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ENVISIONING OUR FUTURE

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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 target audience are 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.

Congratulations and best wishes on the launching of the Philippine Journal of Pathology or PJP. It is a testament to the determination of the Philippine Society of Pathologists to be a globally recognized professional society that we embarked on this journey, that we can finally have a journal we can call our own.

A project initiated by the Board of Governors under the term of Dr. Linda Tamesis, culminating with this term, and spearheaded by Dr. Amado Tandoc III, our editor-in-chief, the road leading to this momentous event has been marked by many challenges but finally we are here.

But many challenges remain so let us remain steadfast in our support of the Philippine Journal of Pathology. I look forward to many more issues. More power to the PSP and PJP!

Januario D. Veloso, MD, FPSP
President, Philippine Society of Pathologists

CATALYST

The Philippine Journal of Pathology (PJP) was envisioned as a biannual publication of the Philippine Society of Pathologists, Inc. (PSP, Inc.), to serve as an avenue for research by Filipino anatomic and clinical pathologists. It was conceived in 1986 through the efforts of Drs. Antonia Cruz-Basa and Generoso Basa who saw the need for an official journal for the society where original pathology research can be published and which shall hopefully stimulate a culture of publication for the pathology community in the Philippines.

Through the years, however, the journal has been published on an irregular basis, primarily due to challenges in both content and sustainability, its last issue released in 2006. Whereas it is the PSP's initiative to support the PJP's regular publication, editorial content was then limited to invited articles and residents' research output during their years of training. Peer review is–at best–internal, and the selection of articles that go into each issue has been largely restricted to the incumbent editorial board/staff.

Ten years hence, we are in a unique and opportune position to address a vital gap in Philippine data, with the increasing importance of research and evidence in the practice of medicine, and the growing awareness of ethical scholarly publication standards and practices.

I firmly refuse to believe that there is nothing worthwhile to publish in the country on laboratory medicine and pathology: I think we all but need a high quality platform, which we can call our own, through which research efforts of pathologists and laboratorians, both seasoned and green, may be shared to the local and international community. I am thankful that our newly established editorial board and advisers have all agreed to our invitation, because they also believe in our vision of a high quality, peer reviewed journal which upholds international standards, for the Filipino pathologist and laboratorian.

We included in this issue the first batch of articles that passed the rigorous editorial process from submission to editorial board deliberation, from double blind peer review to final layout and publication. The harvest for this initial effort is lean, for now, but it is high grade, with all submissions subjected to international standards. All articles are available both in HTML and PDF formats in our official website (http://philippinejournalofpathology.org), open access to all under a Creative Commons BY-NC-SA License, that allows *free sharing, copying and redistributing in any medium and format, and adaptation, in which the material can be built upon by other researchers, under strict terms of giving appropriate credit to the authors and the PJP, for non-commercial purposes*. We will soon have an electronic ISSN to complement our print ISSN to formalize the PJP's online version. As an added feature, all our articles have CrossRef digital object identifiers (DOIs) that will serve as permanent links to our content in the world wide web. We are not predatory like so many fly-by-night journals that are proliferating and preying on authors, as the PJP neither asks for article processing fees nor asks for payment for article downloads and subscriptions.

In this issue, we have incorporated our updated Instructions to Authors, official forms, and Author User Guide for submitting through our Open Journal Systems online editorial management system, as a reference for all. We did not stop there. We also published in this issue the latest version of the **Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals** by the International Committee of Medical Journal Editors (ICMJE), and the standard checklists for various types of research studies from the **Enhancing the Quality and Transparency of health Research** (EQUATOR) Network, an international initiative whose main objective is to improve the reliability and value of scholarly publication of health research through promotion of transparent, complete, and accurate reporting.

It has been a little over a year since we presented the prospects of resurrecting the Philippine Journal of Pathology at the 64th Annual Convention of the PSP. The revival of the journal would not have been possible without the full support of the current and past PSP Presidents, Dr. Januario Veloso and Dr. Linda Tamesis, respectively, the Board of Governors, our dedicated editorial coordinator, and the members of our Core Team with whom we have begun this journey of revival back in 2012.

It only takes a spark, to get a fire going. The first issue of this journal is a push, a catalyst, to create change. You are part of this drive towards elevating our practice and informing the greater research community of pathology research in this part of the globe.

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

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OPEN ACCESS – ORIGINAL ARTICLE

Diagnostic Accuracy of Mean Platelet Volume in the Diagnosis of Acute Coronary Syndromes among Patients with Acute Chest Pain at the Emergency Room of Philippine Heart Center

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ABSTRACT

Introduction. Mean platelet volume (MPV), an index for platelet size, is believed to be associated with acute coronary syndromes (ACS). This study aims to establish the association of MPV and ACS in the local setting and to further evaluate the diagnostic accuracy of MPV in the detection of ACS.

Methodology. Adult patients presenting with chest pain seen at the ER were submitted for complete blood count (CBC). Specimens were processed for MPV and platelet count using Beckman-Coulter ACT 5Diff hematology auto-analyzer. Patients were grouped into ACS and non-ACS. Independent t-test was used for analysis. Diagnostic cut-off point was determined using Receiver Operating Characteristic (ROC) Curve.

Results. A total of 150 adult patients was examined for MPV and platelet counts. There was a significant difference of MPV between the two groups (p value <0.0001). The MPV of patients with ACS was increased at 8.3 fL compared to 7.3 fL in patients not diagnosed with ACS. At cut-off point of 8.4 fL, the positive predictive value and specificity for ACS were 100%, sensitivity of 43.6 and a negative predictive value of 46.2. The number of platelets was increased in non-ACS group.

Conclusion. The MPV of acute chest patients diagnosed with ACS was significantly higher compared to patients not diagnosed with ACS. Increased MPV at 8.4 fL was highly specific and predictive of ACS. However, the sensitivity and negative predictive value were low. The platelet count of non-ACS group was increased.

Key words : Mean platelet volume, MPV, Acute coronary syndromes, ACS

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INTRODUCTION

The clinical use of mean platelet volume (MPV) is unknown.[1] Although it is routinely measured in hematological auto-analyzers for more than a decade, many laboratories do not usually include this in the final report of complete blood count. The primary reason is the lack of standardization of this value.[1,2] Another reason is the limited evidence that this measurement adds any valuable information in the clinical situation.[1]

However, there are increasing data attributing MPV with acute coronary syndromes. Mean platelet volume, as an index of platelet size and function, have been found to be increased in patients having disease conditions under the spectrum of acute coronary artery syndromes (ACS).[2-7] The major reason for this is that increased platelet activity plays a crucial role in the development of acute myocardial infarction.[2] Though there are well-established risk factors identified in the formation of atherosclerosis that bring about ACS, myocardial infarction (MI) could only and likely to happen if there are large and hyperactive platelets in the circulation.[2,3]

The effective screening of patients at the emergency room for acute coronary syndrome remains a challenge. Currently, cardiac coronary related diseases continue to be the leading cause of morbidity in most of the countries and the number one cause of deaths since the beginning of twentieth century.[3] The association of increased MPV with a critical disease like ACS may possibly emerge this measurement as a simple and accessible test to estimate platelet

activity. This will further help to stratify cardiovascular risk among patients with acute coronary syndromes.[4]

The main objective of this study is to determine whether there is an association between mean platelet volume and the diagnosis of acute coronary syndromes in a local setting. We further aim to estimate the diagnostic accuracy of MPV in the detection of acute coronary syndromes in patients with acute chest pain.

METHODOLOGY

This is a diagnostic accuracy study approved by the Philippine Heart Center- Institutional Ethics Review Board conducted at the emergency room (ER) and ER Point-of-care testing (POCT) satellite laboratory of the Philippine Heart Center with data gathered from May 12, 2012 to July 22, 2012 and from June 1, 2013 to August 31, 2013. Included in the study were consecutive adult (≥21 years old) patients with chest pain and informed consent was obtained. Excluded were chest pain patients having serious concurrent illnesses where increase platelet count and mean platelet volume were expected as a result of reactive process from a known injury such as trauma, gastrointestinal bleeding, hyperthyroidism, hematopoietic malignancy, (platelet count >1,000 x 109/L)[8] and sepsis. Patients whose specimens for CBC are not processed within 15 minutes from blood extraction are likewise not included in the study.

The sample size computed is n= 150 at 95% confidence level with a relative error of 15% and assumed ACS rate among patients presenting with chest pain at the emergency room of 34.3% based on the previous study done by Lamorena et al. on ER patients presenting with symptoms suggestive of ACS in the Philippine Heart Center.[9] The variables of the study included mean platelet volume and platelet count as measured by Beckmann Coulter ACT 5 Diff *(Beckmann-Coulter, Inc, Fullerton California)* and the diagnoses of these patients.[10]

In the emergency room, the charts of all adult patients who came in due to acute chest pain were reviewed every 8-hour shift for chief complaint and laboratory tests ordered. Informed consent was performed on all eligible patients and was submitted for complete blood count. At least 4 ml of blood was extracted through venipuncture and was collected using a lavender-top (ethelynediaminetriacetic acid/EDTA) vacutainer *(BD, Franklin Lakes, NJ USA)*. The collected specimen in the lavender-top was fed through the manual-loading probe of Beckman Coulter ACT 5Diff hematology auto-analyzer. The result generated by the machine for platelet count and mean platelet volume were recorded. The reference standard followed for all the parameters of complete blood count was based on the Standard Operating Procedure Manual of the Department of Laboratory Medicine (DLM) of Philippine Heart Center. Smears of each of the specimen were performed and stained with Wright's stain to verify and confirm the results generated by the automated analyzer. CBC tests of all qualified patients were performed by licensed medical technologists and trained staff of the Point-of-Care-Testing Section of DLM. Out of 172 patient charts reviewed, 150 patients were included in the study. Informed consent was unable to be performed in twelve (12) patients and ten patients could not be submitted for CBC because they were discharged immediately. The clinical diagnosis of the patients as written by the cardiology fellow trainee on duty at the emergency room was recorded. Admitted patients were followed-up for the final diagnosis upon discharge and were the recorded diagnosis for such patients. Diagnoses of patients at the emergency room were based on their medical chart as written by the cardiology fellow trainee who examined them. The basis for the diagnosis of ACS and non-ACS was based solely on what is written by the cardiology fellow trainee at the emergency room. All of the hospital staff involved in the study, including the cardiology fellow trainees and the medical technologists, was blinded during the entire study.

Data was described as mean ±SD or frequency and percent distribution comparing the ACS and non-ACS patients. To determine if there is a statistically significant difference of the mean platelet volume and platelet count of these two groups, Independent t-test was used. Cut-off points of MPV were computed with their respective scores of diagnostic accuracy parameters (sensitivity, specificity, positive and negative predictive values), as determined by the area under the curve of Receiver Operating Characteristic (ROC) curve.

RESULTS

A total of 150 adult patients with chest pain were included in the study and 101 of these patients are diagnosed with acute coronary syndrome. The rest of the chest pain patients in the non-ACS category had heterogeneous diagnoses. These wide variety of diagnoses range from pulmonary problems such as chronic obstructive pulmonary disease, pleural effusion, pneumonia, and pulmonary tuberculosis to other cardiac causes like congestive heart failure, valvular heart diseases and pericardial effusion, and even non-cardiac etiology particularly costochondritis.

Table 1 shows the baseline characteristics of the patients included in the study. Patients with ACS tend to be older. The mean age under ACS category was 62 years old compared to 56 years old in non-ACS group. Majority of the population in both groups were males. Hypertension was the most common risk factors seen in 58 patients and significantly associated with the diagnosis of ACS. There were 14 patients who had previous myocardial infarction or stroke and showed a notable relationship with ACS. Aspirin was by far the most common anti-platelet medication used by patients in the study.

The mean platelet volume of patients diagnosed with ACS was remarkably higher than patients who were not diagnosed with acute coronary syndrome at p value <0.0001 (Table 2). The ACS patients had an average MPV of 8.3 compared to only 7.3 mean MPV among non-ACS patients. On the other hand, the platelet number of patients not diagnosed with ACS was increased than patients who had a diagnosis of ACS (Table 3). Patients who were not diagnosed with acute coronary syndrome had an average platelet number of 269, 000 in contrast to 235,000 in ACS patients.

The computed cut-off points of mean platelet volume predictive in the diagnosis of acute coronary syndrome are shown in Table 3. The overall diagnostic accuracy of MPV, calculated as the area under the curve by the ROC curve revealed a significant test with a value under the curve of 0. 868 and p value less than 0.0001. At 8.4 fL cut-off result, positive predictive value is 100% and a 46.2% negative predictive value. A lower value of 7.3 fL MPV shows a higher negative predictive value of 75% and 78% positive predictive value. The cut-off points of MPV between 7.6 fL and 7.8 fL showed fairly acceptable parameters of diagnostic accuracy in detecting acute coronary syndromes. A cut-off value of 7.8 fL satisfactorily predict the diagnosis of ACS having the sensitivity of 80.2%, specificity of 75.5%, positive predictive value of 87.1% and negative predictive value of 64.9 %. Alternatively, a value of 7.7 fL showed relatively similar values of diagnostic accuracy.

Table 1. Baseline characteristics of patients in the study according to diagnosis of chest pain (PHC, 2013)

Characteristics	ACS (n=101)		Non ACS (n=49)		Total		p
	N	%	N	%	N	%	
Age							
Mean + std. dev		62.63 ¬+ 12.446		56.63 + 18.289		60.67 + 14.822	**0.04***
Sex							
Male	73	72.3	38	77.6	111	74.0	0.49**
Female	28	27.7	11	22.4	39	26.0	
Risk factors							
Hypertension	58	57.4	10	20.4	68	45.3	**<0.00****
Smoking	41	40.6	20	40.8	61	40.7	1.00**
Diabetes mellitus	27	26.7	8	16.3	35	23.3	0.22**
Dyslipidemia	7	6.9	2	4.1	9	6.0	0.71***
Previous MI / Stroke	14	13.9	0	-	14	9.3	**0.00*****
Medication							
Aspirin	24	23.8	7	14.3	31	20.7	0.26**
Clopidogrel	6	5.9	3	6.1	9	6.0	1.00***
Warfarin	0	-	1	0.2	1	0.7	0.33***
Fondaparinux	1	1.0	0	-	1	0.7	1.00***

*Independent t-test (significant p value<0.05) **Chi Square test (significant p value <0.05) ***Fisher's Exact Test (significant p value <0.05)

Table 2. Mean platelet volume and platelet count according to diagnosis of chest pain (PHC, 2013)

Characteristics	ACS (n=101)		Non ACS (n=49)		Total		p
	N	%	N	%	N	%	
MPV (fL)							
Mean + std. dev		8.279 + 0.7356		7.276 + 0.5356		7.951 + 0.8238	**<0.00***
Platelet Count							
Mean + std. dev		235.75 + 74.651		269.53 + 98.156		246.79 + 84.241	**0.02***

*Independent t-test (significant p value <0.05)

Table 3. Cut-off points of mean platelet volume predictive of ACS diagnosis

Platelet Volume (fL)	ACS	Non-ACS	Sn* %	Sp* %	PPV* %	NPV* %	Kappa Test
>8.4	44	0	43.6	100.0	100.0	46.2	0.335 +
<8.4	57	49					0.052
Total	101	49					**<0.0001**
>7.8	81	12	80.2	75.5	87.1	64.9	0.535 +
<7.8	20	37					0.072
Total	101	49					**<0.0001**
>7.7	83	14	82.2	71.4	85.6	66.0	0.525 +
<7.7	18	35					0.073
Total	101	49					**<0.0001**
>7.6	85	18	84.2	63.3	82.5	66.0	0.479 +
<7.6	16	31					0.077
Total	101	49					**<0.0001**
>7.3	93	25	92.1	49.0	78.8	75.0	0.451 +
<7.3	8	24					0.078
Total	101	49					**<0.0001**

*Sn=Sensitivity, Sp=Specificity, PPV=Positive Predictive Value, NPV=Negative Predictive Value

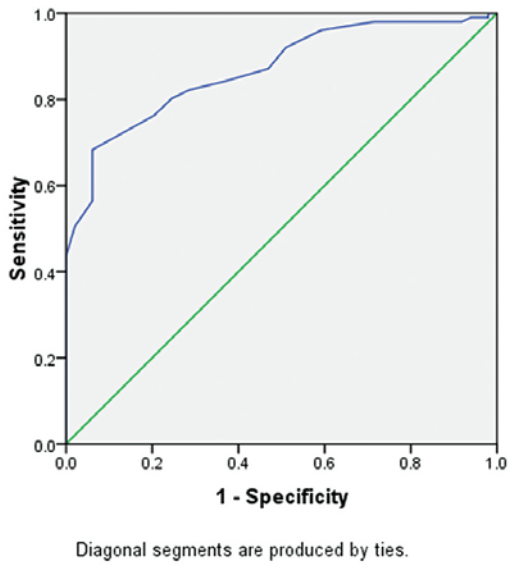

Figure 1. The ROC curve of MPV values in association with the diagnosis of ACS (AUC=0.868, Cut-off values 8.4, 7.8, 7.7, 7.6, 7.3).

Figure 1 is the ROC curve showing a significant association between mean platelet volume and the diagnosis of acute coronary syndrome at p value <0.0001. The value under the area is 0.868.

DISCUSSION

This study showed that there was an association between MPV and the diagnosis of ACS in patients having chest pain at the ER. The MPV of patients who were diagnosed with acute coronary syndrome was significantly higher compared to patients not diagnosed with ACS. The computed average MPV of patients under ACS group was 8.3 fL while non-ACS patients had 7.3 fL. Cut-off points predictive of ACS are calculated based on the area under the curve of the ROC curve revealed a significant test. An MPV of 8.4 fL was highly specific for the diagnosis of ACS.. While the MPV of ACS patients are increased, the number of platelets was significantly decreased compared to non-ACS group. ACS patients are older and composed mostly of males. Hypertension and patients who had previous MI or stroke were also significantly associated with the diagnosis of acute coronary syndromes.

The size and density of the platelets are markedly heterogeneous in the human circulation. The functional activity of platelets is correlated with their sizes.[1, 3, 7] Larger platelets are more likely to be reactive because it contained more granules or active substances that may have important role in coagulation and eventually in thrombus formation and even atherosclerotic plaques.[2] It is in this proposition that the critical role of the platelets in the pathogenesis of acute coronary syndromes sets in.[3] This assumption has even made more substantial by the fact that various drugs used in the management of acute coronary syndromes have anti-platelet activities.[3]

With the observation that the activity of the platelets depends on its size and MPV is reliable index of platelet size, many recent studies have hypothesized that there is an increased MPV in patients with acute coronary syndromes.[4] Though there were several data have already supported this claim, the reason for the increased in the platelet size is not fully understood.[2] Previous studies have provided several possible explanations. Some authors believed that physiological changes of body metabolism and secretion of biologically active substances as a result of aging or complications of diabetes mellitus and obesity might play a role.[2] Others are convinced that toxins derived from tobacco smoking and the actual processes during the development of acute coronary events may possibly contribute to the stimulation of bone marrow to produced more larger platelets.[2] More recent study had shown that platelet size and activity potentially in some ways is genetically determined.[2] This genomic-wide association study had identified three specific genetic features strongly associated with increased mean platelet volume.[2] Notice that most of these explanations are all attributed to the known risk factors of acute coronary syndromes such as aging, obesity, diabetes mellitus and cigarette smoking. Consequently, most the available studies had shown positive correlation between increased MPV and these risk factors.[2,3,7]

However, in a large-scale study made by the group of Klovaite, increased MPV is associated with myocardial infarction independent of other known risk factors after an extensive multifactorial adjusted analysis.[2] This finding is also supported by the study made by Lippi et al. where they concluded that MPV is a useful marker for the risk stratification of ACS patients admitted at the emergency room.[4]

The value of MPV predictive of acute coronary syndromes varies from one study to the other. In this study, the cut off points between 7.6 fL to 7.8 fL had shown satisfactory results of all parameters in diagnostic accuracy of MPV predictive of acute coronary syndromes. This finding is relatively similar to the 7.8 fL cut-off point established by Klovaite et al. with the highest risk of developing myocardial infarction. Higher values are noted in the other studies ranging from 8.0 fL to 10.3 fL.[3-7] The group of Khandekar have recorded an average MPV of 10.3 fL among MI patients, Lippi and his group showed 8.0 fL cut-off in their result and Mercan et al. have documented values ranging from 8.9 fL to 10.1 fL dependent upon the severity of ischemic conditions in the spectrum of ACS.[3,4,7] It is also noted that these values are fairly within the range of recommended normal range of MPV in textbooks of laboratory medicine.[8] Meaning, the increased MPV observed in this study and other previous studies are still within the range of normal platelet-size and not an abnormally large-sized and non-functional platelets usually found in patients with hematolymphoid lesions.[8]

An additional finding in this study is the increased number of platelets of the non-ACS group compared to that of ACS patients. Theoretically, this result may be explained by consumption of the platelets during the development of acute coronary event.[2] Thereby, decreasing the actual number of free platelets in the circulation.[2] In the study made by Klovaite et al., platelet count is not associated with increased risk of myocardial infarction. Either the platelet count is decreased or increased in ACS patients; the number of platelets is not significantly associated with ACS based on previous studies.[2] The limited number of patients under the non-ACS group may have contributed to this finding. This insufficient number of otherwise control or normal group might be one of the limitations of this study.

Another potential limitation of this study is the sample size. Sample collection was limited by procedures such as CBC not routinely done for chest pain patients at the emergency room and patients complaining of chest pain without significant electrocardiographic findings were quickly discharged. Hence, there were patients who failed to be included in the study. Some of the patients had vague diagnosis because of limited diagnostic work-up brought about by financial constraint. Other probable clinical factor that may affect MPV like the onset of chest pain is not recorded in many of the medical charts of patients included in the study.

Even with a limited population size, the association of increased MPV and diagnosis of acute coronary syndromes is clearly established. From this initial finding, many questions are expected to come out and the existing data presented in this study need to be validated in a larger population. Further investigation in the sizes of the platelets corresponding to the severity of ischemia under the spectrum of disease conditions in ACS would also be beneficial. To explore the correlation of MPV with other known risk factors of ACS such as hypertension, cigarette smoking, diabetes mellitus and others is likewise valuable in the effective risk assessment of chest pain patients at the emergency room. Since platelet activity depends on platelet size as measured through MPV, increased MPV among ACS patients also mean that this test might possibly be an acceptable screening tool in identifying patients who are good candidate for a more expensive platelet function tests like Anti-Xa, P2Y12 and aspirin assays, which are all available in this institution.

CONCLUSION

The MPV of chest patients diagnosed with acute coronary syndromes was significantly higher compared to patients not diagnosed with ACS. In contrast, the number of platelets in non-ACS group was elevated than ACS patients. The calculated average MPV of patients with ACS was 8.3 fL while non-ACS patients had 7.3 fL. Cut-off points predictive of ACS were computed based on the area under the curve of the ROC revealed a significant test (AOC is equal to 0.868 95% CI (0.812-0.924) p = <0.0001). At cut-off point of 8.4 fL, the positive predictive value was highly specific at 100% and a low negative predictive value at 46%.

AUTHOR DISCLOSURE

The authors do not have any relevant financial interest in the equipment, products or their respective manufacturers and companies mentioned in this study. Furthermore, there were no funding received from the suppliers or manufactures of all the products included in this study.

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APPENDIX

Definition of Terms

Acute coronary syndrome (ACS) – a spectrum of clinical conditions ranging from ST-elevation myocardial infarction (MI) to non-ST segment elevation and unstable angina. (Taken from the ACC/AHA 2002 Guideline Update for the Management of Patients with UA and NSTEMI: A report of the ACC/AHA Task force on Practice Guidelines).[11]

a. ST- elevation MI – presence of a clinical syndrome of acute ischemia with either chest pain or a crescendo pattern of ischemic pain on minimal exertion, plus electrocardiographic ST-segment elevation and/or biomarker evidence of acute ischemic injury (elevated troponin or CK-MB);
b. Non-ST-elevation MI – presence of a clinical syndrome of acute ischemia with either chest rest pain or a crescendo pattern of ischemic pain on minimal exertion, plus electrocardiographic changes and/or biomarker evidence of acute ischemic injury (elevated troponin or CK-MB);
c. Unstable angina – at least one of the following features: (1) angina pectoris occurring at rest (or with min exertion) and usually lasting more than 20 min (if not interrupted by nitroglycerin), (2) being severe and described as frank pain and of new onset (within 1 month), and (3) occurring with a crescendo pattern (more severe, prolonged, or frequent than previously)

1. Non-Acute Coronary Syndrome (Non-ACS) – refers to clinical conditions of patients having chest pain at the ER other than what are included in the definition of acute coronary syndrome.
2. Mean platelet volume (MPV) – is the arithmetic mean of the extrapolated histogram of the platelets.[10]
3. Platelet count – is the actual count of platelets that are determined using a 64 channel pulse-height analyzer.[10]

Paneth Cells in Colonic Adenomas: Association with Higher Adenoma Burden*

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St. Luke's Medical Center, Quezon City

ABSTRACT

Introduction. The association of Paneth cells with colorectal neoplasms has been demonstrated in several studies and case reports. The frequency of Paneth cell-containing adenomas ranges from 0.2 to 39% in the various published studies. Although adenomas with Paneth cells have already been recognized before, there are no studies in the Philippines that have been done to evaluate their clinicopathologic features. This study was performed to evaluate the clinicopathologic features of Paneth cell-containing adenomas and their association with adenoma burden.

Methodology. A total of 326 colorectal adenomas from 133 patients diagnosed consecutively from April 2013 to June 2013 at St. Luke's Medical Center, Quezon City, Philippines, were reviewed. These were checked for the presence of Paneth cells within the adenomatous crypts. The differences in gender and location were analyzed using one tail z-test, while the association of Paneth-cell containing adenomas with adenoma burden was analyzed using univariate odds ratio at 95% confidence interval.

Results. The frequency of Paneth cell-containing adenomas in this study of 326 adenomas is 15% (50 of 326 adenomas). There was no statistical significance in the occurrence of the lesion between male and female patients (32% vs. 15%; p=0.2041). There was also no statistical difference in their occurrence in the proximal and distal colon (18% vs. 14%; p=0.1723). The odds of having multiple adenomas for patients with Paneth cell-containing adenomas are 3.16 times higher than those patients without Paneth cell-containing adenomas (15 patients with one adenoma, 23 patients with more than one adenoma; p=0.0037).

Conclusion. This study has demonstrated the increased odds of harboring multiple adenomas in patients with Paneth-cell containing adenomas. This may be attributed in part to the fact that there have been recent studies revolving around Paneth cells that have shown that an established pathway of colorectal tumorigenesis, the APC/Wnt/β-catenin pathway, regulates differentiation towards this cell lineage.

