical distribution.

Type D: regionally defined membership organisations: from at least three countries within one continental or sub-continental region; management and policy-making organs reflect a well-balanced geographical distribution.

Type F: organisations having a special form: no criteria for membership; structure is non-formal, unconventional, or unusual.

DISCUSSION

Process evaluation and improvement stage of ISO 15189:2012

The PEI stage comprises Subclauses 4.8, 4.9, 4.10, 4.11, 4.12, 5.6.3 and 5.6.3 of ISO 15189:2012, containing 252/1515 (17 %) CRs. Additional international organisations (Table 1) were identified to provide further relevant guidance documents to support the implementation. The complete list of selected international organisations' (Table S1), recommended guidance documents (Table S2) and additional resources (Table S3) that could support the PEI stage implementation are presented. It is highly recommended that this update is used in conjunction with the published updates (13,15).

Subclause 4.8 (Resolution of complaints) of ISO 15189:2012

Subclause 4.8 of ISO 15189:2012 specifies that the medical laboratory must implement a documented procedure for complaints management practices.

Implications for implementers: the medical laboratory should update its practices by consulting with relevant guidance documents on good practice. The documented procedure should be updated in consultation with relevant International Standards [International Standards ISO 10001:2018 (16), ISO 10002:2018 (17), ISO 10003:2018 (18), ISO 10004:2018 (19)].

Implications for internal auditors: the internal audit process must ensure all relevant records are maintained effectively.

Subclause 4.9 (Identification and control of nonconformities) of ISO 15189:2012

Subclause 4.9 of ISO 15189:2012 specifies that the medical laboratory must manage nonconformities in all aspects of the quality management system by the implementation of a documented procedure.

Implications for implementers: no additional notes.

Implications for internal auditors: no additional notes.

Subclause 4.10 (Corrective action) of ISO 15189:2012

Subclause 4.10 of ISO 15189:2012 specifies that the medical laboratory must implement corrective action practices by the implementation of a documented procedure.

Implications for implementers: no additional notes.

Implications for internal auditors: no additional notes.

Subclause 4.11 (Preventive action) of ISO 15189:2012

Subclause 4.11 of ISO 15189:2012 specifies that the medical laboratory must implement preventive action practices by the implementation of a documented procedure.

Implications for implementers: no additional notes.

Implications for internal auditors: no additional notes.

Subclause 4.12 (Continual improvement) of ISO 15189:2012

Subclause 4.12 of ISO 15189:2012 specifies that the medical laboratory must participate in appropriate continual improvement activities pertaining to the quality management system effectiveness.

Implications for implementers: no additional notes.

Implications for internal auditors: no additional notes.

Subclause 4.14 (Evaluation and audits) of ISO 15189:2012

Subclause 4.14 of ISO 15189:2012 specifies that the medical laboratory must implement evaluation and internal audit processes to ensure continuous conformity to the quality management system.

Implications for implementers: the medical laboratory should take user feedback seriously, especially negative criticism (20,pp.422-423). User feedback can provide valuable insights into the medical laboratory's performance, as specified in Subclause 4.14.3 (Assessment of user feedback) of ISO 15189:2012 (2,pp.16-17).

The medical laboratory must take reasonable steps to conduct risk management in consultation with the latest guidance [International Standard ISO 22367:2020 (21)], as specified in Subclause 4.14.6 (Risk management) of ISO 15189:2012 (2,p.17). Ways to eliminate or minimise the risk should be evaluated according to suitability and effectively implemented.

Implications for internal auditors: the internal audit process must ensure all relevant practices are aligned with the latest auditing management guidance document [International Standard ISO 19011:2018 (22)], as specified in Subclause 4.14.5 (Internal audit) of ISO 15189:2012 (2,p.17). It is also important for the medical laboratory to employ suitably qualified internal auditors to conduct audits, as specified in Subclause 5.1.2 (Personnel qualifications) of ISO 15189:2012 (2,p.19).

Subclause 5.6.3 (Interlaboratory comparisons) of ISO 15189:2012

Subclause 5.6.3 of ISO 15189:2012 specifies that the medical laboratory must participate in interlaboratory comparison programmes appropriate to the examination process.

Implications for implementers: the medical laboratory should consult a guide relating to selection and use of proficiency testing schemes for a limited number of participants (23) prepared by the International Union of Pure and Applied Chemistry and the Cooperative on International Traceability in Analytical Chemistry, if applicable.

Implications for internal auditors: the internal audit process must ensure the performance of interlaboratory comparisons undergoes a review process with relevant staff.

Subclause 5.6.4 (Comparability of examination results) of ISO 15189:2012

Subclause 5.6.4 of ISO 15189:2012 specifies that the medical laboratory must conduct comparative analysis relating to examination results.

Implications for implementers: no additional notes.

Implications for internal auditors: no additional notes.

CONCLUSIONS

This update has provided further references to support the implementation of the medical laboratory quality management system, especially in the PEI stage of ISO 15189:2012. The PEI stage implementation plays a vital role for the medical laboratory because the level of fulfilment of CRs reflects directly on the internal ability to implement corrective action, preventive action and improvement activities. It is particularly important to note that 17/25 (68 %) CRs of Subclause 4.15.2 of ISO 15189:2012 are directly correlated to CRs of the PEI stage (9). In sum, ensuring reasonably practicable implementation of the PEI stage can ensure the relevant processes are regulated by specifications set by the medical laboratory.

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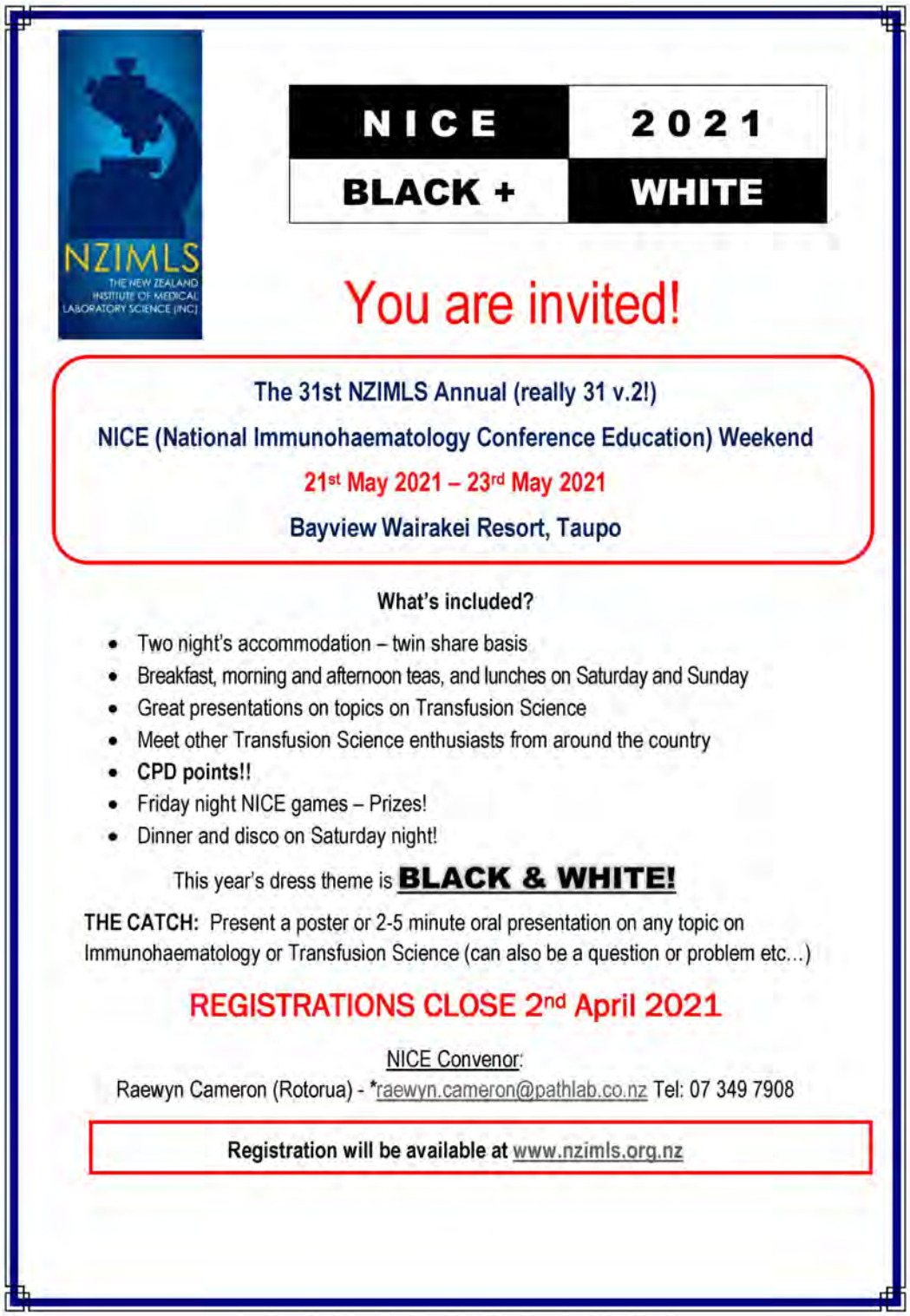
REFERENCES

1. L Van Hulle (Editor). Yearbook of International Organizations 2019 - 2020: Guide to Global Civil Society Networks. 56th edn. Vol. 1. Organization descriptions and cross‑references. Koninklijke Brill, Leiden, 2019: 2894 pp.
2. International Organization for Standardization. Medical laboratories - Requirements for quality and competence. 3rd edn. ISO 15189:2012. International Organization for Standardization, Geneva, 2014.
3. International Organization for Standardization, International Electrotechnical Commission. ISO/IEC directives, part 1: consolidated ISO supplement - Procedures specific to ISO. 11th edn. ISO/IEC DIR 1:2020. International Organization for Standardization, Geneva, 2020.
4. Özkan FZ, Pakdil F, Altuğ T. Accreditation of conformity assessment activities. In: Dahlgaard‑Park SM, Ed. The SAGE Encyclopedia of Quality and the Service Economy. Vol. 1. SAGE Publications, Thousand Oaks, 2015: 1‑5.
5. Pallas J, Bartlett J. Accreditation and certification. In: Carroll CE, ed. The SAGE Encyclopedia of Corporate eputation. Vol. 1. SAGE Publications, Thousand Oaks, 2016: 5‑7.
6. Mok D. ISO 15189:2012 implementation checklists for conformity assessment by accreditation bodies: a comparative analysis. *N Z J Med Lab Sci* 2017; 71: 84‑99.
7. Mok D, Chowdhury S. Document review checklists for ISO 15189:2012 internal auditing: an applied tool for medical laboratories. *Aust J Med Sci* 2016; 37: 48‑55.
8. Mok D, Lim E, Bingham A. Identification of ISO 15189:2012 conformance requirements for medical laboratory internal auditing. *Aust J Med Sci* 2015; 36: 2‑14.
9. Mok D, Nabulsi R, Chowdhury S. Management review input checklist for ISO 15189:2012 internal auditing: an optimisation guide for medical laboratories. *N Z J Med Lab Sci* 2020; 74: 17‑21.
10. Gandellini G, Pezzi A, Venanzi D. Strategy for Action—II: Strategy Formulation, Development, and Control. SpringerBriefs in business. Springer‑Verlag Mailand, Milan, 2013.
11. Gandellini G, Pezzi A, Venanzi D. Strategy for Action—I: the Logic and Context of Strategic Management. SpringerBriefs in business. Springer‑Verlag Mailand, Milan, 2012.
12. Thompson AA Jr, Peteraf MA, Gamble JE, Strickland III AJ. Crafting and Executing Strategy: the Quest for Competitive Advantage: Concepts and Cases. 22nd edn. McGraw‑Hill Education, New York, 2019.
13. Mok D, Ang E. ISO 15189:2012 implementation: an update of related international standards and guidance documents for medical laboratory quality management. *N Z J Med Lab Sci* 2016; 70: 42‑66.
14. Mok D, Chowdhury S. The strategic management stage of ISO 15189:2012 management system standard: an implementation update. *N Z J Med Lab Sci* 2019; 73: 111‑115.
15. Mok D, Chowdhury S. The process control, design and planning stage of ISO 15189:2012 management system standard: an implementation update. *N Z J Med Lab Sci* 2020; 74: 103‑106.
16. International Organization for Standardization. Quality Management — Customer Satisfaction — Guidelines for Codes of Conduct for Organizations. 2nd edn. ISO 10001:2018. International Organization for Standardization, Geneva, 2018.
17. International Organization for Standardization. Quality Management — Customer Satisfaction — Guidelines for Complaints Handling in Organizations. 3rd edn. ISO 10002:2018. International Organization for Standardization, Geneva, 2018.

18. International Organization for Standardization. Quality management — Customer Satisfaction — Guidelines for Dispute Resolution External to Organizations. 2nd edn. ISO 10003:2018. International Organization for Standardization, Geneva, 2018.
19. International Organization for Standardization. Quality Management — Customer Satisfaction — Guidelines for Monitoring and Measuring. 2nd edn. ISO 10004:2018. International Organization for Standardization, Geneva, 2018.
20. SD Hill (Editor). Encyclopedia of Management. 8th edn. Vol. 1. Cengage, Farmington Hills, 2019: 1218 pp.
21. International Organization for Standardization. Medical Laboratories — Application of Risk Management to Medical Laboratories. ISO 22367:2020. International Organization for Standardization, Geneva, 2020.
22. International Organization for Standardization. Guidelines for Auditing Management Systems. 3rd edn. ISO 19011:2018. International Organization for Standardization, Geneva, 2018.
23. Kuselman I, Fajgelj A. IUPAC/CITAC guide: selection and use of proficiency testing schemes for a limited number of participants—chemical analytical laboratories (IUPAC Technical Report). *Pure Appl Chem* 2016; 82: 1099‑1135.



ORIGINAL ARTICLE

BRCA2 mutations associated with invasive lobular breast carcinoma

Husam A Khalil

ABSTRACT

Objective: The study was designed to investigate BRCA2 mutations in Syrian familial breast cancer cases.
Methods: Twenty-five early onset invasive breast cancer patients from different Syrian families (24 females and 1 male) and 10 healthy women as a control group were included in the study. All participants were matched in age (28 to 49 years). 64% of the patients had a significant family history of breast cancer. Twenty-one of the breast cancer patients had the ductal molecular subtype of breast carcinoma while the remaining four patients had the lobular subtype of the disease. The clinical profiles of volunteer patients were compared including tumour hormone receptor expression DNA was extracted from peripheral leukocytes using venous blood samples obtained from all study participants and then polymerase chain reaction (PCR) was performed to amplify specific regions: exon 9 and exon 11 of BRCA2 gene. PCR products were sequenced to investigate any genetic variants present.
Results: Sequencing of PCR products revealed five variants including two-point mutations and three deletions. To our knowledge, the identified mutations have not previously been reported.
Conclusions: BRCA2 mutations may be more likely to be correlated with lobular subtype of invasive breast carcinoma. Also, invasive lobular carcinoma may tend to be associated with hormone receptor positive tumours, and positive estrogen and progesterone receptors.
Key words: invasive lobular carcinoma, invasive ductal carcinoma, BRCA2, Syria, subtype.

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INTRODUCTION

Invasive breast cancers have two major histological subtypes, invasive ductal carcinoma and invasive lobular carcinoma (1). Invasive ductal carcinoma is the most common subtype forming about 90% of all invasive breast tumours, while invasive lobular carcinoma is responsible of about 10% of invasive breast carcinomas (1). Lobular and ductal subtypes are different concerning risk factors, although these differences are underestimated as physicians tend to treat all subtypes in the same way.

In comparison to ductal tumours, lobular tumours tend to be diagnosed at older ages with both a bigger tumours size and a more progressed tumours stage. Also, lobular tumours are often more hormone receptor positive (95% of cases express estrogen receptor and 60 to 70% of cases express progesterone receptor) (2-4), while they tend to be HER2 (type 2 receptors of human epidermal growth factor) receptor negative (4). This suggests a better association of lobular tumours with hormone related risk factors of breast cancer (1). Clinical features of invasive lobular carcinoma tumours show a good prognosis as they are often low grade, although they can be strongly metastatic (5). There have been several reports confirming familial clustering of invasive lobular tumours (6).

When tested, some high-penetrance breast cancer susceptibility genes show a significant association with molecular subtype of breast cancer. Germline mutations in BRCA1 and TP53 are predominantly correlated with invasive ductal carcinoma, while BRCA2 mutations are associated with both lobular and ductal carcinomas (7,8). The relative risk of breast cancer for a post-menopausal woman receiving combined hormonal replacement therapy is higher for that of invasive lobular carcinoma than for invasive ductal carcinomas, as suggested by several studies (9,10).

METHODS

A selected group of 25 familial breast cancer patients (24 females and 1 male) from different Syrian families who attended the Syrian Arab Red Crescent Hospital in Damascus, Syria for treatment during the last six months of 2018 were recruited for a prospective cross-sectional study.

An age-matched control group of 10 healthy women who had neither cancer, nor a family history of any type of cancer, were included in the study (age: 28-49 years). The study was approved by Scientific Research Ethics Committee at the Faculty of Pharmacy, University of Damascus, Syria and all participants gave written informed consent.

All patients had one at least of the following criteria:

- having an early onset age at diagnosis (before the age of 50).
- having bilateral breast cancer
- presence of breast and/or ovarian cancer in two or more relatives (on the same side of the family, either father or mother)
- male breast cancer (11).

All breast cancer patients had early onset invasive breast carcinoma, and 21/25 (84%) patients had a ductal molecular subtype of breast cancer while 4/25 (16%) of patients had the lobular subtype of the disease. Clinical data of breast cancer patients, including their hormone receptor status, were collected from their medical records in the Syrian Arab Red Crescent Hospital (Table1). The responsible clinician had been informed of cases in which a harmful genetic variant was detected in the patient. Venous blood samples were collected using EDTA as anticoagulant. All samples were frozen at -80°C and stored until time of DNA extraction.

Genomic DNA was isolated from peripheral leukocytes using a blood DNA isolation kit (Fermentas/ThermoFisher Scientific, Massachusetts, USA). Isolated DNA was checked for both purity and yield using spectrophotometric assays. PCR was performed after validation on isolated DNA using specific primers (Eurofins, UK). The primers 9F (forward) and 9R (reverse) were adopted from Bensam *et al*. (12) to amplify exon 9 of BRCA2 gene, while primers 11F (forward) and 11R (reverse) were designed using Oligo Analyzer 3.1 tool (http://eu.idtdna.com/calc/analyzer) to amplify region from 32,340,082 to 32,340, 423 in exon 11 of BRCA2 gene. Table 2 shows primer sequences of targeted regions in this study.

Table 1. Clinical data of breast cancer patients.

Patient	Age at diagnosis (years)	Tumours		Receptor status			Pathological type	Lymph node metastasis	Family history
		Grade (1-3)	Size	ER	PR	Her2			
1	47	1	10 mm	+	+	+	Invasive lobular carcinoma	+	-
2	30	2	25 mm	-	-	+	Invasive ductal carcinoma	+ + +	+ +
3	39	1	23 mm	-	-	-	Invasive ductal carcinoma	+	+
4	49	3	35 mm	-	-	-	Invasive ductal carcinoma	-	+
5	44	2	30 mm	-	+	+	Invasive ductal carcinoma	+	+ +
6	29	2	27 mm	+	+	+	Invasive ductal carcinoma	+	+
7	33	3	15 mm	+	-	+	Invasive ductal carcinoma	+ + +	-
8	35	3	70 mm	-	-	+ + +	Invasive ductal carcinoma	+ +	+ +
9	42	2	40 mm	-	-	-	Invasive lobular carcinoma	+ +	+ +
10	35	2	55 mm	+	+	-	Invasive lobular carcinoma	+	-
11	45	3	65 mm	+	+	-	Invasive ductal carcinoma	+	+ +
12	48	2	25 mm	-	-	+	Invasive ductal carcinoma	+	+
13	44	3	10 mm	+	+	-	Invasive ductal carcinoma	-	+
14	37	2	22 mm	-	-	++	Invasice ductal carcinoma	-	-
15	37	3	20 mm	+	+		Invasive lobular carcinoma	++	+
16	44	3	50 mm	-	+	+	Invasive ductal carcinoma	++	-
17	39	3	40 mm	-	-	+	Invasive ductal carcinoma	++	-
18	47	2	35 mm	++ ++	++ ++	++ +	Invasive ductal carcinoma	+++	+
19	42	2	23 mm	-	-	+	Invasive ductal carcinoma	++	-
20	41	3	30 mm	-	-	+	Invasive ductal carcinoma	-	+++
21	43	1	20 mm	-	-	++ +	Invasive ductal carcinoma	+	-
22	48	2	44 mm	-	-	+	Invasice ductal carcinoma	++	++
23	42	1	20 mm	-	-	++	Invasive ductal carcinoma	+	++
24	49		75 mm	++	++	-	Invasive ductal carcinoma	++	-
25	28	3	40 mm	-	-	-	Invasive ductal carcinoma	+	++

Table 2. 5´-3´sequences of primers 9F, 9R for exon 9 of BRCA2 gene and primers 11F and 11R for exon 11 of the same gene.

Primer	5'-3' sequence	GC content	Annealing temperature °C	Product size (bp)	Reference
Exon 9F	CATCACACTACTCAGGATGACA	45.45%	60°C	490	(1)
Exon 9R	GCATGGTGGTGCATGCTTGTA	52.38%			
Exon 11F	AATGATGAATGTAGCACGC	42.1 %	56°C	341	Designed
Exon 11R	GTCTGAATGTTCGTTACT	38.9 %			

PCR was performed in a final volume of 25 µL containing 100 ng of genomic DNA, 10× TBE buffer, DNTPs, $MgCl_2$, Taq polymerase, and primers at 95°C for 5 min, followed by 35 cycles of 30 seconds at 95°C, 20 seconds at annealing temperature, 30 seconds at 72°C, and then one cycle for final extension at 72°C for 7 min. Table 3 shows the components of the final reaction mixture and their corresponding quantities.

PCR products were sequenced using Big Dye Terminator v3.1 Cycle Sequencing Kit and ABI PRISM® 310 Genetic Analyzer (Applied Biosystems, USA). Sequencing was achieved using 9F, 9R, 11F and 11R primers and products were read using Geneious program and the obtained sequences were analyzed and compared to the NCBI reference sequence of the homo sapiens BRCA2 repair associated (BRCA2), mRNA (NM_000059.3) using BLAST tool at the website National Center for Biotechnology Information (NCBI).

Detected variations were documented and analysed using database concerning the previously identified mutations of BRCA genes (BRCA Mutation Database). IBM SPSS Statistics 18 was used for statistical analysis. A P-value < 0.05 was regarded as statistically significant.

Table 3. Components of the final reaction mixture and their corresponding quantities.

Volume	Concentration	
×	100 ng/µl	DNA Template
2.5 µl	10x	TBE Buffer
2 µl	2.5 mmol	DNTPs
1 µl	25 mmol	$MgCl_2$
0.2 µl	5 U/µl	Taq Polymerase
1 µl of working stock solution	10 µmol	Forward Primer
1 µl of working stock solution	10 µmol	Reverse Primer
up to 25 µl		Nuclease free water

RESULTS AND DISCUSSION

Exon 11 mutations were found in two out of 25 of breast cancer patients. Three mutations were also found in exon 9 of BRCA2 gene in three out of 25 of breast cancer patients. The detected mutations involved two deletions in exon 11 of BRCA2 gene; NM_000059.3:c.6206delT and NM_000059.3:c.6283delT, one deletion in exon 9 of BRCA2 gene; NM_000059.3:c.1203delA and two substitutions in exon 9 of BRCA2 gene; NM_000059.3:c.1014T>C and NM_000059.3:c.1147A>T. All detected sequence variants were named based on HGVS nomenclature rules.

To our knowledge, the identified mutations have not been identified previously. BRCA2 mutations were more frequent in lobular subtype of invasive breast carcinoma than in ductal subtype of the disease (three out of five BRCA2 mutation carrier patients had an invasive lobular carcinoma), although ductal molecular subtype was the most prevalent among breast cancer patients in our study. Mutations of BRCA2 were significantly associated with the lobular subtype of invasive breast carcinoma (Pearson Chi Square, P=0.003) as shown in Tables 4 and 5. Our results are consistent with those of Mavaddat *et al*. (13), as they found that lobular tumours were more frequent among BRCA2 mutation carriers compared to BRCA1 mutation carriers. These authors concluded that lobular tumours were more likely to be associated with BRCA2 mutations.

Table 4. Cross-tabulation of BRCA2 gene mutation * Molecular subtype of breast cancer.

			Mutation of BRCA2		
			Normal	Mutant	Total
Molecular subtype of breast cancer	Invasive ductal carcinoma*	Count	19	2	21
		Expected count	16.8	4.2	21.0
		% within mutation of BRCA2	95.0%	40.0%	84.0%
	Invasive lobular carcinoma**	Count	1	3	4
		Expected count	3.2	.8	4.0
		% within mutation of BRCA2	5.0%	60.0%	16.0%
Total		Count	20	5	25
		Expected count	20.0	5.0	25.0
		% within mutation of BRCA2	100.0%	100.0%	100.0%

* Invasive ductal carcinoma. **Invasive lobular carcinoma.

Table 5. Chi-Square test.

	Value	df	Asymp. significance (2-sided)	Exact significance (2-sided)	Exact significance (1-sided)
Pearson Chi-Square	9.003[a]	1	.003		
Continuity correction[b]	5.376	1	.020		
Likelihood ratio	7.313	1	.007		
Fisher's exact test				.016	.016
Linear-by-linear Association	8.643	1	.003		
N of valid cases	25				

[a]Three cells (75.0%) have expected count less than 5. The minimum expected count is 0.80. [b]Computed only for a 2x2 table.

