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IN THIS ISSUE:

The Philippine Journal of Pathology (PJP) is an open-access, peer-reviewed, English language, medical science journal published by the Philippine Society of Pathologists, Inc. Committee on Publications. It shall serve as the official platform for publication of high quality original articles, case reports or series, feature articles, and editorials covering topics on clinical and anatomic pathology, laboratory medicine and medical technology, diagnostics, laboratory biosafety and biosecurity, as well as laboratory quality assurance. The journal's primary audience are Filipino laboratorians, diagnosticians, laboratory managers, pathologists, medical technologists, and all other medical and scientific disciplines interfacing with the laboratory. The PJP follows the **ICMJE Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals, EQUATOR Network Guidelines,** and **COPE Guidelines.** The PJP does not charge any article processing or submission fees from authors. It does not charge any subscription fees or download fees to access content.

Welcome to the June 2023 issue of the Philippine Journal of Pathology. Congratulations to the editorial staff for another publication.

The Board of Governors of the Philippine Society of Pathologists would like to encourage our junior and regular members to continuously submit papers for publication, be it a research work or interesting case reports or series. Let our society recognize your output. We may all learn a thing or two from your scholarly work.

Three years after the COVID-19 pandemic, we have reverted to our normal life style. Most of the restrictions during the pandemic have been lifted. Mobility of people is evident. We can start collaborating with colleagues for ideas and topics and to gather clinical data in order to produce quality publication.

The Philippine Society of Pathologists will always support the endeavor of the PJP editorial team in achieving its goal of publishing wonderful and relevant articles related to Pathology and Laboratory Medicine.

Let us prove to the world that the Filipinos can deliver high-caliber and timely publication.

Mabuhay tayong lahat!

Alan T. Koa, MD, FPSP
President, Philippine Society of Pathologists, Inc.

Embracing the Era of Generative AI: Transforming Scientific Publishing in Laboratory Medicine and Pathology

Scientific publishing has long been the backbone of knowledge dissemination in laboratory medicine and pathology. Researchers and clinicians rely on peer-reviewed journals to share their discoveries, advancements, and diagnostic insights. However, with the rapid emergence of generative artificial intelligence (AI) models, we find ourselves standing at the precipice of a transformative era in scientific publishing. As we navigate the implications of this technology, it is crucial to critically examine its potential impact on laboratory medicine and pathology, understanding both the benefits and challenges it presents.

Enhanced Data Analysis and Interpretation

Generative AI models, powered by deep learning algorithms, possess the ability to analyze vast amounts of data with remarkable efficiency. This technology has the potential to revolutionize data analysis in laboratory medicine and pathology, offering faster and more accurate insights. By training on extensive datasets, AI algorithms can identify patterns, recognize anomalies, and even predict disease outcomes. This enhanced analytical capacity promises to elevate the quality of research and accelerate the pace of scientific discovery.

Accelerated Research and Development

The integration of generative AI in scientific publishing has the potential to fuel innovation and expedite the research and development process. With AI-driven automation, laboratory experiments and data analysis can be streamlined, saving time and resources. Researchers can leverage these technologies to conduct virtual experiments, simulate complex scenarios, and generate hypotheses. This accelerated pace of research and development will undoubtedly contribute to a deeper understanding of diseases, leading to more effective diagnostic methods and therapeutic interventions.

Quality Assurance and Standardization

Scientific publishing in laboratory medicine and pathology relies on rigorous quality assurance and standardization processes. Generative AI has the potential to address some of the challenges associated with reproducibility and variability in research. By automating certain aspects of data analysis and interpretation, AI models can provide consistent and standardized results, reducing the potential for human error. Moreover, the integration of AI systems into the peer-review process can help identify inconsistencies, detect data manipulation, and ensure a higher level of scientific rigor.

Ethical Considerations and Bias Mitigation

While the promises of generative AI are enticing, we must also address the ethical concerns and potential biases associated with this technology. AI models are only as good as the data they are trained on, and biases present in the training data can propagate into their outputs. In laboratory medicine and pathology, it is imperative to ensure that AI algorithms are trained on diverse and representative datasets to mitigate the risk of biased results. Transparency, explainability, and ethical oversight are essential in the development and deployment of generative AI models to maintain scientific integrity and trust.

Preserving Human Expertise and Collaboration

It is important to emphasize that generative AI should be seen as a tool that complements human expertise, rather than a replacement for it. The unique insights, creativity, and intuition of laboratory medicine and pathology professionals remain invaluable in the scientific publishing process. Collaboration between AI models and human experts can lead to groundbreaking discoveries that would not be possible without either component. It is crucial to foster interdisciplinary collaborations that encourage the integration of generative AI while preserving the essence of human intelligence and critical thinking.

Out of scientific curiosity at the potential of Generative Artificial Intelligence (AI) and in light of the statement from the World Association of Medical Editors (which is published in this issue), I wrote this editorial using ChatGPT May 24 Version, with the prompt "what are the implications/impact of Generative AI to scientific publishing in laboratory medicine and pathology."

Generative AI holds immense potential to transform scientific publishing in laboratory medicine and pathology, offering enhanced data analysis, accelerated research, and improved quality assurance. It is a remarkable technology that holds a lot of promise, but it should be emphasized that it is only as good as its user. It is only a tool at this point, not capable of generating its own thoughts and opinions, and most certainly cannot be held accountable as an author.

As part of its commitment to the Filipino pathologist as the platform for laboratory practice in the country, the Philippine Journal of Pathology should update its editorial policies and publishing processes to incorporate and accommodate the increasing use of Generative AI in future research and manuscript writing.

Amado O. Tandoc III, MD, FPSP
Editor-in-Chief

Chatbots, Generative AI, and Scholarly Manuscripts

WAME Recommendations on Chatbots and Generative Artificial Intelligence in Relation to Scholarly Publications

Revised May 31, 2023

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INTRODUCTION

This statement revises our earlier "WAME Recommendations on ChatGPT and Chatbots in Relation to Scholarly Publications" (January 20, 2023). The revision reflects the proliferation of chatbots and their expanding use in scholarly publishing over the last few months, as well as emerging concerns regarding lack of authenticity of content when using chatbots. These Recommendations are intended to inform editors and help them develop policies for the use of chatbots in papers published in their journals. They aim to help authors and reviewers understand how best to attribute the use of chatbots in their work, and to address the need for all journal editors to have access to manuscript screening tools. In this rapidly evolving field, we will continue to modify these recommendations as the software and its applications develop.

A **chatbot** is a tool "[d]riven by [artificial intelligence], automated rules, natural-language processing (NLP), and machine learning (ML)…[to] process data to deliver responses to requests of all kinds."[1] Artificial intelligence (AI) is "the ability of a digital computer or computer-controlled robot to perform tasks commonly associated with intelligent beings."

"Generative modeling is an artificial intelligence technique that generates synthetic artifacts by analyzing training examples; learning their patterns and distribution; and then creating realistic facsimiles. **Generative AI** (GAI) uses generative modeling and advances in deep learning (DL) to produce diverse content at scale by utilizing existing media such as text, graphics, audio, and video."[3,4]

Chatbots are activated by a plain-language instruction, or "prompt," provided by the user. They generate responses using statistical and probability-based language models.[5] This output has some characteristic properties. It is usually linguistically accurate and fluent but, to date, it is often compromised in various ways. For example, chatbot output currently carries the risk of including biases, distortions, irrelevancies, misrepresentations, and plagiarism – many of which are caused by the algorithms governing its generation and heavily dependent on the contents of the materials used in its training. Consequently, there are concerns about the effects of chatbots on knowledge creation and dissemination – including their potential to spread and amplify mis- and disinformation[6] – and their broader impact on jobs and the economy, as well as the health of individuals and populations. New legal issues have also arisen in connection with chatbots and generative AI.[7]

Chatbots retain the information supplied to them, including content and prompts, and may use this information in future responses.[8] Therefore, scholarly content that is generated or edited using AI would be retained and as a result, could potentially appear in future responses, further increasing the risk of inadvertent plagiarism on the part of the user and any future users of the technology. Anyone who needs to maintain confidentiality of a document, including authors, editors, and reviewers, should be aware of this issue before considering using chatbots to edit or generate work.[9]

Chatbots and their applications illustrate the powerful possibilities of generative AI, as well as the risks. These Recommendations seek to suggest a workable approach to valid concerns about the use of chatbots in scholarly publishing.

A note on changes introduced since the previous WAME Recommendations

- *A new recommendation (#4) has been added to the four original principal recommendations: 1) Only humans can be authors; 2) Authors should acknowledge the sources of their materials; 3) Authors must take public responsibility for their work; 4) Editors and reviewers should specify, to authors and each other, any use of chatbots in evaluation of the manuscript and generation of reviews and correspondence; and 5) Editors need appropriate digital tools to deal with the effects of chatbots on publishing.*
- *In addition, this revision acknowledges that chatbots are used to perform different functions in scholarly publications. Currently, individuals in scholarly publishing may use chatbots for: 1) simple word-processing tasks (analogous to, and an extension of, word-processing and grammar-checking software), 2) the generation of ideas and text, and 3) substantive research. The Recommendations have been tailored for application to these different uses.*

WAME RECOMMENDATIONS ON CHATBOTS AND GENERATIVE ARTIFICIAL INTELLIGENCE IN RELATION TO SCHOLARLY PUBLICATION

WAME Recommendation 1: *Chatbots cannot be authors.* Journals have begun to publish articles in which chatbots such as Bard, Bing and ChatGPT have been used, with some journals listing chatbots as co-authors. The legal status of an author differs from country to country but under most jurisdictions, an author must be a legal person. Chatbots do not meet the International Committee of Medical Journal Editors (ICMJE) authorship criteria, particularly that of being able to give "final approval of the version to be published" and "to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved."[10] No AI tool can "understand" a conflict-of-interest statement, and does not have the legal standing to sign a statement. Chatbots have no affiliation independent of their developers. Since authors submitting a manuscript must ensure that all those named as authors meet the authorship criteria, chatbots cannot be included as authors.

WAME Recommendation 2: *Authors should be transparent when chatbots are used and provide information about how they were used.* The extent and type of use of chatbots in journal publications should be indicated. This is consistent with the ICMJE recommendation of acknowledging writing assistance[11] and providing in the Methods detailed information about how the study was conducted and the results generated.[12]

> **WAME Recommendations 2.1:** *Authors submitting a paper in which a chatbot/AI was used to draft new text should note such use in the acknowledgment; all prompts used to generate new text, or to convert text or text prompts into tables or illustrations, should be specified.*

> **WAME Recommendation 2.2:** *When an AI tool such as a chatbot is used to carry out or generate analytical work, help report results (e.g., generating tables or figures), or write computer codes, this should be stated in the body of the paper, in both the Abstract and the Methods section. In the interests of enabling scientific scrutiny, including replication and identifying falsification, the full prompt used to generate the research results, the time and date of query, and the AI tool used and its version, should be provided.*

WAME Recommendation 3: *Authors are responsible for material provided by a chatbot in their paper (including the accuracy of what is presented and the absence of plagiarism) and for appropriate attribution of all sources (including original sources for material generated by the chatbot).* Authors of articles written with the help of a chatbot are responsible for the material generated by the chatbot, including its accuracy. Noting that plagiarism is "the practice of taking someone else's work or ideas and passing them off as one's own,"[13] not just the verbatim repetition of previously published text. It is the author's responsibility to ensure that the content reflects the author's data and ideas and is not plagiarism, fabrication or falsification. Otherwise, it is potentially scientific misconduct to offer such material for publication, irrespective of how it was written. Similarly, authors must ensure that all quoted material is appropriately attributed, including full citations, and that the cited sources support the chatbot's statements. Since a chatbot may be designed to omit sources that oppose viewpoints expressed in its output, it is the authors' responsibility to find, review and include such counterviews in their articles. (Of course, such biases are also found in human authors.) Authors should identify the chatbot used and the specific prompt (query statement) used with the chatbot. They should specify what they have done to mitigate the risk of plagiarism, provide a balanced view, and ensure the accuracy of all their references.

WAME Recommendation 4: *Editors and peer reviewers should specify, to authors and each other, any use of chatbots in the evaluation of the manuscript and generation of reviews and correspondence. If they use chatbots in their communications with authors and each other, they should explain how they were used.* Editors and reviewers are responsible for any content and citations generated by a chatbot. They should be aware that chatbots retain the prompts fed to them, including manuscript content, and supplying an author's manuscript to a chatbot breaches confidentiality of the submitted manuscript.

WAME Recommendation 5: *Editors need appropriate tools to help them detect content generated or altered by AI. Such tools should be made available to editors regardless of ability to pay for them, for the good of science and the public, and to help ensure the integrity of healthcare information and reducing the risk of adverse health outcomes.* Many medical journal editors use manuscript evaluation approaches that were not designed to deal with AI innovations and industries, including manipulated plagiarized text and images and papermill-generated documents. They have already been at a disadvantage when trying to differentiate the legitimate from the fabricated, and chatbots take this challenge to a new level. Editors need access to tools that will help them evaluate content efficiently and accurately. This is of particular importance to editors of medical journals where the adverse consequences of misinformation include potential harms to people.

We encourage comments and feedback from WAME Members and other readers. Please contact us at chris@chriszielinski.com.

Competing Interests

All of the authors report that they have no competing interests aside from any affiliations as editors.

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Prevalence of Somatic BRCA1 and BRCA2 Mutations in Ovarian Cancer among Filipinos Using Next Generation Sequencing*

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ABSTRACT

Introduction. Ovarian cancer is one of the leading causes of mortality in women. In 2020, 5,395 (6.2%) of diagnosed malignancies in females were ovarian in origin. It also ranked second among gynecologic malignancies after cervical cancer. The prevalence in Asian /Pacific women is 9.2 per 100,000 population. Increased mortality and poor prognosis in ovarian cancer are caused by asymptomatic growth and delayed or absent symptoms for which about 70% of women have an advanced stage (III/IV) by the time of diagnosis. The most associated gene mutations are Breast Cancer gene 1 (BRCA1) which is identified in chromosome 17q21 and Breast Cancer gene 2 (BRCA2) identified in chromosome 13. Both proteins function in the double-strand DNA break repair pathway especially in the large framework repair molecules. Olaparib is a first-line drug used in the management of ovarian cancer. It targets affected cells by inhibition of poly (ADP-ribose) polymerase (PARP) activity which induces synthetic lethality in mutated BRCA1/2 cancers by selectively targeting tumor cells that fail to repair DNA double-strand breaks (DSBs).

Objectives. The study aims to determine the prevalence of pathogenic somatic mutations in BRCA1 and BRCA2 among patients diagnosed of having ovarian cancer, to characterize the identified variants into benign/ no pathogenic variant identified, variant of uncertain significance (VUS), and pathogenic, and to determine the relationship of specific mutations detected with histomorphologic findings and clinical attributes.

Methodology. Ovarian cancer tissues available at the St. Luke's Medical Center Human Cancer Biobank and formalin-fixed paraffin-embedded (FFPE) tissue blocks diagnosed as ovarian cancer from the year 2016 to 2020 were included. Determination of the prevalence of somatic BRCA1 and BRCA2 mutations using Next Generation Sequencing (NGS).

Results. A total of 60 samples were processed, and three samples were excluded from the analysis due to an inadequate number of cells. In the remaining 57 samples diagnosed ovarian tumors, pathogenic BRCA1/2 variants were identified in 10 (17.5%) samples. Among the BRCA1/2 positive samples, 3 (5.3%) BRCA1 and 7 (12.3%) BRCA2 somatic mutations were identified.

Conclusion. Identification of specific BRCA1/2 mutations in FFPE samples with NGS plays a big role in the management of ovarian cancer, particularly with the use of targeted therapies such as Olaparib. The use of this drug could provide a longer disease-free survival for these patients. Furthermore, we recommend that women diagnosed with ovarian cancer should be subjected to genetic testing regardless of the histologic subtypes or clinical features. Lastly, genetic testing should be done along with proper genetic counseling, especially for patients who are susceptible to these mutations.

Key words: ovarian cancer, BRCA somatic mutations, BRCA1, BRCA 2, NGS

INTRODUCTION

Ovarian cancer is one of the leading causes of mortality in women. In 2020, 5,395 (6.2%) of diagnosed malignancies in females were ovarian in origin. It also ranked second among gynecologic malignancies after cervical cancer.[1] The prevalence in Asian /Pacific women is 9.2 per 100,00 population. Increased mortality and poor prognosis in ovarian cancer are caused by asymptomatic growth and delayed or absent symptoms[2] for which about 70% of women have an advanced stage (III/IV) by the time of diagnosis. There are several histologic types of ovarian cancer, each with distinct characteristics. Among the different types, surface epithelial tumors, particularly high-grade serous carcinoma, are the most aggressive subtype. It is also the most diagnosed surface epithelial tumor.[3] One of the most

significant risk factors for the occurrence of this tumor is family history.

High-grade serous carcinoma is a genetically unstable malignancy that carries different mutations. The most associated gene mutations are Breast Cancer gene 1 (BRCA1) which is identified in chromosome 17q21 and Breast Cancer gene 2 (BRCA2) identified in chromosome 13. Both proteins function in the double-strand DNA break repair pathway, especially in the large framework repair molecules.[4] A mutated BRCA1/2 gene that is inherited from either parent is defined as germline mutation. While a mutated gene that occurs in a single body cell after birth and cannot be inherited is defined as somatic mutation. Age discrepancy also plays a role in the onset of disease between BRCA1/2, with BRCA1 patients having an increased risk after age 40 and BRCA2 patients after age 50. Somatic mutations were reported in 5-9% and 3-4% of BRCA1 and BRCA2 genes, respectively.[5] Recent advances in the clinical trials for targeted therapy included Olaparib (a poly (ADP-Ribose) polymerase 1 (PARP1) inhibitor). Olaparib is a first-line drug used in the management of ovarian cancer. It targets affected cells by inhibition of poly (ADP-ribose) polymerase (PARP) activity which induces synthetic lethality in mutated BRCA1/2 cancers by selectively targeting tumor cells that fail to repair DNA double-strand breaks (DSBs).[6] This drug provides therapeutic benefits for germline as well as somatic BRCA mutations.

This study aims to determine the prevalence of pathogenic somatic mutations in BRCA1 and BRCA2 among patients with ovarian cancer. We also aim to characterize the identified variants into benign/no pathogenic variants.

METHODOLOGY

A retrospective study was conducted. Sixty (60) cases of ovarian cancer tissues available in St. Luke's Medical Center Human Cancer Biobank and formalin fixed paraffin embedded (FFPE) tissue blocks diagnosed as ovarian cancer from 2016 to 2020 were included in the study. Samples included were primary ovarian cancer with tumor tissue and ovarian masses with metastatic gynecologic origin (fallopian, endometrial, and cervical). All pertinent clinical information from the databank were retrieved and collated. Determination of the prevalence of somatic BRCA1 and BRCA2 mutations using Next Generation Sequencing (NGS) was done. Sample size was calculated based on the estimation of the population proportion. Assuming that the prevalence of ovarian BRCA mutation is 28%[6] with a maximum allowable error of 7.5% and a reliability of 90%, the sample size required was 60.

Genomic DNA was extracted from formalin fixed paraffin embedded (FFPE) ovarian cancer tumor blocks. Four (4) sections, "10 μm thickness" were cut from the blocks. These sections were deparaffinized and DNA was extracted using the QiaAMP DNA MiniKit®. Briefly, 200 μl of the buffy coat was lysed using the lysis solution (Buffer AL) and proteinase K was added to degrade proteins. Cells were incubated at 560C for 10 mins or until complete lysis. To precipitate the isolated DNA, ethanol (EtOH) was added to each sample. Wash Buffers (AW1 and AW2)

Table 1. Pathogenic somatic mutations in BRCA1 and BRCA2 genes

		N	%
Somatic Mutation	Yes	10	17.5
	No	47	82.5
	Total	57	100.0
Mutation	BRCA1 somatic	3	5.3
	BRCA2 somatic	7	12.3
	No pathogenic mutation/ possible germline	47	82.5
	Total	57	100.0

Table 2. Clinicopathologic features and somatic mutations

Characteristics	Somatic mutation		p
	Yes, n=10 n (%)	No, n=47 n (%)	
Age group			.786[a]
Less than 50	4 (40.0)	21 (44.7)	
50 and above	6 (60.0)	26 (55.3)	
Diagnosis			.530[a,b]
Serous carcinoma (High-grade)	5 (50.0)	17 (36.2)	
Endometrioid carcinoma	1 (10.0)	14 (29.8)	
Clear cell carcinoma	3 (30.0)	7 (14.9)	
Mucinous carcinoma	0 (0.0)	5 (10.6)	
Mixed carcinoma	1 (10.0)	3 (6.4)	
Undifferentiated malignancy	0 (0.0)	1 (2.1)	

Results are based on nonempty rows and columns in each innermost subtable.
[a]More than 20% of cells in this subtable have expected cell counts less than 5. Chi-square results may be invalid.
[b]The minimum expected cell count in this subtable is less than one. Chi-square results may be invalid.

were added separately to the spin columns to facilitate the removal of contaminants. To elute purified genomic DNA (gDNA) Buffer AE was added to the spin columns. Using Nanodrop® v1000 spectrophotometer the DNA quality and quantity of the extracted eluent were assessed. A final working concentration of 50 ng/μl of gDNA was used for each sample.

RESULTS

A total of 60 samples were processed, and three samples were excluded from the analysis due to the inadequate number of cells. In the remaining 57 samples diagnosed with ovarian cancer, pathogenic BRCA1/2 variants were identified in 10 (17.5%) samples. Among the BRCA1/2 positive samples, 3 (5.3%) BRCA1 and 7 (12.3%) BRCA2 somatic mutations were identified while 47 samples (82.5%) had no pathogenic or possibly germline mutations (Table 1).

Of the 10 samples that showed somatic mutation, 60% samples were noted in age 50 and above with most of the cases presenting with high-grade serous carcinoma (50%) (Table 2).

Somatic mutations in the BRCA2 gene were more frequently found in patients diagnosed at age 50 and above compared to younger individuals – 71.4% (5/7) versus 28.6% (2/7), respectively. While somatic mutations in the BRCA1 gene were more frequently found in younger patients compared to older individuals – 66.7% (2/3) versus 33.3% (1/3). High-grade serous carcinoma was the most common epithelial ovarian neoplasm presenting with somatic mutations, and these were identified in 3 samples for the BRCA2 gene and 2 samples for the BRCA1 gene (Tables 3 and 4).

Table 3. BRCA1 and BRCA2 somatic mutations in different age groups

		Mutation						
		BRCA1 somatic		BRCA2 somatic		No pathogenic mutation/ possible germline		p
		n	%	n	%	n	%	
Age group	Less than 50	2	66.7	2	28.6	21	44.7	
	50 and above	1	33.3	5	71.4	26	55.3	
	Total	**3**	**100.0**	**7**	**100.0**	**47**	**100.0**	.519[a]

Results are based on nonempty rows and columns in each innermost subtable.
[a]More than 20% of cells in this subtable have expected cell counts less than 5. Chi-square results may be invalid.

Table 4. BRCA1 and BRCA2 somatic mutations in different histologic subtypes

		Mutation						
		BRCA1 somatic		BRCA2 somatic		No pathogenic mutation/ possible germline		p
		n	%	n	%	n	%	
Diagnosis	Serous carcinoma (High-grade)	2	66.7	3	42.9	17	36.2	
	Endometrioid carcinoma	0	0.0	1	14.3	14	29.8	
	Clear cell carcinoma	0	0.0	3	42.9	7	14.9	
	Mucinous carcinoma	0	0.0	0	0.0	5	10.6	
	Mixed carcinoma	1	33.3	0	0.0	3	6.4	
	Undifferentiated malignancy	0	0.0	0	0.0	1	2.1	
	Total	**3**	**100.0**	**7**	**100.0**	**47**	**100.0**	.429[a,b]

Results are based on nonempty rows and columns in each innermost subtable.
[a]More than 20% of cells in this subtable have expected cell counts less than 5. Chi-square results may be invalid.
[b]The minimum expected cell count in this subtable is less than one. Chi-square results may be invalid.

DISCUSSION

In this study, we detected the frequency of somatic BRCA1/2 mutations in ovarian cancer patients. The use of tumoral tissues can detect the presence of both germline and somatic mutations but germline variants are primarily detected through blood samples or buccal swabs. Molecular analysis of the BRCA1/2 genes revealed that out of the 57 samples, 10 (17.5%) of which demonstrated the presence of somatic mutations. In one study, our percentage is higher – 17.5% versus 4.1%.[6] While in another study, as high as 39% of somatic BRCA1/2 mutations were detected.[7] These results can be attributed to varying numbers of samples. However, the prevalence of somatic BRCA1 and BRCA2 mutations in relation to the age of diagnosis was comparable to previous studies. BRCA1 mutations were frequently detected in younger individuals and BRCA2 mutations were more associated with older individuals. This study also identifies that serous carcinoma (high-grade) was the most common epithelial tumor associated with BRCA1/2 mutations comprising 66.7% and 42.9%, respectively. Somatic mutations in the BRCA2 gene were also noted in clear cell carcinoma (3 samples, 42.9%) and endometrioid carcinoma (1 sample, 14.3%). Goodheart et al., demonstrated that clear cell carcinoma showing BRCA2 mutations has shown to have a better prognosis compared to clear cell carcinoma with wild-type mutations.[5]

The guidelines from the American Society of Clinical Oncology and the European Molecular Genetics Quality Network (EMQN) recommend genetic testing for BRCA1 and BRCA2 mutations in every patient diagnosed with ovarian cancer. With the application of NGS as a standard diagnostic tool, we can detect the presence of mutations in each patient.[6] Olaparib, a PARP inhibitor is used as a drug for cases with BRCA1/2 germline as well as somatic mutations. SOLO-1 trial data from the 5-year follow-up demonstrated that Olaparib reduced the risk of disease progression or death by 67 percent. At 5 years, 48.3 percent of patients on Olaparib remained free from disease progression versus 20.5 percent of those who received a placebo (Society of Gynecologic Oncology 2021 Annual Meeting).[7-11]

CONCLUSION AND RECOMMENDATIONS

Identification of specific BRCA1/2 mutations in FFPE samples with the use of NGS plays a big role in the management of ovarian cancer, particularly with the use of targeted therapies such as Olaparib. The use of this drug could provide a longer disease-free survival for these patients. Furthermore, we recommend that women diagnosed with ovarian cancer should be subjected to genetic testing regardless of the histologic subtypes or clinical features. Lastly, genetic testing should be done along with proper genetic counseling, especially for patients who are susceptible to these mutations.

STATEMENT OF AUTHORSHIP

The authors certified fulfilment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

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Baseline Complete Blood Count and Cell Population Data as Prognostic Markers for In-Hospital Mortality among COVID-19 Patients admitted at the Philippine General Hospital from March 2020 to January 2022

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ABSTRACT

Introduction. Complete blood count (CBC) and cell population data (CPD) are hematologic parameters used in several clinical scenarios including infection and neoplastic processes. In the setting of COVID-19 infection, there is relative paucity of data in their use as possible prognostic markers.

Objective. We aim to evaluate the utility of the baseline CBC and CPD as prognostic markers for in-hospital mortality among COVID-19 patients admitted in Philippine General Hospital from March 2020 to January 2022.

Methodology. This is a case-control study. Expired patients served as cases, and recovered patients served as controls. Data from eligible patients including age, sex, admitting COVID diagnosis with severity, final disposition, baseline CBC and CPD results were collected from the hospital medical records and hematology section of the Department of Laboratories. Statistical analyses were done to determine the prognostic value of these parameters for in-hospital mortality.

Results. Among the different CBC and CPD parameters, the study shows total white blood cell (WBC) count, absolute neutrophil count (ANC), absolute eosinophil count (AEC), and neutrophil-lymphocyte ratio (NLR) were statistically significant predictors for in-hospital mortality. For total WBC count, at a cut off 9.9×10^9/L, the sensitivity and specificity is 70.9% and 66.2%, respectively. For ANC, at a cut off of 7.3×10^9/L, the specificity is 76.4% and the specificity is 68.2%. At a cut off of 7.62, the NLR shows a sensitivity of 76.4% and specificity of 70.1%. For AEC, at a cut off of 0.006×10^9/L, the sensitivity is 53.3% and the specificity is 87.3%. AEC predicts towards the direction of survival rather than to the direction of in-hospital mortality.

Conclusion. The total WBC count, ANC, and NLR were statistically significant predictors for in-hospital mortality, while AEC predicts towards the direction of survival. The sensitivities and specificities of the cut off for these parameters were less than ideal. Correlation with clinical and other laboratory parameters is still recommended. For future studies, the authors recommend monitoring CBC and CPD parameters at different time points during the patients' hospital course.

Key words: COVID-19, hematology, blood cell count, complete blood count, prognosis, cell population data

INTRODUCTION

Coronavirus Disease 2019 (COVID-19) is an ongoing pandemic caused by the Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2). As of January 2022, the World Health Organization (WHO) has recorded more than 300 million cases globally with more than 5.5 million deaths. The Philippines has recorded more than 3 million cases with more than 52,000 deaths in the same period.[1]

The clinical spectrum of COVID-19 ranges from asymptomatic illness to severe, life-threatening disease. While the ultimate outcome of patients depends on various factors, identification of prognostic parameters to determine which patients could progress to critical disease may aid in early intervention measures.

Complete blood count (CBC) is an inexpensive and widely available test in most hospitals and diagnostic facilities. Several hematologic changes have been reported in COVID-19, and most changes are associated with the white blood cell component. 20-40% of patients present with leukopenia, while 3-24% have leukocytosis.[2] Strong association was found between lymphopenia and severe COVID-19.[3] Neutrophilia has also been reported in patients with severe manifestations.

Other studies show mild thrombocytopenia in 5-21% of COVID-19 patients.[2] However, studies also show that significant thrombocytopenia is associated with higher mortality risk.[4] Hemoglobin changes in COVID-19 infection are variable and conflicting.[5] Reduced levels in a meta-analysis have been documented in severe cases.[6]

A combination of these parameters has also been studied to evaluate disease severity in COVID-19 patients. An elevated neutrophil to lymphocyte ratio (NLR) was a marker for increased mortality and severity.[7] Platelet to lymphocyte ratio (PLR) is significantly increased in critical patients as compared to those with lesser severity of infection. Conversely, a decreased lymphocyte to monocyte ratio (LMR) is observed among severe cases.[8,9]

The postulated mechanism for lymphopenia is direct invasion by the virus into lymphocytes through ACE2 receptors.[10] Lactic acidosis, a common finding in COVID-19 infection, may also result in decreased lymphocyte proliferation.[11] Neutrophilia may be virally induced, or secondary to a concomitant bacterial infection.[12] Secondary hemophagocytic lymphohistiocytosis from COVID-19 causes excessive proliferation and activation of macrophages, and in turn results in a cytokine storm. The surge in inflammatory cytokines damages hematopoietic progenitors and also reduces platelet production.[13]

Some hematology analyzers can generate Cell Population Data (CPD) values through Volume, Conductivity, and Scatter (VCS) Technology. This technology enables assessment of cellular volume, cell surface structure, cytoplasmic chemical composition, and nuclear topography. Changes among these parameters reflect the morphological adaptation of cells to various triggers and changes in the internal milieu.[14]

The Unicel DxH 900 (Beckman Coulter, Miami, FL, USA), the analyzer used in Philippine General Hospital (PGH), can generate CPD as a research feature. Values for volume, conductivity, axial light loss (AL2), low-angle light scatter (LALS), median-angle light scatter (MALS), lower median angle light scatter (LMALS), and upper median angle light scatter (UMALS) can be generated for each CBC run.

Studies have utilized these parameters in the setting of sepsis[15] and differentiation between viral and bacterial infections in children.[16] Few studies to date have utilized these parameters in the COVID-19 setting. From a diagnostic perspective, monocyte volume served as the best discriminator between COVID-19 and non-COVID-19 patients, with a sensitivity of 89.7% and specificity of 60.5%.[17]

In terms of prognosis, a study showed that neutrophils have increased volume and decreased conductivity, while lymphocytes show increased conductivity, among fatal COVID cases.[18]

In this study, we describe our findings on baseline CBC and CPD as prognostic markers for in-hospital mortality among COVID-19 patients admitted in a tertiary government hospital.

METHODOLOGY

This study was submitted to the University of the Philippines – Manila Research Ethics Board for approval prior to implementation.

Research design
This is a case-control study investigating the prognostic utility of baseline CBC and CPD findings in predicting in-hospital mortality among COVID-19 patients admitted in PGH from March 2020 to January 2022. Expired patients served as cases, and recovered patients served as controls.

Sampling
Purposive sampling was done for this study by employing an inclusion and exclusion criteria. All patients admitted in PGH from March 2020 to January 2022 that fulfill the inclusion and exclusion criteria were included in the study.

Inclusion and exclusion
The study included patients more than 18 years old, admitted with a primary clinical suspicion of COVID-19 (i.e., symptoms compatible with COVID-19), confirmed by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) done in PGH; with a baseline CBC test with corresponding CPD parameters done in PGH using the Unicel DxH 900; and with a final disposition as to "Discharged" or "Expired" based on the Hospital Medical Records.

