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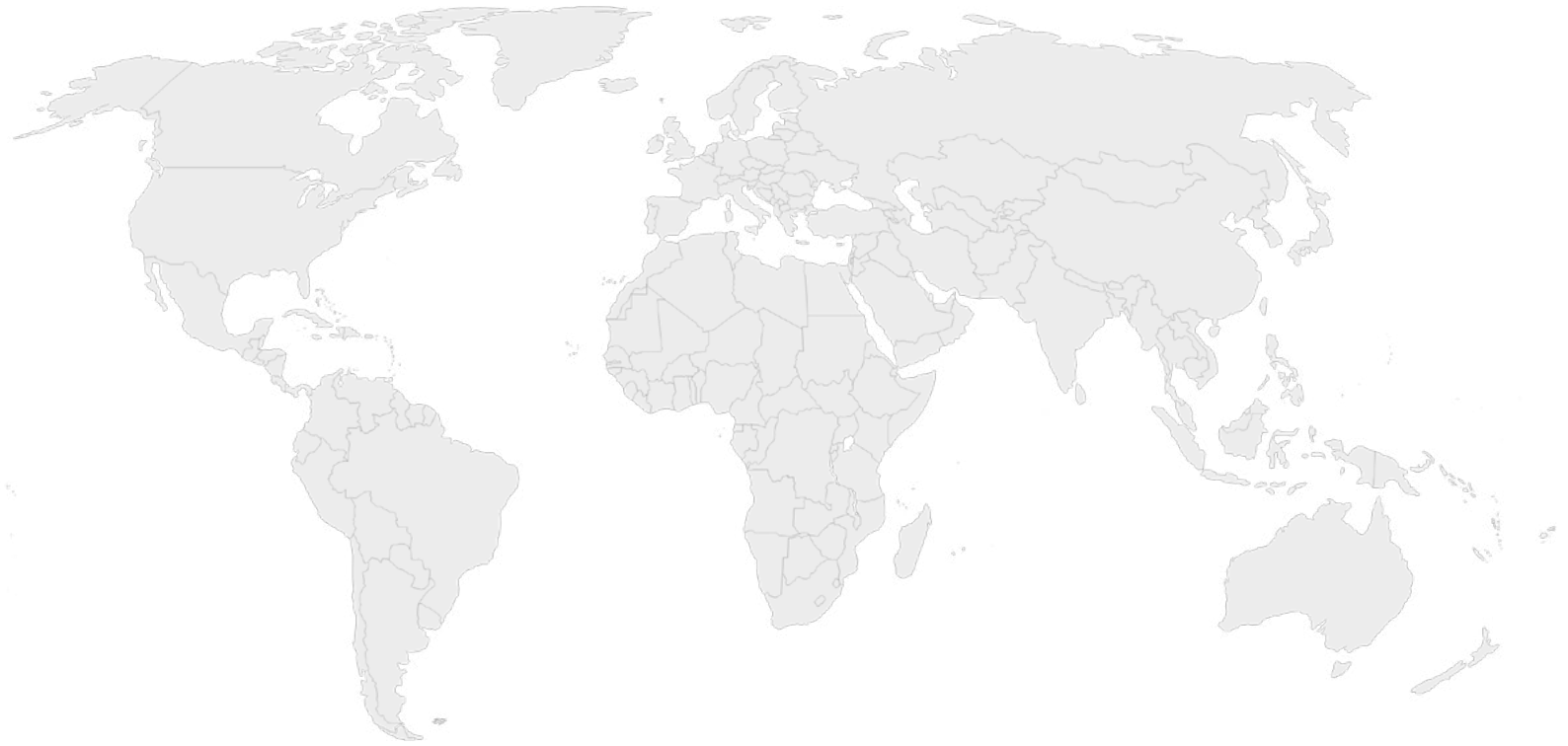

INFORMATION FOR CONTRIBUTING AUTHORS

In order to cope with the increase in submissions to the eJIFCC we decided to advance our manuscript handling process, and we subscribed for the Editorial Manager manuscript handling system. Each author has to register for the program. For the registration there are two possible ways: with an ORCID ID, or with an email address. To finalize the registration, a few questions will be asked that will be necessary for later communication and work-flow handling. The following questions can be expected during the end of the registration: workplace, contact details (email address, phone number), professional qualifications and availability lists. You will receive a system message about the successful registration. If you encounter any problems, or registration fails, log in to the site or if you would like to initiate the deletion of your account, you will be able to communicate it to us by using the email at the bottom of this page. After a plagiarism and a general technical check by the Editoral Office, manuscripts will be forwarded for evaluation to Reviewers. If your manuscript is not found acceptable you will receive detailed commnents that may help you to publish your work elsewhere. If your manuscript requires revision you will always be provided a deadline that should be strictly kept. Once your manuscript gets accepted you will receive the proof two weeks prior to publication.

Instructions for authors
Manuscript preparation: Microsoft Word is preferred, with the use of 12-point font, 2cm margins and double spacing.

The title page should list title and short title, authors (first name, last name), each authors affiliation, including e-mail addresses, corresponding authors contact information, key words.

All manuscript should be provided with structured Abstract not longer than 250 words. This information should proceed the text of the manuscript.

Tables should be created in a common word-processing or spreadsheet format and included within the text of the manuscript, close to the first location where they are referenced. The figures must be submitted as independent images or files, with high enough resolution for publishing and printing. All tables and figures must include a short title, with any further explanatory text to be included at the bottom of either.

The maximum number of references for a review article is 80, and 40 for original papers. Intext reference numbers should be included paranthetically before punctuation. The actual references shall be presented and punctuated consistently and are to be listed sequentially following the main text, with their numbers unlinked.
All references should be provided in the Vancouver style.

Manuscripts should provide any required Authors Disclosures. All studies involving human subjects must indicate that they are in compliance with the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki.

For any further questions please contact:
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Dear Readers,

This is the last issue of the eJIFCC that I edit in my capacity as Editor-in-Chief. My second 3-year term, that I had the chance to hold in the Communication and Publication Division of the IFCC, will terminate by the end of 2023. These six years have been quite an experience for me, and I never regretted that I undertook this – sometimes fairly challenging – task. First and foremost, I thank my predecessor Dr. Gábor L. Kovács under whose editorship the eJIFCC was successfully indexed by PubMed Central and simultaneously become a member of the Committee of Publication Ethics (COPE). These two milestones, and later inclusion of eJIFCC in the Scopus database greatly enhanced the Journals' visibility. I specially thank the continuous and enthusiastic work of my Assistant Editor, Dr. Harjit Pal Bhattoa (Hungary). The journal, the Editorial Board and the Editor always enjoyed the full support of Dr. Khosrow Adeli and Dr. Tahir Pillay who served as IFCC Communication and Publication Division chairs in the past 6 years. I am also grateful to all Guest Editors who contributed in assembling thematic issues that were of exceptional interest to many laboratory specialists.
Indexing of the eJIFCC in the Web of Science database is currently pending. Once indexed the eJIFCC would be allotted its impact factor and perhaps further enhance its visibility and ranking. Nonetheless, SCIMAGO journal ranking, in the Biochemistry (medical) category, lists eJIFCC as a Q2 category journal, and it achieved a CiteScore of 5.3 for the period 2019-2022. The continuous improvement is a reflection of the increasing number of extensively cited publications. Based on the Scopus metrics, between 2018-2023 the following eJIFCC publications received the highest citations (as of December 1, 2023).

1. Bonneau E, Neveu B, Kostantin E, Tsongalis GJ, De Guire V. How close are miRNAs from clinical practice? A perspective on the diagnostic and therapeutic market (2019, Issue 2) **197 citations**

2. Di Resta C, Galbiati S, Carrera P, Ferrari M. Next-generation sequencing approach for the diagnosis of human diseases: Open challenges and new opportunities. (2018, Issue 1) **91 citations**

3. Szilágyi B, Fejes Zs, Pócsi M, Kappelmayer J, Nagy B. Jr., Role of sepsis modulated circulating microRNAs (2019, Issue 2) **45 citations**

The number of submissions amplified tremendously in the past years. In the first half of my term, we mostly had thematic issues with only a handful of free communication submissions annually, that went up to 84 submissions until December 1, 2023 and these numerous manuscripts were submitted from 50 different countries. We always wished to encourage the developing countries in getting their noteworthy manuscripts published after a thorough peer review and this is well reflected in the number of accepted manuscripts. During the past six years the top 10 publishing countries were the followings: Argentina, Canada, Hungary, India, Italy, Nepal, Pakistan, Spain, UK, USA. The handling of the vast number of submissions, and to more effectively process them required two changes in the past years. In February 2021, we started using the Ithenticate software to screen for any potential plagiarism and in January 2023 the Editorial Manager system was introduced to better manage the submissions. As the number of submissions increased, so did the rejection rate and in 2023 the rejection rate was 61%. From 2018 until June 2023, Insoft Digital (Canada) was our publisher and I am grateful for their long and professional service. IFCC decided to change the web-format that became effective from July 2023 when the publishing tasks of the eJIFCC were transferred to the Digiwedo Digital Bureau (Netherlands) who brought a new fresh look to our journal. Both publishers have greatly improved the professional image of the journal. I am particularly thankful for the secretarial and many further organizational aids received from Mrs. Silvia Colli-Lanzi at the IFCC Office in Milan and the two secretaries at my workplace in the Department of Laboratory Medicine at the University of Debrecen, Ildikó Kópis (2018-2020) and Valentina László (2021-2023) for their valuable and highly professional assistance. I wish the new editor and the laboratory experts worldwide success in their personal and professional lives.

Debrecen, Hungary, December 1st, 2023

János Kappelmayer

POC testing and reporting of sodium, and other small molecules need modified IFCC source/type designations to improve operational efficacy and for clinically accurate, unambiguous reporting from LIMS and HIS.

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Abstract

There seems to be a lack of clarity of meaning/understanding of names and symbols and ultimately of quantitative results of certain critical tests performed as a professional in the laboratory and healthcare environment and in the POC environment. Multiple observations of this phenomenon are derived from interactions by the author as a consultant for manufacturers of multicomponent blood gas systems, and through them to the end-users in hospitals, clinics, and point-of-care locations as well as with IT professionals in the health care industry. This causes unnecessary and time-consuming questioning of reported information in the critical care environment. We would recommend wider use of IFCC designations for quantified measurands and most especially specimen type/source plus incorporation of that information as a part of the quantity and name on analytical systems information management (ASIM)[I], as well as by subsequent levels of information management including LIMS[II] and HIMS/IIMS[III]

Additionally, we suggest some modifications of the format and symbols to address the technology that uses a 'whole blood'(B) specimen to quantify concentrations of measurands, especially electrolytes in plasma and plasma/water. Both the sensors and the mode of system calibration are impactful for the clinical utility of the final value. The sensors, because of their 'membrane' detect a measurand in the plasma which is in equilibrium with the whole blood- without the need for other separation. Furthermore, since most, if not all, manufacturer's systems use calibration based on a CLSI[IV] -Inspired, NIST-developed Standard Reference Material[V] a reference material designed to ensure no difference among electrolyte results on patients with normal plasma water (i.e., Central Laboratory vs POC). Symbol modifications would include designating internal actions/calibration/calculations taken by the instrumentation that can aid in interpretation of values. These would be placed after the specimen type (e.g. (B)). Also, certain applications of such data to further calculations outside POC and Central Laboratory purview might be facilitated by the placement of the complete set of qualifiers as a parenthetical subscript to the quantity and measurand name/symbol, but this topic may be pursued elsewhere. We would promote wider consultation between

the IFCC and both member organizations and related medical professional organizations on these issues. Similar consultation should also exist between each central laboratory and institutional clinical professionals using the information and with the IT professionals who write the code to make the information available for laboratory, POCT and institution wide use.

[I] ASIM-Analytical System Information Management- The software/hardware package used by measuring devices in a linked analytical chain that allows for complete patient/specimen information and quality management data to be available independent of a LIMS.
[II] LIMS-Laboratory Information Management Systems.
[III] HIMS Hospital Information Management Systems, IIMS-Institutional Information Management Systems
[IV] CLSI- Clinical and Laboratory Standards Institute, Malvern, PA, USA, (Formerly NCCLS.
[V] SRM No.956d; Sodium in serum, National Institute of Standards and Technology OR National Bureau of Standards; U.S. Department of Commerce: Gaithersburg, MD.

‘New’ technology-‘Old’ physiology: Use of what may be referred to as ‘enhanced’ blood gas analyzers (eBGA’s)[VI] has brought about more frequent calls to the laboratory concerning result discrepancy especially for sodium ion and hemoglobin, among others. This assertion is based on personal observations made in my consulting practice for manufacturers and seems to center on differences between values obtained from central laboratory versus those from intensive care units (ICU) or Emergency departments. on specimens taken at the same time[VII]. I will focus here on just the electrolytes. Individual callers to the central laboratory about differences in values charted and the name of the value reported (e.g., ‘bicarbonate’ or ‘plasma bicarbonate’-are they the same thing?) may be assuaged after a brief investigation that finds the patient has a hyperlipidemia, dehydration, or more rarely a macroglobulinemia. However minor the delay (unless one is put on ‘hp;d’), there is still an obvious delay in finalizing evaluation and treatment of a patient. In addition, during our work with IT professionals as part of product development, we have observed that the naming of different measurands in the same manner makes it difficult to communicate with them as they design new reporting software. They, like many laboratory scientists and direct caregivers, are unfamiliar with the nuances in information about the changes imposed by the rapid advances of measurement technology. A simple example might be whether there is a difference between total carbon dioxide, carbon dioxide content and the molar concentration of carbon dioxide?[VIII]. When added up over time, these issues have the potential for economic and operational impact on the patient support team and are a quality management system issue. Consider, too, that when outside quality systems evaluators or governmental regulators assess testing patterns, there is likely to be a much broader requirement to evaluate patient populations and instruments performance -a costly exercise for both laboratory staff and direct-caregivers. To improve result quality pro-actively, I might suggest some additions and slight changes to the symbolic reporting schemes currently recommended by the IFCC[IX]. For the blood gases, one is accustomed to using a parenthetic notation for specimen type and its source, following the quantity type and measurand identifier, Thus, for oxygen tension in arterial blood ideally one writes **pO_2(aB)** as a **complete name** instead of the **‘oxygen tension of arterial blood’**. This IFCC-based system of symbolic naming is fully adequate for those components of ‘blood gas’ that are, and necessarily measured in whole blood. However, the measurement technology intervened with the advent of the concurrent use in a ‘blood gas’ analyzer, of electrolytes and other small molecules.

[VI] ‘Enhanced’ blood gas analyzers are those designed in a linked analytical stream for measurands beyond the basic pH, pO2 and pCO2,. including related entities such as electrolytes, and oximetry in various combinations.
[VII] Shives Prakash, Shailesh Bihari, Zahn Y Lim, Santosh Verghese Hemant, and Andrew D Bursten) Concordance between point-of-care blood gas analysis and laboratory autoanalyzer in measurement of hemoglobin and electrolytes in critically ill patients; 2018 Jul; 32(6): e22425. Published online 2018 Mar 3.
[VIII] Answer-Nuanced differences only but watch out for reporting units.
[IX] Other additions might also be suggested for specimen type modifiers, based on the use and specimen types submitted-all leading to less ambiguity in the symbols-labels going to the patient record such as (vmB) for mixed venous blood-frequently used for follow-on calculations outside the laboratory. Here I will focus on just one issue.

Analytical overview. In the last several decades of the 20th century, the single most common first step in measurement of ‘blood’ electrolytes required a **quantitatively diluted** specimen of serum/plasma (i.e., the blood cells were already separated). This was followed by a determination of the intensity of the color characteristic in a reacted solution or in a gas- flame when the diluted specimen was aerosolized then injected into a flame of flammable gas. Ion-selective electrodes (ISE’s) were also used in place of the spectrophotometer or flame photometric sensor in systems designed for multi-specimen processing. Calibrators of precisely known concentration in water or buffered salt solution, **diluted similarly** to the plasma specimen were used to relate light intensity to concentration. **The flame emission technology (FAES)** specifically had been used for decades and was the basis of the reference ranges used by most clinicians. Dilution was required for both analytical and functional reasons. Calibrators were made in aqueous solutions and diluted similarly to the serum/plasma specimen with the same aqueous solution. **Ion selective electrodes (ISE’s)** first introduced for clinical use around the same period as the FAES becoming the reference method, had a sensitivity range that could be used on diluted

specimens but also enabled measurement on undiluted specimens. Elimination of the dilution step reduces pre-analytical preparation and enables faster turn-around time on individual specimens, but it was observed that the direct measurement resulted in a bias between undiluted ISE (frequently referred to as 'direct') and the reference method, especially for sodium. The observed consequence of ISE's being used on either plasma/serum alone, on whole blood or in blood gas systems would, on the average be a difference of about 7%. So, at a practical level, if ISEs were to be incorporated into 'blood gas systems', it was necessary to get the electrolytes, especially sodium, to agree, since confusion as to result meaning had no place in a critical environment. **Dilute before analysis.** The dilution is the crux of the issues associated with the technology. Initially the phenomenon was ascribed to simple methodological differences, but it then became recognized that it was because the ISEs were measuring the electrolytes of the medium in which they were dissolved-water-not in the serum/plasma/whole blood whereas the other methods involving dilution based the results on a reference calibrator made from water and pure sodium chloride. Measurement of the same specimen of blood, serum/plasma, resulted in an average of approximately 7% bias relative to the reference method (gravimetry or FAES), methods which had been used to develop reference intervals and had served as the standard for these critical measurands for decades. While this applies to all analytes dissolved in the plasma water, the significant effect is on sodium because of its typical value (140 +/- 5 mmol/L) and narrow acceptable range . A 7% bias represents the entire acceptable range[X]

[X] With a typical value for normal sodium of 140 +/- 5 mmol/L, a seven percent difference spans the whole range.

'Undiluted' (or direct) analysis. While research at the time had shown that differences between the direct, undiluted ISE and dilution (FAES) were not the result of measurement bias but rather to a pre-analytical bias induced by typical total protein concentration in the blood serum/plasma. Since most reported values were on systems that diluted the specimen first, whether in manual-procedure environments or on the multi-specimen analyzers most were calibrated based on a diluted aqueous standard and in general agreed with the FAES- reference method. Since the key was in the water concentration, any analysis using 'dilution' would agree with each other, even ones using ISE's. In addition to the analytical implications, there is a serious clinical implication- what about specimens containing significant high or low protein levels or high lipid levels! The ISE could explain the factitious low results for Na/K in macroglobulinemia and lipidemia's especially those found in uncontrolled diabetes[XI].

[XI] A substantial set of references on all these points is found in the references of the CLSI documents referred to.

Consensus Standardization. As is recognized today, the ISE technology introduced decades ago measures the electrolytes (and other small molecules) on both diluted and undiluted plasma. The Clinical and Laboratory Standards Institute (CLSI[XII] committees charged with examining the situation both analytically and clinically, developed a proposed, a tentative, and finally an 'Approved' standard, having published each for public review- a process taking in total several years. (See Bibliography). NIST, using the recommendations developed by CLSI[XIII], then developed the Standard Reference Material (SRM 956d), available for use now, so that all systems can be calibrated to the same value for sodium, whether the specimen is diluted or not before analysis. In summary, the recommendation was to standardize all systems to avoid clinical confusion. This standardization was set to make specimens with normal plasma water concentrations agree without regard to method. This was done with the constructive agreement with the National Institute of Technology (NIST), since they prepare precise standards materials with protein/lipid amounts such that plasma water was 'normal'. By using this approach, confusion would be limited, and more importantly routine Na/K could be done on any system and the issue of discrepancy would not be an error in the method, but rather a significant difference in plasma water, the result of clinically pertinent conditions. CLSI, however, made no recommendation on reporting results as the morphing of 'blood gas' technology to use outside the laboratory was not considered at the time.

[XII] At the time CLSI was called the National Committee for Clinical Laboratory Standards (NCCLS)

[XIII] Recall that all major manufacturers were participants in the CLSI process.

Solving our own problem in POC testing: The Point

When all reporting of analytical values is the responsibility solely of the laboratory the solution is simplified. For example, if the result is from a high-volume system, likely a measurement involving dilution, one simply uses the appropriate IFCC symbol- but what is it? A bicarbonate would be $c\mathrm{HCO_3^-}$ and a sodium would be $c\mathrm{Na^+}$. Simple, but insufficient. Consider that most authorities require that any value reported be identified by source and type. Why not have that information symbolically within the name of the quantity and measurand as placed on the patient record instead of in the record in various places? A bicarbonate reported from a blood gas system[XIV] would then be $\boldsymbol{c\mathrm{HCO_3}}$**(aBp)**[XV] [*i.e.*-molar concentration of bicarbonate in arterial blood plasma]. For a sodium measured on the same specimen, the symbol would be ***c*Na(aBp)**. The designation of 'p' for plasma is based on what the analyzer does, not on the specimen type or source. Now there is no question about the source? Not quite--- given the differences in results possible in hospitalized populations. Consider that the eBGA is done immediately near the patient but that other specimens collected at the same time go to the main laboratory for less urgent testing that includes electrolytes, glucose, etc. as a

part of the 'panel' for metabolic screen. So, when fully charted there may be **two sets of electrolytes on specimens collected at the same time**-one typically done on a diluted specimen of plasma (central laboratory) one on an undiluted specimen of the plasma of the whole blood (POC, ICU, ED, *etc.*). They will usually agree but if not, how does one differentiate I believe the differentiation must begin with more complete naming within the IFCC system. The only complete pertinent symbol should include the notation with the reported result that shows the undiluted nature of the measurement itself, and thus the least ambiguous name for the reported quantity/entity. Since this occurs *post facto* to the specimen nature itself, additional designations should follow the 'B' if blood is the material. So, we can now have a final symbol for the sodium or bicarbonate which will be clear in its clinical and analytical meaning as shown in the table.

[XIV] Blood gas analyzers from the 1960's through the current eBGA's have been reporting 'plasma bicarbonate'. While the issue is user understanding, the solution is proper result labelling.

[XV] Eliminate the charge symbol for simplicity, or even use 'Bicarb' instead of the chemical symbol.

	Result Source	***Bicarbonate***	***Sodium***
1	**POCT**	$c\mathrm{HCO_3}$ ($\mathrm{aBp_u}$)	$c\mathrm{Na}(\mathrm{aBp_u})$
2	**Central lab (best symbol)**	$c\mathrm{HCO_3}$ ($\mathrm{vBp_d}$)	$c\mathrm{Na}(\mathrm{aBp_d})$
3	**Central Lab (Acceptaable)**	$c\mathrm{HCO_3}$ (vB)	cNa(vB)
4	**Central Lab (?)**	$c\mathrm{HCO_3}$	cNa+
Symbols in rows 1 and 2 when accompanied by the measured value and unit, exemplify unambiguous reporting. (The 'u' represents undiluted, the 'd' diluted, and 'v'; venous.)			

In each symbol shown in the table, the '$\mathrm{p_u}$' or '$\mathrm{p_d}$' follows the 'B' to represent that the measurand is reported as a concentration in undiluted or diluted plasma. These examples clearly show the measurand, the specimen source and type, and that it was reported as a molar concentration in plasma that was diluted or undiluted[XVI]. That is, there should be no question about the meaning of the value reported- the necessary information is part of the name itself. Once coded into analytical system by manufacturers as well as by or LIMS suppliers, and in institutional software, this is a simple approach to providing complete and pertinent information in critical situations and minimizing confusion between results provided by different analytical systems.

[XVI] That is a new symbol '$\mathrm{p_u}$' for plasma undiluted and for plasma, diluted '$\mathrm{p_d}$'.

In conclusion, these suggestions are both simple to implement and analytically sound and help to address the ambiguity and diagnostic/therapeutic advantage of direct ISE technology. The direct measuring BGA/Electrolyte system will be giving the physiologically correct result in all conditions. However, most electrolyte measurements on most patients the economical choice would be the large processing systems typically using a specimen dilution method which almost always is clinically sufficient and dependable. With the complete, unambiguous naming of the quantity, measurand and specimen characteristics, clinical assessment in critical situations will be enhanced.

Some Supplemental Thoughts: While from a scientific perspective some further suggestions are less important than the preceding, some may be critical to get full acceptance and use by the medical-professional users,

- Use standard typeface especially for the measurand symbol or name since special characters or 'object' files are not widely available/known.
- Other post-collection qualifiers, [XVII]should also be added following the '$\mathrm{p_u}$'.
- Recognize that since most measurements using eBGA's will be on arterial blood, elimination of the 'aB' part of the symbol should be a laboratory/institution option allowed for in software coding, while absolutely retaining other source/type symbols as well as a symbol indicating unknown source.
- Expand the audience for the discussion to include not only the IT personnel who will implement this in hospital wide reporting systems but also to other medical specialties who may use the information for their own follow-on calculations (10, 14, 15).
- Parenthetic symbols may be subscripts to the quantity and measurand name especially if the quantity is used in follow-on calculations done by other professionals in the critical care team. From our examples - $c\mathrm{HCO_{3\ (aBpu)}}$ and $c\mathrm{Na_{(aBpu)}}$.