A significant correlation was also found between lobular molecular subtype of breast tumours and positivity of hormone receptors (estrogen receptors and progesterone receptors), (Pearson Chi Square, P=0.044, as shown in Tables 6 and 7). Our results are consistent with Cristofanili *et al.* (14) who found that hormone receptor positive tumours were more frequent in invasive lobular carcinoma patients than in invasive ductal carcinoma patients Also, our results are consistent with Arpino *et al.* (15) who found that lobular breast cancers were more likely to be hormone receptor positive, both estrogen receptor and progesterone receptor are positive.

Table 6. cross-tabulation of positivity of hormone receptors * Molecular subtype of breast cancer.

			Molecular subtype of breast cancer		Total
			Invasive lobular carcinoma	Invasive ductal carcinoma	
Positivity of hormone receptors (ER, PR)	ER+ , PR+	Count	3	5	8
		Expected count	1.3	6.7	8.0
		% within molecular subtype of breast cancer (invasive ductal carcinoma or invasive lobular carcinoma)	75.0%	23.8%	32.0%
	ER- , PR-	Count	1	16	17
		Expected count	2.7	14.3	17.0
		% within Molecular subtype of breast cancer (invasive ductal carcinoma or invasive lobular carcinoma)	25.0%	76.2%	68.0%
Total		Count	4	21	25
		Expected count	4.0	21.0	25.0
		% within molecular subtype of breast cancer (invasive ductal carcinoma or invasive lobular carcinoma)	100.0%	100.0%	100.0%

ER: estrogen receptor. PR: progesterone receptor.

Table 7: Chi-Square test.

	Value	df	Asymp. significance (2-sided)	Exact significance (2-sided)	Exact significance (1-sided)
Pearson Chi-Square	4.046[a]	1	.044		
Continuity Correction[b]	2.036	1	.154		
Likelihood Ratio	3.792	1	.051		
Fisher's Exact Test				.081	.081
Linear-by-Linear Association	3.884	1	.049		
N of Valid Cases	25				

[a]Two cells (50.0%) have expected count less than 5. The minimum expected count is 1.28.

[b]Computed only for a 2x2 table.

Our data adds to the growing body of evidence suggesting that BRCA2 mutations may be more likely to be correlated with the lobular subtype of invasive breast carcinoma. Our data also suggests that invasive lobular carcinoma may tend to be correlated with hormone receptor positive tumours (positive estrogen and progesterone receptors). Therefore, a stronger association of this subtype of breast cancer with hormonal risk factors (endogenous and exogenous estrogen prolonged exposure) as compared to the ductal subtype may be predicted. Lobular breast cancer is associated with balletic mutations in the CDH1 gene.

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REFERENCES

1. Dossus L, Benusiglio PR. Lobular breast cancer: incidence and genetic and non-genetic risk factors. *Breast Cancer Res* 2015; 17: 37.
2. Li CI, Uribe DJ, Daling JR. Clinical characteristics of different histologic types of breast cancer. *Br J Cancer* 2005; 93: 1046–1052.
3. McCart Reed AE, Kutasovic JR, Lakhani SR, Simpson PT. Invasive lobular carcinoma of the breast: morphology, biomarkers and 'omics. *Breast Cancer Res* 2015; 17: 12.
4. Arpino G, Bardou VJ, Clark GM, Elledge RM. Infiltrating lobular carcinoma of the breast: tumor characteristics and clinical outcome. *Breast Cancer Res* 2004; 6: R149–R156.
5. Shin SJ, Lal A, De Vries S, et al. Florid lobular carcinoma in situ: molecular profiling and comparison to classic lobular carcinoma in situ and pleomorphic lobular carcinoma in situ. *Hum Pathol* 2013; 44: 1998–2009.
6. Xin LJJ, Eng LG. A review of invasive lobular carcinoma of the breast: Should it be treated like invasive ductal carcinoma? *Integr Cancer Sci Therap* 2016; 3: DOI: 10.15761/ICST.1000211..
7. Tan MH, Mester JL, Ngeow J, et al. Lifetime cancer risks in individuals with germline PTEN mutations. *Clinical Cancer Res* 2012; 18: 400–407.
8. van Lier MG, Wagner A, Mathus-Vliegen EM, et al. High cancer risk in Peutz-Jeghers syndrome: a systematic review and surveillance recommendations. *Am J Gastroenterol* 2010; 105: 1258–1264.
9. Flesch-Janys D, Slanger T, Mutschelknauss E, et al. Risk of different histological types of postmenopausal breast cancer by type and regimen of menopausal hormone therapy. *Int J Cancer* 2008; 123: 933-941.
10. Fournier A, Fabre A, Mesrine S, et al. Use of different postmenopausal hormone therapies and risk of histology- and hormone receptor-defined invasive breast cancer. *J Clin Oncol* 2008; 26: 1260-1268.
11. Dobricić J, Branković-Magić M, Filipović S, Radulović S. Novel BRCA1/2 mutations in Serbian breast and breast-ovarian cancer patients with hereditary predisposition. *Cancer Genet Cytogenet* 2010; 202: 27-32.
12. Bensam M, Hafez E, Awad D, et al. Detection of new point mutations of BRCA1 and BRCA2 in breast cancer patients. *Biochem Genet* 2013; 52: 15-28.
13. Mavaddat N, Barrowdale D, Andrulis IL, et al. Pathology of breast and ovarian cancers among BRCA1and BRCA2 mutation carriers: results from the Consortium of Investigators of Modifiers of BRCA1/2(CIMBA). *Cancer Epidemiol Biomarkers Prev* 2012; 21: 134–147.
14. Cristofanili M, Gonzalez-Angulo A, Sneige N, et al. Invasive lobular carcinoma classic type: response to primary chemotherapy and survival outcomes. *J Clin Oncol* 2005; 23: 41-48.
15. Arapino G, Bardou VJ, Clark GM, Elledge RM. Infiltrating lobular carcinoma of the breast: tumor characteristics and clinical outcome. *Breast Cancer Res* 2004; 6: R149-R156.

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Their decision will be final and no correspondence will be entered into.

ORIGINAL ARTICLE

Antibacterial effects of unconjugated and silver nanoparticle-conjugated drugs that are clinically used against central nervous system disorders

Ruqaiyyah Siddiqui, Abdulkader Masri*, Ayaz Anwar and Naveed Ahmed Khan*

ABSTRACT

Background: The emergence of drug resistance and limited development of newer antibacterials is of serious concern. Furthermore, the invention of novel composites, specifically those that can efficiently traverse the blood-brain barrier to access the central nervous system are scarce. Nanotechnology is a strategy that can be employed to enhance drug delivery and efficacy against infections due to multi-drug resistant bacteria. Given challenges of drug discovery and development, drug repurposing is a useful avenue. Hence, it is logical to test antibacterial effects of currently marketed drugs for central nervous system (CNS) diseases.

Methods: Here, we selected drugs that are clinically used for CNS disorders and are effective in penetrating the blood-brain barrier. These were phenobarbitone, phenytoin, and levetiracetam. These drugs were tested in their available formulation and following conjugation with silver nanoparticles against neuropathogenic *Escherichia coli* and methicillin-resistant *Staphylococcus aureus*. The drugs were successfully conjugated with silver and formulations were verified using ultraviolet-visible spectrophotometry, atomic force microscopy, and Fourier transformation infrared spectroscopy.

Results: The results showed that selective drug-conjugated silver nanoparticles and silver nanoparticles displayed bactericidal effects when compared to the drugs alone. Of note, drug-conjugated silver nanoparticles inhibited bacterial-mediated host cell death. When incubated with human cells, both the drugs and drug-conjugated silver nanoparticles showed minimal toxic effects.

Conclusions: These findings suggest that drug repurposing is a viable approach and currently available CNS drugs should be tested following conjugation with nanoparticles against CNS pathogens, as they are clinically approved with established pharmacokinetics and are able to permeate the blood-brain barrier, suggesting their potential translational value.

Keywords: CNS drugs, antibacterial, silver nanoparticles, gold nanoparticles.

*These authors contributed equally to this work.

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INTRODUCTION

The emergence of drug resistance is a major concern in the treatment of bacterial infections leading to increased morbidity and mortality, despite advances in antimicrobial chemotherapy and supportive care (1-3). In particular, involvement of the central nervous system (CNS) can result in complications with serious consequences. Even with treatment, neonatal meningitis can lead to developmental disability (1-5), suggesting the need to develop more effective therapies. Other bacterial pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA), are frequently associated with pneumonia, urinary tract, gastrointestinal tract, and wound infections (6-9). Again, emergence of drug resistance, and possible involvement of the CNS, leads to poor prognosis, highlighting the need to find new therapeutic agents. As the discovery of novel drugs is a laborious and expensive process and new agents must be tested for unanticipated side effects through several layers of clinical trials (10), repurposing of drugs is a viable option. It is logical to elucidate the effects of currently marketed drugs for CNS diseases against bacterial pathogens and was the purpose of our study.

Nanosized particles have been reported to be efficient therapeutic substances that are able to cope with organ barriers, such as the blood-brain barrier (11). Due to their miniscule size and the utilisation of smaller concentrations, minimum side effects are anticipated (12). Nanoparticles can pass by transcytosis of capillary endothelial cells into the CNS or into other critical tissues (13). Furthermore, metal nanoparticles, such as silver nanoparticles (AgNPs), have been postulated as the next generation of antimicrobials ascribed to their recognised attraction for the DNA of microbes and production of reactive chemical species containing oxygen (14).

The purpose of our study was to seek the antibacterial effects of clinically available CNS drugs against Gram-negative neuropathogenic *E. coli* K1 and Gram-positive MRSA as model organisms (15). Clinically used drugs that are commercially available for CNS disorders were assessed for their antibacterial effects as they are recognised for efficient blood-brain barrier penetration. The drugs selected are known to target ion channels and included: phenobarbitone (Luminal), phenytoin (Dilantin), and levetiracetam (Spritam). These were conjugated with silver (Ag) to formulate drug conjugated AgNP and tested for antibacterial effects against the selected Gram-negative and Gram-positive pathogenic bacteria, and their cytotoxicity against host cells was tested.

MATERIALS AND METHODS

All chemicals and reagents were obtained from Sigma Laboratories (Poole, Dorset, England, UK), unless otherwise stated.

Cultivation of bacteria

Bacteria used in this study included, *E. coli* K1 strain E44 (018:K1:H7); obtained from the cerebrospinal fluid of a patient suffering from meningitis (Malaysian Type Culture Collection, MTCC 710859). MRSA (MTCC 381123) was obtained from blood cultures provided by the Luton & Dunstable Hospital NHS Foundation Trust, Luton, England, UK. Bacterial stocks were maintained at 4°C and sub-cultured every two weeks on nutrient agar plates prior to experimentation (16).

Clinically used drugs and their nanoparticle formulation using metal conjugation

The following drugs were selected for the study: phenobarbitone, phenytoin, and levetiracetam (barbiturate, hydantoin, and pyrrolidine respectively) purchased from the local pharmacy. The synthesis of phenobarbitone, phenytoin, and levetiracetam conjugated with silver nanoparticles was accomplished *via* reduction of $AgNO_3$ by sodium borohydride ($NaBH_4$) coupled with the drugs, as described previously (17). Five mL (1 mM) $AgNO_3$ aqueous solution was magnetically mixed with 1 mL (1 mM) water mixture of phenobarbitone for a duration of 10 minutes. Next, 5 µL of 5 mM $NaBH_4$ aqueous solution was supplemented to the reaction mixture as a reducing agent. The formation of phenobarbitone-conjugated AgNPs in the reaction mixture was signaled by the occurrence of a yellow brown color. In the same way, phenytoin and levetiracetam nanoconjugates were produced. To obtain stable suspensions, different volume ratios of metal and drugs solution were used. Phenytoin-conjugated AgNPs-, and levetiracetam-conjugated AgNPs were steadied at 1:1 v/v ratio. Similarly, unchanged AgNPs were produced using the same process, however, without any capping agent. Next, nanoparticles were centrifuged for 30 minutes at 10000 x g to separate unreacted reagents and by-products. Next, supernatants were removed and the nanoconjugate pellets were resuspended in 1 mL water. Following the successful synthesis, nanoconjugates were characterised and verified using ultraviolet visible spectrophotometry (UV-Vis) using a Thermo Scientific evolution 201 spectrophotometer, atomic force microscopy (Agilent 5500, Arizona, USA), and Fourier transformation infrared (FT-IR) (IRTracer-100, SHIMADZU, Japan), as previously described (18).

HeLa cell cultivation

HeLa cervical cancer cells were obtained from the American Type Culture Collection (ATCC®CCL-2). Cells were cultured in Roswell Park Memorial Institute (RPMI 1640) containing 1% of non-essential amino acid, L-glutamine, and penicillin (100 U/ mL)-streptomycin (100µg/mL), and 10% fetal bovine serum, as previously described (19). The cells were cultured at 37°C in a 5% CO_2 incubator with humidity levels of more than 95% until the formation of HeLa cells monolayers, monitored by inverted microscopy. For cytotoxicity and cytopathogenicity assays, cells were seeded on to 96-well plates with an initial inoculum of 10^5 cells/well until uniform monolayers were observed. These cells were used for subsequent cytotoxicity and cytopathogenicity assays.

Cytotoxicity assays

To determine the toxic effects of drugs and their nanoparticle formulations, cytotoxicity assays were performed as described previously (20). Briefly, cells were grown in 96-well plates until confluency as described above. Next, drugs and their nanoparticle formulations were added at various concentrations. The plates were incubated at 37°C for 24 hours in a 5% CO_2 incubator with more than 95% humidity. Following incubation, supernatants were collected from each well and lactate dehydrogenase (LDH) release was estimated using a cytotoxicity detection assay kit (Invitrogen Life Technologies, Carlsbad, CA, USA) (20). LDH is an intracellular enzyme and only released when cells are damaged. The following equation was utilised for cytotoxicity percentage determination: % cytotoxicity = (Abs (490nm) of sample treated well – Abs (490nm) of untreated well) / (Abs (490nm) of cells treated with Triton X-100 – Abs (490nm) of untreated well) × 100.

Cytopathogenicity assays

To determine whether drugs alone, and drug-conjugated AgNPs, exhibit bacterial-mediated host cell cytotoxicity, cytopathogenicity assays were performed (20). Briefly, bacteria were grown overnight at 37°C. Next day, the optical density (OD) of bacterial cultures was adjusted to 0.22 at OD_{595nm} (the McFarland standard) that equates to 10^8 colony-forming units per mL (CFU/mL). The precise number of bacterial CFU was determined by plating on nutrient agar plates using serial dilution assay as described previously (18). Next, 10 µL of bacterial cultures (approximately 10^6 CFU) was incubated with and without drugs alone, or drug conjugated AgNPs at various concentrations in 1.5 mL centrifuge tubes for two hours at 37°C. For negative controls, untreated bacteria were incubated with phosphate buffered saline (PBS). Likewise, for the positive control, bacteria were incubated with 100 µg/mL of gentamicin. Next, pre-treated and untreated bacteria were inoculated onto human cells grown in 96-well plates. The plates were incubated at 37°C for 24 hours in a 5% CO_2 incubator with more than 95% humidity. Following incubation, supernatants were collected and LDH release was determined using a cytotoxicity detection assay kit (20). The following equation was utilised for cytotoxicity percentage determination: % cytotoxicity = (Abs of sample treated well – Abs of untreated well) / (Abs of cells treated with Triton X-100 – Abs of untreated well) × 100.

Bactericidal assays

The antibacterial potential of drugs alone, and drug conjugated AgNPs were determined using bactericidal assays (19). The OD_{595nm} of overnight grown bacterial cultures were adjusted to 0.22 (equal to 10^8 CFU/mL). Next, 10 µL of bacterial cultures (approximately 10^6 CFU) were incubated with drugs alone or drug conjugated AgNPs, at various concentrations in 1.5 mL centrifuge tubes for two hours at 37°C. For negative controls, bacteria were incubated with PBS. Likewise, for the positive control bacteria were incubated with 100 µg/mL of gentamicin. Next, bacterial CFU were enumerated using serial dilution assay by plating 10 µL of each dilution on nutrient agar plates. Plates were incubated at 37°C for 24 hours and colonies enumerated the following day.

Statistical analysis

The data are demonstrative of the mean ± standard error of at least three independent experiments performed in duplicate. Statistical significance for differences was accomplished using a 2-sample t-test; two-tailed distribution, comparing the mean of two different experiments repeated using similar conditions. P values of <0.05, <0.01, and <0.005 were employed for analysis.

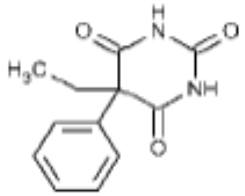
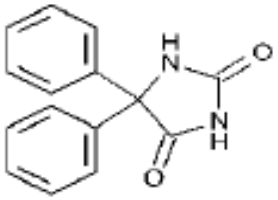
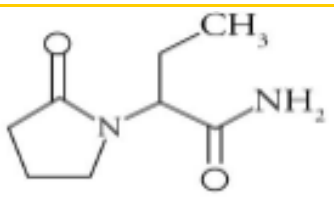
RESULTS

Characterisation of phenobarbitone, phenytoin, and levetiracetam conjugated silver nanoparticles by UV-Vis, AFM, and FT-IR spectroscopy

Drugs conjugated with AgNPs were characterised. Drugs conjugated with AgNPs included: phenobarbitone-AgNPs, phenytoin-AgNPs, and levetiracetam-AgNPs. The spectra of UV-Vis spectra of the drugs-nanoconjugate revealed a typical surface plasmon resonance (SPR) band for AgNPs at 410, 430, and 440 nm respectively, which indicates the formation of the nanoconjugate (Figure 1). Atomic force microscopy was used to determine the shape and size of the drug-conjugated AgNPs. The three nanoconjugates were observed to be sphere-shaped with a wide size distribution (20-250 nm) attributed to the quick reduction by using $NaBH_4$ (Figure 2).

The relative FT-IR spectral evaluation of phenytoin alone with phenytoin-AgNPs was conducted to detect the stabilising chemical functional groups in phenytoin as a representative case. Distinctive signals corresponding to the chemical groups present in phenytoin appeared in the FTIR spectrum for phenytoin. There were peaks located at1601and 1510 cm^{-1}, caused by the stretching mode (C=C) of the aromatic ring. The amide group (C=O) appeared as separate peaks at 1640 and 1688 cm^{-1}. The peak at 3322 cm^{-1} was due to the N-H stretch, while the peak at 1400 cm^{-1} was due to N-H bending (21). Conjugation of phenytoin to the AgNPs caused a shift in the stretch N-H extending changed from 3322 to 3410, whereas the N-H bending signal was remarked with a minor shift at 1365 cm^1, suggesting the participation of the chemical functional group (N-H) which exists in the hydantoin ring in the stabilisation of the AgNPs.

Table 1. Structures and brief mode of action of Phenobarbitone, Phenytoin, and Levetiracetam

Drugs	Uses and Mechanism of action	Structure
Phenobarbital Molar mass: 232.239 g/mol	Phenobarbitone Used as antiepileptic, anti-seizures and no antibacterial effects reported yet	
Phenytoin Molar mass: 252.268 g/mol	Phenytoin is an anti -seizure medication, there are studies showed antibacterial activity of iodinated phenytoin and phenytoin derivatives (N-substituted acyclic nucleoside analogues) but not for standard phenytoin	
Levetiracetam Molar mass: 170.212 g/mol	Levetiracetam used to treat epilepsy. and no antibacterial effects reported yet	

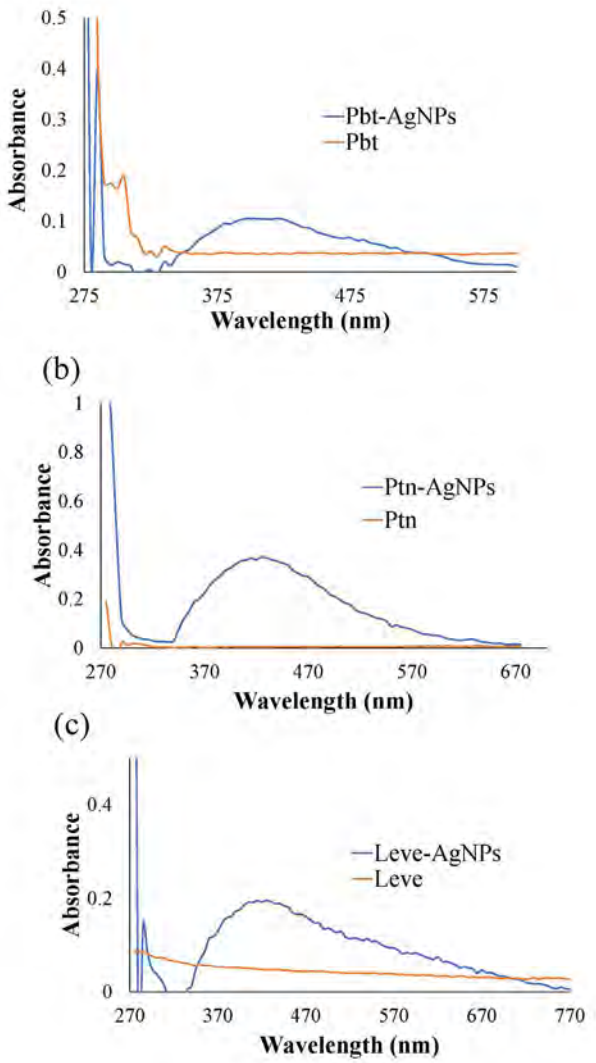


Figure 1. UV-visible spectra of drugs conjugated AgNPs and AuNPs. UV-visible spectra were recorded at UV-Vis spectrophotometer. Drugs conjugated AgNPs showed characteristic surface plasmon resonance band between 400-450 nm.

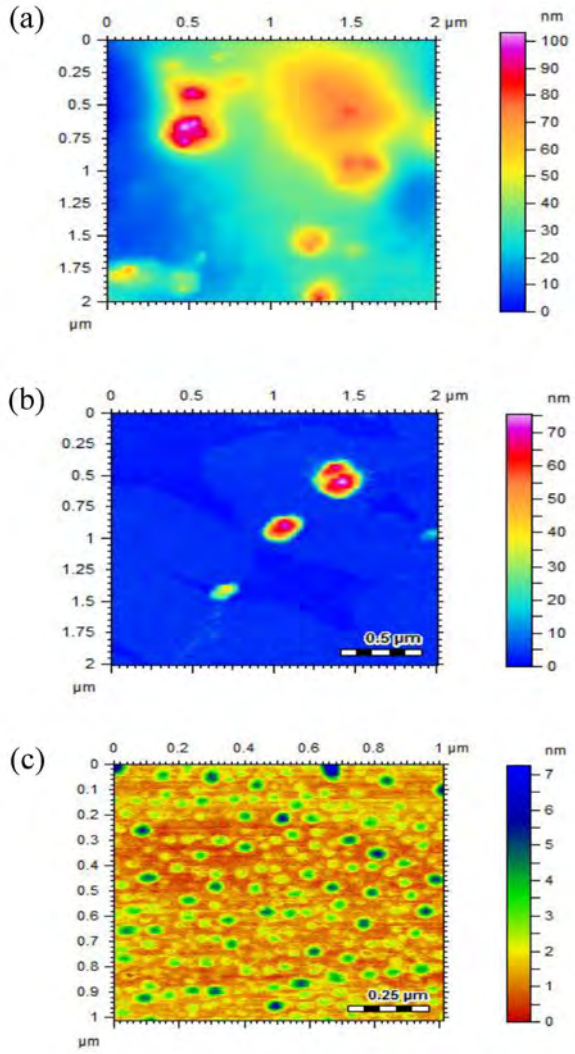


Figure 2. Atomic force microscopy topographic images; (a) corresponds to phenobarbitone-AgNPs, (b) phenytoin-AgNPs and (c) levetiracetam-AgNPs were recorded at AFM operated in tapping mode with silicon nitride cantilever.

CNS drugs and their metal nanoconjugate displayed minimum cytotoxicity

To determine the toxic effects of phenobarbitone, phenytoin, and levetiracetam alone, as well as their AgNPs, cytotoxicity assays were performed by incubating drugs with HeLa cells. Results revealed that drugs alone, as well as their metal nanoconjugates, exhibited minimum cytotoxic effects when tested against human cells, at both concentrations tested (Figure 3). The drugs alone produced less than 10 % HeLa cell cytotoxicity while toxicity of their metal nanoconjugates and AgNPs alone was approximately 15%. This is considered as minimum toxicity, as Triton X-100 exhibits 100% cytotoxicity.

Drugs and their metal nanoconjugates reduced host cells cytopathogenicity

Next, we determined the effects of CNS drugs alone and their metal nanoconjugates on *E. coli* K1 and MRSA-mediated host cell death using cytopathogenicity assays. In the absence of drugs, both bacteria produced severe host cell cytopathogenicity (more than 70 % cell death) within 24 h (Figure 4). None of the drugs tested alone inhibited bacterial-mediated cell death. However, when metal nanoconjugates were formulated, drug conjugated AgNPs exhibited significantly reduced host cell death at both concentrations ($P<0.05$). Importantly, 10 µM of both phenobarbitone-AgNPS and phenytoin-AgNPS inhibited host cell death at levels of less than 20% cytopathogenicity (Figure 4). Notably, similar findings were observed against both Gram-negative *E. coli* K1 and Gram-positive MRSA suggesting broad-spectrum activity of drugs nanoconjugate formulations. Importantly, metal nanoconjugates did not reduce bacterial-mediated host cell death to negligible levels (Figure 4) probably due to the fact that metal nanoconjugates alone also produced host cell cytotoxicity (Figure 3).