Patients with incidental diagnoses of COVID-19 after admission for another disease, or those with concomitant acute inflammatory conditions on admission (i.e., acute infection) not consistent with COVID-19 infection, were excluded.

Data collection procedures
A list of patients admitted in the PGH COVID-19 ward from March 2020 to January 2022 was requested from the Medical Records Division and was screened according to the inclusion and exclusion criteria. Eligible patients were assigned unique code numbers. The age, sex, admitting COVID diagnosis (including disease severity), and final disposition ("Discharged"/ "Expired"), and baseline CBC results of the patients were collected. The corresponding CPD values for the baseline CBC were requested from the Hematology Section of the Department of Laboratories. The CPD values include the mean and standard deviation of Neutrophil, Lymphocyte, Monocyte, Eosinophil and Early Granulated Cell – AL2, LALS, UMALS, LMALS and MALS.

RESULTS

The study population included 235 patients – 168 of which were survivors while 67 died from the disease. Among the survivors, 83 were male and 85 were female, with a median age of 56. Severity of disease on admission among this group are as follows: moderate (109), severe (49), and critical (3). Among the in-house mortality group, 43 were male and 24 were female, with a median age of 63. Severity of disease on admission among this group are as follows: moderate (31), severe (29), and critical (3). The difference in clinicodemographic characteristics of the participants is not homogenous between those who survived and those who died in-hospital by Mann-Whitney U test. Propensity score matching in a 2:1 ratio between in-hospital mortalities and survivors was done using a logit model for in-hospital mortality with the following covariates: age, sex, and COVID-19 disease severity. Caliper matching without replacement was used, with an a priori caliper width set at 0.20 times the SD of the propensity score. Only 162 participants were then included in the data analysis. We can note that the propensity score matching has addressed the heterogeneity of the included participants in the case and control groups (Table 1).

A point-biserial correlation analysis was done between the CBC and CPD parameters and severity of COVID-19 disease on admission. Because of the low representation of the Critical group, it has been grouped together with Severe for this analysis. The following parameters showed significantly weak correlation with disease severity on admission: absolute lymphocyte count, r=-0.25, $p<0.01$; monocyte LMALS, r=0.25, $p=0.001$; monocyte MALS, r=0.22, $p=0.004$; and PLR, r=0.38, $p<0.001$. The absolute lymphocyte count has an inverse relationship with disease severity on admission while the other three parameters have a direct relationship with disease severity. The rest of the blood parameters have negligible or without evidence of correlation with COVID-19 disease severity on admission (Table 2).

The following blood parameters show significantly higher median among in-hospital mortality than among survivors: total WBC count $p<0.001$, absolute neutrophil count (ANC) $p<0.001$, neutrophil volume $p<0.001$, lymphocyte MALS $p=0.003$, lymphocyte UMALS $p=0.012$, monocyte LALS $p=0.043$, and NLR $p<0.001$. In contrast, the following blood parameters show significantly lower median among in-hospital mortality than among survivors: neutrophil conductivity $p=0.009$, neutrophil LMALS $p=0.019$, absolute lymphocyte count (ALC) $p=0.004$, lymphocyte LALS $p=0.033$, lymphocyte ALL $p=0.002$, absolute eosinophil count (AEC) $p<0.001$, eosinophil volume $p=0.002$, early granulocyte conductivity $p=0.017$, early granulocyte MALS $p=0.053$, platelet count $p=0.003$, and LMR $p=0.005$. The rest of the blood parameters have no significant difference in median values (Table 3).

The ROC curve analysis showed that the AUC of the following blood parameters are good predictors of mortality: total WBC count (0.7, 95% CI), ANC (0.7, 95% CI), AEC (0.7, 95% CI), and NLR (0.7, 95% CI). The rest of the blood parameters are poor predictors mortality (95%CI crossing 0.6000) or have no evidence of predicting ability (95%CI crossing 0.5000). Parameters with AUC significantly higher than 0.6000 proceed to cut off determination (Table 4).

For total WBC count, at a cut off 9.9×10^9/L, the sensitivity and specificity is 70.9% and 66.2%, respectively. For ANC, at a cut off of 7.3×10^9/L the specificity is 76.4% and the specificity is 68.2%. At a cut off of 7.62, the NLR shows a sensitivity of 76.4% and specificity of 70.1%. For AEC, at a cut off of 0.006×10^9/L, the sensitivity is 53.3% and the specificity is 87.3%. The latter parameter, however, predicts towards the direction of survival rather than to the direction of in-hospital mortality (Table 5).

DISCUSSION

The in-hospital mortality group in the study shows significant higher WBC counts with concomitant higher ANC. While these parameters did not show correlation with disease severity on admission, the study suggests they are possible markers for poor outcome. Neutrophilia has been historically documented in sepsis and bacteremic states as an early manifestation of immune cell response to severe infection. In the setting of COVID-19 infection, neutrophilia is correlated with the hyperinflammatory state and cytokine storm associated with the disease. The neutrophilia is documented not only in the bloodstream but also in lung tissue where they contribute to further tissue damage.[19]

The NLR is also shown as a predictor for mortality in the study. This reflects not only the increase in neutrophils, but also a decrease in ALC. The median ALC has been shown in this study to be significantly lower among the

Table 1. Clinicodemographic profile of all the participants included in the study, and the propensity score-matched participants that were included in the data analysis

Clinicodemographic profile	All included subjects			1:2 Propensity score-matched		
	In-hospital mortality n = 67	Survivor n = 168	p-value	In-hospital mortality n = 55	Survivor n = 107	p-value
Age, years, median (IQR)	56 (25)	63 (22)	<0.001	61 (20)	60 (15)	0.657
Sex, count (%)			0.040			0.805
Male	83 (49.40%)	43 (64.18%)		34 (61.82%)	64 (59.81%)	
Female	85 (50.60%)	24 (35.82%)		21 (38.18%)	43 (40.19%)	
Disease severity on admission, count (%)			0.024			0.940
Moderate	109 (67.70%)	31 (49.21%)		30 (54.55%)	61 (57.01%)	
Severe	49 (30.43%)	29 (46.03%)		24 (43.64%)	44 (41.125)	
Critical	3 (1.86%)	3 (4.76%)		1 (1.82%)	2 (1.87%)	

Table 2. Point-biserial correlation analysis between CBC and CPD parameters with disease severity

Blood cell parameters	Correlation coefficient	90% CI	p-value
Total WBC count	-0.0362	-0.1651, 0.0940	0.648
Neutrophil			
Absolute count	0.0081	-0.1217, 0.1377	0.918
Volume	-0.1893	-0.3114, -0.0611	0.016
Conductivity	0.0454	-0.0848, 0.1741	0.566
Median-angle light scatter	0.0055	-0.1243, 0.1351	0.944
Upper median-angle light scatter	-0.0123	-0.1418, 0.1176	0.877
Lower median-angle light scatter	0.0305	-0.0996, 0.1595	0.700
Low-angle light scatter	-0.0464	-0.1751, 0.0838	0.557
Axial light loss	0.0773	-0.0530, 0.2049	0.328
Lymphocyte			
Absolute count	-0.2547	-0.3721, -0.1292	0.001
Volume	-0.0335	-0.1625, 0.0966	0.672
Conductivity	0.1258	-0.0040, 0.2524	0.111
Median-angle light scatter	0.1048	-0.0252, 0.2314	0.184
Upper median-angle light scatter	0.1455	0.0161, 0.2701	0.065
Lower median-angle light scatter	0.1470	0.0177, 0.2716	0.062
Low-angle light scatter	-0.0766	-0.2043, 0.0537	0.333
Axial light loss	0.1003	-0.0298, 0.2271	0.204
Monocyte			
Absolute count	-0.1081	-0.2345, 0.0219	0.171
Volume	-0.1085	-0.2349, 0.0215	0.169
Conductivity	0.0886	-0.0416, 0.2158	0.262
Median-angle light scatter	0.2241	0.0972, 0.3438	0.004
Upper median-angle light scatter	0.1062	-0.0238, 0.2327	0.179
Lower median-angle light scatter	0.2553	0.1299, 0.3737	0.001
Low-angle light scatter	0.1831	0.0547, 0.3055	0.020
Axial light loss	0.1670	0.0381, 0.2904	0.034
Eosinophil			
Absolute count	-0.0620	-0.1902, 0.0683	0.433
Volume	-0.0910	-0.2267, 0.0483	0.282
Conductivity	0.0814	-0.0579, 0.2176	0.336
Median-angle light scatter	0.0575	-0.0817, 0.1946	0.497
Upper median-angle light scatter	0.1020	-0.0372, 0.2372	0.227
Lower median-angle light scatter	0.0520	-0.0872, 0.1893	0.539
Low-angle light scatter	-0.0128	-0.01512, 0.1260	0.879
Axial light loss	0.0526	-0.0867, 0.1898	0.535
Early granulocyte			
Absolute count	-	-	-
Volume	-0.1752	-0.3078, -0.0360	0.039
Conductivity	0.0253	-0.1153, 0.1648	0.768
Median-angle light scatter	0.0468	-0.0939, 0.1857	0.584
Upper median-angle light scatter	0.0069	-0.1334, 0.1469	0.936
Lower median-angle light scatter	0.0580	-0.0928, 0.1965	0.498
Low-angle light scatter	0.0187	-0.1217, 0.1584	0.827
Axial light loss	0.1066	-0.0340, 0.2431	0.212
Platelet count	0.1345	0.0049, 0.2597	0.088
Neutropil:lymphocyte ratio	0.1691	0.0398, 0.2927	0.032
Platelet:lymphocyte ratio	0.3776	0.2603, 0.4839	<0.001
Lymphocyte:monocyte ratio	-0.1260	-0.2516, 0.0038	0.110

in-hospital mortality group compared to the survivor group. Together with the ANC, lymphopenia also suggests disease progression and poor outcome. The cause of lymphopenia is hypothesized to be due to viral-induced apoptosis.[20] Another theory is that viral attachment induces ACE-2 receptor expression on the lymphocyte's surface which increases the probability of being a target of the virus.[21] Both these parameters result in an increased NLR which has shown predictive ability for mortality.

The role of eosinophils in COVID-19 disease is largely unknown. According to a study, the findings of eosinopenia, together with neutrophilia and lymphopenia, is consistent among COVID-19 patients.[22] Eosinopenia is also uncommonly found in other conventional viral infections. Tan et al noted that eosinophils were inversely related to the severity of the disease. Furthermore, eosinophil counts returned to normal levels upon discharge.[23] The results from this study showed that AEC is associated with disease survival.

CPD parameters with higher median among the in-hospital mortality group compared to the survivors in the initial analysis include total WBC count, ANC, neutrophil volume, lymphocyte MALS, lymphocyte UMALS, monocyte LALS, and NLR. These changes are brought about by the activation and alterations in internal complexity of these cells in response to a trigger (i.e., infection). However, none of these parameters were statistically significant on further analysis. Because of the novelty of these parameters, only a few cohorts have studied its application in the setting of COVID disease. One study compared

Table 3. Initial screening of possible admission CBC and CPD parameters as predictors of in-hospital mortality among admitted COVID-19 patients

Blood cell parameters	In-hospital mortality n = 55, Median (IQR)	Survivor n = 107, Median (IQR)	p-value
Total WBC count	11.4 (7)	7.5 (5.8)	<0.001
Neutrophil			
Absolute count	9.9 (7.33)	5.13 (5.15)	<0.001
Volume	148 (13)	142 (8)	<0.001
Conductivity	144 (7)	145 (6)	0.009
Median-angle light scatter	135 (9)	138 (9)	0.096
Upper median-angle light scatter	136 (7)	137 (5)	0.742
Lower median-angle light scatter	127 (12)	132 (13)	0.019
Low-angle light scatter	153 (22)	156 (19)	0.504
Axial light loss	133 (11)	135 (22)	0.294
Lymphocyte			
Absolute count	0.8 (1.1)	1.14 (0.8)	0.004
Volume	88 (7)	88 (8)	0.819
Conductivity	116 (4)	115 (5)	0.292
Median-angle light scatter	74 (9)	71 (7)	0.006
Upper median-angle light scatter	75 (14)	71 (11)	0.012
Lower median-angle light scatter	65 (6)	63 (4)	0.072
Low-angle light scatter	34 (4)	35 (4)	0.033
Axial light loss	58 (10)	63 (33)	0.002
Monocyte			
Absolute count	0.68 (0.61)	0.69 (0.39)	0.911
Volume	176 (13)	173 (11)	0.051
Conductivity	124 (5)	124 (5)	0.953
Median-angle light scatter	90 (7)	90 (5)	0.271
Upper median-angle light scatter	99 (9)	98 (5)	0.331
Lower median-angle light scatter	77 (9)	76 (5)	0.147
Low-angle light scatter	87 (16)	79 (19)	0.008
Axial light loss	115 (10)	116 (43)	0.216
Eosinophil			
Absolute count	0 (0)	0.04 (0.16)	<0.001
Volume	147 (14)	153 (16)	0.002
Conductivity	153 (10)	151 (8)	0.053
Median-angle light scatter	195 (15)	198 (13)	0.285
Upper median-angle light scatter	205 (24)	208 (12)	0.066
Lower median-angle light scatter	180 (17)	182 (13)	0.718
Low-angle light scatter	154 (25)	160 (19)	0.101
Axial light loss	122 (24)	123 (12)	0.658
Early granulocyte			
Absolute count	-	-	-
Volume	168 (20)	163.5 (20)	0.092
Conductivity	133 (5)	135 (5)	0.017
Median-angle light scatter	142 (7)	144 (7)	0.053
Upper median-angle light scatter	153 (10)	156 (8)	0.087
Lower median-angle light scatter	127 (10)	128.5 (10)	0.129
Low-angle light scatter	116 (16)	118.5 (22)	0.822
Axial light loss	138 (15)	142 (38)	0.101
Platelet count	212 (182)	282 (194)	0.003
Neutropil:lymphocyte ratio	12.29 (14.61)	4.47 (6.27)	<0.001
Platelet:lymphocyte ratio	255.19 (400.8)	232.46 (244.2)	0.916
Lymphocyte:monocyte ratio	1.25 (1.45)	1.73 (1.13)	0.005

these research parameters among COVID-19 ICU and Non-ICU patients. The study showed that in spite of the striking differences in the morphology of neutrophils, lymphocytes, and monocytes, these research parameters did not show any differences between the two groups.[20] Of note, the latter study used a different analyzer from the one used in this research. One study noted that a subset of their study population with severe and or fatal disease demonstrated increase volume and decreased conductivity of neutrophils, and increased conductivity of lymphocytes.[22] These contradictory results suggest that further studies may still need to be done on the usefulness these research parameters in the setting of COVID.

Important limitations of the study include non-measurement of vaccination status and actual treatment received by the study population. A study by Graña et al showed high-certainty evidence of a reduction in severe or critical COVID-19 cases compared to placebo after vaccination with the following: BNT162b2, mRNA-1273, Ad26.COV2.S, and BBV152. These vaccines are included in the Philippine vaccination drive against COVID-19. Efficacy rates of these vaccines were noted in the range of 76.3% to 98.2% (95% CI).[24] Among hospitalized cases, including non-critical and critical admissions, vaccination has been shown to markedly reduce adverse outcomes including mortalities.[25] Treatment practices may have also shifted as new knowledge on management are being

Table 4. Receiver operating characteristic curve analysis of admission CBC and CPD parameters as predictors of in-hospital mortality among admitted COVID-19 patients

Blood cell parameters	AUC	95% CI
Total WBC count	0.7098	0.6110, 0.8085
Neutrophil		
Absolute count	0.7486	0.6538, 0.8435
Volume	0.6738	0.5657, 0.7819
Conductivity*	0.6480	0.5432, 0.7527
Lower median-angle light scatter*	0.6106	0.5057, 0.7156
Lymphocyte		
Absolute count*	0.5574	0.4399, 0.6750
Median-angle light scatter	0.6540	0.5433, 0.7647
Upper median-angle light scatter	0.6774	0.5665, 0.7883
Low-angle light scatter*	0.5634	0.4553, 0.6715
Axial light loss*	0.6396	0.5350, 0.7443
Monocyte		
Low-angle light scatter	0.6463	0.5461, 0.7466
Eosinophil		
Absolute count*	0.7048	0.6271, 0.7825
Volume*	0.6379	0.5308, 0.7450
Early granulocyte		
Conductivity*	0.6486	0.5408, 0.7563
Platelet count*	0.6352	0.5283, 0.7421
Neutropil:lymphocyte ratio	0.7125	0.6128, 0.8122
Lymphocyte:monocyte ratio*	0.5495	0.4296, 0.6695

*Predicts towards the direction of survival rather than to the direction of in-hospital mortality.

constantly updated,[26] where in triaging of cases and management is based on disease severity on admission. Given the association between vaccination status and disease severity, as well as disease severity on admission and patient management, disease severity on admission served as surrogate marker for these unmeasured factors. The possible confounding effects of disease severity were addressed by propensity score matching, as previously described.

Ultimately, four parameters from this study showed significant results in predicting in-hospital mortality among COVID-19 patients: total WBC count, ANC, AEC, and NLR. At best, the ANC has the highest sensitivity and specificity of 76.4% and 68.2%, respectively, at a cut off of 7.3 x 10⁹/L, followed by total WBC count with 70.9% and 66.2%, respectively, at a cut off of 9.9 x 10⁹/L. Results from a study using the same analyzer showed an AUC of 0.744 ($p<.001$) with a sensitivity of 69% and specificity of 71% for neutrophil counts at a cut off of 5.6 x 10⁹/dL in predicting admission for COVID-19.[22] Another study that compared hematologic parameters between non-severe and severe COVID-19 infected groups, the authors noted that for WBC at a cut off of 7.5, the sensitivity is 65% and the specificity is 53.5%; for Neutrophil count, at a cut off of 4.65, the sensitivity is 75% and the specificity is 60%; lastly, for NLR, at a cut off of 2.98, the sensitivity is 75% and the specificity is 61%.[27]
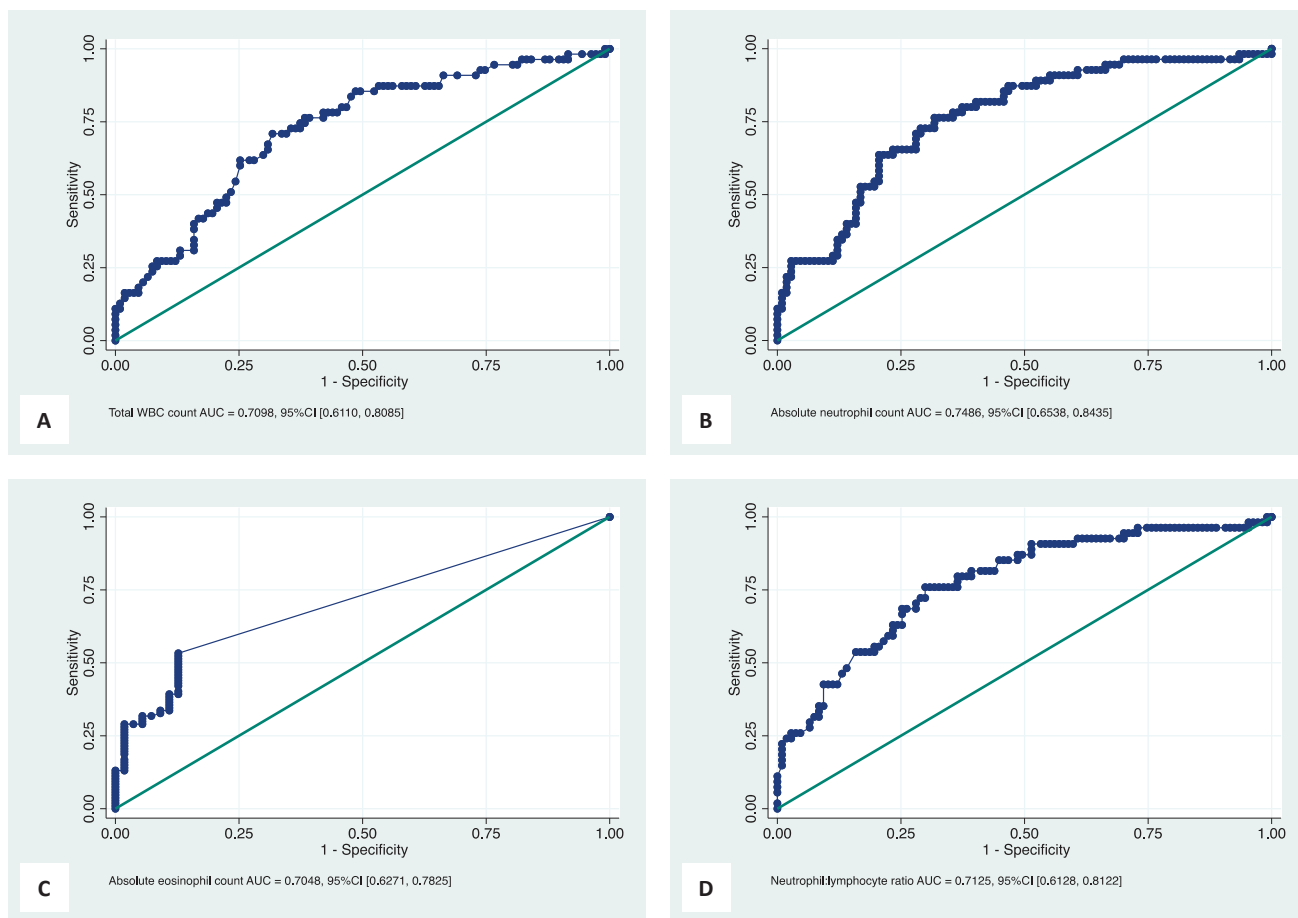

Figure 1. Receiver operating characteristic curves of admission CBC and CPD parameters of in-hospital mortality among admitted COVID-19 patients. **(A)** total WBC count; **(B)** absolute neutrophil count; **(C)** absolute eosinophil count; **(D)** neutrophil:lymphocyte ratio.

Table 5. Cut-off analysis of admission CBC and CPD parameters as predictors of in-hospital mortality among admitted COVID-19 patients

Blood cell parameters	Cut-off (x 10⁹/L)	Youden index	Sensitivity		Specificity	
			Estimate	95% CI	Estimate	95% CI
Total WBC count	9.9	0.391	70.9%	57.1, 82.4	66.2%	58.5, 76.9
Absolute neutrophil count	7.3	0.461	76.4%	63.0, 86.8	68.2%	58.5, 76.9
Absolute eosinophil count*	0.006	0.425	53.3%	43.4, 63.0	87.3%	75.5, 94.7
Neutropil:lymphocyte ratio	7.62	0.481	76.4%	63.0, 86.8	70.1%	60.5, 78.6

*Predicts towards the direction of survival rather than to the direction of in-hospital mortality.

While comparable with results of other studies, the performance of these parameters as early prognostic markers for in-house mortality appears to be less than ideal. Analysis of only the baseline sample may be insufficient to predict the ultimate outcome of patients. Additional monitoring of CBC and CPD parameters taken at various points during admission may give a better picture on their role in predicting patient outcomes. Nonetheless, the study may provide evidence that some these parameters show promise as prognostic markers. Correlation with other laboratory parameters and most importantly clinical context remains the gold standard in patient management.

CONCLUSION AND RECOMMENDATION

This study shows that baseline CBC and CPD parameters show weak correlation with disease severity on admission. The total WBC count, ANC, and NLR are statistically significant predictors for in-hospital mortality, while AEC predicts towards the direction of survival. The sensitivities and specificities of the cut off for these parameters are less than ideal. Correlation with clinical and other laboratory parameters is still recommended. For future studies, the authors recommend monitoring CBC and CPD parameters at different time points during the patients' hospital course.

STATEMENT OF AUTHORSHIP

The authors certified fulfilment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

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Profiling of Genetic Mutations among Adult Filipino Patients Diagnosed with Acute Myeloid Leukemia using Fluorescence In Situ Hybridization from 2014 to 2021: A Single-Institution Study*

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ABSTRACT

Introduction. Among patients with Acute Myeloid Leukemia (AML), the karyotype at diagnosis is an important prognostic indicator for predicting outcomes. Several studies have been done to identify the most common cytogenetic abnormalities seen in patients in other countries, however, limited studies have been done in our setting.

Objective. The study aims to determine the most common abnormalities present among patients with AML referred for Fluorescence in situ Hybridization (FISH) at the National Kidney and Transplant Institute.

Methodology. The study included 131 adult patients with a mean age of 46. Fluorescence in situ Hybridization was used to identify the following cytogenetic abnormalities: t(8;21), 11q23 (MLL), 16q22 (CBFB-MYH11), t(15;17) (PML/RARA), t(9;22) (BCR/ABL), 7q31 deletion, and Monosomy 7.

Results. FISH was negative in 40% (n=53) of patients. 7q31 deletion is the most frequently identified cytogenetic abnormality among patients with a single abnormality (n=17, 13%) present and is the most frequently identified abnormality among patients with multiple abnormalities (n=26). 7q31 deletion is more frequently observed among patients between the ages 51 to 60 years old and among patients with AML with monocytic differentiation. 22% (n=29) of patients have multiple abnormalities, with the most common abnormalities to occur together are 7q31 deletion and t(8;21) (n=20, 15%). Patients with negative results and patients with multiple cytogenetic abnormalities are commonly seen within the 41 to 50 age group.

Conclusion. The current study provides a single-institution view of the cytogenetic abnormalities among adult Filipino patients with AML using FISH. Further investigation on the clinical history of these patients, with correlation with other methods, as well as epidemiologic studies are needed to better understand the similarities and differences seen from previously reported incidences.

Key words: acute myeloid leukemia, fluorescence in situ hybridization, cytogenetics, profiling, hematology, Filipino

INTRODUCTION

Acute Myeloid Leukemia (AML) is a hematologic malignancy that is characterized by increased blasts of myeloid lineage in the bone marrow to the point of detection in the peripheral blood and overwhelms the synthetic capacity of the bone marrow.[1] It is the most common acute leukemia among adults, and the incidence increases with age. The age-adjusted incidence of AML for all races in 2018 is 4.3 per 100,000 persons with a higher male-to-female ratio (5.2 : 3.6).[2]

It is a heterogeneous disease not only regarding morphology and clinical presentation but in the sense that they entail genetic alterations and epigenetic changes in the hematopoietic cells that regulate its growth and differentiation that can be detected through molecular and cytogenetic methods.[3] Various structural and numeric cytogenetic aberrations have been identified which has diagnostic and prognostic implications.[4–6] These rearrangements result in fusion genes that encode for an abnormal chimeric protein required for

leukemic transformation. Some of these alterations have characteristic immunophenotypes, like t(15;17) which results in Acute Promyelocytic Leukemia (APL). Moreover, these alterations have changed our view on how we classify AML as several of these cytogenetic abnormalities have become essential diagnostic criteria for certain subtypes of AML, bypassing the required 20% blast cut-off previously set by the World Health Organization (WHO) Classification of Hematolymphoid Tumors.[7]

These cytogenetic findings thus become an important prognostic indicator used in the clinical management of patients with AML.[8] Pretreatment karyotype is an important prognostic risk factor for achieving complete remission, disease-free survival, and overall survival in adult and pediatric patients with AML, hence, detection of these genetic abnormalities is now included in the routine diagnostic workup of newly diagnosed patients with AML.[9,10] Cytogenetics are also used to stratify patients into distinct prognostic groups to provide risk-adapted chemotherapy protocols.[11] Cytogenetic profiling of AML has been undertaken among patients in other countries,[1,3,12–15] but none so far has been done among Filipinos.

This investigation aims to determine the local prevalence of cytogenetic abnormalities as detected by FISH among patients referred to the National Kidney and Transplant Institute Medical Laboratory (NKTIML) from January 2014 to December 2021.

METHODOLOGY

Study design
This research is a retrospective cross-sectional study which utilized data from the FISH studies performed at the Fluorescence In situ Hybridization and Cytogenetics Section of NKTIML.

Study population
The study included all FISH studies done at the NKTIML from the years 2014 to 2021, adhering to the following criteria: 1) Adult patients (Ages 18 and older) referred to the NKTIML for AML panel by FISH; 2) Diagnosed with AML according to the WHO Classification which includes bone marrow biopsy and/or flow cytometry studies.

Specimens failing to adhere to the criteria, as well as those affected by the following circumstances were excluded: 1) Patients with FISH studies for other malignancies; 2) Patients with clinical diagnosis with AML which cannot be proven through bone marrow biopsy and flow cytometry; 3) Patients with FISH studies for AML where no clinical information or diagnosis is available.

Method sampling
Total enumeration sampling was done. The NKTIML logbooks and laboratory database, accessible through laboratory information system, were reviewed for FISH studies and any bone marrow biopsy and/or flow cytometry studies.

Data collection
All data were collected over three (3) months by the principal investigator under close monitoring by a consultant pathologist. Data collection took place in the FISH and Cytogenetics Section and access to materials was limited to the investigators and medical technologists assigned to the FISH section. The results of the FISH studies of samples that fit the inclusion criteria were sub-classified where appropriate: Negative FISH, t(8;21), MLL (11q23), CBFB-MYH11 (16q22), t(15;17), t(9;22), 7q31 deletion, Monosomy 7 and multiple cytogenetic abnormalities (defined as having more than one cytogenetic abnormality). Patients were divided into seven age groups.

Ethical considerations
Patient confidentiality was ensured during data collection and encoded using numerical patient identifiers. This research protocol adheres to international ethical standards as provided by the International Conference on Harmonization Good Clinical Practice guidelines (ICH GCP) and National Ethical Guidelines for Health and Health-Related Research. Permission to access relevant laboratory records and medical information databases was secured upon approval of the chairperson of the Department of Pathology and Laboratory Medicine and head of the FISH section.

Statistical analysis
The percentage of cytogenetic abnormalities in the different age groups was computed. Chi-square test was used to analyze the difference in cytogenetics among the different age groups. A *p*-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Patient characteristics
Between January 2014 to 2021, 131 patients were included in the study. The average age of the patients included in this research is 46 (SD 16.15). There were 74 males and 57 females. There were 69 bone marrow samples and 62 peripheral blood samples. The average percent increased blast is 53% (SD 22.8). AML was the most common diagnosis among patients included in the study, followed by AML with monocytic differentiation (Table 1).

Cytogenetic abnormalities
Cytogenetic abnormalities seen among patients with AML are shown in Table 2. Among AML patients, no mutations were detected in 53 patients (40%). Cytogenetic abnormalities were detected in 60% (n=78) of patients. There were 29 patients (22%) with multiple abnormalities. The most common single mutation was 7q31 deletion (n=17, 13%), followed by t(8;21) (n=16, 12%), and t(15;17) (n=10, 8%). One patient (1%) with MLL (11q23), 4 patients (3%) with t(9;22), and 1 patient (1%) with Monosomy 7. There were no patients seen who harbor the CBFB-MYH11 (16q22) mutations. Among patients with multiple abnormalities, 7q31 deletion was still the most common mutation seen (n=26, 20%). Whereas the most common mutations seen together were t(8;21) + 7q31 deletion (n=20, 15%).

Table 3 shows the age-specific proportions of the cytogenetic abnormalities. The peak incidence of AML was 41 to 50 years old, with a mean age of 46. Most of the patients with negative FISH were between 41 to 50 years old. The

Table 1. Demographic profile of the patients

Patient's profile	Count (%), Mean ± SD
Age, mean ± SD	46 ± 16.15
Sex	
Male	74 (56.49%)
Female	57 (43.51%)
Blasts (%), mean ± SD	53 ± 22.8
Specimen	
Bone marrow	69 (52.67%)
Peripheral blood	62 (47.33%)
Diagnosis	
AML†	58 (44.27%)
AML† with monocytic differentiation	46 (35.11%)
APL‡	10 (7.63%)
AML† Minimally differentiated	4 (3.05%)
AML† with Myelomonocytic differentiation	1 (0.76%)
AML† with Erythroid Differentiation	1 (0.76%)
AML† vs. APL‡	1 (0.76%)
AML† with monocytic differentiation vs. APL‡	4 (3.05%)
AML† with Aberrant B-lymphoid expression	1 (0.76%)
Mixed Phenotype Acute Leukemia (Myeloid + B-Lymphoid)	1 (0.76%)
AML† with myelofibrosis	1 (0.76%)
AML† with history of Chronic Myelogenous Leukemia	1 (0.76%)
AML† with history of RAEB§ Type I	1 (0.76%)
AML† with history of Breast cancer	1 (0.76%)

†AML, Acute Myeloid Leukemia
‡APL, Acute Promyelocytic Leukemia
§RAEB, Refractory Anemia with Excess Blasts

Table 2. Cytogenetic findings in AML patients

Cytogenetic subtype	All AML patients (N=131)	
	Count (n)	Percent (%)
Negative FISH	53	40
Single abnormalities	49	38
t(8;21)	16	12
t(9;22)	4	3
7q31 deletion	17	13
(16q22)	0	0
(11q23)	1	1
t(15;17)	10	8
Monosomy 7	1	1
Multiple abnormalities	29	22
t(8;21) + 7q31 del	20	15
11q23 + 7q31 del	2	1
11q23 + t(9;22)	2	1
t(8;21) + 11q23 + 16q22 + 7q31 del	1	1
11q23 + 16q22	1	1
t(8;21) + 7q31 del + Monosomy 7	1	1
t(15;17) + 7q31 del	1	1
t(9;22) + 7q31 del + Monosomy 7	1	1
Total	**131**	**100**

AML, Acute Myeloid Leukemia

t(8;21), 7q31, t(15;17), and multiple abnormalities were significant in the Chi-square test for the difference. There is a significant difference between the number of AML patients in each age group. Patients between 41 to 50 years old also exhibited a higher number of patients diagnosed with t(8;21) and the highest number of cases with multiple abnormalities. Patients between 51 to 60 have the highest number of 7q31 deletions. The age group 21 to 30 has the highest number of patients diagnosed with t(15;17).