We hope that these thoughts will stimulate some discussion and action within the broad audience affected. While the focus here is on the electrolytes, some other modifying symbols useful in POC will be the subject of further communications.

[XVII] For oxygen concentration, the dissolved plus the hemoglobin bound-each determined separately in the system, then added together is a 'total' oxygen formerly 'oxygen content'. The latter term being appropriate for the 100-year-old technology from which it came. (9) I would eschew the use of 't' alone for 'total' due to the potential confusion for time or temperature which could potentially be part of the same report.

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The Biochemistry Behind Cognitive Decline: Biomarkers of Alzheimer's Disease

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Keywords

Alzheimer's disease, Biomarkers, Cerebrospinal fluid, Amyloid β peptide, Tau protein, Neurofibrillary tangles

Abstract

Alzheimer's disease (AD) is the most prevalent type of dementia. Pathologically, the disease is marked by neurofibrillary tangles (NFT), which are aberrant accumulations of the tau protein that develop inside neurons, and extracellular plaque deposits of the amyloid β peptide (Aβ). These pathological lesions are present in the brain before the beginning of clinical manifestations. However, despite advancements in the comprehension of AD pathophysiology, timely and accurate clinical diagnosis remains challenging. Therefore, developing biomarkers capable of detecting AD during the preclinical phase holds enormous promise for precise diagnosis since detecting the disease early is crucial because it enables interventions when treatments may be more effective. This article intends to provide a comprehensive review of AD biomarkers, discussing their significance, classification, and recent developments in the field.

Abbreviations:
NFT: neurofibrillary tangles, APP: amyloid precursor protein, PSEN1: presenilin1, PSEN2: presenilin2, SPECT: single photon emission computed tomography, CT: computed tomography, FDG-PET: 18F-fluorodeoxyglucose-positron emission tomography, MRI: magnetic resonance imaging, CSF: cerebrospinal fluid, BBAD: blood biomarkers in AD

Introduction:

Alzheimer's disease (AD) was discovered more than a century ago. It is the most common neurodegenerative disorder in older adults, which results in loss of memory, language, visuospatial abilities, and other mental functions (1). Worldwide, the case rate of AD doubles every five years after age 65. In addition, it is expected that there will be 115 million cases by 2050 (2). People suffering from the disease's latter stages are bedridden and need care 24 hours daily. AD is eventually fatal. According to researchers, early AD diagnosis will be crucial to halting, slowing, or delaying the illness. Therefore, much attention is being put on understanding AD's pathophysiology and establishing early diagnosis and efficient intervention due to the disease's severe economic and societal costs.

Pathophysiologically, AD is characterized by extracellular deposits of the protein beta-amyloid (also known as beta-amyloid plaques) and the buildup of a particular type of protein tau (also known as tau tangles) inside neurons. These changes lead to the destruction of neurons that cause memory loss and other symptoms of AD (3). Physicians typically rely on clinical symptoms for diagnosing AD. However, neuronal loss and neuropathologic lesions are already evident in many brain regions when AD is clinically diagnosed (3). A key objective of biomedical research is identifying preclinical markers of AD (i.e., biomarkers) that permit early diagnosis and intervention. These biomarkers enable clinicians to recognize individuals at risk before observable cognitive decline, thereby allowing for potentially more effective early interventions to preserve cognitive function. This article aims to provide a concise and current overview of AD biomarkers, focusing on their importance in early detection, diagnosis, and treatment monitoring and discussing the challenges associated with their application.

Understanding AD:

Risk factors:

AD, a complex neurodegenerative condition, has been related to a number of risk factors. While age remains the most critical risk factor, research has shown many other characteristics contributing to an individual's susceptibility to the disease. The major other unmodifiable risk factor is genetic, with one APOE ε4 allele raising the risk of developing AD threefold, and two APOE ε4 alleles increasing the risk up to twelvefold. Modifiable risk factors for AD include hypertension, diabetes, hypercholesterolemia, smoking, alcohol consumption, obesity, and diet. On the other hand, physical activity, education, entertainment, and social interaction have all been proven protective factors (4). Table 1 summarizes some of the major risk factors for AD.

Table 1: AD risk factors

Risk factor	**Description**
Age	The main risk factor for AD is advancing age, with 10–30% prevalence in the population older than 65, with an incidence at least doubling every ten years after age 60 (5).
Sex	Women are more likely to develop AD.
Genetics	- Family history of AD - The existence of the APOE ε4 allele - Trisomy 21 and family history are risk factors for early-onset dementia.
Lifestyle Factors	Cardiovascular health, which involves hypertension and elevated cholesterol, smoking, obesity, diabetes, dietary habits, physical inactivity, and lack of intellectual and social activity (6).
Environmental Factors	Exposure to toxins or certain chemicals and traumatic brain injuries.
Mental Health	Conditions like depression and chronic stress increase the risk of AD.
Sleep Disorders	Sleep disturbances may contribute to cognitive decline.

Abbreviations: AD: Alzheimer's disease; APOE ε4: apolipoprotein E-ε4;

Pathophysiology:
AD is marked by brain shrinkage and abnormal deposits called plaques and neurofibrillary tangles (NFT). Plaques are microscopic lesions characterized by a spherical shape, consisting of an extracellular core composed of amyloid beta (Aβ) peptide. At the same time, NFT are intracytoplasmic structures and consist of twisted coupled spiral fibrillary proteins known as tau, found within neurons. The amyloid precursor protein (APP) is a membrane protein that is found mostly in synapses. In standard non-amyloidogenic processing, APP undergoes cleavage by α-secretase, followed by γ-secretase. Subsequently, the resulting fragments are processed and removed correctly. In individuals diagnosed with AD, the amyloidogenic processing pathway involves the sequential action of β-secretase and γ-secretase on APP, resulting in the generation of amyloid-β (Aβ) fragments, with a specific emphasis on Aβ1-42. Extracellular plaques arise as a result of the increased accumulation in conjunction with reduced clearance processes. Aβ deposits form around meningeal and cerebral blood vessels, as well as gray matter. Tau is a protein that stabilizes the microtubule in neuronal axons in a healthy state. Due to the extracellular Aβ aggregation in AD, tau is hyperphosphorylated, which leads to tau aggregates and polymerization into fibrillar structures that destabilize microtubules and produce NFT, which are found within both glial and neuronal cell types in the affected cortical and subcortical brain regions. The presence of these pathogenic proteins and free radicals leads to the activation of microglia, neuroinflammation, damage to mitochondria, oxidative stress, deficits in neurotransmitters (specifically acetylcholine), malfunction in synaptic activity, and finally, the loss of synapses and neurons, which leads to memory loss and cognitive deterioration(5–8).

Challenges in Clinical Diagnosis:
While our understanding of AD biology has evolved significantly, diagnosing the disease remains challenging. The medical diagnosis of AD relies on conducting neuropsychological assessments, which typically involve evaluating memory loss and cognitive decline and carefully excluding other dementias commonly occurring with advancing age, among them cerebrovascular disease, dementia with Lewy bodies, cerebral tumor, normal pressure hydrocephalus, frontotemporal lobar degeneration, or depression (9,10). The clinical identification of AD exhibits limited reliability, particularly in the initial phases of the disease. Based on autopsy validation, the clinical diagnosis of AD compared to non-AD conditions demonstrates an overall accuracy rate of 78% (11). Misdiagnosis of AD is especially common in its initial phases when signs are subtle or mild, and in primary care, in which over half of patients having cognitive impairment are not detected or appropriately diagnosed (12). This misinterpretation leads to inadequate care and treatment, retarded or erroneous interventions, and incorrect data regarding condition and outcome (13). Neuropathological alterations occur years before clinical manifestations of AD. Pathological NFT composed of phosphorylated tau protein accumulate in brain cells during the presymptomatic stages of AD. In addition, distinct isoforms of amyloid-β (Aβ) peptide deposits accumulate in the extracellular space. These proteins are secreted into the CSF, where they are detectable (14). Efforts have been undertaken in recent years to develop reliable biomarkers and sophisticated imaging techniques to aid in early and accurate AD diagnosis, thereby addressing a significant need in clinical practice.

AD biomarkers:
Biomarkers: Definition and Types:
A biomarker is a measurable characteristic that signals healthy physiological functions, pathologic biological events or biological reactions to an exposure or intervention, which include therapeutic responses. Biomarkers can consist of molecular, histologic, radiographic, or physiological traits. Biomarkers fall into the following categories: susceptibility/risk, diagnostic, monitoring, prognostic, and predictive biomarkers (15). The perfect AD biomarker should meet several requirements, including 1) being able to identify AD with high specificity and sensitivity; 2) the aptitude to recognize the earliest stages and monitor the evolution of AD; 3) usefulness for assessing medical effectiveness; and 4) the need for specimens which could be collected quickly, numerous times, in a noninvasive manner, and affordably (16). Biomarkers for AD include genetic markers (17), neuroimaging markers such as PET scans and MRI (18), and biochemical markers such as amyloid beta and tau proteins (19).

Genetic Biomarkers:
The genes AβPP, PSEN1, and PSEN2 have been significantly associated with the development of early-onset AD, which often manifests before age 65 and is exceptional (5% of AD cases). On the other hand, late-onset AD (the most prevalent form) has primarily been linked to the apolipoprotein E-ε4 (APOE ε4) gene, which is suggested to elevate AD risk essentially by modulating Aβ accumulation (20,21). Genetic AD biomarkers (mutations in AβPP, PSEN1, and PSEN2) are just helpful in detecting familial AD (more than 95% of AD cases are isolated and lack mutations in these three genes). On the other hand, the APOE ε4 mutation is a known risk factor for late-onset AD, but it is not a reliable genetic biomarker for diagnosis. Because of lower prices and faster analysis, testing for AD-associated genes using focused sequencing methods such as Sanger or next-generation sequencing became increasingly popular compared to complete-exome sequencing over time. Nevertheless, genetic testing for AD is not usually recommended. It is sometimes used in families with rare early-onset forms of AD (6).

Neuroimaging-based Biomarkers in AD:

Human in vivo neuroimaging provides a deeper comprehension of the pathophysiology of AD. These examinations are essential for identifying non-AD etiologies contributing to cognitive loss (e.g., strokes or cerebral tumors) and providing diagnostic support for AD (22). MRI and CT allow visualization of gray matter, white matter, and CSF. They help characterize supportive features for diagnosing AD, especially brain atrophy. They also permit the elimination of non-AD causes of cognitive decline (22). The PET scan is another neuroimaging technique commonly used in AD research and diagnosis. It works with radiolabeled tracers specific to Aβ, such as Flutemetamol, Pittsburg compound B, and F-florbetapir (23,24). Other Functional neuroimaging techniques for identifying dysfunctional brain regions include functional MRI (fMRI), which examines blood flow in the brain, and SPECT, which investigates brain perfusion as a measure of brain metabolism. Despite their contributions to our comprehension of AD, these neuroimaging techniques have limitations that must be considered—specifically, the requirement for costly equipment and specialized training.

CSF Biomarkers in AD:

Because the brain's extracellular space is in intimate contact with the CSF, modifications in the physiology of nervous system may be detected in the CSF. The two most well-known neuropathologic signs of AD are Aβ deposits and tau protein NFT. CSF biomarkers that have been established reflect the pathophysiology of these two features. When comparing AD patients to normal controls, Aβ1-42 concentrations in CSF are lower, although total tau (T-tau) and phosphorylated tau (P-tau) concentrations are higher. This decline in Aβ1-42 in the CSF of Alzheimer's patients occurs because Aβ accumulates into plaques, trapping the peptide in cerebral tissue, resulting in a decreased capacity for Aβ to diffuse into the CSF (25). Tau found in CSF may be associated with the passive discharge of intracellular content from dead cells. Still, multiple studies show that tau secretion also involves active cellular processes (26). In contrast to Aβ, NFT appear later in the progression of AD (27). Moreover, high CSF tau has been linked with rapid transformation from mild cognitive impairement to AD, tissue injury, and the likelihood of poor clinical outcome (28–30). T-tau is not specific to AD but indicates brain degeneration or injury. It is elevated in the brains of various neurodegenerative disease patients (25). On the contrary, increased P-tau, which denotes hyperphosphorylated tau protein, is associated with NFT formation in the brain (31,32). Aβ1-42 and tau Protein can be measured using immunological techniques such as ELISA and electrochemiluminescence and non-immunological techniques such as mass spectrometry (MS). Multiple studies supported by evidence have shown that using the CSF Aβ1-42/1-40 ratio is more effective than the total amount of CSF Aβ1-42. This approach enhances the accuracy of diagnoses. Similarly, in terms of PET scan consistency, using CSF P-tau/Aβ1-42 or T-tau/Aβ1-42 ratios is more reliable compared to relying exclusively on T-tau or P-tau, respectively, or Aβ1-42 alone (33,34). However, the significant inter-laboratory variability in analyte concentrations can restrict the usefulness of CSF biomarker assays. Therefore, it is necessary to enhance assay development quality control specifications to assure minimal total calibration variation and strict variability limits between lots(19). For this reason, The Alzheimer's Association Quality Control program for CSF biomarkers, which includes 84 laboratories worldwide, and the Working Group for CSF proteins of the International Federation of Clinical Chemistry (IFCC) have initiated work to create uniform procedures and unify levels between assay techniques to tackle these problems and adjust results over laboratories (24). According to a recent meta-analysis (35), clinicians can use CSF biomarkers as a valuable supplementary diagnostic test when evaluating patients with cognitive disorders. Specifically, CSF biomarkers enhanced physicians' assurance of diagnosing AD and impacted patient management. It is advisable to include CSF AD biomarkers as a standard practice in assessing patients with mild cognitive impairment and dementia. They are suggested for accurate and timely diagnosis, differential diagnosis, and predicting the probability and progression of neurological deterioration (36). The kits and methods that are often used for measuring CSF Biomarkers of AD are included in Table 2.

Blood biomarkers in AD (BBAD):

Given the invasiveness and cost of CSF and neuroimaging biomarkers of AD, there is a pressing need to explore and develop reliable BBAD. Despite the common perception that AD is a brain disease, it has been shown that AD is a systemic condition which manifests in peripheral tissues beyond the central nervous system (CNS) throughout the earlier stages of the disease. Furthermore, biomolecules are continuously exchanged between the circulation and CSF (37). The latest innovations in BBAD for identification, prognosis, and monitoring therapy include plasma Aβ1-42/1-40 ratio, P-tau levels, serum neurofilament light chain, and glial fibrillary acidic protein (38). However, several difficulties must be addressed before BBAD may be considered a routine component of clinical therapy. Interference of circulating blood proteins and the reduction in the concentration of proteins and other analytes as they travel from the brain tissue to the CSF and into the circulation pose a significant quantitative identification challenge (39). Moreover, there are few available prospective studies in which plasma samples were collected continuously over an extended period, and clinical efficacy was calculated from a predetermined cut point. Before adopting a specific BBAD or any combination of BBAD in clinical practice, it is recommended that such data be gathered through clinical studies (38). The kits and methods that are often used for measuring BBAD are included in Table 3.

Table 2: Kits and methods that are often used for measuring CSF

Fluid	Biomarker	Commerical kits	Technique	Label	
				USA	EU
CSF	Aβ42	INNOTEST, Fujirebio	ELISA	RUO	CE Marked
		Lumipulse, Fujirebio	CLEIA	RUO	CE Marked
		Elecsys, Roche Diagnostics	ECLIA	BDD	CE Marked
		Euroimmun, PerkinElmer	ELISA	N/A	CE Marked
		TECAN, Ibl-international	ELISA	RUO	CE Marked
		ADMark, Athena Diagnostics	ELISA	LDT	N/A
	Aβ42/Aβ40 ratio	Lumipulse, Fujirebio	CLEIA	FDA approved	CE Marked
		Euroimmun, PerkinElmer	ELISA	N/A	CE Marked
		TECAN, Ibl-international	ELISA	RUO	CE Marked
		ABtest-IA, Araclon Biotech	ELISA	RUO	CE Marked
	P-tau-181	INNOTEST, Fujirebio	ELISA	RUO	CE Marked
		Lumipulse, Fujirebio	CLEIA	RUO	CE Marked
		Elecsys, Roche Diagnostics	ECLIA	BDD	CE Marked
		Euroimmun, PerkinElmer	ELISA	N/A	CE Marked
		TECAN, Ibl-international	ELISA	N/A	CE Marked
		ADMark, Athena Diagnostics	ELISA	LDT	N/A
	T-tau	Lumipulse, Fujirebio	CLEIA	RUO	CE Marked
		Elecsys, Roche Diagnostics	ECLIA	BDD	CE Marked
		Euroimmun, PerkinElmer	ELISA	N/A	CE Marked

Table 3: Kits and methods that are often used for measuring BBAD

Fluid	Biomarker	Commerical kits	Technique	Label	
				USA	EU
Plasma	Aβ42/ Aβ40 ratio	HISCL β-Amyloid, Sysmex	CLEIA	N/A	CE Marked
	P-tau-181	Simoa Advantage V2Kit, Quanterix	Single molecule array (SiMoA)	BDD	N/A
	Panels: Aβ42/ Aβ40 ratio, APOE ε4	PrecivityAD, C_2N Diagnostics	LC-MS/MS	BDD	CE Marked
	P-tau-181, APOE ε4	Elecsys Amyloid Plasma Panel, Roche Diagnostics	Elecsys immunoassays	BDD	N/A

Abbreviations: CSF: Cerebrospinal fluid; Aβ42: Amyloid β-protein 42 ; ELISA: enzyme-linked immunosorbent assay ; RUO : Research Use Only ; CE : *Conformité Européenne* ; CLEIA: Chemiluminescence enzyme immunoassay ; ECLIA: Electrochemiluminescence immunoassay ; BDD: Breakthrough Devices Program ; N/A: not available ; LDT: Laboratory Developed Test ; Aβ40: Amyloid β-protein 40 ; FDA: Food and Drug Administration ; APOE ε4: apolipoprotein E-ε4; LC-MS/MS: Liquid chromatography–tandem mass spectrometry

Future Directions in AD Biomarkers:

Many studies are being conducted in the area of AD biomarkers; prospects include the discovery of biomarkers that can evaluate each stage of disease pathogenesis and enable a precise diagnosis of the condition in its earliest stages. In addition, recent advances in developing proteomics, metabolomics, mass spectrometry, and using exosomes and investigating microRNA profiles revealed promising prospects for blood-based biomarkers as AD screening tools (40). Blood cell-derived biomarkers are an additional area of research with promising potential. Changes in peripheral cells such as platelets, lymphocytes, and erythrocytes have been observed in AD, rendering them potential biomarkers for studying neuronal pathology (41). Blood platelets, which share biochemical properties with neurons, can be used as a basis for comprehending the pathology of AD (42). Through the blood-brain barrier, lymphocytes, essential to neuroinflammatory processes, migrate from the circulation to the Alzheimer's brain. The combination of these neuroinflammatory elements in the blood and the alterations observed in the lymphocytes of patients with AD has the potential to serve as blood cell-based biomarkers for condition (43). The presence of molecules such as β-amyloid peptide, heat shock protein 90, band three protein, and calpain 1 in erythrocytes indicates their potential as preclinical biomarkers. Furthermore, red blood cell morphology is significantly altered in AD (44). However, despite these promising features, additional research is required to explore their diagnostic potential thoroughly (41).

Conclusion:

AD is a form of dementia characterized by irreversible progression and lengthy prodromal phases. Utilizing minimally invasive diagnostic tests that assess biomarkers might present the most effective option for diagnosing AD in its early stages instead of relying only on clinical evaluations. The use of biomarkers for AD diagnosis has gained popularity over the past two decades. However, their efficiency in early AD diagnosis and routine screening is questioned due to their employment of invasive procedures, high expense, and measurement uncertainty. PET scan and CSF markers are more often utilized diagnostic biomarkers in clinical studies; however, they have practical issues (e.g., expense and access). As sensitive and novel technical approaches are developed, and research design is given more significant thought, possibilities for biomarkers for AD will be carefully assessed.

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The researcher has no conflict of interest to declare.

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Hyperautomation in Healthcare: perspectives from a joint IFCC – EHMA session

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Automation, Artificial intelligence, Efficiency, Patient Safety, Data, Process

Abstract

Introduction

This article provides an exploration of hyperautomation's transformative potential in healthcare, building upon the insights gained from the joint session hosted by the European Health Management Association (EHMA) (1) and the Division on Emerging Technologies of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (2) at the EHMA congress in Rome on June 6th, 2023. In a rapidly evolving landscape driven by emerging technologies, healthcare stands at the forefront of change (3,4). This article delves into the implications of hyperautomation for health managers, examines its applications in healthcare processes and laboratory medicine, and addresses the important aspects of providing healthcare professionals with the necessary digital skills.

Streamlining Processes and Maximizing Efficiency

Hyperautomation in healthcare holds the promise of transform healthcare processes, empowering managers to streamline operations for enhanced efficiency and accuracy (Figure 1). By harnessing the capabilities of artificial intelligence (AI), machine learning algorithms, and robotic process automation (RPA), healthcare providers can automate tedious manual and pre-analytical tasks, such as sample handling and blood tests. This automation liberates valuable time and resources that can be redirected towards more critical tasks, ultimately elevating the overall quality of patient care. The value of automation is well established in clinical laboratories and the amplification effect is expected from AI at several points (efficiency, prevention of troubleshooting, maximization of the use of staff and resources, design of next generation central laboratory…) (5). Hyperautomation goes beyond mere automation; it adds substantial value to healthcare organizations by

enabling predictive maintenance, enhancing production, and improving quality. Collaborative robots, known as cobots, work alongside human professionals, elevating the standard of care delivered. Through the reconfiguration of business and care pathways, hyperautomation facilitates the creation of intelligent laboratories and drives the development of data-driven processes, thereby revolutionizing decision-making.

Data integration and augmentation of care Services

One of the standout applications of hyperautomation is its ability to augment care services for physicians. Through seamless data integration and analysis, AI algorithms offer personalized treatment recommendations, expanding the scope of healthcare services beyond conventional boundaries. In a recent report, AI algorithm utilizing multiple data sources including clinical, socioeconomic, and behavioral data was developed to predict patients at highest risk for readmission and provide care navigators to prevent rehospitalization. The study showed that the patients had overall 21.0% less adjusted incidence of 30-day rehospitalization compared with matched control encounters, or 69 fewer rehospitalizations per 1000 encounters. These data highlight that the coordination of patient's care continuum is critical for safe and effective transition of care (6). Additionally, the use of cutting-edge technologies like drones for the secure and timely delivery of blood samples enhances the overall management of healthcare services, ensuring patients receive the care they need when they need it (7).

Living in Smart Healthcare Environments

The integration of hyperautomation paves the way for smart healthcare environments, revolutionizing the patient experience. Through interconnected technologies, patients can receive proactive feedback, creating a more engaging and efficient healthcare journey. Atrial fibrillation (AF) is a good example of this new journey where digital companion can help to risk estimation. Smartphone applications have been evaluated as a stand-alone interpretation tool for 12-lead ECG in primary care. Recent data showed that for AF the sensitivity and specificity were 97% and 99%, respectively, in primary care setting (8). High-risk patients can therefore be advised to connect with healthcare professionals for further investigation.

Another example could be through one of the most common chronic diseases, Chronic Obstructive Pulmonary Disease (COPD) new communication tool between healthcare professionals, the patient and his or her caregivers, as well as the method of identifying and verifying new knowledge generated on an ongoing basis in diagnostic and therapeutic processes were used in COPD (9). The patient engagement and elements of artificial intelligence reduces the significant clinical risk of therapy. Intelligent workflows and data-driven processes facilitate the incorporation of user feedback from both patients and healthcare providers. This results in improved accuracy, heightened customer satisfaction, and valuable business intelligence.

Prerequisites and Challenges

For successful implementation of hyperautomation in healthcare, certain prerequisites must be met. High-quality, structured, and standardized data, along with interoperability, are crucial for accurate information exchange. Multistakeholder engagement, involving clinicians, physicians, and patients, is paramount for technology adoption and acceptance. Competency development and education among users, with a focus on a human-centered approach, are vital for seamless integration. Leadership, vision, and a training framework that fosters a data culture and innovation are essential for driving the adoption of these advanced technologies.