Silver nanoparticles conjugation with phenobarbitone, phenytoin, and levetiracetam enhanced their antibacterial effects

Bactericidal assays were performed to determine antibacterial properties of drugs alone, and their nanoconjugate formulations at different concentrations. When bacteria were incubated alone, no bactericidal effects were observed (Figure 5). In the presence of gentamicin, 100% bacterial kill was observed (Figure 5). When *E. coli* K1 was incubated with drugs alone only limited effects were observed (Figure 5). However, drug conjugated AgNPs showed significant selective bactericidal effects against *E. coli* K1 ($P<0.05$). For example, phenobarbitone-AgNPs showed bactericidal activity compared to the PBS control while phenytoin and levetiracetam conjugated drugs gave CFU very similar to the PBS control. However, all conjugated drugs showed a degree of bactericidal activity against MRSA. Overall, these results imply that the conjugated drugs had an inhibitory effect on the bacteria but may not exhibit bactericidal effects. Notably, Ag alone also exhibited antibacterial properties against *E. coli* K1 (Figure 5).

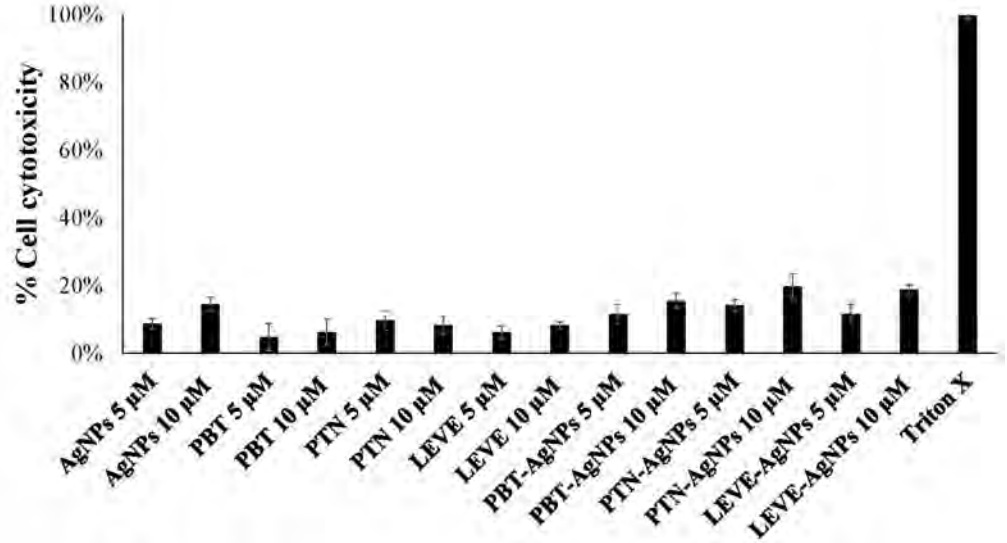
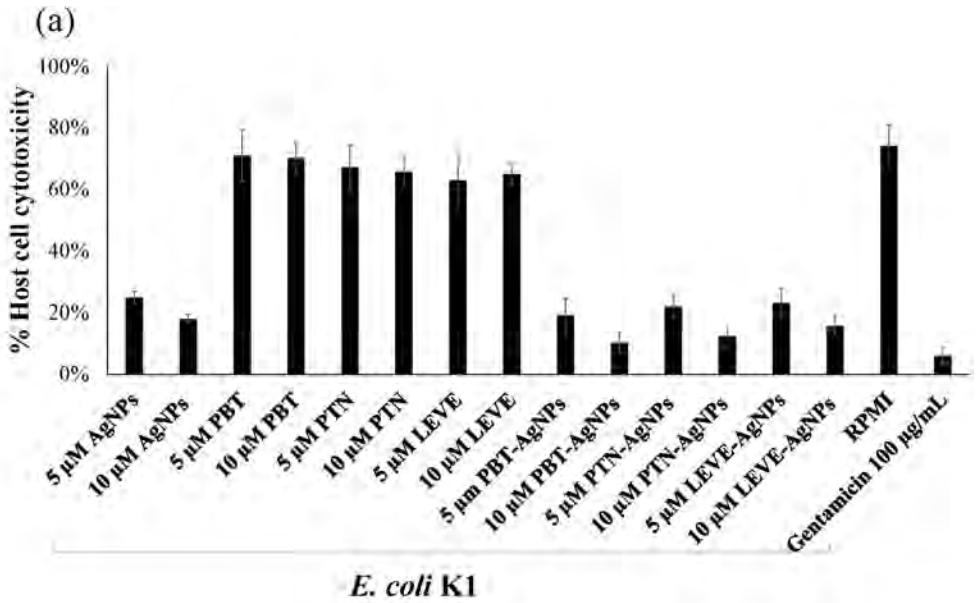


Figure 3. Central nervous system drugs and their silver nanoparticles exhibited minimal cytotoxicity against HeLa cells. Results are representative of at least three experiments performed in duplicate and presented as the mean ± standard error.



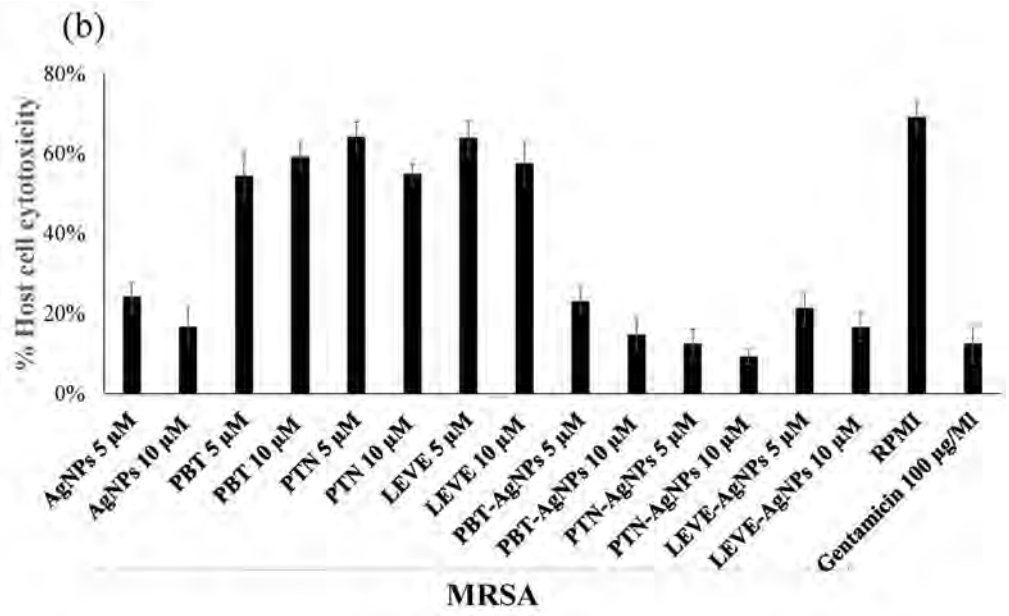


Figure 4. Central nervous system drugs and their metal conjugated exhibited selective bacterial-mediated host cell death. Results are representative of at least three experiments performed in duplicate and presented as the mean ± standard error.

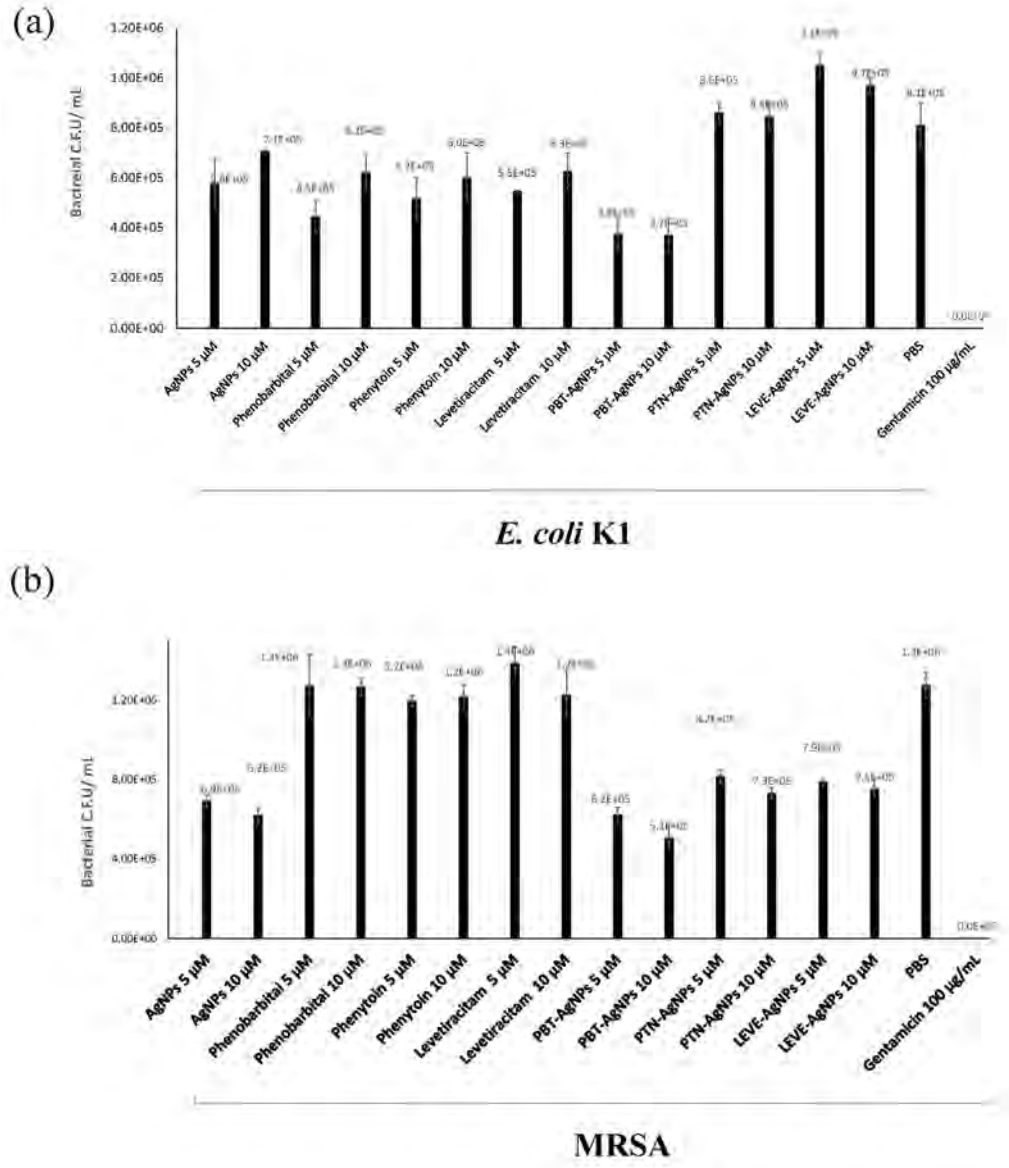


Figure 5. Central nervous system drugs and their metal conjugated exhibited selective bactericidal effects. Results are representative of at least three experiments performed in duplicate and presented as the mean ± standard error.

DISCUSSION

To expedite discovery of new compounds, drug repurposing has become a useful avenue in our search to find effective drugs (10), especially against life-threatening infections. For example, *E. coli* K1 meningitis is a major cause of morbidity and mortality in neonates, second only to group B *Streptococcus* (22,23). Importantly, more than 50% of patients develop lifelong neurological implications involving developmental delay, seizure disorders, physical disability, hearing loss, and hydrocephalus (24). Similarly, Gram-positive MRSA is known to produce meningitis and presents a significant in successful treatment (18). Hence, there is a continued need to develop and find new drugs.

The overall aim of our study was to test the antibacterial effects of drugs currently used against neurological disorders as these are considered effective in crossing the blood-brain barrier to reach the CNS to target pathogens. Phenobarbitol, selected in our study, is used for controlling spasms in epileptic and dipolar patients (25,26). The intravenous dose of phenobarbitone is advised at 15-18 mg/kg (27). However, its main side effects include sedation and hypnosis (25). Phenytoin is used against seizures (28), while levetiracetam is used to treat epilepsy. These are taken orally as an instant or extended release design or via intravenous administration (29,30). There are limited previous studies on the antibacterial activity of CNS drugs (31). Previous attempts have been to modify the molecular structure of some of the drugs. For example, the replacement of the free N-1 in the phenytoin part, as well as phenytoin derivatives, their glycoside, oxadiazolyl, and some acyclic analogs provided compounds with enhanced inhibitory behaviour against *E. coli, S. aureus, and Streptomyces* spp. (32-35). Other studies reported that phenytoin or levetiracetam held by silica core iron oxide diminishes the appearance of seizures in mice (11,36,37). These studies suggest that marketed drug modifications can lead to their enhanced medicinal efficiency.

In our study, for the first time, we used phenobarbitone, phenytoin, and levetiracetam and formulated metal nanoconjugates to enhance their antibacterial effects. Our results clearly revealed that selected Ag-conjugated drug nanoparticles exhibited potent bactericidal effects as well as inhibiting bacterial-mediated host cell cytotoxicity. Although Ag alone also exhibited antibacterial effects, our studies demonstrate that drugs repurposing, together with their conjugation with Ag nanoparticles, is a valuable approach in the search for new antibacterial compounds.

It is noteworthy that nanoparticle toxicity is still not fully understood (38). While much of the function of nanoparticles is due to their core structure, the surface coating describes much of their biological activity (39). This damage may vary depending on nanoparticles physicochemical properties, time and dose, and different target cells. Moreover, the type of surface coating plays a vital protection role against cell damage by regulating cellular nanoparticles uptake. Also, capping agents are crucial to avoid aggregation and improve the solubility of the nanosystem and can be used as a site for bioconjugation of the nanoparticle with molecules (38). Overall, phenobarbitone-AgNPs showed potent bactericidal activity compared to the PBS control while phenytoin and levetiracetam conjugated drugs gave CFU very similar to the PBS control. However, all conjugated drugs showed a degree of bactericidal activity against MRSA implying that the conjugated drugs had an inhibitory effect on the bacteria but may not exhibit bactericidal effects.

In conclusion, our results showed that CNS drugs alone showed potent killing against MRSA and limited killing for *E. coli* but significant inhibition of its cytopathogenic and bactericidal effects. Our data suggests that drug repurposing is a viable approach and currently available CNS drugs should be tested following conjugation with nanoparticles against CNS pathogens.

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REFERENCES

1. Wiles TJ, Kulesus RR, Mulvey MA. Origins and virulence mechanisms of uropathogenic Escherichia coli. *Exp Mol Pathol* 2008; 85: 11-19.
2. Bingen E, Bonacorsi S, Brahimi N, et al. Virulence patterns of Escherichia coli K1 strains associated with neonatal meningitis. *J Clin Microbiol* 1997; 35: 2981-2982.
3. Welch WD, Martin WJ, Stevens P, Young LS. Relative opsonic and protective activities of antibodies against K1, O and lipid A antigens of Escherichia coli. *Scand J Infect Dis* 1979; 11: 291-301.
4. Griffiths MJ, McGill F, Solomon T. Management of acute meningitis. *Clin Med (Lond)* 2018; 18: 164-169.
5. Hasbun R. Update and advances in community acquired bacterial meningitis. *Curr Opin Infect Dis* 2019; 32: 233-238.
6. Todar K. Todar's Online Textbook of Bacteriology. 2008; http://textbookofbacteriology.net/MRSA.html (accessed on 4 June 2020).
7. Greenwood D, Slack R, Barer M, Irving W (Editors). Medical Microbiology, 17th edition, 2012; Elsevier Health Sciences. pp752.
8. Murray PR, Rosenthal KS, Pfaller MA. Medical Microbiology, 8th edition, 2015; Elsevier Health Sciences. p848.
9. Elliott T, Casey A, Lambert PA, Sandoe J. Medical Microbiology and Infection, 2012; John Wiley & Sons. p288.
10. Anwar A, Khan NA, Siddiqui R. Repurposing of drugs is a viable approach to develop therapeutic strategies against central nervous system related pathogenic amoebae. *ACS Chem Neurosci* 2020; 11: 2378-2384.
11. Rosillo-de la Torre A, Zurita-Olvera L, Orozco-Suárez S, et al. Phenytoin carried by silica core iron oxide nanoparticles reduces the expression of pharmacoresistant seizures in rats. *Nanomedicine (Lond)* 2015; 10: 3563-3577.
12. Kumari A, Yadav SK, Yadav SC. Biodegradable polymeric nanoparticles based drug delivery systems. *Colloid Surf B Biointerfaces* 2010; 75: 1-18.
13. Akter M, Sikder MT, Rahman MM, et al. A systematic review on silver nanoparticles-induced cytotoxicity: physicochemical properties and perspectives. *J Adv Res* 2017; 9: 1-16.
14. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bacteria. *J Colloid Interface Sci* 2004; 275: 177-182.

15. Aguilar J, Urday-Cornejo V, Donabedian S, et al. Staphylococcus aureus meningitis: case series and literature review. *Medicine (Baltimore)* 2010; 89: 117-125.
16. Anwar A, Shah MR, Muhammad SP, et al. Thio-pyridinium capped silver nanoparticle based supramolecular recognition of Cu (I) in real samples and T-lymphocytes. *New J Chem* 2016; 40: 6480-6486.
17. Ahmed D, Shah MR, Perveen S, Ahmed S. Cephradine coated silver nanoparticle their drug release mechanism, and antimicrobial potential against Gram-positive and Gram-negative bacterial strains through AFM. *J Chem Soc Pak* 2018; 40: 388-398.
18. Khan NA, Osman K, Goldsworthy GJ. Lysates of Locusta migratoria brain exhibit potent broad-spectrum antibacterial activity. *J Antimicrob Chemother* 2008; 62: 634-635.
19. Masri A, Anwar A, Khan NA, et al. Antibacterial effects of quinazolin-4 (3h)-one functionalized-conjugated silver nanoparticles. *Antibiotics (Basel)* 2019; 8: 179.
20. Anwar A, Siddiqui R, Hussain MA, et al. Silver nanoparticle conjugation affects antiacanthamoebic activities of amphotericin B, nystatin, and fluconazole. *Parasitol Res* 2018; 117: 265-271.
21. Dharani S, Rahman Z, Ali SFB, et al. Quantitative estimation of phenytoin sodium disproportionation in the formulations using vibration spectroscopies and multivariate methodologies. *Int J Pharm* 2018; 539: 65-74.
22. Yousuf FA, Yousuf Z, Iqbal J, et al. Interactions of neuropathogenic Escherichia coli K1 (RS218) and its derivatives lacking genomic islands with phagocytic Acanthamoeba castellanii and nonphagocytic brain endothelial cells. *Biomed Res Int* 2014; 2014: 265424.
23. Naeem W, Liaqat F, Shafee M, et al. Multidrug resistance in pathogenic Escherichia coli; a public health concern. *Pure Appl Biol* 2019; 8: 2104-2118.
24. Korczak B, Frey J, Schrenzel J, et al. Use of diagnostic microarrays for determination of virulence gene patterns of Escherichia coli K1, a major cause of neonatal meningitis. *J Clin Microbiol* 2005; 43: 1024-1031.
25. Dwivedi J, Devi K, Asmat Y, et al. Synthesis, characterization, antibacterial and antiepileptic studies of some novel thiazolidinone derivatives. *J Saudi Chem Soc* 2016; 20: S16-S20.
26. Schwarz JR. The mode of action of phenobarbital on the excitable membrane of the node of Ranvier. *Eur J Pharmacol* 1979; 56: 51-60.
27. Ortinski P, Meador KJ. Cognitive side effects of antiepileptic drugs. *Epilepsy Behav* 2004; 5 Suppl 1: S60-S65.
28. Zakusov VV, Ostrovskaya RU, Kozhechkin SN, et al. Further evidence for GABA-ergic mechanisms in the action of benzodiazepines. *Arch Int Pharmacodyn Ther* 1977; 229: 313-326.
29. Anderson GD, Saneto RP. Current oral and non-oral routes of antiepileptic drug delivery. *Adv Drug Deliv Rev* 2012; 64: 911-918.
30. Siddiqui R, Aqeel Y, Khan NA. The development of drugs against Acanthamoeba infections. *Antimicrob Agent Chemother* 2016; 60: 6441-6450.
31. Sangeetha P, Siva T, Balaji R, Tharini K. Synthesis of phenytoin compound using microwave technology and evaluation of its antibacterial activity. *World J Sci Res* 2016; 1: 26-30.
32. Ali OM, Amer HH, Mosaad AA, Abdel-Rahman AH. Synthesis and antimicrobial activity of new phenytoin derivatives and their acyclic nucleoside analogs. *Chem Heterocycl Comp* 2012; 48: 1043-1049.
33. Ali OM, El-Sayed WA, Eid SA, et al. Antimicrobial activity of new synthesized [(oxadiazolyl) methyl] phenytoin derivatives. *Acta Pol Pharm* 2012; 69: 657-667.
34. Ateş O, Kocabalkanli A, Saniş GO, et al. Synthesis and antibacterial activity of 5-aryl-2-[(alpha-chloro-alpha-phenylacetyl/alpha-bromopropionyl) amino]-1, 3, 4-oxadiazoles and 2-[(5-aryl-1, 3, 4-oxadiazol-2-yl) imino]-5-phenyl/methyl-4-thiazolidinones. *Arzneimittelforschung* 1997; 47: 1134-1138.
35. Esiobu N, Hoosein N. An assessment of the in vitro antimicrobial effects of two antiepileptic drugs–sodium valproate and phenytoin. *Antonie Van Leeuwenhook* 2003; 83: 63-68.
36. Goto H, Isobe T, Senna M. Controlled dissolution of phenytoin by hybridizing with silica nanoparticles. *J Nanoparticle Res* 1999; 1: 205-213.
37. Shahabadi N, Hadidi S, Shiri F. New water-soluble Fe_3O_4@SiO_2 magnetic nanoparticles functionalized with levetiracetam drug for adsorption of essential biomolecules by case studies of DNA and HSA. *J Biomol Struct Dyn* 2019; 38: 283-294.
38. de Lima R, Seabra AB, Durán N. Silver nanoparticles: a brief review of cytotoxicity and genotoxicity of chemically and biogenically synthesized nanoparticles. *J Appl Toxicol* 2012; 32: 867-879.
39. Lewinski N, Colvin V, Drezek R. Cytotoxicity of nanoparticles. *Small* 2008; 4: 26-49.

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ORIGINAL ARTICLE

Validation of a pan-cancer targeted next generation sequencing panel in New Zealand

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ABSTRACT

Objectives: High throughput next generation sequencing of multiple genes in a selection of different sample types provides diagnostic laboratories with the ability to accurately detect variants implicated in a wide range of diseases, including cancer. Creating a mutational profile of a comprehensive list of genes heavily implicated in cancer aids in efficient and tailored care regimes for patients, allows for further research, and provides an outlook on clinical trial eligibility. The clinical importance of being able to provide a sequencing solution of this magnitude in New Zealand is extremely valuable. Turnaround times will be considerably decreased due to limiting the outsourcing of overseas diagnostic tests, and an increase in the efficiency of subsequent patient care in New Zealand is predicted.

Methods: 78 clinical oncology specimens, either bone marrow, peripheral blood, or formalin-fixed, paraffin-embedded tumour samples, were used to aid in the validation of the AmpliSeq for Illumina Cancer Hotspot Panel v2 for diagnostic use. Formalin-fixed, paraffin-embedded samples were treated with 2µl of Uracil-DNA glycosylate (UDG) to improve sequencing quality. Pathogenic variants detected in formalin-fixed, paraffin-embedded samples that have not been previously reported have been confirmed by Sanger sequencing.

Results: All detected variants were concordant with previously reported results from other laboratories overseas. Variant allele frequencies in the OncoSpan gDNA reference standard are concordant with variant allele frequencies observed in-house.

Conclusions: The AmpliSeq for Illumina Cancer Hotspot Panel v2 has been validated for diagnostic use at the Wellington Regional Genetics Laboratory, Wellington Hospital.

Key words: Molecular, oncology, sequencing, diagnostics.

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INTRODUCTION

Next generation sequencing broadly describes technology that possesses the ability to sequence millions of DNA templates in parallel. Current technology available for next generation sequencing has developed significantly over the past decade, progressing from traditional dideoxy Sanger sequencing to second and third generation sequencing techniques (1). Second generation sequencing techniques employ the enzymatic or mechanical shearing of DNA into short fragments, termed short reads, which are then sequenced in a massively parallel setting (2). These DNA fragments are then clonally amplified, resulting in a great degree of genomic coverage (3). Third generation sequencing, in comparison, acts by reading nucleotide sequences at a single molecule level. This methodology does not require DNA fragmentation, and input material is defined as a long read (4).

Currently, diagnostic laboratories around the world have implemented second generation sequencing techniques for diagnostic purposes, since third generation sequencing methods feature a considerably higher rate of error which is inappropriate for molecular diagnostics (5).

Achieving massive parallel sequencing by second generation methods occurs through clonal amplification of DNA libraries generated through an array of commercially available workflows, followed by cyclical reversible termination or single nucleotide addition. Sequencing by synthesis, the process by which fluorescently labelled dNTPs are incorporated into a DNA strand during synthesis, yields a high rate of error free reads and produces a high percentage of good quality base calls. This type of sequencing chemistry dominates the short-read sequencing industry and is predominantly carried out on a flow cell platform (6). Next generation sequencing technology is becoming increasingly more affordable for use in diagnostic practices, particularly in oncology genetics. The molecular profiling of tumours through next generation sequencing has become a common diagnostic method in clinical oncology and provides essential pharmacogenetic information imperative to the offering of efficient and tailored patient care plans (7, 8). Information on the different variants a patient has can elucidate whether or not certain chemotherapies will act effectively, and what drugs to avoid administering (9).

Multi-gene panels commercially available for use in molecular oncology diagnostics, such as the AmpliSeq for Illumina Cancer Hotspot Panel v2, are well suited for clinical use to aid in the guidance of advanced treatment and to help predict prognostic information. Somatic mutations, such as *BRAF* V600E, are associated with poor prognosis in patients with colorectal cancer, and resistance to *EGFR* monoclonal antibody inhibitors such as cetuximab or panitumumab (10). However, in patients with melanoma, the *BRAF* V600E mutation is associated with a greater response to *BRAF* inhibitor medications, such as vemurafenib (11).

This study looked at the implementation of a targeted next generation sequencing panel at the Wellington Regional Genetics Laboratory and compared results to those previously reported externally with the goal of validating the panel for diagnostic use.