Tables 4 and 5 summarize the cytogenetic abnormalities per morphologic subtype. There were only 3 subtypes that have p-values since the rest had very few cases to be analyzed separately. t(8;21) was seen in 11 cases of AML. t(15;17) was seen in 7 cases of APL (including microgranular variants) and 3 cases in whom APL was considered versus AML and AML with monocytic differentiation. 7q31 deletion is seen in 7 cases of AML with monocytic differentiation. Of note, two patients with known Chronic Myelogenous Leukemia in leukemic transformation, both retained t(9;22). Fourteen patients with multiple abnormalities were AML, and 12 patients with multiple abnormalities were diagnosed with AML with monocytic differentiation. The t(8;21) + 7q31 deletion were the most common cytogenetic abnormalities to occur together (n=20) and is seen most commonly among patients with AML (n=11) and AML with monocytic differentiation (n=7).

The study was conducted to determine the most common cytogenetic abnormalities seen among patients with AML using FISH studies performed at our institution. Karyotyping at the time of diagnosis is essential, not only to the pathologist to confirm the diagnosis, but also to the clinician, whose decision to start treatment, as well as, to stratify patients into prognostic groups, relies on this information.

In the current study, the peak incidence of AML occurs at 41-50 years with a mean age of 46 years old which is younger than in the study by Byun et al (51 years old)[12] but older as in the studies performed by Elnaggar (36.5 years old)[1] and Meng (39 years old).[3]

Unlike in the studies done by Elnaggar, Meng, Byun et al., and Shaikh, 7q31 deletion (13%), is as common as the t(8;21) (12%).[1,3,12,14] Across different studies, the current study shows a higher percentage of patients with

Table 3. Age-specific proportions of cytogenetic abnormalities

Cytogenetic subtype	count (n)	group (≤20)	Age group (21-30)	Age group (31-40)	Age group (41-50)	Age group (51-60)	Age group (61-70)	Age group (71-80)	Age group (>80)	p
Negative FISH	53	3	9	7	11	8	8	5	2	0.214185
t(8;21)	16	0	3	2	6	3	2	0	0	0.035994*
t(9;22)	4	0	1	1	1	0	1	0	0	0.779774
7q31 deletion	17	1	2	3	2	7	2	0	0	0.014415*
(16q22)	0	0	0	0	0	0	0	0	0	NA
(11q23)	1	0	0	0	0	1	0	0	0	NA
t(15;17)	10	0	4	3	1	1	1	0	0	0.025164*
Monosomy 7	1	0	1	0	0	0	0	0	0	NA
Multiple cytogenetic abnormalities	29	1	2	5	11	6	2	2	0	0.001841*
Total	131	5	22	21	32	26	16	7	2	

*p<0.05. is considered significant in 95% CI.

Table 4. Cytogenetic abnormalities per morphologic subtype

Cytogenetic Abnormalities per Subtype	t(8;21)	11q23	16q22	t(9;22)	t(15;17)	7q31 deletion	Monosomy 7	Total	p
AML[†]	11	0	0	2	0	8	1	**22**	0.00000*
APL[‡]	0	0	0	0	6	0	0	**6**	0.00000*
AML[†] with monocytic differentiation	5	0	0	0	0	7	0	**12**	0.00002*
APL[‡] Microgranular variant	0	0	0	0	1	0	0	**1**	NA
AML[†] vs APL[‡] Microgranular variant	0	1	0	0	0	0	0	**1**	NA
AML[†] with monocytic differentiation vs. APL[‡] Microgranular variant	0	0	0	0	2	0	0	**2**	NA
APL[‡] Microgranular variant vs. AML[†] with monocytic differentiation	0	0	0	0	1	0	0	**1**	NA
AML[†] Est case of CML[§]	0	0	0	1	0	0	0	**1**	NA
AML[†] with previous diagnosis of breast cancer	0	0	0	0	0	1	0	**1**	NA
AML[†] with minimal differentiation	0	0	0	0	0	1	0	**1**	NA
Mixed phenotype Acute Leukemia (B/Myeloid) Est CML[§]	0	0	0	1	0	0	0	**1**	NA
Total	16	1	0	4	10	17	1	49	

*p<0.05 is considered significant in 95% CI.
[†]AML, Acute Myeloid Leukemia
[‡]APL, Acute Promyelocytic Leukemia
[§]CML, Chronic Myelogenous Leukemia

Table 5. Cytogenetic abnormalities per morphologic subtype in patients with multiple abnormalities

Cytogenetic Abnormalities per Subtype	t(8;21) + 7q31	11q23 + 7q31	11q23 + t(9;22)	t(8;21) + 11q23 + 16q22 + 7q31	t(8;21) + t(9;22) + 7q31	t(8;21) + 7q31 + Monosomy 7	11q23 + 16q22	t(15;17) + 7q31	t(9;22) + 7q31 + Monosomy 7	Total	p
AML[†]	11	0	1	0	0	1	0	0	1	**14**	0.0001*
APL[‡]	0	0	0	0	0	0	0	1	0	**1**	NA
AML[†] with monocytic differentiation	7	2	0	1	1	0	1	0	0	**12**	0.0001*
AML[†] with minimal differentiation	1	0	0	0	0	0	0	0	0	**1**	NA
AML[†] with aberrant B lymphoid expression	1	0	0	0	0	0	0	0	0	**1**	NA
Total	20	2	1	1	1	1	1	1	1	29	-

*p<0.05 is considered significant in 95% CI.
[†]AML, Acute Myeloid Leukemia
[‡]APL, Acute Promyelocytic Leukemia

chromosome 7 abnormalities compared to other studies where the more common findings are translocations t(8;21),[3,12,14] t(15;17)[1] and Trisomy 8.[15,16] 7q31 deletion is also the most common mutation seen among patients with multiple abnormalities seen in 26 cases, similar to the study done by Byrd et al in 2002, wherein deletions involving 7q rarely occur as isolated aberrations.[16] The aberration is also seen significantly among patients with AML with monocytic differentiation. Now the literature regarding the immunophenotype and morphology among patients with 7q31 deletions or mutations in chromosome 7 is limited and requires further study. A study done by Chen, Wood, and Cherian, analyzed the flow cytometry parameters among Myelodysplastic Syndrome (MDS), Myeloproliferative neoplasms (MPN), and AML patients with monosomy 7 and 7q deletions. An increase in CD14 expression on maturing granulocytic cells was seen more frequently in myeloid neoplasms with monosomy 7 than in 7q deletions. CD14 is a GPI-anchored protein expressed among monocytes.[17] 7q31 deletions are associated with a poorer prognosis among patients with AML[16] and in the recent WHO Classification are linked with Myelodysplastic Syndrome and Acute Myeloid Leukemia as well as secondary forms of AML and MDS.[11,18] The mechanism on how mutations in chromosome 7 drive tumorigenesis is still poorly understood and it is hypothesized that a possible tumor suppressor gene that resides in the long arm of chromosome 7 is lost among patients with Monosomy 7 or in 7q deletions.[19] Several studies have tried to investigate such a phenomenon. McNerney et al., demonstrated that CUX1, a tumor suppressor gene in the long arm of chromosome 7 is inactivated among patients with AML.[20]

A negative FISH result was seen in 40% of the population. This is a similar finding to the study done by Byun et al. (42.3%),[12] however, is lower than in the study done by Meng (69.6%)[3] and Byrd (48%).[16] This could mean that either there are no cytogenetic abnormalities present or that there are cytogenetic abnormalities present that are not included in the FISH panel currently done in our institution. In our institution, commercially available probes (Abbott Laboratories, Abbott Park, Illinois) were used. These are available for panel testing consisting of t(8;21), MLL (11q23) rearrangement, CBFB-MYH11 (16q22), t(15;17), t(9;22), 7q31 deletion, and Monosomy 7, while other available markers such as -5/5q deletion, ETV6 mutations, TP53 deletion, and 9q34 rearrangements can be ordered individually. A review article by Gonzales and Mikhail lists other recommended FISH markers, mainly associated with intermediate to poor risk among patients with AML, such as Trisomy 8, MLL gene (11q23) fusion partners, inv(3)(q21q26) or t(3;3)(q21;q26) with MECOM (EVI1) aberrant expression, and t(6;9)(p22.3;q34) with DEK-NUP214 fusion, but these are currently not available in our institution.[21] The NCCN also recommends karyotyping, multiplex gene panels, and next-generation sequencing analysis to develop a more comprehensive diagnostic and prognostic assessment.[11] Patients with multiple abnormalities comprise approximately one-fifth of the population, ranging from 2 up to 4 mutations. A complex karyotype, defined as having ≥3 abnormalities cannot be assumed since one method of detection was used. There are, however, three patients (2%) in the population that meet this requirement which is seen lower in frequency than in the study done by Byun et al. (12.5%)[12]

and Shaikh (9%).[14] An investigation on the clinical history of these patients, when correlated with other molecular and cytogenetic studies can give us more information to better understand the pathogenesis, epidemiology and clinical and laboratory features. Further, the differences in demographic characteristics, ethnicity, socio-economic, environmental, and genetic factors may also be explored.

CONCLUSION AND RECOMMENDATIONS

The current study provides a single-institution view of the cytogenetic abnormalities among adult patients with AML using FISH. The results of the study showed that the most frequent cytogenetic abnormalities are 7q31 deletion followed by t(8;21) as the most common mutations seen among patients with single mutations whereas 7q31 deletion is the most frequent abnormality seen overall among patients with multiple mutations. The study also found 7q31 to be frequent among patients with AML with monocytic differentiation. Further investigation on the clinical history of these patients, with correlation with other methods as well as epidemiologic studies can be done in the future to confirm the findings of the study and provide more information to better understand the possible underlying mechanisms.

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Evaluation of the Effectiveness of Lean Six Sigma Approach for SARS-CoV-2 RT-PCR Turnaround Time (TAT) Improvement at a Hospital-Based Tertiary Laboratory

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ABSTRACT

Objectives. This study aims to evaluate the effectiveness of the Lean Six Sigma approach in improving procedure for (TAT) of reverse transcriptase polymerase chain reaction (RT-PCR) for SARS-CoV-2 testing at The Medical City. Specific objectives of the study are to determine the following: 1) baseline sigma and average TAT (in hours); 2) post-implementation sigma and average TAT (in hours) 3) compare if there is a significant improvement between baseline and post-implementation sigma and average TAT (in hours) 4) effect on workflow efficiency.

Methodology. Lean Six Sigma method for quality improvement was applied using DMAIC: Define, Measure, Improve, and Control. The root causes identified were lack of manpower, equipment, space, and manual and complex processes. Then, process wastes were identified, and corresponding proposed solutions were sustained in the control phase, such as standardization and the use of automation. Measurement of turn-around time and six sigma of the process were performed for evaluation.

Results. Results showed a significant improvement in the TAT in RT-PCR results, with most results released within 24 hours. The pre-Lean Six Sigma data on TAT were as follows: 24.88% released within 24 hours; 65.14% released within 24-48 hours; 3.56% released within 48-72 hours, and 6.42% released in more than 72 hours. The post Lean Six Sigma TAT were as follows: 95.32% released within 24 hours; 4.29% released within 24 to 48 hours; 0.13% released within 48-72 hours, and 0.12% released more than 72 hours. The computed sigma post-implementation was increased from 3.56 to 4.82. The p-value was calculated using the chi-square test, and the computed chi-square statistic is 1894.1021. The p-value is <0.00001 and the result is significant at $p<.05$. Although there is a significant decrease in the volume of samples post implementation due to the changing COVID-19 situation, real time TAT was improved. It also resulted to increased workflow efficiency with the use of lesser manpower with more appropriate utilization.

Conclusion. Applying the Lean Six Sigma method to improve quality processes in the laboratory is shown to be practical, cost-effective, and straightforward.

Key words: Lean Six Sigma, SARS-CoV-2, turnaround time

INTRODUCTION

Background

When the novel coronavirus (COVID-19) pandemic hit the Philippines in the year 2020, there were only a few laboratories capable of performing reverse transcriptase polymerase chain reaction (RT-PCR) testing for SARS-CoV-2. During this time, there were significant delays in the release of results, as laboratory healthcare workers were overwhelmed by the surge of specimens from all over the country. One of the biggest challenges that our laboratory faced was the tedious manual process covering the different phases of testing. All results were manually encoded, including government regulatory requirements such as line lists and certifications. There was increased utilization manpower and even misutilization because even those with different job descriptions such as pathologists, residents, medical technologists, allied medical professionals were performing encoding tasks beyond the working hours. In these situations, a management tool such as the Lean Six Sigma (LSS) approach can be useful for quality improvement.

Since the late 1990s, the application of LSS in the manufacturing industry has come a long way.[1,2] Six Sigma is a quality management strategy that makes efforts to improve the quality of processes, utilizing the DMAIC process (Define, Measure, Analyze, Improve, and Control).[3] The "Lean" concept, on the other hand, is also a powerful quality-improvement tool that focuses on providing "value" and improving performance by systematically eliminating waste or non-value-added activities, from the process.[1,3] Combined, LSS becomes even more effective, with increase in popularity In the field of healthcare, even in laboratory medicine. This might be due to the fact that quality in healthcare is hard to measure but using this approach provides metrics to be able to make better assessments. In addition, oftentimes it is also hard to justify allocation of resources for process improvement without an evidence-based or data driven approach.

We used LSS to improve the quality of SARS-COV-2 RT-PCR testing in our laboratory. We aim to evaluate the over-all effectiveness of this approach in improving the TAT and workflow efficiency of RT-PCR procedure for SARS-CoV-2 testing at The Medical City.

METHODOLOGY

Population and sample
The study population included all samples submitted for SARS-CoV-2 RT PCR testing from April 2020 to May 2021. There are 73,998 tests within this period.

Inclusion and exclusion criteria
The study includes all SARS-CoV-2 RT PCR testing samples, whether oropharyngeal, nasopharyngeal, nasal/oropharyngeal swab, sputum, ETA, and other body fluids with correctly labeled samples, placed in correct containers and with filled up case investigation form (CIF). Samples with incorrect containers, mislabeled specimens, no CIF, and discrepancies in CIF were excluded in the study.

DMAIC process
The study was conducted within the Clinical Pathology section of the Department of Laboratory Medicine and Pathology, The Medical City. A Lean Six Sigma process involved the DMAIC process as follows:
1. **Define phase**, a project charter was made which includes the project objectives, importance, the scope, responsibilities of each member, budget/ resources, expectations/ assumptions, and timelines. A SIPOC diagram (suppliers, input, process, output, and customer) was performed to outline and give the appropriate scope of the process involved (Figure 1). Voice of the customer included informal feedback from the internal and external customers regarding the TAT of RT-PCR results.
2. **Measure phase**. The study included all samples submitted for SARS-CoV-2 RT PCR testing from April 2020 to May 2021 at The Medical City. Data were extracted from the laboratory database, which records information about the patient's assigned accession number, date of specimen received in the laboratory, and date result was released. Data collected were encoded and tabulated using Microsoft Excel 2019. Patients were identified only through their control numbers. TAT was categorized as to whether a sample was released within the following number of hours: within 24 hours, 24 to 48 hours, 48 to 72 hours, and more than 72 hours. Baseline sigma was calculated, as well. A detailed process map was done to lay out the entire process, which included the exact time of each step (Figure 2).
3. **Analyze phase**. The Analysis stage was conducted by brainstorming and a fishbone analysis diagram. From the detailed process map, the wastes were identified. Tools such as the fishbone diagram (cause and effect diagram) were utilized during the brainstorming to identify all the possible causes (Figure 3). All the reasons were analyzed using the effort impact diagram (Figure 4).
4. **Improvement phase**. The effort impact diagram (Figure 4) showed the best solutions to the problem, which was implemented using various tools. The 5S and the lean approach were utilized, which include auto-stop and error-proofing using automation.
5. **Control phase**. Implementation of the control and feedback system. In this stage, the corrective actions were evaluated to determine whether they led to performance improvements in the analysis process. A quality control plan was implemented to improve the TAT and the process continuously.

The project was divided into the following phases: a) process/procedure standardization; b) evaluation of corrective actions: evaluation involved data analysis, brainstorming activities, identification, and control of key performance indicators; and c) continuous improvement:

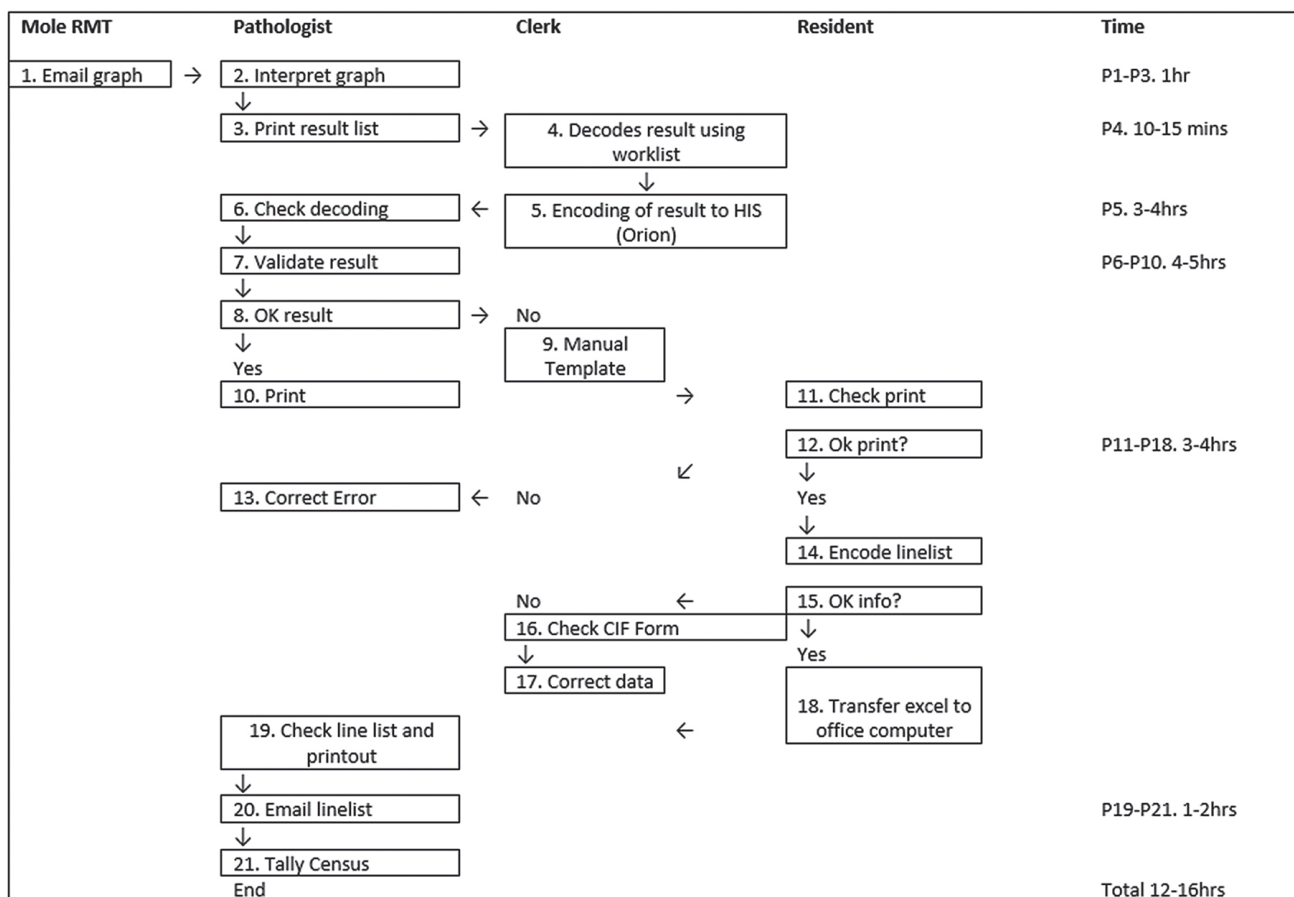| Supplier	Input	Process	Output	Customer
Messenger	CIF forms	Pre-encoding	Draft of line list Patient profile Charges	Residents
Molecular Medical Technologist	Graphs from PCR results	Interpretation	Excel with interpretation	Clerk
Molecular Medical Technologist and Consultant Pathologists	Worklist Excel results Orion Profile of patients	Typing	Typed results in Orion	Consultant Pathologist
Clerk	Worklist with results	Validation	Printed results	Residents
CIF encoders and clerk	Draft line list Worklists/reports	Line lists	Line list for Positive patients Line list for Negative patients	DOH

Figure 1. SIPOC diagram (post analysis).

Mole RMT	Pathologist	Clerk	Resident	Time
1. Email graph →	2. Interpret graph			P1-P3. 1hr
	3. Print result list →	4. Decodes result using worklist		P4. 10-15 mins
	6. Check decoding ←	5. Encoding of result to HIS (Orion)		P5. 3-4hrs
	7. Validate result			P6-P10. 4-5hrs
	8. OK result → No	9. Manual Template		
	Yes			
	10. Print →		11. Check print	
			12. Ok print?	P11-P18. 3-4hrs
			Yes	
	13. Correct Error ← No	↙	14. Encode linelist	
	No ←	16. Check CIF Form	15. OK info?	
		17. Correct data	Yes	
			18. Transfer excel to office computer	
	19. Check line list and printout		←	
	20. Email linelist			P19-P21. 1-2hrs
	21. Tally Census			
	End			Total 12-16hrs

Figure 2. Detailed process map (post analysis).

EQUIPMENT
- Insufficient equipment
- Lack of PCR machine and biosafety cabinet

PROCESS
- Manual encoding
- Complex procedures
- Contamination in process

PEOPLE
- Insufficient manpower
- Insufficient skilled molecular medical technologist
- Health status of medical technologist

Long TAT for COVID RT-PCR results

MATERIAL
- PCR consumables used (quality and availability)
- Stability of reagents and kits used

ENVIRONMENT
- Limited space for expansion
- Ideal flow of specimen not in place

MANAGEMENT
- Lack of budget support for manpower and budget for supplies
- High volume of reports to be submitted
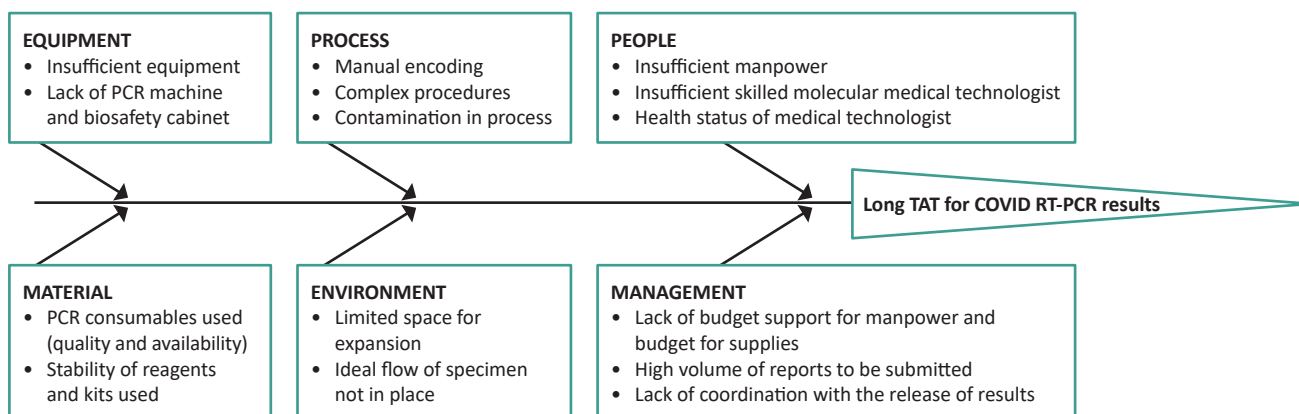- Lack of coordination with the release of results

Figure 3. Fishbone analysis diagram.

staff training system, automation of the process through the Laboratory Information System (LIS).

Analysis

After data were collected and encoded using Microsoft Excel 2019, TAT was categorized as whether a sample was released within the following number of hours: within 24 hours, 24 to 48 hours, 48 to 72 hours, and more than 72 hours. The TAT is determined by subtracting the date the specimens were received from the date the results were released. Six sigma computation was performed by counting the defects, and opportunities and computing the process yield, using the formulas:

Formula 1: Yield = (1-DPO) x 100
Formula 2: DPO = D / N x O

Where:
O = determine the number of defect opportunities per unit.
N = determine the number of units processed.
D = determine the total number of defects made (Include defects caused and later fixed)

TAT analysis was performed for all RT-PCR tests from the start of operations defined as "pre-Lean Six Sigma" data (from April to November 2020) and compared with

Table 1. Lean Six Sigma table

Sigma level	Defects per million opportunities	Percentage yield
1	691,462	31
2	308,537	69
3	66,807	93.3
4	6,210	99.38
5	233	99.977
6	3.4	99.99966

data gathered after the Lean Six Sigma improvement phase (December 2020 to May 2021) labeled as "post-Lean Six Sigma" data. Refer to the Sigma Table (Table 1) to determine the Sigma in the process. To compare if there is a significant improvement between baseline and post-implementation sigma and average TAT (in hours), we calculated the p-value using the chi-square test with the formula below. We used <72 hours and >72 hours in computing for the chi-square test, since 72 hours was the prescribed time of release of SARS-CoV-2 RT PCR results by DOH at the start of pandemic.

$$\chi^2 = \sum_{i=1}^{c} \sum_{j=i}^{r} \frac{\left(Observed\ value_{ij} - Expected\ value_{ij}\right)^2}{Expected\ value_{ij}}$$

Where different values of *P* indicate the different hypothesis interpretations, are given below:

$P \le 0.05$: Hypothesis rejected.
$P > .05$: Hypothesis Accepted.

RESULTS

The pre-Lean Six Sigma data on TAT were as follows: 24.88% released within 24 hours; 65.14% released within 24-48 hours; 3.56% released within 48-72 hours, and 6.42% released in more than 72 hours (Table 2). The post Lean Six Sigma TAT were as follows: 95.32% released within 24 hours; 4.29% released within 24 to 48 hours; 0.13% released within 48-72 hours, and 0.12% released more than 72 hours. There was a significant improvement in the TAT, with most results released within 24 hours. The baseline sigma was also computed at 3.56, and after implementation of Lean Six Sigma, it increased to 4.82 (Table 3).

To compare significant improvement between baseline and post-implementation on average TAT (in hours), we calculated the p-value using the chi-square test. The computed chi-square statistic is 1894.1021 (Table 4). There was a significant difference in the (TAT) of RT-PCR for SARS-CoV-2 testing after applying the Lean Six Sigma Approach.

DISCUSSION AND CONCLUSION

The root causes of the long TAT identified during the Lean Six Sigma implementation were insufficient manpower, space, equipment, manual processes, complex procedures, and high workload volume (Figure 3). Among these, manual encoding, insufficient equipment, and lack of manpower were identified to give the highest impact on the long TAT of results, hence they were shown the highest priority for improvement (Figure 4). TAT has significantly improved due to eliminating the wastes by utilizing auto-stop and error-proofing techniques by automating manual processes using the LIS. The 5-S was also executed to organize the workplace, maximize flexibility, minimize motion, and generally eliminate workplace waste.

After applying Lean Six Sigma, there was a significant improvement in the TAT, with most results released within 24 hours compared with the pre-implementation TAT of
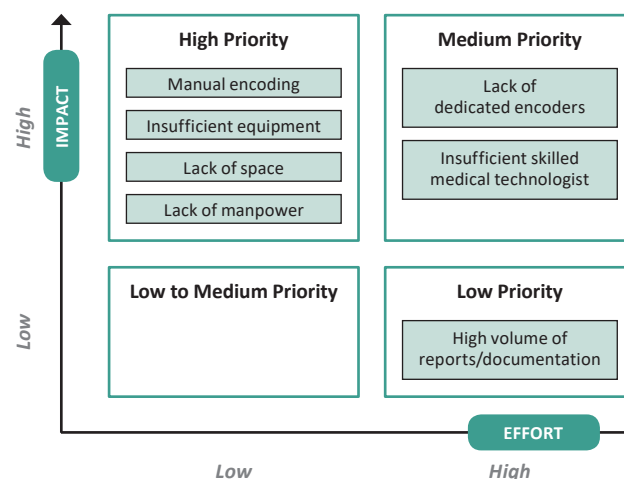

Figure 4. Effort impact diagram.

Table 2. Average TAT of RT-PCR for SARS-CoV-2, at The Medical City, pre-lean six sigma" data (from April to November 2020) and "post-lean six sigma" (December 2020 to May 2021)

TAT	Total number of cases released from April 2020 to December 2020	Percent of cases released %	Total number of cases released from January 2021 to May 2021	Percent of cases released %
Within 24 hours	10988	24.88%	28444	95.32%
24 to 48 hours	28764	65.14%	1320	4.42%
48 to 72 hours	1572	3.56%	40	0.13%
>72 hours	2834	6.42%	36	0.12%
Total	44158	100.00%	29840	100.00%

Table 3. Lean Six Sigma computation "pre" and "post" implementation

	Pre-Lean Six Sigma	Post-Lean Six Sigma
O	3	3
N	44158	29840
D	2834	36
DPO	0.02139	0.0004
Yield	97.86%	99.96%
Process Six Sigma	3.53	4.85

Table 4. TAT pre- and post-implementation of Lean Six Sigma approach

TAT of results released	Pre-LSS	Post-LSS	Total
Within 72 hours	41,324	29,804	71,128
More than 72 hours	2,834	36	2,870
Total	44,158	29,840	73,998

The computed chi-square statistic is 1894.1021. The *p*-value is <.00001, and the result is significant at *p*<.05. Degree of freedom (df) = 1.

48 hours. Six sigma of the process was also increased to 4.82 post-implementation from 3.53 pre-implementation. This can be interpreted using the sigma scale of 1 to 6. A performance that is close or higher than 6 indicates world-class performance with only 3.4 defects per million opportunities or a yield of 99.99966%. On the other hand, a low sigma level of 1 indicates 691,462 defects per million opportunities of 31% yield (Table 1). A low sigma value and a value less than three are considered unstable and unacceptable. This would likely cost a laboratory a lot of money, time, and effort to maintain the quality of test results.[4] Inclusion of six sigma allows for a quantifiable scale where quality can be measured, and improvement can be better monitored, especially when we want to see even small improvements in the process. The study results are comparable with other studies, where a significant positive change in TAT was observed after initiating and implementing the Lean process throughout their laboratory.[2,5,6] However, the impact of the Lean Six Sigma on TAT was reduced due to the significant drop in the number of samples received post implementation.

One of the limitations of this study is the unstable situation of the pandemic causing the lack of control in the volume of samples sent. The lower number samples sent may have contributed also to the improvement in TAT as well but the target TATs were achieved with more ease post implementation using lesser manpower. Previously, there were eight support staff, two residents, and two pathologists helping with the clerical work (a total of 12 people). After implementing the automated processes, this was reduced to eight administrative staff performing all the clerical work. These staff were also able to manage all the tasks other than producing results, such as answering concerns, emails, auditing, and even helping different sections in the laboratory. Typographical errors were also eliminated as most of the tasks, such as results generation, were automated through the laboratory information system. These results were similar to other studies where after implementation of Lean Six Sigma, the key performance metrics and workforce utilization has improved hence reducing staff and teams' idle time, resulting to cost reduction.[5] Although low volume sample during post implementation reduced the impact of LSS on TAT, there was notable improvement in manpower utilization and workflow which resulted in cost savings and improved customer satisfaction.[7,8] The study supports that Lean Six Sigma is an effective tool in improving processes in a workplace and can be highly adaptable in the laboratory setting.

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STATEMENT OF AUTHORSHIP

The authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

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SARS-CoV-2 RT-PCR Ct Value and Laboratory Tests: Clinicopathologic Characteristics among Adult Filipino Inpatients diagnosed with COVID-19 in a Tertiary Medical Center

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ABSTRACT

Introduction. The role of the laboratory during the COVID-19 pandemic is not limited to just diagnosis of the disease, but also in clinical decision-making, by providing information on relevant laboratory biomarkers. Clinicians also use Ct value to guide patient management. There are limited studies available locally regarding the significance of Ct value and pertinent laboratory biomarkers in COVID-19 patients. This study aimed to assess the aforementioned laboratory data, along with the clinicopathologic characteristics of affected patients, and determined if this information may be useful for robust clinical decision-making.