Key words : adenoma, Paneth cells, colon

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INTRODUCTION

It was in 1872 that the Paneth cell was first recognized by Schwalbe and further studied in detail by Paneth.[1] As part of the innate immune system, antimicrobial products, such as defensins, are elaborated by Paneth cells.[1] These substances are present in the small intestine, particularly α-defensins.[2] The normal distribution of these cells in the gastrointestinal tract is from the duodenum to the ileum.[2] They are primarily seen at the base of the crypts of Lieberkuhn.[2] As stated by Andreu and colleagues (2008), "Paneth cells are filled with large apically located granules and have ultrastructural hallmarks (an extensive endoplasmic reticular and well-developed golgi) of prototypical secretory cells."[3] Paneth cells also secrete other products apart from α-defensins. These include antimicrobial proteins and peptides lysozyme, Reg3 γ, and secretory phospholipase A_2.[2]

The intestinal epithelium is in a constant state of proliferation, thereby continuously producing cells of all lineages.[3] The cellular mechanisms that drive this process have been linked to several molecular pathways, among the most crucial of which is the Wnt/β-catenin signalling. Commonly associated with sporadic colorectal cancers, the driving mutations of this pathway primarily involve the inactivation of the APC gene.[3] The unregulated proliferation of progenitor cells in inactivating mutations of the APC gene is due to the cytoplasmic accumulation of β-catenin. This leads to the latter being translocated to the nucleus where transcription factors activate the target genes of the pathway. Recently, it has also been discovered that differentiation towards the Paneth cell lineage also requires activation through the Wnt/β-catenin signaling pathway.[3]

The association of Paneth cells with colorectal neoplasms has been demonstrated in several studies and case reports. Rubio et al. reported a case of a 61-year-old man who had an adenoma with high-grade dysplasia that showed predominantly Paneth cells in the lower half of the villi and clusters of Paneth cells in the villous structures.[4] Similarly, Szumilo et al. presented a case of a large polypoid and ulcerated tumor arising from the hepatic flexure of the colon in a 76-year-old man who presented with hypogastric pain, constipation alternating with diarrhea, distension and weight loss. The tumor was diagnosed as a moderately differentiated adenocarcinoma. Paneth cells were then incidentally identified as part of the tumor. However, the authors concluded that they could not ascertain the impact of these neoplastic Paneth cells on prognosis since there are only a few reported cases in the available literature.[5]

The frequency of Paneth cell-containing adenomas ranges from 0.2 to 39% in the various published studies.[1] Although adenomas with Paneth cells have already been recognized before, there are no studies in the Philippines to the author's knowledge that have been done to evaluate their clinicopathologic features. The aims of the current study include the following: (1) to determine the frequency of Paneth-cell containing adenomas diagnosed consecutively at the St. Luke's Medical Center, Quezon City, Philippines, from April 2013 to June 2013; (2) to determine if gender and age are associated with the development of Paneth-cell containing adenomas; (3) to determine if the proximal location of the adenoma is associated with the presence of Paneth cells; and (4) to determine if the risk of harboring synchronous colorectal adenomas is associated with the presence of Paneth-cell containing adenomas.

METHODOLOGY

Study Population and Pathologic Evaluation of Colorectal Adenomas

The study population consisted of 326 colorectal adenomas from 133 patients diagnosed consecutively during a three-month period (April 2013 to June 2013) in St. Luke's Medical Center, Quezon City, Philippines. The slides were stained with routine Hematoxylin and Eosin. Seventy-nine patients had 1 adenoma, while 54 patients had two or more adenomas. The adenomas were classified as either tubular, tubulovillous or villous, depending on the percentage of villous architecture (less than 25%, 25-75% and more than 75%, respectively). Seven adenomas were classified as sessile serrated adenomas and one was diagnosed as filliform serrated adenoma. The author reviewed the adenomatous tubules within these adenomas. These were then evaluated for the presence of Paneth cells. In routine hematoxylin and eosin staining, the Paneth cells were easily recognized by their cytoplasmic features, which contained large, eosinophilic granules. An adenoma that has a dysplastic Paneth cell within its crypts can be classified as a Paneth cell-containing adenoma.[1]

The gender and age of the patients, as well as the location of the adenomas, were obtained from histopathology reports. The number of adenomas for each patient was reviewed with the endoscopy reports whenever discrepancies were identified. One patient was not included in the study because the number of adenomas could not be ascertained even after reviewing both the histopathology and endoscopy reports. Two patients underwent colonoscopy with biopsy twice. Regarding the location of the adenoma, the splenic flexure was used as the indicator to differentiate the proximal from the distal lesions (proximal if above the splenic flexure; distal if below the splenic flexure).[1]

Since we were limited by the information given in the histopathology report, several parameters could not be assessed adequately and, thus, narrowed the scope of our study. The sizes of the adenomas were not considered because some specimens were received piecemeal and their endoscopy reports did not state their actual sizes. It was also not taken into regard whether the entire colon was inspected during the endoscopic procedure and whether all polyps seen during endoscopy were biopsied and sent to histopathology. The clinical history, including those with a history of colorectal adenocarcinoma, familial adenomatous polyposis, or inflammatory bowel diseases, was not taken into account. Since there were only a few cases that demonstrated high-grade dysplasia, we did not take this into consideration as well.

Statistical Analysis

The sample size was determined using Epi Info 6.04d software (CDC, Atlanta, GA) with 95% confidence interval, power of 80%, and odds ratio of 3.12 based on the study of Pai et al. The minimum number of samples was 114. Adenomas from male and female patients with and without Paneth cells were analyzed using one tail z-test. Similarly, the location of the adenoma was also compared using one tail z-test. To determine the association of Paneth-cell containing adenomas with tumor burden, univariate odds ratio at 95% confidence interval was used.

RESULTS

The study population consisted of 133 patients with 326 adenomas. There were 73 male patients and 60 female patients.

The frequency of Paneth cell-containing adenomas in the 326 adenomas reviewed is 15% (50 of 326 adenomas). Of the 73 male patients, 23 (32%) had Paneth cell-containing adenomas (Figure 1). On the other hand, 15 (25%) of 60 female patients presented with Paneth cell-containing adenomas (Figure 1). However, there is no significant difference in the occurrence of Paneth cell-containing adenomas between male and female patients ($p = 0.2041$).

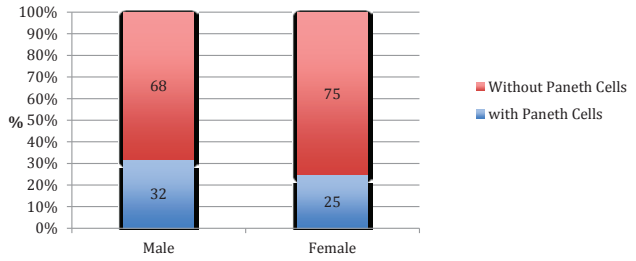

Figure 1. Distribution of subjects according to gender and presence of Paneth cells.

For patients with Paneth cell-containing adenomas, there is a sharp increase seen in patients aged 60-69 years. While for those patients without Paneth cell-containing adenomas, the peak is observed among patients aged 50-59 years.

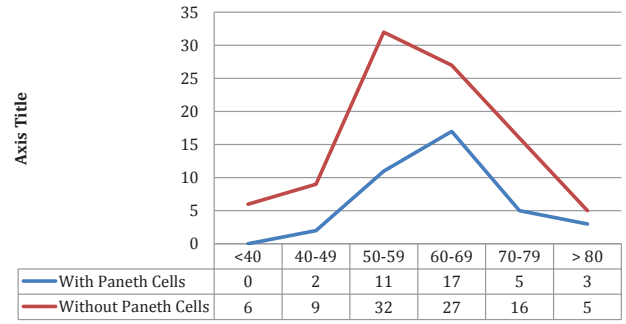

Figure 2. Frequency distribution of patients according to age group and presence of Paneth cells.

The Paneth cell-containing adenomas were also assessed according to their location. Most of the adenomas in this population were located at the distal colon (63% of adenomas).

Table 1. Distribution of adenomas per site

Site	No Paneth cell containing adenomas	With Paneth cell containing adenomas	Total number of adenomas per site	% Paneth cell-containing adenomas per site
Proximal	86	19	105	18%
Distal	178	29	207	14%
Unspecified	12	2	14	14%
Total	276	50	326	

Out of the 326 adenomas, 50 were classified as Paneth cell-containing adenomas. Thirty eight percent (38%) of the Paneth cell-containing adenomas were located at the proximal colon, while 58% of the Paneth cell-containing adenomas were located at the distal colon. However, looking at the proportion of Paneth cell-containing adenomas by location, 19 of the 105 adenomas (18%) in the proximal colon showed the presence of Paneth cells (Figure 3). In contrast, only 14% (29 of 207 adenomas) of the adenomas in the distal colon were classified as containing Paneth cells. Two of the Paneth cell-containing adenomas were excluded since they did not have the biopsy site specified. In spite of the increased proportion of Paneth cell-containing adenomas in the proximal colon, it was found that there was still no significant difference based on their site of occurrence ($p = 0.1723$).

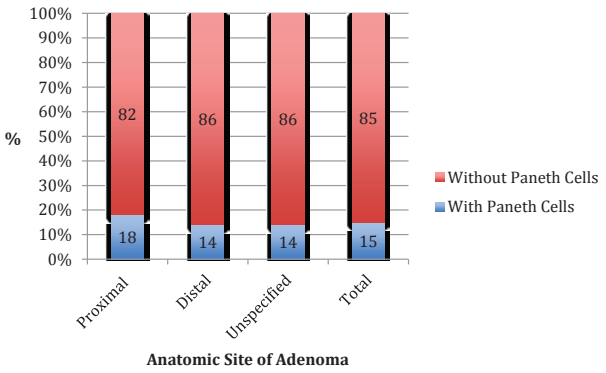

Figure 3. Proportion of Paneth cell-containing adenomas per site.

Among the 133 patients in the study population, 38 (29%) had Paneth cell-containing adenomas. Among these 38 patients, 15 had only one adenoma, while 23 had two or more adenomas (Table 2).

Table 2. Correlation of paneth cell-containing adenoma with adenoma burden

	Patients with More than One Adenoma	Patients with Only One Adenoma	Total
With Paneth-Cell containing Adenoma	23	15	38
Without Paneth-Cell containing Adenoma	31	64	95
% Paneth Cell-containing adenomas	43%	19%	
Total	54	79	133

Based on the above results, an odds ratio of 3.16 (confidence interval: 1.4-6.8) was computed. Thus, the odds of having multiple adenomas for patients with Paneth cell-containing adenomas are 3.16 times higher than those patients without Paneth cell-containing adenomas. Looking at the confidence interval, this is a significant finding ($p = 0.0037$).

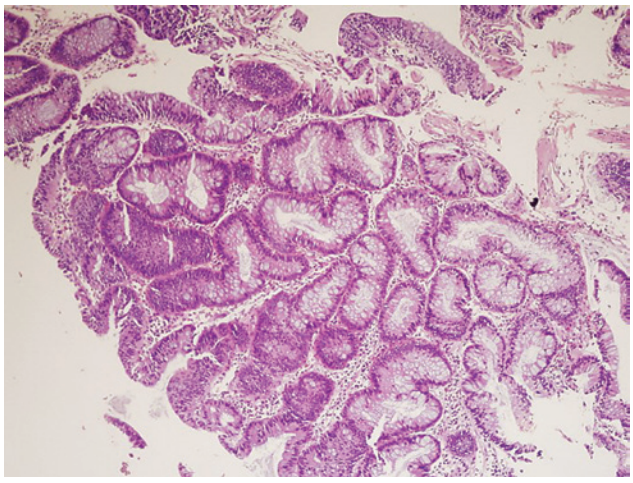

Figure 4. Tubular adenoma featuring at least low-grade dysplasia. The nuclei are pseudostratified, hyperchromatic and elongated. The architecture is predominantly tubular (40x, H&E).

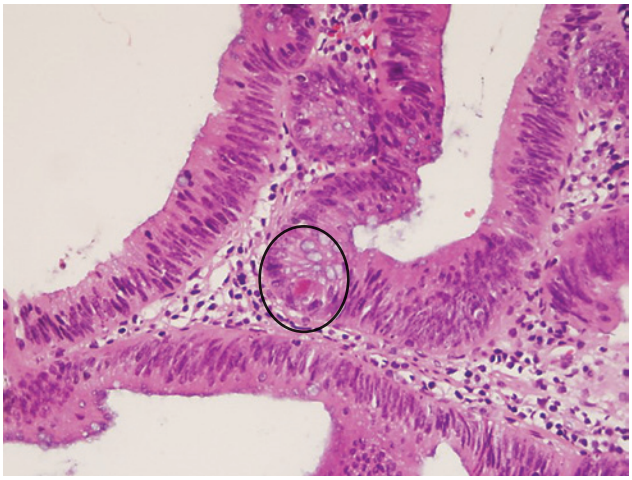

Figure 5. Paneth cell-containing adenoma was defined as the presence of a dysplastic Paneth cell(s) in the adenomatous crypts.[1] Occasionally, they can be found singly scattered along the length of the dysplastic glands (100x, H&E).

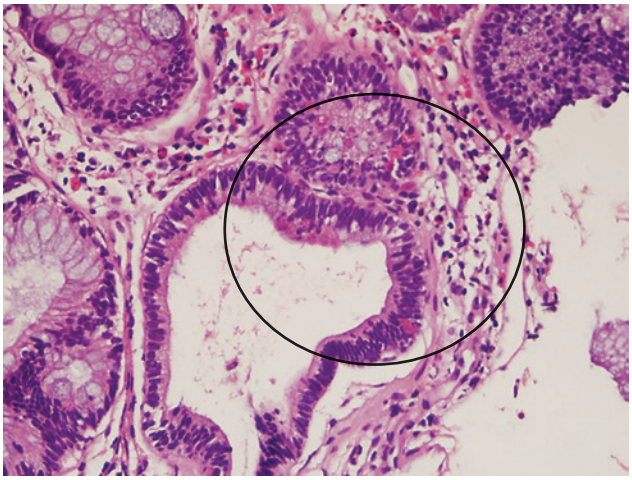

Figure 6. Paneth cells were also found clustered in small groups within the adenomatous crypts (100x, H&E).

DISCUSSION

Paneth cells can normally be found in the small bowel, appendix, and proximal colon. Here, they have an important role in innate intestinal immunity.[1] These cells express α-defensins, which, in the small intestine, aids in the elimination of Gram-positive and Gram-negative bacteria.[2] Apart from this function, Paneth cells have also been found to play a role in idiopathic inflammatory bowel diseases (IBD).[1] They serve as indicators of chronic injury in colitis, a role shared with mucous (pyloric) gland metaplasia.[8]

Epithelial neoplasms, such as adenomas, have been known to be occasionally associated with Paneth cell differentiation.[7] Four decades of case reports have demonstrated this association.[1]

The frequency of Paneth cell-containing adenomas in this study of 133 patients is 15%, which is consistent with the reported frequency of 0.2 to 39%. No significant difference was observed in the occurrence of Paneth cell-containing adenomas between male and female patients.

Although there is a peak in the occurrence of Paneth cell-containing adenomas among patients in their 50's and 60's, it is difficult to ascertain whether this finding is significant since, expectedly, most patients who undergo colonoscopy with biopsy belong to that age group. Perhaps a study that could obtain a population that is more evenly distributed among the different age groups would provide more comparable data regarding the association of increasing age with developing Paneth cell-containing adenomas. Pai et al. did not find any statistically significant association between age and the occurrence of these lesions.

It is not entirely surprising that the incidence of Paneth-cell containing adenomas is higher in the proximal colon than it is in the distal colon since Paneth cells are normal constituents of the former. Although there is an increased proportion of Paneth-cell containing adenomas among lesions of the proximal colon observed in this study, further analysis has shown that the difference in its occurrence in the proximal and distal colon was not statistically significant. These findings are in contrast to three other studies that have shown that Paneth cell-containing adenomas are more common in the proximal colon.[1,9,10]

Finally, analysis of the 133 patients in the study showed that the odds of having more than one adenoma for patients with Paneth cell-containing adenomas is higher than in those patients without Paneth cell-containing adenomas. In their study of 460 polyps from 200 patients, Pai et al. found that there is an increase in polyp burden in association with the presence of a Paneth cell-containing adenoma.[1] This may possibly be explained by recent investigations between the association of the APC/Wnt/β-catenin pathway and the differentiation and function of Paneth cells. Intestinal homeostasis and the maintenance of intestinal stem cells is controlled by the APC/Wnt/β-catenin pathway.[7] Furthermore, through the activation of this pathway, there is expansion of the crypt compartment. This is brought about by the stimulation of cell proliferation and the inhibition of cell migration and differentiation towards the enterocyte, goblet and enteroendocrine lineages. However, through its influence on transcription factors, the pathway also promotes differentiation towards Paneth cells.[7] This role has been shown in an experimental study on mice by Andreu et al. wherein they demonstrated that Paneth cell differentiation is also regulated by β-catenin signalling, apart from its well-known function in intestinal proliferation.[3] Consequently, the finding of Paneth cells in colorectal neoplasms may be linked to mutations in the APC/Wnt/β-catenin pathway since, in a similar fashion, the latter are also present in the formation of colorectal tumors.[7] Of note, none of the serrated colonic polyps (sessile serrated adenomas and filliform serrated adenoma) contained Paneth cells. These lesions harbor a different set of mutations (ex. microsatellite instability, DNA hypermethylation), which can be assumed to be part of the reason why they did not show any Paneth cell differentiation.

CONCLUSION

In summary, neoplasms of the colon, particularly adenomas, may harbor Paneth cells.[7] Although no statistically significant association with gender and site were observed in this paper regarding the presence of Paneth cell-containing adenomas, the trend favoring male patients and its occurrence in the proximal colon can already be seen, and perhaps, by increasing the population size, more significant results could have been obtained. Also, acquiring a population that is more evenly distributed among the different age groups would allow one to see if there is, in fact, an association with developing these lesions as age increases. More importantly, however, this study has demonstrated the increased odds of harboring multiple adenomas in patients with Paneth-cell containing adenomas. This may be attributed in part to the link between differentiation towards this cell lineage and the APC/Wnt/β-catenin pathway. As firmly established in scientific literature, the APC/Wnt/β-catenin pathway is responsible for the formation of a majority of colorectal tumors. However, since to the author's knowledge, there is only one other study that has delved into the association of Paneth cell-containing adenomas and tumor burden,[1] more research, preferably with a larger and more representative population, is still required to confirm these findings. Likewise, the significance of these findings and its impact on clinical practice, such as its possible effect on the interval of surveillance for colonoscopy, has yet to be determined.

AUTHOR DISCLOSURE

The authors declared no conflicts of interest.

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OPEN ACCESS – ORIGINAL ARTICLE

Intraoperative Frozen Section Assessment of Sentinel Lymph Nodes in Breast Cancer: Six-Year Experience in a Tertiary Hospital*

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ABSTRACT

Introduction. To determine the reliability of intraoperative frozen section (FS) assessment of sentinel lymph nodes (SLN) in breast cancer patients and describe the factors affecting its evaluation.

Methodology. Records of 245 breast cancer patients with FS of SLNs from December 2007 to December 2013 were retrieved and analyzed. The effect of discordant FS examination and pathology findings on axillary lymph node (ALND) dissection was then evaluated.

Results. Of the total 616 SLNs evaluated, 85 (13.80%) SLNs were positive on FS, with the majority having a histological diagnosis of invasive ductal carcinoma of no special type (62.04%). Overall identification rate was 98.36%. Frozen section biopsies had good correlation with permanent sections, with a sensitivity (Sn) of 92.39%, specificity (Sp) of 100%, and a positive predictive value (PPV) of 100%. Negative cases on FS but were found positive on permanent sections were all cases of micrometastases, giving a false negative rate of 1.31% and negative predictive value (NPV) of 98.68%. Validation with ALND showed Sn of 100%, Sp of 50%, NPV of 100%, and PPV of 37.17%.

Conclusion. The 6-year data on intraoperative FS reliably evaluated the SLN status of breast cancer patients with a negligible false negative rate. Factors affecting its effectiveness include the predictors of nodal involvement, multilevel sectioning, and size of metastases.

Key words: sentinel node biopsy, breast cancer, axillary lymph node dissection

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INTRODUCTION

With the advent of sentinel lymph node biopsy (SLNB) by frozen section (FS), trends in breast cancer surgery have shifted towards breast-conserving treatment and avoidance of axillary lymph node dissection (ALND) for better quality of life outcomes. In principle, injection of a radioactive isotope and vital blue dye around the area of the tumor allows localization of the first node to receive lymphatic flow which, in principle, is the sentinel lymph node (SLN). The node is then biopsied and examined by routine histopathologic techniques and evaluated for metastasis. If the node is free of metastasis, then it is likely that locoregional spread has not occurred and further ALND is avoided.[1]

The practice of SLNB has been extensively studied since its introduction into clinical practice in the 1990's. The procedure is an extremely sensitive and specific method for predicting whether metastasis has occurred in regional lymph nodes.[2] The sensitivity of intraoperative FS in identifying nodal metastases within SLNs has been reported to vary within the range of 44% to 95%, with most studies reporting the sensitivity to be between 60-75%.[3] Advances in histopathologic methods for SLNs allow safe and accurate identification of early breast cancer without axillary node involvement, and SLN is now widely accepted and recommended by the American Society of Clinical Oncology (ASCO)[4] as it has shown greater benefit in reducing post-operative morbidity and complications like lymphedema, pain, numbness, and limited shoulder movement, which translates to better quality of life (QoL) outcomes.[5] In line with this, numerous studies have emerged in recent years validating its said advantages. The first large prospective randomized control trial, the Axillary Lymphatic Mapping

Against Nodal Axillary Clearance (ALMANAC) trial, compared both procedures for comprehensive and repeated quality of life assessments over 18 months.[6] Similar observations and conclusions on patient outcomes were reported in the National Surgical Adjuvant Breast and Bowel Project (NSABP).[6-10]

Contemporary practice in our setting has since been influenced by promising clinical data. However, studies on the effectiveness of intraoperative FS with SLNB in our country remain few. Here we report the cumulative six year experience at The Medical City and evaluated the sensitivity, specificity, and positive and negative predictive values of intraoperative FS of SLNs. Furthermore, we analysed the impact of discordant FS examination and pathology findings on axillary lymph node dissection.

METHODOLOGY

Sample size was computed using the OpenEpi open source calculator using the equation: Sample size n = [DEFF*Np(1-p)]/[($d^2/Z^2_{1-\alpha/2}$*(N-1)+p*(1-p)]. Computed Sample size at 95% confidence level is 237. Statistical power was likewise determined by open source calculator using the determined sample size of 237 and Alpha error level of 5% with a resulting statistical power of 45.8%.

Between December 2007 to December 2013, a total of 616 sentinel lymph node biopsies (SLNB) were performed at our institution with the following inclusion criteria: a) Clinical Stage 1, 2A or 2B histologically confirmed Invasive Breast Carcinoma with clinically negative axillary lymph node (ALN); and b) Ductal carcinoma in situ (DCIS) requiring Mastectomy. Patients, likewise, did not have prior chemotherapy or hormonal therapy. On the other hand, exclusion criteria or contraindications were: a) Clinical Stage 3 or 4 Invasive Breast Carcinoma; b) Fine needle aspiration biopsy (FNAB) confirmed ALN positive for metastasis; and c) Women who have undergone extensive breast surgery such as breast reduction or augmentation, as well as extensive axillary surgery such as excision of axillary tumors. SLN was identified using vital blue dye and gamma probe methods as per protocol and submitted for histopathologic examination. All lymph nodes were subjected to FS wherein imprints and tissue sections were taken for examination and intraoperatively reported as either positive or negative for tumor metastasis. Subspecialties (Nuclear Medicine, Surgery, and Pathology) involved in the study followed a protocol which was agreed upon by a concensus within each department. This study was granted an approval from our Institutional Review Board in accordance with the guidelines of the International Conference on Harmonization of Good Clinical Practice (ICH-GCP).

Validation

Validation of sentinel node status was done by comparing the number of SLN positives and negatives with ALND outcomes. Starting from January 2011, twenty eight (28) of these cases were identified to have a scheduled ALND in spite of a negative SLNB, as indicated in their histopathology forms. These were done upon agreement with their surgeon.

The remaining tissue samples, including those submitted for FS, were subsequently processed on paraffin section. One four (4) micrometer thick section for each lymph node was mounted on a single glass slide. Three such sections (levels) for each lymph node were taken, with an average distance of 40-50 micrometers apart–corresponding to three (3) levels, and stained using hematoxylin and eosin stain (H&E).

Patients

Population age ranged from 30 to 81 years old with a mean of 55.5 years, predominantly female gender 243/245 (99.19%). Most patients were histologically diagnosed as Invasive Ductal Carcinoma 152/245 (62.04%). The other histologic types: Ductal Carcinoma in situ 27/245 (11.02%), Invasive Lobular Carcinoma 4/245 (1.63%) and others (Mucinous, Metaplastic, Apocrine, Invasive Cribriform and Tubular carcinoma) were identified as well 62/245 (25.30%). One hundred sixty six (166) patients were staged as T1 (7.76%) and seventy two (72) as T2 (29.39%), which were the most frequent tumor stages. Lymphovascular space invasion is noted in 60 patients (24.49%).Tumor biomarker status was also recorded based on the Estrogen receptor (ER), Progesterone receptor (PR),and Human epidermal growth factor receptor2 (HER2) immunohistochemical (IHC) results. Details of patient characteristics are listed on Table 1.

Table 1. Descriptive characteristics of sentinel lymph node, patients and tumor

Variable	Frequency
Age (years)	
Range	31 to 80
Median	53
Mean	53.8
Sex	
Male	2 (0.81%)
Female	243 (99.19%)
LVSI	
Yes	60 (24.89%)
No	185 (75.11%)
HISTOLOGIC TYPE	
Invasive Ductal Carcinoma, NST	152 (62.04%)
Ductal carcinoma In situ	27 (11.02%)
Mucinous Carcinoma	8 (3.27%)
Invasive Lobular Carcinoma	4 (1.63%)
Metaplastic Carcinoma	3 (1.22%)
Apocrine Carcinoma	2 (0.82%)
Invasive Cribriform Carcinoma	1 (0.41%)
Tubular carcinoma	1 (0.41%)
Other types	47 (19.18%)
Tumor Size*	
Tis	12 (11.88%)
T1mic	4 (3.96%)
T1a	9 (8.91%)
T1b	6 (5.94%)
T1c	29 (28.71%)
T2	28 (27.72%)
T3	4 (3.96%)
Unknown	9 (9.91%)
Estrogen Receptor	
Positive	162 (66.39%)
Negative	32 (13.11%)
No data	50 (20.49%)
Progesterone Receptor	
Positive	146 (59.84%)
Negative	48 (19.67%)
No data	50 (20.49%)
HER2	
Positive	68 (27.87%)
Negative	76 (31.15%)
Equivocal	26 (10.66%)
Unknown	65 (26.64%)

Note: LVSI = Lymphovascular space invasion; *Tumor classification based on the 7th American Joint Committee on Cancer (AJCC)

RESULTS

Sentinel lymph nodes (SLN) were successfully identified in 240 of 245 patients with the use of both blue dye and radiolabeled gamma probe, with an identification rate of 98.36%. Lymph nodes submitted for FS ranged from 1 to 13 with an average of 2.6 lymph nodes per examination. This totalled to 616 SLN submitted for FS and subsequent routine paraffin sections; the majority of cases were negative on FS (531 SLN or 86.20%) with 524 true negatives on paraffin sections. All positive SLNs on FS were likewise positive on

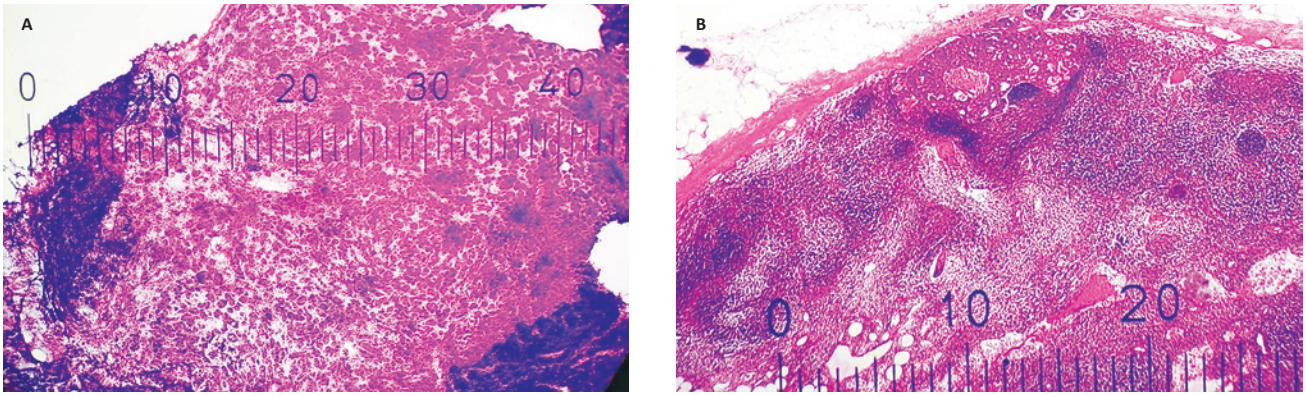

Figure 1. Sentinel lymph nodes with macrometastasis and micrometastasis. **(A)** Photomicrograph of a macrometastasis (>2 mm) in a sentinel lymph node (100x, H&E); **(B)** Photomicrograph of a micrometastasis (<2 mm) in a sentinel lymph node (400x, H&E).

paraffin sections (85 TP, 0 FP). These results yielded a sensitivity of 92.39% (CI 84.94 – 96.97) and a specificity of 100% (CI 99.29 – 100). Our study shows that intraoperative FS has a 98.68% negative predictive value when confirmed with subsequent paraffin sections (See Table 2).