MATERIALS AND METHODS

The DNA samples used in this study were referred to the Wellington Regional Genetics Laboratory or Wellington Southern Community Laboratories following informed patient consent.

Study design
78 clinical oncology specimens: 19 bone marrow samples, 36 peripheral blood samples, and 23 formalin-fixed, paraffin-embedded samples were analysed in this study. Of the 78 specimens, several were inter- and intra-run duplicates to assess reproducibility of results. In addition, sequencing of the OncoSpan gDNA reference standard (Horizon Discovery, USA) was executed to ensure variant allele frequencies were accurately represented. Samples were processed with the AmpliSeq for Illumina Cancer Hotspot Panel v2 (Illumina, Australia) and sequenced in multiple iSeq 100 (Illumina) instrument runs.

DNA extraction and sample quality assessment
Bone marrow and peripheral blood samples were extracted according to the manufacturer's protocol using the Qiagen DNA Midi Kit (Qiagen, Australia) via the QIASymphony SP DNA extractor (Qiagen). 1ml of bone marrow or peripheral blood was processed, and DNA was eluted in a total volume of 200µl. Formalin-fixed, paraffin-embedded samples had DNA extracted via the AmpliSeq for Illumina Direct FFPE DNA kit (Illumina). De-waxed tissue sections were extracted via a modified version of the manufacturer's protocol using a scalpel instead of a pipette tip to increase the quantity of tissue for extraction, and DNA was eluted in an approximate volume of 30µl. Formalin-fixed, paraffin-embedded DNA was then treated with 2µl of UDG (Thermo Fisher Scientific, New Zealand) to remove uracil lesions in the DNA, thereby reducing the risk of false-positive single nucleotide variant calls (12).

Following extraction, DNA from bone marrow and peripheral blood was assessed for quality and purity using an Implen N60 nanophotometer (Total Lab Solutions, New Zealand). All samples showed an A260/280 ratio of ~2.0, and an A260/230 ratio of ~1.8. DNA extracted from formalin-fixed, paraffin-embedded samples was not quality assessed due to low elution volumes. Extracted DNA from bone marrow and peripheral blood samples was quantified for double-stranded DNA using the Qubit dsDNA BR assay (Thermo Fisher Scientific), whereas DNA from formalin-fixed, paraffin-embedded samples was quantified using the Qubit dsDNA HS assay (Thermo Fisher Scientific). 10ng of DNA was used as a starting quantity for amplicon library preparation.

Library preparation
Sequencing amplicon libraries were prepared using the AmpliSeq for Illumina Cancer Hotspot Panel v2 and the AmpliSeq for Illumina Library Prep Kit (Illumina) according to the manufacturer's instructions. The panel covers hotspot regions targeting approximately 2800 mutations identified in the Catalogue of Somatic Mutations in Cancer (COSMIC) database from 50 cancer-related genes: *ABL1, AKT1, ALK, APC, ATM, BRAF, CDH1, CDKN2A, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, KRAS, MET, MLH1, MPL, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, SRC, STK11, TP53*, and *VHL*. Prior to sequencing, it was essential to identify the limitations of the panel and which areas of the 50 genes are covered. Coverage data for each of the 50 genes can be found in the Supplementary Table 1 (online at www.nzimls.org.nz/journals_recent).

A probe set containing pairs of oligonucleotides specific to the targeted regions of the panel was hybridised to each clinical sample. Amplicons were generated by PCR amplification after addition of a DNA polymerase and DNA ligase to the DNA-probe mix. Following amplicon generation, primer dimers were digested, and amplicons were partially digested to facilitate dual-index adapter ligation to each library pool for sample multiplexing and sequence cluster generation. Library pools were cleaned up twice using Agencourt AMPure XP beads (Beckman Coulter, New Zealand). Library quality was assessed on an Agilent 4150 Tape Station (Agilent Technologies, Australia), which provides information on the library size, concentration, and molarity of each sample. Libraries were pooled in equal volumes at a concentration of 50pM for loading onto the iSeq 100.

Sequencing and data analysis
Pooled libraries were sequenced on an iSeq 100 using a 2 x 151 base paired-end sequencing design. FASTQ file generation from raw read data was completed on-board. Alignment of paired-end reads to the hg19 genome assembly was executed with the Burrows-Wheeler Aligner. Identification of variants from aligned reads was conducted with a somatic variant caller algorithm designed by Illumina. Variant call format files generated during secondary analysis were uploaded to BaseSpace Sequencing Hub (Illumina) for further analysis. Variants were called and annotated using Variant Interpreter (Illumina) and filtered according to quality, variant frequency, and pathogenicity. Since most samples run through this workflow were known to contain pathogenic variants, this filtering method was sufficient to confirm the presence or absence of externally reported pathogenic variants. Further statistical analyses were executed by Prism 8.4.3 (GraphPad, USA).

Confirmatory studies via Sanger sequencing
To confirm detected variants in previously unreported cases, verification via Sanger sequencing was conducted. The *BRAF* V600E variant was detected in three formalin-fixed, paraffin-embedded cases via sequencing with the AmpliSeq for Illumina Cancer Hotspot Panel v2. The remaining extracted DNA was sequenced using the Big Dye Terminator v3.1 sequencing kit (Thermo Fisher Scientific) and an Applied Biosystems 3500xL Genetic Analyser (Thermo Fisher Scientific). The following primers were used - (5'-3'): M13-F ACGACGTTGTAAAACGAC, M13-R CAGGAAACAGCTATGACC, BRAFexon15-F CACGACGTTGTAAAACGACTCTTCATGAAGACCTCACAG, and BRAFexon15-R CAGGAAACAGCTATGACC AGCCTCAATTCTTACCATCC (Integrated DNA Technologies, USA). Thermo-cycling was performed according to the manufacturer's protocol.

RESULTS
The AmpliSeq for Illumina Cancer Hotspot Panel v2 consists of 207 amplicons representing the covered hotspot regions of 50 oncogenes and tumour suppressor genes. Paired end reads of 151 base pairs in length were generated, giving bidirectional sequences that provide high-quality alignment and enable detection of DNA rearrangements with precision.

Amplicon depth of coverage
Amplicon coverage values for each sample were calculated on-board the iSeq 100. Data was extracted from the instrument and the mean coverage depth for each amplicon was calculated. Mean coverage depths for the 207 amplicons ranged from 1494x to 8805x (Figure 1), and all samples had a uniformity of coverage (Pct >0.2*mean) value of ≥95%. The mean depth of coverage for all sequenced amplicons met the in-house minimum depth of coverage criteria of ≥500x.

Sensitivity
To examine the ability of the panel to detect low-frequency variants, a variety of cases were selected for inclusion featuring a range of variant allele frequencies. During data analysis, variant allele frequencies detected in house were directly compared to variant allele frequencies reported externally. A cut-off value was assigned during sequencing run setup and was set at the manufacturer's recommendation of 5%. Variants that fall below this frequency can be false-positive calls, especially in cases which feature a high degree of DNA deamination, such as formalin-fixed, paraffin-embedded samples.

Alongside the comparison of variant allele frequencies obtained in-house to previously reported variant allele frequencies, the use of the well-characterised OncoSpan gDNA reference standard provided confidence that in-house variant allele frequencies were accurate. The OncoSpan reference standard features a range of 386 variants across 152 cancer-related genes, with 52 of these variants present at ≤20% for limit of detection (LOD) determination. Table 1 provides a comparison of the established variant allele frequencies in the OncoSpan reference and the variant allele frequencies detected in-house. The data shows that our LOD is at least 4% for single nucleotide variants and 8% for indels. Further investigations surrounding our true LOD are underway.

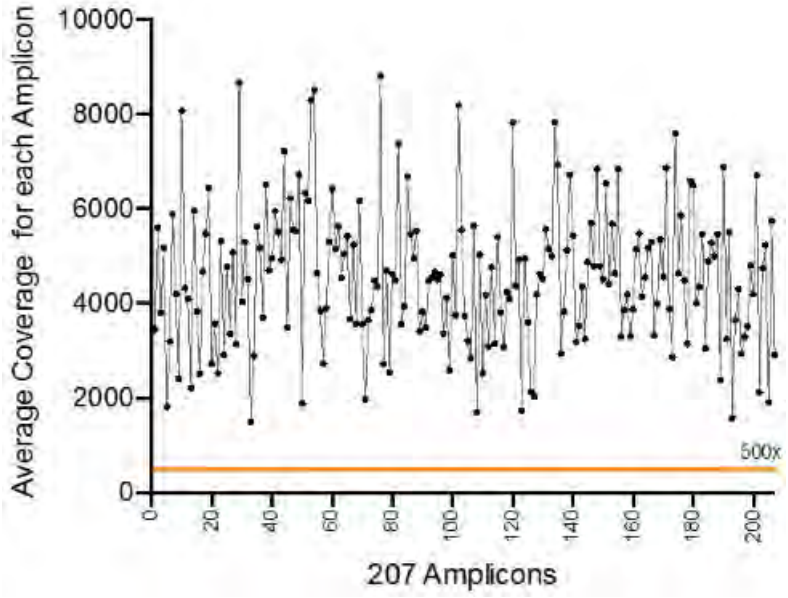

Figure 1. Mean coverage depths for each of the 207 amplicons that feature on the AmpliSeq for Illumina Cancer Hotspot Panel v2. Minimum coverage depth of 500x represented by the orange line.

Table 1. OncoSpan variant allele frequencies compared with in house variant allele frequencies.

Variant	dbSNP ID	OncoSpan VAF (%)	In-house VAF (%)
NRAS c.181C>A (p.Q61K)	rs121913254	9.0	11.2
CTNNB1 c.98C>A (p.S33Y)	rs121913400	25.0	30.8
CTNNB1 c.133_135del (p.S45del)	rs587776850	8.0	13.6
PIK3CA c.1633G>A (p.E545K)	rs104886003	12.0	10.1
PIK3CA c.2740G>A (p.G914R)	rs587776932	31.0	31.7
PIK3CA c.3140A>G (p.H1047R)	rs121913279	20.0	21.1
PDGFRA c.2472C>T (p.V824=)	rs2228230	18.0	14.7
KIT c.2477A>T (p.D816V)	rs121913507	10.0	11.1
KDR c.1416A>T (p.Q472H)	rs1870377	12.0	17.2
APC c.4479G>A (p.T1493=)	rs41115	39.0	31.6
EGFR c.2155G>A (p.G719S)	rs28929495	24.0	25.7
BRAF c.1799T>A (p.V600E)	rs113488022	6.0	11.6
RET c.2307G>T (p.L769=)	rs1800861	57.0	63.3
KRAS c.38G>A (p.G13D)	rs112445441	13.0	14.2
KRAS c.35G>A (p.G12D)	rs121913529	4.0	6.9
TP53 c.215C>G (p.P72R)	rs1042522	91.0	96.3

VAF: variant allele frequency.

Precision

A variety of bone marrow, peripheral blood, and formalin-fixed, paraffin-embedded samples with a range of variants were processed in replicate to account for both inter- and intra-run variables that may confound reproducibility. Previously reported pathogenic variants were detected in all duplicates and showed similar variant allele frequencies. Table 2 provides data on the variant allele frequencies generated from the inter-run replicates to assess reproducibility. The peripheral blood samples (one for the *TP53* variant and one for the *JAK2* and *IDH1* variants) were sequenced in separate runs, each run by different scientists, and all returned variant allele frequencies concordant with the external report.

Table 3 summarises data generated from intra-run replicates to assess repeatability. Both peripheral blood and formalin-fixed, paraffin-embedded samples were assessed. Formalin-fixed, paraffin-embedded samples typically yield DNA with lower amplification efficiency than that of bone marrow or peripheral blood DNA, yet similar variant allele frequencies were generated in each set of duplicates.

Table 2. Inter-run reproducibility.

Variant	dbSNP ID	VAF (%)
TP53 c.736A>G (p.M246V)	rs483352695	40.9
		40.0
		39.3
		41.7
		42.1
JAK2 c.1849G>T (p.V617F)	rs77375493	41.1
		43.6
IDH1 c.395G>A (p.R132H)	rs121913500	43.3
		38.8

VAF: variant allele frequency.

Table 3. Intra-run repeatability.

Variant	dbSNP ID	Replicate 1 VAF (%)	Replicate 2 VAF (%)	Mean VAF (%)
BRAF c.1799 T>A (p.V600E)	rs113488022	30.7	31.1	30.9
CDKN2A c.172C>T (p.R58*)	rs121913387	50.3	55.9	53.1
MET c.3029C>T (p.T1010I)	rs56391007	51.4	49.4	50.4
TP53 c.376-2A>G (p.?)	rs786202799	26.2	26.6	26.4
JAK2 c. 1849G>T (p.V617F)	rs77375493	13.7	12.6	13.2
IDH1 c.395G>A (p.R132H)	rs121913500	10.6	11.7	11.2

VAF: variant allele frequency.

Concordance with clinical results

The reported results of the majority of sequenced samples obtained from other laboratories were concordant with the results obtained in this study. Some formalin-fixed, paraffin-embedded cases had no previously reported variants and were sequenced due to interest from clinical anatomical pathology staff at Wellington Southern Community Laboratories. As such, the variants detected in these samples by the AmpliSeq Cancer Hotspot Panel v2 have been validated by Sanger sequencing. Full validation of a panel of this scope requires a minimum of 59 samples with both inter- and intra-run duplicates, and having concordant results in at least 59 samples provides statistical significance at a 95% confidence interval that the called variants are real (13). One called variant featured a variant allele frequency discrepancy when compared to the external report, which could be attributed to the different technology and methods used.

Analysis of samples

A 5% sensitivity cut-off was implemented prior to somatic variant calling, as variants with a variant allele frequency of <5% are commonly confounded by errors in either the PCR or sequencing stages of the laboratory workflow. In practice, low frequency variants would be reported depending on the mode of pathogenicity, with the report stating that the variant was called at a low frequency and may be confounded.

The somatic variant caller algorithm assigns quality scores to each variant called and provides information regarding base call accuracy. Any called variants that featured <95% called bases at Q30 (99.9% base call accuracy) were filtered out of the analysis as low Q-score percentages are associated with increased false-positive calls. Genes were masked according to the original clinical referral, and variants passing quality filters had their variant allele frequencies compared to previous reports to ensure concordance.

Five formalin-fixed, paraffin-embedded samples failed to meet our quality control criteria, likely due to the poor quality of the samples due to their age and length of time in fixation. These cases were excluded from the study, as in practice these would need to be repeated or reported as of insufficient quality to accurately interpret called variants.

DISCUSSION

Next generation sequencing technology allows high sensitivity detection of mutations in a massively parallel manner. Targeted panels, such as the AmpliSeq for Illumina Cancer Hotspot Panel v2, are often implemented for diagnostic purposes to interrogate multiple genes concurrently. As such, they are cost-effective, feature a streamlined and robust laboratory workflow, and hold the ability to multiplex samples thereby decreasing the time required to report results (14,15).

DNA from bone marrow, peripheral blood, and formalin-fixed, paraffin-embedded samples were used to aid in the validation of this panel for diagnostic use. All but one of the detected variants and their variant allele frequencies were concordant with previous reports from overseas laboratories. Since some formalin-fixed, paraffin-embedded samples used in the validation procedure have not been previously reported, confirmation of the detected pathogenic variants was required by Sanger sequencing. Typically, Sanger sequencing methods can detect variants at as low as approximately a 20% variant allele frequency, therefore this method of confirmation was suitable for the confirmation of detected variants in formalin-fixed, paraffin-embedded samples (16). Whilst five formalin-fixed, paraffin-embedded samples failed to meet our in-house quality control criteria due to poor sample quality, the sequencing success rate of formalin-fixed, paraffin-embedded samples treated with UDG was acceptable.

The ability held by next generation sequencing to sequence in parallel a massive number of short reads with great depth and coverage is unrivalled. Targeted panels containing a set of clinically relevant genes are low in cost, provide a fast turnaround time, feature simple workflows, and are increasingly easy to implement into diagnostic laboratories. The AmpliSeq for Illumina Cancer Hotspot Panel v2 is an example of a commercially available panel featuring amplicon sequencing, which is ideal for single nucleotide variant analysis (17). Panels such as these are fundamental in providing clinicians with a highly specific genetic profile for their patients, especially for those with cancer. However, as with most methods of diagnostic testing, there are limitations with the use of targeted hotspot panels. One of the major caveats noted is the gene coverage, since not all exons of the specified genes are covered in the AmpliSeq for Illumina Cancer Hotspot Panel v2. Discovering which exons of genes are covered in the panel was an arduous task, and information regarding this is not readily available. This creates a potential issue where, if no pathogenic variants are detected, an inaccurate reflection of the true genetic landscape of the patient is obtained. If a targeted panel of this scale calls no pathogenic variants, reflex testing via a larger targeted panel, such as the AmpliSeq for Illumina Myeloid Panel, would be a viable option.

The validation and implementation of targeted sequencing panels, such as the AmpliSeq for Illumina Cancer, is a large step in the right direction towards providing patients in New Zealand with results at a greater speed. This will allow clinicians to formulate care plans much sooner than previously possible due to a reduction in outsourcing of diagnostic sequencing solutions.

In conclusion, this study has demonstrated that the AmpliSeq for Illumina Cancer Hotspot Panel v2 is a robust amplicon sequencing solution fit for use in a diagnostic laboratory. The panel was able to accurately sequence a range of sample types and call variants at variant allele frequencies comparable with those reported externally. Whilst the coverage of regions targeted by the panel is less than that of other panels commercially available, implementation of the AmpliSeq for Illumina Cancer Hotspot Panel v2 provides a solid foundation to providing a comprehensive diagnostic oncology sequencing solution in Wellington, New Zealand. The AmpliSeq for Illumina Cancer Hotspot Panel v2 has been accredited through an external review by International Accreditation New Zealand (IANZ) for diagnostic use in the Wellington Regional Genetics Laboratory.

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REFERENCES

1. Meldrum C, Doyle MA, Tothill RW. Next-generation sequencing for cancer diagnostics: a practical perspective. *Clin Biochem Rev* 2011; 32: 177-195.
2. Meyerson M, Gabriel S, Getz G. Advances in understanding cancer genomes through second-generation sequencing. *Nat Rev Genet* 2010; 11: 685-696.
3. Shendure J, Ji H. Next-generation DNA sequencing. *Nat Biotechnol* 2008; 26: 1135-1145.
4. van Dijk EL, Jaszczyszyn Y, Naquin D, Thermes C. The third revolution in sequencing technology. *Trends Genet* 2018; 34: 666-681.
5. Besser J, Carleton HA, Gerner-Smidt P, et al. Next-generation sequencing technologies and their application to the study and control of bacterial infections. *Clin Microbiol Infect* 2018; 24: 335-341.
6. Cummings CA, Peters E, Lacroix L, et al. The role of next-generation sequencing in enabling personalized oncology therapy. *Clin Transl Sci* 2016; 9: 283-292.
7. Schwartzberg L, Kim ES, Liu D, Schrag D. Precision oncology: who, how, what, when, and when not? *Am Soc Clin Oncol Educ Book* 2017; 37: 160-169.
8. Wakai T, Prasoon P, Hirose Y, et al. Next-generation sequencing-based clinical sequencing: toward precision medicine in solid tumors. *Int J Clin Oncol* 2019; 24: 115-122.
9. Freedman AN, Klabunde CN, Wiant K, et al. Use of next-generation sequencing tests to guide cancer treatment: results from a nationally representative survey of oncologists in the United States. *JCO Precision Oncol* 2018; 2: 1-13.
10. Cheng Y-d, Yang H, Chen G-q, Zhang Z-c. Molecularly targeted drugs for metastatic colorectal cancer. *Drug Des Devel Ther* 2013; 7: 1315-1322.
11. Ravnan MC, Matalka MS. Vemurafenib in patients with BRAF V600E mutation–positive advanced melanoma. *Clin Ther* 2012; 34: 1474-1486.
12. Kim S, Park C, Ji Y, et al. Deamination effects in formalin-fixed, paraffin-embedded tissue samples in the era of precision medicine. *J Mol Diagn* 2017; 19: 137-146.
13. Jennings LJ, Arcila ME, Corless C, et al. Guidelines for validation of next-generation sequencing-based oncology panels: a joint consensus recommendation of the Association for Molecular Pathology and College of American Pathologists. *J Mol Diagn* 2017; 19: 341-365.
14. Gao M, Callari M, Beddowes E, et al. Next Generation-Targeted Amplicon Sequencing (NG-TAS): an optimised protocol and computational pipeline for cost-effective profiling of circulating tumour DNA. *Genome Med* 2019; 11: 1.
15. Dilliott A, Farhan S, Ghani M, et al. Targeted next-generation sequencing and bioinformatics pipeline to evaluate genetic determinants of constitutional disease. *J Vis Exp* 2018; 134: 57266.
16. Sandmann S, de Graaf AO, Karimi M, et al. Evaluating variant calling tools for non-matched next-generation sequencing data. *Sci Rep.* 2017; 7: 43169.
17. Xu H, DiCarlo J, Satya RV, Pet al. Comparison of somatic mutation calling methods in amplicon and whole exome sequence data. *BMC Genomics* 2014; 15: 244.

ORIGINAL ARTICLE

Haematology medical laboratory professionals' opinions on the Clinical Laboratory Scientist role in New Zealand

Lauren N Eddington

ABSTRACT

Objectives: Clinical Scientists and Doctors of Clinical Laboratory Science in the United Kingdom and the United States of America, respectively, are examples of medical laboratory professionals with further qualifications who work at the interface of the laboratory and clinical medicine. There is no specific such role currently established throughout New Zealand, however, a further 'Clinical Laboratory Scientist' position could be considered in future. This study aimed to gauge haematology medical laboratory scientists' opinions on the Clinical Laboratory Scientist in New Zealand.

Methods: An online questionnaire was created, validated, and disseminated by email to 42 haematology laboratories throughout New Zealand. Eligible participants were medical laboratory staff that reported on patients' blood films. Questions covered potential responsibilities of a Clinical Laboratory Scientist, the usefulness of the role, and participant interest in the role using a checkbox format and Likert scales.

Results: 36 completed surveys were returned. Responsibilities thought to be appropriate for this role included educating laboratory scientists, requesting further laboratory testing, and commenting and consulting on result interpretation for clinicians. Responsibilities involving patient contact were not supported. Results showed participants thought a Clinical Laboratory Scientist role would be useful in New Zealand laboratories with neutral personal interest in fulfilling the role.

Conclusions: Participants thought that a Clinical Laboratory Scientist would be useful in New Zealand laboratories.

Key words: Clinical laboratory scientist; medical laboratory scientist; medical laboratory professional; Doctorate of Clinical Laboratory Science; clinical scientist; questionnaire.

N Z J Med Lab Sci 2020; 74: 215-219.

INTRODUCTION

Two separate examples of specialist laboratory positions that allow laboratory staff to be better integrated into the clinical care team include the 'Clinical Scientist' in the United Kingdom (UK) (1), and the Doctorate of Clinical Laboratory Science in the United States of America (2) These roles require different further qualifications have different specific purposes, but both reduce the gap between the laboratory and the clinician. Laboratory positions such these may be referred to in the literature as a 'Clinical laboratory Scientist' (3), which will be the title used throughout this article.

At the interface of the laboratory and medical teams, a suitably qualified Clinical Laboratory Scientist could assist by providing consult regarding laboratory testing (4). The number of available laboratory tests are increasing (5) and clinicians can often be uncertain about ordering and interpreting test results (6). Recommendations from the laboratory can be helpful, reducing time to diagnosis, length of hospital stay, decreasing further testing (7), preventing misdiagnosis, and reducing unnecessary test costs (7,8). However, the frequency of laboratory consults is low (6). A Clinical Laboratory Scientist could also have a wider impact on improving laboratory efficiency by researching laboratory utilisation and directing laboratory operations on a larger scale (2,8).

Training programs for medical laboratory scientists who want to fulfil further positions include the Doctorate of Clinical Laboratory Science in the United States of America (2), and for New Zealand scientists, the Fellowship of the Faculty of Science from The Royal College of Pathologists Australasia [FFSc(RCPA)] is available (9). Although the educational programs towards this position have set competencies for their students to meet, the equivalent job descriptions have not been defined (2,9). It has been stated that professional Doctorate graduates will have the unusual position of proving their worth to an employer on the job (10). A further laboratory position in the UK is that of a Clinical Scientist, which has clear qualifying pathways and differing standards of proficiency as compared to biomedical scientists (1).

Further research must be done to determine whether a Clinical Laboratory Scientist position has a place in New Zealand medical laboratories, and if it does, what that role will look like. This study examined current haematology medical laboratory professionals' views on the value of this position in New Zealand, and the potential responsibilities of someone in that role.

METHODS

Questions regarding opinions on the Clinical Laboratory Scientist role in New Zealand were asked as part of a larger questionnaire for the author's tertiary study in late 2019. Ethical approval was given by the London Metropolitan University and Southern Community Laboratories, Dunedin. The questionnaire was created by the author and subsequently validated by three experts in laboratory haematology. Questions retained had the agreement of at least two out of three experts using the 4-scale content validity index, equating to a modified kappa statistic of 0.47, which has been labelled as 'fair' (11-13). The questionnaire is on-line as supplementary materials at www.nzimls.org.nz under the heading: Journal, current issues.

Participants were asked to select job responsibilities that they thought were appropriate for a Clinical Laboratory Scientist to perform from a list, with options taken from responsibilities noted in either the Clinical Laboratory Scientist course competencies (14), position paper (2), or the RCPA science Fellowship in haematology training handbook (9). The final portion of the questionnaire posed questions about the Clinical Laboratory Scientist role using 5-point Likert scales, ranging from strongly disagree to strongly agree. This included

questions on the perceived usefulness of the role in New Zealand, and on the participant's personal interest in becoming a Clinical Laboratory Scientist. There were opportunities throughout the questionnaire for participants to write further comments.