Impact on Data Quality and Ethics

The adoption of hyperautomation in healthcare brings forth important considerations regarding data quality, algorithms, and the mitigation of biases associated with AI-driven decision support systems. Ensuring that future systems rely on accurate and reliable data is paramount. Leadership and training play a pivotal role in establishing a data-driven culture, promoting ethical practices, and addressing potential liability concerns.

Opportunities and Risks

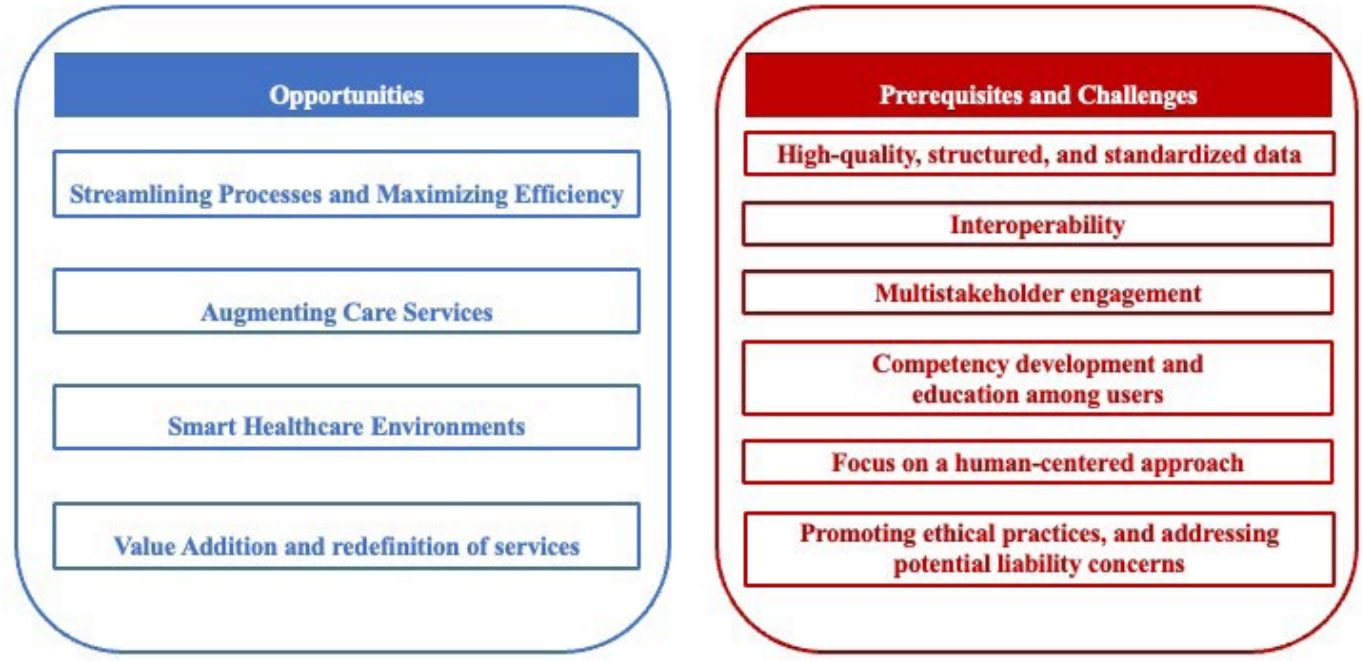
Hyperautomation in healthcare presents a wealth of opportunities, including streamlined organizational processes, improved access to care, enhanced quality of care, and the seamless integration of primary and specialized care. It empowers healthcare professionals with greater control, fosters preventive care, and enhances collaboration among them. Quick wins can be achieved through local network opportunities with general practitioners and pharmacies, which are closely connected to citizens and can effectively showcase the benefits of hyperautomation. However, careful management of risks related to patient safety, ethics, and liability is imperative.

Conclusion

In conclusion, hyperautomation is poised to be a transformative force in healthcare. By seamlessly integrating AI, machine learning, and robotic process automation, it equips health managers to optimize processes, enhance efficiency, improve the accuracy of healthcare operations, and ultimately elevate patient safety and experiences. As healthcare continues to evolve, embracing hyperautomation will be crucial in shaping a more efficient, responsive, and patient-centered future.

Declaration of no conflict of interest:
None.

Figure 1: Opportunities and challenges of hyperautomation



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The Role of Serum NT-proBNP for Predicting Left Ventricular Systolic Dysfunction in Hospitalized Patients in Sri Lanka

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Abstract

Background/aims:
Only a few studies have addressed the role of NT-proBNP in identifying Left Ventricular Systolic Dysfunction (LVSD) in South Asian populations. Therefore, the current study was aimed at assessing the use of serum NT-proBNP in predicting LVSD in a hospitalized population in Sri Lanka.

Methods:
A random sample of 278 individuals referred for echocardiography at a major Teaching Hospital consented for venous blood samples to be collected for serum NT-proBNP assay by sandwich ELISA. Based on the ejection fraction (LVEF) and fractional shortening (FS), participants were differentiated as LVSD (LVEF<50%, FS≤ 29%) and non-LVSD individuals (LVEF>60%). According to inclusion/exclusion criteria, the final study sample consisted of 100 LVSD patients and 41 non-LVSD individuals.

Results:
The mean ages of the LVSD and non-LVSD groups were 69.1 (±6.2 years) and 71.4 (±2.4 years) (p=0.066) respectively. The median NT-proBNP value (with IQR) among LVSD patients (528.2 pg/mL,355.2–924.2) was comparatively higher than that of non-LVSD individuals (207.3 pg/mL,177.5–343.0). Strong correlations of NT-proBNP level with LVEF (Spearman *rho*= -0.84, p<0.001) and FS (*rho*= -0.81, *p*<0.001) suggested that serum NT-proBNP concentration increases in parallel to deteriorating left ventricular functions. The AUROC of serum NT-proBNP for differentiating LVSD was 0.859 (95% CI:0.79 – 0.92) and the optimal cut-off level for predicting LVSD was 265pg/mL, with 90% sensitivity and 70% specificity.

Conclusion:
Current Sri Lankan study revealed a considerable correlation of serum NT-proBNP level with LVSD and utilizing such an assay for screening will facilitate adequate evidence to rule-out LVSD among high-risk residents.

Introduction

The high incidence and prevalence of different cardiovascular diseases (CVDs) are due to the significant presence of risk factors in the respective communities, for instance, chronic Heart Failure (HF); has been observed mostly in communities where there are increasingly ageing populations (1). According to the 2013 American Heart Association (AHA) HF guidelines (page e153), HF is a "complex clinical syndrome caused by structural or functional impairment of ventricular filling or ejection of blood" (2). Left Ventricular Systolic Dysfunction (LVSD) precede the incidence of HF (3) (4) because of the impaired left ventricular function with reduced ejection fraction (LVEF) during systole (5). Approximately 50% of LVSD patients exhibit no apparent clinical manifestations making early diagnosis difficult (6). In Sri Lanka, CVDs are the leading cause of death among patients with non-communicable diseases (NCDs). Such incidents are augmented by the rapid expansion of the ageing community, the sedentary lifestyle of the local residents, unplanned urbanization and the effects of globalization (7). The impact on the quality of life of affected individuals is further complicated by the unequal distribution of healthcare facilities and poor affordability of high-level healthcare by the public in South Asia (8). Therefore, the most critical and vulnerable individuals face both delays in the identification of cardiovascular risks at an early stage and the benefit from advanced definitive diagnostic procedures such as 3D echocardiography. However, such consequences could be prevented to a considerable extent by utilizing alternative screening methods. Although previous studies have emphasized that considering serum N-terminal pro-Brain Natriuretic Peptide (NT-proBNP) measurement and electrocardiography (ECG) abnormalities is a cost-effective, early diagnostic methods for detecting LVSD (9), in Sri Lanka serum NT-proBNP test is still not widely used as a routine clinical diagnostic test in the state health sector.

NT- pro-BNP is an active type of neurohormone belonging to the Natriuretic Peptides (NP) produced by the cardiomyocytes of the left ventricle and then secreted into the blood to induce diuresis, natriuresis and vasodilation. Further, these neurohormones suppress fibrosis, hypertrophy and remodeling of heart muscle after myocardial infarction (10). NT-pro-BNP is more stable in blood in fact, three days in whole blood and up to 24 hours in EDTA samples (11) and exhibits a wider detection range than other NPs (12). NT-pro-BNP also exhibits less inter-patient variability (13), and accurately differentiates normal and impaired LVEF. Therefore, it can also be used to detect lesser degrees of LVSD (14).

In contrast, it was noted that the blood NT-pro-BNP levels vary according to comorbidities, age, gender, genetics, assay method. Therefore, its usage alone as a screening tool for detecting LVSD is debated and deciding a clear universal cut-off value is a challenge (15). As South Asians differ from western ethnicities in terms of anthropology, genetics, and socioeconomic background that affect health status, it is doubtful whether the NT-proBNP reference ranges and cut-off level, as determined in the previous western studies, can directly be applied to the South Asian communities. Although the use of NT-proBNP is beneficial for screening LVSD or HF, in Sri Lanka, serum NT-proBNP is still not widely used as a routine clinical diagnostic test. Therefore, the current study aimed specifically to assess the use of serum NT-pro-BNP in predicting LVSD in a hospitalized Sri Lankan population.

Materials and Methods

Study design, study population and the study sample

We conducted a hospital based prospective study to assess the associations of NT-proBNP with echocardiography findings and the utility of serum NT-pro-BNP in predicting LVSD in a high risk hospitalized population. This population were those referred for echocardiography to assess left ventricular function with a history of acute coronary syndrome (ACS), or other cardiovascular disorders/risk factors such as Hypertension, Hypercholesterolemia, Diabetes Mellitus (DM), Heart Block, Carditis. It also included patients with undefined chest pain, dyspnea, fever, or post-covid syndrome, admitted to the Teaching Hospital in Peradeniya, Sri Lanka. There were about 1000 echocardiography tests performed in each three-month period; hence the estimated sample size for the study was 278 individuals in total (16) who were selected by random sampling.

Blood sample collection, serum separation, and storage

Initially, with the informed written consent obtained from each selected individual referred for echocardiography, a venous blood sample of 3 ml was withdrawn into a clot activator tube; serum was then separated and stored at -80ºC freezer until analysis of NT-pro-BNP.

The Enzyme-Linked Immunosorbent Assay (ELISA) procedure for serum NT-pro-BNP level analysis

The serum samples were analyzed for NT-pro-BNP using the sandwich enzyme-linked immunosorbent assay (ELISA) kits (11) (17) imported from Wuhan Elabscience Biotechnology Company Ltd, Wuhan, Peoples Republic of China (Catalog No: E-EL-H6126). This ELISA kit uses Biotinylated Detection Antibody specific for human NT-proBNP and Avidin-Horseradish Peroxidase (HRP) conjugate to detect NT-proBNP. The skilled professional who carried out the ELISA procedure was blinded to the study participants' demographic and clinical data, and echocardiography findings. The analytical sensitivity of the ELISA method was 0.09 ng/mL (90 pg/mL) with a detection range of 0.16 -10 ng/mL (160 -10,000 pg/mL). The manufacturer claims that there is no significant cross-reactivity or interference

between Human NT-pro-BNP and analogues. The coefficient of variation (CV) is <10%. The ELISA kits were stored below 4°C until unpacking and all reagents were brought to room temperature (~25 °C) and the microplates were preheated (~25 °C) for 15 min, before the use. All the laboratory standards were maintained throughout the ELISA procedure, as instructed in the kit manual. The estimation of raw NT-proBNP values was based on the average optical density (OD) of raw absorbance in each microplate well which was then matched with standard curve formulated for each ELISA plate. All the NT-proBNP measurements less than 160pg/ml were considered as "results below the detection range".

The Echocardiography

On the same day of, or the day after the blood sample collection, each individual underwent echocardiography to identify left ventricular function. The medical officer who performed the echocardiography was blinded to the selected individuals' serum NT-proBNP values. The echocardiography studies were performed by Toshiba XarioXG SSA-680A echocardiography machine in 2D mode when the patient was in the left lateral position. The echocardiography report consisted of several measurements (e.g, LVEF, End Diastolic Volume, End Systolic Volume, diagnoses made through the presence of left ventricular hypertrophy (LVH), left ventricular dilatation, good/impaired left ventricular systolic function, good/impaired left ventricular diastolic function, good/impaired right ventricular function, the function of atrial valve/mitral valve, or the presence of congenital heart diseases).

The definition of LVSD

Based on echocardiography findings, the selected individuals were grouped as LVSD patients and non-LVSD individuals. LVSD was defined as LVEF ≤ 50% and Fractional Shortening (FS) ≤ 29% (18) (19) (20) while those with LVEF ≥ 60% who did not suffer from any concurrent major cardiovascular disorders (ACS, MI, Heart Block, Carditis, etc.) or have multiple risk factors for cardiovascular disorders (e.g. Hypertension, Hypercholesterolemia, Diabetes Mellitus (DM)) within the same patient, were considered as non-LVSD individuals. The following types of patients were excluded from the study sample.

- Patients diagnosed as having HF with preserved EF (HFpEF) or left ventricular diastolic dysfunction (LVDD), and valvular heart diseases.
- Patients whose LVEF was between 51% - 59% as we wanted to select LVSD patients and non-LVSD individuals according to the stated definition of LVSD.
- Patients diagnosed with bi-ventricular dysfunction and concurrent valvular heart disease.
- Patients diagnosed with Acute Kidney Injury, Chronic Kidney Disease or End Stage Renal Disease.
- Patients already on beta-blocker therapy
- Patients whose NT-proBNP level could not be detected by ELISA method or whose raw NT-proBNP level is less than the detection range of used ELISA kit.

Selected individuals were then interviewed for their demographic information, medical/surgical history, and clinical records.

Ethical Approval

The study was performed in accordance with the declaration of Helsinki, thus the ethical clearance (AHS/ERC/2018/097) for the study was obtained from the Ethics Review Committee of the Faculty of Allied Health Sciences, University of Peradeniya, Sri Lanka. The purpose of the study, potential benefits to the patient and to the society, the steps of data collection were explained in lay terms and the written informed consent from each participant was obtained prior to the venipuncture and echocardiography.

The statistical analysis

The 95% Confidence Interval (CI) and p=0.05 were used as the significance level to describe the results of the statistical analysis. Since the serum NT-pro-BNP level of the study population did not meet the parametric assumptions, nonparametric statistical tests were considered for data analysis. Comparison of NT-pro-BNP levels was done using independent samples Mann Whitney u test, median values (with interquartile range (IQR) and Kruskal -Wallis H test. Since the ELISA method was used for serum analysis, and there were no clear NT-pro-BNP cut-off values related to the ELISA method in Sri Lankan adult population, it was necessary to decide on an appropriate serum NT-pro-BNP level to differentiate LVSD in the current study population. Therefore, a receiver operating characteristic (ROC) curve was drawn in respect of serum NT-pro-BNP level and LVSD and the area under ROC curve (AUROC), varying degrees of sensitivities and specificities of NT-pro-BNP levels were considered to determine the optimum cut-off level to discriminate LVSD (LVEF < 50%) (21). The correlations of NT-pro-BNP level with continuous variables (eg: echocardiography measures, age, routine blood investigations, blood pressure, etc.) were determined by Spearman's correlation coefficient (*rho*) and binary logistic regression was considered to determine the independent predictors of NT-pro-BNP above that of the proposed cut-off level. All the statistical analysis was performed by IBM SPSS version 26.

Results

The associations of clinico-epidemiological characteristics, comorbidities, and predisposing risk factors with LVSD

Of the 278 individuals who provided blood samples and underwent echocardiography, 78 were excluded as per the inclusion/exclusion criteria. Of the rest, serum NT-pro-BNP values could not be estimated at all in 27 samples and 32 samples indicated raw NT-proBNP level less than the lower detection limit (i.e. 160 pg/ml). Individuals with both types of samples were excluded from further data analysis. Therefore, from the remaining, 100 individuals were confirmed as LVSD patients, and 41 individuals were non-LVSD individuals. The baseline clinico-epidemiological characteristics of the study sample are

as depicted in table 1. No statistically significant difference was observed between the LVSD group and the non-LVSD group in terms of age distribution and gender. Several cardiovascular risk factors were observed in LVSD patients including prior episodes of coronary heart disease (CHD) in a major proportion (75%). Based on the NYHA functional classification of HF, 10% of the LVSD patients and 97% (n=40) of the non-LVSD individuals belonged to class I followed by 51% of the LVSD patients and 3% (n=1) of the non-LVSD individuals in class II (Table 1).

Table 1: Baseline demographic and clinical characteristics of the LVSD and non-LVSD groups

Baseline demographic and clinical characteristics		**LVSD group (N=100)**	**Non-LVSD group (N=41)**	***p* value**
Age in years		69.3 (±6.1)	71.4 (±2.4)	0.066**
Gender	Male	63	22	0.303*
	Female	37	19	
Alcohol Consumption	Current Alcohol Consumer	17	9	0.158*
	Ex-Alcoholic	25	4	
	Non-Alcoholic	58	28	
Tobacco Smoking	Current Smoker	7	4	0.204*
	Ex-Smoker	25	6	
	Non-Smoker	68	31	
Clinical manifestation	Shortness of Breath (SOB)	45	1	<0.001*
	SOB on Exertion	74	4	<0.001*
	Paroxysmal Nocturnal Dyspnea	70	2	<0.001*
	Orthopnea	73	5	<0.001*
	Lower leg swelling	48	3	<0.001*
	Fatigue	62	9	<0.001*
NYHA HF Classification	Class I	10	40	<0.001*
	Class II	51	1	<0.001*
	Class III	31	-	
	Class IV	8	-	
Hypertension (BP> 140/90 mmHg)		69	3	<0.001*
Coronary Heart Disease		75	-	<0.001*
Hypercholesterolemia		60	2	<0.001*
Diabetes Mellitus		32	3	0.002*

The frequency data for each categorical variable have been presented as number (n). The data for each continuous variable have been presented as mean ±SD. **p* value as derived by Pearson's chi squared test. ***p* value as derived by independent sample *t* test.

The routine blood investigation findings, blood pressure measurements, echocardiography measurements and ECG findings were also significantly higher among LVSD patients than that of non-LVSD individuals (Table 2).

Table 2: The average routine blood investigation findings, blood pressure measurements, echocardiography measurements and selected ECG parameters in LVSD and non-LVSD individuals

Investigation parameter	LVSD group (N=100)	Non-LVSD group (N=41)	*p* value
Hb level (g/dL)	11.65 (±1.6)	13.9 (±1.17)	<0.001*
Serum Creatinine (µmol/L)	90.08 (±27.7)	74.7 (±14.7)	0.001*
Blood Urea Nitrogen (mmol/L)	8.27 (±4.2)	5.08 (±2.45)	<0.001*
Serum K+ (mmol/L)	4.37 (±0.6)	4.0 (±0.24)	0.001*
C-Reactive Protein (mg/L)	46.28 (±45.6)	93.5 (±55.7)	<0.001*
Systolic BP (mmHg)	145.5 (± 18.0)	117.2 ±9.6	<0.001*
Diastolic BP	90.4 (±11.5)	75.9 ±8.0	<0.001*
Mean Arterial Pressure (mmHg)	107.4 (±13.9)	89.7 ±7.8	<0.001*
LVEF (%)	35.47 (±8.2)	62.3 ±1.4	<0.001*
FS (%)	18.35 (±3.9)	33.09 ±2.1	<0.001*
LV Mass (g)	237.38 (±46.1)	143.1 ±18.23	<0.001*
End Diastolic Volume (mL)	169.1 (±37.1)	79.3 ±14.1	<0.001*
End Systolic Volume (mL)	111.2 (±38.6)	29.9 ±5.8	<0.001*
QRS Duration (ms)	137.5 (±16.8)	107.3±9.5	<0.001*
Corrected QT interval (ms)	465.1(± 19.8)	425.7 ±24.8	<0.001*
Goldberger's 1st criterion (mV)	3.86(±0.8)	2.7 ±0.5	<0.001*

The data for each continuous variable have been presented as mean ±SD. **p* value as derived by independent sample *t* test.

The distribution of serum NT-pro-BNP level in the study sample

The range of raw NT-proBNP among LVSD patients (n=100) was 164.52 – 6697.74 pg/mL with the mean NT-proBNP of 1073.0 pg/mL (±1345.74). NT-proBNP levels were undetectable in 27 samples while 32 samples contained raw NT-proBNP level less than the lower detection limit (160 pg/ml) of used ELISA kits. Individuals with both types of samples were excluded from further data analysis. Therefore, the range of raw NT-proBNP among non-LVSD individuals (n=41) was 160.0 – 688.84 pg/mL with the mean NT-proBNP of 277.29 pg/mL (±145.39). Considering the skewed distribution, the median NT-proBNP (with IQR) was 528.2 pg/mL (355.2 – 924.2) in the LVSD group and 207.36 pg/mL (177.52 – 343.0) among the non-LVSD individuals. The Mann Whitney U test revealed that the mean rank of NT-proBNP level in the LVSD group (85.73) was significantly different (p<0.001) than that of non-LVSD group (35.09) (Figure 1).

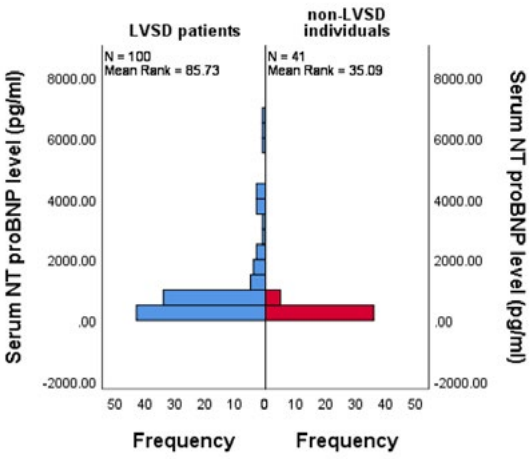

Figure 1. Independent samples Mann Whitney u test results for comparing the NT-proBNP levels in LVSD and non-LVSD groups

The Kruskal -Wallis H test (H (3) = 94.9, p<0.001) suggests that the NT-proBNP levels significantly increases through the increasing severity levels of LVSD (Figure 2). Similarly, the serum NT-proBNP levels increases significantly through the ascending levels of NYHA HF classes (H (3) = 81.4, p<0.001), and the NT-proBNP level in the NYHA HF class I is significantly different than that of NYHA HF class III and IV (Figure 2).

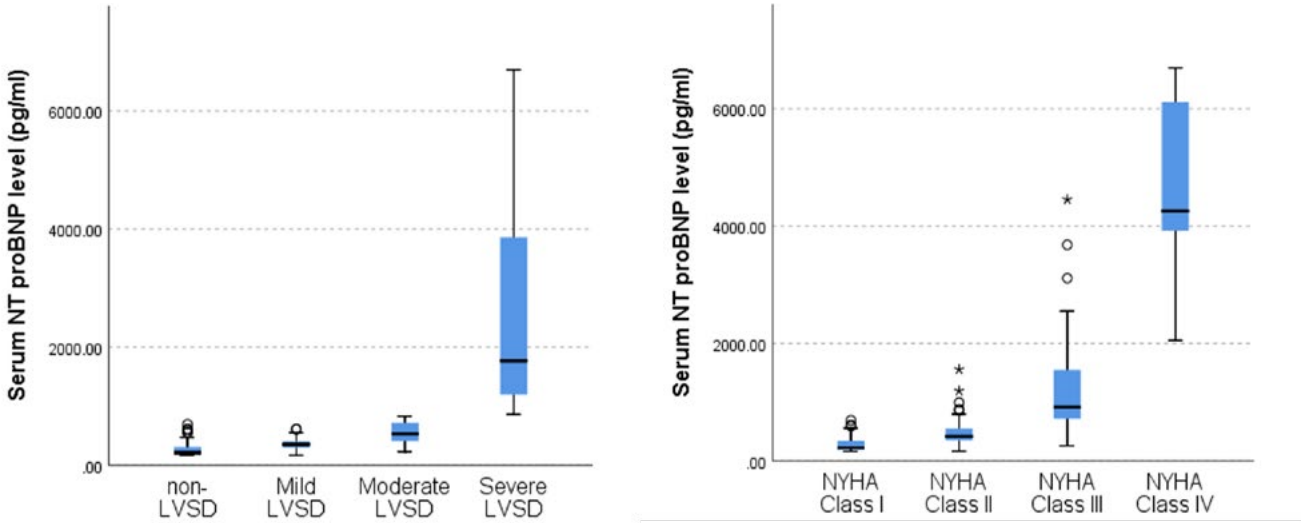

Figure 2: Median NT-proBNP levels among the LVSD severity categories: mild (LVEF= 40%-50%), moderate (LVEF= 30%-39%) and severe LVSD (LVEF= < 29%) categories (left). Median NT-proBNP levels among the NYHA HF classes (right). The probability values were derived from the independent samples Kruskal -Wallis H test. Boxes represent interquartile range (Q1 – Q3), while whiskers represent minimum and maximum values.