Consequently, these results were validated by eighty one cases that underwent subsequent ALND, wherein 53 cases had positive SLNs and 28 cases had negative SLNs. Out of the 53 positive SLNs, 50.1% or 27 cases were found to be negative for metastasis on subsequent ALND, while 49.16% or 26 cases were found to be positive on subsequent ALND. Out of the 28 cases with negative SLNs, all or 100% were confirmed to be negative on subsequent ALND. There were no false negatives by ALND out of the 81 cases observed in this subset. This corresponds to 100% sensitivity, 50% specificity, 100% NPV, and 47.17% PPV of intraoperative FS when compared to ALND results.

The tumor deposits were evaluated upon routine paraffin examination and categorized either as micrometastases (<2.0 mm) or macrometastases (>2.0 mm) (See Figure 1). FS of SLN detected mostly macrometastases in 67 (78.82%) of the 85 positive cases. Micrometastases were also detected in 18 cases (21.18%). The ALN dissection performed on the 81 patients with positive SLN yielded 53 patients (65.43%) confirmed positive on ALND, while 28 patients (34.67%) were negative. Eighty two (82) of the SLN positive patients were histologically diagnosed as Invasive Ductal Carcinoma, while 6 were classified as Ductal Carcinoma in situ. Breast cancers staged as T2 comprised 27.72% of the cases, with LVSI noted in 24.89% (See Table 3).

DISCUSSION

Currently, our centre has an overall identification rate for SLN biopsy at 98.36%.Validation of sentinel node status was done by comparing the number of SLN positive and negatives with ALND outcomes. Our results demonstrate that out of the 53 positive SLNs, 50.1% or 27 cases were found to be negative for metastasis on subsequent ALND, and out of 28 negative SLNs, 100% were confirmed negative in ALND. This finding support the concept of sentinel lymph node as the first lymph node or group of nodes encountered in the lymphatic drainage of the breast. Aside from this, it validates that the technique done by the surgeons in identification of sentinel node is acceptable because all the negative sentinel nodes were indeed negative on the subsequent axillary node dissection.

It can be concluded from our results that proper technique and meticulous screening with intraoperative FS of SLNB reliably identifies locoregional metastasis. Identification of these metastasis-positive nodes through SLN technique allows the surgical practitioner to harvest positive nodes only and avoid aggressive ALND. Also important to note, given the high sensitivity for negative SLNs, subsequent ALND may not be performed which may spare the patient from other morbidities.

According to studies, variables affecting the procedure are the following: age, pathological tumor size, histology, year of accrual, and method of detection.[11] Predictors of further nodal involvement are tumor size, lymphovascular space invasion (LVSI) and lobular histology.[12]

A positive FS can save the patient a second reoperation for completion axillary lymph node dissection and a negative FS

Table 2. 616 Sentinel lymph nodes submitted for frozen section

	Positive	Negative	
Positive	TP 85	FP 0	PPV 100%
Negative	FN 7	TN 524	NPV 98.68%
	Sensitivity 92.39%	Specificity 100%	

Table 3. Descriptive characteristics of sentinel lymph node

Variable	Frequency
Total Number of submitted SLN for 245 patients	616
Range	1-19
Mean	
No. of Positive SLN	85 (13.80%)
Micrometastasis (<2.0mm)	9 (10.59%)
Macrometastasis (>2.0mm)	27 (31.76%)
No. of Negative SLN	531 (86.20%)
Validation by Axillary Dissection	81
SLN Positive Patients with Axillary Dissection	25
Positive ALN	26 (32.5%)
Negative ALN	54 (42.86%)
LVSI	
Present	20 (80.0%)
Absent	5 (20.0%)
Tumor Deposit Size (in mm)	
Range	0.1-26
Mean	6.05
Tumor size of Positive SLN cases	
T1a	1 (1.80%)
T1b	1 (1.80%)
T1c	15 (26.79%)
T2	28 (50.00%)
T3	4 (7.14%)

Note: SLN = Sentinel Lymph Node; ALN = Axillary Lymph Node; LVSI = Lymphovascular space invasion

can spare the patient from ALND completely, avoiding all the associated morbidities. This puts a lot of pressure on the decision making done during intraoperative FS because it can adversely affect outcome and influence management. Our data showed that there is good correlation between intraoperative FS and permanent paraffin H&E sections with a sensitivity of 92.39%, and specificity and positive predictive value of 100%.

Discordant FS results were noted on seven SLN negative cases (8.23%) where micrometastases were noted only on permanent sections (7/85). The false negative rate was 1.31% and NPV was 98.68%. Those were observed during the early course of introduction of this method at our hospital. As we gained experience, multiple levels (2) or step sections were done on each of the submitted SLN for FS, which eliminated our false negative results since October of 2010. Still, other studies have reported higher false negativity at 11%[2] and discordance rate of FS at 17%.[13] Therefore, limitations inherent to the procedure should always be taken into consideration. Frozen section may fail to detect micrometastases. Apart from doing multi-levels on FS, immunohistochemistry (IHC), particularly antibodies to cytokeratin have improved the identification of SLN. Even though this technique was not included in our protocol, studies have shown that IHC has been reported to upstage the disease in approximately 10% of patients with negative SLN.[2] This can improve identification of micrometastasis and isolated tumor cells that maybe difficult to identify even with routine H&E technique.

Submitted SLN for FS range from 1 to 13 lymph nodes, with an average of 2. A study concluded that this may either be due to the migration of dye or isotope from the true SLN to secondary lymph nodes or a normal anatomic variation in which the lymphatics of a given site in the breast drain simultaneously.[2]

CONCLUSION

Intraoperative FS can reliably evaluate the SLN status of women with early breast cancer but it may fail to detect micrometastases. Factors affecting the effectiveness of intraoperative FS of SLN include the predictors of nodal involvement (size of tumor, histology, lymphovascular space invasion), number of step sections, and size of metastases.

AUTHOR DISCLOSURE

The authors declared no conflicts of interest.

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OPEN ACCESS – ORIGINAL ARTICLE

Biological Risk Assessment: Zika Virus Detection at the Research Institute for Tropical Medicine

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Research Institute for Tropical Medicine

ABSTRACT

Background. Biosafety is the application of containment principles and risk assessment. Risk assessment is an essential component of a biological risk management program. It determines the most appropriate mitigation control measure to minimize the risk of Laboratory Acquired Infections (LAIs). In the laboratory response to an emerging disease-causing pathogen such as Zika virus, the risk for laboratory exposure and infection must be assessed.

Objectives. We have conducted biosafety risk assessment of the Research Institute for Tropical Medicine's (RITM) Virology Laboratory to identify the hazards, characterize the risks, determine laboratory compliance with biosafety standards and the competence of the laboratory personnel involved as part of the institutional preparedness for disease outbreak investigation and surveillance of Zika virus. The information gathered shall guide the selection of appropriate mitigation control measures for the prevention of LAIs.

Methodology. We utilized the Biosafety for Microbiological and Biomedical Laboratories (BMBL) 5th Edition guidelines in conducting risk assessment. Risk characterization was performed by determining the likelihood and the consequence of the identified biological risk and plotting it in a diagram using Microsoft Excel. Risk characterization result of ZikV was compared using the risk assessment tool, BioRAM©, developed by Sandia National Laboratory.

Results. The RITM Virology laboratory is generally compliant to the basic biosafety standards. Laboratory staff has established competence and experience in handling specimens for diagnostic test by ELISA and PCR. The risk of infection with ZikV is found to range from very low to low, however, the risk of acquiring other blood-borne pathogens brought by handling serum samples is found to be higher.

Conclusion. We have analyzed the risk of acquiring Zika at the RITM Virology laboratory as part of the Institute's overall preparedness, through biological risk assessment process as described in BMBL 5th Edition. The risk of acquiring ZikV infection while performing diagnostic tests range from very low to low. The risk of acquiring other blood-borne pathogens is higher compared to the risk of infection to the pathogen being assessed. Mitigation control measures against direct contact and percutaneous exposure must be implemented and monitored. This risk assessment strategy will further strengthen RITM laboratory's capacity to respond to infectious disease threats and increase staff confidence in dealing with infectious materials in the laboratory.

Key words: Zika virus, biosafety risk, risk assessment, biosafety, biohazards, likelihood, consequence, BioRAM©

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INTRODUCTION

Zika virus (ZikV) has emerged as a global public health threat over the last decade, with the accelerated geographic spread of the virus noted during the last 5 years.[1] The first major outbreak outside Africa occurred in 2007 in the Yap Islands of Micronesia,[2] another large outbreak in 2013 in French Polynesia,[3] and Brazil in 2015.[4] The World Health Organization (WHO) has recently declared Zika virus as a public health emergency of international concern (PHEIC), due to its rapid spread and the increase in Zika-associated newborn microcephaly cases.[5]

In the Philippines, the first recorded case of ZikV was in Cebu in 2012 and none after that.[6] Recently, the Department of Health (DOH) reported a case of a traveller from the Philippines being diagnosed of a Zika infection upon her return to the United States.[7]

The Research Institute for Tropical Medicine, the research arm of the Department of Health, houses the National Reference Laboratory for Dengue and other Arboviruses. This laboratory is equipped to perform Polymerase Chain Reaction (PCR) testing for suspected ZikV cases in the Philippines. An important component in laboratory-surveillance preparedness is the biological risk assessment of the laboratory to ensure compliance to biosafety standards. It determines the most appropriate containment required to mitigate the risk of Laboratory Acquired Infections (LAIs).

The risk assessment process identifies the hazardous characteristics of an infectious or potentially infectious pathogen or biological agent, the activities that could brace a mean towards unintentional exposure, the likelihood that such exposure could lead to an acquired infection, and its probable consequences.[8] The information identified by this process provides a clear guide for the selection of appropriate laboratory biological safety levels (practices, safety equipment and physical containment/facilities) in order to minimize the risk of exposure.

METHODOLOGY

We utilized the Biosafety for Microbiological and Biomedical Laboratories (BMBL) 5th Edition guidelines in conducting biological risk assessment. This process includes identifying the hazard of the agent and the procedure, determining the compliance of the facility with the standards, verifying competence of staff who will be involved in the performance of procedures, and lastly, the review of the process and findings with the biosafety experts of the institution. The consequence of exposure to other blood-borne pathogens, which may be contained in the samples, was described but not detailed in this report.

Risk characterization was performed by determining the likelihood and the consequence and plotting it in a risk matrix diagram using Microsoft Excel. Likelihood is the probability of the occurence of unwanted event, while consequence pertains to its severity. We have assigned values 1, 2, 3, 4 and 5 for the likelihood and consequence. For both likelihood and consequence we have agreed that 1 should be the lowest semiquantitative value, while 5 should be assigned as the highest. We have compared our risk characterization result using the BioRAM© risk assessment tool developed by Sandia National Laboratory.

The process, results and the findings for compliance and non-compliance were reviewed and verified by a senior Certified Biosafety Officer of the Institute.

RESULTS

Identification of the Agent Hazard

Zika virus or ZikV is an arthropod-borne human pathogen first identified in 1947 in Uganda's rhesus monkeys.[9] It is a positive sense, single stranded RNA virus of the family Flaviviridae, genus Flavivirus. ZikV has a 10,749-nt genome and is closely related to Spondweni virus.[10] It was detected in humans in 1952 in Uganda and Tanzania. Subsequent outbreaks Zika Virus disease in Africa, America Asia and the Pacific has been reported. The virus has high potential for ongoing geographic expansion into countries where Aedes aegypti moquitoes are present. The primary transmission is through the bite of these specific species of mosquitoes that spread dengue and chikungunya viruses.[11] Reports of non-vector-borne include possible Zika virus transmission during pregnancy or when mother is infected at the time of delivery and spread of the virus through blood transfusion and sexual contact.[12] The most common symptoms of Zika virus disease are fever, rash, joint pain, and conjunctivitis. The illness is usually mild with symptoms lasting from several days to a week. Severe disease requiring hospitalization is uncommon.[13] However ZikV infection could lead to Guillain-Barré syndrome and pregnant women giving birth to babies with birth defects (microcephaly) and poor pregnancy outcomes based on previous investigations.[14]

Zika Virus is categorized as a Risk Group 2 pathogen and is not a select agent both for Centers for Disease Control and United States Department of Agriculture. The infectious dose is unknown and there has been no documented report of direct transmission of the virus in hospital or laboratory setting handling patients and clinical specimen infected with the virus. The virus is susceptible to autoclave temperature of 121°C, 1% bleach, 70% ethanol, and 2% gluteraldehyde organic solvent detergents. No vaccine is available and treatment is supportive.

Identification of the Procedure Hazards

Clinical specimens received at the Clinical Laboratory shall be transported to the Virology Annex-1 Laboratory, where aliquots of 200 µL shall be obtained for PCR testing and for posible serology by ELISA. Testing shall be performed at the Virology Annex-2 laboratory and samples that are positive shall be stored at the Institution's Biobank facility. The activities and potential modes of exposure specific to the procedure to be conducted are summarized in Table 1.

Table 1. Activities and potential modes of exposure specific to the procedure to be conducted

Activity	Exposure
Donning Doffing PPE	Direct contact with contaminated / reused PPE
Specimen Reception / Opening of Transport Boxes to check identity and appropriateness of samples submitted	Direct contact with Clinical Specimen due to broken primary container, improperly sealed containers, leaking container or contaminated container
Reception of specimen and Transport Boxes from Clinical Laboratory	Possible contact exposure from contaminated material (request form , pens, door knobs and transport boxes)
Transport of specimen from Clinical Laboratory to Virology Laboratory	Possible contact exposure from contaminated material (request and transport boxes)
Encoding of patient information, work sheets, logbooks and printing of specimen labels (barcode)	Possible contact exposure from contaminated material (request)
Re-opening of transport boxes to check identity and appropriateness of samples submitted	Direct contact with clinical specimen including respiratory samples
Sorting of specimen according to pre assigned specimen ID	Direct contact with clinical Specimen from the primary and secondary container
Centrifugation of blood specimen	Possible direct contact with blood and blood-borne pathogens
	Spills and splashes in processing infectious materials
Opening of primary container and obtaining aliquot sample by pipetting for testing and storage	Direct contact with clinical specimen from the lid and caps of primary containers
	Accidental spills and splashes in processing infectious materials
Transport of specimen to Annex Laboratory	Possible contact exposure from contaminated material (request and transport boxes)
	Possible tripping due to small and obstructed space
Homogenization, vortex mixing and pipetting and centrifugation of serum for RNA Extraction and ELISA	Spills and splashes in processing infectious materials
Shipping fresh and inactivated specimen for Reference laboratory confirmation (WHO-Hong Kong)	Possible contact exposure from contaminated material (primary and/or secondary container)

Compliance of the RITM Virology Laboratory with BSL-2 standards

The WHO recommends a minimum of Biosafety Level 2 for practices, containment, equipment and facility for handling infectious or potentially infectious material for Zika virus diagnosis. We have determined RITM Virology laboratory's compliance using the WHO Biosafety checklist. The findings are summarized in Figure 1.

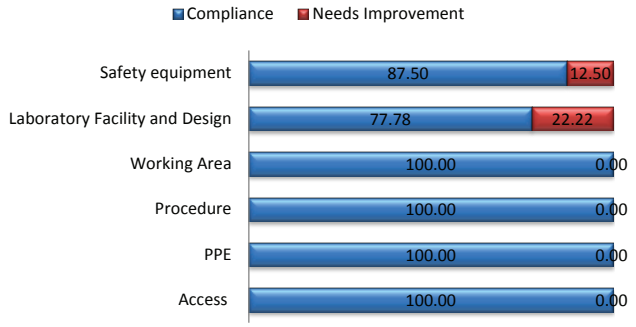

Figure 1. Compliance with WHO biosafety guidelines for basic laboratory (BLS1 and BLS2).

Competence of Laboratory Personnel

The protection of laboratory workers, other personnel working within and outside the laboratory, the general public and the environment will depend ultimately on competence, compliance and commitment of laboratory workers to biological safety. We have determined the proficiency of laboratory personnel who will be tasked to work with ZikV.

A record of staff's name, age, gender, birth date, civil status, educational background and trainings related to biological safety, infectious substance shipping and technical skills in handling and laboratory diagnosis of infectious diseases was obtained.

Twenty-two (22) Virology Department personnel will be involved in specimen reception and processing, RNA extraction and testing, and results validation and reporting. Six personnel will be first line responders while the remaining personnel are reserved to respond as part of surge capacity plan. 73% are females, and 26% are males. The age ranges from 20 to 55 years. Majority are licensed Medical Technologists by profession and are civil service eligible. Staff had undergone local training on Biosafety and infectious substance shipping (Figure 2). Those who have been trained were certified shippers of infectious substances. Laboratory personnel are technically competent in laboratory diagnosis of human sample by PCR and ELISA. Those involved in the molecular testing are certified proficient in the performance of Polymerase Chain Reaction (PCR).

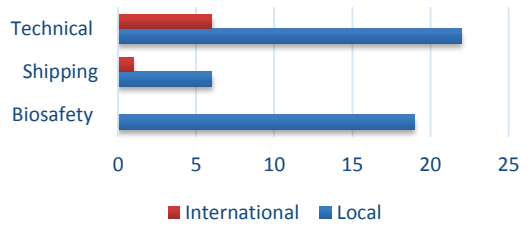

Figure 2. Trainings of laboratory personnel.

Risk Characterization

The risk of infection with ZikV ranges from very low to low (Figure 3), while the risk of infection with other blood-borne pathogens is found to be higher (Figure 4).

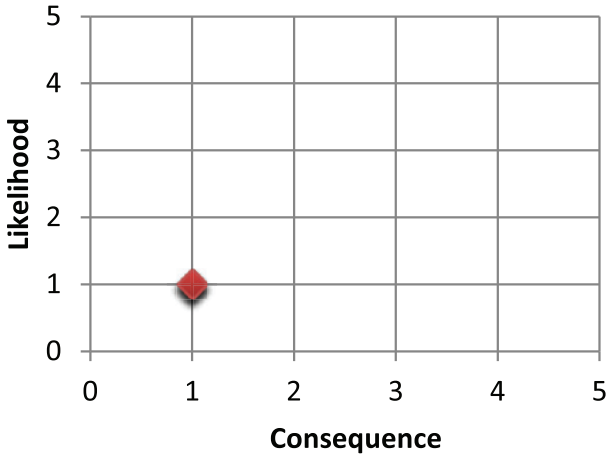

Figure 3. Risk matrix for acquiring zika infection.

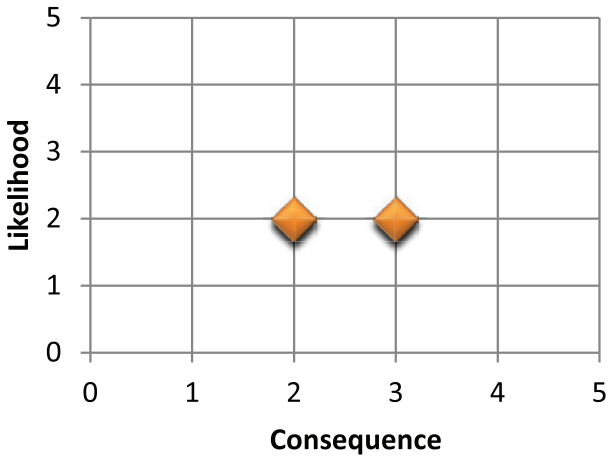

Figure 4. Risk matrix for acquiring other blood-borne pathogens infection processing human blood samples.

BioRAM© Model

The BioRAM© Model has been utilized to identify biosafety risk of ZikV exposure to individuals in RITM Virology Laboratory, to the community and animals in the community while performing laboratory-based investigation and surveillance. BioRAM© reported very low biosafety risk (Figure 5).

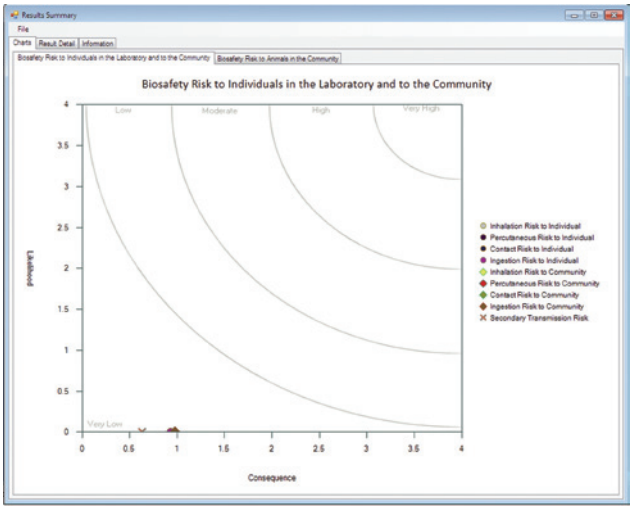

Figure 5. BioRAM© result for Zika.

DISCUSSION

The initial assessment of the risk has been performed by identifying the hazard of the agent and the procedure. We have determined the hazard of the agent by its capacity to infect and cause disease in a susceptible human host, its host range, the severity of disease it causes, the infectious dose, its stability in the environment, the mode of transmission and the availability of preventive measures and effective treatments. Compiled reports of laboratory acquired infections could also be a strong basis of the initial assessments, however, no reports have been documented specific for Zika infection in the laboratory. Due to the limited information about ZikV, we relied on references found in the Internet to gather information. After reviewing all related and available information that supports the identification of the agent's hazard, the hazards of the laboratory procedures were identified.

The risk of LAI with ZikV as characterized in this risk assessment is low. Factors that have influenced low likelihood and consequence of ZikV infection includes the transmission requirement for an arthropod vector, the procedure to be conducted that has minimal potential exposure risk and the absence of reported LAI related TI ZikV. The reproductive cycle of ZikV follows that of other known flaviviruses like Dengue and Chikungunya. ZikV requires mosquito vectors from the genus Aedes. Transmission occurs when an infected vector feeds on a host with an incubation time of around 10 days.

We have enumerated all laboratory procedures related to ZikV diagnosis at the RITM. The principal probable exposure hazard that we have identified is through direct contact. The procedures also do not require the use of sharps, live animals and insect vectors for inoculation and culture, thus minimized the personnel's exposure to the virus. Possible use of sharps, during blood collection was considered. Caution must be observed as percutaneous transmission via blood transfusion is being investigated. Currently, there have been no reports of transmission via direct contact with contaminated material. While the exposure risk to ZikV virus is low in the laboratory setting, the risk of exposure to other blood-borne pathogens was found to be higher than the pathogen being assessed. Since human blood and serum are the optimum specimen for diagnosis, the risk of exposure to other blood-borne pathogens must be considered. To manage worst case consequences of exposure to these pathogens, a separate risk assessment should be conducted for each suspected pathogen.

The WHO recommends a minimum of Biosafety Level-2 practices, containment, equipment, containment and facility for handling ZikV. In this risk assessment, we found that the laboratory is compliant to the requirements with the following recommendations. However, the Virology laboratory must work on its administrative controls and further improve the facility. Improper placement of supplies and equipment along the corridors and aisles are physical hazards. Accidental tripping due to obstructed walkways could lead to physical injury or potential exposure to infectious material if accidents occur during specimen manipulation. Windows must be fitted with arthropod screens that could be opened in case of emergencies. It also must develop its procedure for decontaminating equipment prior to repair and maintenance.

Since this risk assessment is only limited to the RITM Virology Laboratory, it is recommended that biological risk assessment be conducted in other laboratories included in the response (Clinical Laboratory and Entomology laboratory). The Clinical Laboratory serves as the central specimen reception facility in charge of specimen collection within RITM, while Entomology Laboratory are involved in vector studies.

Risk assessment is fundamental in biological risk management program. The biological risk management program includes risk assessment, mitigation and monitoring the performance. Rapid risk assessment should be done as often as the introduction of new pathogen, technique, equipment, personnel, facility, procedure, practices, and/or mitigation control measure that may influence biological safety in laboratory. If done correctly, risk assessment, could provide effective allocation of resources to mitigate risk, identify training needs and supervision, evaluate procedural changes and exchange of work flow with other laboratories, and comply with standards and regulations.

Risk assessment must be documented. Risk must be communicated to all at stake personnel. Compliance with biosafety standards must be verified at least annually or as often as need arises. Initial risk assessment result must be reviewed prior conducting follow up risk assessment and when monitoring implemention of mitigation control measures.

Biosafety officers should lead in conducting the risk assessment. Technical staff, laboratory supervisor and subject matter experts must be involved as the quality of risk assessment result is dependent upon the exchange of ideas and findings. The laboratory head and biosafety officer are responsible for the implementation of biosafety recommendations and mitigation controls based on risk assessment. The institute is in charge of the biosafety administrative controls. Safe laboratory working environment, biological safety and the general welfare of all employees and researchers involved in Zika Virus activities and laboratory surveillance must be ensured.

CONCLUSION

Zika virus is an emerging public health threat. Laboratory diagnosis and surveillance of Zika is a critical component of response. However, biosafety is indispensable and must be considered. The process of doing the risk assessment is a vital strategy to ensure biological safety of laboratory personnel involved. We have analyzed and assessed the risk of acquiring Zika in RITM Virology laboratory as part of the overall preparedness. In this process we have documented that, the risk of acquiring ZikV infection while performing diagnositic test ranges from very low to low. The specimen to be collected and handled for ZikV diagnosis is human serum sample. The risk of acquiring other blood-borne pathogens is higher compared to the risk of infection to the pathogen being assessed. Mitigation control measures against direct contact and percutaneous exposure must be implemented and monitored. The laboratory is generally compliant with WHO basic laboratory biosafety standards required for ZikV laboratory diagnosis. Its staff are technically proficient for the procedure and are trained in biosafety. RITM is employing a documented risk assessment strategy as part of its biological risk management program. This risk assessment strategy will further strengthen laboratory capacity to respond to infectious disease threats and increase staff confidence in dealing with infectious materials in the laboratory.

AUTHOR DISCLOSURE

The authors declared no conflicts of interest.

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Rare Mammary Lesions: A Radiologic-Pathologic Correlation

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ABSTRACT

Appearances can be deceiving and this pictorial essay illustrates the imaging appearance of breast lesions which may or may not appear as classic for malignancy. These cases are considered unusual, interesting and uncommonly encountered, thus providing an avenue for better collaboration and as a teaching point for both radiologists and pathologists.

Key words: breast neoplasms, angiosarcoma pseudoangiomatous stromal hyperplasia, granulomatous mastitis

INTRODUCTION

The presentation of unusual breast lesions is quite challenging, knowing that a wide array of benign and malignant lesions may be encountered in practice. Familiarity with their radiologic appearances as well as the pathologic findings are important since the detection of such lesions can impact their clinical management.

Rare breast lesions have mammographic and sonographic features which appear similar to those of breast carcinomas and as such warrant tissue biopsy to obtain a diagnosis.

The four cases included in this report range from benign to malignant lesions that are considered as rare. However, in those that are benign, their imaging features may be similar to those of carcinomas. In light of this, the concomitant pathologic findings augment the imaging, hence, the subsequent diagnosis alters the management.

METHODOLOGY

This is a case series of four (4) patients with rare breast lesions which have imaging studies that were seen and performed at the Breast Center of St. Luke's Medical Center, Quezon City -one with both digital mammography and ultrasound while the rest only have ultrasound. Their ages range from 24 to 51 years old. The clinical histories were reviewed with particular attention to their initial presentaton as well as their imaging finding. Core-needle biopsies were done and specimens sent to the Institute of Pathology of St. Luke's Medical Center which were then interpreted by a dedicated breast pathologist.