The questionnaire was created using the platform Survey Monkey 2015 (15) and disseminated to points of contact in 38 eligible laboratories by email. Eligible laboratories were New Zealand haematology laboratories that were participants in the Waikato morphology quality control program. Staff in these laboratories who report on patients' blood films were eligible to take part. The questionnaire was available for three weeks, with two reminder emails sent. Results were analysed using Microsoft Excel 2016 and IBM SPSS Statistics v26.

RESULTS

75 attempts were made to begin the questionnaire, resulting in 36 (48%) completed surveys that were included in the study. The majority, 16/36 (44%), of participants worked in laboratories where the daily volume of full blood count samples was 500-1000. Only 5/36 (14%) worked in larger laboratories running 1000-2000 full blood counts daily. Years of laboratory work experience of the participants ranged from 1 to 49.

Results regarding responsibilities that participants thought appropriate for a Clinical Laboratory Scientist are shown ranked by the percentage of participants that selected each option in Table 1. Responsibilities with over 33/36 (90%) agreement were educating laboratory scientists, applying laboratory techniques, and requesting further laboratory testing.

Participant opinions on the usefulness of the Clinical Laboratory Scientist and personal interest in pursuing the role are shown in Figure 1. The two Likert scale results were statistically transformed into the two new overall scales shown in the figure. The 'usefulness' Likert scale returned a Cronbach's alpha of 0.884, and the 'personal interest' scale Cronbach's alpha was 0.933. This showed that both scales are reliable with all eight questions included (16). The usefulness data showed a mean of 4.04, equating to the 'agree' category with an SD of 0.76. The personal interest mean is 3.4, equating to 'neither agree nor disagree', with an SD of 0.86. Individual item results are shown in Figure 2.

Table 1. Scientist's selections of job responsibilities appropriate for the Clinical Laboratory Scientist.

Responsibility	Percentage of respondents
Educate laboratory scientists	97%
Competently apply laboratory techniques	92%
Request further laboratory testing	92%
Consult on result interpretation for clinicians	89%
Educate clinicians on laboratory issues	83%
Provide interpretive comments on lab test results	83%
Decline unnecessary lab tests	81%
Consult on test selection	78%
Consult on development of laboratory services	75%
Audit laboratory systems	72%
Provide direction to the laboratory operation	72%
Formulate differential diagnoses	67%
Consult on specimen collection	58%
Analyse laboratory utilisation	58%
Identify signs and symptoms of medical conditions	56%
Educate clinicians on point of care devices	53%
Conduct research	50%
Attend grand rounds	33%
Educate patients on lab test interpretation	31%
Educate patients on point of care devices	28%
Suggest patients for clinical trials	22%
Attend ward rounds	19%
Take medical histories	17%
Perform specimen collections	8%

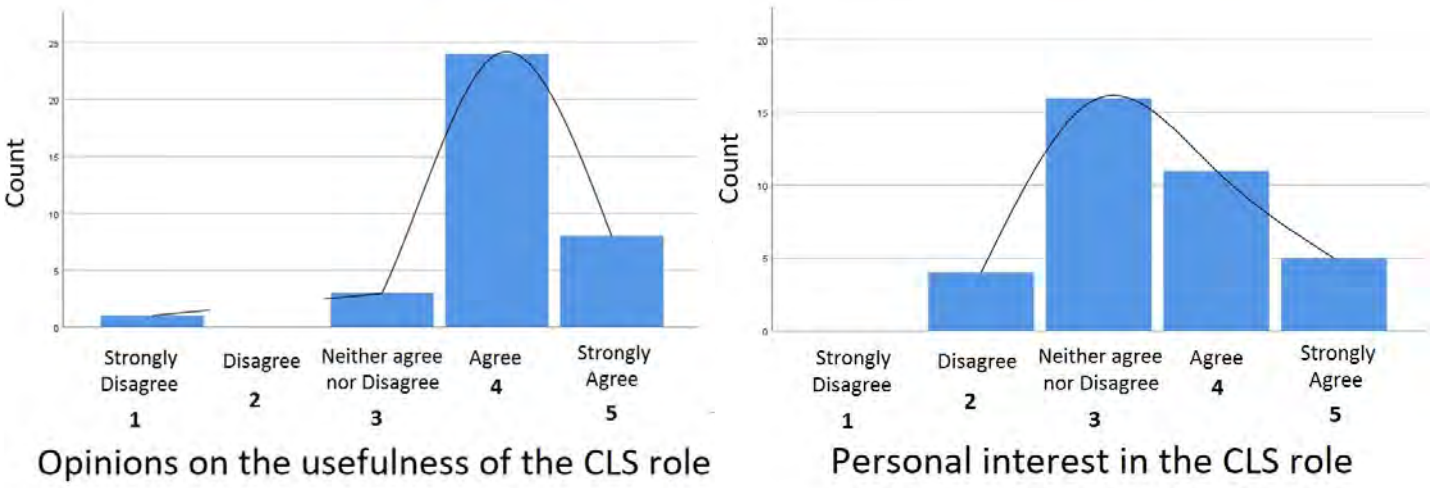

Figure 1. Haematology medical laboratory professionals' overall opinions on the usefulness of, and personal interest in, the Clinical Laboratory Scientist.

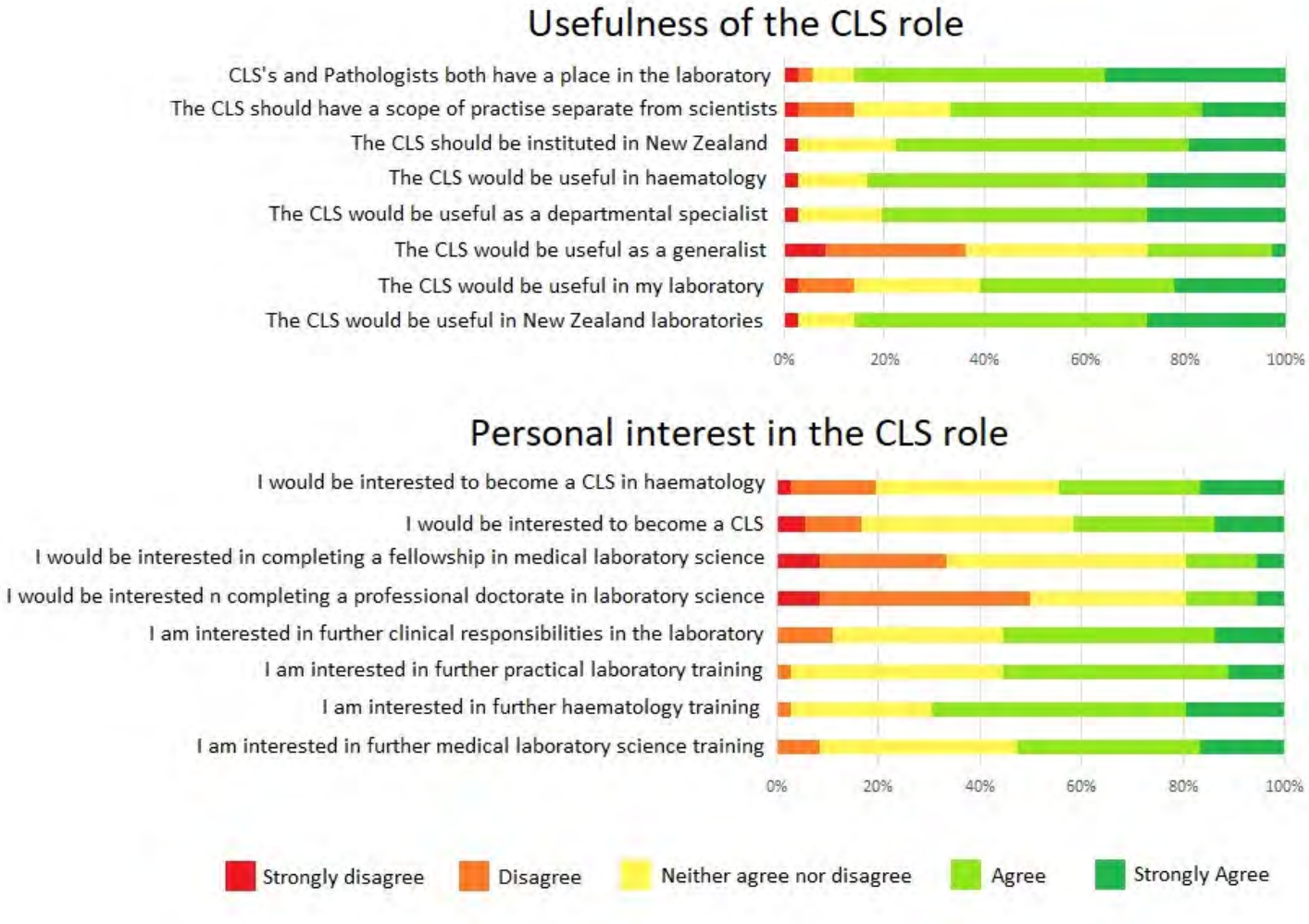

Figure 2. Individual Likert item responses from haematology laboratory professionals regarding the usefulness of, and personal interest in, the Clinical Laboratory Scientist role.

DISCUSSION

The Clinical Laboratory Scientist role

Responses from the 'usefulness' Likert scales reflected a positive opinion of the Clinical Laboratory Scientist role by scientists. Results indicated a preference for the Clinical Laboratory Scientist in New Zealand to be a departmental specialist, as opposed to a generalist across a variety of laboratory departments. Comments both for and against the role were provided by participants. One participant commented that given how 'stretched the clinical side of haematology is' that the role would be useful. Another wrote that it would be less useful in their larger teaching laboratory where many registrars are available to give input. It was noted that the role already exists to a degree in an informal manner with technical advisors, or scientists who liaise with doctors often. Along these lines, one participant stated: '*If a Clinical Laboratory Scientist role is introduced it will severely undermine the role of the Medical Laboratory Scientist that are currently operating in New Zealand*.' Although the results returned were positive, there was still mixed opinions and points for further discussion raised.

Responsibilities of the Clinical Laboratory Scientist

Nine potential responsibilities returned results of over 75% agreement from participants. The most approved option was 'educating laboratory scientists'. Consulting responsibilities were commonly selected, including consulting on result interpretation for clinicians, test selection, and on the development of laboratory services. Providing interpretive comments on laboratory test results was also supported, which is already part of the job description of a Medical Laboratory Scientist in New Zealand (17). Scientists also selected that the Clinical Laboratory Scientist to be able to 'competently apply laboratory techniques.'

The results show majority agreement among respondents that a Clinical Laboratory Scientist should be able to both decline unnecessary testing and request further laboratory testing. Scientists reducing unnecessary testing would provide a financial benefit (8); however, this may raise medical, ethical, and legal issues. Potential responsibilities for the Clinical Laboratory Scientist based outside of the laboratory itself were met with poor enthusiasm from respondents. This included attending grand rounds and ward rounds, educating patients on test results and point of care devices, and performing specimen collections.

Personal Interest in the Clinical Laboratory Scientist role

Personal interest in fulfilling this role was neutral overall. Numerous participants explained this was related to age or the late stage of their career with one noting '*If I was a lot younger, I would have answered them differently and would possibly be keen to pursue this avenue*.' Specific Likert items asked for interest in completing a professional Doctorate in laboratory science as in America, or a Fellowship in medical laboratory science as available in Australia. Both showed a low agreement of 7/36 (20%). 18/36 (50%) of participants disagreed or strongly disagreed regarding being interested in completing a professional Doctorate program, this is in comparison to 12/36 (33%) showing disinterest in a Fellowship program. Limitations here are that professional doctorates are not well-known qualifications in New Zealand, and questions regarding the qualification routes as in the UK were not included.

A new scope of practise for the Clinical Laboratory Scientist

If the role of a Clinical Laboratory Scientist was to be instituted as a separate job title in New Zealand, it raises an issue of whether a new scope of practise would need to be instituted by the Medical Sciences Council of New Zealand. One item in the questionnaire specifically asked 'The Clinical Laboratory Scientist should have a scope of practise separate from scientists' and 67% of participants agreed or strongly agreed. An opposing point raised by one respondent noted '*There is nothing stopping the Medical Laboratory Scientist in New Zealand expanding their roles above and beyond what they are doing currently within both the existing legal and contractual frameworks that exist within New Zealand*.' The current scope of practise for the practise of medical laboratory science in New Zealand includes 'collect, test, and analyse human biological material to support patient diagnosis, management and treatment…selection of appropriate samples and preparation for testing and analysis … Scientists analyse and interpret laboratory results and report their findings to referring clinicians. In certain circumstances they also advise of the need for further relevant testing.' It also notes that medical laboratory science teaching, quality management, and research are under the scope of a scientist (17). Many of the potential roles for a clinical laboratory scientist posed in the questionnaire can therefore be interpreted to be within the current scope of a Medical Laboratory Scientist. Responsibilities of the Clinical Laboratory Scientist role would have to be substantially different from a Medical Laboratory Scientist to necessitate a new scope of practise in New Zealand. Expanded practise amendments to annual practising certificates may be an option for broadening role responsibilities (18).

Limitations

Limitations of the questionnaire include the long length of the survey which may have been off-putting to participants, and the dissemination method which relied on recipients forwarding an email link. The Likert scale questions were all phrased in a positive manner, which may have biased participants to respond in the affirmative instead of negating the question. As the survey was voluntary, those who decided to participate may have been biased to those who had strong views on the Clinical Laboratory Scientist role. The survey included some terms which may have been misinterpreted, such as 'professional Doctorate' which is not common in New Zealand, Fellowship, and generalist. Explanations of terms would have been beneficial.

This study was restricted to haematology scientists and does not reflect the views of scientists from all departments, laboratory management staff, the Medical Sciences Council, or clinicians whose input would be required for a more complete discussion about this position within New Zealand. Further aspects to consider include appropriate entrance qualifications, certification, and remuneration, which were not within the scope of this study.

CONCLUSIONS

Participants in this study showed an agreement that a Clinical laboratory Scientist would have a place in New Zealand haematology laboratories. Preferences were that the role would be for a departmental specialist. Responsibilities of this position in New Zealand could include educating colleagues, and consulting on result interpretation and test selection. Participants believed the Clinical Laboratory Scientist should request and decline laboratory testing when needed, however, this would bring up legal and ethical issues that would need to be addressed. The need for a separate scope of practise for Clinical Laboratory Scientist in New Zealand remains debatable. Participants indicated that they would encourage a separate scope of practise; however, it appears that the anticipated responsibilities of a Clinical Laboratory Scientist in New Zealand from this study would still be within the current scope of a Medical Laboratory Scientist.

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REFERENCES

1. Health and Care Professions Council, London, UK. Standards of proficiency; 2019. Available at: https://www.hcpc-uk.org/standards/standards-of-proficiency/. Accessed 2 Aug 2019.
2. American Society for Clinical Laboratory Science, McLean, VA, USA. Advanced Practice: Doctorate in Clinical Laboratory Science Position Paper; 2016. Available at: https://www.asClinical Laboratory Scientist.org/position-papers/178-advanced-practice-doctorate-in-clinical-laboratory-science/154-advanced-practice-doctorate-in-clinical-laboratory-science. Accessed 30 May 2019.
3. Zinder O. Educating a new generation of clinical laboratory scientists. *Clin Chim Acta* 2002; 319: 149-153.
4. Leibach EK. The Doctorate in Clinical Laboratory Science: A view of the process of integration into healthcare. *Clin Lab Sci* 2007; 20(2): 69-71.
5. Bennett A, Garcia E, Schulze M, et al. Building a laboratory workforce to meet the future: ASCP Taskforce on the Laboratory Professionals Workforce. *Am J Clin Pathol* 2014; 141: 154-167.
6. Hickner J, Thompson PJ, Wilkinson T, et al. Primary care physicians' challenges in ordering clinical laboratory tests and interpreting results. *J Am Board Fam Med* 2014; 27: 268-274.
7. Laposata ME, Laposata M, Van Cott EM, Buchner DS, Kashalo MS, Dighe AS. Physician survey of a laboratory medicine interpretive service and evaluation of the influence of interpretations on laboratory test ordering. *Arch Pathol Lab Med* 2004; 128: 1424-1427.
8. Eddington, L. An interview with Brandy Gunsolus. First graduate of the Doctorate of Clinical Laboratory Science. *N Z J Med Lab Sci* 2019; 73: 134-135.
9. The Royal College of Pathologists of Australasia, NSW, Australia. Faculty of Science Trainee Handbooks 2019. Available at: https://www.rcpa.edu.au/Trainees/Curriculum#item12. Accessed 20 May 2019.
10. Rutgers, School of Health Related Professions, Department of Clinical Laboratory Sciences, McLean, VA, USA. Clinical Laboratory Scientist Proposal- Concept Document Sample; 2012. Available at: https://www.asClinical Laboratory Scientist.org/images/Clinical Laboratory Scientist_Toolkit/10_Clinical Laboratory Scientist_Proposal-Concept-Document_Sample.Pdf. Accessed 20 May 2019.
11. Cicchetti DV, Sparrow SA. Developing criteria for establishing interrater reliability of specific items: Application to assessment of adaptive behaviour. *Am J Ment Defic* 1981; 86: 127-137.
12. Fleiss J. Statistical Methods for Rates and Proportions (2nd ed.); 1981. John Wiley, New York, USA.
13. Polit DF, Tatano Beck C, Owen SV, Is the CVI an acceptable indicator of content validity? Appraisal and recommendations. *Res Nurs Health* 2007; 30: 459-467.
14. The American Society for Clinical Laboratory Science. McLean VA. Doctor of Clinical Laboratory Science Tool kit: A Resource for Educators; 2012. Available at: https://www.asClinical Laboratory Scientist.org/careers-asClinical Laboratory Scientist/dClinical Laboratory Scientist. Accessed 30 May 2019.
15. SurveyMonkey. Available at: https://www.surveymonkey.com/.
16. Gliem JA, Gliem RR. Calculating, Interpreting, and Reporting Cronbach's Alpha Reliability Coefficient for Likert-Type Scales. *Midwest Research to Practice Conference in Adult, Continuing, and Community Education, Columbus*. 2003: 82-88. Available at: https://scholarworks.iupui.edu/handle/1805/344. Accessed 30 May 2029.
17. Medical Sciences Council of New Zealand. Notice of Scopes of Practise and Prescribed Qualifications for the Practise of Medical Laboratory Science. *New Zealand Gazette* 2015 [Cited 5 August 2019] 135, Available at: https://www.medicalsciencescouncil.org.nz/assets_MedicaLLaboratorySci entist/Uploads/Medical LaboratoryScientist-Scopes-and-Quals-Dec-2015.pdf. Accessed 5 August 2019,
18. Medical Sciences Council of New Zealand. New Zealand: Policy and Guidelines: Expanded Practise for Registered Medical Laboratory Science Practitioners; 2018. Available at: https://www.MedicalSciencesCouncil.org.nz/assets_Medical Laboratory Scientist/Uploads/RP-Medical SciencesCouncil006-Expanded-Practice-Medical Laboratory Scientist-Feb2019.pdf. Accessed 2 Aug 2029.

THE FIRST FORENSIC CONVICTION?

In 1247 a Chinese official, Song Ci, wrote a book "The Washing Away Wrongs" based on elements of forensic analysis. In the book was the first known recorded application of forensic entomology. A man was found murdered on the roadside with multiple slashes. An official tested various blades on a cow carcass and concluded the murder weapon was a sickle. The victim had no apparent enemies and all his possessions were in tact, ruling out robbery. The victim owed money to a moneylender who became the prime suspect but had a good alibi. To resolve the case the official had 70 men stand in line with their sickles. Within seconds the moneylenders sickle attracted swarms of flies despite being apparently clean but the flies were attacked to the unseen traces of blood. The moneylender confessed to the murder.

SHORT COMMUNICATION

Evaluation of a new *Legionella longbeachae* urine antigen test in patients diagnosed with pneumonia

Roslyn Podmore and Mona Schousboe

ABSTRACT

Early differentiation of Legionella pneumonia from other forms of bacterial pneumonia is vital for correct treatment options. Rapid urine antigen tests are available for the detection of *Legionella pneumophila* serogroup 1 antigens, but not for the detection of *L. longbeachae*. Canterbury Health Laboratories, in collaboration with the Staten Serum Institute Diagnostica, Denmark, developed a lateral flow immunochromogenic prototype urinary antigen test. Using urine collected from 133 patients with pneumonia, the *L. longbeachae* urine antigen test was assessed, yielding a sensitivity of 59.1% and a specificity of 82.2%. This study suggests that this prototype urine antigen test would be a useful tool in the diagnosis of *L. longbeachae* pneumonia.
Key words: *Legionella longbeachae*, urine antigen test, polymerase chain reaction.

N Z J Med Lab Sci 2020; 74: 220-221.

INTRODUCTION

Legionella pneumonia is clinically indistinguishable from other forms of bacterial pneumonia. As Legionella pneumonia requires non-empiric antimicrobials, correct and early treatment is vital for favourable outcomes. For this reason, diagnostic testing, in initial phases of disease, is important for the commencement of optimal antibiotic treatment.

Worldwide *Legionella pneumophila* is the most common cause of Legionella pneumonia. However, in New Zealand the majority of Legionella pneumonia infections are caused by *L. longbeachae* (David Harte, ESR, CDC New Zealand, unpublished data). Culturing remains the gold standard for the diagnosis of Legionella infections (1), however, PCR on respiratory specimens is more sensitive (2). Rapid urine antigen tests are available for the detection of *L. pneumophila* serogroup 1 antigens, but not for other *L. pneumophila* serogroups, nor for *L. longbeachae*. Canterbury Health Laboratories in partnership with the Staten Serum Institute Diagnostica, Denmark collaborated in the development of a lateral flow urine antigen test for the rapid detection of *L. longbeachae* urinary antigens.

METHODS AND MATERIALS

Between 2015 and 2018, urines from 133 patients with pneumonia were tested with a new lateral flow immunochromogenic urine antigen test (prototype LL20170529). The SSI assay can detect both *L. pneumophila* and *L. longbeachae*; however, only *L. longbeachae* detection was evaluated in this study.

All patients had a Legionella polymerase chain reaction (PCR) completed (4). Of the 133 respiratory samples tested by PCR, 88 were *L. longbeachae* positive, 12 *L. pneumophila* positive, 9 *Legionella spp*. positive and 24 *Legionella* PCR negative.

133 urine samples were concentrated 25 times using BJP prochem clinical concentrators (Vivaproducts, Canada and USA), then three drops of urine concentrate and two drops of buffer were mixed in a tube. The lateral flow strip was then inserted into the tube and together incubated at room temperature for 15 minutes (Figure 1). The strip was then removed, with coloured lines read and interpreted manually and results recorded. Results of the *L. longbeachae* urine antigen tests were compared with the PCR results.

RESULTS

In this study, the urine antigen test for *L. longbeachae* yielded a positive result for 52 of the 88 positive PCR respiratory specimens, producing a sensitivity of 59.1%. There were two false positive results and six cross reactions with other *Legionella spp*., (three *L. pneumophila*, two *L. micdadei* and one *Legionella spp*.) giving a specificity of 82.2%. However, if the cross reactions with other Legionella species are included then the specificity increased to 95.6%. Although this prototype is primarily designed to detect *L. longbeachae* and *L. pneumophila sg 1* it is evident there is some cross reactivity with other *Legionella spp*. The results are displayed in Table 1.

In practice, because the strip has an indicator for both *L. pneumophila sg 1* (blue line) and *L. longbeachae* (purple line), aberrant reactions are apparent using the colour and intensity of the reactions as a guide. Any line that is not a clear blue or purple can be discounted.

CONCLUSIONS

This study found that the SSI *L. longbeachae* urine antigen test prototype assay was an extremely useful tool for the early detection of *L. longbeachae*, despite the low sensitivity achieved. In patients with Legionella pneumonia it is estimated that less than 50% are able to produce sputa for testing (4), thus any test with a sensitivity above 50% will increase the number of patients diagnosed. In addition, urine samples have the advantage of being easily obtainable. The comparative rapidity of the urine antigen test compared with sputum PCR is also an advantage.

Using this dual test, as opposed to other urine antigen tests, which detect only *L. pneumophila* sg 1, should ensure a more accurate indication of the prevalence of *L. longbeachae* worldwide. This study suggests that this prototype urine antigen test would be a useful tool in the diagnosis of *L. longbeachae* pneumonia, especially for laboratories where a urine antigen test is the only method available for the diagnosis of Legionella pneumonia. It is hoped that further development of the assay will result in improved sensitivity for this *L. longbeachae* urine antigen test.