Methodology. In this retrospective analytic study, we identified 325 out of 1,049 adult Filipino inpatients diagnosed with COVID-19 and analyzed their Ct values and pertinent laboratory biomarkers such as neutrophil and lymphocyte count, platelet count, LDH, ferritin, procalcitonin, CRP, AST/SGOT, ALT/SGPT, PT/INR, and D-dimer, and correlated them with the severity of the disease.

Results. Two hundred twenty (67.7%) patients had non-severe disease, while 105 (32.3%) had severe disease. Lower Ct values of ORF1ab (median = 26.4) and N (median = 24.8) genes were seen in the severe group compared to the non-severe group and were found to be significant ($p<0.001$). Laboratory markers (neutrophil, platelet counts, LDH, ferritin, procalcitonin, CRP, AST, PT/INR, and D-dimer) were associated with severe COVID-19. On the other hand, ALT was not associated with severe disease.

Conclusion. The laboratory biomarkers together with Ct value and overall clinical picture may provide valuable information to physicians for more robust clinical decision-making.

Key words: COVID-19, cycle threshold, laboratory biomarkers, SARS-CoV-2, RT-PCR

INTRODUCTION

COVID-19 is a disease caused by a novel beta coronavirus, SARS-CoV-2, which was first discovered in Wuhan, China last December 2019 and has subsequently spread all over the globe.[1] Based on the World Health Organization (WHO) situation report as of March 8, 2023, the Philippines has had 4,077,302 confirmed cases, with 98.14% recovered cases and 1.86% fatalities.[2] Among the severe (13%) and critical (5.5%) cases, the most affected were individuals aged 60-69 years (26%) followed by those aged 70-79 years (22%), and around 14% of deaths involved those aged 80 years and up.[2]

Currently, both globally and in the Philippines, the most widely used method to confirm the diagnosis of COVID-19 is by detecting viral nucleic acids in respiratory tract samples with real time reverse transcriptase polymerase chain reaction (RT-PCR).[3] The WHO recommends that SARS-CoV-2 RT-PCR tests detect certain viral genes, such as *ORF1ab* (Open Reading Frame) and *N* (nucleocapsid), which were discovered early in the viral genome, alongside *S* (spike) and *E* (envelope) genes.[4] These genes in particular code for major structural proteins in the SARS-CoV-2 virus, which help to differentiate it from other members of the coronavirus family.[5] The data obtained by RT-PCR test is reported as cycle threshold (Ct) value, which

represents the number of times an amplification of a target gene occurs prior to detection.[6,7] Ct values in theory can represent an indirect measurement of viral load, and their relationship is inversely proportional, such that a higher viral load would be represented by a lower Ct value.[6–8] The RT-PCR for the detection of SARS-CoV-2 viral RNA is a qualitative test, i.e., a Ct value less than the determined cut-off value is considered positive and the absence of a Ct value or more than the set cut-off is considered as negative.[6] Although RT-PCR for COVID-19 testing is a qualitative test, the majority of the clinicians use the Ct value in the management of patients.[9,10] Current data suggest that a lower Ct value may be associated with poor prognosis, abnormal biomarkers, and generally a worse clinical outcome, though this is not consistent, as Ct values can be affected by other variables inherent to the test itself or by individual patient variables.[7,8,11]

Of note is the multitude of variables that affect the Ct value. The majority of these variables are pre-analytic, which can include patient factors, disease factors, sampling methods, specimen transport and age.[12] With regards to sampling technique, the laboratory cannot distinguish whether it is of an anterior nasal swab, a mid-turbinate swab, or a combined oro/nasopharyngeal swab. The lack of a standardized sampling method may contribute to the variability of the Ct value because of the differences in sample quality, with lower sensitivities found in nasal swabs, throat swabs, and saliva. RNA material concentration varies in specimen type and therefore has an influence on the Ct value.[12,13]

The clinical picture of COVID-19 varies widely, and can range from asymptomatic persons, or those with a mild upper respiratory tract illness, up to severe or critical cases of acute respiratory distress requiring mechanical ventilation with high morbidity and mortality (up to 94%).[3,14]

Correlation with the patient's history is important when dealing with Ct values. It was reported that the highest viral load belonged to the presymptomatic stage. Thus, the timing of specimen collection must be noted by the clinician when interpreting the Ct value.[15] Another study has shown that Ct values are highest among asymptomatic infections, with the values decreasing as the patients become presymptomatic and symptomatic. This supports the idea that Ct values reflect viral load and disease severity.[16]

In addition, some patients may exhibit prolonged viral shedding, with low Ct values persisting particularly in patients with more severe disease, the elderly and immunocompromised population, and in those without proper antiviral treatment. Those with more severe disease had detectable Ct values up to 28 days after the onset of symptoms.[17] It is postulated that a less robust immune response in the elderly and immunocompromised populations may contribute to the persistence of the virus, and hence the persistence of detectable viral particles upon testing.[17,18]

There are also some cases wherein patients who meet hospital discharge criteria and are sent home, still have positive RT-PCR results. Their Ct values gradually increase until they test negative. However, for some patients, symptoms recur, and Ct values also remain low, suggesting re-infection. Hence, post-discharge patient monitoring is important in curbing the spread of COVID-19.[19]

In the diagnosis of COVID-19, clinical assessment of patients are important, and this is supplemented by the provision of biomarkers or laboratory markers which provide clinicians with objective information that can impact patient care.[20] Different biomarkers are recommended for use, including hematologic, immunologic, inflammatory, coagulation, and biochemical markers, which reflect the underlying pathophysiology of SARS-CoV-2 infection.[14,20] With the emergence of studies involving biomarkers in COVID-19 disease, it is being increasingly recognized that this is not confined to the respiratory system, but instead is a disease that involves multiple organ systems.[14,20,21] The role of the laboratory during this time is not only limited to the detection of this novel disease entity, but also to aid patient management by reporting of pertinent laboratory parameters or biomarkers. These biological substances can be objectively measured and evaluated, and give clinicians insight into disease progression, patient prognosis, and therapeutic monitoring of medical and pharmacological interventions.[14,22,23]

To our knowledge, there are limited studies available locally regarding the significance of Ct value and pertinent laboratory biomarkers in COVID-19 patients.[7] In this study, we assessed these laboratory data along with the clinicopathologic findings of affected patients and determined if this information may be useful for clinicians for more robust clinical decision-making.

METHODOLOGY

Population and sampling

In this retrospective study, we included adult (19 years old and above) Filipino inpatients diagnosed with COVID-19 by RT-PCR in The Medical City, a tertiary hospital in Pasig City with a 500-bed capacity, from April 2020 to December 2020. Pediatric patients (aged 18 years and below), asymptomatic COVID-19 patients, patients without available laboratory work-up, and those who consulted the institution on an outpatient basis were excluded from the study (total excluded, n = 724).

The patient demographic data, clinical diagnosis and other pertinent medical history were obtained by review of electronic medical records. The initial SARS-CoV-2 RT-PCR result (including the Ct values of *ORF1ab* and *N* genes) and other laboratory workup were retrieved using the laboratory information system (LIS).

RNA extraction for all samples was performed using the *GeneFinder EX-MATE 32* instrument. The corresponding extraction kit is the *GeneFinder* Viral DNA/RNA Extraction kit. Samples were treated with a lysis buffer in order to free the nucleic acids, which then get bound to the magnetic particles. Washing was done to separate the unneeded lysed cellular components, leaving only the desired eluate containing RNA. The eluate was then processed for RT-PCR.

The test kit used for the RT-PCR assay was Sansure Biotech, which targets viral ORF1ab and N genes. Fluorescent dyes were used in order to distinguish the amplifications of the different targets. The dyes used were FAM for *ORF1ab* gene, ROX for *N* gene, and Cy5 for *RNase P* gene (internal control). The assay has a manufacturer-declared positive agreement rate (sensitivity) of 94.34% (95% CI: 84.34 ~ 98.82%) and a negative agreement rate (specificity) of 98.96% (95% CI: 96.31 ~ 99.87%). All samples were run in the *Bio-rad CFX thermal cycler* instrument. The entire process was performed in accordance with the manufacturer's recommendations. Batch runs of RT-PCR were considered valid if the negative controls had no amplifications for all three targets, and if the positive controls had amplifications in all targets with Ct values of 40 or lower. Individual samples were considered valid (have adequate extracted RNA) if they have amplification of their internal control (Cy5 channel) with Ct value of 40 or lower. A positive result was rendered if the sample had any amplification with Ct value or 40 or lower in either FAM or ROX channel. Negative results were rendered for samples that had no amplification in both FAM and ROX channels.[24]

A cut-off of 30 was used to classify Ct value, wherein Ct values below 30 were considered low, and Ct values above 30 were considered high.[25,26] Other laboratory parameters included in the study were based on the Interim Guidance on the Clinical Management of COVID-19 Version 3.1 document, which recommended the following parameters to support the diagnosis of COVID-19: neutrophil count, lymphocyte count, platelet count, lactate dehydrogenase (LDH), ferritin, procalcitonin, c-reactive protein (CRP), aspartate aminotransferase (AST/SGOT), alanine amino-transferase (ALT/SGPT), PT/INR, and D-dimer.[3]

The identified patients included in the study were further subdivided into two groups, depending on the clinical severity of the disease.[3] The non-severe group consisted of patients who did not require critical care and/or mechanical ventilation, and with O_2 saturation ≥92% at room air upon admission.[3] The severe group comprised patients requiring critical care and/or mechanical ventilation, and with O_2 saturation <92% at room air upon admission. This study was given a full review and approved by the institutional board review of The Medical City.

Analysis
The demographic data for each case, which included patient age, sex, co-morbid illness, and respiratory status were determined by frequency, percentage, median and interquartile range. The age of both groups was subjected to Mann-Whitney U test, while sex and co-morbid illnesses were subjected to Chi-square and Fisher-exact test. Laboratory data for both groups were determined by frequency, percentage, median and interquartile range

and subjected to Mann-Whitney U and Chi-square test where appropriate.

RESULTS

We identified a total of 325 patients diagnosed with COVID-19. There were 154 (47.4%) women and 171 (52.6%) men with a median age of 62 years (range, 19 to 94 years). Two hundred twenty (67.7%) patients were classified as non-severe, and 105 (32.3%) patients were classified as severe. Among the severe cases, 69 (65.7%) ended in mortality, while 36 (34.2%) recovered. All 220 (67.6%) patients in the non-severe group recovered. Patient age was found to be significantly associated with disease severity ($p< 0.001$), while patient sex had no association ($p=0.068$) (Table 1).

All laboratory parameters, including Ct value, were found to be associated with disease severity ($p<0.001$). Lower Ct values of *ORF1ab* (median = 26.4) and *N* (median = 24.8) genes were seen in the severe group compared to the non-severe group. For hematologic parameters, the severe group showed increased neutrophils (81%) and decreased lymphocytes (8%) compared to the non-severe group, while platelet count remained within the normal range for both groups. Platelet count remained within range for both groups, however, there were generally lower counts in the severe group (median = 215, $p=0.001$). The coagulation parameter of PT/INR was found to be within the normal range for both groups, while an elevated D-dimer test was more commonly found in the severe group (n=58/105, 55.2%). Tests for liver enzymes showed elevation of AST/SGOT in the severe group compared to the non-severe group, while ALT/SGPT remained within range. Markers of inflammation – including LDH, ferritin, procalcitonin, and CRP – were all found to be more elevated in the severe group compared to the non-severe group. A tabulated summary of these findings is seen in Table 2.

Nearly all patients (n=311/325, 95.7%) had community-acquired pneumonia (CAP). The most common co-morbid illness was diabetes mellitus (n=93/325, 28.6%), followed by hypertension (n=89/325, 27.4%). There was also a variety of other illnesses which included conditions such as acute kidney injury (AKI) (n=20/325, 6.2%), acute respiratory distress syndrome (ARDS) (n=49/325, 15.1%), septic shock (n=36/325, 11.1%), and others (n=116/325, 35.7%) – heart failure, myocardial infarction, acute gastroenteritis, asthma, chronic obstructive pulmonary disease, Alzheimer's disease, acquired immunodeficiency syndrome, bipolar disorder, breast cancer, lung cancer, pancreatic cancer, multiple myeloma, T-cell Acute Lymphoblastic Lymphoma, cerebrovascular disease, hypothyroidism, multinodular goiter, intracerebral hemorrhage, pulmonary tuberculosis, urinary tract infection, acute pyelonephritis, and

Table 1. Patient demographic characteristics

Characteristics	All patients (n = 325)	Non-severe (n = 220)	Severe (n = 105)	p
Age, years	62 [12]	59 [20]	68 [22]	<0.001#
Sex				
Male	154 (47.4%)	111 (50.5%)	43 (41.0%)	0.068$
Female	171 (52.6%)	109 (49.5%)	62 (59.0%)	
Median [Interquartile Range]; Frequency (%); #Mann-Whitney U-test, $Chi-square test				

Table 2. Ct value and pertinent laboratory parameters among patients with COVID-19

Characteristics	Reference range	All patients (n = 325)	Non-severe (n = 220)	Severe (n = 105)	p
ORF1ab Gene	≤40	29.0 [8.4]	30.1 [7.7]	26.4 [6.6]	<0.001[#]
N Gene	≤40	27.2 [8.2]	28.3 [7.8]	24.8 [6.8]	<0.001[#]
Neutrophil	56–66%	76 [17]	72 [17]	81 [11]	<0.001[#]
Lymphocyte	22–40%	18 [17]	22 [17]	9 [11]	<0.001[#]
Platelet count	140–440 x 10^9/L	245 [122]	258 [118]	215 [121]	<0.001[#]
D-dimer	0.0–0.50 ug/mL	0.7 [1.2]	0.6 [0.7]	1.3 [2.2]	<0.001[#]
Not elevated	<0.50 ug/mL	79 (24.3%)	61 (27.7%)	18 (17.1%)	<0.001[$]
Elevated	≥0.50 ug/mL	126 (38.8%)	68 (30.9%)	58 (55.2%)	
PT/INR	0.8–1.1	1.0 [0.1]	1.0 [0.1]	1.0 [0.1]	0.003[#]
LDH	120–246 U/L	369 [218]	326 [164]	487 [268]	<0.001[#]
Ferritin	17.9–464.00 ng/mL	792.5 [1,241.0]	647.0 [907.0]	1,350.0 [1,868.0]	<0.001[#]
Procalcitonin	0.0–0.50 ng/mL	0.1 [0.5]	0.1 [0.2]	0.5 [3.0]	<0.001[#]
Not elevated	<0.50 ng/mL	223 (68.6%)	172 (78.2%)	51 (48.6%)	
Elevated	≥0.50 ng/mL	77 (23.7%)	25 (11.4%)	52 (49.5%)	<0.001[$]
CRP	1.00–3.00 mg/L	86.6 [117.5]	68.5 [109.7]	137.9 [175.3]	<0.001[#]
AST/SGOT	17.0–59.0 U/L	61 [52]	53 [43]	75 [70]	<0.001[#]
ALT/SGPT	0.0–50.0 U/L	46 [49]	45 [47]	48 [57]	<0.341[#]

median [interquartile range]; frequency (%); [#]Mann-Whitney U-test; [$]Chi-square test

Table 3. Co-morbid illnesses present in patients with COVID-19

Co-morbid illness	All patients (n = 325)	Non-severe (n = 220)	Severe (n = 105)	p
Diabetes mellitus	93 (28.6%)	60 (27.3%)	33 (31.4%)	0.259[$]
Hypertension	89 (27.4%)	53 (24.1%)	36 (34.3%)	0.037[$]
ARDS	49 (15.1%)	5 (2.3%)	44 (41.9%)	<0.001[*]
Septic shock	36 (11.1%)	1 (0.5%)	35 (33.3%)	<0.001[*]
AKI	20 (6.2%)	6 (2.7%)	14 (13.3%)	<0.001[*]
CAP - High risk	104 (32.0%)	15 (6.8%)	89 (84.8%)	
CAP - Moderate risk	202 (62.2%)	186 (84.5%)	16 (15.2%)	<0.001[*]
CAP - Low Risk	5 (1.5%)	5 (2.3%)	0 (0.0%)	
Other illnesses	116 (35.7%)	86 (39.1%)	29 (27.6%)	0.028[*]

Frequency (%); [$]Chi-square test; [*]Fisher-exact test

Table 4. Respiratory status of patients with COVID-19

Respiratory status	All patients (n = 325)	Non-severe (n = 220)	Severe (n = 105)
Room air (ward)	99 (30.5%)	99 (45.0%)	0 (0.0%)
Nasal cannula	114 (35.1%)	114 (51.8%)	0 (0.0%)
Face mask	7 (2.2%)	7 (3.2%)	0 (0.0%)
Room air (ICU)	15 (4.6%)	0 (0.0%)	15 (14.3%)
Intubated	86 (26.5%)	0 (0.0%)	86 (81.9%)
Tracheostomy	4 (1.2%)	0 (0.0%)	4 (3.8%)

Frequency (%); [$]Chi-square test

myocarditis. There was also a higher percentage of patients with co-morbid illness in the severe group (Table 3). The presence of co-morbid illness, except for diabetes mellitus, was found to be significantly associated with disease severity (Table 3).

The majority of patients in the non-severe group were placed on oxygen support via nasal cannula (n=114/325, 35.1%), or tolerated room air (n=99/325, 30.5%), while majority of patients in the severe group were intubated (n=86/325, 26.5%) (Table 4).

DISCUSSION

This study finds that Ct values are lower in the severe group compared to the non-severe group for both *ORF1ab* and *N* genes (Table 2, Figure 1). In addition, the severe group had worse respiratory status, since these patients needed ICU admission, intubation, or tracheostomy (Table 4). These patients were in the older age group (median = 68 years, interquartile range [IQR] = 22), and had worse clinical outcomes consisting of High-Risk CAP, ARDS, AKI,

and septic shock (Table 3). This appears to be consistent with data reported from a metanalytic study by Rao et al.[7] Their findings state that lower Ct values (median = 34.79) were seen in patients who died compared to those who survived (median = 37.43); lower Ct values were also present in patients who had severe disease progression (Ct value = 24); and that lower Ct values were associated with an increased risk of mortality.[7] In addition, Ct values below 30 were found to be associated with a higher risk of severe disease and hospitalization in comparison to those patients with Ct-value higher than 30.[25,26] However, it should be noted that the studies described in the meta-analysis involved mostly hospitalized adult patients, and may be subject to a population bias.

Another meta-analysis however, found no association between COVID-19 disease and Ct value.[25,27] Their findings were more variable, wherein the Ct value was either increased or decreased among hospitalized patients; did not differ among patient groups with differing disease severity; and the Ct values were not associated with risk of hospitalization.[25,27] The reason for these findings may be

due to several factors, majority of which are pre-analytic. These factors include timing of taking patients' sample, the adequacy of the swab material, and the type of sample included in each study.[7,25-27] There are also factors inherent to the PCR process itself which can affect the Ct value. These factors include the presence of inhibitors within the patient's swab sample, the PCR test kit used, the kit reagents, and the efficiency of RT-PCR procedure.[8,26-28] Furthermore, though Ct value is used as a surrogate marker for viral load in the absence of a viral culture, they may not have a linear relationship due to the aforementioned pre-analytic and analytic factors described.[6,26] Majority of RT-PCR kits available locally for COVID-19 are qualitative in nature, as complex procedures are required to standardize quantitative PCR tests.[9] Due to the heterogenous findings on the correlation of Ct values with clinical outcomes and laboratory parameters among patients with COVID-19, we maintain that qualitative reporting of Ct value is sufficient for rendering a diagnosis. The reporting of Ct value may be considered on a case-to-case basis, with particular emphasis that clinicians should correlate these values with their overall clinical and laboratory assessment per patient.[9,26,27]

For the pertinent laboratory parameters, abnormal values were present in both groups of patients, however the inflammatory biomarkers, coagulation studies, hematologic markers and liver enzymes were more elevated in the severe group (Table 2). Specifically for hematologic markers, patients with severe COVID-19 disease had higher neutrophil count (median = 81%, IQR = 11) and lower lymphocyte count (median = 9%, IQR = 11) (Figure 2). Findings of neutrophil and lymphocyte counts are similar to other studies, and it is suggested that lymphocyte counts below 0.8×10^9/L and neutrophil counts higher than 3.5×10^9/L reflect a poor clinical outcome and are associated with COVID-19 disease severity.[14,22] Besides the differential count, the determination of Neutrophil-Lymphocyte ratio (NLR) is of interest to clinicians, as a higher NLR was found to be associated with worse clinical outcomes in inflammatory conditions, some cancers, and as a predictor of cardiovascular mortality.[22,29,30] It is proposed that infection with the SARS-CoV-2 virus triggers activation of the innate immune system, manifested by neutrophilia and elevated acute phase reactants, which was found in our study.[29,30] Therefore in the local setting, it is recommended to determine complete blood counts of COVID-19 patients, in order to compute the NLR for prognostication and clinical management.

The coagulation markers routinely tested in COVID-19 patients in the local setting include PT/INR and D-dimer, and in the current study this was found to be associated with severe disease (Table 2, Figure 5).[3] Platelet counts were lower in the severe group ($p<0.001$) (Table 2, Figure 3), the median D-dimer value was higher (median = 1.3 ug/mL, IQR = 2.2), while PT/INR values were similar between groups (median = 1.0, IQR = 0.1). This is similar to the findings in a meta-analysis, wherein patients with severe disease exhibit thrombocytopenia, significant elevation in D-dimer, but with variable findings of PT/INR.[14,22,31] In particular, the abnormalities of coagulation have been associated with those COVID-19 patients who are in the ICU setting.[14,32] These findings point to an underlying coagulopathy caused by the virus, whose pathophysiology
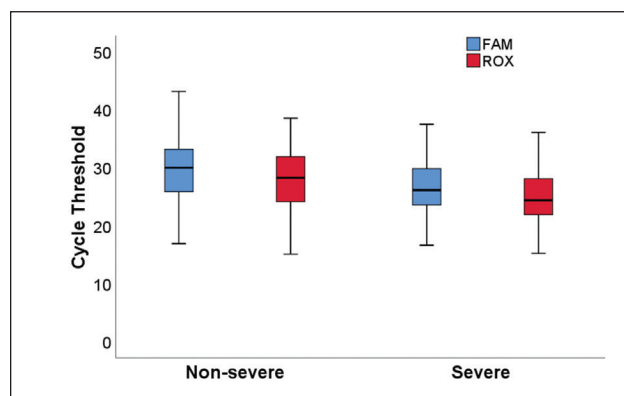

Figure 1. Cycle threshold values in non-severe and severe groups of patients with COVID-19.
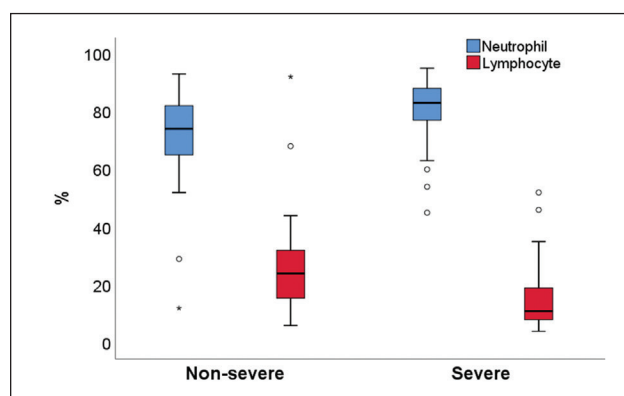

Figure 2. Neutrophil and Lymphocyte counts of non-severe and severe patients with COVID-19.
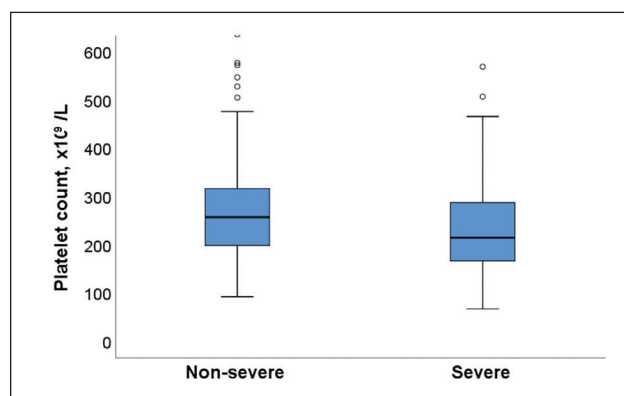

Figure 3. Platelet counts among non-severe and severe patients with COVID-19.

is not yet well understood.[22,30,33] It is proposed that one of the mechanisms of coagulopathy is viral infection of the vascular endothelial cells, causing injury and activation of the fibrinolytic system with microthrombi formation particularly in the pulmonary circulation.[32] In addition, D-dimer is associated with various critical illnesses such as venous thromboembolism (VTE), disseminated intravascular coagulopathy (DIC); and elevated levels are widely recognized as a poor prognostic marker.[6,22,33] Therefore, it is recommended to test for these coagulation parameters in the local setting.

Several acute phase reactants (APRs) are recommended locally to be tested in patients with COVID-19, which include CRP, Ferritin, LDH and Procalcitonin.[3] This study finds that all patients with COVID-19 exhibit elevated levels of these reactants, which is expected due to the inflammatory response against the virus (Figures 4 and 5).[6,34] Higher levels of these APRs were found to be associated with more severe disease (Table 2), which is similarly reported in other studies.[7,14,22,34,35] A study by Sayit et al., report the following findings: that a CRP value higher than 20.42 mg/L can predict the severity of COVID-19 disease; elevated LDH is a strong predictor of lung injury in COVID-19 as it is released from the cytoplasm of necrotic cells; Ferritin levels increase due to stimulation by proinflammatory cytokines and leakage from damaged cells; and that elevated Procalcitonin levels are related to a 5-fold higher risk of severe COVID-19.[34] It is of benefit to clinicians to monitor these APRs as they correlate with disease severity and prognosis.

In the local setting, it is recommended to test for liver function markers as COVID-19 may affect this organ, hence the inclusion of AST and ALT.[3] The current study finds that AST is associated with more severe disease ($p<0.001$), while no association is seen in ALT ($p= 0.341$) (Table 2, Figure 6). The significant increase in AST compared to ALT may be due to the presence of the mitochondrial isoenzyme of AST, which has a long half-life (87 hours), and may also be due to the fact that AST is not limited to the liver and can be found in other organs such as the heart.[6,14,26] In addition, it is widely recognized that the SARS-CoV-2 virus may cause damage to other organs which express the ACE2 receptor, as this is the point of attachment of the virus.[14,26] This receptor is expressed in organs such as the lungs, liver, heart, and kidneys, hence the work-up for organ involvement in COVID-19 disease should not be limited to liver function tests. Other studies report that patients with severe COVID-19 disease present with abnormal cardiac markers such as elevated troponins, and with abnormalities in kidney function like elevated creatinine.[14,22,34] Therefore there is merit for clinicians to expand their laboratory work-up where appropriate in order to facilitate care for COVID-19 patients with multiple organ involvement.

For the co-morbid illnesses, the most common in this population was diabetes mellitus (n=93/325, 28.6%) followed by hypertension (n=89/325, 27.4%) (Table 3). This is the opposite of the profile of other studies, which found hypertension (32.5% of patients) to be more common than diabetes mellitus (24.10% of patients).[26,35] In the current study, the median age of involved Filipino patients is 62 (IQR = 12), which is older than the population in a similar study by Yormaz et al., which was performed in Turkey (average age = 56.3 years).[35] Older age and co-morbid conditions (particularly hypertension and diabetes) are found to be associated with more severe disease (Table 1, Table 3), and are predisposed to prolonged viral shedding.[26] Lower Ct values have been found in older patients with COVID-19, and this has been attributed to their slower immune response due to immunosenesence.[6,26] In addition, older patients tend to have more frequent co-morbid illnesses, therefore the age factor should be taken into consideration as it is correlated with more severe outcomes.[26,35]

Limitations of the study include the retrospective design, which analyzed the laboratory parameters and Ct values of patients at a single point in time. Trends or changes of Ct value and laboratory parameters were not determined. Another limitation was not accounting for the timing of nasopharyngeal swabs for SARS-CoV-2 and timing of blood collection in relation to the day of illness in the study. This may act as a confounder because drawing any kind of association between Ct values and other lab test results requires that specimen collection for all of them be done at the same time. As mentioned earlier, a factor that could
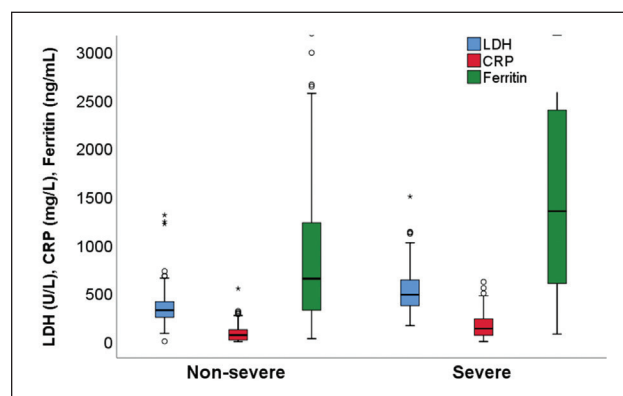

Figure 4. Acute Phase Reactants among non-severe and severe patients with COVID-19.
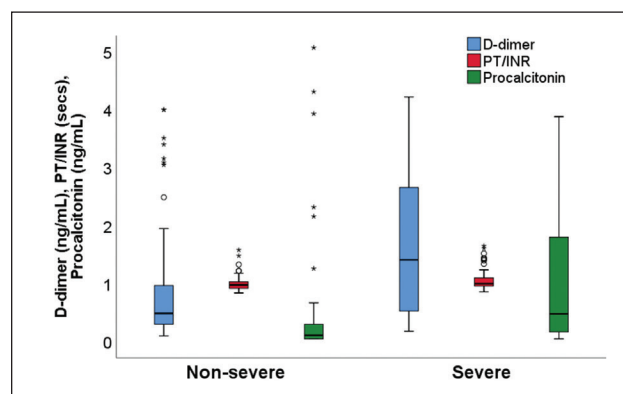

Figure 5. Coagulation markers and procalcitonin among non-severe and severe patients with COVID-19.
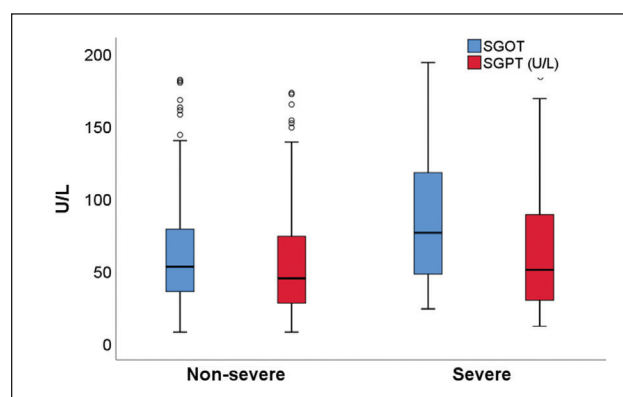

Figure 6. Liver enzymes among non-severe and severe patients with COVID-19.

influence Ct values is the time interval between collection and processing (age of the specimen). A long interval before processing could affect the stability of the viral RNA and thus lead to high Ct values or false negatives.[12] Factors that could affect this time interval include stat requests from doctors, availing of tests for non-clinical purposes (e.g., travel, employment), and requests for home swabbing, among others. When such factors are not accounted for, it is possible for them to behave as confounders, as these various situations are related to both the Ct values and the clinical picture of the patient. Interpretations of the laboratory values must be taken into the proper clinical context. The study was carried out during the period when variants of the SARS-CoV-2 virus were not yet identified. Different variants of SARS-CoV-2 can also confound the outcomes, as they could have different properties from each other in terms of infectivity, replication, immunogenicity, pathophysiology or severity. The study is also limited to the symptomatic adult population who were hospitalized and does not represent the entire spectrum of the disease. Asymptomatic patients were not included as the majority had no laboratory workup performed. Multivariate analysis to account for confounding factors and potential biases was not done as this was beyond the scope of the study.

The authors recommend that further investigation should be undertaken in the manner of pursuing correlational studies, or prospective cohort studies, taking into account the timing of specimen collection with the correlation of clinical parameters. This is of interest, especially in the light of newly developing medical interventions, mass population vaccinations, and the recognition of Post COVID-19 syndrome.[36] Pursuing studies to include non-hospitalized patients may be of benefit to adequately represent how COVID-19 affects the local community, and to guide policies on containment and spread of the virus. There is also limited data on COVID-19 in pediatric patients, and looking into this population may be of interest to facilitate pediatric patient care. Investigation of various biomarkers of organ involvement and how these change in COVID-19 patients may also provide further insight into the pathophysiology of the infection and aid patient management and therapeutic decision-making.

CONCLUSION

Laboratory biomarkers such as neutrophil and lymphocyte counts, LDH, ferritin, procalcitonin, CRP, D-dimer, PT/INR, and AST are associated with severe COVID-19 disease. Lower Ct-values, older age, and the presence of co-morbid illness are also associated with severe COVID-19 disease. The qualitative reporting of SARS-CoV-2 results as positive or negative is sufficient for diagnosis of the disease, and with the currently available data, the reporting of Ct values may be considered on a case-to-case basis by clinicians to aid in patient management decisions. The Ct values should be interpreted with caution, given the multiple pre-analytic and analytic factors which may affect the result. Instead, the patient's overall clinical profile, laboratory biomarkers and Ct value should be taken as a whole to guide therapeutic decision-making.