DISCUSSION

Sarcomas of the breast are extremely uncommon, constituting less than 1% of all malignant breast tumors and rarely present as primary breast malignancies. The most common breast sarcomas are: phyllodes tumor, previously referred to as cystosarcoma phylloides, and osteogenic sarcoma belong to malignancies of stromal origin.[1] According to the WHO classification of breast tumors, phyllodes tumors are classified as fibroepithelial tumors, in the same class as fibroadenoma, low-grade periductal stromal sarcoma, and mammary hamartoma while osteosarcoma belongs to tumors classified as mesenchymal tumors which include hemangioma, angiosarcoma, and granular cell tumor to name a few.[2]

Benign phyllodes tumours are characterized by few if any mitoses, moderate to marked cellular overgrowth, and slight to moderate cellular pleomorphism. Low-grade malignant or borderline lesions include a zone of microscopic invasion around their borders, an average of two to five mitoses per 10 high-power field, and moderate stromal cellularity that is heterogeneously distributed in hypocellular areas. Malignant phyllodes tumors show a marked degree of hypercellular stromal overgrowth with more than five mitoses per 10 high-power field, and have an invasive border.[3]

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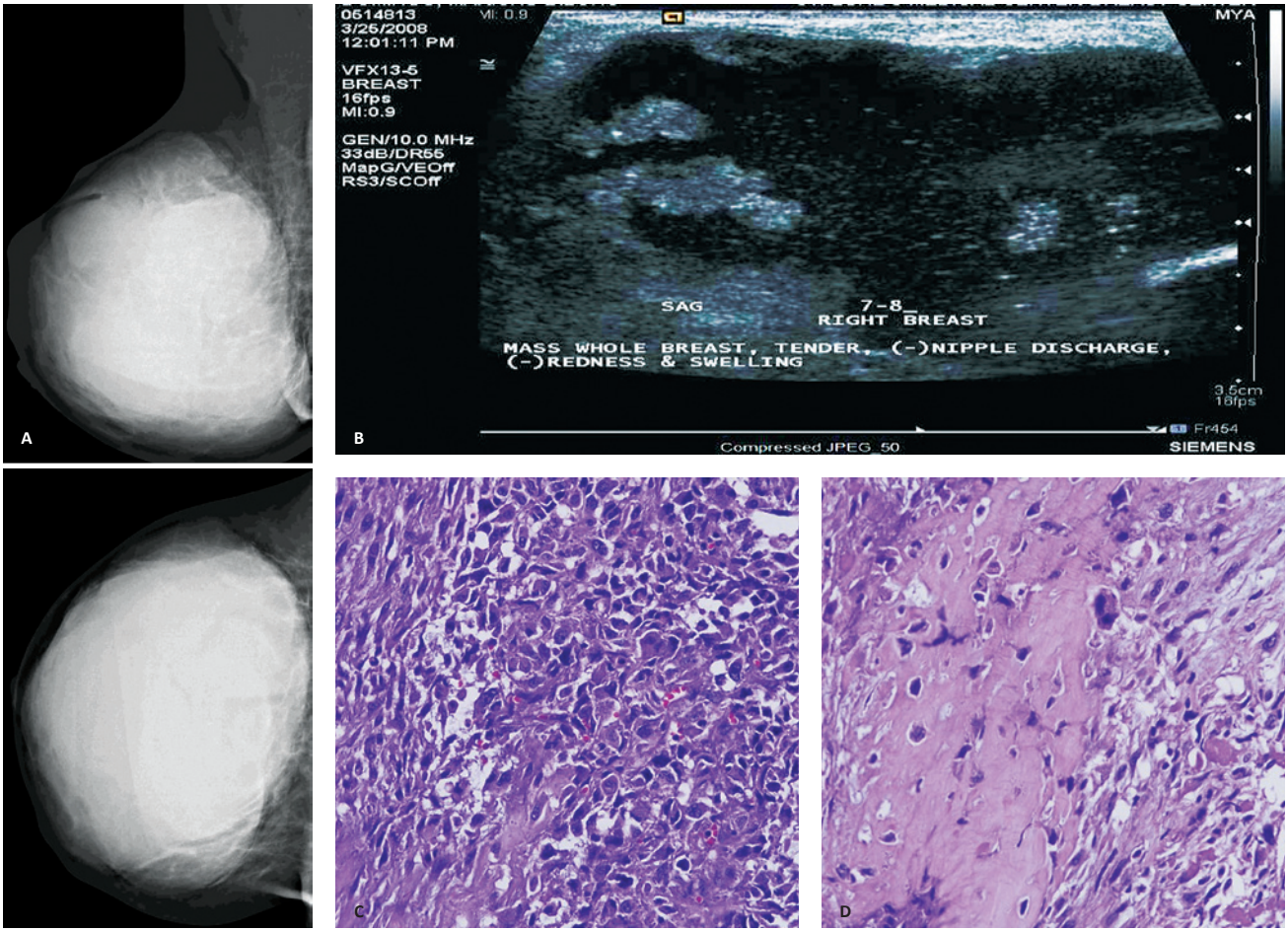

Figure 1. Malignant phyllodes of the breast in a 51-year-old woman with a few month's history of gradually enlarging right breast mass with no history of previous irradiation to the breast/chest region. **(A)** Mediolateral oblique (MLO) and craniocaudal (CC) mammograms demonstrate the large lobulated mass of high density occupying the entire right breast; **(B)** Ultrasound (US) image shows a portion of the mass to be complex - with solid and cystic components. The cystic component demonstrates medium to high level echoes; **(C)** High-power magnification (400x, H&E) of the cellular component shows it to be made of pleomorphic cells with average mitotic count of 10-12 per hpf; **(D)** High power magnification of a slide showing a focus with osteosarcomatous features.

Mammographically, phyllodes tumors usually present as focal masses and may have lobulated margins. The tumor may manifest initially as a large mass or may show a rapid increase in size and on ultrasound, a phyllodes tumor may resemble a fibroadenoma or may have a variable appearance with internal heterogeneity, cystic changes, and posterior enhancement.[4]

Primary osteogenic sarcoma can occur in extraosseous locations and has been reported in the breast. They are rare but can occur in an area that was previously irradiated and as many as 40% of these tumors are preceded by fibroadenomas or cystosarcoma phyllodes.[5] Osteosarcomatous differentiation in phyllodes tumors is uncommon.[6] Metastasis occurs by blood rather than by lymphatic spread. Complete excision without axillary dissection is advised.

Pseudoangiomatous stromal hyperplasia (PASH) is a benign lesion that is classified as a mesenchymal tumor of the breast.[2] The lesion is commonly seen in premenopausal women or those receiving hormone therapy, hence, the lesion is likely related to fluctuations of hormone levels. The clinical manifestation of PASH is myriad, ranging from insignificant incidental microscopic changes in the breast to focal palpable or nonpalpable masslike nodules (nodular PASH) to diffuse breast involvement.[4] On imaging, US images may show a hypoechoic circumscribed mass that resembles a fibroadenoma.[7]

The most striking histologic finding is a complex pattern of empty anastomosing slitlike spaces within the stroma. These slitlike spaces resemble the vascular spaces in lesions such as low-grade angiosarcoma and may be mistaken for such (hence the name *pseudoangiomatous*), from which PASH must be histologically differentiated.[4, 7]

A short-term follow-up imaging may be done or, alternatively, surgical excision may be performed immediately. The prognosis is generally good, with a reported recurrence rate of approximately 10%.[7]

Angiosarcoma is a malignancy of endovascular origin. Primary angiosarcoma can arise anywhere in the body. When it occurs in the breast, it affects women in their 3rd and 4th decade and accounts for one in 1700-2300 cases of primary breast cancer.[8] It arises in the breast more often than in any other organ.[3]

The size and location of palpable masses vary from small cutaneous nodules (the cutaneous subtype) to large lumps that constitute

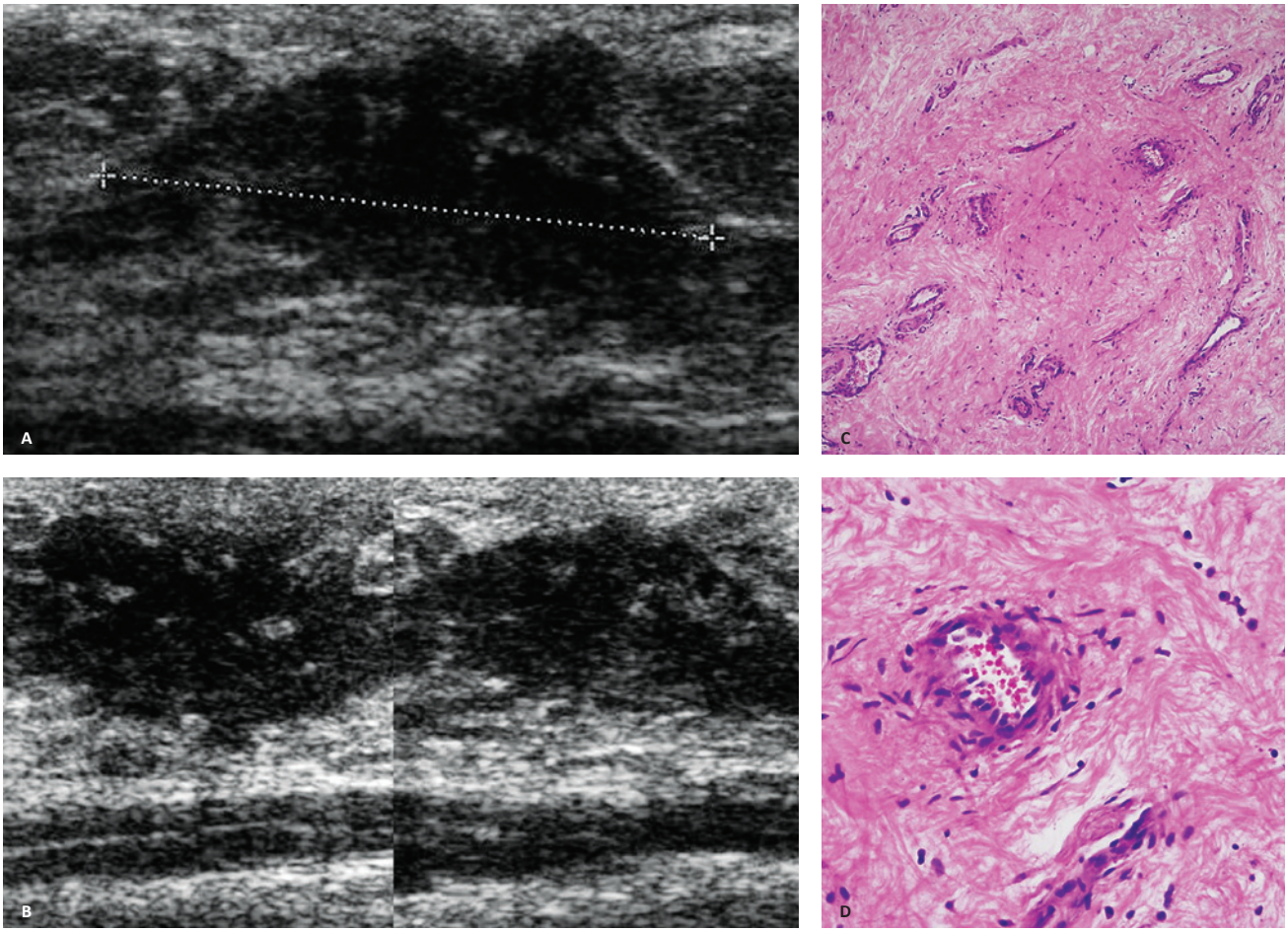

Figure 2. PASH in a 24-year-old woman who presented with palpable masses in both breasts of 3 years duration. **(A,B)** Ultrasound (US) images demonstrate an irregular hypoechoic solid mass punctuated with hyperechoic foci within the lesion; **(C)** Low-power magnification (100x, H&E) shows increased vascularity within the cellular stroma; **(D)** High-power magnification shows a blood vessel surrounded with typical spindle-shaped cells with normal nuclei (400x, H&E).

the entire breast (the cutaneous subtype). The majority of cases exhibit skin changes. Advance disease is typically marked by edema and ulcerative lesions in the breast. Radiologic findings are often nonspecific. Mammograms my appear completely normal in 33%. Ultrasound reveals a lesion that may appear hypoechoic, hyperechoic, or a heterogeneous region, with or without acoustic shadowing.[8]

Angiosarcoma is divided into low and high histologic grades. Grade is prognostically siginificant.[3] The cells of low-grade angiosarcoma resemble endothelium both morphologically and functionally. The cells are typically spindle-shaped and have large, oval nuclei with vesicular chromatin pattern. They form irregular, anastomosing vascular channels, and sometimes seem to connect to dermal capillaries. High-grade angiosarcoma is characterized by larger cells, pleomorphic nuclei, prominent nucleoli, and a high degree of mitotic activity. Hemorrhage into the surrounding stroma, known as "blood lakes," is also common and could account for the sometimes heterogeneous appearance of angiosarcomas on ultrasound.[3,8]

Angiosarcoma although a rare malignancy has a very poor prognosis. It is almost uniformly fatal with rapid metastatic spread and survival beyond 5 years is extremely rare.[1] Aggressive surgical resection is advocated as the treatment of choice.

Granulomatous mastitis is a very rare inflammatory disease of unknown origin that can clinically mimic carcinoma. It generally manifests as a distinct, firm to hard mass that may involve any part of the breast. The mammographic features are variable, from normal findings to masses with benign or malignant features and focal asymmetric density. The US appearance of multiple clustered, often contiguous tubular hypoechoic lesions is often an uncommon manifestation whose features resemble carcinoma.[9]

At pathologic analysis, granulomatous mastitis manifests as a non-caseating, nonvasculitic granulomatous inflammatory reaction centered on lobules. Granulomatous being the term adopted in the absence of a specific etiologic agent.[3] Even with the absence of an etiologic agent, it is imperative to take into consideration a pathogenic cause such as tuberculosis which is prevalent in our country. Although the diagnosis of mammary tuberculosis is difficult since acid-fast bacteria are not detected in most cases, it is usually based on inflammatory and granulomatous findings at FNA cytologic analysis or biopsy. Primary treatment consists of excisional biopsy.[9]

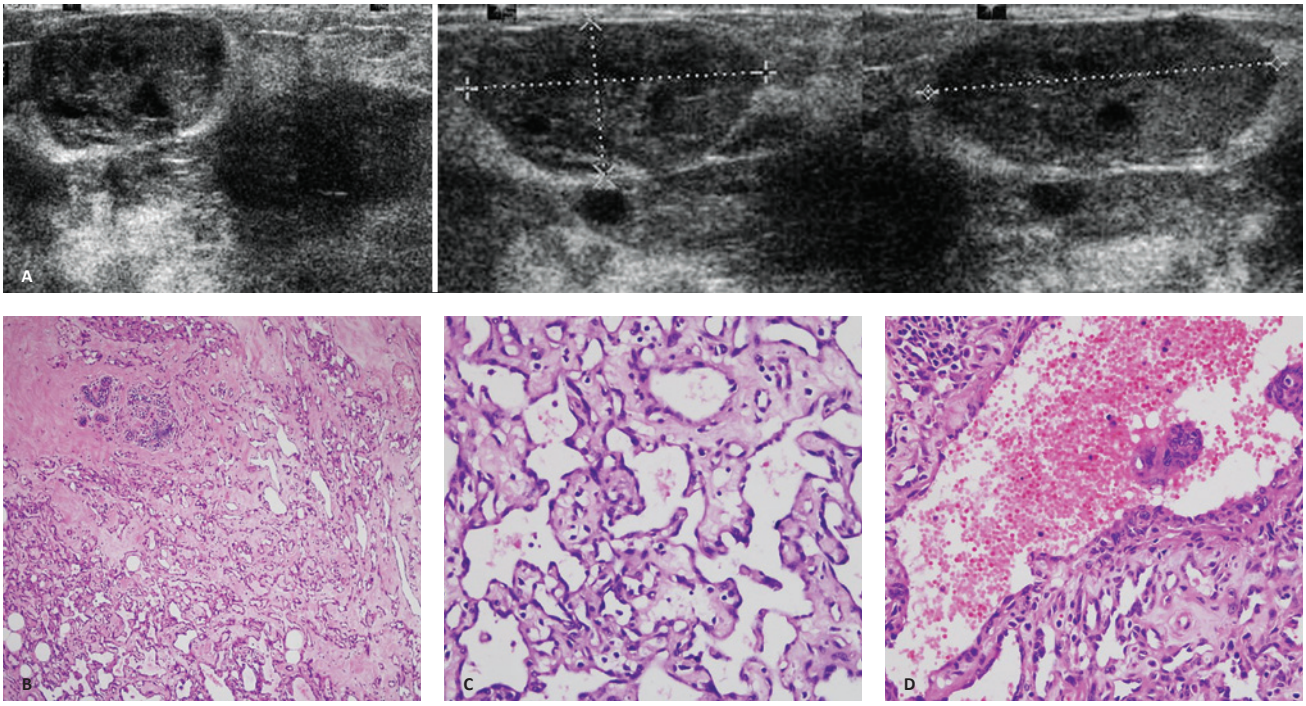

Figure 3. Angiosarcoma of the breast, bilateral in a 32-year-old female who presented initially with a bleeding right breast mass with no history of any previous irradiation to the breast/chest area. **(A)** US images show fairly circumscribed complex solid nodules with heterogeneous echogenicity, one of which shows few hypoechogenicities within the masses; **(B)** Low-power magnification (100x, H&E) shows anastomosing blood vessels; **(C)** High-power magnification (400x, H&E) demonstrate atypical spindle-shaped cells lining the vascular channels; **(D)** HPO view of the typical hemorrhagic "blood lakes" of angiosarcoma (400x, H&E).

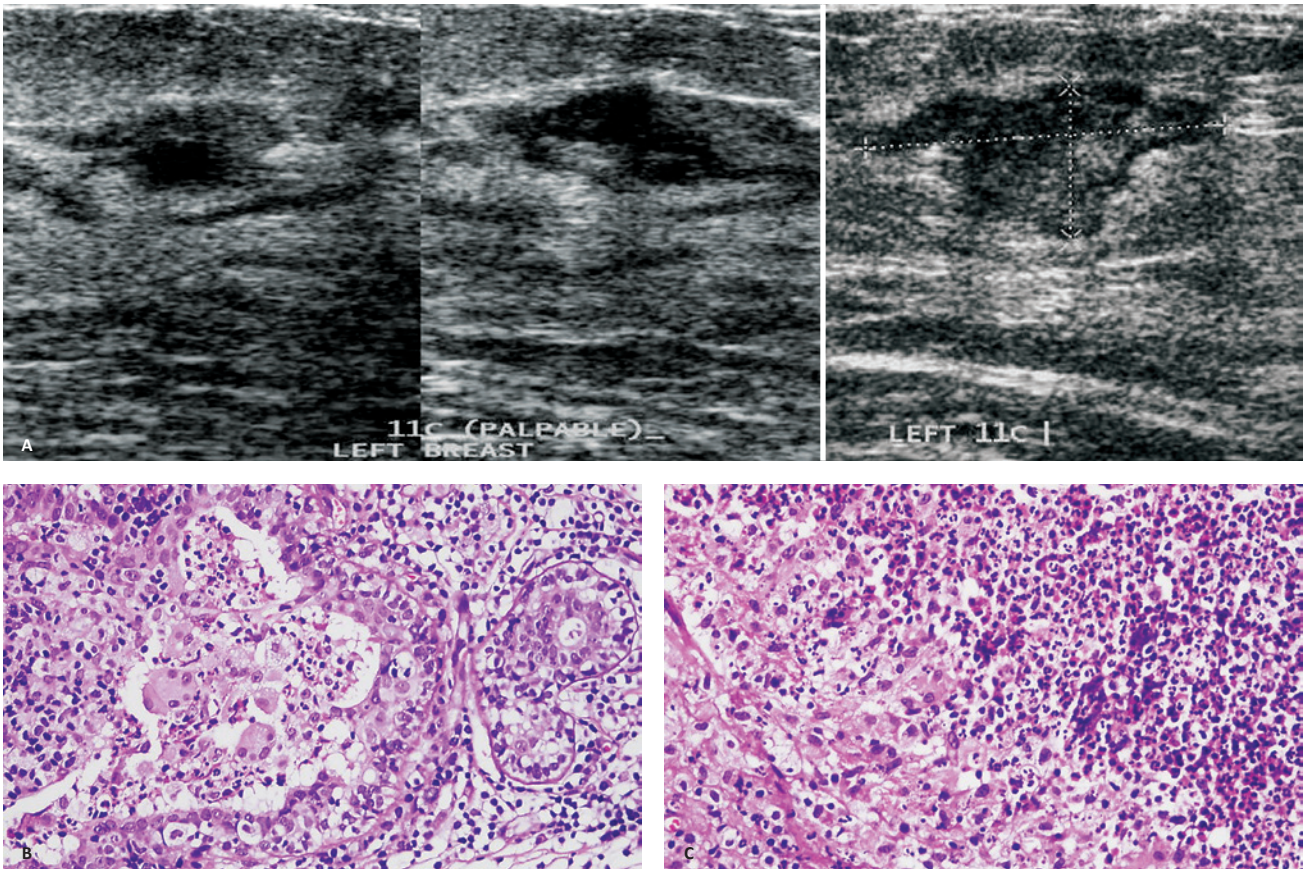

Figure 4. Granulomatous lobular mastitis in a 34-year-old woman who presented with a palpable mass on the left breast. **(A)** US images demonstrate an irregular hypoechoechogenicity; **(B)** High-power magnification (400x, H&E) showing multinucleated giant cells within the terminal ductal-lobular unit; **(C)** Low-power magnification (100x, H&E) of an area with proliferation of neutrophils indicating abscess with disruption of the basement membrane.

CONCLUSION

Radiologic-pathologic correlation is crucial in the diagnosis of the spectrum of breast diseases. The relation of the underlying pathology of a lesion explains its imaging appearance. However, as these cases have demonstrated, lesions that initially present as malignancy may actually be benign or vice versa. Close collaboration between radiologists and pathologists is copacetic and greatly influences clinical decisions and management.

AUTHOR DISCLOSURE

The author wrote this case report while she was a fellow of breast imaging at the Breast Center of St. Luke's Medical Center–Quezon City. There are no other conflicts of interest declared.

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Hepatocellular-Cholangiocarcinomas in Non-alcoholic Fatty Liver Disease: Evidence for Stem Cell Origin

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ABSTRACT

The authors present two cases of combined hepatocellular-cholangiocarcinoma in a background of non-cirrhotic, non-alcoholic fatty liver disease (NAFLD). The increasing incidence of NAFLD and the subsequent recognition of it being a pre-malignant condition even in the absence of significant fibrosis or established cirrhosis has led to the investigations of the different pathways involved in NAFLD-associated hepatocarcinogenesis, including speculations regarding the possibility that many derive from pre-malignant hepatocellular adenomas, tumors also increasingly associated with NAFLD, or reflect malignant transformation of mature hepatocytes through genetic and epigenetic alterations reflecting inflammatory changes in NAFLD. However, NAFLD, like most chronic liver diseases, leads to progressive activation of resident hepatobiliary stem/progenitor cells that are thought to give rise to malignant tumors in other settings. In particular, combined hepatocellular-cholangiocarcinomas (with and without stem cell features) are thought to reflect malignant transformation of these activated progenitors. Our two cases of combined hepatocellular-cholangiocarcinomas suggest that malignant transformation of hepatobiliary stem/progenitor cells in NAFLD are also a possible pathway to malignancy, even in the absence of established cirrhosis.

Key words: hepatocellular-cholangiocarcinoma, non-alcoholic fatty liver disease (NAFLD), steatohepatitis, stem cell

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is now the most common form of chronic liver disease in industrialized countries.[1] The histologic changes seen in patient livers include steatosis, steatohepatitis, and steatofibrosis. Many patients progress to cirrhosis and its associated complications of liver failure and hepatocellular carcinoma (HCC). Studies indicate that development of HCC in cirrhotic patients with NAFLD has a yearly incidence of 2%-5%.[2] Recently, reports of HCC developing in NAFLD in the absence of cirrhosis have started to surface.[3-4] Currently, individuals who have NAFLD without underlying cirrhosis are not screened routinely for HCC at most centers because of the assumed low risk of cancer development, though such cases of pre-cirrhotic HCC raise questions in this regard.

Hypotheses about the pathogenesis of non-cirrhotic, NAFLD-associated HCC have been suggested. One proposal suggests that these cases developed from malignant transformation of hepatocellular adenoma (HCA), particularly the telangiectatic subtype and those with β-catenin mutation, though whether HCC and HCA develop simultaneously or successively has not been clearly resolved.[2,5] Some studies suggest that tumor suppressor genes such as phosphatase and tensin homolog (PTEN), promyelocytic leukemia and p53 play an important role in the development of steatosis with associated liver cell damage.[6-7] It follows that the loss of tumor suppression could promote formation of HCC, even

without cirrhosis. Another mechanism that may contribute is the dysregulation of bile acid metabolism, which may induce hepatocyte apoptosis promoting HCC in the setting of steatosis.[8] These various hypotheses all generally point toward tumor development from pre-existing hepatocytes that undergo malignant transformation.

We present two cases of combined hepatocellular-cholangiocarcinoma (HCC-ChC) developing in a setting of NAFLD, one without significant scarring and the other with "incomplete septal cirrhosis" (possibly regressed fibrosis). The combination of histomorphology and immunostaining (stains summarized in Table 1) confirmed the tumor diagnoses. The presence of mixed hepatobiliary tumors, one containing an overt stem cell variant, raises the possibility that some hepatobiliary malignancies in non-cirrhotic NAFLD arise through the activation and malignant transformation of pluripotent liver stem/progenitor cells.

Table 1. Antibodies

Antibody	Differentiation	Company	Titer
Hep Par 1	Hepatocytic lineages	Dako	1:400
Keratin 19	Cholangiocytic and Stem / Progenitor cell lineages	Dako	1:100
EpCAM	Cholangiocytic and Stem / Progenitor cell lineages	Leica	1:250
CD56	Stem / Progenitor cell lineages, Neuroendocrine differentiation	Leica	1:250
Arginase-1	Hepatocytic lineages	Sigma	1:8000
Keratin 7	Cholangiocytic and Stem / Progenitor cell lineages	Dako	1:1000
Canalicular CD10	Hepatocytic lineages	Leica	1:250
Canalicular CEA (polyclonal)	Hepatocytic lineages	Dako	1:6000

CASE 1

A 49-year-old-woman, from the island of St. Vincent, but residing in New York City, was referred to Hepatology clinic for serum alkaline phosphatase elevation and sonogram with fatty liver. She denied any symptoms of fatigue, weight loss, pruritis, abdominal pain or distention. Past medical history revealed hypertension, dyslipidemia and pre-diabetes. Family history was significant for sarcoidosis. On physical exam, she was obese (body mass index of 31.0 kg/m^2), had peri-orbital swelling and was in no acute distress. She had no stigmata of chronic liver disease or hepatosplenomegaly.

Pertinent laboratory tests included serum alkaline phosphatase (ALP) of 223 U/L (normal: 38-126), serum aspartate aminotransferase (AST) 37 U/L (normal: 15-46), serum alanine aminotransferase (ALT) 66 U/L (normal: 13-69), GGT 275 U/L (normal: 0-51) and angiotensin converting enzyme (ACE) 85 U/L (nl: 8-57). Total cholesterol >240 mg/dl (normal:<200 mg/dl) and low density lipoprotein (LDL) >160 mg/dl (normal:<100) were significantly elevated. Serologic studies for hepatotropic viral infection and autoimmune disease were negative. Ceruloplasmin and alpha-1-antitrypsin levels were within normal range. A computed tomography (CT) of the abdomen revealed fatty infiltration of the liver with no demonstration of hepatic mass. Ultra-sound guided liver biopsy was performed to evaluate the patient's chronic liver disease. Following confirmation of NAFLD (and exclusion of sarcoidosis), she was referred to a nutritionist and recommended to lose weight.

For approximately two years thereafter, the patient had close follow up with her primary care physician in Beth Israel Medical Center's Hepatology Clinic. Regularly scheduled blood work showed persistent elevation of her ALP and ALT levels with the range of 89-340 U/L and 66-289 U/L, respectively. She remained asymptomatic. After more than 2 year interval, a follow up CT scan of the abdomen and pelvis revealed a large, peripherally enhancing, hypodense lesion replacing much of the posterior segment of the right lobe of the liver with intrahepatic infiltration of the segment to the level of the porta hepatis. An MRI soon after confirmed the hepatic mass measuring 4.8 x 5.2 x 6.6 cm.