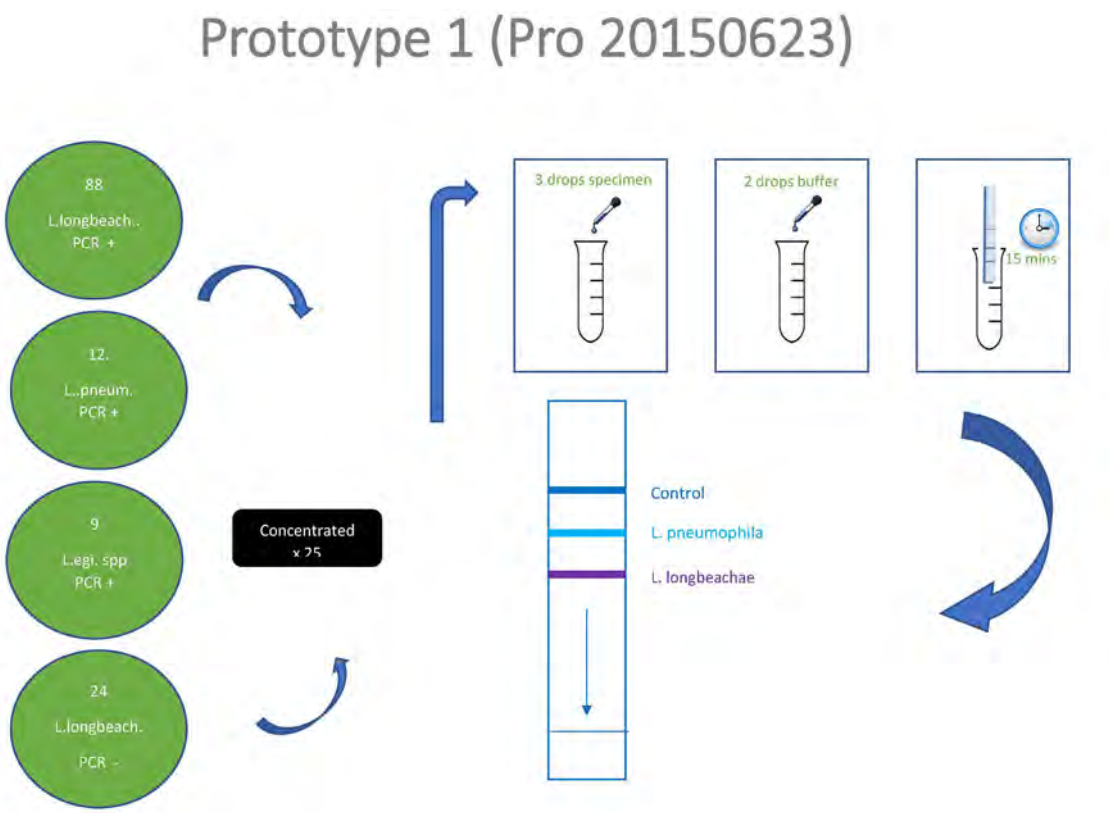

Figure 1. Urine antigen test flow diagram.

Table 1. Results of *L. longbeachae* urine antigen test compared with PCR.

	Legionella PCR Results			
	***L. longbeachae* n=88**	***L. pneumophila* n=12**	***L. Species* n=9**	***Negative* n=24**
***L. longbeachae* UAT**				
Positive	52	0	6	0
Negative	36	12	3	24
% sensitivity	**59.1%**			
% specificity	**82.2%**			

n= number of strains tested. UAT: urine antigen test.

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CONFLICTS OF INTEREST

Staten Serum Diagnostica provided the kits used in the study and provided partial funding for attendance to a Legionella conference for presentation of the data.

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REFERENCES

1. Pierre DM, Baron J, Yu VL, Stout JE. Diagnostic testing for Legionnaires' disease. *Ann Clin Microbiol Antimicrob* 2017: 16: 59.
2. Murdoch DR, Podmore RG, Anderson TP, et al. Impact of routine systemic polymerase chain reaction testing on case findings for Legionnaires' disease: a pre-post comparison study. *Clin Infect Dis* 2013; 57: 1275-1281.
3. Reischl U, Linde HJ, Len N, et al. Direct detection and differentiation of Legionella spp. and Legionella pneumophila in clinical specimens by dual-colour real-time PCR and melting curve analysis. *J Clin Microbiol* 2002; 40: 3814 – 3817.
4. Woodhead MA, Macfarlane JT. Legionnaire's disease: a review of 79 community acquired cases in Nottingham. *Thorax* 1986; 41: 635-640.

CASE STUDY

Cold autoimmune haemolytic anaemia as the presenting feature in a patient with angioimmunoblastic T-cell lymphoma an illustrative case

Shirley May Gates and Elizabeth Lombard

ABSTRACT

We present an interesting case of cold autoimmune haemolytic anaemia (CAIHA) as the presenting feature in a patient with angioimmunoblastic T-cell lymphoma (AITL). In CAIHA cold agglutinins cause symptoms due the agglutination of red blood cells in the cooler parts of the body and subsequent haemolysis in the warmer parts of the body. The development of cold agglutinins may be primary or secondary to a wide range of conditions including chronic lymphoproliferative conditions, although more commonly involving B-lymphocytes.

Our patient presented with CAIHA and was investigated for an underlying cause. Bone marrow investigation demonstrated a small B-cell clone in keeping with a monoclonal B-cell lymphocytosis. No evidence of a concurrent lymphoproliferative process was present on available material; however, radiological studies indicated extensive lymphadenopathy and a lymph node biopsy was subsequently obtained. The histological and immunohistochemical features indicated a diagnosis of AITL. Diagnosis was confirmed by clonal T-cell gene rearrangement on molecular studies.

This case demonstrates the need for a full investigation in patients with CAIHA to exclude the less common causes.

Key words: Lymph node, angioimmunoblastic, cold agglutinin, cold auto-immune haemolytic anaemia.

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INTRODUCTION

Cold autoimmune haemolytic anaemia (CAIHA) is a type of anaemia in which cold agglutinins can cause clinical symptoms related to the agglutination of red blood cells in the cooler parts of the body and haemolysis in the warmer parts (1). Historically, AITL has been poorly understood and challenging to diagnose (2) and treat (3).

Cold agglutinins typically cause red cell autoagglutination at a temperature of 4°C. They are usually driven by an IgM auto-antibody. In cooler parts of the body, the large IgM molecule is able to form bridges between adjacent red cells causing them to clump together. The IgM binds the red cell membrane, coating the cell surface with C3b and activating the complement cascade. As the blood recirculates back to the warmer body core, IgM releases its binding, and the coated red blood cells lose surface membrane through the action of macrophages in the liver and spleen, with eventual destruction and uptake of damaged red blood cells. This results in extravascular haemolysis. Intravascular haemolysis also occurs due to direct damage to the red cell membrane by activation of the complement cascade (4).

The causes of CAIHA are described and classified as either primary cold agglutinin disease (CAD) and secondary cold agglutinin syndrome (CAS). CAD is a clinical entity defined by the absence of any underlying or associated disease. Patients with CAS also have cold haemolytic anaemia, but this is secondary to another disease such as an underlying infection or lymphoproliferative condition (5). The offending IgM cold antibodies are produced by lymphocytes and interestingly in humans, appear to be a reflection of our primitive vertebrate ancestry (5). The direct antiglobulin test (DAT) is used to demonstrate the presence of immunoglobulin and/or complement fragments on the red cell surface [5].

CAD and CAS secondary to lymphoproliferative conditions are both autoimmune diseases in which the monospecific DAT is strongly positive for the complement fragment C3d and associated with a cold agglutinin titre of ≥64 at 4°C. Monospecific IgM DAT is usually negative because the red cells lose the cold agglutinins before they can be identified. However, when antigen-bound, IgM is a strong activator of the complement system, explaining the retention of the complement fragment on the surface. While monospecific IgG DAT may also be positive in CAD and CAS, this is usually weak and IgG does not activate complement as strongly as IgM (5).

Angioimmunoblastic T-cell lymphoma (AITL) is an uncommon and aggressive disease. In the USA only 1-2% of non-Hodgkin lymphomas are due to AITL, however, it is the second most common type of T-cell lymphoma. It is more common in elderly persons with a male predominance (6). The term “angioimmunoblastic” is derived from the words “angio” which refers to blood vessels, and “immunoblast’”, or activated lymphocytes (6). AITL is associated with a dysfunctional immune system, displaying features of both a hyperactive immune system and also immunodeficiency (3). Diagnosis is complex and patients may be in an advanced stage at presentation (7). Until relatively recently, AITL was poorly understood and thus difficult to diagnose and treat. However, since 2002 progress has been made due to the use of molecular biology and specific cell markers, such as CD10 and PD-1 (2,8).

The case presented here demonstrates that the diagnosis of AITL still remains complex. The prognosis is poor with a high relapse rate (6). Treatment usually consists of chemotherapy, radiation, and supportive care (6).

CASE REPORT

In June 2017, a 67-year-old female was referred to our hospital with a suspected haemolytic anaemia. Historically she was known to have red cell agglutination. She subsequently developed haematuria, polymyalgia, fevers, night sweats, a body rash, and fatigue with a 13 kg weight loss over the preceding eight months (B-symptoms). She had shingles in 2014 and a possible history of Hepatitis A infection. Physical examination revealed no cervical lymphadenopathy, but bilateral inguinal lymphadenopathy and palpable splenomegaly were present. A CT scan showed extensive central and peripheral lymphadenopathy and lytic lesions in the vertebra, suggestive of a possible lymphoma.

A full blood count (FBC) was performed using a Sysmex XT Haematology Analyser. Biochemistry was performed on a Cobas Biochemistry 6000 Analyser. A strong cold agglutinin was present and the FBC specimen had to be warmed for 60 minutes at 37ºC prior to analysis. Results indicated anaemia, with increased levels of C-reactive protein (CRP) and lactic dehydrogenase (LDH) (Table 1). The blood film still showed red-cell autoagglutination despite being warmed to 37ºC for one hour. Polychromasia was present, which is consistent with cold autoimmune haemolytic anaemia. The white blood cell morphology and differential were normal with just the occasional immature granulocyte present (Figure 1).

Table 1. Full blood count and selected biochemistry and serology results on admission.

	Results	Reference range
HB g/L	91	115-155
WBC 10^9/L	7.70	4-11
Platelets 10^9/L	289	150-400
RDW %	19.9	12.0-14.6
WBC differential	Normal	
CRP mg/L	64	1-5
LDH U/L	428	120-250
Serum Protein Electrophoresis	Polyclonal gammopathy	
EBV serology	IgG positive IgM negative	
CMV serology	IgG positive IgM negative	

A direct antiglobulin test (DAT) was performed using Bio-RaD Monospecific DATS (IgG/C3d/Ctl) DC-Screening Cards. Cells had to be washed 15 times in warm buffered saline prior to analysis to get interpretable results. IgG DAT was strongly positive (4+) as was C3d DAT (3+). This indicated the presence of a warm autoantibody as well as the morphologically identified cold agglutinin. A specimen submitted to New Zealand Blood Services for auto-absorption of antibodies showed no underlying alloantibody.

A serum specimen was referred for serological tests. Results for Epstein-Barr virus (EBV) and Cytomegalovirus virus (CMV) indicated positivity for IgG for both, while IgM was negative. This is consistent with past infection. Serological tests for hepatitis were negative.

Cell markers on a subsequent bone marrow sample showed a chronic lymphocytic leukaemia phenotype with very low-level involvement. Of note is that no CD10+ T-cell population was present and the bone marrow showed no morphological evidence of AITL. However, there were marked reactive features. A lymph node biopsy was then performed.

Histological findings on the lymph node indicated a loss of normal architecture with a diffuse infiltrate of lymphocytes and increased vascularity (Figure 2), accompanied by expanded follicular dendritic networks (Figure 3). The subcapsular sinus was intact. There was a population of cells that were positive for CD4, CD10 and PD-1 on immunohistochemistry (Figure 4). CD3 was also positive. Scattered large B cells (CD20 positive on immunohistochemistry) were present. EBER in-situ hybridisation on the lymph node biopsy was positive in these B-cells (Figure 5), indicating that they wereinfected with EBV.

A specimen referred for T-cell receptor gene rearrangement by PCR indicated a monoclonal pattern. No other molecular studies were performed. Flow cytometry on the lymph node biopsy showed a population of CD4 positive cells that also expressed CD10. These cells showed loss of CD7 in 50% of cells. This is consistent with AILT, but should be correlated with histology and other clinical findings.

These findings taken in conjunction with the clinical findings of polyclonal gammopathy, fever, weight loss and a skin rash confirmed the diagnosis of AILT.

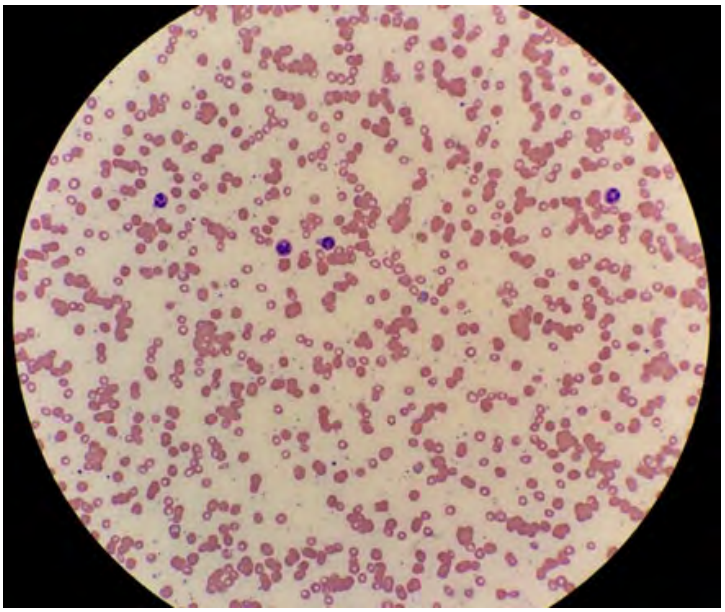

Figure 1. Blood film made from the warmed FBC specimen. The red blood cells show marked autoagglutination. Magnification x 100.

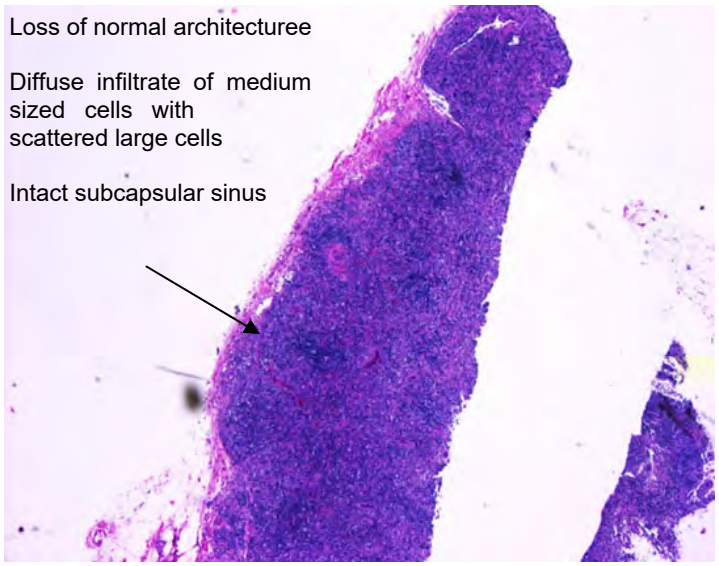

Figure 2. Lymph node biopsy (H &E stain; magnification x20).

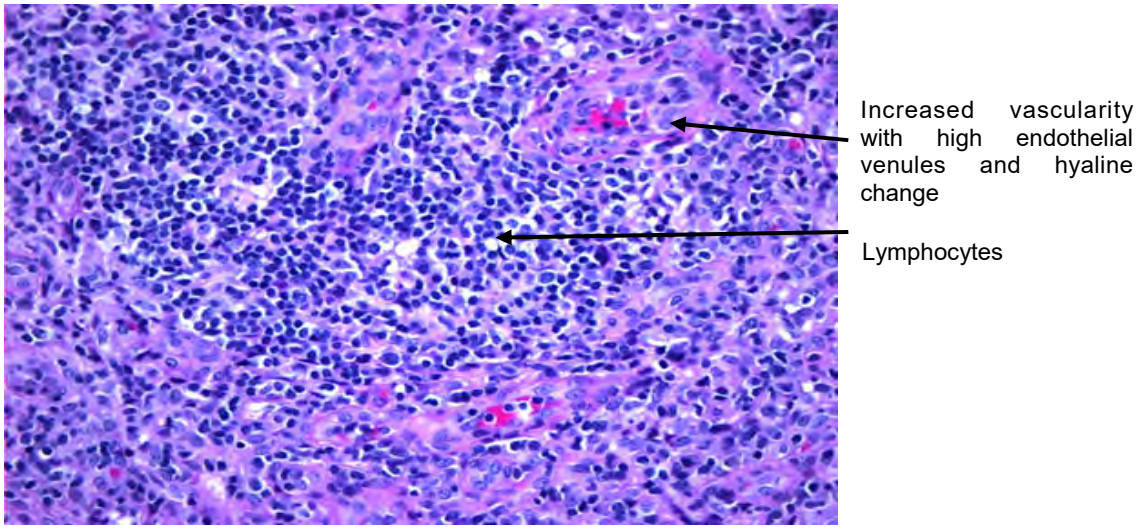

Figure 3. Lymph node biopsy showing proliferation of follicular dendritic cells. (Magnification x100).

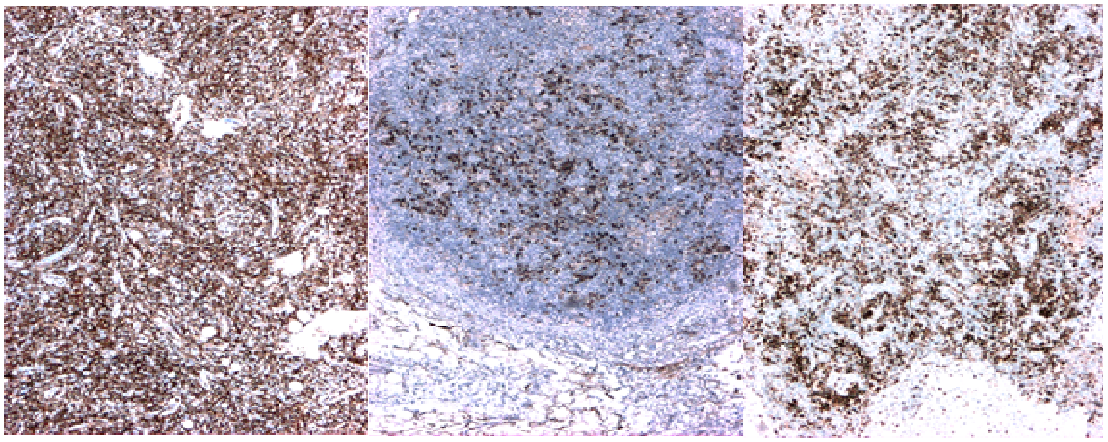

Figure 4. Lymph node biopsy showing positive expression of selected cell markers. The brown staining indicates positivity. Magnification x40).

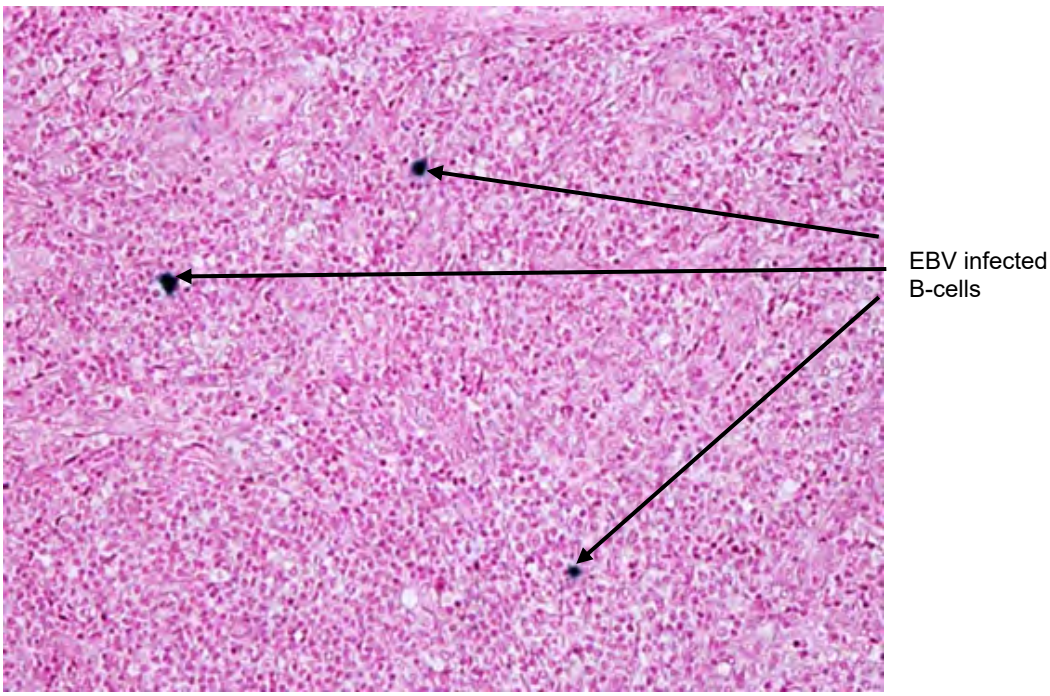

Figure 5. Lymph node biopsy showing the presence of EBV infected B-cells (Magnification x100).

DISCUSSION

Cold autoimmune haemolytic disease is caused by antibodies that act in cooler temperatures. There is agglutination of the red blood cells and extravascular and intravascular haemolysis, resulting in anaemia. Cold AIHA can be primary or secondary. Secondary causes include infections, autoimmune disease, and lymphoid malignancies. Vast majority of lymphoid malignancies causing cold AIHA are B-cell lymphomas. According to some studies, unspecified lymphoproliferative conditions cause approximately 9% of CAIHA. AITL will most likely fall into this group, although this is not specified in the related article (1).

The clinical features of AITL can include lymphadenopathy, hepatomegaly, splenomegaly, weight loss, fever, skin rash, fatigue, polyclonal hypergammaglobulinemia fatigue, and often autoimmune manifestations such as joint inflammation, muscle pain, haemolytic anaemia, and thrombocytopenia (1-3,6,7,9-11). There is also a frequent history of EBV infection (3,6,9). All these features were displayed by our patient.

In AITL, lymph nodes display either partial or complete destruction of the normal architecture due to a polymorphic infiltrate with proliferation of high endothelial venules and follicular dendritic cells (2,11). High endothelial venules are the post capillary venules found in secondary lymphoid tissue such as lymph nodes. They regulate the migration of lymphocytes in and out of the blood stream while actively proliferating lymphocytes congregate in the germinal centre in the middle of lymph nodes (12). The follicular dendritic cells form a stable network through which resting recirculating B-lymphocytes migrate, while antigen-activated B-lymphocytes are intercepted and proliferate to form the germinal centres (12). Proliferation of the follicular dendritic cells can be extensive in advanced cases of AITL (9). Follicular dendritic cells express the markers CD21, CD23 and CD35 (10).

Recent advances in the identification of specific cell markers have aided the diagnosis of AITL. In AITL, the neoplastic T-cells in lymph nodes display an aberrant expression of CD10 and express PD-1(Programmed Death-1) (8). Profiling by gene expression suggests that these neoplastic cells are follicular helper $T_{(FH)}$ cells originating from the germinal centre (3). These T-cells are usually CD4 and PD-1 positive (3), while CD10 is normally only expressed by germinal centre B-cells and B-cell precursors (8). A population of B-cells infected with Epstein-Barr virus (EBV) is frequently present in AITL (3,6,9).

Latent EBV infection is widespread world-wide (13). The question has arisen as to whether reactivation of EBV occurs as a result of immunodeficiency associated with AITL, or whether the virus is playing a central role in its early pathogenesis (3).

Although perhaps best known as a causative agent of infectious mononucleosis, there is also an association between EBV and a wide range of lymphoproliferative conditions and malignant lymphomas, such as Burkitt Lymphoma (3,13). The dysfunctional immune system which characterises AITL, gives rise to various clinical conditions due to the associated immunodeficiency (3). A postulated pathogenesis is outlined in Figure 6.

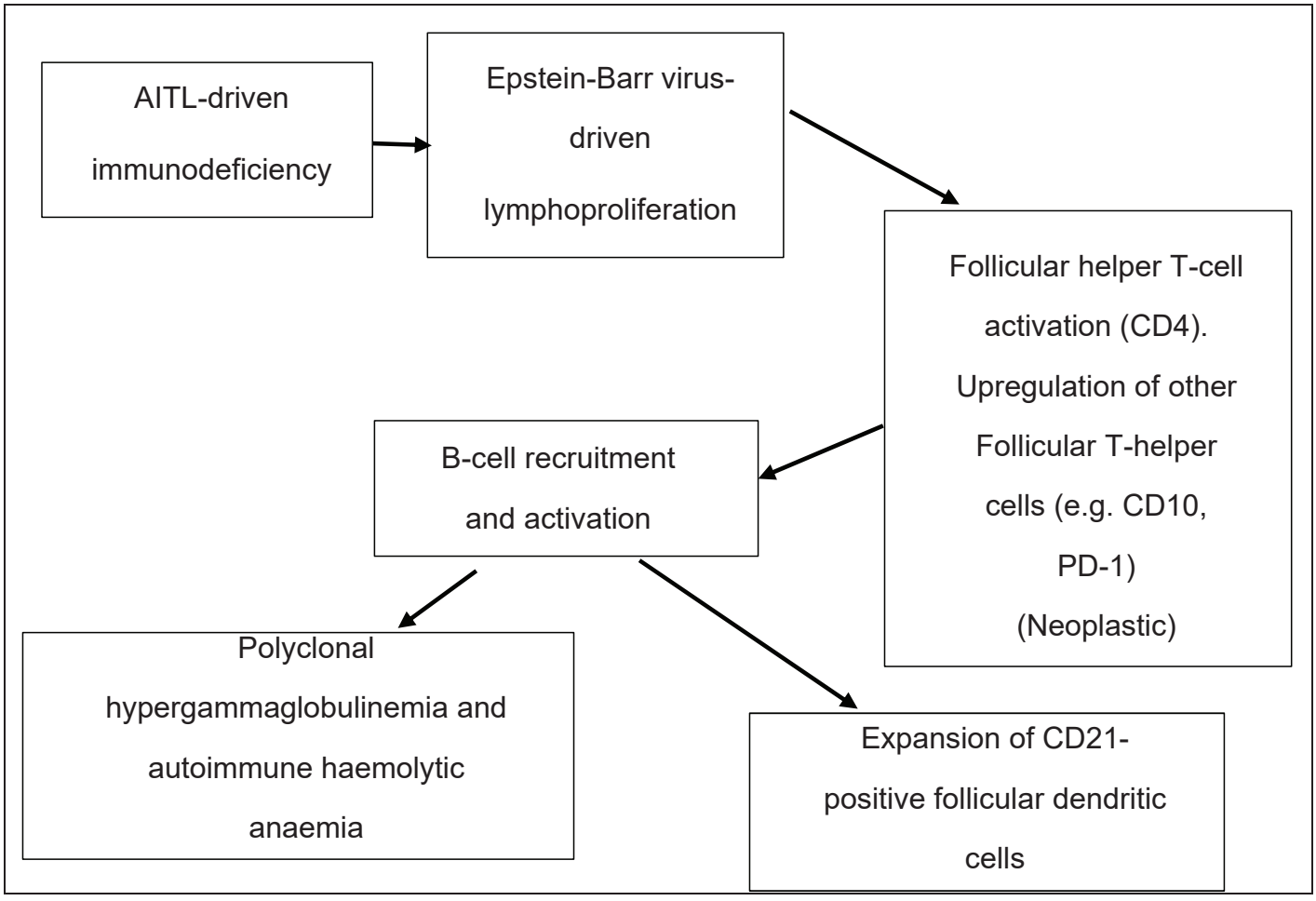

Figure 6. AITL pathogenesis simplified.