The NT-proBNP cut-off values and the predictive values to identify LVSD patients.

As depicted in Figure 3, The AUROC of NT-proBNP was 0.859 (95% CI: 0.79 – 0.92) which is significant in the discrimination of LVSD patients from non LVSD individuals. Based on the coordinates of ROC, 265 pg/mL presented with 90% sensitivity and 70% specificity. Therefore, with regard to the selected high-risk study sample, 265 pg/ml is considered as the optimum cut-off value of serum NT-proBNP in differentiating LVSD patients from non-LVSD individuals.

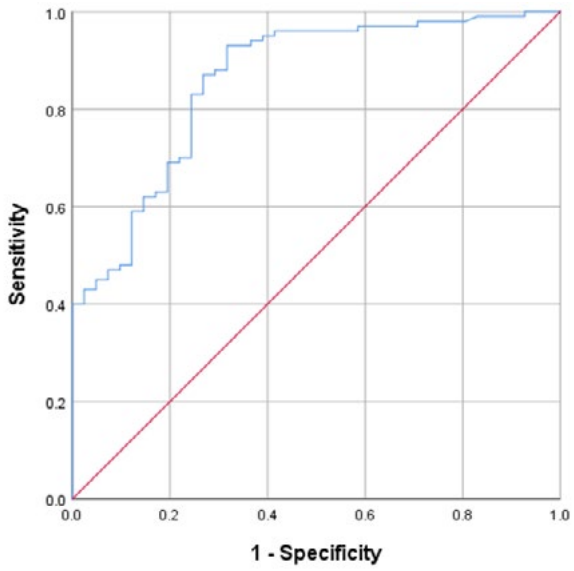

Figure 3. The ROC curve for NT-proBNP to predict LVSD in the selected elderly population. The AUROC is 0.859 (with SE = 0.034 and p < 0.001)

The correlations of serum NT-proBNP level with echocardiography, ECG, age and other covariates

Strong correlations of serum NT-proBNP level with LVEF (*rho* = -0.840, p <0.001), FS (*rho* = -0.815, p <0.001) and LV mass were (*rho* = 0.685, p <0.001) observed suggesting that NT-proBNP concentration in blood increases in parallel to deteriorating left ventricular functions and increasing left ventricular mass. Similarly, NT-proBNP level indicated strong positive correlations with ECG parameters such as corrected QT interval (*rho* = 0.69, p <0.001), Goldberger's 1st criterion (*rho* = 0.68, p <0.001) and QRS duration *(rho* = 0.68, p <0.001). Systolic blood pressure (*rho* = 0.72, p <0.001) and diastolic blood pressure (*rho* = 0.65, p <0.001) also indicated strong positive correlations with increased serum NT-proBNP level. It was also found that higher NT-proBNP levels are moderately correlated with lower hemoglobin level (*rho* = - 0.37, p <0.001). The older the study participants, the higher the NT-proBNP level was observed to be (*rho* = 0.40, p <0.001).

Though there were several clinical parameters that strongly correlated with NT-proBNP level, the binary logistic regression analysis revealed that elevated NT-proBNP level > 265pg/ml in the current study sample is independently associated with the age, LVEF, PND, orthopnea, history of CHD and LV mass only. Table 3 below presents the estimates of coefficients related to NT-proBNP level and associated covariates obtained by logistic regression.

Table 3: The statistically significant independent predictors of NT-proBNP level > 265 pg/ml based on binary logistic regression.

Covariate	Unstandardized Coefficients	SE	Wald	df	*p* value	*Exp(B)*	95% CI for EXP(B)	
LVEF	0.44	0.16	7.83	1	0.005	1.56	1.14	2.13
Age	-0.40	0.10	14.02	1	<0.001	0.66	0.54	0.82
PND	-2.32	1.17	3.91	1	0.048	0.09	0.01	0.97
Orthopnea	-2.45	1.23	3.93	1	0.047	0.08	0.008	0.97
CHD	-3.96	1.76	5.03	1	0.025	0.02	0.001	0.60
LV Mass	-0.05	0.02	7.04	1	0.008	0.94	0.90	0.98

SE= Standard Error, df= degree of freedom, Exp (B) = Odds ratio

Discussion

Our study is the first Sri Lankan study attempting to establish the associations of serum NT-proBNP level with demographic and echocardiography findings of LVSD patients. As hypothesized, the NT-proBNP level is significantly associated with impaired left ventricular function in the selected high risk study sample.

The skewed distribution of NT-proBNP level towards the lower LVEF values has been reported in several previous studies (17) (22) (23) where the mean, median and mean rank of serum NT-proBNP level were significantly higher in the LVSD group than the non-LVSD group. In a local study conducted in 2016, the mean NT-proBNP level in HF cases was relatively higher than in the current study. Though our study yielded a lower median NT-proBNP level than the previous local study, the range of NT-proBNP distribution in both studies was similar. One explanation of the different findings in two Sri Lankan studies is that NT-proBNP level is assay method dependent (24). In the previous study, this was measured by the minividas® auto analyser (25) whilst in the present study, the ELISA method was used. Use of the ELISA method for assessing NT-proBNP is not affected by the presence of different analogues such as bilirubin, hemoglobin, rheumatoid factor, triglycerides and biotin etc in the blood samples (11). A previous study that considered the ELISA method for assessing NT-proBNP emphasized that there is a 97% certainty of normal LVEF if NT-proBNP level is below the proposed cut-off level (ie: 97.2 pg/mL) in the particular study (17). In a study conducted in India in 2012, the mean NT-proBNP level was 1503.33pg/mL in left ventricular failure patients (26) which is slightly higher than the mean NT-proBNP observed in the present LVSD group. A study based in Pakistan which included congestive HF patients, reported a mean NT-proBNP level of 10 000pg/mL (27), obviously an extreme cut-off level when compared to the present study. Our study exhibited a considerable variance of serum NT-proBNP level through both the LVSD severity stages and the NYHA HF classes. Other studies have also shown increasing NT-proBNP median levels through worsening left ventricular function (17) and towards the higher NYHA HF classes (14). The results of the Dallas heart study also support that NT-proBNP level is significantly associated with the presence of LVSD (28). However, the current recommendation is that when a patient presents with a higher NYHA HF class, as evidenced by the symptoms and activity tolerance, the advanced diagnostic methods such as echocardiography should be employed directly rather than relying on NT-proBNP level (29). The main echocardiography parameters including LVEF and FS exhibited a strong negative correlation with the serum NT-proBNP, allowing for speculation that when the LVEF and FS decline, the serum NT-proBNP level gradually rises. This finding may be supported by the theory that declining LVEF and FS reflect the impaired function of the left ventricle resulting from more anatomical alterations of the myocardium with accelerating cardiomyocyte growth and increasing cardiomyocyte stretch (3)

(30). As a compensatory response to such structural alterations, higher concentrations of NT-proBNP are secreted into the blood, by the cardiomyocytes of the left ventricle (31). Similar patterns of strong negative correlations of NT-proBNP level with LVEF, as found in the present study, have also been explained by several other Western researchers (13) (32). Apart from the correlations of NT proBNP level with LVEF and FS, the findings of the present study indicated that the NT proBNP level was also significantly correlated with the abnormal ECG parameters such as corrected QT interval, Goldberger's 1st criterion and QRS duration. Therefore, it can be speculated that NT proBNP level and ECG characteristics are in accordance in predicting LVSD.

Burke and Cotts in 2007 suggested that since NT-proBNP level is affected by various comorbidities and demographic factors, thus appropriate reference values for the specific individual groups must be determined (33). In the current study, the AUROC clearly distinguishes LVSD patients from non-LVSD individuals. Similarly, in the Pakistani study, the AUROC of NT-proBNP was 0.99 for diagnosing congestive HF (27). Consistent with the current study finding, the study by Verdu et al (2012) also found an AUROC of 0.94 for NT-proBNP to exclude HF (24) whilst two other overseas studies (4) (12) have also shown similar AUROCs.

In a previous Sri Lankan study, the identified optimal cut-off level of plasma NT-proBNP to exclude HF was lower (82.7pg/ml) (25); nevertheless, the relevant sensitivity, and specificity were not found to compare with the current study. In the Pakistani study, NT-proBNP level > 300pg/ml was 100% sensitive and 42% specific for identifying congestive HF (27) It has also been reported that NT-proBNP <300pg/ml is ideal for ruling out acute HF with a negative predictive value (NPV) of 99% (34). In the study by Verdu et al (2012) the optimal cut-off level to exclude HF was 280pg/ml (24). The NT-proBNP level of 150pg/ml exhibited a sensitivity of 94%, specificity of 40%, positive predictive value (PPV) 48% and NPV 92% (22). An NT-proBNP level of 100pg/ml was 88% sensitive for LVSD; however, the related AUROC was 0.77 (95% CI, 0.71 -0.82) for LVEF <50% (35). The most compelling explanation for such differences in cut-off levels in various studies is that NT-proBNP level is assay dependent (36) resulting in NT-proBNP cut off level applicable only for the selected method of assay. The report of Hammerer-Lercher et al (2004) also highlighted that there is a diversity of natriuretic peptide assays resulting in different cut-off levels, such that, importantly, the published cut-off/ reference levels in literature are applicable only to the method where such studies were based (37). Apart from the age, weight and comorbidities, the diagnostic method also influences the performance characteristics leaving it a challenge when deciding universal reference values for NT-proBNP measurement (38). NT-proBNP level is also affected by the medications such as β blockers and diuretics, so it is advisable to test for NT-proBNP before the start of such medications, if facilities are available. However, the proposed cut-off level in the present study falls within the range of different cut-off levels as presented in the contemporary overseas studies. However, it cannot be claimed that the proposed cut-off level is the most suitable for screening for LVSD in the general population, because this study is limited to a high risk hospitalized population in Sri Lanka. Therefore, validation of the findings of this study should be carried out in a selected general population, perhaps outside the hospital setting to determine its applicability in the community screening. The ELISA procedure used in this study was only to obtain the serum NT-proBNP level rather than assessing the diagnostic performance of the used ELISA kits. Different studies have resulted in varying NT-proBNP levels, depending on the sample type (serum, plasma, tissue) and the assay procedure. Therefore, before utilizing a specific method of analysis, it is recommended that the diagnostic performance along with appropriate cut-off levels for each of target communities should be determined. In the present study, the advantages of assessing NT-proBNP such as availability, accessibility, and with no expertise needed for interpreting the results over echocardiography were considered for the applicability of NT-proBNP measurement in the local clinical services, regardless of their cost effectiveness. Therefore, a comprehensive validation study is recommended to evaluate the cost-effectiveness of assessing NT-proBNP in predicting LVSD. During the course of this study, we did not interfere with the pharmacological management of selected individuals; thus, the effects of medications on NT-proBNP level were not established. Equally, age standardized NT-proBNP cut-off levels must also be determined in a future study.

Conclusion

Against the Sri Lankan background of inconsistency of resources in the local health services, access to echocardiography and expertise in performing echocardiography is not always possible. Although previous studies in Western countries have reported that NT-proBNP measurement is a cost-effective strategy superseeding echocardiography, in Sri Lanka this blood investigation is still an expensive test which is performed mostly in the private sector laboratories. However, the findings of this current Sri Lankan study found that the NT-proBNP level exhibits significant diagnostic performances for LVSD patients, suggesting that NT-proBNP assessment is an appropriate test to be introduced at the local public sector hospitals. Providing the facilities to perform the NT-proBNP test at such hospitals will facilitate adequate evidence towards the early detection and the ruling out of LVSD. The NT-proBNP assay can also be used for the screening of vulnerable individuals in the community, especially at the NCD prevention clinics for periodic evaluation of 'at risk' groups such as those aged 50 years or over, living with uncontrolled hypertension, CHDs, hypercholesterolemia, and DM. By so doing those with LVSD can be ruled out or further referral for advanced diagnostic studies can be ensured at an early stage of the disease. Having observed significant correlations of NT-proBNP with other screening methods such as ECG, it is asserted that combining NT-proBNP measurement with ECG abnormalities increase the possibility for identifying

and ruling out LVSD when the patients present to health care settings that have limited diagnostic resources.

What is Already Known?

Previous studies of Western countries have reported that NT-proBNP measurement is a cost-effective strategy over echocardiography in predicting LVSD. However, there is a lack of information regarding the use of NT proBNP in South Asian region.

What this Study Adds?

The findings of this foremost Sri Lankan study found that the NT-proBNP level exhibited a significant diagnostic performance for LVSD patients in Sri Lanka, suggesting that NT-proBNP assessment is a valuable test to be introduced to the state health sector, in order to rule out LVSD at an early stage of the disease.

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Declaration of Conflict of interests

The authors of the project declare that there is no conflict of interest with regard to the content of this manuscript.

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A novel score-based approach by using routine laboratory tests for accurate diagnosis of spontaneous bacterial peritonitis (SBP) in cirrhotic patients

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Keywords
SBP, Peritonitis, Cirrhosis, Laboratory

Abstract

Summary
Background: Spontaneous Bacterial Peritonitis (SBP) poses a significant risk to cirrhosis patients with ascites, emphasizing the critical need for early detection and intervention. This retrospective observational study spanning a decade aimed to devise predictive models for SBP using routine laboratory tests. Additionally, it aimed to propose a novel scoring system to aid SBP diagnosis.

Methods: Data analysis encompassed 229 adult cirrhotic patients hospitalized for ascites between 2012 and 2021. Exclusions eliminated cases of secondary ascites unrelated to liver cirrhosis. Patients were categorized into SBP-positive (n=110) and SBP-negative (n=119) groups. Comparative analysis of demographic details and various laboratory indicators (Neutrophil-to-Lymphocyte Ratio (NLR), Mean Platelet Volume (MPV), C-Reactive Protein (CRP), Platelet (PLT), Alanine Transaminase (ALT), Aspartate Amino Transferase (AST), Potassium (K), Sodium (Na), Total Bilirubin (TB) and International Normalized Ratio (INR) was performed between the groups. The study presented effective SBP prediction models for prompt diagnosis and treatment: a multivariate logistic regression model and a simple scoring system.

Findings: The study advocates early diagnosis and rapid treatment for all cirrhotic patients with ascites, regardless of cirrhosis stage. Furthermore, it recommends initiating SBP treatment for patients scoring 2-3 in the proposed scoring system while excluding SBP findings for those scoring zero.
Conclusion: Combining age, sex, and specific laboratory tests (MPV, NLR, CRP, TB, and INR) within random forest models and a simple scoring system enables swift and accurate SBP diagnosis.

1. Introduction

Ascites, a prevalent and severe complication of chronic liver diseases, notably cirrhosis, imposes a significant burden of morbidity and mortality (1). Cirrhosis, characterized by progressive liver tissue fibrosis, stands as a leading cause of liver-related morbidity and mortality globally (2). It commonly originates from chronic liver injuries induced by factors such as viral hepatitis, excessive alcohol intake, nonalcoholic fatty liver disease (NAFLD), autoimmune liver diseases (3-5). During the early stages of cirrhosis, patients might remain asymptomatic or exhibit non-specific symptoms like fatigue, weight loss, and abdominal discomfort. However, disease progression leads to complications like ascites, hepatic encephalopathy, and variceal bleeding (6). Notably, individuals with cirrhosis are more vulnerable to bacterial infections, with up to 35% developing infections post-hospitalization (2). Ascites, the abnormal accumulation of fluid in the abdominal cavity, represents the most common complication of cirrhosis. It develops due to factors such as portal hypertension and renal sodium retention. The onset of ascites significantly impacts the quality of life and prognosis for cirrhotic patients (7). Among the life-threatening infections in cirrhotic patients with ascites, Spontaneous Bacterial Peritonitis (SBP) stands prominent. SBP results from bacterial translocation from the gut to the peritoneum, often due to compromised immune function (8-12). Its classic symptoms include fever and abdominal pain, though these might be absent in some cases (13). Swift diagnosis and treatment are pivotal for SBP, as mortality rates range from 10% to 50%, contingent on various factors (13). Traditional SBP diagnosis relies on ascitic fluid analysis through invasive procedures like paracentesis. To overcome the limitations of invasive testing, research has explored non-invasive markers, including neutrophil-to-lymphocyte ratio (NLR), mean platelet volume (MPV), Platelet-to-lymphocyte ratio (PLR), and C-reactive protein (CRP). (14-18) This article reviews the critical importance of early diagnosis and management of ascites and SBP in cirrhotic patients, emphasizing non-invasive markers to expedite diagnosis.

2. Scientific Background

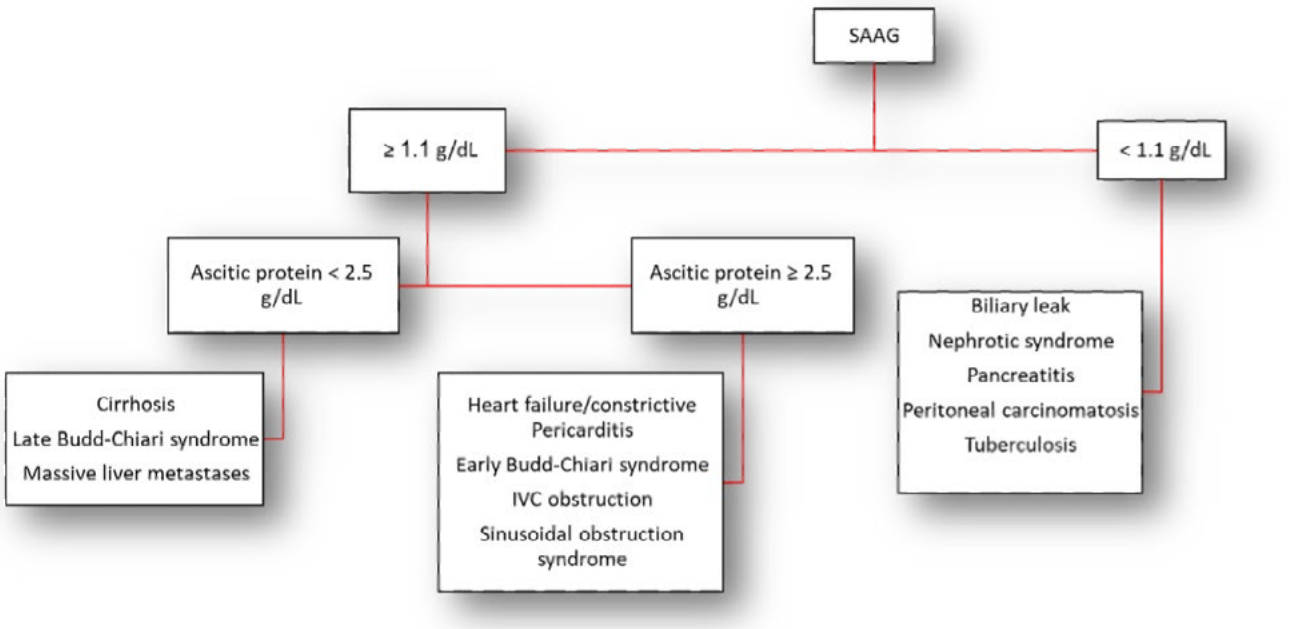
Cirrhosis, marked by liver fibrosis, represents a progressive liver disease with diverse etiologies. Although the liver can function initially despite cirrhosis, disease progression can culminate in liver failure and life-threatening complications (2). These complications encompass ascites, hepatic encephalopathy, and variceal bleeding (3-5). Effective management of cirrhosis involves addressing underlying causes, such as antiviral therapy for viral hepatitis or lifestyle modifications for non-alcoholic fatty liver disease. In advanced stages, liver transplantation might become necessary (3-5) Ascites emerges as a frequent consequence of cirrhosis, affecting approximately 60% of patients within ten years of diagnosis (19). It stems from portal hypertension-induced sodium retention and carries a high mortality rate, particularly when refractory to medical treatment (19). Timely diagnosis and management significantly enhance patient outcomes (7). Diagnosing ascites involves puncturing ascitic fluid to measure albumin levels, neutrophil counts, and culture for infection (20). Ascites etiology can also be discerned based on serum-ascites albumin gradient (SAAG) levels (21).

SBP stands as a common and life-threatening infection in cirrhotic patients with ascites. It correlates with a compromised immune system, bacterial translocation, and systemic inflammation, SBP diagnosis typically relies on invasive procedures like paracentesis (13). Mortality rates for SBP vary, yet early diagnosis and appropriate treatment are pivotal in reducing morbidity and mortality (13). Strategies for SBP prevention encompass prophylactic antibiotics and interventions to diminish bacterial translocation (8-12). Diagnosing SBP often necessitates invasive surgical puncture, leading to potential treatment delays. Therefore, the identification of reliable and non-invasive markers for early diagnosis holds crucial significance (22). Promising markers encompass NLR, MPV, PLR, CRP, total bilirubin, and INR (18, 16-17, 14-15, 23-26). Neutrophil-to-Lymphocyte Ratio (NLR), calculated by dividing the neutrophil count by the lymphocyte count, emerges as an indicator of immune system balance. Elevated NLR exhibits promise in diagnosing SBP (27). Mean Platelet Volume (MPV), associated with platelet activation, has been under study as a potential non-invasive marker for SBP diagnosis, showing promising results (28).C-Reactive Protein (CRP), synthesized during inflammation, has demonstrated diagnostic and prognostic value in SBP detection (22). Elevated INR and bilirubin levels are associated with an increased risk of SBP and higher mortality rates (23-25). Utilizing non-invasive markers like these offers potential benefits in early SBP diagnosis, ensuring timely intervention and improved patient outcomes.

3. Methods

The study employed computer algorithms constructed using fixed codes for diagnoses and laboratory tests. These algorithms aimed to maximize accuracy in extracting data for patients meeting the inclusion criteria. The first step involved gathering diagnoses and demographic data through the medical center's computerized medical record, utilizing one of the described algorithms. Next, the study extracted results from bacteriological laboratory cultures for ascites fluid sent between 2012 and 2021, totaling 408 cultures. Subsequently, patients with creatinine levels exceeding 5 mg/dL (79 patients) were excluded due to dialysis dependency, categorized as secondary ascites. Among the remaining 329 patients, the Serum Ascites Albumin Gradient (SAAG) was calculated using the Kasper et al. model (21) to isolate cases of ascites due to liver cirrhosis. Patients with a SAAG $\geq$ 1.1 and an ascites protein $<$ 2.5 were included. Further data extraction included laboratory test results, demographic information, diagnoses, and background diseases using the established algorithms. This encompassed details such as age, gender, length of hospitalization, days of survival after hospitalization commencement, and mortality within 30 days post-hospitalization. The study population was stratified into two groups: one with a positive diagnosis for Spontaneous Bacterial

Peritonitis (SBP) (N=110) and a control group with a negative SBP diagnosis (N=119). Lastly, a statistical analysis was conducted on the data generated by the algorithms to compare the two study groups. This analysis aimed to evaluate any potential relationships between various laboratory indicators and SBP, employing statistical prediction models outlined in the results chapter. The dataset consisting of 229 samples was split into a training set (172 samples, or 75% of the data) and a testing set (57 samples, or 25% of the data). The training set was used to build the models, while the testing set was reserved for evaluating their performance on unseen data. The R package 'caret' was employed to perform model training and validation.



Kasper, D. L., Fauci, A. S., Hauser, S. L., Longo, D. L., Jameson, J. L., & Loscalzo, J. (2019). Preface Harrison's Manual of Medicine. Asia Book Registry.

Figure 1: Etiology of ascites according to SAAG Kasper model values with numerical data (Kasper et al., 2019)

4. Results

4.1 Demographic data

Table 1: Descriptive statistics of the demographic data by the SBP group

	No SBP (N=119)	**SBP (N=110)**	**Total (N=229)**	***p* value**
Age	70.57 (12.06)	69.15 (13.59)	69.89 (12.81)	0.401
Gender				0.059
Female	50 (42.0%)	33 (30.0%)	83 (36.2%)	
Male	69 (58.0%)	77 (70.0%)	146 (63.8%)	

Notes: t-test for independent samples was used to test differences in continuous variables and Fisher exact test for categorical variables.

The SBP group comprised 70% males with a mean age of 69.2 (SD = 13.6), while the group without SBP consisted of 58% males with a mean age of 70.6 (SD = 12.1). As per Table 1, no statistically significant differences in age and gender were observed between the two groups.