Patient underwent a right hepatic lobectomy.

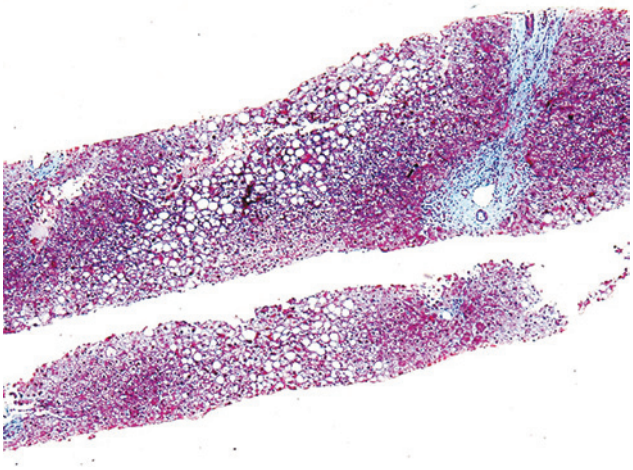

Figure 1. Liver tissue cores with steatosis and absence of pericellular fibrosis, (Trichrome stain, 20x, H&E).

Histopathology

Needle biopsy

Multiple cores of liver tissue were submitted for evaluation. All of the cores showed a moderate degree of steatosis (Figure 1). Histologic steatohepatitis (i.e. hepatocyte ballooning with or without Mallory-Denk bodies, neutrophilic infiltration) was not present. Trichrome stain showed mildly increased portal stroma, but no features more specific for steatofibrosis. Hemosiderosis was absent with Prussian blue stain. Periodic acid-Schiff stain following diastase digestion showed no alpha-1-antitrypsin globules. Prominent ductular reactions, large cell change, and small cell change were not identified.

Tumor resection

The specimen was a partial hepatectomy of the right lobe. Grossly, the tumor was pale brown to cream in color and fibrotic in consistency. It measured 7 cm in greatest dimension, was irregular in shape and had ill-defined borders.

The histologic appearance of the non-tumoral liver showed an absence of the steatosis present in the prior biopsy specimen. Trichrome stain confirmed the mildly increased portal tract stroma, but again showed no steatofibrosis. Prominent ductular reactions, large cell change, and small cell change were not identified.

The histology of the mass is predominantly composed of malignant glands admixed with nests of tumor cells exhibiting hepatocellular morphologies (Figure 2). The glandular elements show a spectrum of moderate to poorly differentiated regions with some islands floating in mucin. Significantly, the nests of tumor cells with hepatocellular differentiation, have focal, peripheral small cells with increased nucleus:cytoplasmic ratio and nuclear hyperchromasia as have been identified in so-called "combined HCC-ChC with stem cell features, typical subtype" (Figure 2A)

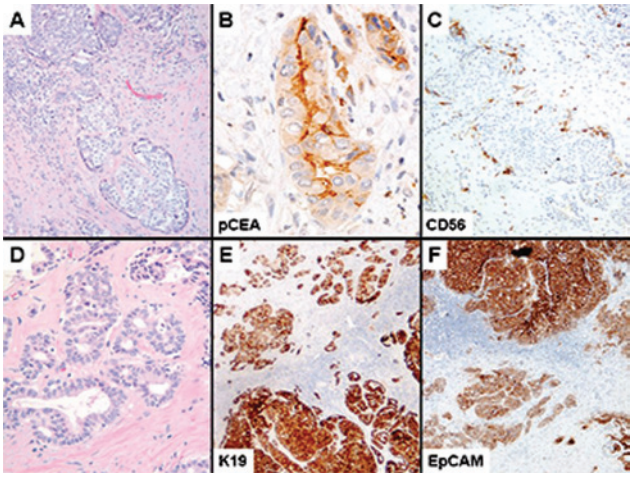

Figure 2. Case 1 tumor morphology **(A)** hepatocellular carcinoma admixed with **(D)** cholangiocarcinoma; **(B)** Positive canalicular staining with polyclonal CEA in HCC; **(E)** Keratin 19 with diffuse cytoplasmic staining in cholangiocarcinoma; **(C)** CD56 and **(F)** EpCAM highlight the stem/progenitor cell differentiation.

Immunohistochemistry

Needle biopsy

Immunostains for K19 and EpCAM did not demonstrate prominent ductular reactions or EpCAM positive hepatocytes.

Tumor resection

By immunohistochemistry, the areas with hepatocellular differentiation exhibited canalicular staining with polyclonal anti-CEA antibodies, but were negative for CD10, HepPar1 and arginase-1. Diffuse strong staining for keratins 7 and 19 and EpCAM were present throughout all forms of the tumor. The glandular components also showed cytoplasmic and luminal staining with polyclonal anti-CEA antibodies. The cuboidal cells surrounding the hepatocyte-like nests stain positively for CD56, a typical finding of the "typical" form of mixed hepatobiliary stem cell tumors.[9,10]

CASE 2

A 63-year-old male from Hong Kong presented in the clinic because of dizziness and vomiting. He is a known diabetic and hypertensive who presented with left sided weakness a few months prior to the present admission. On physical examination, he was conscious and alert with a blood pressure of 160/90 mmHg. Neither nystagmus nor other signs of cerebellar dysfunction were recognized. The lungs were clear and the abdomen was non-tender. There was no hepatomegaly or stigmata of chronic liver disease. His body mass index (BMI) was 23.9 kg/m^2. Laboratory tests showed elevated blood urea nitrogen, creatinine, glucose and triglycerides. Serum liver enzymes were not elevated nor were there serologic markers of hepatotropic viral infection or of autoimmune disease.

Upon sonographic assessment of the kidneys, an incidental, enhancing liver mass was found. The mass was seen next to the diaphragm, stomach and heart and was thus deemed technically too difficult to proceed with needle biopsy. The presence of a 2 cm mass with smooth outline was also confirmed via CT scan, revealing its close proximity to the surface and the risk of biopsy-associated rupture. Segmental resection of the bulging mass at segment 2 was done instead.

After the uneventful operation, patient was compliant with follow up visits and medications until three years when diabetic nephropathy worsened. He underwent dialysis treatment and suffered from another event of stroke. At this time, he started to complain of numbness, back pain and dysuria. Work up showed metastatic tumor foci, compressing the vertebra at T4 and T5 level.

Histopathology

The submitted specimen is a segmental resection with a bulging tumor on the surface. The 4 cm tumor was pale brown with alternating areas of hemorrhage. No necrotic or cirrhotic regions identified.

The non-tumoral liver parenchyma contained within the resection specimen shows incomplete septal cirrhosis (probably regressed cirrhosis) with features indicative of diabetes associated, non-alcoholic fatty liver disease, namely central-portal fibrous septa (Figure 3). Residual steatosis and histologic features of steatohepatitis are not identified. Prominent ductular reactions, large cell change, and small cell change were not present.

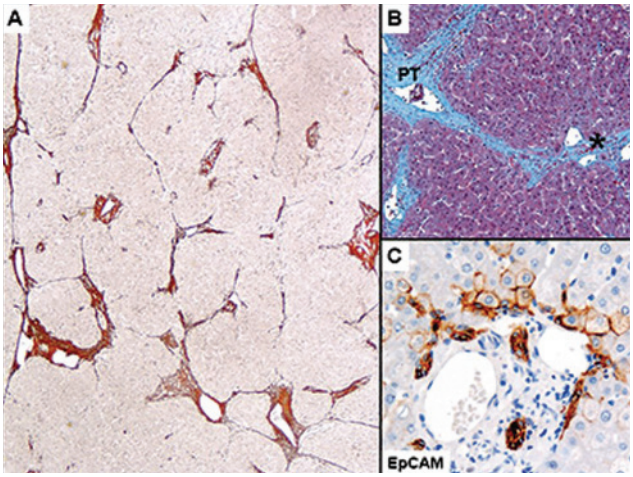

Figure 3. **(A)** Non-tumoral liver in case 2 with incomplete septal cirrhosis; **(B)** Trichrome stain highlighting septal fibrosis; **(C)** Positive membrane staining with EpCAM supporting stem cell features.

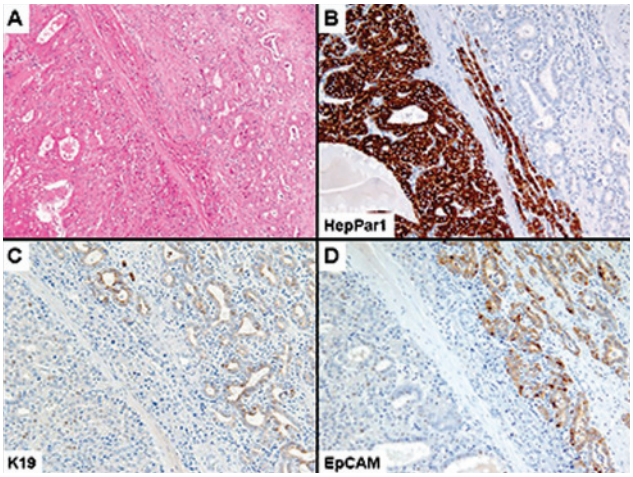

Figure 4. **(A)** Case 2 tumor morphology (Hematoxylin); **(B)** HepPar1 highlights the HCC component of the tumor; **(C)** Keratin 19 highlights the cholangiocarcinoma component of the tumor. **(D)** No stem cell features supported by a negative EpCAM stain.

Histologically, the tumor cells are predominantly seen in pseudoacini and trabecular configurations exhibiting atypia with eosinophilic cytoplasm (Figure 4). Admixed in the surrounding desmoplastic stroma are irregular glands lined by columnar epithelium with large nuclei and prominent nucleoli. Stem cell features are not present. The tumor is seen extending to the subcapsular area and invading the vascular wall.

Immunohistochemistry

Immunostaining for EpCAM highlighted focal ductular reactions, sometimes with adjacent, clustered, EpCAM positive hepatocytes, indicative of stem/progenitor cell mediated regeneration, albeit to a small degree, of hepatic parenchyma (Figure 3C).[11] Immunostains of the tumor are presented in Figure 4. The areas with hepatocellular differentiation exhibited canalicular staining for CD 10 and with polyclonal anti-CEA antibodies and strong and diffuse punctate staining for HepPar1. Stains for keratins 7 and 19 and for EpCAM were negative in these areas. Glandular components, on the other hand, stain positive for keratins 7 and 19, EpCAM and CEA (cytoplasm and membrane), but were negative for arginase-1 and HepPar1.

DISCUSSION

NAFLD is the hepatic manifestation of the metabolic syndrome, a cluster of conditions that are related by obesity, insulin resistance, dyslipidemia and elevated blood pressure.[12] Patients with NAFLD may show a spectrum of histologic features including steatosis, steatohepatitis and steatofibrosis, with some progressing to the complications of cirrhosis and hepatocellular carcinoma.

The clinical course of NAFLD is related to its histology at the time of diagnosis. Mild steatosis on presentation will usually connote a benign prognosis. Presence of steatohepatitis indicates an increased likelihood of disease progression that may lead to significant fibrosis. Statistically, 26%-37% of patients with steatohepatitis demonstrate progression to fibrosis over time, with up to 9% progressing to cirrhosis and 2%-5% to hepatocellular carcinoma.[2,13-16] This natural history is reflected in the increasing incidence of HCC paralleling the epidemic of obesity in the United States, suggesting that NAFLD is a key factor linking obesity and HCC.[17-20]

Moreover, increasing reports of HCC arising in non-cirrhotic patients with NAFLD raise the possibility that carcinogenesis occurs in NAFLD even in the absence of advanced liver disease or cirrhosis. Although uncommon, these cases show that cirrhosis is not necessary for the progression to HCC in patients with NAFLD. In this regard, data concerning contributory risk factors for its development have also become subjects of discussion. Obesity and diabetes are the two most prevalent risk factors mentioned in the literature that are associated with HCC in the background of NAFLD.[21-22]

Several hypotheses have been proposed in previous reports for the mechanisms of NAFLD to HCC, particularly in pre-cirrhotic stages. Malignant transformation of HCA is one suggested pathway and has been reported with a frequency of 4.2%.[23] HCA, though usually benign, represents a monoclonal tumor proliferation that has an inherent risk of undergoing malignant changes, more or less depending on the recently defined subtype.[24] Among the different subtypes, HCA with β-catenin mutation and inflammatory HCA are the subgroups most associated with malignant progression.[2,25] It is the latter form that has been presented as a precursor lesion to HCC in some pre-cirrhotic NAFLD cases.[2]

Some investigators suggest that the prolonged exposure of hepatocytes to the toxic effects of accumulated fats is the culprit.[7,26] These studies suggest that non-esterified free fatty acids (FFA) induce lipoapoptosis via the activation of the c-Jun N-terminal kinase (JNK) signaling pathway.[27-29] The activation of the JNK pathways plays a pivotal role in the succeeding molecular events leading to hepatocyte apoptosis. Thus, it can be said that circulating levels of FFA correlates with liver disease severity as well as with molecular events increasing the likelihood of hepatocyte malignant transformation.

Tumor suppressor genes have also been implicated by some authors who suggest that p53, promyelocytic leukemia and PTEN genes play an important role in the development of steatosis and steatosis-induced liver cell damage.[6-7] Another potential mechanism relates to dysregulated bile acid metabolism in NAFLD which has been reported to induce apoptosis and may promote HCC in the setting of hepatic steatosis.[8]

Our cases now suggest yet another pathway for the emergence of malignancy in non-cirrhotic NAFLD. Combined HCC-ChC (with or without stem cell features) accounts for <1% of all liver carcinomas.[9] Even without the histologic and immunophenotypic demonstration of a cell compartment with stem cell features, most combined HCC-ChC are recognized as the result of malignant transformation of a bipotent hepatobiliary stem cell.[10] The finding of combined HCC-ChC with stem cell features in the background of non-cirrhotic NAFLD raises the possibility that some hepatobiliary malignancies in this disease may arise through the activation and malignant transformation of liver stem/progenitor cells in that setting.

The development of steatohepatitis and the inflammatory cascade are likely to provide the clues to the carcinogenic potential of fatty liver disease.[1] The accumulation of free fatty acids potentiates the vulnerability of the liver to the byproducts of inflammation. Increase fatty acids propels the cytochrome P4502EI producing reactive oxygen species (ROS) and lipid peroxidation.[30] Overproduction of these molecules depletes the anti-oxidant mechanisms causing cellular injury and oxidative stress that may lead to hepatic hyperplasia. Contributory to the inflammatory cascade and hepatocyte apoptosis is the activation of JNK1, a protein kinase also activated by ROS and free fatty acid accumulation.[1] ROS together with sustained activation of JNK1 may bring about hyperplasia[31-32] and increase in several genes important for hepatic proliferation.[33] Furthermore, IL-6, which is markedly elevated in NAFLD, is also a potent promoter of hepatocarcinogenesis.[34-35]

Steatosis via the ROS and toxin production also affects the liver's inherent replicative capacity, causing its arrest leading to hepatocellular senescence.[36] In effect, it thereby also stimulates hepatocellular stem/progenitor cell expansion – creating the ductular reaction – which by default becomes the source of regenerating hepatocytes.[37] If the mutational events take place within the stem cell compartment directly or within their transit amplifying progeny, hepatobiliary progenitors in the ductular reaction, this pathway can lead to emergence of a combined HCC-CHC. The steatosis, thus, in some patients, may act as catalyst, through production of the above described molecules and cell/tissue reactions necessary to stimulate both stem cell activation, exposure of such cells to mutagenic events, and ultimately tumorigenesis.

CONCLUSION

In conclusion, we present these two cases of combined hepatocellular-cholangiocarcinoma, one with overt stem cell features, as evidence for malignant transformation of hepatic stem/progenitor cells in the setting of NAFLD as one pathway of development, even when established cirrhosis is not present. These findings further underscore the increasing concern regarding the development of malignancies in non-cirrhotic NAFLD, inclusive of combined HCC-CHC in which the CHC component may indicate a particularly poor prognosis.

AUTHOR DISCLOSURE

The authors declared no conflicts of interest.

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External Quality Assessment Scheme for Transfusion Transmissible Infections among Blood Service Facilities in the Philippines, 2015

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ABSTRACT

The External Quality Assessment Scheme (EQAS) for Blood Screening Serology aims to raise standards and assess the phases of laboratory testing of blood units.

In 2015, the National Blood Program listed a total of 200 Blood Service Facilities (BSF), 147 of which, enrolled for EQAS. These participants were given an EQAS panel designed to check the capacity of a BSF to detect the 5 transfusion transmitted infections (HIV, HBV, HCV, Syphilis and Malaria). Panels should be tested how a blood unit is routinely screened to mimic the actual laboratory process. This allows the NRL and participant to check and validate the entire blood unit screening process.

Test results were analyzed by OASYS Canada using the ISO 13528:2005 Robust Statistics method (Huber's Method) to identify outliers. Data analysis from the test event showed a significant number of participants that reported aberrant results due to errors related to random or systematic errors. This also showed deviations from standard practice recommended by the Department of Health as well as a comparison of different test platforms for blood screening.

Ultimately, the data gathered from the EQAS are used to improve on policies for blood screening and set recommendations for the safety of the Philippine blood supply.

Key words: blood transfusions, human immunodeficiency virus, hepatitis, syphilis, malaria, external quality assurance scheme, transfusion transmissible infections

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INTRODUCTION

The EQAS for Blood Screening Serology provided by the Transfusion Transmissible Infections – National Reference Laboratory (TTI-NRL) of the Research Institute for Tropical Medicine aims to raise standards and assess the phases of laboratory testing on blood units to determine inter-laboratory comparison. The NRL-Australia cites the importance of EQAS as this provides objective evidence of quality through its capability to: (1) review kit and assay performance through monitoring consistency and accuracy of test results, (2) check on lab performance through comparison of different laboratory data, (3) identify random and systematic errors that needs to be managed, and (4) identification of laboratory's training needs.[1]

The EQAS panel is designed to check the capacity of a Blood Screening Facility (BSF) to detect the 5 common TTI. Samples of known reactivity to HIV, HBV, HCV, Syphilis and Malaria are to be tested in the same way as how a blood is routinely screened in the BSF as this mimics the routine samples received and screened. This allows the NRL and the participant to check and validate the blood unit screening process from receipt of samples up to release of results. As stated in DOH Department Circular No. 2013-0132, blood screening for TTI should only be done by licensed HIV proficient Medical Technologist and that all BSF are required to enroll for EQAS as per DOH Department Memorandum No. 2009-0086B.

METHODOLOGY

Panel Composition

The serology EQAS panel for program code HVHT4320 consists of twenty (20) pooled plasma samples obtained from blood donors from different regions of the country. Each pooled sample was prepared by mixing similar volumes of at least two samples that had similar antibody and antigen profiles. All samples were subjected to filtration prior to aliquoting. The samples were aliquoted and their homogeneity confirmed. Representative samples were tested following shipment to participants to confirm their stability. The serology profile for HIV, HBV, HCV, Syphilis of each sample were identified using Chemiluminiscensce (ChLIA), Enzyme Immunoassay (EIA), Rapid Plasma Reagin (RPR), Particle Agglutination (PA) and Western Blot (WB).

Program code MLRA415 consists of five (5) blood smears. The samples were obtained from Malaria patients in Palawan and prepared by the NRL for Malaria and other Parasites of the Research Institute for Tropical Medicine.

Participants

The Multimarker Blood Serology EQAS panel ID HVHT4320 and Malaria Microscopy EQAS Panel ID MLRA415 were distributed to 147 participants nationwide. These participants enrolled for the EQAS 2015 Program with a corresponding registration fee to cover expenses for the test event.

Majority of the participants are private institutions followed closely by government institutions and the remainders are from the Philippine Red Cross. Figure 1 shows the distribution of BSF by region.

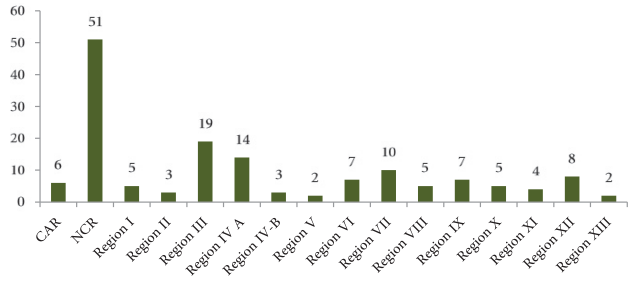

Figure 1. Regional distribution of participants.

Data Analysis

For data analysis, the TTI-NRL made use of the online informatics system (OASYS) developed and operated by Oneworld Accuracy Systems, Canada.

Participants were asked to enter assay results as well as assay interpretations in the online informatics system. Results reported by participating BSF for assay interpretations and final status were compared with the relevant reference results for qualitative evaluation. An assay interpretation that is different from the reference result is marked as aberrant.

The test results from the participating BSF were also sorted into peer groups. A peer group is defined as a set of laboratories that utilize the same test format and test kit/assay for screening TTI. The ISO 13528:2005 Robust Statistics method (Huber's Method) was used to identify outlying results (numerical test results found to be statistically different from other test results reported by participants that tested the same sample in the same assay) for the created peer groups. The said method uses the mean as an estimator. Outlying test results were removed from statistical calculation.[2]

RESULTS AND DISCUSSION

The predominant testing platform used by most participants was ChLIA followed by EIA, which is in concordance with what is being recommended by DOH DC No. 2013-0132. A significant number of participants are also using Rapid Diagnostic Test (RDT) kits for screening, which is not recommended for blood screening for the reason that these tests are often not as sensitive as EIA or instrument-based tests and can lead to false negative results in samples with low titres.[3]

One participant used an expired reagent for testing one analyte and 10 participants failed to indicate the expiry dates of their assay reagent kits. Six participants were identified to have reported results that were due to data entry error or clerical error (e.g. reactive test results were interpreted as negative or vice versa).

For the HVHT4320 serology panel, 19% of 147 BSF reported aberrant results. Out of the 11,760 total number of results entered by the BSF, 11,722 (99.68%) were correctly identified and 38 (0.32%) were marked as aberrant. Out of the 0.32% aberrant results, 21 (0.18%) and 17 (0.14%) results were reported as false negative and false reactive respectively (Figure 2). Distribution of platform per TTI among aberrant results for the initial panel is shown in Table 1. These aberrant results were either due to data entry errors, sample mix-up or sample carry-over (particularly where an instrument was used in assay set-up).

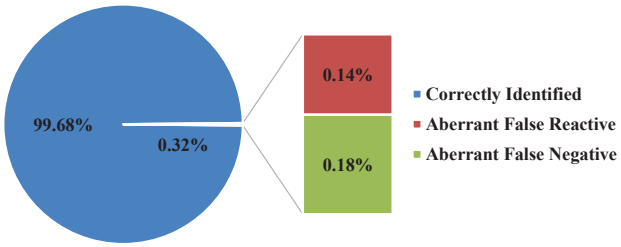

Figure 2. Percentage of aberrant results for HVHT4320 1st panel.

In testing the HVHT4320 initial panel, these criteria must be met for a BSF to be classified as having an unsatisfactory performance: (a) At least one false negative result; (b) At least twenty percent (20%) false positive results. In accordance with these criteria, corresponding BSF, were given an investigation checklist to assist them in identifying their errors and make the necessary

Table 1. Number of aberrant results per transfusion transmissible infections testing platform (HVHT4320 1st Panel)

Platform	HIV		HBV		HCV		Syphilis		Total
	False Negative	False Reactive	False Negative	False Reactive	False Negative	False Reactive	False Negative	False Reactive	Aberrant
ChLIA	3 (7.89%)	2 (5.26%)	3 (7.89%)	4 (10.53%)	2 (5.26%)	2 (5.26%)	0 (0%)	1 (2.63%)	17 (44.74%)
EIA	1 (2.63%)	0 (0%)	1 (2.63%)	4 (10.53%)	2 (5.26%)	3 (7.89%)	1 (2.63%)	0 (0.00%)	12 (31.58%)
RDT	0 (0%)	0 (0%)	5 (13.16%)	0 (0%)	0 (0%)	1 (2.63%)	2 (5.26%)	0 (0%)	8 (21.05%)
RPR	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	1 (2.63%)	0 (0%)	1 (2.63%)
Total Aberrant	4 (10.53%)	2 (5.26%)	9 (23.68%)	8 (21.05%)	4 (10.53%)	6 (15.79%)	4 (10.53%)	1 (2.63%)	38 (100.00%)

corrective actions and/or troubleshooting methods. A 2nd set of the HVHT4320 panel were given to the BSFs for retesting if the identified unsatisfactory performance was due to a testing error. BSFs with aberrant results due to transcription errors were only given an investigation/troubleshooting checklist and a written recommendation. Five (5) BSFs were identified with transcription errors. Six (6) BSFs were given a second set of samples where only 3 were able to report each assay interpretation correctly.

Due to lack of accessibility to a good amount of inexpensive positive malaria blood samples, the NRL opted to provide a set of Blood Smears of known Malaria status to assess the capacity of the BSF to detect the presence of malaria parasite *(qualitative identification only indicating presence or absence of the parasite)*. Although most, if not all, of the BSF perform platforms such as EIA and RDT for Malaria testing, it must be noted that according to DOH DC No. 2013-0132, a BSF should have the minimum capacity to detect the presence of malaria parasite through its gold standard, Malaria Microscopy. Presently, Malaria EIA kits in the country are neither evaluated nor regulated. The NRL for Malaria and other Parasites is in the process of evaluating these kits in partnership with the TTI-NRL. The microscopic diagnosis technique remains the gold standard for laboratory confirmation of malaria.[4]

For the MLRA415 panel, 12% of participants reported aberrant results and out of these, 9% reported false detection of human Plasmodia and 3% reported having false negative slides. This may be attributed to the fact that technicians are not proficient in reading malaria smears.

Figure 3 shows the distribution of grades of the BSFs. BSFs were evaluated and graded as follows:

- Excellent – 100% acceptable results on the initial panel (all final results were correctly identified in comparison with the reference results);
- Very Satisfactory – Less than 100% acceptable results on the initial panel without being given a second panel for retesting. (A second panel is given to the BSF if upon comparison with the TTI-NRL reference result, there is at least one false negative or at least 20% false positive results reported);
- Satisfactory – 100% acceptable results on retesting of second panel (all final results are correctly identified in comparison with the reference result); or had an aberrant result in the initial panel due to clerical error (provided that the BSF identified the clerical error upon run through of the EQAS Investigation Checklist);
- Poor – BSF did not follow minimum requirements of testing as per DOH – DC 2013-0132 or; less than 100% acceptable results on retesting of second panel (in comparison with the reference result, there is at least one false negative or at least 10% false positive results reported); or had an aberrant result in the initial panel that is due to clerical error which the BSF failed to identify upon run through of the EQAS Investigation Checklist.

According to DOH Memorandum 2009-0086B, EQA Participation and Proficiency testing with 2 or more consecutive failures, unsatisfactory, unacceptable results shall comply with the guidelines of the respective NRL. As an added quality assurance activity, the TTI-NRL conducts site-visits and assessment to BSF that attained satisfactory results and below. A detailed summary report and necessary recommendations are given to the BSF and the DOH for necessary actions.

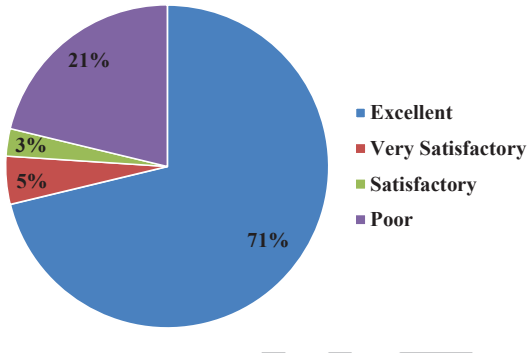

Figure 3. Distribution of grades for the EQAS 2015 test event.

CONCLUSION

Since the DC No. 2013-0132 was implemented in 2013, some BSFs still do not acknowledge or comply with the recommendations provided therein. Stringent measures should be enforced by each BSF for the safety of our national blood supply.

It is recommended that BSFs check and monitor their testing performance to identify aberrant results and perform appropriate corrective actions. The use of assay test kits evaluated by the STD/AIDS Cooperative Central Laboratory (SACCL) and recommended by the National Blood Program, adherence to the manufacturer's protocols, strict internal quality control procedures and critical supervisory review are measures to avoid technical deficiencies. A second person should also check the assay results independently prior to reporting as this can resolve data entry errors.