The 2016 World Health Organization has now classified AITL under nodal T-cell lymphoma with follicular T-helper phenotype (14). A review by Lunning *et al*. indicated that between 1975-1999, 61% of AITL patients had bone marrow involvement, with 28-70% between 2007-2016 (15).In a retrospective analysis of 115 newly diagnosed AITL patients, Hong *et. al.* estimated the 5-year survival rate at about 45%, with bone marrow involvement a significant adverse risk factor (16). A study by Gerlach *et al*., using matched pairs of bone marrow and lymph node biopsies from 37 patients, confirmed the unfavourable prognosis associated with bone marrow involvement, but noted that the survival rate depended on the infiltration pattern in the bone marrow (17). Three patterns were identified. Patients displaying an interstitial/micronodular pattern with eosinophils survived significantly longer than patients with either an interstitial/micronodular pattern without marked eosinophilia, or those with a diffuse pattern with eosinophilia (17).

Monoclonal B-cell lymphocytosis is defined as a monoclonal B-cell population <5 $\times 10^9$/L and is found in the bone marrow of 5-10% of apparently healthy persons over the age of 60 years. The significance of this phenomenon is uncertain, as it is unclear whether these are just incidental findings or the forerunner of disease. However, any diagnosis of lymphoma should be confirmed clinically (17).

Our patient exhibited a monoclonal B-cell population in the bone marrow and AITL in the lymph nodes. One could postulate that the CLL phenotype found in the bone marrow may have contributed to the immunodeficiency associated with the AITL. It is also possible that bone marrow clonal populations may be underdiagnosed if flow cytometry is not routinely performed. Patients with AITL have a poor prognosis (6). Death may be the result of opportunist infections arising from immunologic compromise rather than progressive lymphoma(3).

Treatment usually consists of a mixture of chemotherapy, radiation, and supportive care, such as blood transfusions and antibiotics (6). Stem cell transplant is an option for some patients receiving chemotherapy (6). During admission, our patient received steroid treatment. This reduced the neck swelling and symptomatic lymphadenopathy and partially resolved the haemolytic anaemia. The patient later chose alternative care rather than conventional treatment. She is now deceased.

In conclusion, cold antibodies are not an uncommon finding in a haematology laboratory, but these often appear to be weak and associated with an underlying infection. However, sometimes they do reflect a serious clinical condition, requiring further investigation and targeted treatment. This case report illustrates that the diagnosis of AITL is based on a combination of clinical, radiological, laboratory, molecular and histopathological findings. Cold antibodies as the first presenting feature further emphasises the importance of thoroughly investigating a patient with CAIHA to exclude an underlying condition.

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REFERENCES

1. Brugnara C, and Berentsen S. Cold agglutinin disease. *UpToDate* 2020. https://www.uptodate.com/contents/cold-agglutinin-disease.
2. Attygalle A, Al-Jehani R, Diss TC, et al. Neoplastic T- cells in angioimmunoblastic T-cell lymphoma express CD10. *Blood* 2002; 99 :627-633.
3. Dunleavy K, Wilson WH, Jaffe ES. Angioimmunoblastic T-cell Lymphoma: pathobiological insights and clinical implications. *Curr Opin Hematol* 2007; 14: 348-353.
4. Swiecicki PL, Hegerova LT, Gertz MA. Cold agglutinin disease. *Blood* 2013; 122: 1114–1121.
5. Berentsen S. New insights in the pathogenesis and therapy of cold agglutinin-mediated autoimmune hemolytic anemia. *Front Immunol* 2020; 11; 590.
6. Angioimmunoblastic T-cell lymphoma. 2018. http:// www.dovemed.com/diseases-conditions/ angioimmunoblastic-t-cell-lymphoma.
7. Federico M, Rudiger T, Bellei M, et al. Clinicopathologic characteristics of angioimmunoblastic T-cell lymphoma: analysis of the international peripheral T-cell lymphoma project. *J Clin Oncol* 2013 ;31: 240-246.
8. Dorfman DM, Brown JA, Shahsafaei A, Freeman GJ. Programmed death -1 (PD-1) is a marker of germinal center-associated T-cells and angioimmunoblastic T-cell lymphoma. *Am J Surg Pathol* 2006 30: 802-810.
9. Dogan A, Attygalle AD, Kyriakou C. Angioimmunoblastic Cell Lymphoma. *Br J Haematol* 2003; 121: 681-691.
10. Khokhar FA, Payne WD, Talwalkar SS, et al. Angioimmunoblastic T-cell Lymphoma in bone marrow: a morphologic and immunophenotypic study. Hum Pathol 2010; 41: 79-87.
11. Iannitto E, Ferreri AJM, Minardi V, et al. Angioimmunoblastic T-cell lymphoma. *Crit Rev Oncol Hematol* 2008; 68: 264-271.
12. The Free Dictionary. 2009. https://medical-dictionary.thefreedictionary.com.
13. Shannon-Lowe C, Rickinson AB, Bell AI. Epstein-Barr virus-associated lymphomas. *Philos Trans B Soc Lond Biol Sci* 2017; 372(1732): 20160271.
14. Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood* 2016; 127: 2375-2390.
15. Lunning MA, Vose JM. Angioimmunoblastic T-cell lymphoma: the many-faced lymphoma. *Blood* 2017; 129: 1095-1102.
16. Hong H, Fang X, Wang Z, et al. Angioimmunoblastic T-cell lymphoma: a prognostic model from a retrospective study. *Leuk Lymphoma* 2018; 59: 2911-2916.
17. Gerlach M Juskevicius D, Vela V, et al. Bone marrow infiltration of angioimmunoblastic T- cell lymphoma: identification and prognostic impact of histologic patterns and diagnostic application of ancillary phenotypic and molecular analyses. *Arch Pathol Lab Med* 2020; 144: 602-611.

EDUCATIONAL ARTICLE

Comparison between Medical Laboratory Science degrees at the University of Otago, New Zealand and Uppsala University, Sweden: a synopsis

Birgitta Tomkinson

Before the comparison between the universities, it is important to note that the structure of labour in a diagnostic laboratory is different in New Zealand compared to Sweden. In Sweden the majority of employees are Biomedical Laboratory Scientists (Medical Laboratory Scientists equivalent), with some other employees in the laboratory assistant or molecular biologist categories.

At Uppsala University, and in Sweden, the Degree of Bachelor of Biomedical Laboratory Science is three years full time study, but science (mathematics, chemistry, physics and biology) from upper secondary school is required for admission, which is also grade dependent. Comparing the workload for the students, the academic year is shorter in New Zealand compared to Sweden, and therefore the workload for the students taking four years in New Zealand would correspond to three years in Sweden.

The advantage of the Otago First Year Health Science programme is that the students are prepared for academic studies and most will continue and obtain their degree. In Uppsala, extra students are admitted to the program in order to compensate for losses during the first year, which is a strain on resources. It also makes it difficult to plan how many students we will have during the second and third year. A disadvantage with the Otago Health Sciences First Year is that the content must provide a suitable background for all health professional degrees and students probably don't identify themselves with the profession until the second year. In Uppsala we focus on providing information regarding their future profession at a very early stage and the content of all papers during all three years is focused on their future profession.

A comparison of the curricula at the two universities shows considerable similarity and for the two papers examined in detail, learning outcomes were the same. A point of difference between the degrees is that at Uppsala there are no grades, just approved (>60%) or fail (<60%) on all exams. To be admitted to third-year the students must be approved on 8 out of 10 exams from the first two years. The students have the right to sit each exam 5 times (twice at Otago). To start the second year, the students must have passed 40 credits out of 60 credits and for access to the third year the limit is 90 credits out of 120 credits. The students can do practical placements just one more time if they fail there, which is the same as Otago. It was noted that Uppsala has more emphasis on progressing students through oral presentation skills and provides more opportunities to improve writing skills.

In New Zealand, the two long placements at accredited laboratories provide a chance for students to be more proficient in their chosen areas and I would expect them to be able to work more independently when they are employed. With three shorter placements, Uppsala students are provided with a broader experience of several workplaces, but it is challenging to meet new colleagues so often and to learn new routines. They are prepared to work in a laboratory environment after their practical tuition, but some training on-site will be necessary before they will be able to work independently at any workplace. The longer research project that students at Uppsala University do probably gives a better research connection to their chosen subject. As a whole I believe that the students' knowledge of research and development in laboratory science is comparable at the two universities.

While there are many similarities between the two degrees, there are also many differences and this is especially true in the area of organisational control, with responsibility for the programme at Uppsala shared between more staff members. It is also a much simpler process to gain approval for course changes at Uppsala. While language and degree structure are a barrier to undergraduate student exchange between the universities, it is hoped that exchange of students studying for postgraduate degrees in Medical Laboratory Science will be possible in the future.

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UPPSALA UNIVERSITET

Abstracts 4th year BMLSc research projects Semester 1, 2020

Evaluation of the BioFire Filmarray-2.0 Meningitis/ Encephalitis and blood culture identification panels

Ghazwan Alashkar[1] and Gayleen Parslow[2]
[1]University of Otago, Dunedin and [2]Southern Community Laboratories, Dunedin

Objectives: Cerebrospinal fluid and bloodstream infections are responsible for high morbidity and mortality. Diagnosis of the causative pathogens is mainly based on microscopy, culturing onto agar media and PCR, which takes a long time leading to prescription of potentially inappropriate antibiotics. Over the past decade, the BioFire Filmarray system has been used in many laboratories along with other methods. This study aimed to assess the performance of the BioFire FilmArray Meningitis/ Encephalitis (ME) panel and the blood culture identification (BCID) panel in a hospital microbiology laboratory.
Methods: Twenty-four monomicrobial blood-culture samples and six positive cerebrospinal fluid samples (one of them was poly-microbial) were tested using the BioFire Filmarray system. Ten samples were spiked, and the others were clinical. Results were compared with those obtained using routine methods, including identification by MALDI-TOF MS. This study took approximately one month to be completed.
Results: Twenty-one/twenty-one (100%) blood cultures containing pathogens included in the BCID panel gave positive results. Three out of three (100%) isolates that were not included in the BCID panel gave negative results. Additionally, all resistance genes in spiked samples were correctly detected; these were *Enterococcus* VanA/B, *Staphylococcus aureus* mecA and *Klebsiella pneumoniae* KPC. In the ME panel, bacterial pathogens in the polymicrobial (one) and monomicrobial (one) samples were correctly detected, and four out of five (80%) of other microbes were detected. A CSF sample containing a pathogen not included in the ME panel gave a negative result.
Conclusions: The BioFire FilmArray system is easy to use. It helps decrease the time to results to about 80 minutes, and it has an overall high specificity and sensitivity when compared with traditional methods. However, it is expensive, some important pathogens are not covered in the panels, it only processes one sample per run, and the possibility of having false negative and false positive results should be taken into consideration.
Acknowledgement: The primary author thanks Gayleen Parslow for providing the results for this study.

Establishing a cost-effective method to perform mass immunohistochemistry validation within a histopathological setting using tissue microarrays

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Objectives: The process of mass immunohistochemistry validation is under rigorous scrutiny in order to become a more streamlined and efficient process within a histopathological setting. The aim of this project was to determine the cost-effectiveness of three different methods in performing mass immunohistochemistry validation within Wellington Southern Community Laboratories (WSCL). A comparison was performed between singular tissue blocks and multi-tissue blocks in accordance with NordiQC guidelines. These methods were further compared to a third method devised by WSCL to best optimise the use of tissue-microarrays and the avoidance of having to use rare and specific tissue types that cannot be sourced easily within WSCL's own tissue bank.
Methods: Each of the three methods were compared at each individual stage of mass immunohistochemistry validation, by measuring the expected resources, labour and logistics required to perform each individual task. These stages started from tissue sourcing, embedding/ tissue-microarray block creation, cutting of sections on to the slides, and finished with immunohistochemistry staining. The total cost of each method was calculated by factoring the above aspects in a holistic manner.
Results: The singular tissue per block method is the most expensive and logistically difficult out of the three methods, brought about by a high degree of labour required to perform microtomy and immunohistochemistry staining, with a high number of slides to stain. The multi-tissue per block method and WSCL tissue-microarray method were fairly comparable in terms of the cumulative cost, with the former slightly cheaper than the latter. However, the WSCL method was deemed superior in a logistical sense as there were less specialized and rare tissues required for the process.
Conclusions: The WSCL method of mass immunohistochemistry validation was superior to the other two methods when factoring in both cost-effectiveness and logistics.

Validation of a new biotin-tolerant Roche NT-proBNP assay with comparison to the current Roche assay

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

Objectives: N-terminal pro B-type natriuretic peptide (NT-proBNP) is a clinically important cardiac marker that requires accurate laboratory results for the assessment of acute coronary syndromes, left ventricular dysfunction, and heart failure. Increasing use of biotin supplements has raised concerns regarding biotin interference in the current Roche NT-proBNP (proBNP II) immunoassay. Thus, this study aimed to validate a new, biotin-tolerant Roche NT-proBNP (PBNP2) assay and to compare its performance to the current Roche assay.
Methods: Serum NT-proBNP measurements using both current and new Roche assays were performed on 20 serum samples from 20 patients following the manufacturer's instructions on the Roche Cobas 8000 e602 system.
Results: The new Roche assay showed a statistically significant mean difference of 27.5 pmol/L (95% Confidence Interval [CI] 2.8-52.2 pmol/L; 95% Limits of Agreement -69.8 to 124.9 pmol/L; p = 0.0309) from the current assay. Median NT-proBNP values of the two assays were statistically different and had a location shift (Hodge-Lehmann shift = 18.3 pmol/L; 95% CI 2.6-37.9 pmol/L; p < 0.0001). Passing-Bablok regression yielded PBNP2 = 1.077 proBNP + 0.415 (n=18; 95% CI for slope 1.060-1.115; 95% CI for intercept -0.1039 – 0.7904), suggesting a slight but significant positive bias (about 7.7%) of the new NT-proBNP assay.
Conclusion: In this study, the new Roche assay showed clinically comparable results with a slight positive bias (about 7.7 %) to that measured on the current assay. Therefore, the new Roche NT-proBNP assay is suggested to replace the current assay owing to its greatly reduced biotin interference and satisfactory analytical performance.

Comparing the effectiveness of the current cervical screening pathway and the proposed pathway

Debbie Mirambel[1] and Michelle Cheale[2]
[1]University of Otago, Dunedin and [2]Canterbury Health Laboratories, Christchurch

Objectives: The National Cervical Screening Programme (NCSP) proposed to replace liquid-based cytology (LBC) with HPV testing as the primary screening step in the cervical screening pathway. This research-based study aims to compare the effectiveness of the current cervical screening pathway and the proposed screening pathway.
Methods: Ten studies were pooled in total, five from the list of technical references provided by the NCSP on their website, and the other five were found by searching keywords "HPV primary testing" in an Otago Library search with selected filters: "Years: 2010-2020; peer reviewed journals; journal articles"
Results: Nine out of ten of the pooled studies found that HPV testing is more effective than LBC testing as a primary screening tool. The one study that did not have the same conclusion suggested that HPV and LBC co-testing should be carried out. One of the studies also found that, despite the effectiveness, Māori women still had a higher rate of incidence of and mortality from cervical carcinomas. Two of the studies predicted that the proposed screening pathway is cost-efficient and is expected to reduce programme costs about 16% by 2035 compared to 2018 costs.
Conclusion: HPV primary testing is more effective than LBC testing, but it is important to explore the effectiveness of co-testing to maximise the sensitivity of the screening pathway but also reduce the false positive results to prevent unneeded anxiety as a result of the misdiagnosis. Educating Māori women on the screening programme is also important in order to decrease the disparities in the incidence of and mortality from cervical carcinoma. The proposed pathway is also cost-effective. However, it is important to remember that the lives of women who rely on the cervical screening programme are more important than money.

Comparison between ten minutes incubation and immediate spin on the ABO/RhD (DVI-, DVI-) + Reverse grouping cards

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Objectives: Various factors can affect red cell serological reactions, including incubation time where a longer incubation period produces stronger antibody-antigen reactions. This project was carried out to compare between immediate spin and 10 minutes room temperature (RT) incubation on the ABO grouping microcolumn gel cards.
Methods: Two sample groups, including patient samples with weak reverse grouping reactions (≤ 2+) and with known cold antibodies, were chosen for this project. Test sample (0.8% red cell suspension/ plasma) was added into respective microtubes containing the appropriate reagent (monoclonal antibodies/ reagent red blood cells) of the BioRad ID-card DiaClon ABO/ RhD (DVI-,DVI-) + Reverse grouping card and left standing for 10 minutes at RT. After centrifugation, reaction intensity was determined according to the position of the agglutinates in the gel. Results were compared with the reaction grades obtained from immediate spin.
Results: 12 of 14 (86%) patient samples with weak reverse grouping reactions demonstrated increased reaction grades after 10 minutes incubation while the other two samples (14%) showed unchanged and decreased reactions. For patients with known cold antibodies, three of six showed increased reaction grades, whereas one sample had a decreased reaction and two had unchanged reaction grades.
Conclusion: Ten minutes incubation was demonstrated to improve antibody-antigen reactions in samples with weak reverse grouping reactions resulting in stronger reactions. However, incubation provides a higher opportunity for cold antibodies to cause agglutination providing stronger reactions leading to false positive results. Limitations, such as insufficient patient samples and variation in incubation time of the IH-1000 analyser, should be considered. Moreover, the increase in reaction was not significant enough to change the current method used at WSCL as increase in incubation period would increase the process time for urgent samples.

Comparison of routine Grifols DG Gel Card technique with conventional Tube IAT method for alloantibody identification

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Objectives: Antibody identification is essential for the provision of compatible blood units. The goal of this study was to compare the routine use of two antibody identification methods, namely Grifols DG Gel Card and Tube IAT, mainly focusing on differences in procedures, result interpretations, and exclusion criteria. The secondary goal was to compare the performance of BioRad and Grifols DG Gel Card methods to evaluate whether the implementation of the BioRad method for routine antibody identification would be beneficial.
Methods: Over a period of one week, patients who received positive antibody screens were tested using three methods (Tube IAT, Grifols DG Gel Card, and BioRad Card) to identify the clinically significant antibodies present.
Results: The results showed that both Grifols DG Gel Card and Tube IAT methods were able to successfully identify the antibodies present in all tested patients. The Tube IAT method failed to exclude some clinically significant antibodies, therefore further testing was required. When comparing the performance of the BioRad and Grifols DG Gel Card methods, the results obtained were essentially similar. Both Grifols DG Gel and BioRad Card methods produced strong reactions and identified antibodies present without additional testing.
Conclusion: Overall, the Grifols DG Gel Card method has certain advantages over the Tube IAT, namely faster test performance, easier result interpretation, and better antibody identification and exclusion. The BioRad Card technique showed nearly identical performance to Grifols DG Gel Card method, thus it was impossible to conclude whether its implementation would further benefit the routine antibody identification. The obtained results are comparable to the results of similar studies. Due to small sample size, a definitive conclusion cannot be given, and more testing is required.

Now is the hour

Fran van Til, Executive Officer

"Now is the hour when we must say goodbye.
Soon I'll be sailing far across the sea.
While I'm away, please remember me.
When you come to Rangiora, you'll find me waiting here."

I so remember being interviewed by the late Barrie Edwards and the late Kevin McLaughlin for the position of Executive Assistant for the New Zealand Institute of Medical Laboratory Technology. First, I had to navigate my way around Christchurch Hospital to the rabbit warren of an antiquated laboratory holding back my unfounded fear of coming face-to-face with a corpse. In all honesty, I cannot remember the finer details of the interview except for Kevin leaning against the wall with his arms folded across his chest and saying "so you know all about computers then?" I have to assume the interview went well, as within a week an office was set up in my home, then I went from the Executive Assistant to the Executive Officer when Barrie stood down as the long-standing secretary.

Little did I know (or Barrie and Kevin), that you would have to put up with me for so many years!

However, it is not about me – it is about you. Your dedication and perseverance ensuring that your work is carried out at the highest possible standard, your willingness to enhance the well-being of all in New Zealand, is a credit to you all.

Let's go back to the beginning. After my encounter with Barrie and Kevin, it was then a briefing with Paul McLeod (then President) at Christchurch Airport to enter a world that I knew nothing of. My introduction to my first Council meeting was by way of the 'larger than life', the late Jim Le Grice. I have been so honoured to have worked with a number of Presidents: Paul McLeod, Dennis Reilly, Anne Paterson, Shirley Gainsford, Les Milligan, Chris Kendrick, Robin Allen, Kevin Taylor, Ken Beechey, Ross Hewett and Terry Taylor. Each have brought different skills and strengths to the Council table. I am astounded at the energy they put into this organisation for the betterment of the members and the profession.

The above have been ably supported by their respective elected Council members (too many to mention) who are willing to pick up the extra portfolios and duties of QMLT examinations, professional affairs, membership, financial, liaison with the Universities (this is only a few areas of responsibility – there are many more). It has been a pleasure working with all of you.

I have worked with so many amazing scientists and technicians who take up the roles of SIG convenors, seminar organisers, examiners and moderators, organisers of the annual conference – conference being my really happy place. All these roles are vital to the NZIMLS and without you being willing to take these extra responsibilities, the organisation would be much poorer, both fiscally, educationally and qualification-wise. To all that I have worked with in these capacities, a big 'thank you'. I have enjoyed meeting and getting to know you all.

Special mention must be made of our diagnostic companies and their representatives. Sponsors and exhibitors are a major resource to any organisation and their events. We are so fortunate as we have a great relationship with these companies in New Zealand. They are both generous with their time and support, and I have been treated to many wonderful occasions with these people. Please know that you are truly appreciated. I have also valued your friendship.

Speakers too are an enormous part of any seminar and conference. It has been awesome meeting and working with these people, both nationally and internationally.

You are being left in good hands - Sharon Tozer is a long-term employee and Jillian Broadbent a long-term contractor. These gorgeous girls bring diverse abilities, excellent skills and experience to the NZIMLS team and I have no doubt that they will continue, within their respective roles, to enhance the obligations of the NZIMLS Council, its members and the profession of medical laboratory science. Thank you both for your all your assistance, tolerance, perseverance and care. I am seriously going to miss working with these two wonderful ladies. We now work closely with Mike Legge particularly since he came on board as a professional advisor. The NZIMLS is fortunate to have such an enthusiastic person involved with a number of professional directives, and I shall miss him also.

One last note – I cannot exit without acknowledging that the NZIMLS Journal is a credit to all those involved. Over the years, it has gone from strength to strength and is a publication worthy of local and overseas recognition. The Journal is the baby of Rob Siebers and he has committed himself to producing a world-class publication. You have been an impassioned Editor Rob, and well done to you. The status of the Journal did not change overnight, rather from long hours of Rob's devotion.

What you in the laboratories have endured particularly over the past couple of years is incomprehensible to most:

- The Christchurch earthquakes
- The Mosque shootings
- The White Island disaster
- And now COVID-19

Through all of these unforeseen events, our medical laboratory science people excelled whilst also dealing with your own circumstances, families and friends. Your professionalism and dedication to your work, and to others particularly at these times, is exceptional. I have met, valued and enjoyed the company of so many of you very remarkable people. I have made lifelong friends who I will continue to treasure. And you will always be very, very special to me.

From my point of view, it has been 30 years of privilege. I am always in awe by the resilience, dedication and professionalism of our members and your work in diagnostic laboratories. It has been a pleasure being the Executive Officer of such a worthy organisation.

The role as Executive Officer has been demanding, yet it is one that I have been very privileged to hold. While it is a wrench leaving the NZIMLS and the office, I am leaving you in excellent hands. I will continue to value my Life Membership of the Institute and watch with keen interest as the NZIMLS continues to serve its members.

A big thank you to Eddy and our children, Tiffany, Devon and Ben. Without their love, encouragement and tolerance, this journey could have been a different story. I am looking forward to spending more time with my family and their activities, friends, maybe some time at the piano and on the tennis court. The garden will definitely benefit!

"Should auld acquaintance be forgot
And never brought to mind?
Should auld acquaintance be forgot
And days of auld lang syne?

For auld lang syne, my dear
For auld lang syne
We'll take a cup o' kindness yet
For auld lang syne"

MINUTES OF THE NZIMLS VIRTUAL ANNUAL GENERAL MEETING HELD THURSDAY 20 AUGUST 2020 at 1.30pm

PRESENT
The President presided over approximately 40 members.

APOLOGIES
Apologies were received from Shirley Gates, Jill Meyer

PROXIES

Fran van Til	1
Sue Melvin	6
Jennifer Sucich	1

MINUTES OF THE AGM HELD 17 August 2019
Proposed by T Barnett, seconded R Siebers
That the minutes of the Annual General Meeting held on 17 August 2019 be received.
Carried

Proposed by T Barnett, seconded R Siebers
That the minutes of the Annual General Meeting held on 17 August 2019 be received as a true and correct record.
Carried

Proposed by T Barnett, seconded S Munroe
That the motions from the minutes of the previous Annual General Meeting held on 17 August 2019 be approved.
Carried

BUSINESS ARISING FROM THE MINUTES
Nil

REMITS AS CIRCULATED
Moved
Seconded: J Sheared
"THAT Policy Decision Number 3 be reaffirmed

Policy Decision No 3 (1972): Council will make and administer awards to members of the Institute, the details of each award will be recorded and may be amended from time to time by resolution of Council. The summary of these details shall be published annually in the Journal.
Carried

Moved
"THAT Policy Decision Number 5 be reaffirmed."