4.2 Laboratory data

Table 2: Descriptive statistics of the Laboratory data by the SBP group

	No SBP (N=119)	SBP (N=110)	Total (N=229)	*p* value
Lymphocytes (abs)	1.46 (1.09)	1.35 (0.68)	1.41 (0.91)	0.384
NEUT (abs)	6.06 (2.68)	7.13 (3.98)	6.57 (3.40)	**0.017**
NLR (ratio)	5.11 (3.14)	8.06 (9.59)	6.56 (7.22)	**0.003**
PLT (1000/uL)	210.55 (86.94)	216.15 (99.60)	213.39 (93.39)	0.664
MPV (fL)	9.29 (1.42)	8.81 (1.35)	9.05 (1.40)	**0.016**
CRP (mg/dl)	34.97 (36.26)	43.39 (41.55)	39.61 (39.36)	0.201
ALT (U/l)	29.47 (19.14)	38.07 (42.02)	33.71 (32.68)	0.069
AST (U/l)	36.03 (35.99)	43.76 (53.27)	39.94 (45.59)	0.254
Potassium (mmol/l)	4.35 (0.72)	4.41 (0.62)	4.38 (0.67)	0.490
Sodium (mmol/l)	137.39 (4.50)	137.47 (5.00)	137.43 (4.75)	0.898
TB (mg/dl)	1.71 (1.83)	2.57 (2.75)	2.14 (2.36)	**0.010**
INR (ratio)	1.38 (0.87)	1.71 (1.42)	1.54 (1.18)	**0.039**

Notes: t-test for independent samples was used to test differences in continuous variables. Spontaneous Bacterial Peritonitis (SBP), Neutrophil to lymphocyte ratio (NLR), Mean Platelet Volume (MPV), C-Reactive Protein (CRP), Platelets (PLT), Alanine Trans Aminase (ALT), Aspartate Amino Transferase (AST), Total Bilirubin (TB), International Normalized Ratio (INR).

(7.13 vs. 6.06, p = .017), NLR (8.06 vs. 5.11, p = .003), TB (2.57 vs. 1.71, p = .010) and INR (1.71 vs. 1.38, p = .039), and lower in MPV (8.81 vs. 9.29, p = .016). No statistically significant differences were present in Lymphocytes, PLT, CRP, ALT, AST, Potassium and Sodium.

4.3 Mortality data

Table 3: Descriptive statistics of the mortality data by the SBP group

	No SBP (N=119)	SBP (N=110)	Total (N=229)	*p* value
Length of stay (days)	7.41 (6.82)	9.18 (13.21)	8.27 (10.45)	0.202
30-days mortality (n = 19)	12 (10.1%)	7 (6.4%)	19 (8.3%)	0.308
30-days Survival days (n = 19)	13.42 (8.37)	10.29 (8.40)	12.26 (8.29)	0.443
Survival days (n = 119)	716.53 (851.63)	709.44 (824.93)	713.02 (834.95)	0.963

Notes: t-test for independent samples was used to test differences in continuous variables and Fisher exact test for categorical variables.

4.4 Predictive models for SBP

4.4.1 multivariate logistic regression

A multivariate logistic regression was first performed to predict SBP based on NLR, MPV, INR, TB and the demographic data age and gender (Table 4).

Table 4: logistic regression model for predicting

Predictors	*Odds Ratios*	*95%CI*	*p*
(Intercept)	8.76	5.15 – 19.22	**0.001**
NLR (ratio)	1.09	1.01 – 2.10	**0.013**
MPV (fL)	0.74	0.63 – 0.82	**0.011**
INR (ratio)	1.30	1.14 – 1.50	**0.009**
TB (mg/dl)	1.15	0.95 – 1.96	0.077
Age	0.98	0.90 – 1.87	0.229
Gender [Male]	1.94	0.98 – 5.55	0.055

Neutrophil to lymphocyte ratio (NLR), Mean Platelet Volume (MPV), International Normalized Ratio (INR), Total Bilirubin (TB).

As presented in table 4, the variables NLR (OR = 1.09, 95%CI: 1.01 – 2.10), MPV (OR = 0.74, 95%CI: 0.63 – 0.82), and INR (OR = 1.30, 95%CI: 1.14 – 1.50) were statistically significantly associated with SBP. The global model was significant with R2Tjur = 0.244.

Table 5 presents a summary of the algorithm performances. It indicates that the random forest model exhibited superior performance compared to both the decision tree model and the standard logistic regression when tested on new data. Specifically, the random forest algorithm outperformed the other models across both the experimental and validation groups, The enhanced efficacy of the random forest algorithm can be attributed to its nature as an ensemble of decision trees. By amalgamating multiple trees, this model excels in capturing intricate data patterns and mitigating overfitting, hence showcasing its ability to generalize well to unseen data.

Table 5: Summary of algorithms performance

	Accuracy [95%CI]	Sensitivity	Specificity	NPV	PPV	P [Acc>NIR]	AUC
Testing data							
Logistic regression	67% [52%-81%]	55%	82%	66%	69%	**0.009**	0.67
Decision tree	86% [71%-97%]	77%	84%	72%	80%	**<0.001**	0.79
Random forest	89% [78%-99%]	85%	95%	79%	82%	**<0.001**	0.83
Training data							
Logistic regression	74% [65%-83%]	65%	85%	74%	75%	**0.003**	0.84
Decision tree	88% [83%-95%]	82%	92%	81%	85%	**<0 .001**	0.92
Random forest	100% [97%-100%]	100%	100%	100%	100%	**<0.001**	1.00

Neutrophil to lymphocyte ratio (NLR), Mean Platelet Volume (MPV), International Normalized Ratio (INR), Total Bilirubin (TB).

4.4.2 Scoring system

The scoring system was created by simulating the laboratory results data set to achieve the maximum accuracy in predicting SBP based on three different laboratory indices, where one point is assigned to each value that is above a predefined result cutoff according to the ROC curves of each index: Cutoff TB ≥ 2.375 mg/dl, NLR ≥ 3.438 and CRP ≥ 30 mg/dl.

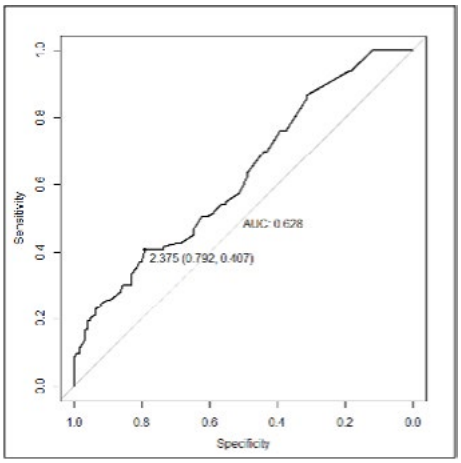


Figure 2A: ROC curve for Total Bilirubin (TB)

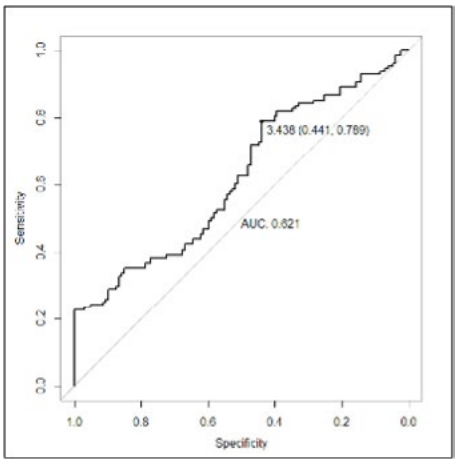


Figure 2B: ROC curve for Neutrophil to lymphocyte ratio (NLR)

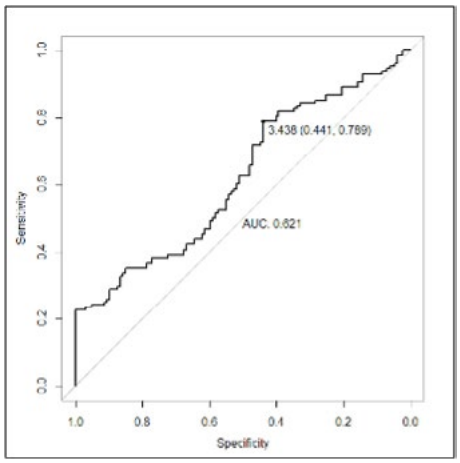


Figure 2C: ROC curve for C-Reactive Protein (CRP)

The ROC curve for the specificity and sensitivity of TB showed that at a score cutoff of TB ≥ 2.375 mg/dl, had a specificity of 79.2% and a sensitivity of 40.7% for predicting SBP (AUC = 0.628; P<0.001). The ROC curve for the specificity and sensitivity of NLR at a Cutoff result of NLR ≥ 3.438, had a specificity of 44.1% and a sensitivity of 78.9% for predicting SBP (AUC = 0.621; P<0.001). The ROC curve for the specificity and sensitivity of CRP showed that at a score cutoff of CRP ≥ 30 mg/dl, had a specificity of 89.3% and a sensitivity of 62% for predicting SBP (AUC = 0.714; P<0.001).

Table 7 presents the distribution of total scores among the study population, ranging from 0 to 3. It illustrates that: 61 patients obtained a score of 0, among whom 59 were negative for SBP, and 2 were positive. This signifies a 97% Negative Predictive Value (NPV) and a 3% Positive Predictive Value (PPV).
70 patients received a score of 1, with 38 testing negative for SBP and 32 testing positive. This shows a 54% NPV and a 46% PPV. 76 patients achieved a score of 2, where 21 were negative for SBP and 55 were positive. This results in a 28% NPV and a 72% PPV. 22 patients obtained a score of 3, among whom 1 was negative for SBP and 21 were positive. This demonstrates a 4% NPV and an impressive 96% PPV.

Table 6: Scoring system for predicting SBP

Lab Variable ≥ cutoff	Scoring points	Else
TB ≥ 2.375	1	0
NLR ≥ 3.438	1	0
CRP ≥ 30	1	0

Table 7: Summary of scoring system performance for the study population

Lab Variable sum of cutoff	No SBP (N=119)	SBP (N=110)	Total (N=229)	NPV	PPV
0	59	2	61	**97%**	3%
1	38	32	70	54%	46%
2	21	55	76	28%	**72%**
3	1	21	22	4%	**96%**

Table 8: The effect of the total score on the risk of having SBP

	SBP		
Predictors	**Odds Ratios**	**CI**	**p**
(Intercept)	0.05	0.01 – 0.17	**<0.001**
sum cat [1]	15.48	4.40 – 98.47	**<0.001**
sum cat [2]	39.81	10.80 – 259.54	**<0.001**
sum cat [3]	399.00	50.63 – 9583.99	**<0.001**
R2 Tjur		0.265	

The results of the scoring model show that the total score has a statistically significant effect on the risk of having SBP ($P < 0.001$). Patients with a score of 1 are 15.48 times more likely to have SPB than patients with a score of 0. Patients with a score of 2 are 39.81 times more likely to have SPB than patients with a score of 0. Patients with a score of 3 are 399 times more likely to have SPB than patients with a score of 0.

5. Discussion

Early detection of Spontaneous Bacterial Peritonitis (SBP) in cirrhotic patients with ascites is vital for effective treatment. Routine lab tests—Neutrophil-to-Lymphocyte Ratio (NLR) (16), Mean Platelet Volume (MPV) (3), C-Reactive Protein (CRP) (9), Total Bilirubin (TB), and International Normalized Ratio (INR) (24)—proved promising in predicting SBP.

NLR emerged as a strong predictor, aligning with prior studies that linked higher NLR values with SBP presence (8,9). In our study, an NLR cutoff of ≥ 3.438 showed significance in detecting SBP (sensitivity: 78.9%, specificity: 44.1%) (8).

MPV, contrary to conventional literature, revealed a significant decrease in SBP patients, aligning with previous studies. (3,16) Similarly, elevated CRP and INR levels were associated with SBP, echoing previous findings of their diagnostic relevance (9,24). TB, less studied in this context, showed potential as a predictor, with a cutoff of ≥ 2.375 mg/dl indicating SBP presence (sensitivity: 40.7%, specificity: 79.2%).

The developed random forest model, leveraging these markers, displayed a high predictive accuracy (sensitivity: 85%, specificity: 95%) in detecting SBP without invasive procedures (29). A scoring system akin to previous models demonstrated effective risk stratification for SBP, correlating scores with SBP probability (30).

The retrospective nature of our single-center study poses limitations in sample size and real-time data. The scoring system's limitations in diagnosing SBP with a single point warrant further refinement, Integration of the random forest model into clinical tools could aid in SBP diagnosis.

To validate findings, prospective studies with larger cohorts are crucial, Implementing the models in diverse medical centers globally could enhance SBP diagnosis and treatment. Recommendations include treating patients scoring 2-3 on the system and considering a prospective study for real-time validation.

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Pharmacological Considerations in the Interpretation of Biochemical Results in Diabetic Patients with Cardiovascular Complications

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Keywords
Diabetes mellitus; cardiovascular complication; pharmacological consideration

Abstract

Diabetes mellitus with cardiovascular diseases is often a multi-systemic disease that requires a multi-therapeutic approach which mostly poses a challenge to laboratory result interpretation. The non-availability of information on many patients due to poor referral, documentation and record keeping has resulted in isolated interpretation of laboratory result of diabetic patients with multisystemic complications. This has led to both analytical and post-analytical errors which has a negative impact on total quality of results. Therefore, this review showed the possible therapeutic treatment of a diabetic patient with cardiovascular disease and how their pharmacological role could affect laboratory result.

Introduction:

There is a high global prevalence of diabetes mellitus (DM) which increases the likelihood of patients presenting with the disease as a comorbid condition alongside other disease entities, and/or its associated cardiovascular complications. These scenarios may necessitate co-management by different physicians frequently resulting in the use of multiple drugs with little recourse to the implications of these sometimes-unplanned drug combinations on the body's metabolic balance, the resultant biochemical result findings, and their interpretation. Interpretation of these laboratory results without foreknowledge of all the medications the patient had been exposed to is a common occurrence especially in high-pressured healthcare systems due to a tight work schedule amongst other possible reasons. Little attention is usually given to comprehensive information on therapeutics before laboratory testing and biochemical result interpretation. This has contributed significantly to post-analytical error, thereby reducing the overall quality of the biochemical test result. There is a need for more attention to be given to the post-analytical testing phase of the laboratory as is with the pre-analytical and analytical phases because the appropriate interpretation of precise and accurate laboratory data will, to a significant extent, determine the clinical utility of laboratory test results in general, and particularly in diabetes mellitus patients on multiple medications. Thus, if the overall clinical utility of the laboratory test result is to be considered,

the total testing process must be the focus, not just the analytical phase considering its significance.(1) There are 422 million diabetes mellitus (DM) patients worldwide with middle and low-income countries contributing higher to the global prevalence. Over the past few decades, the prevalence of DM has risen significantly in nearly all countries and may be considered a growing epidemic.(2) It is predicted that Africa may have the world's largest surge in non-communicable disease (NCD) deaths over the next decade due to the epidemiological transition of disease.(3) In Nigeria, NCDs account for an annual death of about 36 million with cardiovascular diseases having the highest contribution of about 17.3 million deaths while diabetes mellitus is the commonest cause of cardiovascular diseases.(4) This poses a huge challenge to the health sector including the clinical laboratory. Diabetes mellitus is a multi-systemic disease that requires a multidisciplinary management approach which mostly leads to multiple drug therapy and adversely affects laboratory test results. Therefore, this review emphasizes on the importance of the foreknowledge of the therapeutic profile of diabetes mellitus patients in the total laboratory testing process to ensure the provision of clinically useful laboratory results to patients and their clinicians, thus enhancing the quality of life of diabetes mellitus patients, improving the practice of clinicians, and reducing the overall health cost.

Diabetic Complication and Therapeutics:

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in either insulin secretion, insulin action, or both.(5) Diabetic mellitus patients are prone to acute and chronic complications. Many years of inadequately controlled hyperglycaemia lead to multifarious, primary vascular complications that affect small vessels (microvascular), large vessels (macrovascular), or both. The cardiovascular complication commonly associated with diabetes mellitus is associated with various mechanisms which include glycosylation of intracellular and extracellular proteins and lipid in a maillard reaction leading to the formation of advanced glycation end products, increased superoxide production, activation of Protein kinase C, a signalling molecule that increases vascular permeability and causes endothelial dysfunction, accelerated hexosamine biosynthetic and polyol pathways leading to accumulation of sorbitol within tissues. Moreso, associated hypertension and dyslipidaemias, as well as arterial micro- thromboses, proinflammatory and prothrombotic impact impair vascular autoregulation.(6)

The acute complications of DM include diabetic ketoacidosis, hyperosmolar hyperglycaemic non-ketotic coma, and hypoglycaemia which are caused by an acute increase or decrease of glucose in the bloodstream. On the other hand, the chronic complications may lead to cardiovascular diseases via vascular damage due to chronic hyperglycaemia resulting to microvascular and macrovascular complications. Chronic microvascular complications of diabetes comprise of microvascular diseases which include retinopathy, neuropathy nephropathy, encephalopathy, erectile dysfunction, cardiomyopathy and periodontal disease. Macrovascular complications include diabetic myonecrosis, cardiovascular accident, carotid artery stenosis, diabetic foot ulcer, coronary artery disease and female infertility. Additionally, hyperglycaemia reduces the immune cells function and increases inflammation, predisposing the afflicted persons to respiratory infections such as pneumonia and influenza, wound infections, restrictive lung disease, lipohypertrophy and depression. The implication is that most diabetics are frequently on multiple drug therapy that could lead to mis-interpretation of laboratory results if their drug history is not taken into cognisance [Table 1].

Table 1: Most Frequent Laboratory Changes Upon Cardiovascular Drug Treatment

Dysglycemia due to coadministration of antibiotics with hypoglycaemics
Erroneous eGFR due to positive or negative interference of drugs in serum creatinine estimation which may be in vitro or in vivo
Thiazide induced hyperlipidaemia
Changes in serum calcium due to effect of diuretics-loop diuretic is associated with hypercalcemia on the contrary thiazide diuretic is linked to hypocalcaemia
Electrolyte changes associated with diuretics
Dysglyceamia due to effect of statins, thiazides and beta blockers
Drug-induced azotaemia
Hypervolemic hyponatremia in elderly with a state of endogenous or exogenous active ADH secretion such as chronic renal failure, excessive water intake, low salt intake, heart failure, concurrent analgesic use, excessive fluid intake
Hyperuricemia in diuretic therapy
Hypoglycaemia due to phosphodiesterase-5-inhibitor

Impact of Co-Medications on Laboratory Result Interpretation of Diabetes Mellitus Patients:

Anti-hypertensive Medications:

The frequency of hypertension (HTN) among those with diabetes is almost twice that of non-diabetics.(7) This suggests either a common genetic or environmental factor in the pathogenesis or secondary complications of the diseases. Genetic variants in the gene encoding, adrenomedullin, angiotensinogen, apolipoprotein, and α-adducin have been reported to be associated with common conditions such as diabetes, hypertension, dysglycaemia, and/or metabolic syndrome [8]. In genome scans of a Chinese population, the region associated with diabetes was also associated with metabolic syndrome, which is a cluster of diseases including hypertension [9]. Moreover, prolonged hyperglycaemia leads to atherosclerosis which can cause the stiffening of arteries, increasing the peripheral resistance in the vessels and hypertension [10]. Hence, most diabetic patients are also on anti-hypertensive medications and antidiabetic medications concomitantly. Therefore, knowledge of the metabolic impact of anti-hypertensives will be helpful in the interpretation of biochemical profiles and management of diabetes mellitus [Table 2].

Table 2: Anti-Hypertensives-Induced Laboratory Changes in Diabetic Patients with Cardiovascular Complications

DRUGS	ASSOCIATED LABORATORY CHANGES
ACE Inhibitors	Azotaemia [11,12], Hyperbilirubinemia [41], elevates liver enzymes [41], reduces proteinuria [43]
Diuretics	
a. Thiazide	Dyslipidaemia [20-22], hypokalaemia [14], hyponatremia [14], hypomagnesemia [23], hypercalcemia[18],hyperglyceamia[24,25],hyperuricemia[27]
b. Loop	Hyponatremia[13],hypocalcemia[26],hypomagnesemia[23],hyperuricemia[27]
c. Potassium sparing	Hyperkalemia[27], metabolic acidosis[27]
Beta blockers	Dyslipidemia[28,29] ,hyperglycemia[34]
Centrally acting alpha 2 Adrenergic Agonist-methyldopa	Hyperbilirubinemia[37],elevates liver enzymes[37], hypernatremia[37], hyperchloremia[37] hypermagnesemia[37], hyperkalemia[37]

Angiotensin-converting Enzyme Inhibitors (ACEIs) and Angiotensin II Receptor Blockers (ARBs):

In the Action in Diabetes and Vascular Disease: Preterax(perindopril/indapamide) and Diamicron(gliclazide) Modified Release Controlled Evaluation (ADVANCE trial), sudden elevation in serum creatinine after starting perindopril-indapamide were associated with greater risks of subsequent major clinical outcomes. However, the continuation of angiotensin-converting enzyme inhibitor-based therapy reduced the long-term risk of major clinical outcomes, despite acute rise in creatinine[11].Increase in serum creatinine following the use of ACEI is largely attributed to a renin-angiotensin-aldosterone system (RAAS) inhibition-induced intraglomerular pressure decline (i.e.hypofiltration)[12].Discontinuation of ACEI is recommended if patients experience a ≥ 30% acute rise in serum creatinine after the commencement of treatment.Though, the long-term effects of its continuation or discontinuation on major clinical outcomes after such rise in serum creatinine is still unclear[11].

Diuretics:

Therapy with a loop or thiazide diuretics may lead to hypovolaemia, hyponatraemia, hypokalaemia, hypomagnesaemia, hyperuricaemia, hyperglycaemia, metabolic alkalosis and azotaemia[13].Thiazides also alters lipid profile causing an increase in serum total cholesterol, HDL-cholesterol, triglycerides, and bilirubin. Hypokalaemia is a sequela of the aldosterone-mediated actions of the Na/K pump in the collecting tubule of the nephron [14]. Thiazides doubtlessly have more potential to cause hyponatraemia than loop diuretics. Loop diuretics inhibit sodium (Na^+) transport in the renal medulla leading to prevention of the creation of a maximal osmotic gradient. Hence, loop diuretics impairs the renal concentrating ability [14]. On the other hand, the mechanism of action of thiazide diuretics is to decrease sodium reabsorption and therefore decrease fluid reabsorption. This directly causes decreased levels of circulating sodium. If hyponatraemia were to occur, it would happen during the first 2 to 3 weeks of therapy. After which, the patient is in a new steady state in which further sodium and water losses do not occur [14][15].Hypokalaemic metabolic alkalosis occurs due to the increase in aldosterone-mediated potassium and hydrogen ions excretion in the intercalated cells of the collecting tubules[16] [17]. Thiazide -induced hypercalcemia seems to result from increasing calcium reabsorption from the luminal membrane into the interstitium in exchange for sodium. Thiazides also reduce urine calcium levels

and increase blood calcium[18].Thiazide-induced hypokalaemia at the level of the pancreatic beta cells, causes hyperpolarization of the beta cell and decreases insulin secretion, leading to hyperglycaemia[19]. Thiazides act directly on the the organic anion transporter(OAT) 1 on the basolateral membrane of the proximal convoluted tubule to increase urate reabsorption and the OAT 4 on the luminal membrane of PCT where they are exchanged for urate.

The mechanism by which thiazides and thiazide-like diuretics induced hyperlipidaemia is largely unclear. However, thiazide diuretics appear to decrease catabolism while increasing synthesis [20]. Acute administration of high dose thiazide diuretics tend to increase sympathetic nervous activity and circulating noradrenaline that consequently promotes lipolysis, hepatic synthesis of cholesterol and apolipoproteins except Apoproteins A1 and A2 which are important component of HDL-C [20][21] [22]. Thiazide and loop diuretics increase urinary Mg^{2+} excretion which often coexists with hyponatraemia and hypokalaemia [23]. A combination of amiloride with hydrochlorothiazide, however, prevents glucose intolerance more than hydrochlorothiazide only[24][25].Loop diuretics induce natriuresis by inhibiting the Na-K-2Cl transporter in the thick ascending limb of the loop of Henle, causing increased urine calcium wasting and leading to hypocalcaemia with increased parathyroid hormone (PTH) level [26].On the other hand, hydrochlorothiazide and amiloride diminish calcium excretion by increasing calcium reabsorption from the luminal membrane into the interstitium in exchange for sodium at the NaCl transporter in the distal convoluted tubule leading to hypercalcaemia [26]. The potassium-sparing diuretics (amiloride, triamterene, mineralocorticoid receptor antagonists) can induce hyperkalaemia and metabolic acidosis and may also increase serum uric acid concentrations due to increased reabsorption in the proximal tubule [27]. Carbonic anhydrase inhibitors such as acetazolamide, ethoxazolamide, dorzolamide, brinzolamide can cause hypokalaemia and hyperchloremic metabolic acidosis by reversible inhibition of the carbonic anhydrase enzyme that causes decreased hydrogen ion secretion at the renal tubule and increases renal excretion of bicarbonate, potassium, sodium and water. Therefore, diabetes mellitus patients on diuretics should be closely monitored and their biochemical results should be interpreted on the background of their drug therapy.