The testing staff of the BSFs must be theoretically and technically proficient in testing for transfusion transmissible infections as this increases the competence most especially in correlation of test results as well as proper identification of Malaria parasites through intensive Malaria Microscopy Training and Proficiency Testing of Transfusion Transmissible Infections.

The use of RDT kits for blood screening is of inadequate sensitivity compared to Enzyme Immunoassay or instrument-based tests. This can lead to false negative results in samples with low levels of the transfusion transmissible infection. BSFs using two platforms for screening are encouraged not to retest samples on kits of low specificity/sensitivity (e.g. initial screening on EIA or CLIA and retesting on RDT).

The Blood Screening Serology EQAS plays a vital role in the improvement of efficiency of BSFs that ultimately improves the overall quality of the National Blood Program. Active participation of BSFs in this EQAS program will positively strengthen the quality of their service as there will always be room for improvement and development in this system.[5]

AUTHOR DISCLOSURE

The authors declared no conflicts of interest.

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We also thank all participating Blood Service Facilities for their cooperation.

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Oral Verrucous Carcinoma

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Key words: verrucous carcinoma, Ackerman's tumor, oral cavity

A 34-year-old Indian male presented with a white, painless growth on the upper posterior region of the oral cavity since 6 months. Patient had a history of chewing betel quid *(a combination of betel leaf, areca extract and lime)* since 8 years, 7-8 times/day in the lower right buccal vestibule for 10 minutes before spitting them out. Intra-oral examination revealed a proliferative, verruco-papillary growth on the left maxillary alveolar gingiva extending to the palate. The lesion was approximately 3x4 cm in size, well defined with irregular margins (Figure 1). On the basis of clinical features a provisional diagnosis of proliferative verrucous leukoplakia (PVL) was given. Incisional biopsy of the lesion was taken and excised tissue was sent for histopathological examination.

Histopathological examination revealed stratified squamous parakeratinized epithelium with broad acanthotic, elephant foot like rate ridges growing down into the stroma (Figure 2). Numerous cleft like spaces were seen, filled with parakeratin (Figure 3). The final diagnosis of oral verrucous carcinoma was made. Surgical excision of the lesion was done and six months follow up period of the patient was uneventful.

Oral verrucous carcinoma (OVC), a variant of squamous cell carcinoma (SCC), was first described by Lauren V. Ackerman in 1948.[1] OVC has a predilection for male in the sixth decade, with a slow growth rate, and with potential to become invasive if not treated properly. Distant metastasis is rare.[2]

In most cases, verrucous carcinoma, verrucous hyperplasia and proliferative verrucous leukoplakia are clinically indistinguishable from each other so histopathological evidence is necessary to render an appropriate diagnosis. Deeper sections and complete sampling are required not just to distinguish verrucous lesion in general, but to rule out the presence of concomitant conventional squamous cell carcinoma and hybrid squamous cell carcinoma in the sample. The differentiation of verrucous carcinoma with other verruco-papillary benign and malignant processes is difficult although it can be differentiated with keratinizing squamous cell carcinoma on the basis of characteristic histological features[3] (Table 1). The best treatment modality of OVC is surgical resection of the tumor.[4]

Table 1. Histopathological differences between squamous cell carcinoma and verrucous carcinoma

Verrucous carcinoma	Squamous cell carcinoma
Histopathological features	Histopathological features
Epithelium seldom shows dysplastic features.	Epithelium shows high dysplasia.
Elephant foot like rete ridges is seen.	Elephant foot like rete ridges is not seen.
Parakeratin plugging is present.	Parakeratin plugging is usually absent.
Keratin pearls are not seen.	Keratin pearls are seen.
Breach in the basement membrane is absent.	Breach in the basement membrane is present.
Islands of dysplastic epithelium are not seen in the connective tissue.	Islands of dysplastic epithelium are seen in the connective tissue.

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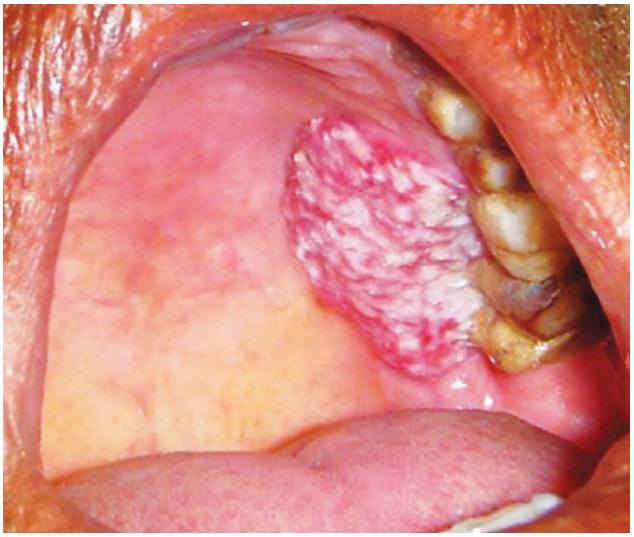

Figure 1. Clinical appearance of the lesion.

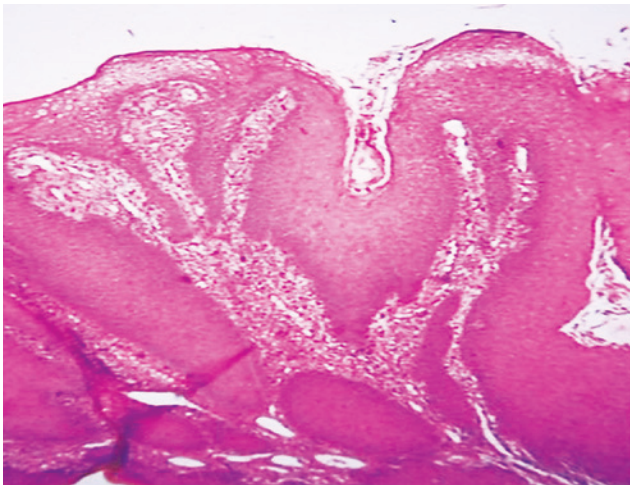

Figure 2. Broad elephant foot like rete ridges and underlying connective tissue stroma. (200x, H&E).

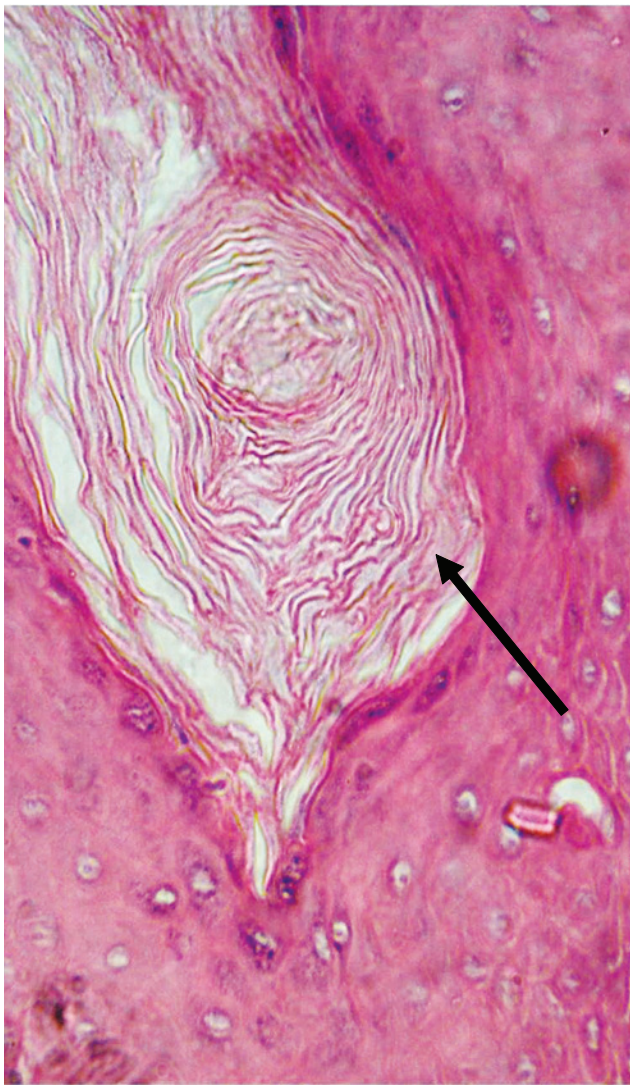

Figure 3. Parakeratin plugging (400x, H&E).

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ICMJE INTERNATIONAL COMMITTEE of MEDICAL JOURNAL EDITORS

Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals

Updated December 2015

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.html). 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 http://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 via e-mail at icmje@acponline.org. 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 development of a number of Separate Statements on editorial policy. The entire Uniform Requirements document was revised in 1997; sections were updated in May 1999 and May 2000. In May 2001, the ICMJE revised the sections related to potential conflicts of interest. In 2003, the committee revised and reorganized the entire document and incorporated the Separate Statements into the text, and revised it again in 2010. Previous versions of this document can be found in the "Archives" section of www.icmje .org. Now renamed "Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals" (ICMJE Recommendations), the document was revised in 2013, 2014, and the current version in 2015.

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. 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. 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, and typically ensures that all the journal's administrative requirements, such as providing details of authorship, ethics committee approval, clinical trial registration documentation, and gathering conflict of interest forms and statements, are properly completed, although these duties may be delegated to one or more coauthors. 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 conflict-of-interest 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.

3. Non-Author Contributors

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").

Because acknowledgment may imply endorsement by acknowledged individuals of a study's data and conclusions, editors are advised to require that the corresponding author obtain written permission to be acknowledged from all acknowledged individuals.

B. Author Responsibilities—Conflicts of Interest

Public trust in the scientific process and the credibility of published articles depend in part on how transparently conflicts of interest are handled during the planning, implementation, writing, peer review, editing, and publication of scientific work.

A conflict of interest 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.

Financial relationships (such as employment, consultancies, stock ownership or options, honoraria, patents, and paid expert testimony) are the most easily identifiable conflicts of interest and the most likely to undermine the credibility of the journal, the authors, and of science itself. However, conflicts can occur for other reasons, such as personal relationships or rivalries, academic competition, and intellectual beliefs. Authors should avoid entering in to 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.

1. Participants

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 their conflicts of interest when fulfilling their roles in the process of article review and publication and must disclose all relationships that could be viewed as potential conflicts of interest.

a. Authors

When authors submit a manuscript of any type or format they are responsible for disclosing all financial and personal relationships that might bias or be seen to bias their work. The ICMJE has developed a Form for Disclosure of Conflicts of Interest 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 conflicts of interest that could complicate their review. Reviewers must disclose to editors any conflicts of interest 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 conflicts of interest or relationships 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 financial interests or other conflicts (as they might relate to editorial judgments) and recuse themselves from any decisions in which a conflict of interest exists. Editorial staff must not use information gained through working with manuscripts for private gain. Editors should publish regular disclosure statements about potential conflicts of interests related to the commitments of journal staff. Guest editors should follow these same procedures.

2. Reporting Conflicts of Interest

Articles should be published with statements or supporting documents, such as the ICMJE conflict of interest form, declaring:

– Authors' conflicts of interest; 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; the decision to submit the report for publication; or a statement declaring that the supporting source had no such involvement; and

– Whether the authors had access to the study data, with an explanation of the nature and extent of access, including whether access is on-going.

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 conflicts of interest detailed in section IIA and B of this document. A growing number of entities are advertising themselves as "medical journals" yet do not function as such ("predatory journals"). Authors should be aware of the integrity, history, practices and reputation of the journals to which they submit manuscripts. Further guidance is available at http://www.wame.org/about/principles-of-transparency-and-best-practice.

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 conflicts of interest.

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 description of their peer-review process.

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 posting in a place which 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.

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 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 conflicts of interest 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 (eg, a sponsoring society).

2. Editorial Freedom

The ICMJE adopts the World Association of Medical Editors' definition of editorial freedom, 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 editorial advisory board to help the editor establish and maintain editorial policy. Editors should seek input as needed from a broad array of advisers, such as reviewers, editorial staff, an editorial board, and readers, to support editorial decisions and potentially controversial expressions of opinion, and 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

When reporting research involving human data, authors should indicate whether the procedures followed have been assessed by the responsible review committee (institutional and national), or if no formal ethics committee is available, were in accordance with the Helsinki Declaration as revised in 2013 (www.wma.net/en/30publications/10policies/b3/index.html). 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 institutional review body explicitly approved the doubtful aspects of the study. Approval by a responsible review committee 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 de-identified, 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. Further guidance on animal research ethics is available from the International Association of Veterinary Editors' Consensus Author Guidelines on Animal Ethics and Welfare (http://veteditors.org/ethicsconsensusguidelines.html).

III. Publishing and Editorial Issues Related to Publication in Medical Journals

A. Corrections 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.

Errors serious enough to invalidate a paper's results and conclusions may require retraction.

B. Scientific Misconduct, Expressions of Concern, and Retraction

Scientific misconduct includes but is not necessarily limited to data fabrication; data falsification including deceptive manipulation of images; 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 such as the Committee on Publication Ethics (COPE) (publicationethics.org/resources/flowcharts) 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 retrac-

tion 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.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 (for example, 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 or an abstract or poster displayed at a scientific meeting. 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.

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.

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 patients en-

rolled, 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. 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 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 (for example, 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).

4. Manuscripts Based on the Same Database

If editors receive manuscripts from separate research groups or from the same group analyzing the same data set (for example, 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 dataset should add substantially to each other to warrant consideration for publication as separate papers, with appropriate citation of previous publications from the same dataset 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.

Sometimes for large trials it is planned from the beginning to produce numerous separate publications regarding separate research questions but using the same original patient 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 or conflicting interests.

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 potential conflicts of interest 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 believe 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 Trial Registration

The ICMJE's clinical trial registration policy is detailed in a series of editorials (see Updates and Editorials [www.icmje.org/update.html] and FAQs [www.icmje.org/faq_clinical.html]).

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 [icmje.org/journals.html] should recognize that the listing implies enforcement by the journal of ICMJE's trial registration policy.

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 cause-and-effect 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, behavioural 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 patient enrollment, but best practice dictates registration by the time of first patient consent.

The ICMJE accepts registration in any registry that is a primary register of the WHO International Clinical Trials Registry Platform (ICTRP) (www.who.int/ictrp/network/primary/en/index.html) 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 20-item trial registration dataset (http://prsinfo.clinicaltrials.gov/trainTrainer/WHO-ICMJE-ClinTrialsgov-Cross-Ref.pdf or www.who.int/ictrp/network/trds/en/index.html) at the time of registration and before enrollment of the first participant. The ICMJE considers inadequate trial registrations missing any of the 20 data fields or those that have fields that contain uninformative information. 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 encourages posting of clinical trial results in clinical trial registries but does not require it. 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 patients enrolled, key 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. If an exception to prospective registration 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.

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, cross-linking, 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 (www.stard-statement.org/). 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.

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.

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.

Conflict of Interest declaration. Conflict of interest 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 conflict of interest 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 conflict of interest declarations on the manuscript title page 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, authors should state at the end of the abstract the data set name, 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 or exempted from the need for review by the responsible review committee (institutional or national). If no formal ethics committee is available, a statement indicating that the research was conducted according to the principles of the Declaration of Helsinki should be included.

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. 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 measured race or ethnicity and justify their relevance.

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 (for example, percentages) but also as the absolute numbers from which the derivatives were calculated, and specify the statistical significance attached to them, if any. 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. 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. 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.

Do not use conference abstracts as references: they can be cited in the text, in parentheses, but not as page footnotes. 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.

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 (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 International Committee of Medical Journal Editors (ICMJE) Recommendations for the Conduct, Re-

porting, Editing and Publication of Scholarly Work in Medical Journals: 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)

Digital images of manuscript illustrations should be submitted in a suitable format for print publication. Most submission systems have detailed instructions on the quality of images and check them after manuscript upload. For print submissions, figures should be either professionally drawn and photographed, or submitted as photographic-quality digital prints.

For X-ray films, scans, and other diagnostic images, as well as pictures of pathology specimens or photomicrographs, send high-resolution photographic image files. Since blots are used as primary evidence in many scientific articles, editors may require deposition of the original photographs of blots on the journal's website.

Although some journals redraw figures, many do not. Letters, numbers, and symbols on figures should therefore be clear and consistent throughout, and large enough to remain legible when the figure is reduced for publication. Figures should be made as self-explanatory as possible, since many will be used directly in slide presentations. Titles and detailed explanations belong in the legends—not on the illustrations themselves.

Photomicrographs should have internal scale markers. Symbols, arrows, or letters used in photomicrographs should contrast with the background. Explain the internal scale and identify the method of staining in photomicrographs.

Figures should be numbered consecutively according to the order in which they have been cited in the text. If a figure has been published previously, acknowledge the original source and submit written permission from the copyright holder to reproduce it. Permission is required irrespective of authorship or publisher except for documents in the public domain.

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Measurements of length, height, weight, and volume should be reported in metric units (meter, kilogram, or liter) or their decimal multiples.

Temperatures should be in degrees Celsius. Blood pressures should be in millimeters of mercury, unless other units are specifically required by the journal.

Journals vary in the units they use for reporting hematologic, clinical chemistry, and other measurements. Authors must consult the Information for Authors of the particular journal and should report laboratory information in both local and International System of Units (SI).

Editors may request that authors add alternative or non-SI units, since SI units are not universally used. Drug concentrations may be reported in either SI or mass units, but the alternative should be provided in parentheses where appropriate.

k. Abbreviations and Symbols

Use only standard abbreviations; use of nonstandard abbreviations can be confusing to readers. Avoid abbreviations in the title of the manuscript. The spelled-out abbreviation followed by the abbreviation in parenthesis should

be used on first mention unless the abbreviation is a standard unit of measurement.

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Manuscripts should be accompanied by a cover letter or a completed journal submission form, which should include the following information:

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A statement of financial or other relationships that might lead to a conflict of interest, if that information is not included in the manuscript itself or in an authors' form. See also Section II.B.

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Contact information for the author responsible for communicating with other authors about revisions and final approval of the proofs, if that information is not included in the manuscript itself.

The letter or form should inform editors if concerns have been raised (e.g., via institutional and/or regulatory bodies) regarding the conduct of the research or if corrective action has been recommended. The letter or form should give any additional information that may be helpful to the editor, such as the type or format of article in the particular journal that the manuscript represents. If the manuscript has been submitted previously to another journal, it is helpful to include the previous editor's and reviewers' comments with the submitted manuscript, along with the authors' responses to those comments. Editors encourage authors to submit these previous communications. Doing so may expedite the review process and encourages transparency and sharing of expertise.

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The manuscript must be accompanied by permission to reproduce previously published material, use previously published illustrations, report information about identifiable persons, or to acknowledge people for their contributions.

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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.

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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.

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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.

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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 (PJP-2015-AT-001)** must be used.

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For submissions to the PJP to be accepted, all authors must read and sign the **PJP Author Form (PJP-2015-AF-001)** 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.

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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 (IMCJE 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.
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- Journal abbreviations should conform to those used in PubMed.
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- 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 or .gif files).
- For photomicrographs, the stain used (e.g. H & E) and magnification (e.g. X400) 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.
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- 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.

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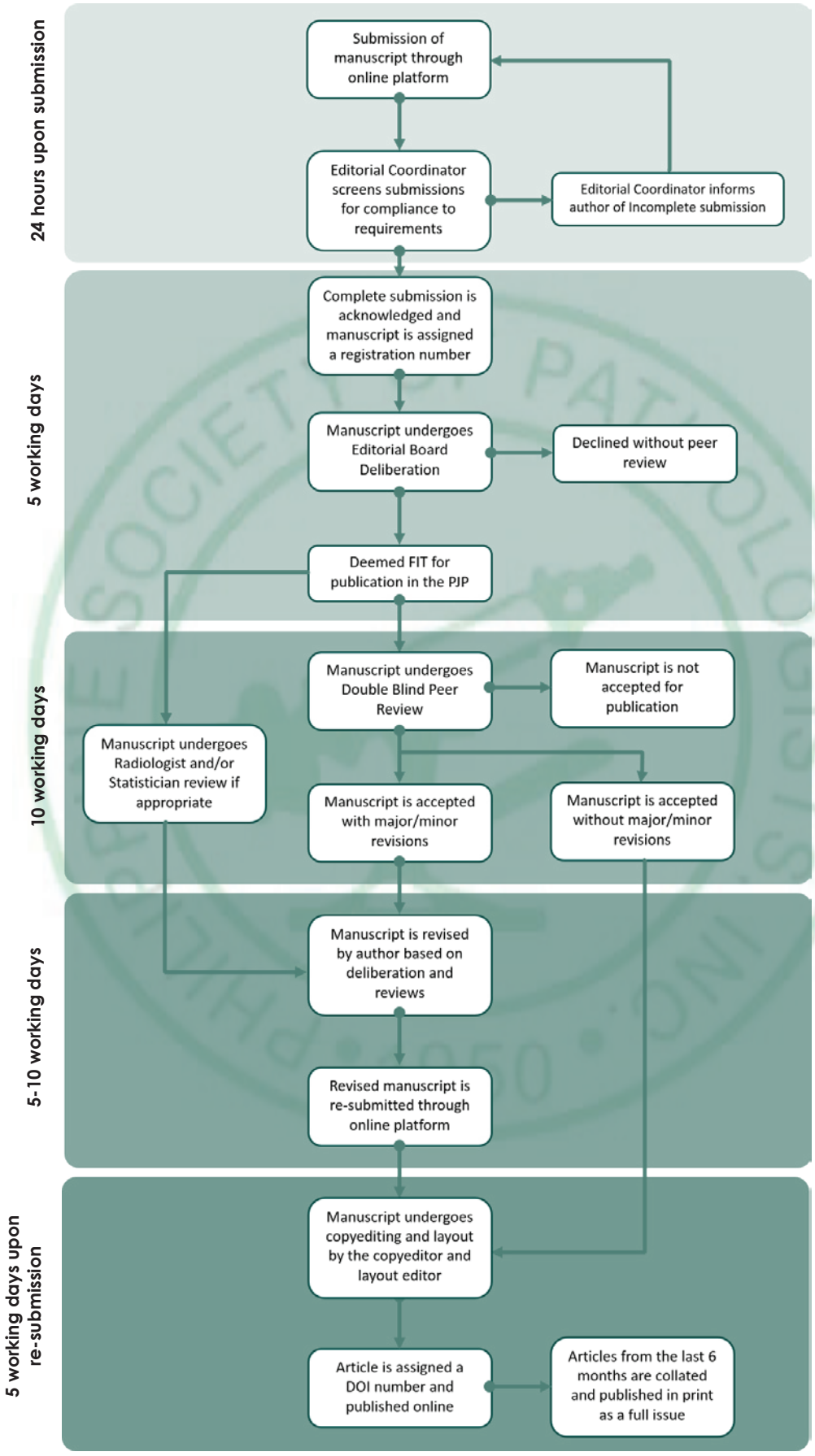

Figure 1. Editorial Process Flow.

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COMPLETE TITLE OF MANUSCRIPT

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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.

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☐ 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.

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

Author Name	Signature	Date (MM/DD/YYYY)

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For case report and image submissions to the PJP to be accepted, the author/s must ensure that patients or patients□legal guardian/relative have provided informed consent to publish information about them in the journal. The completely accomplished PJP Patient Consent Form shall be scanned and submitted along with the manuscript. No case report and image shall be received without the PJP Consent Form.

Name of person described in article or shown in photograph:_____

_____

Subject matter of photograph or article (brief description):

_____

_____

_____

(The Subject matter of the photograph or article is hereafter termed as the "INFORMATION.")

Title of article:

_____

_____

I, _____ , give my consent for this information
[please insert your full name]

about MYSELF/MY CHILD OR WARD/MY RELATIVE relating to the subject matter
[please underline correct description]

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Witness:

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Consolidated criteria for reporting qualitative research (COREQ): A 32-item checklist for interviews and focus groups

No	Item	Guide questions / description
DOMAIN 1: RESEARCH TEAM AND REFLEXIVITY		
Personal Characteristics		
1	Interviewer/facilitator	Which author/s conducted the interview or focus group?
2	Credentials	What were the researcher's credentials? E.g. PhD, MD
3	Occupation	What was their occupation at the time of the study?
4	Gender	Was the researcher male or female?
5	Experience and training	What experience or training did the researcher have?
Relationship with participants		
6	Relationship	Was a relationship established prior to study commencement?
7	Participant knowledge of the interviewer	What did the participants know about the researcher? e.g. personal goals, reasons for doing the research
8	Interviewer characteristics	What characteristics were reported about the interviewer/facilitator? e.g. Bias, assumptions, reasons and interests in the research topic
DOMAIN 2: STUDY DESIGN		
Theoretical framework		
9	Methodological orientation and Theory	What methodological orientation was stated to underpin the study? e.g. grounded theory, discourse analysis, ethnography, phenomenology, content analysis
Participant selection		
10	Sampling	How were participants selected? e.g. purposive, convenience, consecutive, snowball
11	Method of approach	How were participants approached? e.g. face-to-face, telephone, mail, email
12	Sample size	How many participants were in the study?
13	Non-participation	How many people refused to participate or dropped out? Reasons?
Setting		
14	Setting of data collection	Where was the data collected? e.g. home, clinic, workplace
15	Presence of non-participants	Was anyone else present besides the participants and researchers?
16	Description of sample	What are the important characteristics of the sample? e.g. demographic data, date
Data Collection		
17	Interview guide	Were questions, prompts, guides provided by the authors? Was it pilot tested?
18	Repeat interview	Were repeat interviews carried out? If yes, how many?
19	Audio/visual recording	Did the research use audio or visual recording to collect the data?
20	Field notes	Were field notes made during and/or after the interview or focus group?
21	Duration	What was the duration of the interviews or focus group?
22	Data saturation	Was data saturation discussed?
23	Transcripts returned	Were transcripts returned to participants for comment and/or correction?
DOMAIN 3: ANALYSIS AND FINDINGS		
Data analysis		
24	Number of data coders	How many data coders coded the data?
25	Description of the coding tree	Did authors provide a description of the coding tree?
26	Derivation of themes	Were themes identified in advance or derived from the data?
27	Software	What software, if applicable, was used to manage the data?
28	Participant checking	Did participants provide feedback on the findings?
Reporting		
29	Quotations presented	Were participant quotations presented to illustrate the themes / findings? Was each quotation identified? e.g. participant number
30	Data and findings consistent	Was there consistency between the data presented and the findings?
31	Clarity of major themes	Were major themes clearly presented in the findings?
32	Clarity of minor themes	Is there a description of diverse cases or discussion of minor themes?

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CARE Checklist (2013) of Information to include when Writing a Case Report

Topic	Item no.	Checklist item description	Reported on page no.
Title	1	The words "case report" should be in the title along with the area of focus	_____
Key Words	2	2 to 5 key words that identify areas covered in this case report	_____
Abstract	3a	Introduction—What is unique about this case? What does it add to the medical literature?	_____
	3b	The main symptoms of the patient and the important clinical findings	_____
	3c	The main diagnoses, therapeutics interventions, and outcomes	_____
	3d	Conclusion—What are the main "take-away" lessons from this case?	_____
Introduction	4	One or two paragraphs summarizing why this case is unique with references	_____
Patient Information	5a	De-identified demographic information and other patient specific information	_____
	5b	Main concerns and symptoms of the patient	_____
	5c	Medical, family, and psychosocial history including relevant genetic information	_____
		(also see timeline)	
	5d	Relevant past interventions and their outcomes	_____
Clinical Findings	6	Describe the relevant physical examination (PE) and other significant clinical findings	_____
Timeline	7	Important information from the patient's history organized as a timeline	_____
Diagnostic Assessment	8a	Diagnostic methods (such as PE, laboratory testing, imaging, surveys)	_____
	8b	Diagnostic challenges (such as access, financial, or cultural)	_____
	8c	Diagnostic reasoning including other diagnoses considered	_____
	8d	Prognostic characteristics (such as staging in oncology) where applicable	_____
Therapeutic Intervention	9a	Types of intervention (such as pharmacologic, surgical, preventive, self-care)	_____
	9b	Administration of intervention (such as dosage, strength, duration)	_____
	9c	Changes in intervention (with rationale)	_____
Follow-up and Outcomes	10a	Clinician and patient-assessed outcomes (when appropriate)	_____
	10b	Important follow-up diagnostic and other test results	_____
	10c	Intervention adherence and tolerability (How was this assessed?)	_____
	10d	Adverse and unanticipated events .	_____
Discussion	11a	Discussion of the strengths and limitations in your approach to this case	_____
	11b	Discussion of the relevant medical literature	_____
	11c	The rationale for conclusions (including assessment of possible causes)	_____
	11d	The primary "take-away" lessons of this case report	_____
Patient Perspective	12	When appropriate the patient should share their perspective on the treatments they received	_____
Informed Consent	13	Did the patient give informed consent? Please provide if requested	☐ Yes ☐ No

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PRISMA 2009 Checklist of Preferred Reporting Items for Systematic Reviews and Meta-Analyses

Section / Topic	Item no.	Checklist item	Reported on page no.
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	_____
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	_____
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	_____
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	_____
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	_____
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	_____
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	_____
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	_____
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	_____
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	_____
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	_____
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	_____
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	_____
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	_____
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	_____
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	_____
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	_____
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	_____
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	_____
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	_____
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	_____
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	_____
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	_____
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	_____
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	_____
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	_____
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	_____

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097. For more information, visit: www.prisma-statement.org.