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RUNX1::RUNX1T1 Fusion in Pediatric Acute Myeloid Leukemia: A Description of Two Cases

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ABSTRACT

RUNX1::RUNX1T1 is a core-binding factor driving fusion gene which arises from t(8;21)(q22;q22). It is one of the most common chromosomal rearrangements in both pediatric and adult Acute Myeloid Leukemia (AML) with a reported incidence of 15% in children and young adults. There are few case reports documenting *RUNX1::RUNX1T1* translocation in pediatric AML. Although this is generally associated with a favorable prognosis, we report two (2) cases of de novo pediatric AML in the Philippines harboring a *RUNX1::RUNX1T1* translocation, one eventually relapsed while the other attained remission but succumbed to sepsis.

Key words: Pediatric acute myeloid leukemia, Next Generation Sequencing, RUNX1::RUNX1T1 fusion, Berlin-Frankfurt-Münster (BFM-87) protocol, AML 15 Medical Research Council protocol

INTRODUCTION

Hematologic malignances affect approximately 38% of adolescents and young adults worldwide, with leukemias being more prevalent than lymphomas.[1]

Acute Myeloid Leukemia (AML) is defined as the presence of myeloid blast count of more than 20% in a bone marrow or peripheral blood smear.[2] Since morphology alone cannot be used to determine blast lineage, immunophenotyping – either by immunohistochemical staining or by flow cytometry is used to confirm blast lineage.

Flow cytometry, a multi-parametric analytic technique on a cellular basis, assigns the lineage of the progenitor population and facilitates the classification of the acute leukemia.[2] Expression of markers such as CD33, CD13, HLA-DR, CD11c, cMPO, and CD117 characterizes the cell population of interest as belonging to the myeloid lineage. Flow cytometry is also useful in the detection of residual disease and monitoring of therapeutic response. With the acknowledgement of the role of recurrent genetic aberrations in the pathophysiology, prognosis, and treatment of AML, the demand for cytogenetic and molecular genetic testing continues to increase.[3] The presence of certain recurrent genetic abnormalities, such as t(15;17)(q24;q21); *PML::RARA* in acute promyelocytic leukemia, and t(8;21)(q22;q22.1); *RUNX1::RUNX1T1* and inv(16)(p13.1q22) or t(16;16)(p3.1;q22); *CBFB::MYH11* in core binding factor AMLs, may also be used to diagnose AML, even with less than 20% blasts.[4]

In a study by Tamayo et al (2021), most Filipino pediatric cases of de novo AML have a normal karyotype (12/20) and harbors *CBFB::MYH11* (7/20).[5] While a normal karyotype is associated with a generally intermediate prognosis, the same study also shows that cytogenetically normal patients may harbor significant alterations in *CEBPA, FLT3, PML, RARA, TET2, ASXL1, NPM1, RUNX1, RUNX1T1,* and

ZRSR2. It has been documented that a higher total number of mutations in core-binding factors and signaling genes at the time of diagnosis correlate with inferior prognosis and relapse.[6]

RUNX1::RUNX1T1 is the fusion gene generated from translocation t(8;21)(q22;q22). It is one of the most common chromosomal rearrangements with an incidence of 15% in children and young adults.[7] We report two (2) cases of patients with *RUNX1::RUNX1T1* translocation.

CASE 1

A 12-year-old Filipino male presented with a 2-month history of pallor and intermittent fever. The patient had stable vital signs and pale palpebral conjunctiva. Past medical history was unremarkable. There was also no family history of cancer or other hematolymphoid disease. Complete blood count showed anemia (hemoglobin 102.0 g/L, hematocrit 0.28, and red blood cell count 3.4 x 10^{12}/L), thrombocytopenia (platelets 21 x 10^9/L), and presence of blasts (white blood cell count 7.9 x 10^9, Differential count: segmenters 3%, lymphocytes 43% and blasts 54%). These blasts were characterized as having scant cytoplasm, increased nuclear to cytoplasmic ratio,

irregular nuclear membrane, fine chromatin pattern and occasionally conspicuous nucleoli (Figure 1A). Flow cytometry demonstrated 61% myeloblasts with the following immunophenotype: CD13, CD33, CD34, CD117, anti-HLA-DR and cMPO (Figure 1B). These findings were consistent with AML. Cytospin of the patient's cerebrospinal fluid was negative for malignant cells.

Karyotype was 46,XYqh+ (normal male carrying a heteromorphic variant in the long arm of Y chromosome). The Fluorescence In Situ Hybridization (FISH) analysis using MetaSystems Translocation Probe (MetaSystems Asia Co. Ltd.) showed t(8;21)(q22;q22.1); *RUNX1::RUNX1T1* fusion gene in 50.47% of cells (Figure 2). Next Generation Sequencing (NGS) performed using the AmpliSeq™ for Illumina Myeloid Panel (Illumina, San Diego, CA, USA) run on MiSeq platform confirmed *RUNX1::RUNX1T1*. Additionally, alteration in variants of uncertain significance, *KIT* (*Proto-oncogene c-KIT*) M541L and SH2B3 (*Src homology 2 B3*) P242S were detected. Berlin-Frankfurt-Münster (BFM-87) protocol for AML was done and patient was inducted with Cytarabine 100mg/m² and Doxorubicin 25mg/m².[8] After two cycles, a minimal residual disease (MRD) panel showed 10% myeloblasts by flow cytometry. Remission was achieved after three (3) cycles. However, five (5) months after
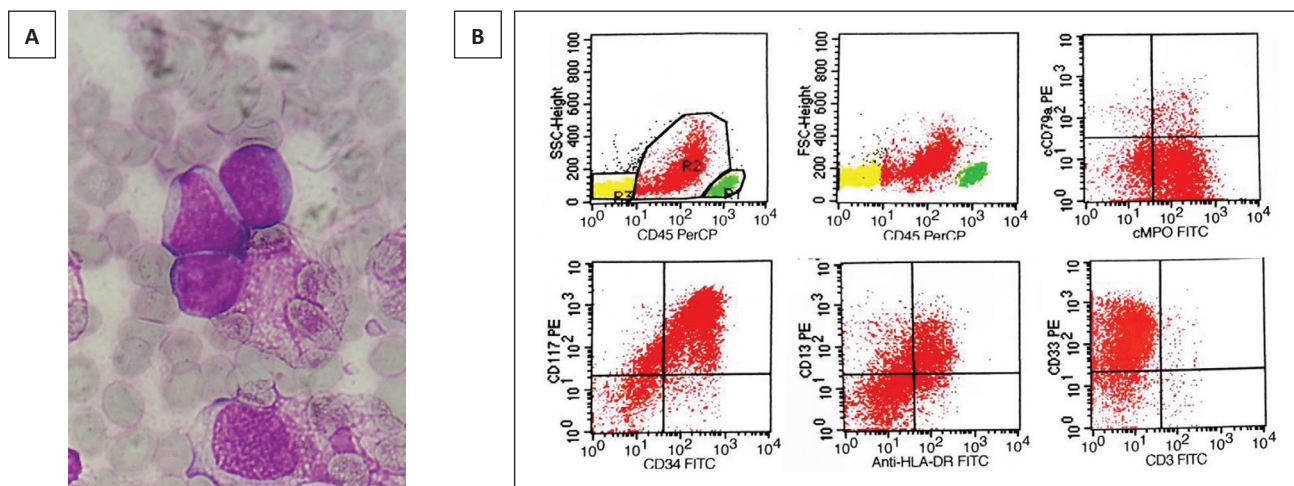

Figure 1. (A) Peripheral blood smear of the patient showing blasts with scant cytoplasm, increased nuclear to cytoplasmic ratio, irregular nuclear membrane, fine chromatin pattern and occasionally conspicuous nucleoli; **(B)** Flow Cytometry shows a dim CD45 blast population with the following immunophenotype: CD13, CD33, CD34, CD117, anti-HLA-DR and cMPO.
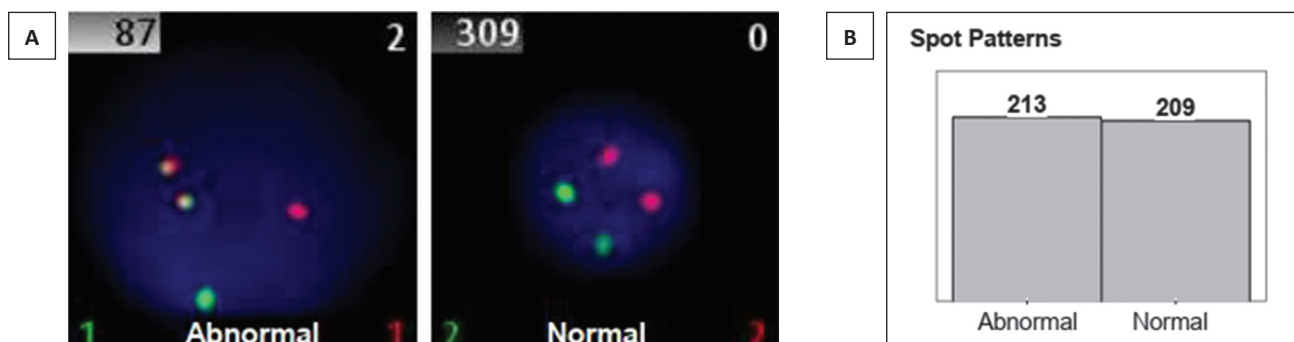

Figure 2. (A) Detection of *RUNX1::RUNX1T1* by Fluorescence in situ Hybridization (FISH). In normal cells, two red signals representing *RUNX1T1* and two green signals representing *RUNX1* are detected. In the abnormal cell containing *RUNX1::RUNX1T1* fusion gene, one red, one green and two red/green (yellow) fusion signals are observed. **(B)** Spot patterns showing 213 out of 422 total cells analyzed or 50.47% having *RUNX1::RUNX1T1* gene (MetaSystems *AML1::ETO* (*RUNX1::RUNX1T1*) DCDF Translocation Probe).

diagnosis, examination of the patient's peripheral blood on routine follow-up showed myeloblasts (white blood cell count 9.6 x 10^9, and blasts 47%), indicating relapse. Salvage therapy was started; however, the patient contracted Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) infection shortly before his 3rd cycle and the patient did not achieve remission. Bone marrow transplant was contemplated but did not push through due to lack of a compatible donor. The patient expired from intracranial hemorrhage 8 months after initial leukemia diagnosis.

CASE 2

An 18-year-old Filipino male presented with a 2-month history of generalized pallor. The patient had stable vital signs and pale palpebral conjunctiva. Other physical examination findings and past medical history were unremarkable. There was also no history of cancer or other hematolymphoid disease in the family. Complete blood count showed anemia (hemoglobin 85.0 g/L, hematocrit 0.25, and red blood cell count 2.7 x 10^{12}/L), thrombocytopenia (platelets 11 x 10^9/L), and presence of immature granulocytes and blasts (white blood cell count 6.8 x 10^9/L, Differential count: segmenters 10%, lymphocytes 60%, monocytes 2%, stabs 3%, myelocytes 3%, metamyelocytes 2% and blasts 20%). These blasts

exhibited a perinuclear hof and large, pink-colored cytoplasmic granules (Figure 3A). Flow cytometry showed 85% myeloblasts with the following immunophenotype: CD13, CD33, CD34, CD117, anti-HLA-DR, and cMPO (Figure 3B). These findings were consistent with AML.

Karyotype was normal (46,XY) but FISH using MetaSystems Translocation Probe (MetaSystems Asia Co. Ltd.) revealed t(8;21)(q22;q22.1); *RUNX1::RUNX1T1* in 56.31% cells (Figure 4). NGS performed using the AmpliSeqTM for Illumina Myeloid Panel (Illumina, San Diego, CA, USA) run on MiSeq platform confirmed *RUNX1::RUNX1T1*. In addition, mutation of *TET2* (*Tet methylcytosine dioxygenase 2*) N281Gfs*2 was also noted. The patient was optimized by giving blood transfusion and chemotherapy was initiated. Patient was part of a trial on Low-dose chemotherapy (Cytarabine 10 mg/m^2 and Doxorubicin 25 mg/m^2) with Granulocyte Colony Stimulation Factor (G-CSF) AML Protocol.[9] After 1 cycle, monitoring by flow cytometry showed low level residual (1% blast). Patient was shifted to BFM-87 protocol and then attained remission after induction. After the late intensification phase, patient presented with dry cough, fever and vomiting, and eventually succumbed to septic shock secondary to febrile neutropenia. Table 1 summarizes the clinical data, karyotype, FISH and NGS result of the two cases.
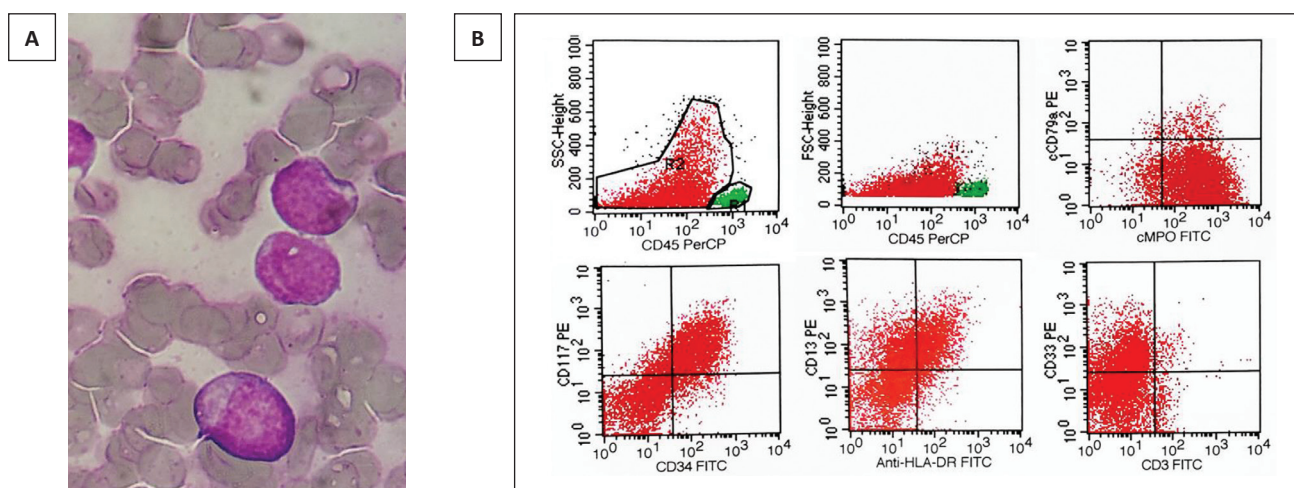

Figure 3. (A) Blasts in the peripheral blood exhibiting perinuclear hof and large, pink-colored granules; **(B)** Flow Cytometry showing 85% myeloblasts with the following immunophenotype: CD13, CD33, CD34, CD117, anti-HLA-DR and cMPO consistent with AML.
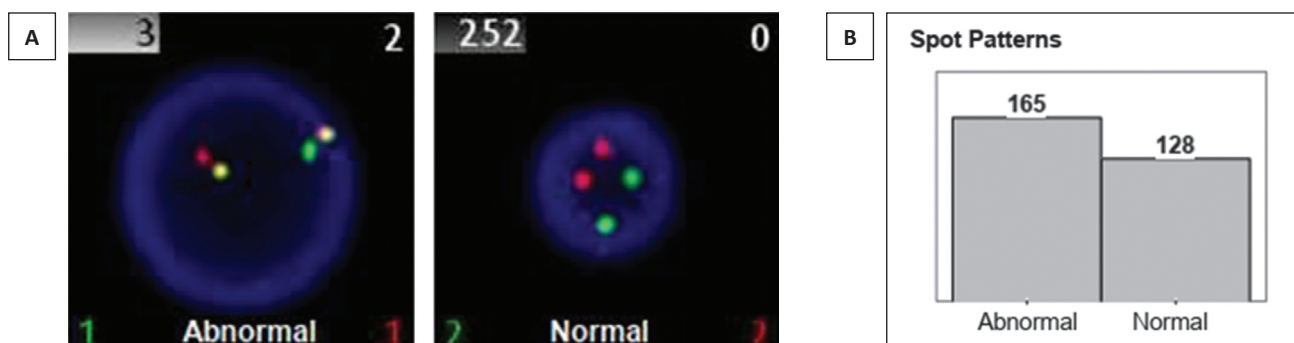

Figure 4. (A) Detection of *RUNX1::RUNX1T1* by Fluorescence in situ Hybridization (FISH). As with the first case, the abnormal cells containing *RUNX1::RUNX1T1* fusion gene show one red, one green and two red/green (yellow) fusion signals. **(B)** Spot patterns showing 165 out of 293 total cells analyzed or 56.31% having *RUNX1::RUNX1T1* gene (MetaSystems *AML1::ETO* (*RUNX1::RUNX1T1*) DCDF Translocation Probe).

Table 1. Clinical data, karyotype, FISH and NGS result of the two cases

| Clinical and molecular data	Case 1	Case 2
Patient age/sex	12/Male	18/Male
WBC at diagnosis	7.9 x 10⁹/L	6.8 x 10⁹/L
% Blast at diagnosis (flow cytometry)	61%	85%
Immunophenotype (flow cytometry)	CD13, CD33, CD34, CD117, anti-HLA-DR and cMPO	CD13, CD33, CD34, CD117, anti-HLA-DR, and cMPO
Karyotype	46,XYqh+	46,XY
FISH result	*RUNX1::RUNX1T1* (50.47%)	*RUNX1::RUNX1T1* (56.31%)
NGS result	*RUNX1::RUNX1T1* KIT M541L SH2B3 P242S	*RUNX1::RUNX1T1* TET2 N281Gfs*2
Initial MRD (flow cytometry) after 1 cycle of chemotherapy	Residual AML (10% blasts)	Low-level residual AML (1% blast)
Treatment Protocol	Berlin-Frankfurt-Münster (BFM-87) protocol	Low dose chemotherapy AML protocol BFM-87 protocol
Course	Attained remission after 3 cycles but relapsed and had SARS-CoV-2 infection. Expired from intracranial hemorrhage.	Attained remission after 1 cycle of BFM-87 protocol. Succumbed to sepsis.
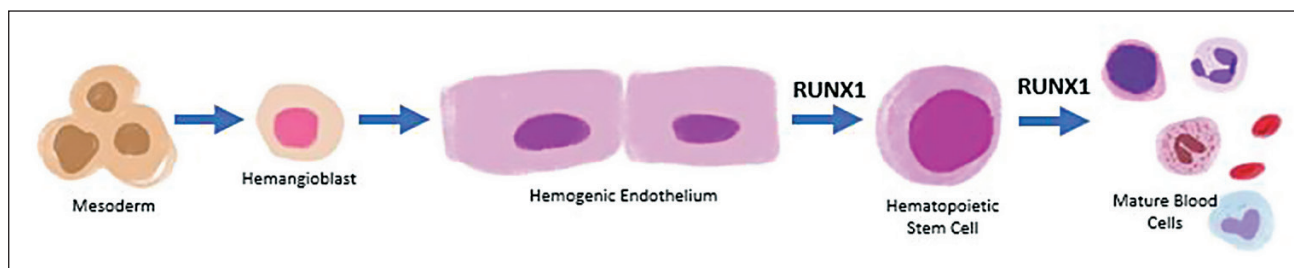
Figure 5. The role of *RUNX1* in the embryonic origin of the blood. The mesoderm cells in the yolk sac transform into hemangioblasts which are the precursor of hemogenic endothelium. *RUNX1* encodes for the transcription factor RUNX1 which was identified as the first specific marker of hemogenic endothelium (HE).[10] HE is now considered as the immediate precursor of hematopoietic stem cells (HSC), and laboratory models show that removal of RUNX1 prevents HE to transform into HSCs.[11] In adult hematopoiesis, RUNX1 also plays a role in the differentiation of mature blood cells from hematopoietic stem cells.

DISCUSSION

Both cases included in this case series were found to harbor a core binding factor driver fusion, the *RUNX1::RUNX1T1* fusion gene. *RUNX1* has been identified as the first hemogenic endothelium marker shown to have a critical function in the earliest stages of blood cell formation[10] (Figure 5). In adult hematopoiesis, the protein it encodes, RUNX1 is expressed by hematopoietic cells and forms a complex with core binding factor ß (CBFß encoded by *CBFB* gene). This DNA-binding heterodimer regulates hematopoietic differentiation.[3] *RUNX1T1* is a translational corepressor expressed in the megakaryocytic and erythrocytic lineages, basophils and eosinophils, and B progenitors.[7]

Similar to wildtype RUNX1, the protein encoded by the resulting *RUNX1::RUNX1T1* fusion gene forms a complex with CBFß, increasing its DNA-binding activity.[7] This competes with wildtype RUNX1 and plays a role in leukemogenesis by influencing cell proliferation, differentiation and self-renewal capacity[12] (Figure 6). Despite being one of the most common translocation in AML, the study by Tamayo et al., demonstrated that only three (3) out of 20 Filipino pediatric AML cases harbor this fusion gene.[5]

Phenotypically, myeloid blasts in patients with AML with *RUNX1::RUNX1T1* translocation express high positivity with HLA-DR and CD34, and less CD33.[13] They may
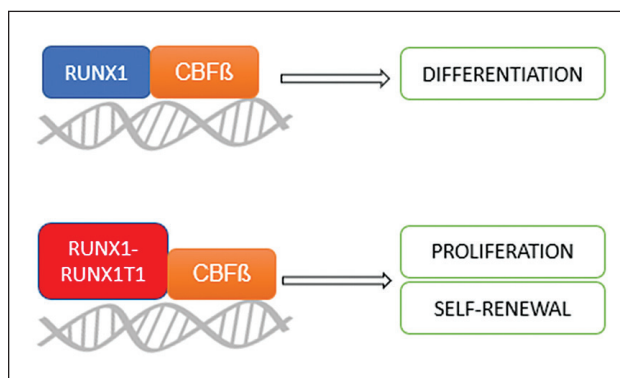

Figure 6. The role of *RUNX1::RUNX1T1* in leukemogenesis. RUNX1 forms a DNA-binding heterodimer with CBFß which promotes hematopoietic differentiation. In the presence of *RUNX1::RUNX1T1* fusion gene, the protein it encodes compete with wildtype RUNX1 and promotes leukemogenic proliferation and self-renewal.

also co-express CD19 or PAX5 and CD56.[13,14] With both of our cases, flow cytometry showed positivity with HLA-DR, CD34 and CD33 and did not express CD19. PAX5 or CD56 were not included in the performed panel. Morphologically, the blasts in the second case showed the characteristic perinuclear hof and abundant, large cytoplasmic pink- or salmon-colored granules, while non-blastic granulocytes showed Auer Rods.[13]

Clinically, pediatric AML commonly arises *de novo* while adult AML is associated with an underlying myeloproliferative neoplasms (MPN) or myelodysplastic syndrome (MDS).[15] Whereas adult AML has the propensity to harbor somatic sequence variance, pediatric AML exhibits chromosomal rearrangements such as *RUNX1::RUNX1T1 fusion gene*.[16] There is evidence that such chromosomal rearrangements may exist in utero, however, these do not all develop into AML.[15] Furthermore, *RUNX1::RUNX1T1* -positive cells may still be detected in patients who have received treatment for AML and has undergone complete remission.[15]

In congruence with the favorable prognosis observed in AML with *RUNX1::RUNX1T1* clinically, the two cases described herein achieved complete remission initially. In core-binding factor driven leukemias, 90% of patients achieve complete remission with chemotherapy, and 70% overall survival.[16] Unfortunately, relapse is seen in 30% of cases, with overall survival being reduced to 51%.[7,12] In the first case, the patient had a relapse after attaining remission with 3 cycles of chemotherapy.

The two patients were given BFM-87 protocol, for which *RUNX1::RUNX1T1* is recognized to have good response.[17] The second patient was part of a trial and was given initially low dose chemotherapy. This regimen is documented to have comparable complete response and overall survival but with less toxicity; and based on 149 AML cases with known molecular alterations, there was no significant difference on the rate of morphologic and molecular remission between the low dose chemotherapy and the standard protocol.[9] The second patient was shifted to the standard BFM-87 protocol when complete remission was not attained after 1 cycle of low-dose chemotherapy. The patient then had complete remission after induction phase and never went into relapse.

There are few case reports documenting *RUNX1::RUNX1T1* translocation in pediatric AML. Totadri et al., reported a case of a 9-year-old male who had an extramedullary presentation of *RUNX1::RUNX1T1* translocated AML.[18] The patient presented with cranial nerve palsies; however peripheral blood smears showed blasts with Auer rods. Patient underwent AML 15 Medical Research Council protocol and cranial radiotherapy and attained a 1-month disease-free survival at the time of publication of the case report.[18] Additionally, Kondo et al., reported a case of a 7-year-old male with *RUNX1::RUNX1T1* translocated AML who achieved complete remission after conventional chemotherapy but relapsed after six (6) months, like in our first case.[19]

RUNX1::RUNX1T1 is also used in MRD monitoring through multiparameter-flow cytometry or molecular techniques. The induction regimen used in chemotherapy is found to be an independent factor influencing the prognostic significance of MRD.[20] A repetitive and sensitive detection of MRD negativity has the best prognostic value.[6] In a study by Hollein et al., 68% of the patients with *RUNX1::RUNX1T1* translocation who underwent allogenic stem cell transplant achieved complete molecular remission (CMR) post transplantation. In patients who relapsed following CMR, transplantation also confers

an increase in overall survival to 69% at 2 years while the median survival of patients who did not undergo transplant is only 5 months.[21]

The *RUNX1::RUNX1T1* fusion gene usually coexists with additional chromosomal mutations such as *KIT, NRAS* and *ASXL1* in leukemogenesis.[12] The first case has a *KIT* M541L mutation, the most common chromosomal mutation associated with core binding factor driver fusion genes. The other gene mutated in the first case is *SH2B3* P242S and the protein it encodes is a negative regulator of *JAK2*. This is present in 13% of secondary AML, 1% of essential thrombocythemia, and 3% of primary myelofibrosis.[22] Studies on the role of *SH2B3* in primary AML are scarce. The second case harbors a concurrent *TET2* (Ten-Eleven Translocation 2) gene mutation, specifically *TET2* N281Gfs*2, which encodes for a protein critical in promoting DNA demethylation and immune homeostasis.[23] The effect of *TET2* in the prognosis of AML is controversial, but has been recurrently detected in the early events of AML pathogenesis.[21,22]

While certain somatic mutations such as somatic mutations of *WT1, ELF1, KMT2C,* and *MLLT10* were associated with primary chemotherapy resistance in pediatric AML, it is unclear if the additional mutations in both cases trumped the prognosis to worse. In the first case, *KIT* mutations have been shown to have no significant effect in the prognosis of pediatric AML, in contrast to the poor prognosis it confers to adult patients.[13,24] It is also not known if the concurrent *TET2* mutation seen in the second case conferred a worse prognosis as discussed, as the patient died of other cause. Nevertheless, genomic testing for this population of AML patients will pave the way for optimizing prognosis and the development of targeted therapies.

In developing countries, other prognostic factors identified that are associated with decreased overall survival includes WBC at presentation and response to induction therapy.[25] Our two cases had a WBC of less than 50×10^9/L at presentation, which is associated with a better overall survival. Both cases were in partial remission after induction chemotherapy, which is associated with less favorable prognosis. In the study done in Pakistan, neutropenic sepsis and bleeding are the most common cause of treatment-associated mortality, similar to our two cases.[25] These complications may have trumped the favorable prognosis associated with *RUNX1::RUNX1T1* translocation to worse.

The findings of this paper is limited to the two (2) cases discussed and cannot be used to generalize the prognostic factors and treatment of AML with *RUNX1::RUNX1T1* translocation and/or additional mutations. In the Philippines, molecular testing in pediatric AML is not routinely done due to limited resources. Further studies are warranted for correlation of the molecular profile of pediatric AML with prognosis and treatment in our setting.

CONCLUSION

Although the presence of *RUNX1::RUNX1T1* translocation is generally considered to confer favorable prognosis in AML, up to 30% of cases relapse, leading to worse outcome

and decreased overall survival. Additional genetic mutations may coexist with *RUNX1::RUNX1T1* translocation, and the effect of these mutations on the prognosis is an evolving field. This emphasizes the significance of genomic testing in patient management, prognostication and in the development of targeted therapies.

ETHICAL CONSIDERATION

Efforts were made to have the patients' relatives sign the consent form for publication, however there was no reply. Initially, consent for the second case was obtained verbally, however, there was no reply when they were contacted to sign the consent form.

STATEMENT OF AUTHORSHIP

The authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

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None.

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Enhancing Autopsy Workflow Through a Downdraft Set Up

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The autopsy or necropsy is a post-mortem procedure that consists of a thorough examination of a corpse by dissection: to determine the cause, mode, and manner of death, and, to evaluate any disease or injury that may be present for research or educational purposes. Autopsy literally means "seeing for oneself." A significant number of major findings cannot be diagnosed without histology; thus, without a biopsy or an autopsy they cannot be diagnosed.

Autopsies can be regarded as a form of quality control. One large review in JAMA suggests that approximately 25% of autopsies reveal a finding that would have changed clinical management, and 5% of autopsies reveal a missed diagnosis that probably affected the outcome. For the past decade, clinical autopsy has not been given proper attention due to various factors including advancements in medical diagnostic technology that deem the need for postmortem examinations to be uncertain. Yet in the recent COVID-19 pandemic, autopsies have been instrumental in the provision of important public health information.[1-4]

All autopsies to be performed must be handled as if they contain an infectious agent (standard precautions). The entire autopsy area and its contents are designated as a biohazard area and posted with appropriate warning signs. Autopsies are ideally performed in negative pressure room suites (i.e., *the pressure in the room is lower than those outside it*) to allow air to flow into the isolation room or autopsy suite, but not escape from this room. Air will naturally flow from areas with higher pressure to areas with lower pressure, thereby preventing contaminated air from escaping the autopsy suite with negative pressure. The internal air is forced out so that negative air pressure is created pulling air passively into the system from other inlets.[5-7]

At the Research Institute for Tropical Medicine (RITM), autopsy services were suspended since the SARS pandemic in 2003 due to inadequate facilities. As recent events have underscored the need for autopsy, efforts to resume its operation have been pursued to include emerging and re-emerging infectious diseases. This article aims to revisit the proceedings in an autopsy and also feature the design of the RITM morgue suite.

The RITM autopsy suite (Figure 1) is a well-ventilated room with negative pressure airflow exhaust system and contains a separate low-traffic isolation room. Whenever possible, autopsies performed on human remains that are potentially infectious should be done in settings that have adequate air-handling system. This includes: 1) a minimum of six (old construction) to twelve (new construction) air changes per hour (ACH), 2) negative pressure relative to adjacent areas as per recommendations for airborne infection isolation rooms (AIIRs), and 3) direct exhaust of air to the outside or passed through a HEPA filter if

Figure 1. Autopsy suite.

air is recirculated. The RITM autopsy suite has a 23-34 ACH (minimum 12 ACH per WHO guidelines) with 12 to 20% differential air on the supply and exhaust air. The inward airflow smoke pattern test also ensures the uni-directional ventilation at the door openings of the suite.[2,3,7-10]

Preceding the autopsy procedure, the pathologist and the team must perform proper hand washing technique before donning of the personal protective equipment (PPE) in the ante room. A biosafety officer will perform a risk assessment for the case and assists in ensuring proper donning. The autopsy team will enter the morgue suite, which is a negative pressure room, that incorporates a ventilation system designed so that air flows from the corridor into the negative pressure room, ensuring that contaminated air cannot escape from the negative pressure room to other parts of the hospital area. The cadaver is placed in the autopsy table.

The autopsy table with downdraft ventilation (CSI Jewett DEM Dyna-Poise, Spire Integrated Solutions) (Figure 2) is used for the postmortem examination, ideal for examining cadavers, especially potentially infectious cases. The downdraft ventilation in an autopsy table facilitates airflow (Figure 3) that will decrease the exposure of the prosector from infectious agents that may be transported airborne. Exhaust systems around the autopsy table direct air (and aerosols) away from healthcare workers performing the procedure (e.g., exhaust downward). This downdraft table can also be rotated through an arc of 180 degrees with elevating mechanism that is of an ergonomic design. The downward exhaust system HEPA filter test of the autopsy table resulted in a percentage of less than 0.005% (0.00014), and the airflow visualization of the downdraft using a smoke test pattern showed a downward direction.

The autopsy suite is equipped with mortuary refrigerators. These are low-temperature refrigerated cabinets composed of condensing and evaporating units, that are used to keep dead bodies. Autopsy tools are stationed near the autopsy table for easy access. Obtained tissue are placed in the pass box, which is used to transfer materials from the suite to the tissue processing area through a controlled environment in order to avoid airborne cross-contamination.

After the procedure the team exits thru the autoclave room and doff their used PPE before taking a shower and exiting thru the designated egress leading to the open space at the side of the morgue. The diener will clean up the dead body and place it back to the refrigerator. He/she will clean and disinfect the suite and turn on ultraviolet (UV) lights.