Beta Blockers:

Monotherapy with beta adrenergic blockers such as atenolol and carvedilol for a period of one month to about three years could lead to increased LDL-C, urea, triglycerides, uric acid, potassium, glucose, and reduces HDL-C [28][29].Beta-blockers have been observed to reduce HDL-C and increase triglycerides. The inhibition of lipoprotein lipase has been implicated in several studies but the exact mechanism is not well understood, however, increase in alpha-adrenergic tone due to β-blockade result in the inhibition of lipoprotein lipase and decreased catabolism of triglycerides [30]. Consequently, circulating serum HDL decreases, while triglycerides increases. On the contrary, some β-blockers such as pindolol increase the activity of lecithin-cholesterol transferase leading to increase in HDL[31] [32]. Azotemia may set in when ACEIs or ARBs cause efferent arteriolar dilatation, thereby decreasing intraglomerular pressure and filtration[33]. Also, beta-blockers can potentially increase blood glucose concentrations and antagonize the action of oral hypoglycaemic drugs[34]. Therefore, a proper history of anti-hypertensive drug use is vital in the laboratory assessment of diabetes mellitus patients, especially at the pre-analytical testing phase. As such, drugs like thiazides and beta-blockers may be stopped three days before the laboratory test depending on the aim of the test.

Centrally-acting Alpha-2 Adrenergic Agonist:

Methyldopa causes a reduction in blood pressure via inhibition of adrenergic neuronal outflow leading to reduced total peripheral resistance and reduced blood pressure[35]. An attenuation of adrenergic neuronal outflow leads to a decrease in circulating norepinephrine levels which may lead to decreased insulin resistance. Thus, such drugs are thought to be beneficial in ameliorating glucose levels in pregnant women with gestational diabetes mellitus(GDM)[36]. However, it has been reported to increase ALP, bilirubin, urea, electrolytes (sodium, chloride, potassium and magnesium) as well as prolactin[37]. Moderate hyperprolactinemia caused by α- Methyldopa, is possibly caused by inhibition of the enzyme aromatic-l-amino-acid decarboxylase, which is responsible for converting L-dopa to dopamine, and by acting as a pseudo neurotransmitter to decrease dopamine synthesis. Though, Methyldopa is considered as the drug of choice in the treatment of non-complicated pregnancy-induced hypertension. However, methyldopa may cause hepatic necrosis through immunological reaction and there is some evidence to support the immunological mechanism. About 5% of nonpregnant women receiving methyldopa have been to have mild hepatitis [38]. Methyldopa may also cause increase in electrolytes by inducing dehydration. This may lead to hypovolemic-induced dyselectrolytemia.

ACE Inhibitors:

ACE inhibitors increase liver enzymes (AST, ALT, ALP), bilirubin, potassium, and Azotaemia (urea, creatinine), and reduce proteinuria. Though the ACE inhibitors are rare causes of clinically apparent liver injury[39]. They are considered first-line drugs for the treatment of hypertension and are considered particularly helpful in renoprotection of diabetes and hypertension[40]. ACE inhibitors have also been associated with acute liver injury that is usually cholestatic and self-limiting. The onset is typically within 1 to 8 weeks of starting the medication. Lisinopril is reported to be the most common cause of liver injury among the ACE inhibitors in clinical practice[41]. This may be misunderstood for a complication of metabolic syndrome such as non-alcoholic liver diseases. Thus, if this class of anti-hypertensive agents is indicated, close monitoring after commencement would be required.

Azotaemia emerges in ACE inhibitors or ARBs induced- efferent arteriolar dilatation, thereby reducing intraglomerular pressure and filtration[42]. Nevertheless, treatment with ACE inhibitors results in renal protection due to a reduction of systemic blood pressure, intraglomerular pressure, an antiproliferative effect, reduction of proteinuria and a lipid-lowering effect in proteinuric patients (secondary due to reduction of protein excretion)[43]. The rise in serum creatinine values usually begins a few days after initiation of ACE inhibitor or an ARB therapy, as it occurs due to reduction or blockade of angiotensin II levels. This results in efferent arteriolar dilatation and decreased effective GFR[44]. Therefore, a review of drug history may prevent a false assumption of new onset of renal insufficiency or worsening renal dysfunction in patients that had azotaemia at the commencement of ACEI regimen. Thus, discontinuation of ACEI may not be necessary unless serum creatinine level rise above 30% over baseline during the first 2 months after initiation of therapy or significant hyperkalemia of ≥5.6 mmol/L develops[45].

2. Anti-dyslipidemic Drugs:

Dyslipidemia is a very common finding in diabetes mellitus patient. It is estimated that 30-60% of patients with T2DM have dyslipidaemia[46]. Hyperglycaemia and postprandial hypertriglyceridemia, low HDL-cholesterol, elevated LDL-cholesterol and the predominance of small-dense LDL-cholesterol particles are typical characteristics of diabetic dyslipidaemia. These lipid profile changes underlie the pathologic link between diabetes mellitus and the increased risk of cardiovascular diseases[47]. To prevent cardiovascular complications most diabetes mellitus patients are placed on statins. Statins alter blood levels of glycated haemoglobin, glucose, AST, ALT, ALP, GGT, bilirubin, and creatinine phosphokinase. Some studies have suggested that statins may cause hyperglycaemia by increasing calcium concentration in the islet cells leading to a decrease in insulin release, or by decreasing GLUT 4-mediated peripheral glucose uptake[48]. Others have proposed that it increases insulin resistance and its secretion after 10 weeks of high-dose therapy[49]. Statin intake causes hyperglycemia. Glycated haemoglobin, a biomarker of long-term glycaemic control and fasting blood glucose are all influenced by statin intake. Although, statin therapy may decrease the risk of atherosclerotic cardiovascular disease but increases the risk of type 2 diabetes[49] [Table 3].

3. Antibiotics:

Hyperglycaemia with an associated immune compromise predisposes diabetics to infections and frequent use of antibiotics. Hyperglycaemia-induced neutrophil dysfunction which alters neutrophilic chemotaxis, phagocytosis and intracellular killing of the bacteria[50]. Also reduction in the quantity and function of lymphocytes in diabetics have been implicated as the major cause of immune compromise. Antimicrobials such as penicillins, aminoglycosides, macrolides, fluoroquinolones, amphotericin B and foscarnet may cause renal loss of potassium leading to hypokalaemia[51][52][53][Table 3].These antimicrobials are said to enhance delivery of sodium to the distal tubule where the reabsorption of sodium is done in exchange for potassium leading to wasting of potassium and hydrogen ions, eventually leading to hypokalaemia and concomitant metabolic alkalosis[54].

Electrolyte and acid–base imbalance have been attributed to aminoglycosides [55]. These are broad spectrum antibiotics commonly used for the treatment of aerobic gram-negative bacteria. They are often used in the treatment of severe infections of the abdomen and urinary tract, bacteraemia and endocarditis amongst others. Aminoglycosides have been linked with reversible tubular dysfunction without an associated change in glomerular filtration rate.and therefore, urine osmolality should be a preferred biochemical test in suspected aminoglycosides dysfunction than glomerular filtration rate determination because it is a potential cause of nephrogenic diabetes insipidus[52].

Normal therapeutic dosages of aminoglycosides have been reported to cause hypomagnesaemia in more than one-third of patients[56]. Hypomagnesaemia occurs early in therapy, as a result of renal magnesium wasting and may produce hypocalcaemia and hypokalaemia [57]. This Gitelman syndrome-like disorder has been associated with aminoglycoside use in many patients. Hypomagnesaemia is underscored by potentially severe symptoms such as neuromuscular and cardiac symptoms and it is also associated with symptoms and signs of metabolic abnormalities like hypocalcaemia, hypophosphatemia, and hypokalaemia[58]. Antibiotics–induced hypomagnesaemia can also occur during therapy with colistin and amphotericin B[51]. Antibiotics such as ciprofloxacin and trimethoprim-sulphamethoxazole interfere with creatinine result. While trimethoprim inhibits creatinine secretion and increases the serum creatinine concentration without affecting GFR, ciprofloxacin interferes with routine creatinine assay by reacting with picric acid giving a false positive result in vitro. Macrolides, metronidazole, penicillins, cephalosporins and tetracyclines(doxycycline) and fluoroquinolones (Ciprofloxacin) cause self-resolving transient increases in liver enzymes such as ALT, AST, LDH, Alkaline Phosphatase (ALP) and bilirubin, as well alters glucose results[59]. Hepatocellular injury or cholestasis can lead to elevation of serum enzyme ;. cases with short onset usually have more marked elevation of ALT levels, with occasional rapid worsening prolonged prothrombin time and early signs of hepatic failure. The mechanism of ciprofloxacin hepatotoxicity is suspected to be due to hypersensitivity which may present as hepatic complication of metabolic syndrome [60]. This mimics the hepatic complication of metabolic syndrome in T2DM. Moxifloxacin was found to be associated the most and ciprofloxacin the least with dysglycaemia. Therefore, caution should be taken in the interpretation of the results of patients on fluoroquinolones because this can be mistaken for poor glycaemic control due to other causes.

Macrolides have been implicated in azotaemia in elderly patients as a patient-associated risk factors.,However,the acute kidney injury caused by azithromycin is reversible[61]. In addition, ceftriaxones, azithromycin and nitrofurantoin but not clarithromycin may cause an increase in both urea and creatinine[62]. Cephalosporins are known potential nephrotoxins at high doses. Cefditoren, Tigecycline, Ertapenem, and Clarithromycin are associated with hypoglycaemia while azithromycin and Nitrofurantoin causes an elevation in serum glucose[63][64]. Amoxicillin alone or amoxicillin-clavulanic acid and cefelexin causes false positive or negative urine glucose[65]. Many antibiotics have been implicated in dysglycaemia when coadministered with hypoglycaemic agents, Hence may be considered during sample collection for glucose, as well as in the interpretation of results viz a viz clinical decisions of these patients[Table 2].

Table 3: Other Drug-Related Laboratory Changes in Diabetic Patients with Cardiovascular Complications

DRUGS	**ASSOCIATED LABORATORY CHANGES**
Fluoroquinolones Trimethoprim,penicillins,macrolides	Positive interference with creatinine assay[59,62]
aminoglycosides	Hypomagnesemia [56], hypocalcemia[57,58], hypophosphateamia[57,58], hypokalemia[57,58]
Penicillins, minoglycosides, macrolides, fluoroquinolones, amphotericin B, Forscanet	Hypokalemia[51-53], metabolic alkalosis[54]
Macrolides, nitroimidazole, penicillins, cephalosporins and tetracyclines and fluoroquinolones	Elevated liver enzymes (ALT,AST,LDH,ALP)[60]and bilirubin[60], dysglyceamia[59,63,64]
Macrolides, ceftriazones, azithromycin and nitrofurantoin	Azotemia[61,62]
Cefditoren,tigecycline,ertapenem and clarithromycin	Hypoglyceamia[63,64]
Azithromycin,Nitrofurantoin	Hyperglyceamia[63,64]
Amoxicillin,amoxicillin-clavulinic acid,cefelexin	False negative or positive glucosuria[65]
Statins	Hyperglycemia,elevated liver enzymes, hyperbilirubinemia, increased creatinine kinase[48,49]
Anti-inflammatory drugs	Hypervolemic hyponatremia[69-71]
Lactulose	Hypernatremia[84]
Cisplastin	Hypomagnesemia[90,91], hypokalemia[90-92]
Cyclosporine	Hyperkalemia[85],hypocalcemia[85] and hypomagnesemia[85]
Calcineurin inhibitors-Tacrolimus,Cyclosporine	Impaired glucose tolerance[85] and dysglyceamia[85]
Vitamin D	Increases creatinine[86], increases eGFR[86]
Sildenafil	Hypoglyceamia[94]

4. Anti-inflammatory drugs:

Overweight/obesity frequently co-exist with osteoarthritis (OA) in Type 2 diabetes mellitus (T2DM). Nearly half (47.3%) of patients with T2DM have some form of arthritis[66]. Although, excess body weight causes mechanical effect on joints which may be an underlying pathophysiological explanation of osteoarthritis of the lower limb. Diabetes mellitus has been reported to play a direct role in the pathophysiology of osteoarthritis via two major pathways involving oxidative stress and insulin resistance resulting to pro-inflammatory cytokines and advanced glycation end products (AGEs) production in joint tissues. This causes a low-grade chronic metabolic inflammation that can lead to structural joint damage. Insulin is a critical negative modulator of synovial inflammation and catabolism, therefore insulin resistance in obese individuals would diminish the suppressive capacity of insulin to production of inflammatory and catabolic mediators that promote OA[67].

Unfortunately, despite the well-recognised adverse effects of Non-Steroidal Anti-inflammatory Drugs (NSAIDs), the frequency of their utilization among diabetic patients with chronic complications such as hypertension, heart failure and chronic kidney disease (CKD), remains high[68]. NSAIDs inhibit cyclooxygenase (COX) (also known as prostaglandin H synthase). They also enhance the actions of Anti-diuretic Hormone (ADH) due to prostaglandin inhibition[69]. In clinical practice, water intoxication and hyponatraemia only occur with the use of NSAIDs in a state of endogenous or exogenous active ADH secretion, such as in elderly or neonatal patients, chronic renal failure, low salt diet, excessive oral water intake or heart failure, or concurrent analgesic use[70]. These scenarios are typical in diabetes mellitus patients with chronic complications. Therefore, knowledge of medication intake during result interpretation in this patient is vital to the prevention of post-analytical errors that may be misleading to clinicians. NSAIDs have been implicated as one of the commonest causes of hyponatraemia in elderly patients with T2DM[71] [Table 2].

5. **Gastrointestinal Drugs:**

Insulin resistance and hyperglycaemia are two major factors which play a significant role in gastrointestinal complications of diabetes mellitus. These complications include gastroesophageal reflux diseases (heartburn, acid reflux regurgitation and angina-pectoris-like pain), gastroparesis (early satiety, bloating, postprandial fullness, nausea, vomiting, or upper abdominal pain), intestinal enteropathy (diarrhoea, constipation, and faecal incontinence), and Non-Alcoholic Fatty Liver Disease (NAFLD). About a hundred million, meticulously organized neurons in the enteric nervous system controls the gut motility via the myenteric nerve plexus, and the absorption and secretion through the submucosal network [72]. The interstitial cells of Cajal (ICC) act as pacemaker, enabling the conveyance of impulses to the smooth muscles [72] Diabetes mellitus can chronically disrupt the GI tract's enteric, autonomic, and somatic nervous systems [72]. In hyperglycaemia, excess glucose molecules are diverted to alternative metabolic pathways like polyol, hexosamine, etc [73]. These glucose molecules attach avidly to fats or proteins leading to the formation of Advanced Glycation End products (AGEs) [73]. Free radicals and oxidative molecules leads to abnormalities in the structure and function of nervous system [74]. The reduced number of ICC, and accompanying damage to the smooth muscle and central nervous system (CNS) cells, may inadvertently cause contractile malfunction of the gut [75]. Furthermore, prolonged hyperglycaemia produces inflammatory changes and osmotic stress with resultant damage to vasa nervorum, contributing further to diabetic neuropathy [76]. Therefore, parasympathetic neural dysfunction due to diabetes resulting in alteration of digestive, secretory, absorptive and motor functions of the gastrointestinal tract leads to myriad of complications. Increased Helicobacter pylori (H. pylori) proliferation in gastrointestinal tract of T2DM is a major predisposing factor to peptic ulcer which may lead to catastrophic consequences such as bleeding and perforation [77]. Diabetes mellitus makes a patient prone to infection and gastroparesis diabeticorum. The duo may lead to microbial overgrowth in the upper gastrointestinal tract of diabetic patients with resultant increase incidence of peptic ulcer diseases and its complications in them. Hence, a strong association between the prevalence of H. pylori colonization and diabetes mellitus has been reported [77]. Therefore, many diabetes mellitus patients with chronic complications may be on some gastrointestinal drugs due to prevalent symptoms of GERD and these may influence laboratory results. Many diabetes mellitus patients are on H2 receptor inhibitors such as Cimetidine, Famotidine and Ranitidine. These drugs are known to decrease the secretion of creatinine by inhibiting it's secretion at the proximal tubule causing a decrease in renal clearance [78]. Cimetidine increase the serum creatinine concentration without affecting the true GFR. Moreover, dyselectrolytemia has been increasingly being associated with Proton pump inhibitors therapies such as hypokalaemia, hyponatraemia, hypomagnesaemia, hypophosphataemia and hypocalcaemia most especially in long-term therapy[79]. The very commonly used creatinine assay, the Jaffe method, is subject to interference by bilirubin and metabolic syndrome-associated pathophysiologic states, such as NAFLD which is often linked to hyperbilirubinemia, may create a negative interference. Bilirubin interferes with negatively the Jaffe method, leading to the under quantitation of creatinine concentrations. The resultant biliverdin generation in alkaline solutions due to oxidation of bilirubin diminishes the absorbance of both the creatinine picrate complex and bilirubin at the absorbance peak of 510 nm and increases the absorbance of biliverdin at the absorbance peak of 620 nm. In spite of better specificity of the enzymatic method, bilirubin can also interfere negatively with it, especially creatinine amidohydrolase based assay (creatininase). This interference may be attributable to the competition between bilirubin and the assay substrate for the H2O2 produced during the reaction. The falsely decreased creatinine results can have significant implications for the clinical management of patients. Moreover, the estimation of

the glomerular filtration rate and the measurements of creatinine clearance are highly dependent on accurate creatinine values which may adversely affect clinical decisions [80]. The most commonly reported gastrointestinal symptoms among diabetes mellitus patients is constipation [81]. The underlying aetiology of constipation in diabetes is multifactorial. In view of increased predisposition to autonomic and enteric neuropathy due to chronic hyperglycemia, there is an increased risk of altered colonic motility underlying constipation in diabetes mellitus patients than in the general population. It should also be noted that anorectal disorders are more common among these patients and can also contribute to constipation [82]. Smooth muscle structure and function, the density of the interstitial cells of Cajal, the health and function of the autonomic and enteric nerves of the colon are all potentially affected. Consequently, leading to alterations in colon motility and microbiome as well as immune and endothelial functions [83]. Lactulose is a bulk laxative used in the treatment of constipation. Hypernatremia has been reported infrequently in portal-systemic encephalopathy treatment with lactulose[84]. Due to its osmotic cathartic effects, the drug may cause faecal water loss in excess of sodium resulting in contraction of ECF volume and hypernatremia. This is usually a neglected cause of hypernatremia as most hypernatremia in diabetes mellitus patients is usually attributed to excessive use of diuretic antihypertensive[Table 2].

6. Immunomodulatory drugs:

Immunosuppressive drugs like calcineurin inhibitors are the cornerstone of immunosuppression for renal transplantation. Tacrolimus and cyclosporine are commonly used for renal transplantation which is more often done in diabetes due to the increased prevalence of diabetic nephropathy and end-stage renal disease. Cyclosporine is known to cause a combination of metabolic side effects including hyperkalaemia, hypercalciuria and hypomagnesaemia, as well as are increased creatinine, uric acid and urea. The calcineurin inhibitors are both known to reduce insulin release leading to decreased glucose tolerance and dyslipidaemia [85] [Table 2]. Vitamin D replacement therapy may be needed in chronic renal failure to prevent or treat renal osteodystrophy. However, it should be noted that vitamin D replacement modifies the production and release of creatinine increasing its blood levels, and reducing estimated glomerular filtration rates. The mechanism is said to be via a short-term vitamin D receptor activation increasing creatinine generation and serum creatinine, but it does not influence the glomerular filtration rate. Therefore, it may negatively affect the chronic renal failure classification which is based on the estimated glomerular filtration rate which in turn may lead to misjudgement of the clinical state of the patient [86] [Table 2].

Other prescribed Drugs

Anti-neoplastic drugs: Many epidemiological studies have associated diabetes mellitus with cancer. The risk of cancer has been said to increase with diabetes. Conversely, some cancers and their therapies have been implicated as a major cause of Diabetes Mellitus. Many studies have provided substantial evidence of associations between T2DM and risks of cancer in the mouth, lungs, gastrointestinal system, kidneys, bladder, thyroid, breast, ovaries, endometrium,white blood cells, glioma, and melanoma [87]. Gastrointestinal cancers such as cancer of the colon, pancreas and liver have been noted to have the highest risk of association with diabetes mellitus [88]. Therefore, some diabetic patients may be on anti-cancer therapy due to diabetes-associated cancer. Some anti-cancer drugs affect laboratory parameters such as electrolytes, creatinine, liver function test, etc. Chemotherapeutic drugs such as olaparib increase the secretion of creatinine in about 37% of individuals [89]. This may be mistaken for diabetic nephropathy if proper drug history is not taken. Cisplatin is commonly known to cause electrolyte imbalance. The electrolyte abnormality caused by cisplatin is mostly linked to hypomagnesaemia due to renal magnesium wasting. Others include hypernatremia, hypokalaemia, hypocalcaemia and hypophosphatemia. [90]. Cisplatin-induced hypomagnesaemia is mainly related to impaired magnesium reabsorption in the proximal tubule. However, cisplatin was depicted to down regulate the TRPM6/EGF pathway resulting in magnesium loss [91]. It causes an impairment of the calcium-sensing receptor leading to hypomagnesaemia. Subsequently, hypomagnesaemia causes an impaired release of parathyroid hormone which then leads to hypocalcaemia. Similarly, renal potassium loss occurs due to hypomagnesaemia resulting in hypokalaemia. Depletion of intracellular magnesium reverses the inactivation of voltage-dependent renal outer medulla potassium channels (ROMK), thus increasing kaliuresis. Kaliuresis is equally exacerbated by increased distal Na delivery or hyperaldosteronemia[92]. Potassium supplementation may fail to correct such hypokalaemia until hypomagnesaemia is corrected. Thus patients receiving platinum drugs can also develop persistent distal tubular dysfunction with a Gitelman-like syndrome characterized by hypocalciuria, hypomagnesaemia and hypokalaemic metabolic alkalosis. Cisplatin induces a syndrome of inappropriate secretion of ADH leading to hyponatraemia. It can decrease 1-α-hydroxylation activity resulting in reduced vitamin D3 levels, hypocalcaemia and a concomitant hypophosphatemia [93]. Drugs for Erectile dysfunction: Diabetics are prone to urinary tract infections (UTIs), cancer, bladder disorders and sexual dysfunction. Erectile dysfunction, is common in men who have diabetes, especially those with T2DM [94]. It is estimated that about 59.39% of men with diabetes have erectile dysfunction [94]. It may be a result of damage to nerves and blood vessels caused by poor long-term blood glucose control. Many diabetes mellitus patients are self-medicating or on sildenafil prescription, especially in developing countries where many myths are associated with impotence. Hypoglycemia has been observed following administration of sildenafil in some patients. This may also give an erroneous picture of good glycaemic control due to the transient effect of the drug. Therefore, a good drug history is very important in the interpretation of glucose results to ensure that the true state of the patient is reflected [Table 2].

Recommendations

Cardiovascular complications in diabetes mellitus patients predispose them to multiple drug use, and the drug history in this patient is paramount in the preparation of the patients for laboratory testing to ensure that an accurate, reliable and timely result is available for the management of patients. In order to achieve this the following should be done:

1. Provision and utilization of an effective Laboratory/ Hospital information system to ensure accessibility to the drug history of the patients.
2. Relevant clinical history including the patients' current drug history should accompany laboratory request forms in places where data collection and easy accessibility is still a challenge.
3. Referral systems should be established and enforced to keep track of patients' information as they move from one health facility to another.
4. Provision of affordable health insurance to improve funding of health care facilities to enable them to provide alternative drugs whenever it becomes necessary in the management of these patients.

Conclusion

Good knowledge of the drug history of diabetic patients will ensure that an accurate, reliable and timely result which is fit for purpose is produced from the laboratory for improved management outcomes

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From Bench to Bytes: Utility of social media platforms by Pathology Residents

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Keywords

Social media,Pathology residents, Impact

Abstract

Background: The rise of virtual media has expanded to various fields and the medical profession has not been immune to its influence. The purpose of our study was to analyse and evaluate the impact of virtual social networks, on the professional growth and career progression of Pathology residents.

Materials and Methods: A cross-sectional survey was undertaken at the section of Chemical Pathology, Department of Pathology and Laboratory Medicine, Aga Khan University, Karachi. An online, questionnaire based on google forms was sent via WhatsApp application to the Pathology residents (n = 30) from July to August 2023. The anonymity of the participants was maintained. The statistical analysis was performed using Microsoft Excel 2019.