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STROBE Statement - Checklist of Items that should be included in Reports of Observational Studies

Section / Topic	Item no.	Recommendation
TITLE		
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract (b) Provide in the abstract an informative and balanced summary of what was done and what was found
INTRODUCTION		
Background / rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
METHODS		
Study Design	4	Present key elements of study design early in the paper
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6	(a) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants (b) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data Sources / measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	Describe any efforts to address potential sources of bias
Study Size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding (b) Describe any methods used to examine subgroups and interactions (c) Explain how missing data were addressed (d) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy (e) Describe any sensitivity analyses
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed (b) Give reasons for non-participation at each stage (c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders (b) Indicate number of participants with missing data for each variable of interest (c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time *Case-control study*—Report numbers in each exposure category, or summary measures of exposure *Cross-sectional study*—Report numbers of outcome events or summary measures
Main Results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (b) Report category boundaries when continuous variables were categorized (c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
DISCUSSION		
Key Results	18	Summarise key results with reference to study objectives
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	Discuss the generalisability (external validity) of the study results
OTHER INFORMATION		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

** Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.*

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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STARD 2015 Checklist of Essential Items for Reporting Diagnostic Accuracy Studies

Section and Topic	No.	Item
TITLE OR ABSTRACT		
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)
ABSTRACT		
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)
INTRODUCTION		
	3	Scientific and clinical background, including the intended use and clinical role of the index test
	4	Study objectives and hypotheses
METHODS		
Study design	5	Whether data collection was planned before the index test and reference standard were performed (prospective study) or after (retrospective study)
Participants	6	Eligibility criteria
	7	On what basis potentially eligible participants were identified (such as symptoms, results from previous tests, inclusion in registry)
	8	Where and when potentially eligible participants were identified (setting, location and dates)
	9	Whether participants formed a consecutive, random or convenience series
Test Methods	10a	Index test, in sufficient detail to allow replication
	10b	Reference standard, in sufficient detail to allow replication
	11	Rationale for choosing the reference standard (if alternatives exist)
	12a	Definition of and rationale for test positivity cut-offs or result categories of the index test, distinguishing pre-specified from exploratory
	12b	Definition of and rationale for test positivity cut-offs or result categories of the reference standard, distinguishing pre-specified from exploratory
	13a	Whether clinical information and reference standard results were available to the performers/readers of the index test
	13b	Whether clinical information and index test results were available to the assessors of the reference standard
Analysis	14	Methods for estimating or comparing measures of diagnostic accuracy
	15	How indeterminate index test or reference standard results were handled
	16	How missing data on the index test and reference standard were handled
	17	Any analyses of variability in diagnostic accuracy, distinguishing pre-specified from exploratory
	18	Intended sample size and how it was determined
RESULTS		
Participants	19	Flow of participants, using a diagram
	20	Baseline demographic and clinical characteristics of participants
	21a	Distribution of severity of disease in those with the target condition
	21b	Distribution of alternative diagnoses in those without the target condition
	22	Time interval and any clinical interventions between index test and reference standard
Test Results	23	Cross tabulation of the index test results (or their distribution) by the results of the reference standard
	24	Estimates of diagnostic accuracy and their precision (such as 95% confidence intervals)
	25	Any adverse events from performing the index test or the reference standard
DISCUSSION		
	26	Study limitations, including sources of potential bias, statistical uncertainty, and generalisability
	27	Implications for practice, including the intended use and clinical role of the index test
OTHER INFORMATION		
	28	Registration number and name of registry
	29	Where the full study protocol can be accessed
	30	Sources of funding and other support; role of funders

This STARD list was released in 2015. The 30 items were identified by an international expert group of methodologists, researchers, and editors. The guiding principle in the development of STARD was to select items that, when reported, would help readers to judge the potential for bias in the study, to appraise the applicability of the study findings and the validity of conclusions and recommendations. The list represents an update of the first version, which was published in 2003.

More information can be found on http://www.equator-network.org/reporting-guidelines/stard.

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CHEERS Checklist - Items to include when Reporting Economic Evaluations of Health Interventions

Section / Item	Item no.	Recommendation	Reported on page no. / line no.
TITLE AND ABSTRACT			
Title	1	Identify the study as an economic evaluation or use more specific terms such as "cost-effectiveness analysis", and describe the interventions compared.	
Abstract	2	Provide a structured summary of objectives, perspective, setting, methods (including study design and inputs), results (including base case and uncertainty analyses), and conclusions.	
INTRODUCTION			
Background and objectives	3	Provide an explicit statement of the broader context for the study. Present the study question and its relevance for health policy or practice decisions.	
METHODS			
Target population and subgroups	4	Describe characteristics of the base case population and subgroups analysed, including why they were chosen.	
Setting and location	5	State relevant aspects of the system(s) in which the decision(s) need(s) to be made.	
Study Perspective	6	Describe the perspective of the study and relate this to the costs being evaluated.	
Comparators	7	Describe the interventions or strategies being compared and state why they were chosen.	
Time horizon	8	State the time horizon(s) over which costs and consequences are being evaluated and say why appropriate.	
Discount rate	9	Report the choice of discount rate(s) used for costs and outcomes and say why appropriate.	
Choice of health outcomes	10	Describe what outcomes were used as the measure(s) of benefit in the evaluation and their relevance for the type of analysis performed.	
Measurement of effectiveness	11a	*Single study-based estimates:* Describe fully the design features of the single effectiveness study and why the single study was a sufficient source of clinical effectiveness data.	
	11b	*Synthesis-based estimates:* Describe fully the methods used for identification of included studies and synthesis of clinical effectiveness data.	
Measurement and valuation of preference based outcomes	12	If applicable, describe the population and methods used to elicit preferences for outcomes.	
Estimating resources and costs	13a	*Single study-based economic evaluation:* Describe approaches used to estimate resource use associated with the alternative interventions. Describe primary or secondary research methods for valuing each resource item in terms of its unit cost. Describe any adjustments made to approximate to opportunity costs.	
	13b	*Model-based economic evaluation:* Describe approaches and data sources used to estimate resource use associated with model health states. Describe primary or secondary research methods for valuing each resource item in terms of its unit cost. Describe any adjustments made to approximate to opportunity costs.	
Currency, price date, and conversion	14	Report the dates of the estimated resource quantities and unit costs. Describe methods for adjusting estimated unit costs to the year of reported costs if necessary. Describe methods for converting costs into a common currency base and the exchange rate.	
Choice of model	15	Describe and give reasons for the specific type of decision analytical model used. Providing a figure to show model structure is strongly recommended.	
Assumptions	16	Describe all structural or other assumptions underpinning the decision-analytical model.	
Analytical methods	17	Describe all analytical methods supporting the evaluation. This could include methods for dealing with skewed, missing, or censored data; extrapolation methods; methods for pooling data; approaches to validate or make adjustments (such as half cycle corrections) to a model; and methods for handling population heterogeneity and uncertainty.	
RESULTS			
Study parameters	18	Report the values, ranges, references, and, if used, probability distributions for all parameters. Report reasons or sources for distributions used to represent uncertainty where appropriate. Providing a table to show the input values is strongly recommended.	
Incremental costs and outcomes	19	For each intervention, report mean values for the main categories of estimated costs and outcomes of interest, as well as mean differences between the comparator groups. If applicable, report incremental cost-effectiveness ratios.	
Characterising uncertainty	20a	*Single study-based economic evaluation:* Describe the effects of sampling uncertainty for the estimated incremental cost and incremental effectiveness parameters, together with the impact Consolidated Health Economic Evaluation Reporting Standards – CHEERS Checklist 3 of methodological assumptions (such as discount rate, study perspective).	
	20b	*Model-based economic evaluation:* Describe the effects on the results of uncertainty for all input parameters, and uncertainty related to the structure of the model and assumptions.	
Characterising heterogeneity	21	If applicable, report differences in costs, outcomes, or costeffectiveness that can be explained by variations between subgroups of patients with different baseline characteristics or other observed variability in effects that are not reducible by more information.	
DISCUSSION			
Study findings, limitations, generalisability, and current knowledge	22	Summarise key study findings and describe how they support the conclusions reached. Discuss limitations and the generalisability of the findings and how the findings fit with current knowledge.	
OTHER INFORMATION			
Source of funding	23	Describe how the study was funded and the role of the funder in the identification, design, conduct, and reporting of the analysis. Describe other non-monetary sources of support.	
Conflicts of interest	24	Describe any potential for conflict of interest of study contributors in accordance with journal policy. In the absence of a journal policy, we recommend authors comply with International Committee of Medical Journal Editors recommendations.	

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The ARRIVE Guidelines (Animal Research: Reporting of In Vivo Experiments)

Section / Topic	Item no.	Checklist item
TITLE AND ABSTRACT		
Title	1	Provide as accurate and concise a description of the content of the article as possible.
Abstract	2	Provide an accurate summary of the background, research objectives, including details of the species or strain of animal used, key methods, principal findings and conclusions of the study.
INTRODUCTION		
Background	3	a. Include sufficient scientific background (including relevant references to previous work) to understand the motivation and context for the study, and explain the experimental approach and rationale.
Objectives		b. Explain how and why the animal species and model being used can address the scientific objectives and, where appropriate, the study's relevance to human biology.
	4	Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.
METHODS		
Ethical statement	5	DIndicate the nature of the ethical review permissions, relevant licences (e.g. Animal [Scientific Procedures] Act 1986), and national or institutional guidelines for the care and use of animals, that cover the research.
Study design	6	For each experiment, give brief details of the study design including: a. The number of experimental and control groups. b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (e.g. randomisation procedure) and when assessing results (e.g. if done, describe who was blinded and when). c. The experimental unit (e.g. a single animal, group or cage of animals). A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out.
Experimental procedures	7	For each experiment and each experimental group, including controls, provide precise details of all procedures carried out. For example: a. How (e.g. drug formulation and dose, site and route of administration, anaesthesia and analgesia used [including monitoring], surgical procedure, method of euthanasia). Provide details of any specialist equipment used, including supplier(s). b. When (e.g. time of day). c. Where (e.g. home cage, laboratory, water maze). d. Why (e.g. rationale for choice of specific anaesthetic, route of administration, drug dose used).
Experimental animals	8	a. Provide details of the animals used, including species, strain, sex, developmental stage (e.g. mean or median age plus age range) and weight (e.g. mean or median weight plus weight range). b. Provide further relevant information such as the source of animals, international strain nomenclature, genetic modification status (e.g. knock-out or transgenic), genotype, health/immune status, drug or test naïve, previous procedures, etc.
Housing and husbandry	9	Provide details of: a. Housing (type of facility e.g. specific pathogen free [SPF]; type of cage or housing; bedding material; number of cage companions; tank shape and material etc. for fish). b. Husbandry conditions (e.g. breeding programme, light/dark cycle, temperature, quality of water etc for fish, type of food, access to food and water, environmental enrichment). c. Welfare-related assessments and interventions that were carried out prior to, during, or after the experiment.
Sample size	10	a. Specify the total number of animals used in each experiment, and the number of animals in each experimental group. b. Explain how the number of animals was arrived at. Provide details of any sample size calculation used. c. Indicate the number of independent replications of each experiment, if relevant.
Allocating animals to experimental groups	11	a. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done. b. Describe the order in which the animals in the different experimental groups were treated and assessed.
Experimental outcomes	12	Clearly define the primary and secondary experimental outcomes assessed (e.g. cell death, molecular markers, behavioural changes).
Statistical methods	13	a. Provide details of the statistical methods used for each analysis. b. Specify the unit of analysis for each dataset (e.g. single animal, group of animals, single neuron). c. Describe any methods used to assess whether the data met the assumptions of the statistical approach.
RESULTS		
Baseline data	14	For each experimental group, report relevant characteristics and health status of animals (e.g. weight, microbiological status, and drug or test naïve) prior to treatment or testing (this information can often be tabulated).
Numbers analysed	15	a. Report the number of animals in each group included in each analysis. Report absolute numbers (e.g. 10/20, not 50%). b. If any animals or data were not included in the analysis, explain why.
Outcomes and estimation	16	Report the results for each analysis carried out, with a measure of precision (e.g. standard error or confidence interval).
Adverse events	17	a. Give details of all important adverse events in each experimental group. b. Describe any modifications to the experimental protocols made to reduce adverse events.
DISCUSSION		
Interpretation/ scientific implications	18	a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature. b. Comment on the study limitations including any potential sources of bias, any limitations of the animal model, and the imprecision associated with the results. c. Describe any implications of your experimental methods or findings for the replacement, refinement or reduction (the 3Rs) of the use of animals in research.
Generalisability/translation	19	Comment on whether, and how, the findings of this study are likely to translate to other species or systems, including any relevance to human biology.
Funding	20	List all funding sources (including grant number) and the role of the funder(s) in the study.

The ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines were developed as part of an NC3Rs initiative to improve the design, analysis and reporting of research using animals – maximising information published and minimising unnecessary studies. The guidelines were published in the online journal PLOS Biology in June 2010 and are currently endorsed by scientific journals, major funding bodies and learned societies. More information can be found on www.nc3rs.org.uk/ARRIVE

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Revised Standards for Quality Improvement Reporting Excellence (SQUIRE 2.0)

No	Item	Guide questions / description
TITLE AND ABSTRACT		
1	Title	Indicate that the manuscript concerns an initiative to improve healthcare (broadly defined to include the quality, safety, effectiveness, patient-centeredness, timeliness, cost, efficiency, and equity of healthcare)
2	Abstract	a. Provide adequate information to aid in searching and indexing b. Summarize all key information from various sections of the text using the abstract format of the intended publication or a structured summary such as: background, local problem, methods, interventions, results, conclusions
INTRODUCTION		**WHY DID YOU START?**
3	Problem Description	Nature and significance of the local problem
4	Available knowledge	Summary of what is currently known about the problem, including relevant previous studies
5	Rationale	Informal or formal frameworks, models, concepts, and/or theories used to explain the problem, any reasons or assumptions that were used to develop the intervention(s), and reasons why the intervention(s) was expected to work
6	Specific aims	Purpose of the project and of this report
METHODS		**WHAT DID YOU DO?**
7	Context	Contextual elements considered important at the outset of introducing the intervention(s)
8	Intervention(s)	a. Description of the intervention(s) in sufficient detail that others could reproduce it b. Specifics of the team involved in the work
9	Study of the Intervention(s)	a. Approach chosen for assessing the impact of the intervention(s) b. Approach used to establish whether the observed outcomes were due to the intervention(s)
10	Measures	a. Measures chosen for studying processes and outcomes of the intervention(s), including rationale for choosing them, their operational definitions, and their validity and reliability b. Description of the approach to the ongoing assessment of contextual elements that contributed to the success, failure, efficiency, and cost c. Methods employed for assessing completeness and accuracy of data
11	Analysis	a. Qualitative and quantitative methods used to draw inferences from the data b. Methods for understanding variation within the data, including the effects of time as a variable
12	Ethical Considerations	Ethical aspects of implementing and studying the intervention(s) and how they were addressed, including, but not limited to, formal ethics review and potential conflict(s) of interest
RESULTS		**WHAT DID YOU FIND?**
13	Results	a. Initial steps of the intervention(s) and their evolution over time (e.g., time-line diagram, flow chart, or table), including modifications made to the intervention during the project b. Details of the process measures and outcome c. Contextual elements that interacted with the intervention(s) d. Observed associations between outcomes, interventions, and relevant contextual elements e. Unintended consequences such as unexpected benefits, problems, failures, or costs associated with the intervention(s). f. Details about missing data
DISCUSSION		**WHAT DOES IT MEAN?**
14	Summary	a. Key findings, including relevance to the rationale and specific aims b. Particular strengths of the project
15	Interpretation	a. Nature of the association between the intervention(s) and the outcomes b. Comparison of results with findings from other publications c. Impact of the project on people and systems d. Reasons for any differences between observed and anticipated outcomes, including the influence of context e. Costs and strategic trade-offs, including opportunity costs
16	Limitations	a. Limits to the generalizability of the work b. Factors that might have limited internal validity such as confounding, bias, or imprecision in the design, methods, measurement, or analysis
17	Conclusions	c. Efforts made to minimize and adjust for limitations a. Usefulness of the work b. Sustainability c. Potential for spread to other contexts d. Implications for practice and for further study in the field e. Suggested next steps
OTHER INFORMATION		
18	Funding	Sources of funding that supported this work. Role, if any, of the funding organization in the design, implementation, interpretation, and reporting

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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section / Topic	Item no.	Description
ADMINISTRATIVE INFORMATION		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry
	2b	All items from the World Health Organization Trial Registration Data Set
Protocol version	3	Date and version identifier
Funding	4	Sources and types of financial, material, and other support
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors
	5b	Name and contact information for the trial sponsor
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)
INTRODUCTION		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention
	6b	Explanation for choice of comparators
Objectives	7	Specific objectives or hypotheses
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)
METHODS: PARTICIPANTS, INTERVENTIONS, AND OUTCOMES		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size
METHODS: ASSIGNMENT OF INTERVENTIONS (FOR CONTROLLED TRIALS)		
Allocation:		
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial

METHODS: DATA COLLECTION, MANAGEMENT, AND ANALYSIS		
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)
METHODS: MONITORING		
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor
ETHICS AND DISSEMINATION		
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions
	31b	Authorship eligibility guidelines and any intended use of professional writers
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code
APPENDICES		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable

**It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.*

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CONSORT 2010 Checklist of Information to include when Reporting a Randomised Trial*

Section / Topic	Item no.	Checklist item	Reported on page no.
TITLE AND ABSTRACT			
	1a	Identification as a randomised trial in the title	_____
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	_____
INTRODUCTION			
Background and objectives	2a	Scientific background and explanation of rationale	_____
	2b	Specific objectives or hypotheses	_____
METHODS			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	_____
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	_____
Participants	4a	Eligibility criteria for participants	_____
	4b	Settings and locations where the data were collected	_____
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	_____
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	_____
	6b	Any changes to trial outcomes after the trial commenced, with reasons	_____
Sample size	7a	How sample size was determined	_____
	7b	When applicable, explanation of any interim analyses and stopping guidelines	_____
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	_____
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	_____
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	_____
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	_____
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	_____
	11b	If relevant, description of the similarity of interventions	_____
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	_____
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	_____
RESULTS			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	_____
	13b	For each group, losses and exclusions after randomisation, together with reasons	_____
Recruitment	14a	Dates defining the periods of recruitment and follow-up	_____
	14b	Why the trial ended or was stopped	_____
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	_____
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	_____
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	_____
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	_____
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	_____
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	_____
DISCUSSION			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	_____
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	_____
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	_____
OTHER INFORMATION			
Registration	23	Registration number and name of trial registry	_____
Protocol	24	Where the full trial protocol can be accessed, if available	_____
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	_____

** We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.*

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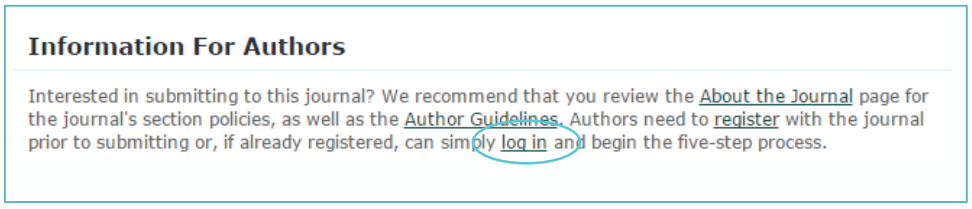
PJP Online Journal System

User Guide for Authors

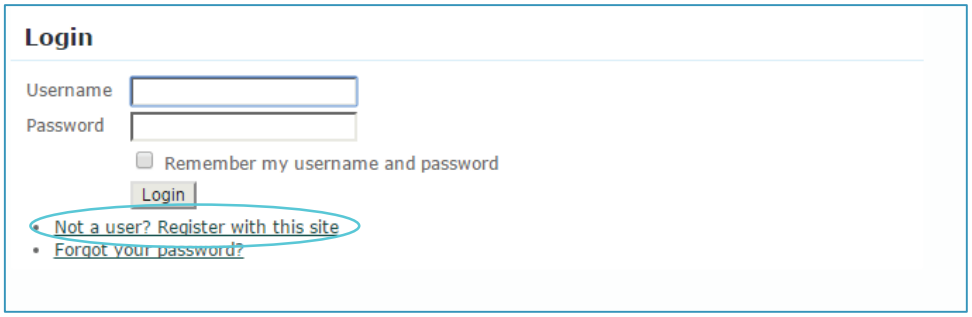
Getting Started

- From the PJP website (http://www.philippinejournalofpathology.org), navigate to 'For Authors'. (add screenshot of PJP landing page, circle 'for authors' on right column).

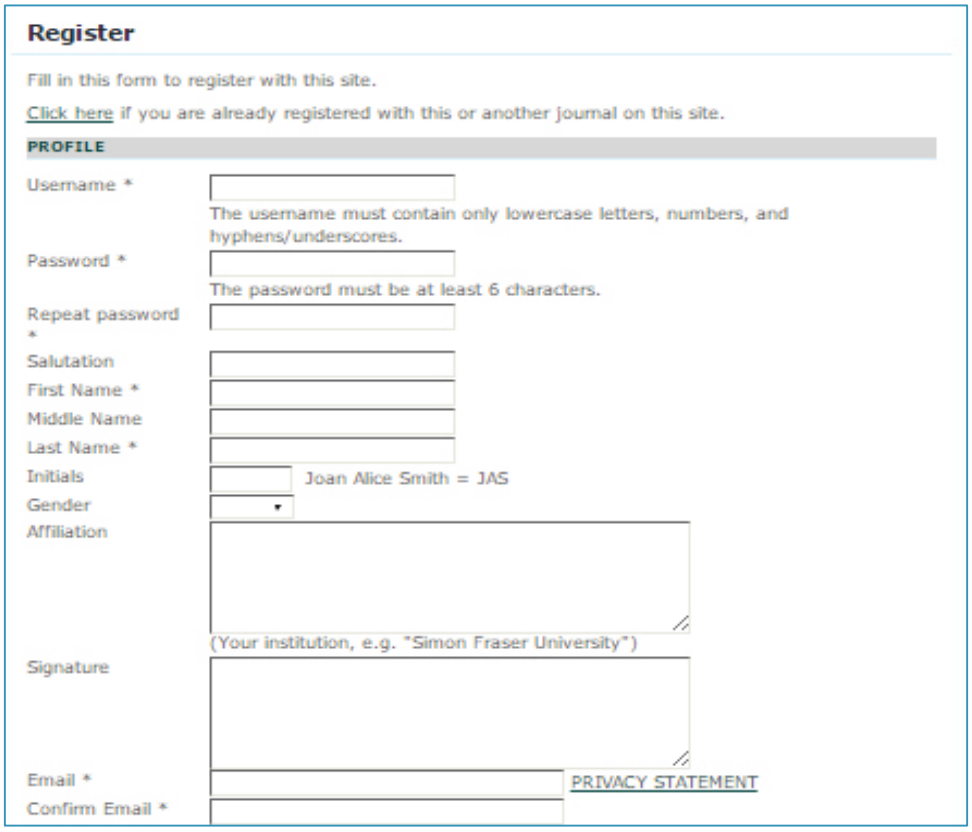
Select 'FOR AUTHORS'.

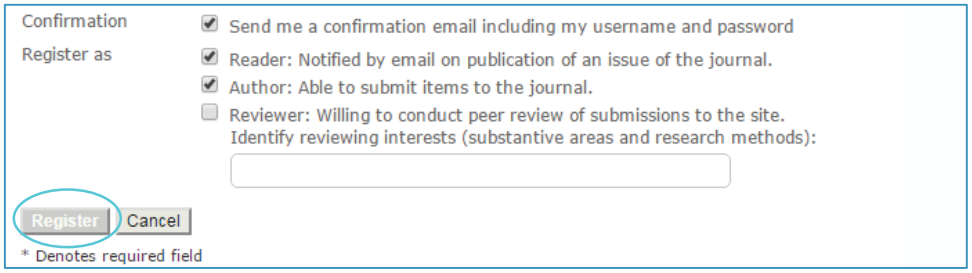


- Log in
- New user:
  - If you are a new user of the PJP website, please register by clicking the link 'Not a user, Register with this site'.

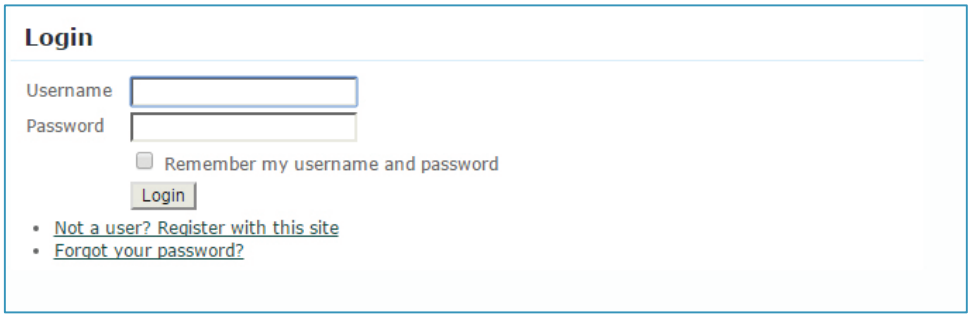


  - Complete the online form then select 'Register'. A confirmation email with your username and password will be sent to your email address.



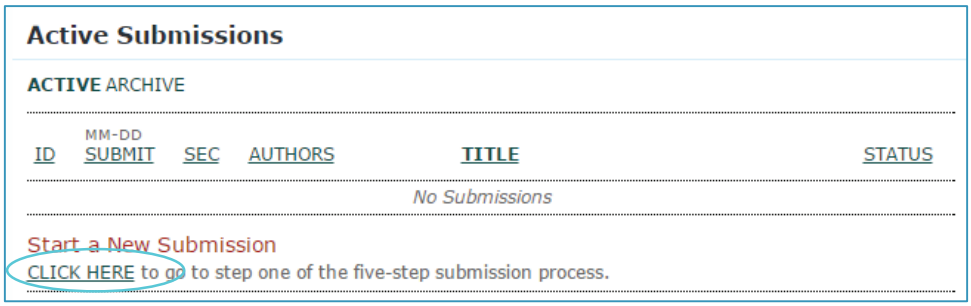


- Existing user:
  - Log in to your OJS account using the username and password from original registration.
  - If you have forgotten your log in details, please click the 'Forgot the password?' and an email will be sent to your registered email address.