The autopsy table is also connected to a waste water drain treatment system, which ensures that the water waste is

Figure 2. Autopsy table with down draft ventilation. The autopsy table is designed for dissection of cadavers and includes a recessed top which eliminates the need for a body tray. The downdraft system provides a safe environment, completely ventilating formalin vapors down and away from the user.
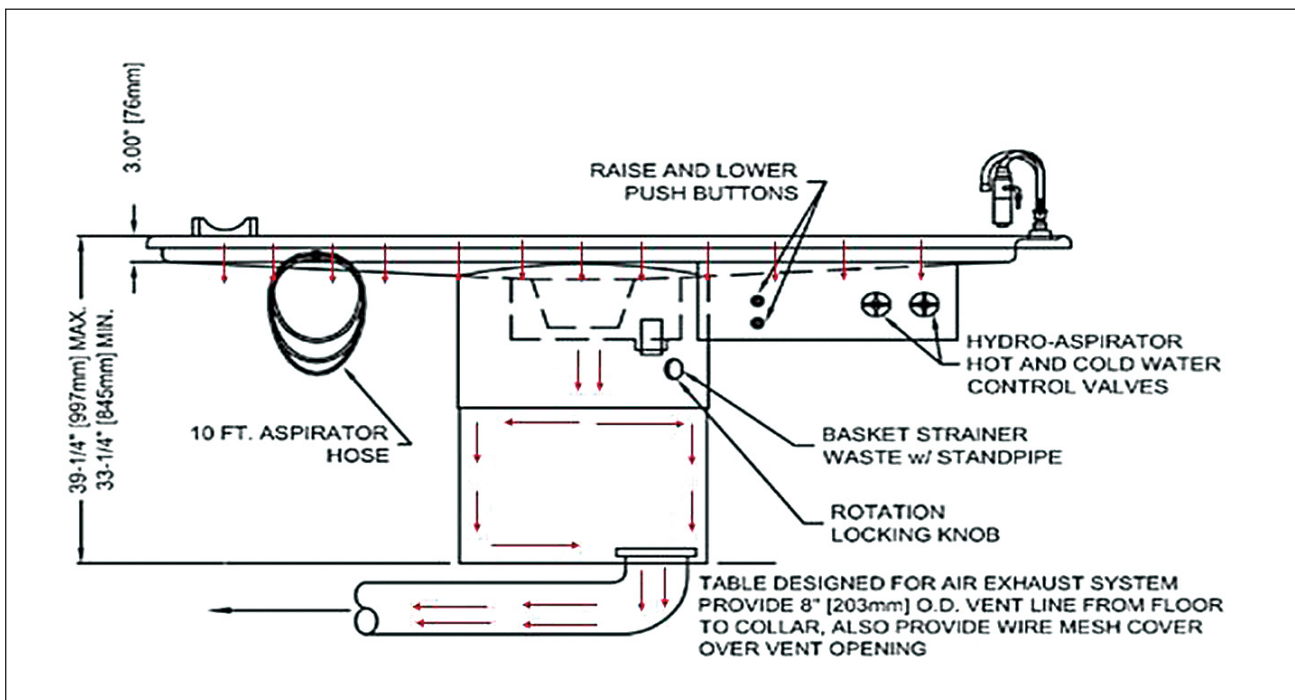

Figure 3. Airflow in downdraft autopsy table. Exhaust systems in and around the autopsy table should direct air (and aerosols) away from the health care worker performing the procedure (e.g., exhaust downward).

disinfected prior to release to the sewage treatment plant (STP). The flow of such system is initiated by trigger switch of the chlorinator into the wastewater within the holding tank (sump pit) then the transfer pump moves the chlorinated waste water into aeration tank. Finally, the effluent pump releases the wastewater into the existing sewer manhole.

Engineering and environmental controls, together with safety procedures, biohazard risk assessment and adequately trained personnel are vital factors in performing autopsies. This is in support of revitalizing the need and performance of autopsies especially with the continued threat of emerging and re-emerging infectious diseases, underscoring its continued relevance in the practice of medicine.

STATEMENT OF AUTHORSHIP

The authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interests.

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Congratulations to the winners of the

72ND PSP ANNUAL CONVENTION RESEARCH COMPETITION

As part of its commitment to advancing local research in pathology and laboratory medicine in the Philippines, the Philippine Society of Pathologists, Inc. held the Annual Research Competition of the 72nd PSP Annual Convention, its first in three years, last 22 April 2023 at the Shangri-La Hotel, Fort Bonifacio, Bonifacio Global City, Taguig. The Annual Convention Research Committee was chaired by Dr. Justine Alessandra Uy, with Dr. Allison Pagarigan and Dr. Amado Tandoc III as members.

A total of 62 pathologists-in-training joined the poster category, while 22 participated in the proffered/platform category. From the entries, 15 posters and 5 oral presentations were chosen as finalists.

Dr. Daphne Ang, Dr. Jared Billena, Dr. Edwin Muñoz, Dr. Sheryl Racelis-Andrada, and Dr. Joshua Uyboco served as judges for the oral presentation. Dr. Jose Jasper Andal, Dr. Kevin Elomina, Dr. Ira Doressa Anne How, Dr. Aldin Legaspi, Dr. Manuelito Madrid, Dr. Pier Angeli Medina, and Dr. Ansarie Salpin were the judges for the poster presentation.

All winners received a trophy and cash prizes at ₱25,000, ₱20,000, ₱15,000, for the first, second, and third place in the oral category, and ₱17,500, ₱15,000, and ₱10,000 for the first, second, and third place in the poster category, respectively.

Congratulations to the winners of the

72ND PSP ANNUAL CONVENTION RESEARCH COMPETITION

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POSTER CATEGORY

1st Place: **Dr. Jasher L. Chua**
Co-Authors: Marvin C. Masalunga and Nelson T. Geraldino
Institution: Philippine General Hospital
Title: **Right Atrial Isomerism: An Autopsy Approach from the Philippines**

2nd Place: **Dr. Jeffrey D. Domingo**
Co-Author: Ma. Cristina D.C. Briones
Institution: St. Luke's Medical Center - Quezon City
Title: **A Case of Extensive Facial Primitive Myxoid Mesenchymal Tumor of Infancy: An Approach to Diagnosis and Review of Literature**

3rd Place: **Dr. Julienne Ross C. Yarra**
Co-Author: Glenda Lyn Y. Pua
Institution: St. Luke's Medical Center - Quezon City
Title: **A Case of Hepatoid Adenocarcinoma of the Colon with Metastasis to the Liver: A Potential Diagnostic Confusion in Patients Presenting with Liver Mass**

PROFFERED/PLATFORM CATEGORY

1st Place: **Dr. Ermine Myrrhlet C. Bañares**
Co-Authors: Rowen T. Yolo and Celestine Marie G. Trinidad
Institution: University of Santo Tomas Hospital
Title: **Validity of the Singapore General Hospital Web-based Phyllodes Tumor Recurrence Risk Assessment Tool among Patient seen in a Tertiary Hospital in Metro Manila, Philippines: A 5-Year Retrospective Cohort Study**

2nd Place: **Dr. Aaron Pierre P. Calimag**
Co-Author: Januario D. Veloso, Jr.
Institution: National Kidney and Transplant Institute
Title: **Profiling of Genetic Mutations in Patients Diagnosed with Acute Myeloid Leukemia Using Fluorescence-in-Situ Hybridization from 2014 to 2021 at the National Kidney and Transplant Institute**

3rd Place: **Dr. Charles Joseph L. Bernardo**
Co-Authors: Daphne C. Ang, Claire Anne Therese M. Hemedez, Jose Jasper L. Andal, Rubi Li, Yancel Mascardo, Alizza Mariel S. Espiritu, Josephine Matudan Babida
Institution: St. Luke's Medical Center - Quezon City
Title: **Prevalence of Somatic BRCA 1 and BRCA 2 Mutations in Ovarian Cancer among Filipinos Using Next Generation Sequencing**
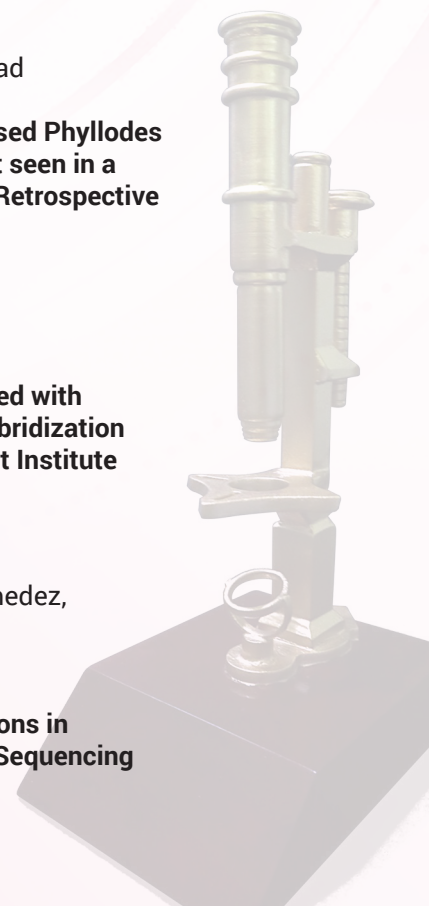
72ND PSP ANNUAL CONVENTION RESEARCH COMPETITION

ORAL PRESENTATIONS – FINALISTS

NAME	INSTITUTION	RESEARCH TITLE

Ermine Myrrhlet N. Bañares
 	
Co-Authors:
  Rowen T. Yolo
  Celestine Marie G. Trinidad
	University of Santo Tomas Hospital	Validity of the Singapore General Hospital Web-based Phyllodes Tumor Recurrence Risk Assessment Tool among patients seen in a Tertiary Hospital in Metro Manila, Philippines: A 5-year Retrospective Cohort Study

Charles Joseph L. Bernardo
 	
Co-Authors:
  Daphne C. Ang
  Claire Anne Therese M. Hemedez
  Jose Jasper L. Andal
  Rubi Li
  Yancel Mascardo
  Alizza Mariel S. Espiritu
  Josephine Matudan Babida
	St. Luke's Medical Center – Quezon City	Prevalence of Somatic BRCA1 and BRCA 2 Mutations in Ovarian Cancer among Filipinos Using Next Generation Sequencing

Aaron Pierre P. Calimag Co-Author: Januario Antonio D. Veloso	National Kidney and Transplant Institute	Profiling of Genetic Mutations in Patients Diagnosed with Acute Myeloid Leukemia Using Fluorescence In-Situ Hybridization from 2014 to 2021 at the National Kidney and Transplant Institute

Rebecca Rivera-Nagtalon
 	
Co-Authors:
  Daphne C. Ang
  Jose Jasper L. Andal
	St. Luke's Medical Center – Global City	A Cross-sectional Study on the Comparison of Saliva-based and Nasopharyngeal/ Oropharyngeal swab-based PCR tests for SARS-CoV-2 Detection in Acutely Ill patients in the Emergency Department of a Tertiary Hospital in the Philippines

Joseph Gary C. Sanchez, Jr. Co-Author: Kathrina S. Perez	Vicente Sotto Memorial Medical Center	The Diagnostic Value of a Grossly Normal Appendix in Determining The Absence of an Appendiceal Mucinous Neoplasm in Appendices Removed During Surgery for Mucinous Ovarian Neoplasms in a Tertiary Hospital in Cebu City from 2003 to 2022

72ND PSP ANNUAL CONVENTION RESEARCH COMPETITION

POSTER PRESENTATIONS – FINALISTS

NAME	INSTITUTION	RESEARCH TITLE

Anna Marielle G. Belmonte
 	
Co-Authors:
    Steven O. Truelen
    Redante D. Mendoza
    Jeffrey S. So
	Makati Medical Center	Unexpected Seminoma in a 34-year-old Phenotypical Female

Josh Matthew B Chen
 	
Co-Authors:
    Leo Francis N. Aquilizan
    Alejandro E. Arevalo
    Claire Anne Therese M. Hemedez
	St. Luke's Medical Center – Quezon City	B-Cell Lymphoblastic Lymphoma in the Cervix of a 44-year-old Female: A Case Report and Review of Literature

Jasher L. Chua
 	
Co-Authors:
    Marvin C. Masalunga
    Nelson T. Geraldino
	Philippine General Hospital	Right Atrial Isomerism: An Autopsy Approach from the Philippines

Joan Marie Diestro Co-Author: Margie Gayapa	West Visayas State University Medical Center	Aberrant β-hCG Expression of Diffuse Large B-cell Lymphoma, Anaplastic Variant Involving the Meckel Diverticulum
Jeffrey D. Domingo Co-Author: Ma. Cristina D.C. Briones	St. Luke's Medical Center – Quezon City	A Case of Extensive Facial Primitive Myxoid Mesenchymal Tumor of Infancy: An Approach to Diagnosis and Review of Literature
Carmela Claire A. Ferrer Co-Author: Januario Antonio D. Veloso	National Kidney and Transplant Institute	Anaplastic Lymphoma Kinase-Positive Large B-cell Lymphoma: A Rare Entity
Carmela Claire A. Ferrer Co-Authors: Pamela R. Delos Reyes-Murillo	National Kidney and Transplant Institute	A Rare Case of Myxoid Adrenal Cortical Carcinoma

Danielle Anne G. Gonong
 	
Co-Authors:
    Carol C. Tan-Hernandez
    Josy Naty M. Venturina-Fano
    Claire Anne Therese M. Hemedez
	Philippine Children's Medical Center	Synchronous Sertoli-Leydig Cell Tumor with Retiform Pattern and Pleuropulmonary Blastoma in a 3-Year-Old with DICER1 Syndrome

72ND PSP ANNUAL CONVENTION RESEARCH COMPETITION

NAME	INSTITUTION	RESEARCH TITLE

Kris Raychelle R. Godoy
 	
Co-Authors:
  Maria Lourdes L. Goco
  Claire Anne There M. Hemedez
  Jose B. Moran
	St. Luke's Medical Center – Quezon City	Mesonephric-Like Adenocarcinoma of the Uterus: A Case Report

Kris Raychelle R. Godoy
 	
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  Ann Margaret V. Chang
  Jose Jasper L. Andal
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	St. Luke's Medical Center – Quezon City	Primary Synovial Sarcoma of the Parotid Gland in a Filipino Female: A Case Report

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Roxanne Joy P. Quiton Co-Author: Jimmy S. Rosales	Ilocos Training Regional and Medical Center	A Rare case of Adult Onset Xanthogranuloma Presenting as Intracranial Mass

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	Philippine General Hospital	Chordoid Glioma: A Case of an Intracranial Mass in a Forty-four year old Female

Geneda Camille F. Sebial-Orteza	Mariano Marcos Memorial Hospital	Growing Teratoma Syndrome: A Rare Tumor Transformation
Julienne Ross C. Yarra Co-Author: Glenda Lyn Y. Pua	St. Luke's Medical Center – Quezon City	A Case of Hepatoid Adenocarcinoma of the Colon with Metastasis to the Liver: A Potential Diagnostic Confusion in Patients Presenting with Liver Mass

Instructions to Authors

The **Philippine Journal of Pathology (PJP)** is an open-access, peer-reviewed, English language, medical and health science journal that is published continuously online and semi-annually in print by the Philippine Society of Pathologists, Inc. (PSP, Inc). All manuscripts must be submitted through the PJP Official Website (Open Journal Systems) (http://philippinejournalofpathology.org). All other correspondences and other editorial matters should be sent via electronic mail to philippinepathologyjournal@gmail.com.

Articles and any other material published in the PJP represent the work of the author(s) and do not reflect the opinions of the Editors or the Publisher. **Articles that do not subscribe to the Instructions to Authors shall be promptly returned.**

ARTICLE SECTIONS

The PJP welcomes manuscripts on all aspects of pathology and laboratory medicine, to include cytology, histopathology, autopsy, forensic pathology, clinical chemistry, clinical microscopy, medical microbiology, parasitology, immunology, hematology, blood banking, medical technology, laboratory diagnostics, laboratory biosafety and biosecurity, laboratory management, and quality assurance.

The PJP accepts original articles, review articles, case reports, feature articles, brief communications, autopsy cases, editorials, or letters to the Editor.

Original articles
The research must have received institutional review board approval that is explicitly stated in the methodology. The abstract should contain no more than 200 words with a structured format consisting of the objective/s, methodology, results and conclusion. A manuscript for original articles should not exceed 25 typewritten pages (including tables, figures, illustrations and maximum of 30 references) or 6000 words.

Reviews
Review articles, both solicited and unsolicited, provide information on the "state of the art." PJP reviews not only summarize current understanding of a particular topic but also critically appraise relevant literature and data sources, describe significant gaps in the research, and future directions. The abstract should be from 50 to 75 words and should not be structured. A manuscript for reviews should not exceed 15 typewritten pages (including tables, figures, illustrations and maximum of 50 references) or 4000 words.

Case Reports
This type of article pertains to single or multiple reports of well-characterized cases that are highly unusual, novel, or rare; or with a unique or variant presentation, evolution or course; or that represent an unexpected or uncommon association of two or more diseases or disorders that may represent a previously unsuspected causal relationship; or that are underreported in the literature. The abstract should be from 50 to 75 words and should not be structured. A manuscript for case reports should not exceed 10 typewritten pages (including tables, figures, illustrations and maximum of 15 references) or 3000 words.

Feature articles
The PJP may feature articles, either as part of an issue theme or a special topic on pathology by a local or international expert or authority. The abstract should be from 50 to 75 words and should not be structured. A manuscript for feature articles should not exceed 25 typewritten pages (including tables, figures, illustrations and maximum of 30 references) or 6000 words.

Autopsy Vault
The PJP highly welcomes articles on autopsy protocols of cases. The article must include a summary presentation of the history, evaluation and work-up, clinical course of a case, followed by the autopsy procedure performed, gross and microscopic findings, discussion, learning points and conclusion. The PJP recognizes the instructional and educational value of articles under this section. The abstract should be from 50 to 75 words and should not be structured. A manuscript for the Autopsy Vault should not exceed 25 typewritten pages (including tables, figures, illustrations and maximum of 30 references) or 6000 words.

Images in Pathology
Images of unique, interesting, or highly educational cases encountered in hematology, cytology, histopathology, or medical microbiology, may be submitted under this section, and may include photomicrographs, gross pictures, machine read-outs, among others. A brief history, the photograph(s) and short discussion of the case. No abstract is required. A manuscript for Images in Pathology should not exceed 500 words, with maximum of 10 references. This is distinct from the Case Report which is a full write up.

Brief Communications
Brief Communications are short reports intended to either extend or expound on previously published research or present new and significant findings which may have a major impact in current practice. If the former, authors must acknowledge and cite the research which they are building upon. The abstract should be from 50 to 75 words and should not be structured. A manuscript for brief communications should not exceed 5 typewritten pages (including tables, figures, illustrations and maximum of 10 references) or 1500 words.

Editorials
Recognized leaders in the field of pathology and laboratory medicine may be invited by the Editor-in-Chief/Editorial Board to present their scientific opinion and views of a particular topic within the context of an issue theme or issues on scholarly publication. No abstract or keywords necessary.

Letters to the Editor
PJP welcomes feedback and comments on previously published articles in the form of Letters to the Editor. No abstract or keywords are necessary. A Letter to the Editor must not exceed 2 typewritten pages or 500 words.

Special Announcements
Special announcements may include upcoming conventions, seminars or conferences relevant to pathology. The Editors shall deliberate and decide on acceptance and publication of special announcements. Please coordinate with the Editorial Coordinator for any request for special announcements.

COVER LETTER
A cover letter must accompany each manuscript citing the complete title of the manuscript, the list of authors (complete names, position/designation and institutional affiliations), with one (1) author clearly designated as corresponding author, providing his/her complete institutional mailing address, institutional telephone/fax number, and work e-mail address. The **PJP Cover Letter Template** must be used.

PJP AUTHOR FORM

For submissions to the PJP to be accepted, all authors must read and sign the **PJP Author Form** consisting of: (1) the Authorship Certification, (2) the Author Declaration, (3) the Statement of Copyright Transfer, and (4) the Statement of Disclosure of Conflicts of Interest. The completely accomplished PJP Author Form shall be scanned and submitted along with the manuscript. No manuscript shall be received without the PJP Author Form.

GENERAL FORMATTING GUIDELINES

- Authors must use the standard PJP templates for each type of manuscript. These templates are aligned with the most current versions of the EQuaToR Network guidelines and checklists (http://equatornetwork.org).
- The manuscript should be encoded on the template using Microsoft Word (2007 version or later version), single-spaced, 2.54 cm margins throughout, on A4 size paper. Preferred fonts may include Century Gothic (template default), Times New Roman, or Arial.
- The manuscript should be arranged in sequence as follows: (1) Title Page, (2) Abstract, (3) Text, (4) References, (5) Tables, and (6) Figures & Illustrations.
- All the sheets of the manuscript should be labelled with the page number (in Hindu-Arabic Numerals) printed on the upper right corner.
- References should pertain directly to the work being reported. Within the text, references should be indicated using Hindu-Arabic numerals in superscripts.

SPECIFIC FORMATTING GUIDELINES

Title and Authors

- The title should be as concise as possible.
- A running title (less than 50 characters) shall also be required. The running title is the abbreviated version of the title that will be placed in the header. The running title should capture the essence of the manuscript title.
- The full name of the author(s) directly affiliated with the work should be included (First name, Middle initial and Last name). The order of authorship shall be the prerogative of the author(s).
- There are 4 criteria for authorship (ICMJE recommendations). These are captured in the **PJP Author Form**.
  - **Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND**
  - **Drafting the work or revising it critically for important intellectual content; AND**
  - **Final approval of the version to be published; AND**
  - **Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.**
- The highest educational attainment or title of the authors should be included as an attachment whenever appropriate (MD, PhD, et cetera).
- Name and location of no more than one (1) institutional affiliation per author may be included.
- If the paper has been presented in a scientific forum or convention, a note should be provided indicating the name of the forum or convention, location (country), and date of its presentation.

Abstract

- **For manuscripts under the "Original Article" section:** the abstract should contain no more than 300 words with a structured format consisting of the following standard headings: objective/s, methodology, results and conclusion.
- **For manuscripts under the "Feature Article," "Review Article," "Case Report," "Brief Communications," and "Autopsy Vault" sections:** the abstract should be no more than 200 words and need not be structured.
- Letters to the Editor and editorials do not require an abstract.

Keywords

At least three (3) keywords but no more than six (6), preferably using terms from the **Medical Subject Headings (MeSH) list of Index Medicus,** should be listed horizontally under the abstract for cross-indexing of the article.

Text

- The text should be organized consecutively as follows: **Introduction, Methodology, Results and Discussion, Conclusion** (IMRaD format), followed by **Disclosures, Acknowledgments** and **References.**
- All references, tables, figures and illustrations should be cited in the text, in numerical order.
- All abbreviations should be spelled out once (the first time they are mentioned in the text) followed by the abbreviation enclosed in parentheses. The same abbreviation may then be used subsequently instead of the full names.
- All measurements and weights should be in System International (SI) units.
- Under **Methodology**, information should be provided on institutional review board/ethics committee approval or informed consent taking (if appropriate).
- **Acknowledgements** to individuals/groups of persons, or institution/s who have contributed to the manuscript but *did not qualify as authors* based on the ICMJE criteria, should be included at the end of the text just before the references. Grants and subsidies from government or private institutions should also be acknowledged.

References

- References in the text should be identified by Hindu-Arabic Numerals in superscript on the same line as the preceding sentence.
- References should be numbered consecutively in the order by which they are mentioned in the text. They should not be alphabetized.
- All references should provide inclusive page numbers.
- Journal abbreviations should conform to those used in PubMed.
- A maximum of six authors per article can be cited; beyond that, name the first three and add "et al."
- The style/punctuation approved by PJP conforms to that recommended by the International Committee of Medical Journal Editors (ICMJE) available at http://www.icmje.org. Examples are shown below:

  One to Six Authors
  Krause RM. The origin of plagues: old and new. *Science*. 1992;257:1073-1078.

Mokdad AH, Bowman BA, Ford ES, Vinicor F, Marks JS, Koplan JP. The continuing epidemics of obesity and diabetes in the US. JAMA. 2001;286(10):1195-1200.
More than Six Authors
Rhynes VK, McDonald JC, Gelder FB, et al. Soluble HLA class I in the serum of transplant recipients. Ann Surg. 1993; 217 (5): 485–9.
Authors Representing a Group
Moher D, Schulz KF, Altman D; for the CONSORT Group. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomized trials. JAMA. 2001;285(15):1987-1991.
Book
Byrne, DW. Publishing your medical research paper: What they don't teach in medical school. Baltimore: Williams & Wilkins, 1998.
World Wide Web
Barry JM. The site of origin of the 1918 influenza pandemic and its public health implications. [Commentary]. JTranslational Med. January 20, 2004;2(3):1-4. http://www.translational-medicine.com/content/2/1/3. Accessed November 18, 2005.

Tables

- Cite all tables consecutively in the text and number them accordingly.
- Create tables preferably using Microsoft Excel with one table per worksheet.
- Tables should not be saved as image files.
- The content of tables should include a table number (Hindu-Arabic) and title in capital letters above the table.
- Place explanatory notes and legends, as well as definitions of abbreviations used below the table. For legends, use small letters (i.e., a, b, c, d).
- Each table must be self-explanatory, being a supplement rather than a duplicate of information in the text.
- Up to a maximum of five (5) tables are allowed.

Figures and Graphs

- Figures or graphs should be identified by Hindu-Arabic Numeral/s with titles and explanations underneath.
- The numbers should correspond to the order in which the figures/graphs occur in the text.
- Figures & graphs should not be saved as image files. For illustrations and photographs, see next section.
- Provide a title and brief caption for each figure or graph. Caption should not be longer than 15-20 words.
- All identifying data of the subject/s or patient/s under study such as name or case numbers, should be removed.
- Up to a maximum of five (5) figures and graphs are allowed.

Illustrations and Photographs

- Where appropriate, all illustrations/photographic images should be at least 800 x 600 dpi and submitted as image files (preferably as .png, .jpeg, .tif, .psd or .pdf files).
- For photomicrographs, the stain used (e.g. H & E) and magnification (e.g. 400X) should be included in the description.
- Computer-generated illustrations which are not suited for reproduction should be professionally redrawn or printed on good quality laser printers. Photocopies are not acceptable.
- All letterings for illustration should be of adequate size to be readable even after size reduction.
- Place explanatory notes and legends, as well as definitions of abbreviations used below the illustration/photograph.
- Up to a maximum of five (5) illustrations/ photographs are allowed.

N.B.: For tables, figures, graphs, illustrations and photographs that have been previously published in another journal or book, a note must be placed under the specific item stating that such has been adapted or lifted from the original publication. This should also be referenced in the **References** portion.

EDITORIAL PROCESS (Figure 1)

- The Editorial Coordinator shall review each submission to check if it has met aforementioned criteria and provide feedback to the author within 24 hours.
- Once complete submission is acknowledged, the manuscript undergoes Editorial Board Deliberation to decide whether it shall be considered or not for publication in the journal. Within five (5) working days, authors shall be notified through e-mail that their manuscript either (a) has been sent to referees for peer-review or (b) has been declined without review.
- The PJP implements a strict double blind peer review policy. For manuscripts that are reviewed, authors can expect a decision within ten (10) working days from editorial deliberation. There may be instances when decisions can take longer: in such cases, the Editorial Coordinator shall inform the authors.
- The editorial decision for manuscripts shall be one of the following: (a) acceptance without further revision, (b) acceptance with minor revisions, (c) major manuscript revision and resubmission, or (d) non-acceptance.
- Accepted manuscripts are subject to editorial modifications to bring them in conformity with the style of the journal. Copyediting and layout shall take five (5) working days, after which the manuscript is published online.
- All online articles from the last six (6) months shall be collated and published in print as a full issue.

EDITORIAL OFFICE CONTACT INFORMATION:

24 hours upon submission

Submission of manuscript through online platform

Editorial Coordinator screens submissions for compliance to requirements

Editorial Coordinator informs author of Incomplete submission

5 working days

Complete submission is acknowledged and manuscript is assigned a registration number

Manuscript undergoes Editorial Board Deliberation

Declined without peer review

Deemed FIT for publication in the PJP

10 working days

Manuscript undergoes Double Blind Peer Review

Manuscript is not accepted for publication

Manuscript undergoes Radiologist and/or Statistician review if appropriate

Manuscript is accepted with major/minor revisions

Manuscript is accepted without major/minor revisions

5-10 working days

Manuscript is revised by author based on deliberation and reviews

Revised manuscript is re-submitted through online platform

5 working days upon re-submission

Manuscript undergoes copyediting and layout by the copyeditor and layout editor

Article is assigned a DOI number and published online

Articles from the last 6 months are collated and published in print as a full issue

Figure 1. Editorial Process Flow.

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For submissions to the PJP to be accepted, all authors must read and sign this PJP Author Form consisting of: (1) the Authorship Certification, (2) the Author Declaration, (3) the Statement of Copyright Transfer, and (4) the Statement of Disclosure of Conflicts of Interest. The completely accomplished PJP Author Form shall be scanned and submitted along with the manuscript. No manuscript shall be received without the PJP Author Form.

COMPLETE TITLE OF MANUSCRIPT

_____
_____
_____

AUTHORSHIP CERTIFICATION

☐ In consideration of our submission to the Philippine Journal of Pathology (PJP), the undersigned author(s) of the manuscript hereby certify, that all of us have actively and sufficiently participated in (1) the conception or design of the work, the acquisition, analysis and interpretation of data for the work; AND (2)drafting the work, revising it critically for important intellectual content; AND (3) that we are all responsible for the final approval of the version to be published; AND (4) we all agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

AUTHOR DECLARATIONS

☐ The undersigned author(s) of the manuscript hereby certify, that the submitted manuscript represents original, exclusive and unpublished material. It is not under simultaneous consideration for publication elsewhere. Furthermore, it will not be submitted for publication in another journal, until a decision is conveyed regarding its acceptability for publication in the PJP.

☐ The undersigned hereby certify, that the study on which the manuscript is based had conformed to ethical standards and/or had been reviewed by the appropriate ethics committee.

☐ The undersigned likewise hereby certify that the article had written/informed consent for publication from involved subjects (for case report/series only) and that in case the involved subject/s can no longer be contacted (i.e., retrospective studies, no contact information, et cetera), all means have been undertaken by the author(s) to obtain the consent.

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AUTHOR DISCLOSURE OF CONFLICTS OF INTEREST

In order to ensure scientific objectivity and independence, the PJP requires all authors to make a full disclosure of areas of potential conflict of interest. Such disclosure will indicate whether the person and/or his/her immediate family has any financial relationship with pharmaceutical companies, medical equipment manufacturers, biomedical device manufacturers, or any companies with significant involvement in the field of health care. Place all disclosures in the table below. An extra form may be used if needed.

Examples of disclosures include but not limited to: ownership, employment, research support (including provision of equipment or materials), involvement as speaker, consultant, or any other financial relationship or arrangement with manufacturers, companies or suppliers. With respect to any relationships identified, author(s) must provide sufficiently detailed information to permit assessment of the significance of the potential conflict of interest (for example, the amount of money involved and/or the identification of any value of goods and services).

AUTHOR NAME	RELATIONSHIP	MANUFACTURER/ SUPPLIER/ COMPANY

All disclosures shall remain confidential during the review process and the nature of any final printed disclosure will be determined by the PJP. If there are no conflicts of interest to disclose, the author(s) should check the box below.

☐ I/We do not have any conflicts of interest to disclose.

Author Name	Signature	Date (MM/DD/YYYY)
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Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals

Updated May 2022

I. ABOUT THE RECOMMENDATIONS

A. Purpose of the Recommendations

ICMJE developed these recommendations to review best practice and ethical standards in the conduct and reporting of research and other material published in medical journals, and to help authors, editors, and others involved in peer review and biomedical publishing create and distribute accurate, clear, reproducible, unbiased medical journal articles. The recommendations may also provide useful insights into the medical editing and publishing process for the media, patients and their families, and general readers.

B. Who Should Use the Recommendations?

These recommendations are intended primarily for use by authors who might submit their work for publication to ICMJE member journals. Many non-ICMJE journals voluntarily use these recommendations (see www.icmje.org/journals-following-the-icmje-recommendations/). The ICMJE encourages that use but has no authority to monitor or

enforce it. In all cases, authors should use these recommendations along with individual journals' instructions to authors. Authors should also consult guidelines for the reporting of specific study types (e.g., the CONSORT guidelines for the reporting of randomized trials); see www.equator-network.org.

Journals that follow these recommendations are encouraged to incorporate them into their instructions to authors and to make explicit in those instructions that they follow ICMJE recommendations. Journals that wish to be identified on the ICMJE website as following these recommendations should notify the ICMJE secretariat at www.icmje.org/journals-following-the-icmje-recommendations/journal-listing-request-form/. Journals that in the past have requested such identification but who no longer follow ICMJE recommendations should use the same means to request removal from this list.

