

# PHILIPPINE JOURNAL OF PATHOLOGY

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To all our PJP readers,

Again I would like to invite all of you to read and enjoy the Philippine Journal of Pathology. This journal does not provide only a space for new knowledge in pathology but this gives you also an opportunity to critique, debate and create dialogue charge to what you have read in other internationally distributed journals.

As we all know, this is an e-based publication which makes this possible for us to be intertwined with each other and be directly involved in continuing knowledge construction.

Our vision is to create a high-quality publication that will be relevant, challenging, and inclusive of a diverse range of perspectives, including pathology residents, researchers, diplomates and fellows undergoing subspecialty trainings, and consultant pathologists who are in academe and who are interested in doing researches. The PJP welcomes original researches, case reports, reviews of the literature, critical commentaries, case studies, book reviews, and even works-in progress.

Our gratitude goes to the editors for excellently continuing this project. We are most delighted that you are joining us as readers, but we will be more than grateful if you join us as contributors.

Thank you and enjoy our Philippine Journal of Pathology.

A handwritten signature in black ink, appearing to read 'B. Espiritu'.

**Bernadette R. Espiritu, MD, FPSP, MMHoA, MIAC**  
*President, Philippine Society of Pathologists, Inc.*

## The Labors of Hercules and Sisyphus



There are times when one thinks a task too great or too burdensome, requiring immense effort.

I imagine that this must be the feeling of a researcher, who, after exhaustive journal searches, laboratory experiments, and data gathering, is finally, *finally* getting down to writing his or her manuscript. A product of so many months, or years, there always is that struggle to get the first paragraph going and from there, the second, the third, and so on.

*Why did we do the study in the first place*, the background and rationale of it all, the related literature and research objectives, these sometimes become difficult to pin down on the blank page. *How did we perform the research, what did we exactly do*, the methods section teeters between too much and too little information, a delicate balance that needs to be achieved to achieve replicability, repeatability.

*Can our results withstand scrutiny*, the data are there, analyzed, waiting to be integrated into the body of the paper, transformed into figures and tables, diagrams and appendices. Then there are the references, the care that comes into making sure that these are cross linked properly, in ascending order, expressed in the appropriate, at times challenging, but required citation formats. All this, in a word, can only best be described as *Herculean*.

At the other end, lies the arduous task of the editor and the publisher, mirroring, in many respects, the labors of the manuscript writer and researcher. *Sisphyean*, this time, as another aspect, perhaps unrealized by

many, emerges – the cyclical pattern of effort upon editorial effort, of inviting and soliciting articles, engaging reviewers and retaining them, processing and polishing manuscripts, to beat the publication cycle and *still* meet standards, each issue a display of consistency, a faithful replication.

Like a colossal rock being rolled slowly up a hill, the labors of publication increase as one reaches the apex: once completed at the top, one witnesses everything reset, rolling down, back to the starting point, with each new volume and issue. It never ends.

Time is not helping at all. There are so many other things to do, slides to read, results to release, laboratories to visit, meetings to attend, a million more things that remunerate much more than this academic exercise. Deadlines are stressful, and if we learned anything at all in our lifetimes, it is to mitigate or eliminate the source of the stress.

I write this right at the cusp of a new issue, our first issue for 2018. Articles have slowly trickled in. Some made it on time, others needing a bit more to pass the bar. Another lean harvest in numbers, but bountiful in new learning and knowledge for pathologists of every generation.

Like Hercules, like Sisyphus, we, researchers and editors, must go on.

For our research needs to be written up and submitted, our output published, built upon and translated to new knowledge, transformed to policy. In the end, this is our obligation as scientists to our people, to humanity even. And there are miles to go before we all get to sleep.

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

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## PHILIPPINE BIOSAFETY & BIOSECURITY SURVEY

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The purpose of this survey is to examine the policies and standards that Philippine laboratorians employ to advance laboratory biosafety and biosecurity in their laboratories. Specifically, we need to (1) characterize the practices, equipment, and facilities used by these laboratorians and (2) examine the effectiveness of existing national and state regulations in the context of the infectious pathogens they handle.

# Frequency of Epidermal Growth Factor Receptor Mutations among Filipino Patients with Non-small Cell Lung Carcinoma in a Private Tertiary Care Setting

Cyril Kim Nee-Estuye-Evangelista, Jose Jasper Andal, Daphne Ang

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## ABSTRACT

**Background.** Epidermal Growth Factor Receptor (EGFR) mutation status has been shown to have a significant prognostic and predictive role in the management of Non-small Cell Lung Carcinoma (NSCLC), significantly prolonging patients' survival. Thus, EGFR mutational analysis before initiation of treatment is now recommended in several clinical practice guidelines. Although EGFR mutation testing in NSCLC has been a part of clinical care in the Philippines, there is little data on the EGFR mutation spectrum among Filipinos.

**Objective.** This study aims to determine the frequency of EGFR mutations among Filipino population diagnosed with NSCLC in a private tertiary care setting.

**Methodology.** A total of 626 tissue samples (444 biopsies, 108 pleural/ascitic fluids, 74 excision/resection), during a 15-month period (January 2015-March 2016) were assessed for the known EGFR driver mutations (exons 18, 19, 20, 21) using the Roche EGFR protocol with the Cobas Quantitative Real Time PCR. Macrodissection was performed as necessary. Available patient demographics were recorded. Statistical analyses were performed using the Fisher's exact test.