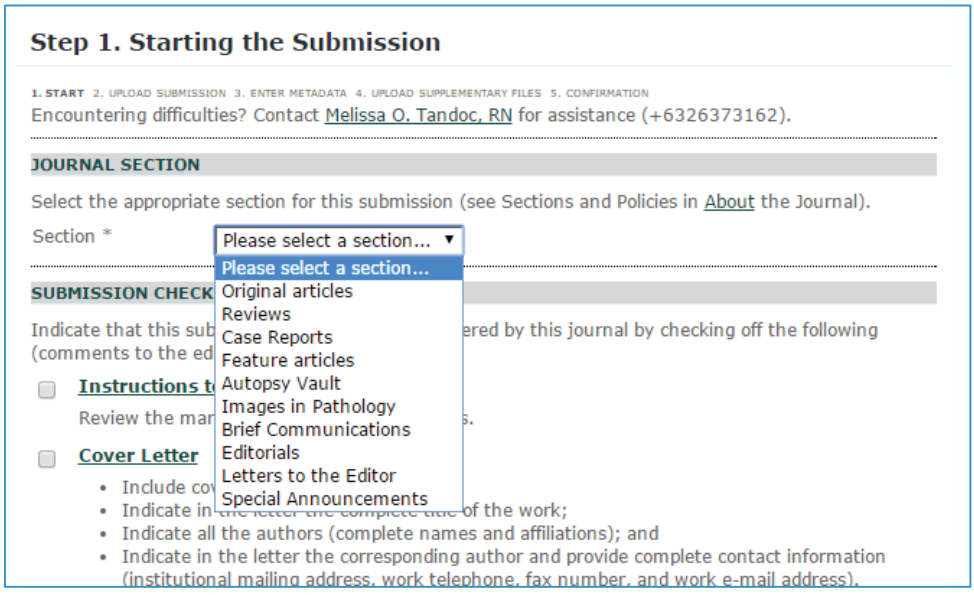
The Submission Process

- To start the submission process, click 'New Submission'

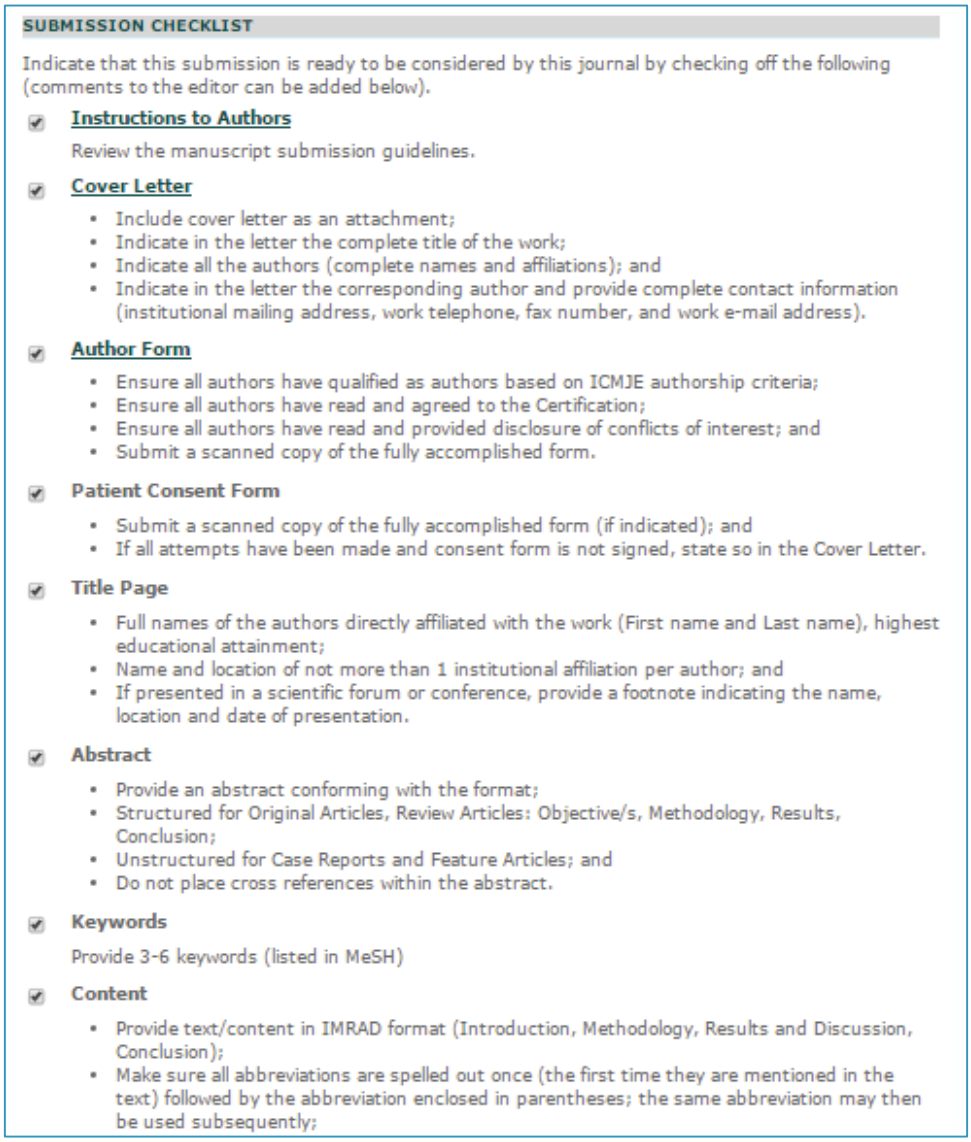


Step 1: Starting the submission

- From the drop-down menu, please select the most appropriate section to describe your submission article type. If you are not sure what section to select, click 'About' to find out more information.



- Please ensure the items listed in the checklist are ready then tick each box.



SUBMISSION CHECKLIST

Indicate that this submission is ready to be considered by this journal by checking off the following (comments to the editor can be added below).

☑ **Instructions to Authors**

Review the manuscript submission guidelines.

☑ **Cover Letter**

- Include cover letter as an attachment;
- Indicate in the letter the complete title of the work;
- Indicate all the authors (complete names and affiliations); and
- Indicate in the letter the corresponding author and provide complete contact information (institutional mailing address, work telephone, fax number, and work e-mail address).

☑ **Author Form**

- Ensure all authors have qualified as authors based on ICMJE authorship criteria;
- Ensure all authors have read and agreed to the Certification;
- Ensure all authors have read and provided disclosure of conflicts of interest; and
- Submit a scanned copy of the fully accomplished form.

☑ **Patient Consent Form**

- Submit a scanned copy of the fully accomplished form (if indicated); and
- If all attempts have been made and consent form is not signed, state so in the Cover Letter.

☑ **Title Page**

- Full names of the authors directly affiliated with the work (First name and Last name), highest educational attainment;
- Name and location of not more than 1 institutional affiliation per author; and
- If presented in a scientific forum or conference, provide a footnote indicating the name, location and date of presentation.

☑ **Abstract**

- Provide an abstract conforming with the format;
- Structured for Original Articles, Review Articles: Objective/s, Methodology, Results, Conclusion;
- Unstructured for Case Reports and Feature Articles; and
- Do not place cross references within the abstract.

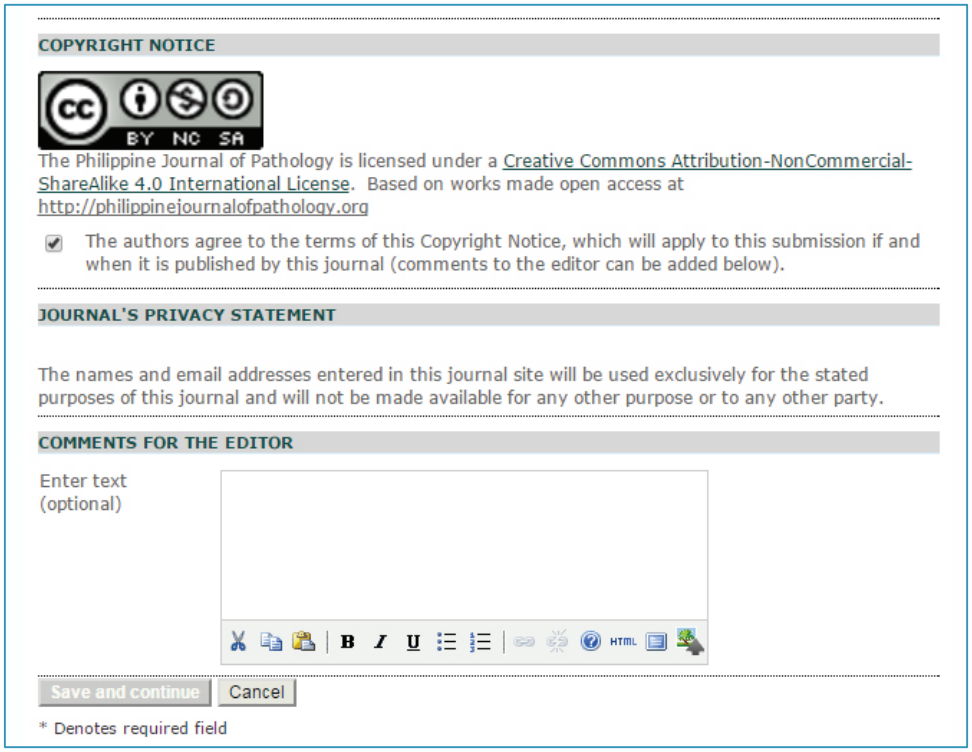
☑ **Keywords**

Provide 3-6 keywords (listed in MeSH)

☑ **Content**

- Provide text/content in IMRAD format (Introduction, Methodology, Results and Discussion, Conclusion);
- Make sure all abbreviations are 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;

- Read the 'Copyright Notice' and add comments to the editor (optional). Select 'Save and continue'.

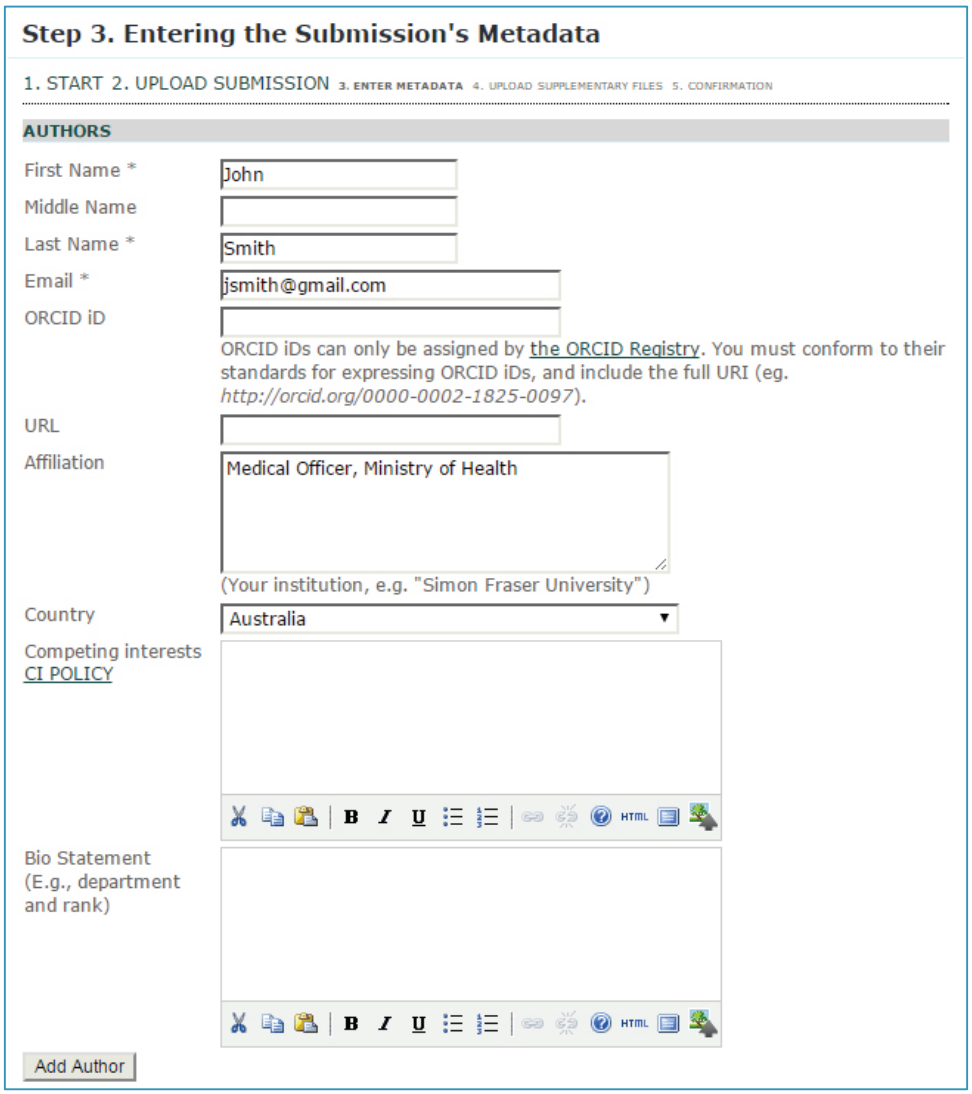


Step 2: Uploading the Submission

- Please follow the instructions on this page to upload your file, then select 'Save and continue'. This is where you upload the **manuscript only**. (You will be asked to upload other required documents at Step 4.

Step 3: Entering the Submission's Metadata

- Complete author(s)'s information as much as you can. Fields marked with * are mandatory. If you have more than one author for your submission, click 'Add author' for each of these.



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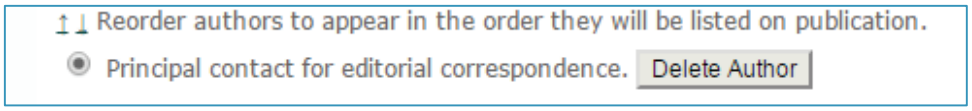

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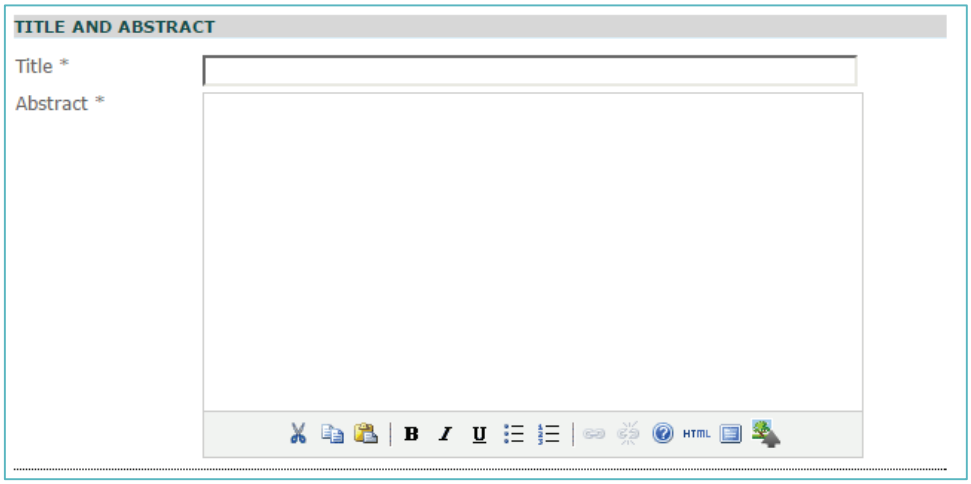

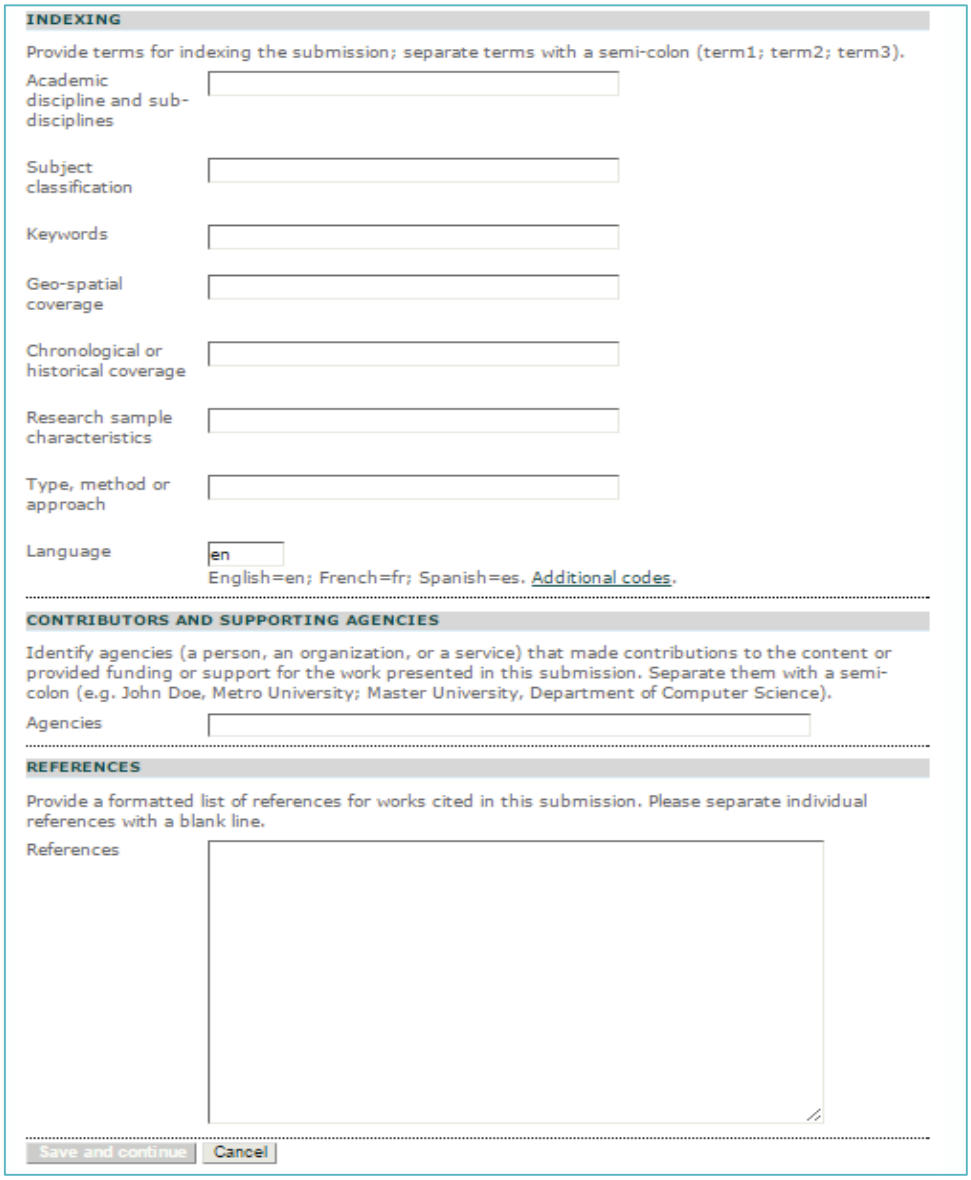

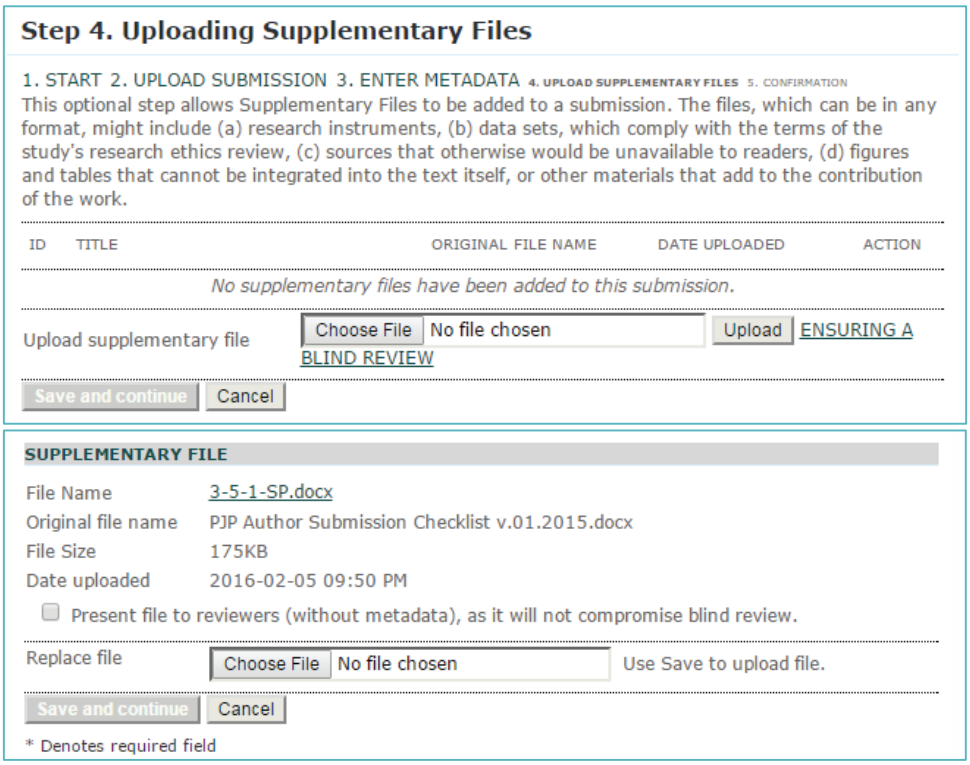

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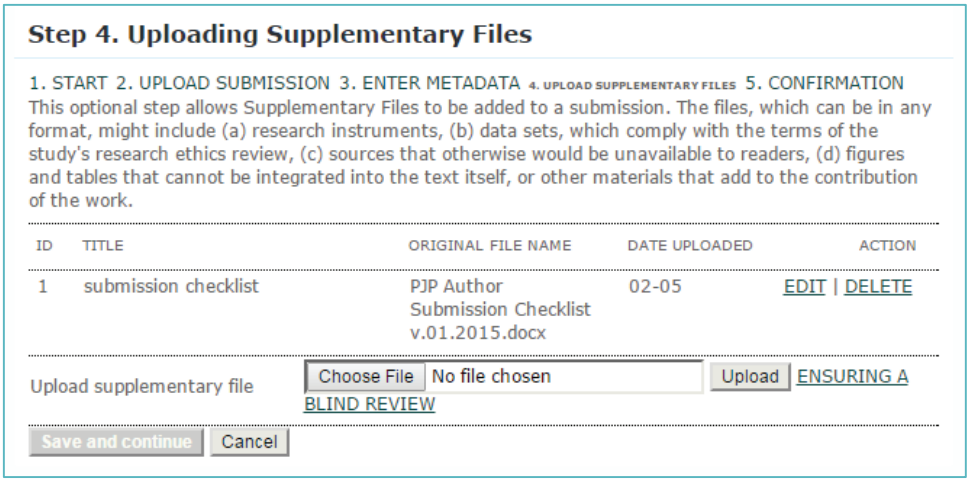

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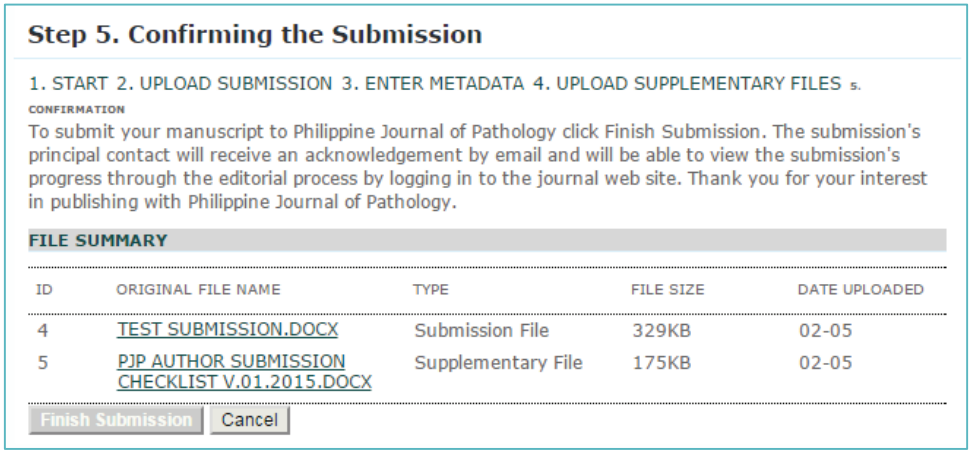

Step 5. Confirming the Submission

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FILE SUMMARY

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4	TEST SUBMISSION.DOCX	Submission File	329KB	02-05
5	PJP AUTHOR SUBMISSION CHECKLIST V.01.2015.DOCX	Supplementary File	175KB	02-05

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Amado O. Tandoc III, MD, DPSP
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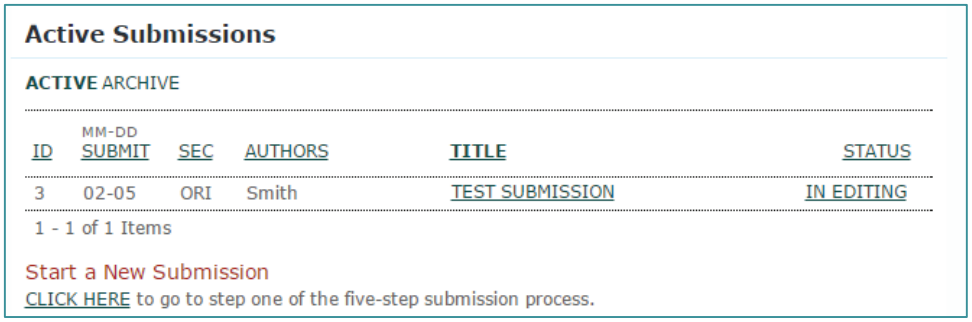

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1 - 1 of 1 Items

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> Please find attached the comments from the peer reviewers. Please take the following actions:
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> 2. Provide a response to each of the reviewers' comments in a separate Word document.
> 3. Upload both the revised manuscript and the response to the reviewers' comments.
>
> The due date for these revisions is Friday, xx month. If you have any queries regarding this please contact me.
>
> Thank you and kind regards,
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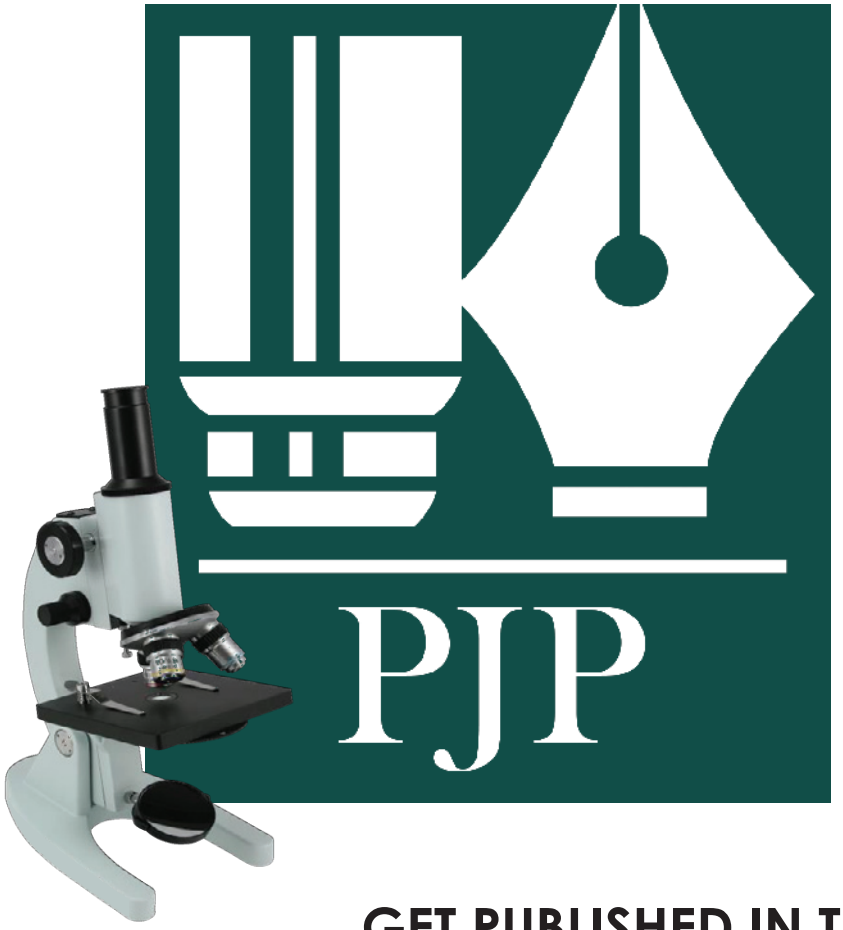

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