The ICMJE encourages wide dissemination of these recommendations and reproduction of this document in its entirety for educational, not-for-profit purposes without regard for copyright, but all uses of the recommendations and document should direct readers to www.icmje.org for the official, most recent version, as the ICMJE updates the recommendations periodically when new issues arise.

C. History of the Recommendations

The ICMJE has produced multiple editions of this document, previously known as the Uniform Requirements for Manuscripts Submitted to Biomedical Journals (URMs). The URM was first published in 1978 as a way of standardizing manuscript format and preparation across journals. Over the years, issues in publishing that went well beyond manuscript preparation arose, resulting in the development of separate statements, updates to the document, and its renaming as "Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals" to reflect its broader scope. Previous versions of the document may be found in the "Archives" section of www.icmje.org.

II. ROLES AND RESPONSIBILITIES OF AUTHORS, CONTRIBUTORS, REVIEWERS, EDITORS, PUBLISHERS, AND OWNERS

A. Defining the Role of Authors and Contributors

1. Why Authorship Matters

Authorship confers credit and has important academic, social, and financial implications. Authorship also implies responsibility and accountability for published work. The following recommendations are intended to ensure that contributors who have made substantive intellectual contributions to a paper are given credit as authors, but also that contributors credited as authors understand their role in taking responsibility and being accountable for what is published.

Because authorship does not communicate what contributions qualified an individual to be an author, some journals now request and publish information about the contributions of each person named as having participated in a submitted study, at least for original research. Editors are strongly encouraged to develop and implement a contributorship policy. Such policies remove much of the ambiguity surrounding contributions, but leave unresolved the question of the quantity and quality of contribution that qualify an individual for authorship. The ICMJE has thus developed criteria for authorship that can be used by all journals, including those that distinguish authors from other contributors.

2. Who Is an Author?

The ICMJE recommends that authorship be based on the following 4 criteria:
1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
2. Drafting the work or revising it critically for important intellectual content; AND
3. Final approval of the version to be published; AND
4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

In addition to being accountable for the parts of the work he or she has done, an author should be able to identify which co-authors are responsible for specific other parts of the work. In addition, authors should have confidence in the integrity of the contributions of their co-authors.

All those designated as authors should meet all four criteria for authorship, and all who meet the four criteria should be identified as authors. Those who do not meet all four criteria should be acknowledged—see Section II. A.3 below. These authorship criteria are intended to reserve the status of authorship for those who deserve credit and can take responsibility for the work. The criteria are not intended for use as a means to disqualify colleagues from authorship who otherwise meet authorship criteria by denying them the opportunity to meet criterion #s 2 or 3. Therefore, all individuals who meet the first criterion should have the opportunity to participate in the review, drafting, and final approval of the manuscript.

The individuals who conduct the work are responsible for identifying who meets these criteria and ideally should do so when planning the work, making modifications as appropriate as the work progresses. We encourage collaboration and co-authorship with colleagues in the locations where the research is conducted. It is the collective responsibility of the authors, not the journal to which the work is submitted, to determine that all people named as authors meet all four criteria; it is not the role of journal editors to determine who qualifies or does not qualify for authorship or to arbitrate authorship conflicts. If agreement cannot be reached about who qualifies for authorship, the institution(s) where the work was performed, not the journal editor, should be asked to investigate. The criteria used to determine the order in which authors are listed on the byline may vary, and are to be decided collectively by the author group and not by

editors. If authors request removal or addition of an author after manuscript submission or publication, journal editors should seek an explanation and signed statement of agreement for the requested change from all listed authors and from the author to be removed or added.

The corresponding author is the one individual who takes primary responsibility for communication with the journal during the manuscript submission, peer-review, and publication process. The corresponding author typically ensures that all the journal's administrative requirements, such as providing details of authorship, ethics committee approval, clinical trial registration documentation, and disclosures of relationships and activities, are properly completed and reported, although these duties may be delegated to one or more co-authors. The corresponding author should be available throughout the submission and peer-review process to respond to editorial queries in a timely way, and should be available after publication to respond to critiques of the work and cooperate with any requests from the journal for data or additional information should questions about the paper arise after publication. Although the corresponding author has primary responsibility for correspondence with the journal, the ICMJE recommends that editors send copies of all correspondence to all listed authors.

When a large multi-author group has conducted the work, the group ideally should decide who will be an author before the work is started and confirm who is an author before submitting the manuscript for publication. All members of the group named as authors should meet all four criteria for authorship, including approval of the final manuscript, and they should be able to take public responsibility for the work and should have full confidence in the accuracy and integrity of the work of other group authors. They will also be expected as individuals to complete disclosure forms.

Some large multi-author groups designate authorship by a group name, with or without the names of individuals. When submitting a manuscript authored by a group, the corresponding author should specify the group name if one exists, and clearly identify the group members who can take credit and responsibility for the work as authors. The byline of the article identifies who is directly responsible for the manuscript, and MEDLINE lists as authors whichever names appear on the byline. If the byline includes a group name, MEDLINE will list the names of individual group members who are authors or who are collaborators, sometimes called non-author contributors, if there is a note associated with the byline clearly stating that the individual names are elsewhere in the paper and whether those names are authors or collaborators.

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Contributors who meet fewer than all 4 of the above criteria for authorship should not be listed as authors, but they should be acknowledged. Examples of activities that alone (without other contributions) do not qualify a contributor for authorship are acquisition of funding;

general supervision of a research group or general administrative support; and writing assistance, technical editing, language editing, and proofreading. Those whose contributions do not justify authorship may be acknowledged individually or together as a group under a single heading (e.g., "Clinical Investigators" or "Participating Investigators"), and their contributions should be specified (e.g., "served as scientific advisors," "critically reviewed the study proposal," "collected data," "provided and cared for study patients," "participated in writing or technical editing of the manuscript").

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Public trust in the scientific process and the credibility of published articles depend in part on how transparently an author's relationships and activities, directly or topically related to a work, are handled during the planning, implementation, writing, peer review, editing, and publication of scientific work.

The potential for conflict of interest and bias exists when professional judgment concerning a primary interest (such as patients' welfare or the validity of research) may be influenced by a secondary interest (such as financial gain). Perceptions of conflict of interest are as important as actual conflicts of interest.

Individuals may disagree on whether an author's relationships or activities represent conflicts. Although the presence of a relationship or activity does not always indicate a problematic influence on a paper's content, perceptions of conflict may erode trust in science as much as actual conflicts of interest. Ultimately, readers must be able to make their own judgments regarding whether an author's relationships and activities are pertinent to a paper's content. These judgments require transparent disclosures. An author's complete disclosure demonstrates a commitment to transparency and helps to maintain trust in the scientific process.

Financial relationships (such as employment, consultancies, stock ownership or options, honoraria, patents, and paid expert testimony) are the most easily identifiable, the ones most often judged to represent potential conflicts of interest and thus the most likely to undermine the credibility of the journal, the authors, and science itself. Other interests may also represent or be perceived as conflicts, such as personal relationships or rivalries, academic competition, and intellectual beliefs.

Authors should avoid entering into agreements with study sponsors, both for-profit and nonprofit, that interfere with authors' access to all of the study's data or that interfere with their ability to analyze and interpret the data and to prepare and publish manuscripts independently when and where they choose. Policies that dictate where authors may publish their work violate this

principle of academic freedom. Authors may be required to provide the journal with the agreements in confidence.

Purposeful failure to report those relationships or activities specified on the journal's disclosure form is a form of misconduct, as is discussed in Section III.B.

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All participants in the peer-review and publication process–not only authors but also peer reviewers, editors, and editorial board members of journals–must consider and disclose their relationships and activities when fulfilling their roles in the process of article review and publication.

a. Authors

When authors submit a manuscript of any type or format they are responsible for disclosing all relationships and activities that might bias or be seen to bias their work. The ICMJE has developed a Disclosure Form to facilitate and standardize authors' disclosures. ICMJE member journals require that authors use this form, and ICMJE encourages other journals to adopt it.

b. Peer Reviewers

Reviewers should be asked at the time they are asked to critique a manuscript if they have relationships or activities that could complicate their review. Reviewers must disclose to editors any relationships or activities that could bias their opinions of the manuscript, and should recuse themselves from reviewing specific manuscripts if the potential for bias exists. Reviewers must not use knowledge of the work they're reviewing before its publication to further their own interests.

c. Editors and Journal Staff

Editors who make final decisions about manuscripts should recuse themselves from editorial decisions if they have relationships or activities that pose potential conflicts related to articles under consideration. Other editorial staff members who participate in editorial decisions must provide editors with a current description of their relationships and activities (as they might relate to editorial judgments) and recuse themselves from any decisions in which an interest that poses a potential conflict exists. Editorial staff must not use information gained through working with manuscripts for private gain. Editors should regularly publish their own disclosure statements and those of their journal staff. Guest editors should follow these same procedures.

Journals should take extra precautions and have a stated policy for evaluation of manuscripts submitted by individuals involved in editorial decisions. Further guidance is available from COPE (https://publicationethics.org/files/A_Short_Guide_to_Ethical_Editing.pdf) and WAME (http://wame.org/conflict-of-interest-in-peer-reviewed-medical-journals).

2. Reporting Relationships and Activities

Articles should be published with statements or supporting documents, such as the ICMJE Disclosure Form, declaring:
- Authors' relationships and activities; and

- Sources of support for the work, including sponsor names along with explanations of the role of those sources if any in study design; collection, analysis, and interpretation of data; writing of the report; any restrictions regarding the submission of the report for publication; or a statement declaring that the supporting source had no such involvement or restrictions regarding publication; and
- Whether the authors had access to the study data, with an explanation of the nature and extent of access, including whether access is ongoing.

To support the above statements, editors may request that authors of a study sponsored by a funder with a proprietary or financial interest in the outcome sign a statement, such as "I had full access to all of the data in this study and I take complete responsibility for the integrity of the data and the accuracy of the data analysis."

C. Responsibilities in the Submission and Peer-Review Process

1. Authors

Authors should abide by all principles of authorship and declaration of relationships and activities detailed in Sections II.A and II.B of this document.

a. Predatory or Pseudo-Journals

A growing number of entities are advertising themselves as "scholarly medical journals" yet do not function as such. These journals ("predatory" or "pseudo-journals") accept and publish almost all submissions and charge article processing (or publication) fees, often informing authors about this after a paper's acceptance for publication. They often claim to perform peer review but do not and may purposefully use names similar to well-established journals. They may state that they are members of ICMJE but are not (see www.icmje.org for current members of the ICMJE) and that they follow the recommendations of organizations such as the ICMJE, COPE, and WAME. Researchers must be aware of the existence of such entities and avoid submitting research to them for publication. Authors have a responsibility to evaluate the integrity, history, practices, and reputation of the journals to which they submit manuscripts. Guidance from various organizations is available to help identify the characteristics of reputable peer-reviewed journals (www.wame.org/identifying-predatory-or-pseudo-journals and www.wame.org/principles-of-transparency-and-best-practice-in-scholarly-publishing).

Seeking the assistance of scientific mentors, senior colleagues, and others with many years of scholarly publishing experience may also be helpful.

Authors should avoid citing articles in predatory or pseudo-journals.

2. Journals

a. Confidentiality

Manuscripts submitted to journals are privileged communications that are authors' private, confidential property, and authors may be harmed by premature disclosure of any or all of a manuscript's details.

Editors therefore must not share information about manuscripts, including whether they have been received and are under review, their content and status in the review process, criticism by reviewers, and their ultimate fate, to anyone other than the authors and reviewers. Requests from third parties to use manuscripts and reviews for legal proceedings should be politely refused, and editors should do their best not to provide such confidential material should it be subpoenaed.

Editors must also make clear that reviewers should keep manuscripts, associated material, and the information they contain strictly confidential. Reviewers and editorial staff members must not publicly discuss the authors' work, and reviewers must not appropriate authors' ideas before the manuscript is published. Reviewers must not retain the manuscript for their personal use and should destroy paper copies of manuscripts and delete electronic copies after submitting their reviews.

When a manuscript is rejected, it is best practice for journals to delete copies of it from their editorial systems unless retention is required by local regulations. Journals that retain copies of rejected manuscripts should disclose this practice in their Information for Authors.

When a manuscript is published, journals should keep copies of the original submission, reviews, revisions, and correspondence for at least three years and possibly in perpetuity, depending on local regulations, to help answer future questions about the work should they arise.

Editors should not publish or publicize peer reviewers' comments without permission of the reviewer and author. If journal policy is to blind authors to reviewer identity and comments are not signed, that identity must not be revealed to the author or anyone else without the reviewers' expressed written permission.

Confidentiality may have to be breached if dishonesty or fraud is alleged, but editors should notify authors or reviewers if they intend to do so and confidentiality must otherwise be honored.

b. Timeliness

Editors should do all they can to ensure timely processing of manuscripts with the resources available to them. If editors intend to publish a manuscript, they should attempt to do so in a timely manner and any planned delays should be negotiated with the authors. If a journal has no intention of proceeding with a manuscript, editors should endeavor to reject the manuscript as soon as possible to allow authors to submit to a different journal.

c. Peer Review

Peer review is the critical assessment of manuscripts submitted to journals by experts who are usually not part of the editorial staff. Because unbiased, independent, critical assessment is an intrinsic part of all scholarly work, including scientific research, peer review is an important extension of the scientific process.

The actual value of peer review is widely debated, but the process facilitates a fair hearing for a manuscript among members of the scientific community. More practically, it helps editors decide which manuscripts are suitable for their journals. Peer review often helps authors and editors improve the quality of reporting.

It is the responsibility of the journal to ensure that systems are in place for selection of appropriate reviewers. It is the responsibility of the editor to ensure that reviewers have access to all materials that may be relevant to the evaluation of the manuscript, including supplementary material for e-only publication, and to ensure that reviewer comments are properly assessed and interpreted in the context of their declared relationships and activities.

A peer-reviewed journal is under no obligation to send submitted manuscripts for review, and under no obligation to follow reviewer recommendations, favorable or negative. The editor of a journal is ultimately responsible for the selection of all its content, and editorial decisions may be informed by issues unrelated to the quality of a manuscript, such as suitability for the journal. An editor can reject any article at any time before publication, including after acceptance if concerns arise about the integrity of the work.

Journals may differ in the number and kinds of manuscripts they send for review, the number and types of reviewers they seek for each manuscript, whether the review process is open or blinded, and other aspects of the review process. For this reason and as a service to authors, journals should publish a clear, transparent description of their peer-review process for all types of manuscripts.

Journals should notify reviewers of the ultimate decision to accept or reject a paper, and should acknowledge the contribution of peer reviewers to their journal. Editors are encouraged to share reviewers' comments with co-reviewers of the same paper, so reviewers can learn from each other in the review process.

As part of peer review, editors are encouraged to review research protocols, plans for statistical analysis if separate from the protocol, and/or contracts associated with project-specific studies. Editors should encourage authors to make such documents publicly available at the time of or after publication, before accepting such studies for publication. Some journals may require public posting of these documents as a condition of acceptance for publication.

Journal requirements for independent data analysis and for public data availability are in flux at the time of this revision, reflecting evolving views of the importance of data availability for pre- and post-publication peer review. Some journal editors currently request a statistical analysis of trial data by an independent biostatistician before accepting studies for publication. Others ask authors to say whether the study data are available to third parties to view and/or use/reanalyze, while still others encourage or require authors to share their data with others for review or reanalysis. Each journal should establish and publish their specific requirements for data analysis and post in a place that potential authors can easily access.

Some people believe that true scientific peer review begins only on the date a paper is published. In that spirit, medical journals should have a mechanism for readers to submit comments, questions, or criticisms about published articles, and authors have a responsibility to respond appropriately and cooperate with any requests from the journal for data or additional information should questions about the paper arise after publication (see Section III).

ICMJE believes investigators have a duty to maintain the primary data and analytic procedures underpinning the published results for at least 10 years. The ICMJE encourages the preservation of these data in a data repository to ensure their longer-term availability.

d. Integrity

Editorial decisions should be based on the relevance of a manuscript to the journal and on the manuscript's originality, quality, and contribution to evidence about important questions. Those decisions should not be influenced by commercial interests, personal relationships or agendas, or findings that are negative or that credibly challenge accepted wisdom. In addition, authors should submit for publication or otherwise make publicly available, and editors should not exclude from consideration for publication, studies with findings that are not statistically significant or that have inconclusive findings. Such studies may provide evidence that, combined with that from other studies through meta-analysis, might still help answer important questions, and a public record of such negative or inconclusive findings may prevent unwarranted replication of effort or otherwise be valuable for other researchers considering similar work.

Journals should clearly state their appeals process and should have a system for responding to appeals and complaints.

e. Diversity and Inclusion

To improve academic culture, editors should seek to engage a broad and diverse array of authors, reviewers, editorial staff, editorial board members, and readers.

f. Journal Metrics

The journal impact factor is widely misused as a proxy for research and journal quality and as a measure of the importance of specific research projects or the merits of individual researchers, including their suitability for hiring, promotion, tenure, prizes, or research funding. ICMJE recommends that journals reduce the emphasis on impact factor as a single measure, but rather provide a range of article and journal metrics relevant to their readers and authors.

3. Peer Reviewers

Manuscripts submitted to journals are privileged communications that are authors' private, confidential property, and authors may be harmed by premature disclosure of any or all of a manuscript's details.

Reviewers therefore should keep manuscripts and the information they contain strictly confidential. Reviewers must not publicly discuss authors' work and must not appropriate authors' ideas before the manuscript is published. Reviewers must not retain the manuscript for their personal use and should destroy copies of manuscripts after submitting their reviews.

Reviewers who seek assistance from a trainee or colleague in the performance of a review should acknowledge these individuals' contributions in the written comments submitted to the editor. These individuals must maintain the confidentiality of the manuscript as outlined above.

Reviewers are expected to respond promptly to requests to review and to submit reviews within the time agreed. Reviewers' comments should be constructive, honest, and polite.

Reviewers should declare their relationships and activities that might bias their evaluation of a manuscript and recuse themselves from the peer-review process if a conflict exists.

D. Journal Owners and Editorial Freedom

1. Journal Owners

Owners and editors of medical journals share a common purpose, but they have different responsibilities, and sometimes those differences lead to conflicts.

It is the responsibility of medical journal owners to appoint and dismiss editors. Owners should provide editors at the time of their appointment with a contract that clearly states their rights and duties, authority, the general terms of their appointment, and mechanisms for resolving conflict. The editor's performance may be assessed using mutually agreed-upon measures, including but not necessarily limited to readership, manuscript submissions and handling times, and various journal metrics.

Owners should only dismiss editors for substantial reasons, such as scientific misconduct, disagreement with the long-term editorial direction of the journal, inadequate performance by agreed-upon performance metrics, or inappropriate behavior that is incompatible with a position of trust.

Appointments and dismissals should be based on evaluations by a panel of independent experts, rather than by a small number of executives of the owning organization. This is especially necessary in the case of dismissals because of the high value society places on freedom of speech within science and because it is often the responsibility of editors to challenge the status quo in ways that may conflict with the interests of the journal's owners.

A medical journal should explicitly state its governance and relationship to a journal owner (e.g., a sponsoring society).

2. Editorial Freedom

The ICMJE adopts the World Association of Medical Editors' definition of editorial freedom (http://wame.org/editorial-independence), which holds that editors-in-chief have full authority over the entire editorial content of their journal and the timing of publication of that content. Journal owners should not interfere in the evaluation, selection, scheduling, or editing of individual articles either directly or by creating an environment that

strongly influences decisions. Editors should base editorial decisions on the validity of the work and its importance to the journal's readers, not on the commercial implications for the journal, and editors should be free to express critical but responsible views about all aspects of medicine without fear of retribution, even if these views conflict with the commercial goals of the publisher.

Editors-in-chief should also have the final say in decisions about which advertisements or sponsored content, including supplements, the journal will and will not carry, and they should have final say in use of the journal brand and in overall policy regarding commercial use of journal content.

Journals are encouraged to establish an independent and diverse editorial advisory board to help the editor establish and maintain editorial policy. To support editorial decisions and potentially controversial expressions of opinion, owners should ensure that appropriate insurance is obtained in the event of legal action against the editors, and should ensure that legal advice is available when necessary. If legal problems arise, the editor should inform their legal adviser and their owner and/or publisher as soon as possible. Editors should defend the confidentiality of authors and peer reviewers (names and reviewer comments) in accordance with ICMJE policy (see Section II.C.2.a). Editors should take all reasonable steps to check the facts in journal commentary, including that in news sections and social media postings, and should ensure that staff working for the journal adhere to best journalistic practices including contemporaneous note-taking and seeking a response from all parties when possible before publication. Such practices in support of truth and public interest may be particularly relevant in defense against legal allegations of libel.

To secure editorial freedom in practice, the editor should have direct access to the highest level of ownership, not to a delegated manager or administrative officer.

Editors and editors' organizations are obliged to support the concept of editorial freedom and to draw major transgressions of such freedom to the attention of the international medical, academic, and lay communities.

E. Protection of Research Participants

All investigators should ensure that the planning, conduct, and reporting of human research are in accordance with the Helsinki Declaration as revised in 2013 (www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/). All authors should seek approval to conduct research from an independent local, regional, or national review body (e.g., ethics committee, institutional review board). If doubt exists whether the research was conducted in accordance with the Helsinki Declaration, the authors must explain the rationale for their approach and demonstrate that the local, regional, or national review body explicitly approved the doubtful aspects of the study. Approval by a responsible review body does not preclude editors from forming their own judgment whether the conduct of the research was appropriate.

Patients have a right to privacy that should not be violated without informed consent. Identifying information, including names, initials, or hospital numbers, should not be published in written descriptions, photographs, or pedigrees unless the information is essential for scientific purposes and the patient (or parent or guardian) gives written informed consent for publication. Informed consent for this purpose requires that an identifiable patient be shown the manuscript to be published. Authors should disclose to these patients whether any potential identifiable material might be available via the Internet as well as in print after publication. Patient consent should be written and archived with the journal, the authors, or both, as dictated by local regulations or laws. Applicable laws vary from locale to locale, and journals should establish their own policies with legal guidance. Since a journal that archives the consent will be aware of patient identity, some journals may decide that patient confidentiality is better guarded by having the author archive the consent and instead providing the journal with a written statement that attests that they have received and archived written patient consent.

Nonessential identifying details should be omitted. Informed consent should be obtained if there is any doubt that anonymity can be maintained. For example, masking the eye region in photographs of patients is inadequate protection of anonymity. If identifying characteristics are deidentified, authors should provide assurance, and editors should so note, that such changes do not distort scientific meaning.

The requirement for informed consent should be included in the journal's instructions for authors. When informed consent has been obtained, it should be indicated in the published article.

When reporting experiments on animals, authors should indicate whether institutional and national standards for the care and use of laboratory animals were followed.

III. PUBLISHING AND EDITORIAL ISSUES RELATED TO PUBLICATION IN MEDICAL JOURNALS

A. Corrections, Retractions, Republications, and Version Control

Honest errors are a part of science and publishing and require publication of a correction when they are detected. Corrections are needed for errors of fact. Matters of debate are best handled as letters to the editor, as print or electronic correspondence, or as posts in a journal-sponsored online forum. Updates of previous publications (e.g., an updated systematic review or clinical guideline) are considered a new publication rather than a version of a previously published article.

If a correction is needed, journals should follow these minimum standards:

- The journal should publish a correction notice as soon as possible detailing changes from and citing the original publication; the correction should be on an electronic or numbered print page that is

included in an electronic or a print Table of Contents to ensure proper indexing.

- The journal should also post a new article version with details of the changes from the original version and the date(s) on which the changes were made.
- The journal should archive all prior versions of the article. This archive can be either directly accessible to readers or can be made available to the reader on request.
- Previous electronic versions should prominently note that there are more recent versions of the article.
- The citation should be to the most recent version.

Pervasive errors can result from a coding problem or a miscalculation and may result in extensive inaccuracies throughout an article. If such errors do not change the direction or significance of the results, interpretations, and conclusions of the article, a correction should be published that follows the minimum standards noted above.

Errors serious enough to invalidate a paper's results and conclusions may require retraction. However, retraction with republication (also referred to as "replacement") can be considered in cases where honest error (e.g., a misclassification or miscalculation) leads to a major change in the direction or significance of the results, interpretations, and conclusions. If the error is judged to be unintentional, the underlying science appears valid, and the changed version of the paper survives further review and editorial scrutiny, then retraction with republication of the changed paper, with an explanation, allows full correction of the scientific literature. In such cases, it is helpful to show the extent of the changes in supplementary material or in an appendix, for complete transparency.

B. Scientific Misconduct, Expressions of Concern, and Retraction

Scientific misconduct in research and non-research publications includes but is not necessarily limited to data fabrication; data falsification, including deceptive manipulation of images; purposeful failure to disclose relationships and activities; and plagiarism. Some people consider failure to publish the results of clinical trials and other human studies a form of scientific misconduct. While each of these practices is problematic, they are not equivalent. Each situation requires individual assessment by relevant stakeholders. When scientific misconduct is alleged, or concerns are otherwise raised about the conduct or integrity of work described in submitted or published papers, the editor should initiate appropriate procedures detailed by such committees as the Committee on Publication Ethics (COPE) (http://publicationethics.org/resources/flowcharts), consider informing the institutions and funders, and may choose to publish an expression of concern pending the outcomes of those procedures. If the procedures involve an investigation at the authors' institution, the editor should seek to discover the outcome of that investigation; notify readers of the outcome if appropriate; and if the investigation proves scientific misconduct, publish a retraction of the article. There may be circumstances in which no misconduct is proven, but an exchange of letters to the editor could be published to highlight matters of debate to readers.

Expressions of concern and retractions should not simply be a letter to the editor. Rather, they should be prominently labelled, appear on an electronic or numbered print page that is included in an electronic or a print Table of Contents to ensure proper indexing, and include in their heading the title of the original article. Online, the retraction and original article should be linked in both directions and the retracted article should be clearly labelled as retracted in all its forms (abstract, full text, PDF). Ideally, the authors of the retraction should be the same as those of the article, but if they are unwilling or unable the editor may under certain circumstances accept retractions by other responsible persons, or the editor may be the sole author of the retraction or expression of concern. The text of the retraction should explain why the article is being retracted and include a complete citation reference to that article.

Retracted articles should remain in the public domain and be clearly labelled as retracted.

The validity of previous work by the author of a fraudulent paper cannot be assumed. Editors may ask the author's institution to assure them of the validity of other work published in their journals, or they may retract it. If this is not done, editors may choose to publish an announcement expressing concern that the validity of previously published work is uncertain.

The integrity of research may also be compromised by inappropriate methodology that could lead to retraction.

See COPE flowcharts for further guidance on retractions and expressions of concern. See Section IV.A.1.g.i for guidance about avoiding referencing retracted articles.

C. Copyright

Journals should make clear the type of copyright under which work will be published, and if the journal retains copyright, should detail the journal's position on the transfer of copyright for all types of content, including audio, video, protocols, and data sets. Medical journals may ask authors to transfer copyright to the journal. Some journals require transfer of a publication license. Some journals do not require transfer of copyright and rely on such vehicles as Creative Commons licenses. The copyright status of articles in a given journal can vary: Some content cannot be copyrighted (e.g., articles written by employees of some governments in the course of their work). Editors may waive copyright on other content, and some content may be protected under other agreements.

D. Overlapping Publications
1. Duplicate Submission

Authors should not submit the same manuscript, in the same or different languages, simultaneously to more than one journal. The rationale for this standard is the potential for disagreement when two (or more) journals claim the right to publish a manuscript that has been submitted simultaneously to more than one journal, and the possibility that two or more journals will unknowingly and unnecessarily undertake the work of peer review, edit the same manuscript, and publish the same article.

2. Duplicate and Prior Publication

Duplicate publication is publication of a paper that overlaps substantially with one already published, without clear, visible reference to the previous publication. Prior publication may include release of information in the public domain.

Readers of medical journals deserve to be able to trust that what they are reading is original unless there is a clear statement that the author and editor are intentionally republishing an article (which might be considered for historic or landmark papers, for example). The bases of this position are international copyright laws, ethical conduct, and cost-effective use of resources. Duplicate publication of original research is particularly problematic because it can result in inadvertent double-counting of data or inappropriate weighting of the results of a single study, which distorts the available evidence.

When authors submit a manuscript reporting work that has already been reported in large part in a published article or is contained in or closely related to another paper that has been submitted or accepted for publication elsewhere, the letter of submission should clearly say so and the authors should provide copies of the related material to help the editor decide how to handle the submission. See also Section IV.B.

This recommendation does not prevent a journal from considering a complete report that follows publication of a preliminary report, such as a letter to the editor, a preprint, or an abstract or poster displayed at a scientific meeting. The ICMJE does not consider results or data contained in assessment reports published by health technology assessment agencies, medical regulators, medical device regulators, or other regulatory agencies to be duplicate publication. It also does not prevent journals from considering a paper that has been presented at a scientific meeting but was not published in full, or that is being considered for publication in proceedings or similar format. Press reports of scheduled meetings are not usually regarded as breaches of this rule, but they may be if additional data tables or figures enrich such reports. Authors should also consider how dissemination of their findings outside of scientific presentations at meetings may diminish the priority journal editors assign to their work.

Authors who choose to post their work on a preprint server should choose one that clearly identifies preprints as not peer-reviewed work and includes disclosures of authors' relationships and activities. It is the author's responsibility to inform a journal if the work has been previously posted on a preprint server. In addition, it is the author's (and not the journal editors') responsibility to ensure that preprints are amended to point readers to subsequent versions, including the final published article. See Section III.D.3.

In the event of a public health emergency (as defined by public health officials), information with immediate implications for public health should be disseminated without concern that this will preclude subsequent consideration for publication in a journal. We encourage editors to give priority to authors who have made crucial data publicly available without delay.

Sharing with public media, government agencies, or manufacturers the scientific information described in a paper or a letter to the editor that has been accepted but not yet published violates the policies of many journals. Such reporting may be warranted when the paper or letter describes major therapeutic advances; reportable diseases; or public health hazards, such as serious adverse effects of drugs, vaccines, other biological products, medical devices. This reporting, whether in print or online, should not jeopardize publication, but should be discussed with and agreed upon by the editor in advance when possible.

The ICMJE will not consider as prior publication the posting of trial results in any registry that meets the criteria noted in Section III.L if results are limited to a brief (500 word) structured abstract or tables (to include participants enrolled, key outcomes, and adverse events). The ICMJE encourages authors to include a statement with the registration that indicates that the results have not yet been published in a peer-reviewed journal, and to update the results registry with the full journal citation when the results are published.

Editors of different journals may together decide to simultaneously or jointly publish an article if they believe that doing so would be in the best interest of public health. However, the National Library of Medicine (NLM) indexes all such simultaneously published joint publications separately, so editors should include a statement making the simultaneous publication clear to readers.

Authors who attempt duplicate publication without such notification should expect at least prompt rejection of the submitted manuscript. If the editor was not aware of the violations and the article has already been published, then the article might warrant retraction with or without the author's explanation or approval.

See COPE flowcharts for further guidance on handling duplicate publication.

3. Preprints

Posting of work as a preprint may influence a journal's interest in or priority for peer review and publication of that work. Journals should clearly describe their policies related to the posting and citing of preprints in their Information for Authors. Authors should become familiar with the policies of journals they wish to submit their work to prior to posting work on a preprint server.

a. Choosing a Preprint Archive

There has been an increase in preprint archives in biomedicine. There are both benefits and harms in dissemination of scientific findings prior to peer review. To maximize potential benefits and minimize potential harms, authors who wish to make preprints of non–peer-reviewed work publicly available should choose preprint archives that have the following characteristics:

- Clearly identify preprints as work that is not peer reviewed;
- Require authors to document disclosures of interest;
- Require authors to indicate funding source(s);

- Have a clear process for preprint archive users to notify archive administrators about concerns related to posted preprints—a public commenting feature is desirable for this purpose;
- Maintain metadata for preprints that are withdrawn from posting and post withdrawal notices indicating the timing and reason for withdrawal of a preprint; and
- Have a mechanism for authors to indicate when the preprint article has been subsequently published in a peer-reviewed journal.

b. Submitting Manuscripts That Are in Preprint Archives to a Peer-Reviewed Journal

Authors should inform a journal if the work submitted to the journal has been posted on a preprint server and provide a link to the preprint, whether the posting occurs prior to submission or during the peer-review process. It is also helpful to indicate in the text of the manuscript, perhaps in the introduction, that a preprint is available and how reviewers can access that preprint. In addition, it is the authors' (and not the journal editors') responsibility to ensure that preprints are amended to point readers to subsequent versions of the work, including the published article. Authors should not post in the preprint archive the published article nor interim versions that are produced during the peer-review process that incorporate revisions based on journal feedback.

c. Referencing Preprints in Submitted Manuscripts

When preprints are cited in submitted manuscripts or published articles, the citation should clearly indicate that the reference is a preprint. When a preprint article has been subsequently published in a peer-reviewed journal, authors should cite the subsequent published article rather than the preprint article whenever appropriate. Journals should include the word "preprint" following the citation information in the reference list and consider indicating that the cited material is a preprint in the text. The citation should include the link to the preprint and DOI if the preprint archive issues DOIs. Authors should be cautious about referencing preprints that were posted and never subsequently published in a peer-reviewed journal, but the time interval of concern will vary depending on the topic and specific reasons for citation.