Results: A total of 25 (83%) Pathology residents participated in the survey. 72% of the participants were females and 28% were males. Chemical Pathology residents constituted the largest proportion at 44%, followed by 24% from Hematology, 24% from Microbiology and 8% from Histopathology. 60% of the trainees acknowledged using social media platforms routinely for professional development. The most used social media platforms were YouTube (48%), Twitter (36%), Facebook (12%) and Instagram (4%). Social media network was perceived the most useful in increasing understanding regarding the profession (60%), strengthening the proficiency in problem-solving (56%) and enhancing critical thinking abilities (52%) whereas it was regarded as somewhat useful in enhancing clinical/professional decision-making skills (52%) and clinical expertise (60%).

Conclusion: Social media is gaining popularity in the realm of medicine and this survey reveals the perspective of Pathology residents on the social media networks and its growing impact on their professional growth.

1. INTRODUCTION:

Social media is the new monarch of the 21st century. From the mere supplementary communication tool, it has transitioned to become the core of modern-day lifestyles. Across the world, social media has facilitated communication, collaboration, networking, and the generation of novel ideas among medical students, physicians, and laboratory professionals (1). Pathologists, the usually behind-the-scenes physicians, are swiftly adapting social media and gaining significant visibility as active users on these platforms (2). This is in part due to the impact of COVID-19 pandemic that prompted numerous pathology departments and residency programs to increase their presence on social media platforms and establish their own social media pages (1).

The rising adoption of social media by the current generation of postgraduate trainees comes with its own set of benefits and drawbacks (3). Nonetheless, when employed effectively, social media can be harnessed to aid in professional growth of pathology residents and the advancements in their career. A plethora of online tools are available to pathologists, encompassing various social media platforms like Facebook, Twitter, and Instagram. These platforms offer diverse educational content, including virtual posts, pictures, newly published articles, and YouTube videos that offer free access to lectures and tutorials (4). Hence, these online platforms allow pathologists to effortlessly apply acquired knowledge to practical clinical situations (4).

The reason why these online social platforms can influence professional development lies in their ability of unparalleled networking – a communication circuitry so rapid, economic and user-friendly that connections can be built, and relationships can be maintained all across the globe in the fraction of time that was possible before. These connections are seemingly important, particularly for residents, to accelerate their professional growth, develop research and scientific collaborations at national levels and share interesting and novel laboratory report findings with their peers and seniors that can broaden their interpretative vision. Furthermore, free online access to the relevant course materials regardless of their geographical location gives equal opportunities to trainee pathologists to thrive professionally.

The possible fusion of digital pathology and artificial intelligence holds the promise of revolutionizing the practices of diagnosticians and could soon mark the third evolution in the field of pathology (5). The disseminated presence of these social media platforms could be the beginning of this revolution, advocating the need for a new generation of pathologists who can effectively handle and perpetuate this novel mode of information.

The aim of this study was to analyze and evaluate the impact of virtual social networks on the professional growth and career progression of Pathology residents at a tertiary care hospital in a developing country.

2. MATERIALS AND METHODS:

A cross-sectional survey was undertaken at the section of Chemical Pathology, Department of Pathology and Laboratory Medicine, Aga Khan University, Karachi. An online questionnaire based on google forms was sent via WhatsApp application to residents (n = 30) of Department of Pathology and Laboratory Medicine at Aga Khan University Hospital, Karachi and peer Chemical Pathology residents working at other center in Pakistan to elicit information regarding the choice of social media platforms, their utilization, the type of preferred content viewed on these platforms and their impact on the professional growth and development of the residents. Responses were collected from July to August 2023.

A team comprising of a Resident Medical Officer and a Chemical Pathology Consultant serving as faculty at the department designed the questionnaire survey. The survey was organized to be completed within a 10-minute timeframe, ensuring it was not overly time-consuming for participants. It comprised of 15 questions divided into three sections. The first part entailed demographics of the participants and questions regarding their educational qualification and years of clinical experience. The subsequent section focused on the selection of social media platforms and their utility in the enhancement of professional development of the trainee pathologists. It included items that inquired the participants of the particular social media applications they relied on for their professional use, the type of online pathology-related content they enjoyed and an average time duration the respondents dedicated to social media networks. The final section of the survey evaluated the perception of residents regarding the impact of virtual media on their professional growth.

Informed consent was taken from the participants at the beginning of the survey. Participation in the survey was entirely optional, and individuals had the choice to opt out and withdraw by not submitting their responses. To maintain confidentiality, the survey did not collect any personal information, such as email addresses, that could potentially identify the participants. The anonymity of the participants was maintained, and no personal information was requested or saved. The statistical analysis was performed using Microsoft Excel 2019. Frequency and percentages were calculated for gender, experience level and designation. While descriptive results based on the responses were also recorded.

This study was conducted in compliance with the ethical principles for medical research involving human subjects, in accordance with the Declaration of Helsinki.

3. RESULTS:

This study provides data from twenty-five residents belonging to the department of Pathology and peer Chemical Pathology residents working at other centers in Pakistan. Laboratory

Medicine at Aga Khan University Hospital (AKUH). After an informed consent, a total of 83% (n = 25) responses were collected. 72% of the participants were females and 28% were males with age ranging between 26-33 years. The years of clinical experience ranged from 1 to 6 years. The postgraduate training in department of Pathology and Laboratory medicine is a 5-year program but a few of the trainees worked as resident medical officers in the department prior to their training and therefore have clinical experience greater than 5 years. Table 1 presents the descriptive attributes of the respondents.

3.1. Choice of Social Media Platforms and their Utilization

The inclination of pathology residents towards online social platforms and their usage was assessed in the first part of the survey. Majority of the respondents i.e., 60% (n = 15) acknowledged using social media platforms routinely for professional development and 52% (n = 13) of the respondents dedicated an average of 3-6 hours daily to social media networks. The most used social media platforms in descending order of frequency were YouTube (48%), Twitter (36%), Facebook (12%) and Instagram (4%) and 48% (n = 12) of the respondents selected YouTube as a more user-friendly and convenient virtual application for professional use followed by Twitter and Facebook. The range of information gathered from this survey, involving the options and usage of social media platforms, is summarized in Figure 1.

3.2. Preferred educational content type on Social Media

The second part of the survey was dedicated to the type of educational content favored by the respondents. 84% (n = 21) of the trainees agreed on the idea of utilizing social media to share pathology related content, with interesting case reports with pictures being voted as the most enjoyable type of educational content on social media (56%) followed by academic videos and research articles.

3.3. Effect of Social Media on Professional Development

The final part of the survey assessed the perception of participants regarding the impact of social media on their professional development. The responses were recorded on a three-point Likert scale (1 = very useful, 2 = somewhat useful, 3 = not at all useful). Social media network was perceived the most useful in increasing understanding regarding the profession by 60% (n = 15) of the participants while the remainder percentage responded neutrally. 56% (n = 14) of the residents agreed that social media platforms helped in strengthening proficiency in problem-solving whereas these online platforms were considered ineffective by a single respondent. The question regarding the enhancement of critical thinking abilities was replied positively by 52% (n = 13) while the other trainees responded neutrally. The virtual platforms were regarded as somewhat useful in enhancing clinical decision-making skills by 52% of the respondents (n = 13) while a solitary participant answered negatively. 60% (n = 15) of the residents were of the view that social media was useful to some extent in improving clinical expertise. The spectrum of data elicited regarding pathology residents' perception of social media's impact on their professional development is illustrated in figure 2. Furthermore, the data was divided into two groups based on the age groups which revealed no significant relationship between ages and perception of social media impact on the professional development, as depicted in table 2.

Table 1: Description of participants (n = 25)

Variables		Number (n)	Percentage (%)
Gender	Males	7	72
	Females	18	28
Section	Chemical Pathology	11	44
	Hematology	6	24
	Microbiology	6	24
	Histopathology	2	8
Educational Qualification	Bachelors of Medicine and Bachelors of Surgery (MBBS)	25	100
Years of clinical experience	1-2 years	15	60
	3-4 years	6	24
	5-6 years	4	16

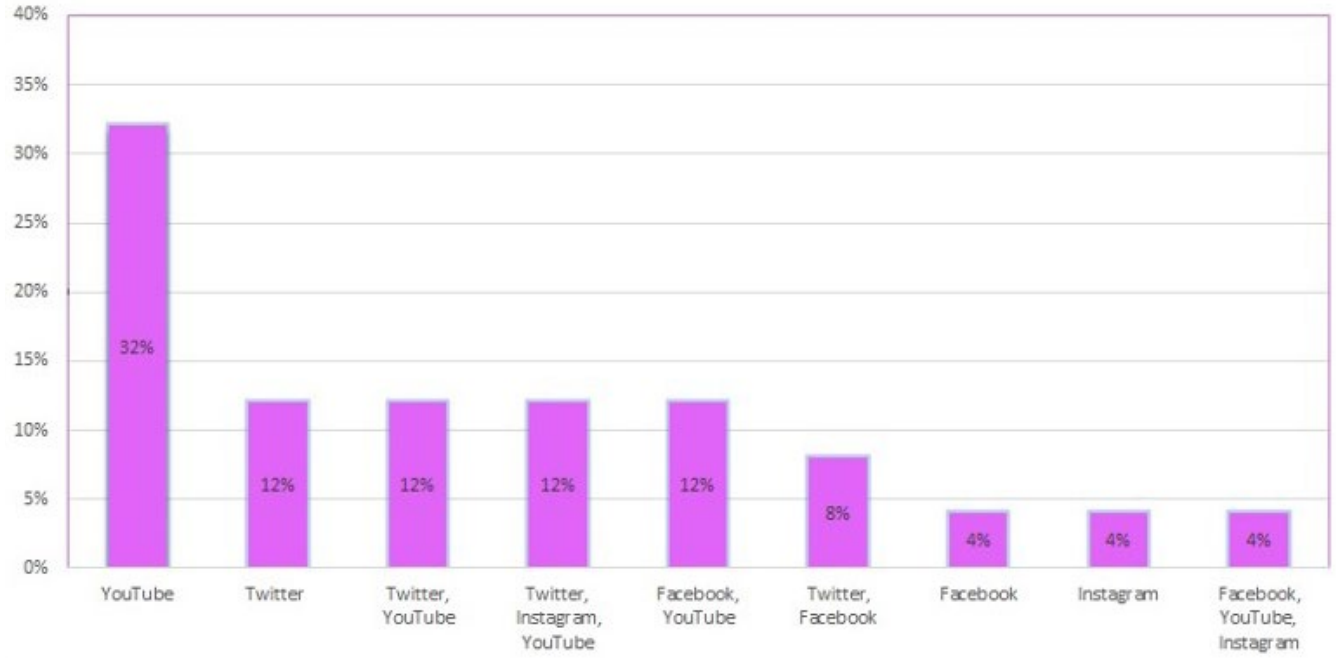

Figure 1a: Social media platforms to facilitate your professional development

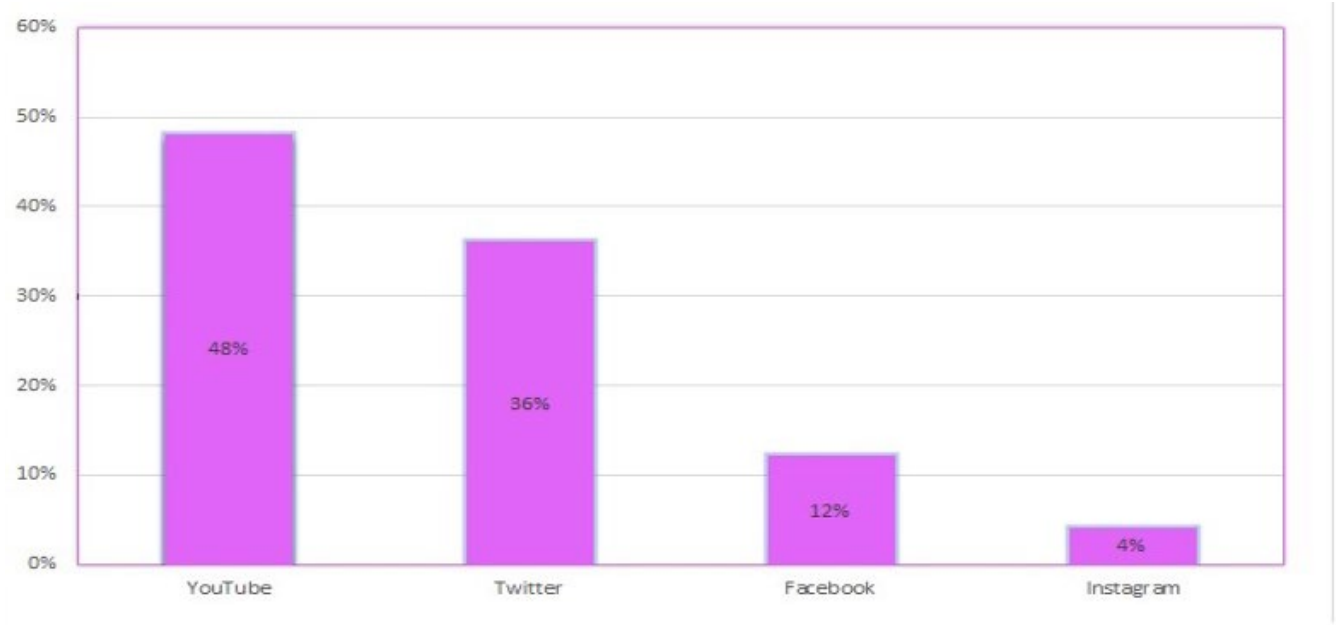

Figure 1b: User-friendly social media platforms for professional use

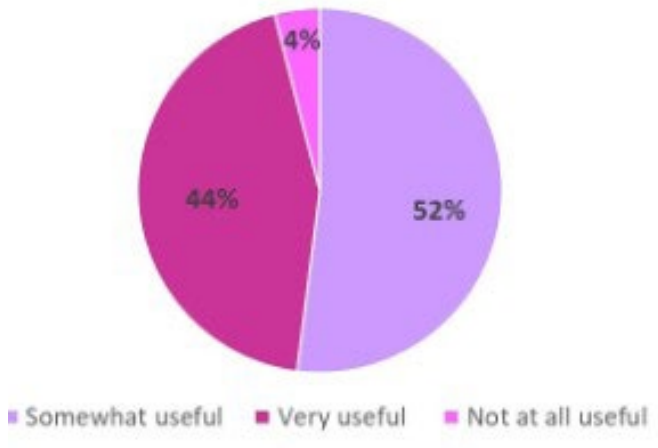

Figure 2a: Enhancing clinical/professional decision making skills

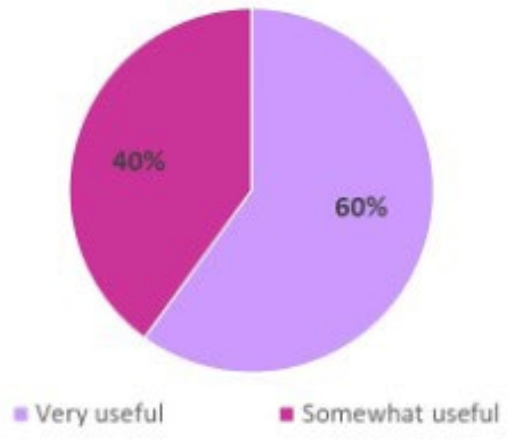

Figure 2b: Increasing understanding of the profession

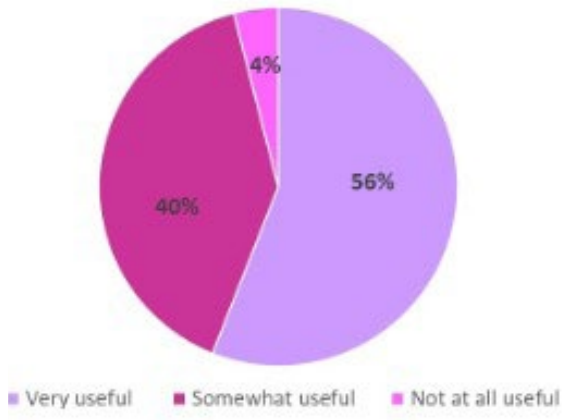

Figure 2c: Strenthening proficiency in problem-solving

Table 2: Perception of social media impact on professional development between the two groups categorized based on ages (n = 25)

	Group 1 (age ≥ 30)	Group 2 (age < 30)
Increasing understanding of the profession	Very useful = 8 Somewhat useful = 3 Not at all useful = 0	Very useful = 7 Somewhat useful = 7 Not at all useful = 0
Enhancing clinical/professional decision-making skills	Very useful = 5 Somewhat useful = 6 Not at all useful = 0	Very useful = 6 Somewhat useful = 7 Not at all useful = 1
Strengthening proficiency in problem-solving	Very useful = 7 Somewhat useful = 4 Not at all useful = 0	Very useful = 7 Somewhat useful = 6 Not at all useful = 1
Enhancing clinical expertise	Very useful = 6 Somewhat useful = 5 Not at all useful = 0	Very useful = 9 Somewhat useful = 5 Not at all useful = 0
Enhancing critical thinking abilities	Very useful = 4 Somewhat useful = 7 Not at all useful = 0	Very useful = 8 Somewhat useful = 6 Not at all useful = 0

4. DISCUSSION:

Social media is a magical wand that has extraordinarily transformed the mundane lifestyles. One of the greatest metamorphoses it has achieved is the interconnectivity between medical professionals across borders and beyond their respective medical disciplines. From connecting medical students to well-established pathology programs and assisting pathology residents in developing connections with distinguished pathologists and experts in the field (6), it has brought the usual 'behind-the-scenes' Pathology physicians in the limelight. Social media platforms have also played a contributing role in developing a sense of kinship among aspiring pathologists and attending physicians. It has enabled relationship-building with the chairs and directors of residency programs and helped discover unexpected fellowship openings (7).

Additionally, social media networks offer pathology residents and fellows to seize the opportunity to showcase their skills of knowledge, leadership and education through their online professional accounts that can serve as an 'online resume'. There are well-known tales of residents who were offered research assignments, positions on editorial boards, speaking invitations and an array of other chances via social media platforms (8).

These virtual platforms have aided numerous collaborative research between pathology residents on national levels. Unnoticed conferences at a residency program or research/educational presentations by a well-renowned speaker at a national level may attract a limited audience of tens or maybe a few hundreds, but this reach can be expanded to involve thousands of mindsets by a handful of social media posts circulating on the internet. The beauty of social media diversity is exemplified by the fact that this audience reach can range from medical students to trainee residents, from practicing pathologists to non-pathologists (8). The magnitude of power that a single click or a single virtual post possesses is intense, and its broadcast is even more enormous.

Social media networks have indeed diminished the geographical distances and erased topographical boundaries. A small-city trainee resident from a developing country can easily have access to the same education material that a Harvard Medical School resident has - this opens doors of equal opportunities for trainees who may lack access to resources in their own settings.

Even though the medical education predominantly relies on textbooks, and they remain the cornerstone of medical curriculum, social media platforms augment the role of these textbooks by imparting free access to knowledge combined with the sound narratives and personal experiences of the senior antecedents - pathologists who have lived their era and who can contribute professionally to the pathologists to come (9). This is strongly substantiated by our survey where majority of the respondents agreed that social media helps in increasing understanding about the profession and strengthens their problem-solving skills. This can be attributed to the interesting case reports with eye-catching

visual illustrations posted on online platforms and groups, as evidenced by most responses in our survey. Residents can stay well-acquainted with the engrossing case scenarios and receive latest updates in their field of interest.

Social media platforms have also birthed the concept of 'e-mentoring', where the diagnostic insights, individual expertise, and pearls of knowledge gained through years of practicing pathology by the older generation can be transmitted to the newer generation as valuable wisdom.

One of the interesting disclosures of our survey was that a greater percentage of residents responded affirmatively to using social media for the enhancement of their professional development in the field of pathology. This optimistic inclination of the young generation of pathologists-to-be calls for a need to create an alumni group where the predecessors and future pathologists can connect in a symbiotic relationship. Established pathologists can benefit by building and fostering global professional connections at an unprecedented pace while the aspiring physicians can nurture on the decades of experiences that are passed on to them like a precious heirloom (10). This is vital for their career progression and the training institute can also benefit from this fluid lattice of virtual connections by an expansive global reach, funding of scholarships and research projects by the alumni, a fostering sense of legacy and tradition and continued learning and career support to the juniors. The pool of educational resource materials available on the conventional social media platforms brings forward yet another challenge - authenticity of the provided information. These resources pertaining to education are non-standardized and unsupervised and this can lead to unreliable dissemination of information among physicians and residents alike. Fortunately, organizations like eJIFCC and EFLM have invested sustained efforts and devotion to standardize the postgraduation education of trainees in the field of laboratory medicine by developing free online learning resources. In addition to the social media platforms discussed here, the electronic journal of the International Federation of Clinical Chemistry and Laboratory Medicine (EJIFCC) and European Medicine of Clinical Chemistry and Laboratory Medicine (EFLM) have invested focused and committed endeavours in postgraduate education. In the area of e-learning, the EFLM Task group for Syllabus course (TG-ESC) has created a curriculum comprising more than 40 modules and spanning over 300 lectures covering a wide range of topics in the field of laboratory medicine (11). The course delivers a set of highly informative online lectures that delve into practical advice and useful resources to help individuals acquire the vital skills and competencies necessary for practicing laboratory medicine. Advancing this distance education and e-learning platform, they introduced live and on-demand webinars on the most sought-after topics of laboratory medicine from expert speakers from across the globe. Moreover, the collaborative effort between the New England Journal of Medicine, Clinical Chemistry, and Area9 in establishing the Learning Lab has yielded the most cutting-edge adaptive learning tool in the field of laboratory medicine. It is freely accessible to educators and trainees and offers over 110 courses. The IFCC e-academy, managed by the IFCC Committee on Internet and Digital Communications (C-IDC) serves as a lead for IFCC member societies in designing educational curricula for postgraduate trainees specializing in laboratory medicine. It also provides freely accessible global educational resources to laboratory professionals and trainees. One of the enriching resources developed by IFCC for e-learning are the webinars and distance learning modules that aid in professional growth. These include a broad-spectrum series of educational topics including advancing healthcare webinar series, POCT webinars, etc.

5. CONCLUSION:

Social media has taken the world by storm and more and more pathologists are making their presence palpable online. Junior pathologists - who are the leaders of tomorrow - can leverage this use of modern-day virtual technology for amelioration of their professional growth.

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Bisalbuminemia: A rare incidental finding in monoclonal gammopathy

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Keywords

Bisalbuminemia, Serum Protein electrophoresis, Capillary electrophoresis, multiple myeloma, rare.

Abstract

Bisalbuminemia is a rare, benign, protein anomaly presenting with two distinct peaks of albumin on serum protein electrophoresis. It reflects the presence of a normal albumin and a modified albumin in the same individual. This condition can be either hereditary or acquired. Bisalbuminemias are more frequently encountered when serum protein electrophoresis is performed with capillary technique, because this offers better resolution compared to the conventional gel electrophoresis. There are very few case reports available in the literature, showing the presence of a bifid albumin peak along with a distinct paraprotein peak in the globulin region in serum protein electrophoresis. Here we are reporting two cases, a 46-year-old male and a 48 year-old male, diagnosed with multiple myeloma, revealing the presence of an extra peak in the albumin region along with a distinct paraprotein band, when the electrophoresis was performed using capillary mode. From these case reports, we wish to reveal the extremely rare nature of this entity and also to acquaint the clinicians and laboratory personnel with this pattern of electrophoretogram.