Policy Decision No 5 (1978): That invitro diagnostic companies should not be approached to aid in the finance of Special Interest Group meetings; companies may be invited to SIG Seminars and although donations may be accepted money is not to be solicited.
Carried

PRESIDENTS REPORT
Proposed by T Barnett, seconded T Taylor
That the President's Report be received.
Carried

T Taylor noted that his report refers to the year ending 31/3/20 so does not include events after that date. He then read his report. Dr David Murdoch and Siouxsie Wiles were thanked for their representation of the profession during these trying times.

Council is currently concentrating on strategic priorities and these were outlined to members.

T Taylor then thanked the Executive and Council for their work during the year, along with J Broadbent, R Siebers, S Tozer and M Legge for their contributions. Also, a big thank you to all members for supporting NZIMLS events during the past year, and the employers for allowing their staff to attend these events.

ANNUAL REPORT
Proposed by T Barnett, seconded T Taylor
That the Annual Report be received.
Carried

FINANCIAL REPORT
Proposed by T Barnett, seconded S Melvin
That the Financial Report be received.
Carried

COUNCIL ELECTIONS:
Not required for 2020. Therefore, the NZIMLS Council for the 2020/2021 term will be:

President:	Terry Taylor
Vice President:	Mary-Ann Janssen
Treasurer / Secretary:	Tony Barnett S
Region 1 Representative:	Sujata Hemmady
Region 2 Representative:	Sean Munroe
Region 3 Representative:	Philip Ibrahim
Region 4 Representative:	John Sheard
Region 5 Representative:	Sue Melvin

HONORARIA
Motion:
Moved R Siebers, seconded T Taylor
That no honoraria to be paid.
Carried

AUDITOR
Motion**:**
Moved T Barnett Seconded T Taylor
That the auditor for 2020/2021 financial year be Nexia New Zealand Limited.
Carried

GENERAL BUSINESS
C Kendrick queried the CPD programme and if NZIMLS has submitted an updated programme to the Medical Sciences Council (MSC). T Taylor responded that NZIMLS have been working over the past few months refining the programme and following the domains as outlined by the MSC. The revised programme is currently before MSC for consideration at their next meeting. Council do not expect there to be any issues.

R Siebers, on behalf of the PPTC, recorded their thanks for the continuing support of NZIMLS Council.

2021 Annual Scientific Meeting
Will be held at the Waipuna Hotel & Conference Centre, Auckland 24-28 August 2021. The convenor is Tracy Camp from LabPlus. This will include the NZIMLS 75th celebration.

2022 Annual Scientific Meeting
T Barnett asked for anyone interested in holding this event to contact the Executive Office

There being no further business, the meeting closed 1.52pm.

Publications by NZIMLS members

This column is to showcase recent international peer-reviewed publications by NZIMLS members. If you have had such a recent publication please provide full details to the Editor at rob.siebers@otago.ac.nz

Pullon BM, Moore JA. Hemoglobin Ottawa (HBA2:c.46G>C) and β+ thalassemia (HBB:c.-138C>T) detected in an Indian male by capillary zone electrophoresis. *Thalassemia Reports* 2020; 10: 8733.

Pullon BM, Moore JA. Hemoglobin Manitoba [α102(G9) Ser→Arg] in Pasifika: Tongan case report. *Hemoglobin* 2020; 1-4: doi: 10.1080/03630269.2020.1785890.

Irinyi L, Hu Y, Hoang MTV, Pasic L, Halliday C, Jayawardena M, Basu I, et al. Long-read sequencing based clinical metagenomics for the detection and confirmation of Pneumocystis jirovecii directly from clinical specimens: A paradigm shift in mycological diagnostics. *Medical Mycology* 2020; 58: 650-660.

Vesty A, McAuliffe G, Roberts S, Henderson G, Basu I. Mycoplasma genitalium antimicrobial resistance in community and sexual health clinic patients, Auckland, New Zealand. *Emerging Infectious Diseases* 2020; 26: 332-335.

Fox-Lewis A, Basu I, Vesty A, Henderson G, Chhibber AV, Thomas M. *Helicobacter cinaedi* bacteremia in a returning traveler. *IDCases* 2020; 21: e00910.

ANSWERS AUGUST 2020 JOURNAL QUESTIONNAIRE (A)

1. Phenotypic tests for detection of carbapenemase production are prone to false negative results due to what?
   Weakly expressing carbapenemases, such as OXA-48-like and OXA-23, multiple resistance mechanisms and detection in non-Enterobacterales.
2. The CARBA PAcE test is based on what test principle?
   Enzyme hydrolyses of an indicator carbapenem and conversion of a pH indicator from yellow to red and shades thereof.
3. Fox P3 has a role in controlling what, acts as a negative regulator of what, and what does it suppress?**Expression of IL-2 and CD25 genes in Tregs. It acts as a negative regulator of cytokine production by CD4 T cells and suppresses the transcription of IL-2 and other cytokine genes.**
4. What are Tregs necessary for?
   Maintaining tolerance to self-antigens by suppressing self-reactive T cells.
5. Why is the hyperinsulinaemic-euglycemic clamp test not useful in clinical application and epidemiological investigation?
   Because it is costly, has complicated application, the method is invasive and time consuming.
6. What are thought to be the benefits of increased levels of haemoglobin F in sickle cell anaemia?
   Ameliorating the sickling process, reducing crisis frequency, and impacting positively on quality of life.
7. What defines the clinical features of sickle cell anaemia?
   Chronic anaemia, haemolysis, sepsis, and recurrent acute vaso-occlusive crisis.
8. Where is the site of bleeding due to subdural haemorrhage and what is it usually the result of?
   Site of bleeding is located between the dura and the brain. It is usually the result of the rupture of bridging veins between the cortex and the discharging sinuses.
9. What is enolase, how does it act, and what is it composed of?
   It is a dimetric cytoplasmic enzyme that acts on the glycolytic pathway and is composed of three distinct subgroups called alpha, beta, and gamma.
10. Which aspects of work practice can be considered in everyday ethical concepts?
    Consent, confidentiality, honesty, conflict of interest, proficiency, medical actionable results, professionalism, and codes of conduct.

ANSWERS AUGUST 2020 JOURNAL QUESTIONNAIRE (B)

1. Why is detection of the nuclear dense fine speckled pattern for DFS70 antibody important? **Because it prompts follow up testing by a DFS70-specific assay. Anti-DFS70 IgG, when detected as an isolated autoantibody, is thought to be an exclusion marker for connective tissue disease.**
2. Sensitivity of microscopy for the diagnosis of fungal infections varies with what?
   With the individual agent, the source and quality of the specimen and the skills and experience of the laboratorian.
3. What would help monitoring extraction efficiency of fungal DNA in clinical samples?
   An exogenous positive extraction control which is phylogenetically unrelated to the target pathogen, yet reasonably reflects the passage of target pathogen through the extraction route.
4. Which *Legionella* species are in the majority associated with Legionnaires' disease in New Zealand?***Legionella longbeachae* and *Legionella pneumophilia*.**
5. What are the risk factors for Legionnaires' disease in adults?
   Smoking, chronic obstructive pulmonary disease, diabetes, a compromised immune system, and being the recipient of a transplant or chemotherapy.
6. In antibody screening patients' plasma is tested against red cells that express which major red cell antigens?
   Rh, Kell, Duffy, Kidd, Lewis, P, MNS, and Lutheran blood group systems.
7. What does alloimmunization to red cell antigens depend on?
   Both genetic and acquired patient factors, as well as the dose and immunogenicity of the antigens.
8. What can a positive direct antiglobulin test be attributed to?
   Autoimmune haemolytic anaemia, incompatible blood transfusion, and some medications.
9. Platelet activation in diabetes increases the risk of what?
   Thrombosis, myocardial infarction, and stroke.
10. What is thought to give rise to platelet activation, what does this promote, and how is this reflected?
    Endothelial dysfunction and direct glycation of platelets are thought to give rise to platelet activation which in turn promotes haemostatic disturbance in diabetes. Platelet activation is reflected in increased mean platelet volume.

Science Digest

Contributed by Michael Legge

Check the radiocarbon dating before buying for Christmas!

Scotch whisky is a significant international export earner for the UK worth over four billion UK pounds per year. Despite the numerous brands "Scotch Whisky" is a protected title under UK and EU laws and the precious liquid is available in two forms: single malt and blends whisky. Either of these whiskies must be aged in oak barrels for a minimum of three years with a barrel capacity of no greater than 700 litres. Due to international demand a market has developed for rare Scotch Whisky with a specific emphasis for the expensive 19th to mid 20th century single malt whiskies. Increasingly this has led to production of fraudulent 'rare' single malt Scotch Whisky.

Aware of this issue, Scottish researchers investigated whether whisky 'fingerprints' could be established to determine the true age of the whisky rather than using biochemical profiles which were not definitive (1). The work was based on measuring anthropogenic radiocarbon decay rates as a dating signal that was produced from the atmospheric nuclear weapons testing during the 1950s and 1960s. This technique has been used in forensic analysis, toxicology, and the wine industry. Reputable Scotch Whisky distillers through-out Scotland provided small whisky samples of known years from their archives. The researchers were then able to use the dates and radiocarbon analysis to provide a calibration curve relating to dates and radiocarbon decay.

Using these data researchers tested eight 'rare' brand single malt whiskies and three were identified as fake: Talisker 1863, Laphroaig 1903, and Ardbeg 1964. Rare single malt Scotch whiskies often sell at auction for up to and over 10,000 UK pounds per bottle, with one bottle of a 1926 whisky fetching 848,750 UK pounds in 2017. With a market estimated at 40 million pounds in 2018 for rare whiskies the authors conclude that the market is very susceptible to fraud, however using radiocarbon decay rates, single malt whiskies can be accurately dated to within 1 to 3 years of manufacture depending on the barley being used.

Complementary therapy glucosamine may offer more than expected?

Glucosamine and chondroitin are frequently associated with complementary therapy to treat joint pain and cartilage deterioration typically found in osteoarthritis. A primary source of glucosamine is crustacean shells which is then chemically modified for use. (These may be contaminated with the muscle protein tropomyosin, a significant allergen cross-reacting with house dust mite allergen). Glucosamine is often sold in combination with chondroitin sulphate, which is widely sourced from bovine, porcine, chicken, or predominantly shark cartilage.

As shellfish reactions are one of the most common allergenic reactions, Australian researchers investigated spontaneous allergic reactions to glucosamine and chondroitin using the Therapeutic Goods Administration Adverse Events Notifications database (2). The study examined 366 spontaneously reported adverse drug reactions (ADR) where the only suspected drugs were glucosamine and chondroitin. Of the 366 ADRs to glucosamine and chondroitin preparations, 71.9% of cases were identified as having hypersensitivity reactions. Of these 92 were classified as mild, 128 as moderate, and 43 cases as severe hypersensitivity reactions. Additionally, 2% of all cases of ADRs had some degree of liver involvement. Four of the cases (1%) were reported as anaphylactic shock. In addition, there was evidence of an interaction with glucose-lowering medication commonly used for treating Type 2 diabetes. Given the mean age of the ADR data was 62.9 ±13.61 for females and 59.4 ±13.19 for males, these are the most likely age group to be treated for Type 2 diabetes, as well as joint disorders, thereby confirming previous published work that complementary therapy has the most severe detrimental effect when taken with conventional medication. The authors conclude that more attention is needed from clinicians when treating patients relating to complementary agents use and that clear labelling relating to ADRs is necessary.

An interesting case of mononucleosis

An 18-year-old female visited her physician complaining of fever, a sore throat, and swelling in her neck. There were exudates on her tonsils but tested negative for streptococcus and for infectious mononucleosis using the "Monospot" test. A full automated blood count was normal. EBV antibodies were negative as were viral capsid antigen IgM and IgG, and EBV associated nuclear antibodies. The illness progressed over the next 10 days and she was admitted to the Emergency Department presenting with a temperature of 38.9^{o}C, lymphadenopathy in her neck, and the tonsils were coated with a white exudate. The second "Monospot" test was negative. A repeat blood count demonstrated a white count of 15x10^{9}/L and a manual differential showed 79% lymphocytes, including 30% reactive lymphocytes. When stained with Wright-stain numerous Downey cells were identified (classified as I, II, and III) that are associated with infectious mononucleosis. When the patient's plasma was tested for viral DNA with EBV, PCR gave a positive result and the patient subsequently made an uneventful recovery.

The authors concluded that routine tests, such as "Monospot", are 80-90% accurate for true positives, but if EBV-PCR is available it is 99% accurate. If EBV-PCR was not available, then a full blood count with manual differential would resolve the outliers (3). As an interesting note the authors indicated that when Downey first described the cells in 1923 a colleague presumed a diagnosis of leukaemia.

A potential treatment for bacterial biofilms from the 10th Century

Although the use of plant compounds and other natural sources continue to be investigated, the modern approach is to identify and isolate what would be the 'active' compound. Historically it has been observed that by using whole plant extracts there is often a synergy between the potential active compound and other unrelated compounds in the plant extract. Prior to the advent of modern medicine, many remedies were plant based and in medieval times an extensive pharmacopeia was prevalent to "cure" many diseases. One of these was Bald's Leechbook which was one medieval source that has survived to modern times and has a recipe for Bald's eye-salve specifically for eye infections.

A UK research group investigated the efficacy of BALD's eye salve in bacterial biofilm related infections (4). The ingredients for this preparation were: garlic, cropleac (interpreted from the old English as either onion or leek) crushed and added to an equal volume of wine and ox gall (bovine bile) all mixed together. In preliminary experiments the onion preparation was identified as being the most effective rather than leeks. When tested against biofilms *of S. aureus,* the preparation was consistent in killing 82.7% of biofilms compared with the controls. When tested against: *P. aeruginosa,*

A. baumannii, E. cloacae, S. maltophilia, S. aureus, S. epididermus and *S. pyogenees* the mixture eradicated all planktonic cultures with the exception of *S. aureus* USA 300, and *S. maltophilia* where a decrease in culture growth was noted. When tested in synthetic wound cultures a 2 to 6 log drop in viable cells was observed for *S. aureus* Newman, *S. aureus* USA 300, *S. epidiymus*, *S. pyogenees* and *A. Baumannii*. No consistent killing was identified for *P. aeruginosa*, *E. cloacae* or *S. melophilia*. When tested individually the ingredients did not have the same effect with the exception of garlic which demonstrated anti-planktonic activity probably by anti-quorum sensing, however, in the synthetic wound analysis it was the combined effect of the ingredients rather than a single component. Interestingly the researchers considered the manual grinding of the garlic and onion (as historically preferred) as an important process for releasing such anti-bacterial compounds and activity.

AUTHOR INFORMATION

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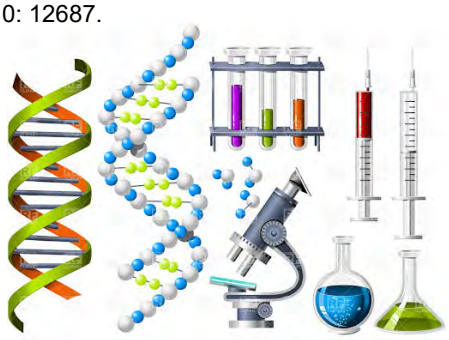
REFERENCES

1. Cook GT, Dunbar E, Tripney BG, Fabel D. Using carbon isotopes to fight the rise in fraudulent whisky. *Radiocarbon* 2020; 62 (1) 51-62.
2. Hoban C, Byard R, Musgrave I. Hypersensitive adverse reactions to glucosamine and chondroitin preparations in Australia between 2000 and 2011. *Postgrad Med J* 2020; 96: 190-193.
3. Feder HM Jr, Rezuke WN. Infectious mononucleosis diagnosed by Downey cells: sometime the old ways are better. *Lancet* 2020; 395: 225.
4. Furner-Pardoe J, Anonye BO, Cain R, et al. Anti-biofilm efficacy of a medieval treatment for bacterial infection requires the combination of multiple ingredients. *Sci Rep* 2020; 10: 12687.



News from the University of Otago Medical Laboratory Science Programme

Visiting Professor

In Februrary, Professor Birgitta Tomkinson, Course Director for Biomedical Laboratory Science at the University of Uppsala, Sweden, arrived on a three-month teaching sabbatical. Unfortunately, her visit was cut short by the COVID-19 pandemic. However, she managed to complete nearly all of the programme arranged for her before lockdown came into effect. One of the aims of her visit was to carry out a comparison between the Otago BMLSc and the equivalent Bachelor of Biomedical Laboratory Science at Uppsala University. A synopsis of her report is as an Educational Article elsewhere in the Journal.

COVID-19

COVID-19 had a major impact on student study in Semester 1, but students responded well to the switch to online learning. Laboratory classes were suspended for Semester 1 papers, but 'make-up' classes have been squeezed into Semester 2 to ensure no essential practical skills are missing. In Semester 1, 4th year students managed to complete nearly half of their placements, with some able to partially, or totally finish, their research projects. Two students were employed during level 4 lockdown to assist in microbiology and molecular diagnostics laboratories. Both found the experience invaluable and were grateful for the opportunity.

Under Alert Level 2 this Semester, extra laboratory streams ensure social distancing can be maintained while lectures and tutorials have been moved online where possible through the use of Zoom conferencing. At the time of writing, 4th year students are still in their Semester 2 placements.

New appointment

In June, we welcomed Cat Ronayne back into the programme. Cat has been appointed as a lecturer with responsibility for Biochemistry and some aspects of Haematology teaching. With many years of both teaching and medical laboratory practice under her belt, we were glad to be able to snatch her away from a holiday which ended in a forest in Spain, where she and her family were under COVID-19 lockdown.

THE Pacific WAY

The Tokelau's

The PPTC received a request from the New Zealand Ministry of Foreign Affairs and Trade to set up a mobile laboratory unit for Tokelau as a measure of preparedness in response to potential COVID-19 outbreaks that could threaten the wellbeing of this small Pacific Nation.

The PPTC worked with a New Zealand based container modification company and successfully arranged the joining of two 20ft containers to house a laboratory setup for Tokelau. This double unit containing an appropriately designed internal infrastructure, and along with newly purchased laboratory equipment and consumables was successfully shipped from Auckland to Samoa and then to Tokolau. On receipt in Tokelau the portable laboratory was then positioned on the Islands hospital campus where water and power were connected.

The PPTC fully equipped the laboratory with general and specialised laboratory equipment and will oversee training of staff on site (once international borders re-open) as well as through a zoom communication platform. The modification of the container system and the procurement of the equipment began on the 4th June and set up was completed ready for transportation to Tokelau on the 24th July. The PPTC's experience and expertise enables it to provide the same service to other countries if there is a need provided that funding is available.

PPTC Staff, Mr Ross Adern (Administrator for Tokelau), NZ Ministry of Foreign Affairs and Trade, Australian AID, and members of the Wellington Tokelau community at an official gathering to celebrate the completion of the portable laboratory before its departure to Tokelau.

Internal structure of the portable laboratory.

Portable laboratory: Front container.

Newly appointed members to the PPTC Board of Trustees

It is of great pleasure that the PPTC welcomes Dr. Dianne Sika-Paotonu and Dr. Vladimir Osipov as newly appointed members of the PPTC Board of Trustees.

Dr. Dianne Sika-Paotonu

Dr. Dianne Sika-Paotonu is currently the Associate Dean (Pacific) at Otago University's Wellington campus and is the first Tongan and Pacific biomedical scientist to be appointed to this role within the Division of Health Sciences at Otago. Dianne completed her PhD in Biomedical Science at Victoria University of Wellington specializing in Immunology based at the Malaghan Institute of Medical Research. While there, Dianne was a member of the cancer vaccines team and undertook work that explored the modification of these vaccines to generate stronger immune responses against cancer.

Dianne has received numerous awards for her PhD and other research work, including the MacDiarmid New Zealand Young Scientist of the Year - Advancing Human Health & Wellbeing, Colmar Brunton Research Excellence Award, Australasian Society of Immunology BD Science Communication award, Asia Pacific Science Technology Studies Networks New Contributions to Science Technology & Innovation Award - Indigenous studies and the Australasian Society of Immunology Buck Memorial Award among others. Dianne was also recipient of the Royal Order Award- the Most Illustrious Royal Order of Queen Salote Tupou III-Commander, awarded by the late King George Tupou V of Tonga in recognition of scientific achievement.

Dianne has strong research interests in areas of health research relevant to Pacific populations living in New Zealand and in the Region and is an Honorary Research Associate with the Wesfarmers Centre for Vaccines & Infectious Diseases, Telethon Kids Institute, Perth, Western Australia and Victoria University of Wellington, and is an Affiliate with the Maurice Wilkins Centre for Molecular Biodiscovery, University of Auckland.

Dr. Vladimir Osipov

Dr. Osipov trained in pathology at the Medical College of Wisconsin, followed by a subspecialty training at the Mayo Clinic, Rochester, USA. He is a Fellow of the American College of Pathologists and the Royal College of Pathologists of Australasia, and is currently Chief Anatomical Pathologist for Southern Community Laboratories based in Wellington as well as Honorary Senior Lecturer at the University of Otago, Wellington. Dr. Osipov's areas of interest include gastrointestinal, skin, lung, urologic, and orthopaedic pathology.

The PPTC External Quality Assessment Programme

The PPTC EQA programme has provided its quality service to the laboratories of the Asia–Pacific region for over 30 years. Since 1990 it has been recognised by the WHO as a collaborating centre for External Quality Assessment in Health Laboratory Services. Funded through the NZ Overseas Development programme, this service is delivered to over 100 regional laboratories.

It is provided to National Pacific laboratories free of charge providing invaluable benefit to their national healthcare, directly impacting patient health outcomes. Close to 22 surveys are dispatched throughout the year covering the seven medical laboratory disciplines.

- Haematology
- Clinical Biochemistry
- Microbiology
- Blood Transfusion Science
- Infectious Disease Serology
- Anatomical Pathology
- Molecular Diagnostics - COVID-19 (SARS-CoV-2). Recently added to the PPTC Programme

Due to the 2019 Coronavirus outbreak, the PPTC was asked by WHO to establish an EQA programme for the SARS-CoV2 disease in the Pacific region. The purpose of the SARS-CoV-2 EQA programme is to provide additional quality assurance for laboratories in the Pacific region, to prove the accuracy of detection rates.

Pacific Joint Incident Management Team

Since early January 2020, the WHO in the Pacific has been working closely alongside Pacific Governments and Ministries of Health, in collaboration with partners to ensure that countries are well prepared to respond to the threat of COVID-19.

The PPTC has been invited to join as a member of the Pacific Joint Incident Management Team (JIMT, Lab cell), and WHO is coordinating with Pacific partners that constitute this team to bring together resources and assist Pacific countries' readiness to delay the spread of the virus and mitigate negative health and socioeconomic impacts of COVID-19.

Activities that this team is involved with include:

- Training and technical guidance on critical preparedness, readiness, and response actions for COVID-19
- Working with government and community sectors
- Procuring critical laboratory and medical supplies
- Communicating with the public and engaging with communities

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 years 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.
Tel: 64-4-389 6294 or 027 2305483

Journal Questionnaire

Due to reduced opportunities to obtain CPD points due to the COVID-19 situation there are two journal questionnaires for the November 2020 issue. You can either do one (A) or the other (B) questionnaire, or both (A & B). There are 5 CPD points per questionnaire, thus if you do both and get at least 8 out of 10 questions right for each questionnaire then you get 10 CPD points. Otherwise, 5 CPD points for either questionnaire A or B.

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 5th March 2021. You must get a minimum of eight questions right per questionnaire to obtain five CPD points. The Editor sets the questions but the CPD Co-Ordinator, Jillian Broadbent, marks the answers. Direct any queries to her at cpd@nzimls.org.nz.

NOVEMBER JOURNAL QUESTIONNAIRE—A

1. Which mechanism has been suggested for lymphopenia in COVID-19 patients?
2. Which laboratory parameters have been suggested as markers for potential progression to critical illness in COVID-19 patients?
3. A 100% increase was seen in which cardiac parameters in COVID-19 patients?
4. An increase in CK-MB in COVID-19 patients is evidence that suggests what heart pathology?
5. SARS-COV-2 is known to have an affinity for which receptor, where is this receptor located, and is a significant increase of what in, in patients infected with COVID-19?
6. What abnormalities in coagulation profiles are associated with COVID-19 severity?
7. Studies have used which laboratory parameters to predict severity and/or mortality from COVID-19?
8. COVID-19 patients with which blood gases parameters have been classified as being severe/critical requiring intensive care management and mechanical ventilation?
9. In addition to the primary injury in spinal cord injury there is a strong association with which diseases, and what does this result in?
10. Transition from lean tissue muscle mass to adipose tissue is associated with, and leads to what?

NOVEMBER JOURNAL QUESTIONNAIRE—B

1. Which laboratory parameters have been employed to further improve the clinical diagnosis of acute appendicitis?
2. Which imaging studies have been used for a more accurate diagnosis of acute appendicitis?
3. Metabolic syndrome is a combination of which cardio-metabolic risk factors?
4. Neck circumference has been shown to correlate with which parameters?
5. Which anthropometric indices are markers of central obesity?
6. In New Zealand the majority of Legionella pneumonia infections are caused by which organism, what is the gold standard for diagnosis of Legionella infections, but which test is more sensitive?
7. Second generation sequencing techniques employ which techniques?
8. Which germline mutations are predominantly correlated with ductal carcinoma, and which mutation with both lobular and ductal carcinomas?
9. In cold autoimmune haemolytic anaemia, cold agglutinins can cause clinical symptoms related to what?
10. The term ‘angioimmunoblastic’ is derived from which words that refer to what?

Answers to the August Journal Questionnaires may be found on page 233 of this Journal

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