4. Acceptable Secondary Publication

Secondary publication of material published in other journals or online may be justifiable and beneficial, especially when intended to disseminate important information to the widest possible audience (e.g., guidelines produced by government agencies and professional organizations in the same or a different language). Secondary publication for various other reasons may also be justifiable provided the following conditions are met:

1. The authors have received approval from the editors of both journals (the editor concerned with secondary publication must have access to the primary version).

2. The priority of the primary publication is respected by a publication interval negotiated by both editors with the authors.
3. The paper for secondary publication is intended for a different group of readers; an abbreviated version could be sufficient.
4. The secondary version faithfully reflects the authors, data, and interpretations of the primary version.
5. The secondary version informs readers, peers, and documenting agencies that the paper has been published in whole or in part elsewhere—for example, with a note that might read, "This article is based on a study first reported in the [journal title, with full reference]"—and the secondary version cites the primary reference.
6. The title of the secondary publication should indicate that it is a secondary publication (complete or abridged republication or translation) of a primary publication. Of note, the NLM does not consider translations to be "republications" and does not cite or index them when the original article was published in a journal that is indexed in MEDLINE.

When the same journal simultaneously publishes an article in multiple languages, the MEDLINE citation will note the multiple languages (e.g., Angelo M. Journal networking in nursing: a challenge to be shared. Rev Esc Enferm USP. 2011 Dec 45[6]:1281-2,1279-80,1283-4. Article in English, Portuguese, and Spanish. No abstract available. PMID: 22241182).

5. Manuscripts Based on the Same Database

If editors receive manuscripts from separate research groups or from the same group analyzing the same data set (e.g., from a public database, or systematic reviews or meta-analyses of the same evidence), the manuscripts should be considered independently because they may differ in their analytic methods, conclusions, or both. If the data interpretation and conclusions are similar, it may be reasonable although not mandatory for editors to give preference to the manuscript submitted first. Editors might consider publishing more than one manuscript that overlap in this way because different analytical approaches may be complementary and equally valid, but manuscripts based upon the same data set should add substantially to each other to warrant consideration for publication as separate papers, with appropriate citation of previous publications from the same data set to allow for transparency.

Secondary analyses of clinical trial data should cite any primary publication, clearly state that it contains secondary analyses/results, and use the same identifying trial registration number as the primary trial and unique, persistent data set identifier.

Sometimes for large trials it is planned from the beginning to produce numerous separate publications regarding separate research questions but using the same original participant sample. In this case authors may use the original single trial registration number, if all the outcome parameters were defined in the original registration. If the authors registered several substudies as separate entries in, for example, ClinicalTrials.gov,

then the unique trial identifier should be given for the study in question. The main issue is transparency, so no matter what model is used it should be obvious for the reader.

E. Correspondence

Medical journals should provide readers with a mechanism for submitting comments, questions, or criticisms about published articles, usually but not necessarily always through a correspondence section or online forum. The authors of articles discussed in correspondence or an online forum have a responsibility to respond to substantial criticisms of their work using those same mechanisms and should be asked by editors to respond. Authors of correspondence should be asked to declare any competing relationships or activities.

Correspondence may be edited for length, grammatical correctness, and journal style. Alternatively, editors may choose to make available to readers unedited correspondence, for example, via an online commenting system. Such commenting is not indexed in MEDLINE unless it is subsequently published on a numbered electronic or print page. However the journal handles correspondence, it should make known its practice. In all instances, editors must make an effort to screen discourteous, inaccurate, or libellous comments.

Responsible debate, critique, and disagreement are important features of science, and journal editors should encourage such discourse ideally within their own journals about the material they have published. Editors, however, have the prerogative to reject correspondence that is irrelevant, uninteresting, or lacking cogency, but they also have a responsibility to allow a range of opinions to be expressed and to promote debate.

In the interests of fairness and to keep correspondence within manageable proportions, journals may want to set time limits for responding to published material and for debate on a given topic.

F. Fees

Journals should be transparent about their types of revenue streams. Any fees or charges that are required for manuscript processing and/or publishing materials in the journal shall be clearly stated in a place that is easy for potential authors to find prior to submitting their manuscripts for review or explained to authors before they begin preparing their manuscript for submission (http://publicationethics.org/files/u7140/Principles_of_Transparency_and_Best_Practice_in_Scholarly_Publishing.pdf).

G. Supplements, Theme Issues, and Special Series

Supplements are collections of papers that deal with related issues or topics, are published as a separate issue of the journal or as part of a regular issue, and may be funded by sources other than the journal's publisher. Because funding sources can bias the content of supplements through the choice of topics and viewpoints, journals should adopt the following principles, which also apply to theme issues or special series that have external funding and/or guest editors:

1. The journal editor must be given and must take full responsibility for the policies, practices, and content of supplements, including complete control of the decision to select authors, peer reviewers, and content for the supplement. Editing by the funding organization should not be permitted.
2. The journal editor has the right to appoint one or more external editors of the supplement and must take responsibility for the work of those editors.
3. The journal editor must retain the authority to send supplement manuscripts for external peer review and to reject manuscripts submitted for the supplement with or without external review. These conditions should be made known to authors and any external editors of the supplement before beginning editorial work on it.
4. The source of the idea for the supplement, sources of funding for the supplement's research and publication, and products of the funding source related to content considered in the supplement should be clearly stated in the introductory material.
5. Advertising in supplements should follow the same policies as those of the primary journal.
6. Journal editors must enable readers to distinguish readily between ordinary editorial pages and supplement pages.
7. Journal and supplement editors must not accept personal favors or direct remuneration from sponsors of supplements.
8. Secondary publication in supplements (republication of papers published elsewhere) should be clearly identified by the citation of the original paper and by the title.
9. The same principles of authorship and disclosure of relationships and activities discussed elsewhere in this document should be applied to supplements.

H. Sponsorship or Partnership

Various entities may seek interactions with journals or editors in the form of sponsorships, partnerships, meetings, or other types of activities. To preserve editorial independence, these interactions should be governed by the same principles outlined above for Supplements, Theme Issues, and Special Series (Section III.G).

I. Electronic Publishing

Most medical journals are now published in electronic as well as print versions, and some are published only in electronic form. Principles of print and electronic publishing are identical, and the recommendations of this document apply equally to both. However, electronic publishing provides opportunities for versioning and raises issues about link stability and content preservation that are addressed here.

Recommendations for corrections and versioning are detailed in Section III.A.

Electronic publishing allows linking to sites and resources beyond journals over which journal editors have no editorial control. For this reason, and because links to external sites could be perceived as implying

endorsement of those sites, journals should be cautious about external linking. When a journal does link to an external site, it should state that it does not endorse or take responsibility or liability for any content, advertising, products, or other materials on the linked sites, and does not take responsibility for the sites' availability.

Permanent preservation of journal articles on a journal's website, or in an independent archive or a credible repository, is essential for the historical record. Removing an article from a journal's website in its entirety is almost never justified as copies of the article may have been downloaded even if its online posting was brief. Such archives should be freely accessible or accessible to archive members. Deposition in multiple archives is encouraged. However, if necessary for legal reasons (e.g., libel action), the URL for the removed article must contain a detailed reason for the removal, and the article must be retained in the journal's internal archive.

Permanent preservation of a journal's total content is the responsibility of the journal publisher, who in the event of journal termination should be certain the journal files are transferred to a responsible third party who can make the content available.

Journal websites should post the date that nonarticle web pages, such as those listing journal staff, editorial board members, and instructions for authors, were last updated.

J. Advertising

Most medical journals carry advertising, which generates income for their publishers, but journals should not be dominated by advertisements, and advertising must not be allowed to influence editorial decisions.

Journals should have formal, explicit, written policies for advertising in both print and electronic versions. Best practice prohibits selling advertisements intended to be juxtaposed with editorial content on the same product. Advertisements should be clearly identifiable as advertisements. Editors should have full and final authority for approving print and online advertisements and for enforcing advertising policy.

Journals should not carry advertisements for products proven to be seriously harmful to health. Editors should ensure that existing regulatory or industry standards for advertisements specific to their country are enforced, or develop their own standards. The interests of organizations or agencies should not control classified and other nondisplay advertising, except where required by law. Editors should consider all criticisms of advertisements for publication.

K. Journals and the Media

Journals' interactions with media should balance competing priorities. The general public has a legitimate interest in all journal content and is entitled to important information within a reasonable amount of time, and editors have a responsibility to facilitate that. However, media reports of scientific research before it has been peer-reviewed and fully vetted may lead to dissemination of inaccurate or premature conclusions, and doctors in practice need to have research reports available in full detail before they can advise patients about the reports' conclusions.

An embargo system has been established in some countries and by some journals to assist this balance, and to prevent publication of stories in the general media before publication of the original research in the journal. For the media, the embargo creates a "level playing field," which most reporters and writers appreciate since it minimizes the pressure on them to publish stories before competitors when they have not had time to prepare carefully. Consistency in the timing of public release of biomedical information is also important in minimizing economic chaos, since some articles contain information that has potential to influence financial markets. The ICMJE acknowledges criticisms of embargo systems as being self-serving of journals' interests and an impediment to rapid dissemination of scientific information, but believes the benefits of the systems outweigh their harms.

The following principles apply equally to print and electronic publishing and may be useful to editors as they seek to establish policies on interactions with the media:

- Editors can foster the orderly transmission of medical information from researchers, through peer-reviewed journals, to the public. This can be accomplished by an agreement with authors that they will not publicize their work while their manuscript is under consideration or awaiting publication and an agreement with the media that they will not release stories before publication of the original research in the journal, in return for which the journal will cooperate with them in preparing accurate stories by issuing, for example, a press release.

- Editors need to keep in mind that an embargo system works on the honor system—no formal enforcement or policing mechanism exists. The decision of a significant number of media outlets or biomedical journals not to respect the embargo system would lead to its rapid dissolution.

- Notwithstanding authors' belief in their work, very little medical research has such clear and urgently important clinical implications for the public's health that the news must be released before full publication in a journal. When such exceptional circumstances occur, the appropriate authorities responsible for public health should decide whether to disseminate information to physicians and the media in advance and should be responsible for this decision. If the author and the appropriate authorities wish to have a manuscript considered by a particular journal, the editor should be consulted before any public release. If editors acknowledge the need for immediate release, they should waive their policies limiting prepublication publicity.

- Policies designed to limit prepublication publicity should not apply to accounts in the media of presentations at scientific meetings or to the abstracts from

these meetings (see Duplicate Publication). Researchers who present their work at a scientific meeting should feel free to discuss their presentations with reporters but should be discouraged from offering more detail about their study than was presented in the talk, or should consider how giving such detail might diminish the priority journal editors assign to their work (see Duplicate Publication).

- When an article is close to being published, editors or journal staff should help the media prepare accurate reports by providing news releases, answering questions, supplying advance copies of the article, or referring reporters to appropriate experts. This assistance should be contingent on the media's cooperation in timing the release of a story to coincide with publication of the article.

L. Clinical Trials

1. Registration

The ICMJE's clinical trial registration policy is detailed in a series of editorials (see News and Editorials [www.icmje.org/news-and-editorials/] and FAQs [www.icmje.org/about-icmje/faqs/]).

Briefly, the ICMJE requires, and recommends that all medical journal editors require, registration of clinical trials in a public trials registry at or before the time of first patient enrollment as a condition of consideration for publication. Editors requesting inclusion of their journal on the ICMJE website list of publications that follow ICMJE guidance (www.icmje.org/journals.html) should recognize that the listing implies enforcement by the journal of ICMJE's trial registration policy.

ICMJE uses the date trial registration materials were first submitted to a registry as the date of registration. When there is a substantial delay between the submission of registration materials and their posting at the trial registry, editors may inquire about the circumstances that led to the delay.

The ICMJE defines a clinical trial as any research project that prospectively assigns people or a group of people to an intervention, with or without concurrent comparison or control groups, to study the relationship between a health-related intervention and a health outcome. Health-related interventions are those used to modify a biomedical or health-related outcome; examples include drugs, surgical procedures, devices, behavioral treatments, educational programs, dietary interventions, quality improvement interventions, and process-of-care changes. Health outcomes are any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events. The ICMJE does not define the timing of first participant enrollment, but best practice dictates registration by the time of first participant consent.

The ICMJE accepts publicly accessible registration in any registry that is a primary register of the WHO International Clinical Trials Registry Platform (ICTRP) (www.who.int/clinical-trials-registry-platform/network/who-data-set) that includes the minimum acceptable 24-item trial registration data set or in ClinicalTrials.gov, which is a

data provider to the WHO ICTRP. The ICMJE endorses these registries because they meet several criteria. They are accessible to the public at no charge, open to all prospective registrants, managed by a not-for-profit organization, have a mechanism to ensure the validity of the registration data, and are electronically searchable. An acceptable registry must include the minimum 24-item trial registration data set (http://prsinfo.clinicaltrials.gov/trainTrainer/WHO-ICMJE-ClinTrialsgov-Cross-Ref.pdf or www.who.int/clinical-trials-registry-platform) at the time of registration and before enrollment of the first participant.

The ICMJE considers inadequate trial registrations missing any of the 24 data fields, those that have fields that contain uninformative information, or registrations that are not made publicly accessible such as phase I trials submitted to the EU-CTR and trials of devices for which the information is placed in a "lock box." In order to comply with ICMJE policy, investigators registering trials of devices at ClinicalTrials.gov must "opt out" of the lock box by electing public posting prior to device approval. Approval to conduct a study from an independent local, regional, or national review body (e.g., ethics committee, institutional review board) does not fulfill the ICMJE requirement for prospective clinical trial registration. Although not a required item, the ICMJE encourages authors to include a statement that indicates that the results have not yet been published in a peer-reviewed journal, and to update the registration with the full journal citation when the results are published.

The purpose of clinical trial registration is to prevent selective publication and selective reporting of research outcomes, to prevent unnecessary duplication of research effort, to help patients and the public know what trials are planned or ongoing into which they might want to enroll, and to help give ethics review boards considering approval of new studies a view of similar work and data relevant to the research they are considering. Retrospective registration, for example at the time of manuscript submission, meets none of these purposes. Those purposes apply also to research with alternative designs, for example observational studies. For that reason, the ICMJE encourages registration of research with non-trial designs, but because the exposure or intervention in non-trial research is not dictated by the researchers, the ICMJE does not require it.

Secondary data analyses of primary (parent) clinical trials should not be registered as separate clinical trials, but instead should reference the trial registration number of the primary trial.

The ICMJE expects authors to ensure that they have met the requirements of their funding and regulatory agencies regarding aggregate clinical trial results reporting in clinical trial registries. It is the authors', and not the journal editors', responsibility to explain any discrepancies between results reported in registries and journal publications. The ICMJE will not consider as prior publication the posting of trial results in any registry that meets the above criteria if results are limited to a brief (500 word) structured abstract or tables (to include trial

participants enrolled, baseline characteristics, primary and secondary outcomes, and adverse events).

The ICMJE recommends that journals publish the trial registration number at the end of the abstract. The ICMJE also recommends that, whenever a registration number is available, authors list this number the first time they use a trial acronym to refer either to the trial they are reporting or to other trials that they mention in the manuscript.

Editors may consider whether the circumstances involved in a failure to appropriately register a clinical trial were likely to have been intended to or resulted in biased reporting. Because of the importance of prospective trial registration, if an exception to this policy is made, trials must be registered and the authors should indicate in the publication when registration was completed and why it was delayed. Editors should publish a statement indicating why an exception was allowed. The ICMJE emphasizes that such exceptions should be rare, and that authors failing to prospectively register a trial risk its inadmissibililty to our journals.

2. Data Sharing

The ICMJE's data sharing statement policy is detailed in an editorial (see Updates and Editorials [www.icmje.org/update.html]).

1. As of 1 July 2018 manuscripts submitted to ICMJE journals that report the results of clinical trials must contain a data sharing statement as described below.
2. Clinical trials that begin enrolling participants on or after 1 January 2019 must include a data sharing plan in the trial's registration. The ICMJE's policy regarding trial registration is explained at www.icmje.org/recommendations/browse/publishing-and-editorial-issues/clinical-trial-registration.html. If the data sharing plan changes after registration this should be reflected in the statement submitted and published with the manuscript, and updated in the registry record.

Data sharing statements must indicate the following: whether individual deidentified participant data (including data dictionaries) will be shared ("undecided" is not an acceptable answer); what data in particular will be shared; whether additional, related documents will be available (e.g., study protocol, statistical analysis plan, etc.); when the data will become available and for how long; by what access criteria data will be shared (including with whom, for what types of analyses, and by what mechanism). Illustrative examples of data sharing statements that would meet these requirements are provided in Table 1.

Authors of secondary analyses using shared data must attest that their use was in accordance with the terms (if any) agreed to upon their receipt. They must also reference the source of the data using its unique, persistent identifier to provide appropriate credit to those who generated it and allow searching for the studies it has supported. Authors of secondary analyses must explain completely how theirs differ from previous analyses. In addition, those who generate and then share clinical trial data sets deserve substantial credit for their efforts. Those using data collected by others should seek collaboration with those who collected the data. As collaboration will not always be possible, practical, or desired, the efforts of those who generated the data must be recognized.

IV. MANUSCRIPT PREPARATION AND SUBMISSION

A. Preparing a Manuscript for Submission to a Medical Journal

1. General Principles

The text of articles reporting original research is usually divided into Introduction, Methods, Results, and Discussion sections. This so-called "IMRAD" structure is not an arbitrary publication format but a reflection of the process of scientific discovery. Articles often need subheadings within these sections to further organize their content. Other types of articles, such as meta-analyses, may require different formats, while case reports, narrative reviews, and editorials may have less structured or unstructured formats.

Electronic formats have created opportunities for adding details or sections, layering information, crosslinking, or extracting portions of articles in electronic versions. Supplementary electronic-only material should be submitted and sent for peer review simultaneously with the primary manuscript.

2. Reporting Guidelines

Reporting guidelines have been developed for different study designs; examples include CONSORT (www.consort-statement.org) for randomized trials, STROBE for observational studies (http://strobe-statement.org/), PRISMA for systematic reviews and meta-analyses (http://prisma-statement.org/), and STARD for studies of diagnostic accuracy (http://www.equator-network.org/reporting-guidelines/stard/). Journals are encouraged to ask authors to follow these guidelines because they help authors describe the study in enough detail for it to be evaluated by editors, reviewers, readers, and other researchers evaluating the medical literature. Authors of review manuscripts are encouraged to describe the methods used for locating, selecting, extracting, and synthesizing data; this is mandatory for systematic reviews. Good sources for reporting guidelines are the EQUATOR Network (www.equator-network.org/home/) and the NLM's Research Reporting Guidelines and Initiatives (www.nlm.nih.gov/services/research_report_guide.html).

3. Manuscript Sections

The following are general requirements for reporting within sections of all study designs and manuscript formats.

a. Title Page

General information about an article and its authors is presented on a manuscript title page and usually includes the article title, author information, any disclaimers, sources of support, word count, and sometimes the number of tables and figures.

Table 1. Examples of Data Sharing Statements That Fulfill These ICMJE Requirements[*]

	Example 1	Example 2	Example 3	Example 4
Will individual participant data be available (including data dictionaries)?	Yes	Yes	Yes	No
What data in particular will be shared?	All of the individual participant data collected during the trial, after deidentification.	Individual participant data that underlie the results reported in this article, after deidentification (text, tables, figures, and appendices).	Individual participant data that underlie the results reported in this article, after deidentification (text, tables, figures, and appendices).	Not available
What other documents will be available?	Study Protocol, Statistical Analysis Plan, Informed Consent Form, Clinical Study Report, Analytic Code	Study Protocol, Statistical Analysis Plan, Analytic Code	Study Protocol	Not available
When will data be available (start and end dates)?	Immediately following publication. No end date.	Beginning 3 months and ending 5 years following article publication.	Beginning 9 months and ending 36 months following article publication.	Not applicable
With whom?	Anyone who wishes to access the data.	Researchers who provide a methodologically sound proposal.	Investigators whose proposed use of the data has been approved by an independent review committee (learned intermediary) identified for this purpose.	Not applicable
For what types of analyses?	Any purpose.	To achieve aims in the approved proposal.	For individual participant data meta-analysis.	Not applicable
By what mechanism will data be made available?	Data are available indefinitely at (*Link to be included*).	Proposals should be directed to xxx@yyy. To gain access, data requestors will need to sign a data access agreement. Data are available for 5 years at a third-party website (*Link to be included*).	Proposals may be submitted up to 36 months following article publication. After 36 months the data will be available in our University's data warehouse but without investigator support other than deposited metadata. Information regarding submitting proposals and accessing data may be found at (*Link to be provided*).	Not applicable

*These examples are meant to illustrate a range of, but not all, data sharing options.

Article title. The title provides a distilled description of the complete article and should include information that, along with the abstract, will make electronic retrieval of the article sensitive and specific. Reporting guidelines recommend and some journals require that information about the study design be a part of the title (particularly important for randomized trials and systematic reviews and meta-analyses). Some journals require a short title, usually no more than 40 characters (including letters and spaces) on the title page or as a separate entry in an electronic submission system. Electronic submission systems may restrict the number of characters in the title.

Author information. Each author's highest academic degrees should be listed, although some journals do not publish these. The name of the department(s) and institution(s) or organizations where the work should be attributed should be specified. Most electronic submission systems require that authors provide full contact information, including land mail and e-mail addresses, but the title page should list the corresponding authors' telephone and fax numbers and e-mail address. ICMJE encourages the listing of authors' Open Researcher and Contributor Identification (ORCID).

Disclaimers. An example of a disclaimer is an author's statement that the views expressed in the submitted article are his or her own and not an official position of the institution or funder.

Source(s) of support. These include grants, equipment, drugs, and/or other support that facilitated conduct of the work described in the article or the writing of the article itself. Inappropriate attribution of funding sources and affiliations are misleading and should be avoided.

Word count. A word count for the paper's text, excluding its abstract, acknowledgments, tables, figure legends, and references, allows editors and reviewers to assess whether the information contained in the paper warrants the paper's length, and whether the submitted manuscript fits within the journal's formats and word limits. A separate word count for the abstract is useful for the same reason.

Number of figures and tables. Some submission systems require specification of the number of figures and tables before uploading the relevant files. These numbers allow editorial staff and reviewers to confirm that all figures and tables were actually included with the manuscript and, because tables and figures occupy space, to assess if the information provided by the figures and

tables warrants the paper's length and if the manuscript fits within the journal's space limits.

Disclosure of relationships and activities. Disclosure information for each author needs to be part of the manuscript; each journal should develop standards with regard to the form the information should take and where it will be posted. The ICMJE has developed a uniform Disclosure Form for use by ICMJE member journals (www.icmje.org/coi_disclosure.pdf), and the ICMJE encourages other journals to adopt it. Despite availability of the form, editors may require disclosure of relationships and activities on the manuscript title page or other Disclosure section in the manuscript to save the work of collecting forms from each author prior to making an editorial decision or to save reviewers and readers the work of reading each author's form.

b. Abstract

Original research, systematic reviews, and meta-analyses require structured abstracts. The abstract should provide the context or background for the study and should state the study's purpose, basic procedures (selection of study participants, settings, measurements, analytical methods), main findings (giving specific effect sizes and their statistical and clinical significance, if possible), and principal conclusions. It should emphasize new and important aspects of the study or observations, note important limitations, and not overinterpret findings. Clinical trial abstracts should include items that the CONSORT group has identified as essential (www.consort-statement.org/resources/downloads/extensions/consort-extension-for-abstracts-2008pdf/). Funding sources should be listed separately after the abstract to facilitate proper display and indexing for search retrieval by MEDLINE.

Because abstracts are the only substantive portion of the article indexed in many electronic databases, and the only portion many readers read, authors need to ensure that they accurately reflect the content of the article. Unfortunately, information in abstracts often differs from that in the text. Authors and editors should work in the process of revision and review to ensure that information is consistent in both places. The format required for structured abstracts differs from journal to journal, and some journals use more than one format; authors need to prepare their abstracts in the format specified by the journal they have chosen.

The ICMJE recommends that journals publish the clinical trial registration number at the end of the abstract. The ICMJE also recommends that, when a registration number is available, authors list that number the first time they use a trial acronym to refer to the trial they are reporting or to other trials that they mention in the manuscript. If the data have been deposited in a public repository and/or are being used in a secondary analysis, authors should state at the end of the abstract the unique, persistent data set identifier; repository name; and number.

c. Introduction

Provide a context or background for the study (that is, the nature of the problem and its significance). State the specific purpose or research objective of, or hypothesis tested by, the study or observation. Cite only directly pertinent references, and do not include data or conclusions from the work being reported.

d. Methods

The guiding principle of the Methods section should be clarity about how and why a study was done in a particular way. The Methods section should aim to be sufficiently detailed such that others with access to the data would be able to reproduce the results. In general, the section should include only information that was available at the time the plan or protocol for the study was being written; all information obtained during the study belongs in the Results section. If an organization was paid or otherwise contracted to help conduct the research (examples include data collection and management), then this should be detailed in the methods.

The Methods section should include a statement indicating that the research was approved by an independent local, regional or national review body (e.g., ethics committee, institutional review board). If doubt exists whether the research was conducted in accordance with the Helsinki Declaration, the authors must explain the rationale for their approach and demonstrate that the local, regional or national review body explicitly approved the doubtful aspects of the study. See Section II.E.

i. Selection and Description of Participants

Clearly describe the selection of observational or experimental participants (healthy individuals or patients, including controls), including eligibility and exclusion criteria and a description of the source population. Because the relevance of such variables as age, sex, or ethnicity is not always known at the time of study design, researchers should aim for inclusion of representative populations into all study types and at a minimum provide descriptive data for these and other relevant demographic variables. Comment on how representative the study sample is of the larger population of interest.

Ensure correct use of the terms sex (when reporting biological factors) and gender (identity, psychosocial or cultural factors), and, unless inappropriate, report the sex and/or gender of study participants, the sex of animals or cells, and describe the methods used to determine sex and gender. If the study was done involving an exclusive population, for example in only one sex, authors should justify why, except in obvious cases (e.g., prostate cancer). Authors should define how they determined race or ethnicity and justify their relevance. In the case where race or ethnicity was not collected, explain why it was not collected. Race and ethnicity are social and not biological constructs; authors should interpret results associated with race and ethnicity in that context. Authors should use neutral, precise, and respectful language to describe study participants and avoid the use of terminology that might stigmatize participants.

ii. Technical Information

Specify the study's main and secondary objectives—usually identified as primary and secondary outcomes. Identify methods, equipment (give the manufacturer's name and address in parentheses), and procedures in sufficient detail to allow others to reproduce the results. Give references to established methods, including

statistical methods (see below); provide references and brief descriptions for methods that have been published but are not well-known; describe new or substantially modified methods, give the reasons for using them, and evaluate their limitations. Identify precisely all drugs and chemicals used, including generic name(s), dose(s), and route(s) of administration. Identify appropriate scientific names and gene names.

iii. Statistics

Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to judge its appropriateness for the study and to verify the reported results. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (such as confidence intervals). Avoid relying solely on statistical hypothesis testing, such as *P* values, which fail to convey important information about effect size and precision of estimates. References for the design of the study and statistical methods should be to standard works when possible (with pages stated). Define statistical terms, abbreviations, and most symbols. Specify the statistical software package(s) and versions used. Distinguish prespecified from exploratory analyses, including subgroup analyses.

e. Results

Present your results in logical sequence in the text, tables, and figures, giving the main or most important findings first. Do not repeat all the data in the tables or figures in the text; emphasize or summarize only the most important observations. Provide data on all primary and secondary outcomes identified in the Methods section. Extra or supplementary materials and technical details can be placed in an appendix where they will be accessible but will not interrupt the flow of the text, or they can be published solely in the electronic version of the journal.

Give numeric results not only as derivatives (e.g., percentages) but also as the absolute numbers from which the derivatives were calculated. Restrict tables and figures to those needed to explain the argument of the paper and to assess supporting data. Use graphs as an alternative to tables with many entries; do not duplicate data in graphs and tables. Avoid nontechnical uses of technical terms in statistics, such as "random" (which implies a randomizing device), "normal," "significant," "correlations," and "sample."

Separate reporting of data by demographic variables, such as age and sex, facilitate pooling of data for subgroups across studies and should be routine, unless there are compelling reasons not to stratify reporting, which should be explained.

f. Discussion

It is useful to begin the discussion by briefly summarizing the main findings, and explore possible mechanisms or explanations for these findings. Emphasize the new and important aspects of your study and put your findings in the context of the totality of the relevant evidence. State the limitations of your study, and explore the implications of your findings for future research and for clinical practice or policy. Discuss the influence or association of variables, such as sex and/or gender, on your findings, where appropriate, and the limitations of the data. Do not repeat in detail data or other information given in other parts of the manuscript, such as in the Introduction or the Results section.

Link the conclusions with the goals of the study but avoid unqualified statements and conclusions not adequately supported by the data. In particular, distinguish between clinical and statistical significance, and avoid making statements on economic benefits and costs unless the manuscript includes the appropriate economic data and analyses. Avoid claiming priority or alluding to work that has not been completed. State new hypotheses when warranted, but label them clearly.

g. References

i. General Considerations

Authors should provide direct references to original research sources whenever possible. References should not be used by authors, editors, or peer reviewers to promote self-interests. Authors should avoid citing articles from predatory or pseudo-journals. When preprints are cited, the citation should clearly indicate that the reference is a preprint (also see Section III.D.3). Although references to review articles can be an efficient way to guide readers to a body of literature, review articles do not always reflect original work accurately. On the other hand, extensive lists of references to original work on a topic can use excessive space. Fewer references to key original papers often serve as well as more exhaustive lists, particularly since references can now be added to the electronic version of published papers, and since electronic literature searching allows readers to retrieve published literature efficiently.

References to papers accepted but not yet published should be designated as "in press" or "forthcoming." Information from manuscripts submitted but not accepted should be cited in the text as "unpublished observations" with written permission from the source.

Published articles should reference the unique, persistent identifiers of the data sets employed.

Avoid citing a "personal communication" unless it provides essential information not available from a public source, in which case the name of the person and date of communication should be cited in parentheses in the text. For scientific articles, obtain written permission and confirmation of accuracy from the source of a personal communication.

Some but not all journals check the accuracy of all reference citations; thus, citation errors sometimes appear in the published version of articles. To minimize such errors, references should be verified using either an electronic bibliographic source, such as PubMed, or print copies from original sources. Authors are responsible for checking that none of the references cite retracted articles except in the context of referring to the retraction. For articles published in journals indexed in MEDLINE, the ICMJE considers PubMed the authoritative source for information about retractions. Authors can identify retracted articles in MEDLINE by searching

PubMed for "Retracted publication [pt]", where the term "pt" in square brackets stands for publication type, or by going directly to the PubMed's list of retracted publications (https://www.ncbi.nlm.nih.gov/pubmed/?term= retracted+publication+[pt]).

References should be numbered consecutively in the order in which they are first mentioned in the text. Identify references in text, tables, and legends by Arabic numerals in parentheses.

References cited only in tables or figure legends should be numbered in accordance with the sequence established by the first identification in the text of the particular table or figure. The titles of journals should be abbreviated according to the style used for MEDLINE (www.ncbi.nlm.nih.gov/nlmcatalog/journals). Journals vary on whether they ask authors to cite electronic references within parentheses in the text or in numbered references following the text. Authors should consult with the journal to which they plan to submit their work.

ii. Style and Format

References should follow the standards summarized in the NLM's Sample References (www.nlm.nih.gov/bsd/ uniform_requirements.html) webpage and detailed in the NLM's *Citing Medicine, 2nd edition* (www.ncbi.nlm. nih.gov/books/NBK7256/). These resources are regularly updated as new media develop, and currently include guidance for print documents; unpublished material; audio and visual media; material on CD-ROM, DVD, or disk; and material on the Internet.

h. Tables

Tables capture information concisely and display it efficiently; they also provide information at any desired level of detail and precision. Including data in tables rather than text frequently makes it possible to reduce the length of the text.

Prepare tables according to the specific journal's requirements; to avoid errors it is best if tables can be directly imported into the journal's publication software. Number tables consecutively in the order of their first citation in the text and supply a title for each. Titles in tables should be short but self-explanatory, containing information that allows readers to understand the table's content without having to go back to the text. Be sure that each table is cited in the text.

Give each column a short or an abbreviated heading. Authors should place explanatory matter in footnotes, not in the heading. Explain all nonstandard abbreviations in footnotes, and use symbols to explain information if needed. Symbols may vary from journal to journal (alphabet letter or such symbols as *, †, ‡, §), so check each journal's instructions for authors for required practice. Identify statistical measures of variations, such as standard deviation and standard error of the mean.

If you use data from another published or unpublished source, obtain permission and acknowledge that source fully.

Additional tables containing backup data too extensive to publish in print may be appropriate for publication in the electronic version of the journal, deposited with an archival service, or made available to readers directly by the authors. An appropriate statement should be added to the text to inform readers that this additional information is available and where it is located. Submit such tables for consideration with the paper so that they will be available to the peer reviewers.

i. Illustrations (Figures)

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