INTRODUCTION

Albumin is the major constituent of human plasma produced in the liver and has a variety of physiological functions. Besides being essential, it constitutes 60 to 65% of total plasma proteins. Its major function is to maintain oncotic pressure along with the transportation of several endogenous and exogenous molecules. Its antioxidant function protects the body from several oxidizing agents (1). Serum protein electrophoresis (SPE) is an investigation performed routinely for the screening of monoclonal gammopathies. It can be performed using various techniques, with capillary electrophoresis being the most sensitive among them, giving more discrete bands. Electrophoresis pattern normally reveals albumin, the largest peak followed by the next five components of globulins labelled as alpha1, alpha2, beta1, beta2, and gamma. The subsets of these proteins and their relative quantity are the primary focus of the interpretation of serum protein electrophoresis (2). Albumin is usually a tall, single, discrete peak on electrophoretogram. Very rarely, we come across samples giving two peaks in the albumin region, which could be completely distinct peaks or partial splitting of the albumin peak. Structurally, the primary sequence of

albumin contains three major regions with three peptide loops each, suggesting that it arose from gene duplication of some ancestral gene in a tandem rearrangement. Variants of albumin differ from the most common allotype, albumin A, by single amino acid substitutions. It is the presence of these variants which gives splitting of the albumin peak. These variants can be rapid or slow migrating compared with albumin A (3). This phenomenon was first described in the year 1955 by Scheurlen in a diabetic German patient. The incidence of these variants is around 1:1000 to 1:3000. The incidence is reported to be high (1:100) in several tribes of North American Indians (1). This condition can be inherited or acquired. Hereditary bisalbuminemia is a relatively rare genetic disorder, usually revealed by chance. The causative genetic lesion is a point mutation of human serum albumin gene, inherited in an autosomal codominant pattern. At least 77 different mutations in albumin are recognized, 65 of which lead to bisalbuminemia (3). The presence of acquired bisalbuminemia have been described in various pathological conditions like diabetes mellitus, Waldenstrom's macroglobulinemia, multiple myeloma, sarcoidosis, Alzheimer's disease, pancreatic pseudocyst, nephrotic syndrome, chronic kidney disease, and also in patients receiving high doses of penicillin (4). Hereditary type is permanent but the acquired form may be transient. Most of the isoforms of albumin have normal function, and most individuals with bisalbuminemia have normal serum concentrations of albumin. Clinically, no pathological consequences have been reported for bisalbuminemia (alloalbuminemia) and it is being of interest only for human genetics due to its rare incidence. In this study, we present two cases of incidentally detected bifid albumin peaks in newly diagnosed patients of multiple myeloma. Out of the 3360 samples run for serum protein electrophoresis (SPE), during the last five years in our hospital, there were only two patients with a bifid albumin peak showcasing the rarity of the scenario.

CASE REPORTS:

Case 1:

A 46 year old male patient presented with complaints of body pains and aches in multiple joints, for which he was evaluated at an outside hospital, where his investigations were unremarkable, except for the presence of severe anaemia and a high total protein. Anaemia was treated with blood transfusion followed by oral supplements. However, the patient had similar complaints of anaemia and polyarthralgia few months later. In view of recurrent anaemia, high total protein, and persistent polyarthralgia, he was sent to our hospital for further management. In our hospital, the results of serum creatinine, calcium and liver function tests were normal, except for high total protein. Serum protein electrophoresis (SPE) and serum free light chain assay were first performed as screening tests to rule out multiple myeloma. The electrophoretogram of the patient was showing a distinct monoclonal (M) band of 23 g/L in the gamma region along with the presence of a small extra peak in the albumin region and serum free light chain assay was suggestive of kappa type of monoclonal gammopathy. Bone marrow aspiration and biopsy followed by flow cytometry were further done, which confirmed the presence of multiple myeloma. For the management of the disease, the patient was given four cycles of chemotherapy with Lenalidomide, Bortezomib, and Dexamethasone (RVD) regimen. During the course of chemotherapy, SPE was repeated to check for the disease progression every 4 months. All of his electrophoretograms had the presence of the same bifid albumin peak which as depicted in the figures 1 and 2. Now the patient is on maintenance therapy with Bortezomib drug.

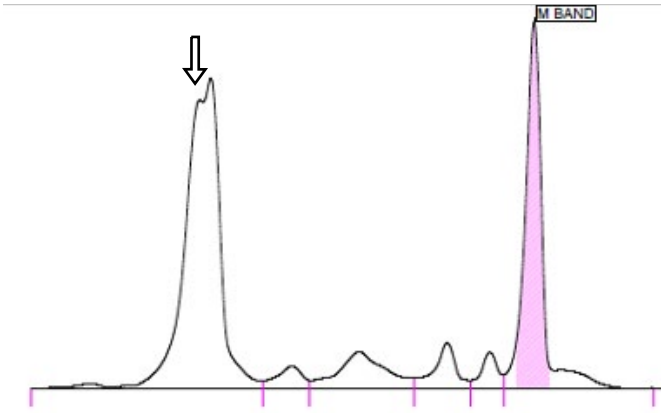

Figure 1: Electrophoretogram of Case-1 at the time of presentation showing bifid albumin along with M band.

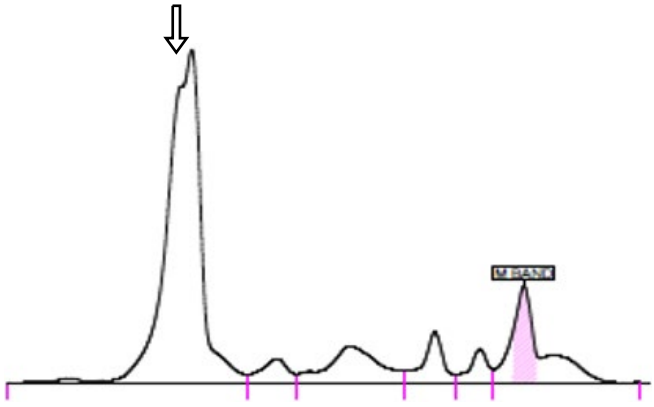

Figure 2: Electrophoretogram of Case-1 after chemotherapy showing bifid albumin peak as well as M band

Case 2:

A 48 year old male patient had complaints of backache and paraparesis. He was evaluated at outside hospital and diagnosed as plasmacytoma in the epidural region. He was treated with decompressive laminectomy with excision of epidural mass followed by 5 weeks of external beam radiation therapy to D5 – L1 vertebrae. In view of suspicion of multiple myeloma, bone marrow aspiration was done, which showed 38% plasma cells, suggestive of plasma cell neoplasm. Skeletal survey was showing multiple lytic lesions in the skull, ribs, femur, humerus and tarsal bones. He was sent to our hospital for further management. At our hospital, the results of serum creatinine, calcium and liver function tests were normal, except for high total protein of 144 g/L, high globulin and altered A:G ratio. Serum free light chain assay was suggestive of kappa type of monoclonal gammopathy. The electrophoretogram was showing a distinct M band of 67 g/L in the gamma region and the albumin region was showing two bands which were not clearly demarcated. From the investigations, he was diagnosed as multiple myeloma and was planned treatment with Lenalidomide, Bortezomib, and Dexamethasone (RVD) Regimen. Electrophoretograms of this patient (figure 3 and figure 4), later during follow up, while on chemotherapy, were all showing the same extra band in the albumin region, which was more distinct, confirming the presence of bisalbuminemia.

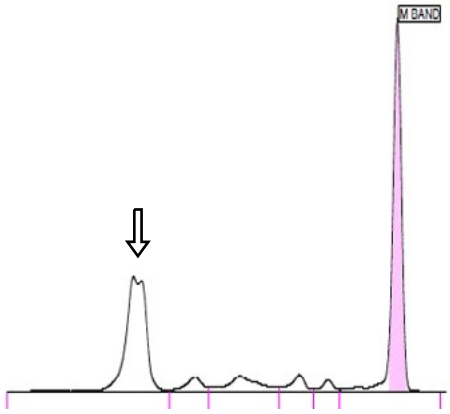

Figure 3: Electrophoretogram of Case-2 at the time of presentation showing bifid albumin along with M band.

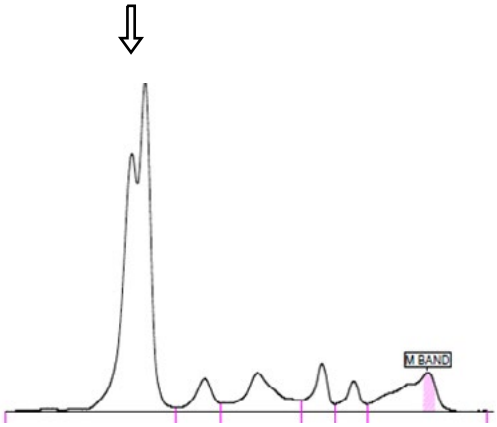

Figure 4: Electrophoretogram of Case-2 after chemotherapy showing bifid albumin peak as well as M band.

Table 1: Investigations performed at the time of presentation in both the cases.

Parameter (Units)	Case-1	Case-2	Reference range
Serum Total Bilirubin (µmol/L)	3.42	6.84	3.42 – 22.2
Serum Creatinine (µmol/L)	60.1	85.7	58.3 – 110.5
Blood Urea (mmol/L)	2.33	4.49	3.16 - 7.18
Serum Total Protein (g/L)	88	144	63 – 82
Serum Albumin (g/L)	45	50	35 – 50
Aspartate transaminase (SGOT) (U/L)	13	21	17.0 - 59
Alanine transaminase (SGPT) (U/L)	12	46	0 - 50.0
Alkaline Phosphatase (ALP) (U/L)	76	83	38 - 126
Serum Free Kappa light chain (mg/L)	112.1	145.5	3.30 – 19.40
Serum Free Lambda light chain (mg/L)	8.64	15.7	5.71 – 26.30
Free Kappa/Lambda (κ/λ) ratio	12.97	9.20	0.26 – 1.65
Hemoglobin (g/L)	48	102	130 – 170

DISCUSSION

Bisalbuminemia is an incidental finding in serum protein electrophoresis and can be identified using electrophoresis only. Bisalbuminemia is a rare disorder characterised by the presence of two distinct fractions of albumin on serum protein electrophoresis. This can be hereditary or acquired and is very uncommon in Indian population (1). As seen in our hospital, out of 3360 patients screened for plasma cell neoplasms using serum protein electrophoresis during the last five years, only two patients had the presence of bifid albumin peaks in their electrophoretograms. The prevalence of bisalbuminemia obtained in our hospital is about 0.06% indicating the rarity of the condition. Albumin variations, either acquired or inherited, should always be on the radar of both clinicians and research scientists. Such new forms can possibly provide data on protein evolution and on the molecular structure and characteristics of the albumin molecule (5). There is no known clinical significance of this finding, though this should not be misinterpreted as an abnormal globulin peak specifically when dealing with suspected or confirmed cases of plasma cell dyscrasia (10). However, some albumin variants may have altered affinity for some hormones, especially thyroxine, metal ions, fatty acids, and drugs, with clinical implication in rare scenarios(9). There is no known association or pathophysiological relationship between bisalbuminemia and multiple myeloma. The recognition of bisalbuminemia in these patients is likely simply due to the fact that serum protein electrophoresis is commonly performed in patients with suspected myelomas. The incidence in myeloma patients is expected be similar to that of the general population (8). The differential diagnosis of an additional band in the same region as albumin includes various conditions. The first among them is that the band might be an artefact of electrophoresis, which usually happens in gel electrophoresis due to air bubbles, distortions of the gel, overloading, etc. Since we have used capillary system for electrophoresis, gel artefacts can be ruled out. Bisalbuminemias are more frequently encountered with the development of capillary electrophoresis, because this technique offers better resolution (9). Secondly, proteins that normally migrate in the same region of albumin may be elevated and mimic the ap¬pearance of bisalbuminemia. These include prealbumin, which is increased after recent food ingestion, alpha 1 acid glycoprotein (an acute phase reactant), and alpha lipoproteins. Moreover, any unusual band in a serum protein electrophoresis may be a paraprotein of monoclonal gammopathy (6). In our case, no discordances were found between the spectrophotometric quantification of albumin in VITROS 5600 dry chemistry systems and the albumin concentration determined by capillary electrophoresis. Bands due to acute phase reactants and food ingestion are temporary and do not recur on electrophoretic run in several different occasions as it recurred in our patients. Paraprotein band is a separate band in the gamma region in both our patients. As the patient is on treatment for myeloma and the M band concentration have decreased, comigration of free light chains and abnormal proteins can be ruled out. Lastly, interferences by radio-opaque agents or medications, which could lead to the appearance in the capillary electropherograms of abnormal peaks, were discarded as explanations because they are visible in the α2-globulin fraction or beta region and do not show a bifid pattern in the albumin region (11). No genetic study was performed in our cases to ascertain the genetic causes of bisalbuminemia, as the primary concern of these patients was monoclonal gammopathy. In a study by Chan PC, the author found that bisalbuminemia is not associated with monoclonal gammopathies, but, is an incidental finding (8). Bisalbuminemia can interfere with the serum protein electrophoresis diagnosis, but, is of little diagnostic or therapeutic significance. This can cause difficulty in the reporting of serum protein electrophoresis diagnosis in multiple myeloma. Hence, it is important to recognise such variant, while interpreting serum protein electrophoresis (7, 8, 12). The experience and expertise in SPE reporting helps ensure the identification of even a faint M-band or spike and distinguish it from the other bands including the albumin variants (12). Moreover, learning about albumin variants can be of great inquisitiveness and might be valuable in assessing their geographical distribution.

CONCLUSION

Bisalbuminemia is an extremely rare entity encountered on serum protein electrophoresis. Its presence should be acknowledged and not mistaken for a paraprotein band, though its presence does not influence the disease process in multiple myeloma.

Authors' contribution

All the authors have contributes to the intellectual content of this paper including Conception of the idea, drafting the article, Critical revision and final approval of the version to be published.

Authors' disclosures or potential Conflicts of interest

No authors disclosed any potential conflicts of interest.

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Suspect the unexpected: A rare association of Autoimmune Hemolytic Anemia and Hemophagocytic Lymphohistiocytosis with Visceral Leishmaniasis: A Case Report and Review of Literature

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Keywords

Autoimmune hemolytic anemia, Hemophagocytic lymphohistiocytosis, Visceral Leishmaniasis

Abstract

Visceral leishmaniasis is a common tropical infection presenting with a myriad of hematological abnormalities. We report an unusual case of an 11-year-old girl suffering from a febrile illness with hepatosplenomegaly and anemia. Laboratory findings included pancytopenia and hyperbilirubinemia. The leishmania antigen rK39 was positive and bone marrow examination revealed hemophagocytosis and amastigote forms of Leishmania donovani. Direct Coombs' test was positive (warm type, IgG) and LDH was elevated. Tests for other infections were negative. A diagnosis of visceral leishmaniasis with autoimmune hemolytic anemia (warm antibody type, IgG) with hemophagocytic lymphohistiocytosis was made. Patient showed response with anti-leishmanial treatment with improvement in clinical condition.

Introduction

Visceral leishmaniasis (VL) is an intracellular protozoal infection caused by Leishmania donovani and transmitted by infected sandfly Phlebotomus argentipes. India, Bangladesh, Nepal, Sudan and Brazil are home to more than 90% of cases of VL in the world.1

Anemia is almost always present in VL and has multifactorial pathogenesis but Coombs' positive hemolytic anemia is rarely reported.2 Likewise, Hemophagocytic lymphohistiocytosis (HLH) secondary to VL is very rare and overlap in the clinical characteristics of VL and HLH can be a diagnostic challenge.3 Here we present a rare case of hemolytic anaemia and HLH in a case of VL.

Case report

An 11-year-old girl resident of Nepal presented with fever and abdominal distension for 2 months. She had anorexia and weight loss with no history of rash, bleeding, jaundice, blood transfusions or contact with tuberculosis. She was developmentally appropriate and unimmunized. This thin built girl had pallor, icterus and bilateral pitting pedal edema with no palpable lymph nodes, bone pains or clubbing. There was firm, non-tender hepatomegaly of 5 cm below costal margin with span of 10 cm and firm splenomegaly of 10 cm below costal margin. There was no free fluid in the abdomen. Other organs were within normal limits.

Laboratory reports revealed hemoglobin 70 g/L, leukocyte count, platelet count 73 x 109/L, reticulocyte count 1.2% and mean corpuscular volume 69 x 1015/L. Peripheral smear showed microcytic hypochromic red blood cells (RBC) with mild anisocytosis without abnormal cells or hemoparasites. Total bilirubin 41 µmol/L (5.1-17 µmol/L) with indirect fraction of 25.6 µmol/L (0.2-0.8 µmol/L), AST 581 U/L, ALT 229 U/L, ALP 747 U/L, serum albumin 20 g/L, international normalized ratio (INR) was 2.3, activated partial thromboplastin time (aPTT) was 45 seconds and D-dimer was positive (>5mg/L). Workup for her prolonged pyrexia was negative for malaria, enteric fever, tuberculosis, HIV, viral hepatitis, rickettsial illness or systemic lupus erythematosus. Bone marrow examination clinched the diagnosis as amastigote forms of Leishmania donovani were seen (Figure 1) along with evidence of hemophagocytosis (Figure 2). rK39 antigen was positive. Diagnosis of HLH was supported by clinical picture, bone marrow findings and the presence of hypertriglyceridemia (4.7 mmol/L), hypofibrinogenemia (0.6 g/L) and elevated serum ferritin (1909 pmol/L). During hospital stay, her hemoglobin and platelet count started falling from 70 g/L and 73 x 109/L at admission to 47 g/L and 35 x 109/L on day 10 of admission, respectively. She was further investigated for the worsening anemia and Direct Coombs' Test (DCT) was strongly positive (4+). Further DCT profile showed presence of Immunoglobulin G. Indirect Coombs' test (ICT) at 37°C was negative. Lactate dehydrogenase (LDH) was elevated (4199 U/L) and urine was positive for hemoglobin, suggesting intravascular hemolysis. Patient was started on injectable Amphotericin B alternate day therapy with supportive therapy (fluids, nutrition, antipyretics, vitamin K, and blood component support). The patient showed gradual response with improvement in general condition and appetite followed by improvement in hematological parameters (hemoglobin of 84 g/L, TLC of 4.5 x 109/L, platelet count of 130 x 109/L), coagulogram (INR of 1.25) and regression of organomegaly (liver 2 cm and spleen 3 cm) by day 10 of therapy. Unfortunately, the patient expired due to a suspected nosocomial intercurrent respiratory infection.

Discussion

Visceral leishmaniasis is a disseminated intracellular protozoal infection caused by the Leishmania donovani. It multiplies in the reticuloendothelial system (liver, spleen, lymph node and bone marrow) leading to enlargement of these organs and bone marrow dysfunction. It manifests as fever, hepatosplenomegaly, pancytopenia and hypergammaglobinemia.4 In advanced illness, ascites with pedal edema (due to hypoalbuminemia), bleeding manifestations (due to thrombocytopenia and coagulopathy) and secondary infections (measles, pneumonia, tuberculosis, dysentery, etc) are common. Anemia in VL occurs due to a combination of factors - hemolysis, hemorrhage, marrow replacement by Leishmania infected macrophages, splenic sequestration of red blood cells, hemodilution, shortened RBC lifespan, hemophagocytosis, marrow suppression by cytokines, reversible myelodysplasia and concomitant infections and malnutrition leading to iron, vitamin B12 and folate deficiency.5,6 Rarely immune hemolysis is seen which is due to immune complex deposition on the red cell surface, mostly as a result of nonspecific adsorption secondary to polyclonal hypergammaglobinemia and in rare cases due to cold or warm antibody. In our patient, hemolysis was immune mediated by warm antibody as suggested by positive DCT at 37°C. Contributing to anemia in our patient also were hemophagocytosis and hypersplenism. We started our patient on Amphotericin B therapy which led to decrease in the DCT titres from 4+ to 2+ at the 10th day of therapy. Our patient required only one packed RBC and no platelet transfusion during hospital stay. HLH is a clinico-pathological condition characterized by uncontrolled and non-malignant proliferation of macrophages and T lymphocytes with cytokine overproduction. It is important to differentiate primary and secondary HLH as treatment of the two differs considerably. Primary HLH requires cytotoxic therapy and bone marrow transplant whereas secondary HLH requires specific therapy of the underlying disease.7 HLH related VL is very rare in childhood.8-10 Our patient had fever, splenomegaly, cytopenia, hypertriglyceridemia, hypofibrinogenemia, increased ferritin and hemophagocytosis on bone marrow, hence meeting the criteria for HLH. Our patient responded to amphotericin B therapy alone with improvement in fever, blood count and splenomegaly. This is the first of its kind case reporting the unique presentation of VL with AIHA and HLH. Although separate associations of AIHA with VL and HLH with VL have been reported, the combination of the three is a novel finding.

Conclusion

The present case demonstrates the unique presentation of visceral leishmaniasis with auto immune hemolytic anaemia and hemophagocytic lymphohistiocytosis. VL should be suspected in cases of febrile illness with hepatosplenomegaly presenting from endemic areas. Bone marrow aspiration is safe but less sensitive in diagnosing VL. rK39 test is a rapid diagnostic test to diagnose VL. Causes of anaemia in VL are diverse and hemolysis should be suspected in case of rapidly falling hemoglobin. HLH should always be suspected in severe infections with disseminated disease. Timely diagnosis is key and prompt institution of anti-leishmanial therapy leads to resolution of the disease in majority of patients.

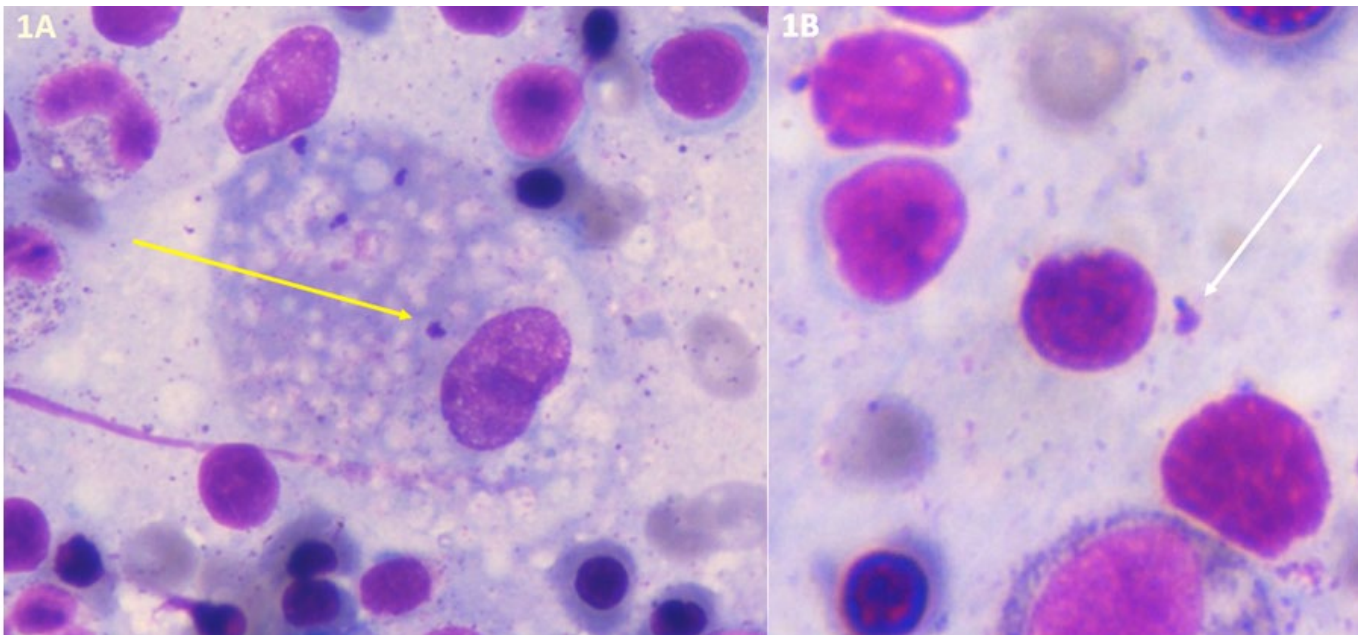

Figure 1A and 1B: Intracellular (yellow arrow) and extracellular (white arrow) amastigote forms of Leishmania donovani on bone marrow aspiration smear (Wright stain, 100x)

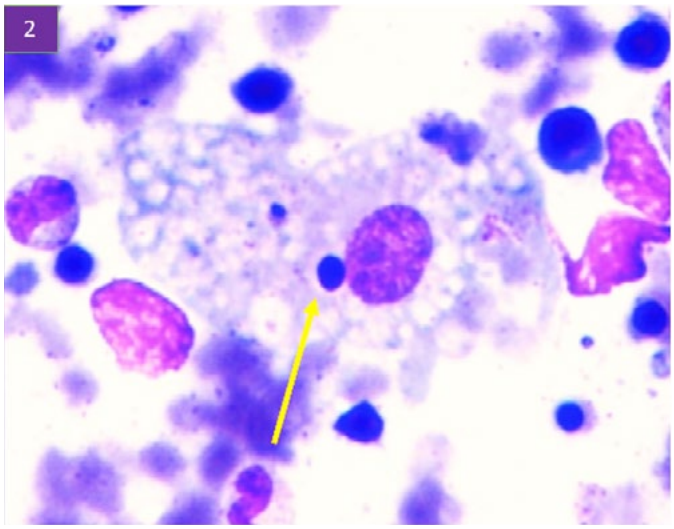

Figure 2: Hemophagocytosis on bone marrow aspirate evidenced by intracellular lymphocyte nucleus in a macrophage (yellow arrow) (Wright stain, 100x)

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