**Results.** In this study, we report the largest EGFR mutation profiling data among Filipino patients with NSCLC, which showed an overall 49.4% EGFR mutation rate. The mutation rates according to histologic types, were as follows: adenocarcinoma (49.9%, n=287/575), squamous cell carcinoma (3.5%, n=9/26), NSCLC NOS (50%, n=10/20), adenosquamous cell carcinoma (66.7%, n=2/3), and adenocarcinoma with neuroendocrine features (50%, n=1/2). Consistent with the literature, we found a significant higher incidence of EGFR mutation among women than men (60.2% vs 39.8%). With regards to individual mutation types, the most common mutations detected were deletions in exon 19 (54.7%, n=168), followed by L858R point mutation in exon 21 (27.4%, n=84).

**Conclusion.** The incidence of EGFR mutations in NSCLC varies across different ethnicity. In previous reports, the frequency of EGFR mutations is approximately 30% (with a range of 22.2% to 64.2%) among the Asian population compared with 20% among the white population. In the Philippines, the incidence of EGFR mutations is sparsely explored. Here we report the largest EGFR mutation profiling data among Filipinos with NSCLC in a tertiary care setting, with a frequency of 49.4%. This prevalence is almost similar to those reported in Asia. EGFR is differentially mutated among NSCLC patients with different gender, as women have significantly higher incidence than men. Hence, this study establishes relevance of routine EGFR mutation testing for all NSCLC patients as part of initial workup at diagnosis and underscores the significant role of EGFR inhibitors as a treatment option among Filipino population.

*Key words: Epidermal growth factor receptor, non-small cell lung carcinoma, Exons 18,19,20 and 21, T790M, polymerase chain reaction*

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## INTRODUCTION

Lung cancer is the leading cause of cancer mortality in the world and is the second most common cause of cancer deaths among Filipinos. Approximately 85% of lung cancer cases are non-small lung cancers (NSCLC).<sup>1</sup> In patients with NSCLC, platinum-based chemotherapy used to be considered as standard first-line treatment.<sup>2</sup> During the recent years, there has been continuous development of new and effective targeted treatment modalities for advanced NSCLC. One of these therapeutic agents are tyrosine kinase inhibitors (TKI) which targets mutant epidermal growth factor receptor (EGFR).<sup>3-4</sup> EGFR mutations play an important role in the pathogenesis of multiple carcinoma, including NSCLC. Activating EGFR mutation promotes tumor

growth and progression, stimulates tumor cell proliferation, inhibits apoptosis and produces angiogenic factors.<sup>5</sup> For patients with advanced NSCLC harboring EGFR mutation, several phase III studies have shown the clinical efficacy of the FDA approved EGFR inhibitors, gefitinib, afatinib and erlotinib, as compared to platinum-based chemotherapy when used as first line of treatment. Also, EGFR mutation status has been shown to have a significant prognostic and predictive role in the management of NSCLC, significantly prolonging patients' survival.<sup>6-8</sup> Not all types of EGFR mutation, however, are responsive to the first-generation tyrosine kinase inhibitors. For NSCLC patients with the common EGFR mutations, namely exon 19 deletion and exon 21 L858R, the response rate to TKIs (gefitinib and erlotinib) is approximately 60%. Several studies also suggested that patients with exon 19 deletion mutation might be more sensitive to targeted therapy than with exon 21 L858R. On the contrary, mutation in exon 20 (T790M) have been associated with resistance to first generation TKIs. EGFR T790M mutations usually occurs as a resistance mutation after first generation TKI therapy. For patients with EGFR T790M mutations, treatment with osimertinib may be effective and nazartinib (EGF816) is promising for the majority of them.<sup>9</sup> Thus, an accurate EGFR mutational analysis before initiation of treatment and repeat EGFR mutation testing at relapse, are now recommended in several clinical practice guidelines.<sup>10</sup>

The incidence of EGFR mutations in NSCLC varies across different ethnicities. In previous reports, the frequency of EGFR mutations is approximately 30% (range of 22.2% to 62%) among the Asian population compared with 20% among the white populations.<sup>11,12</sup> In the Philippines, although EGFR mutation testing in NSCLC has been a part of clinical care setting, there is little data on the EGFR mutation spectrum among Filipinos. Here, in this study we determine the EGFR mutation status in 626 NSCLC patients of Filipino ethnicity and correlate with different variables like age, gender, and histologic types.

## METHODOLOGY

This is a retrospective study of Filipino patients diagnosed with NSCLC who were referred to our institution for EGFR mutation testing from various hospitals across the Philippines over a 15-month period (January 2015-March 2016). The EGFR mutational status is performed when a diagnosis of adenocarcinoma, non-small cell carcinoma favors squamous cell carcinoma, adenosquamous carcinoma and non-small cell carcinoma, NOS are rendered. The histopathological diagnoses were mostly made based on the histomorphology with or without immunohistochemical staining performed with TTF-1, p63, Napsin A, CK7, CK20. In distinguishing adenocarcinoma from squamous cell carcinoma, or a lung primary vs. metastatic, 2 or more immunohistochemical stains were used. 78 of 626 patients were diagnosed as primary lung carcinoma using panel of immunohistochemical stains (positive for CK7, TTF1 and Napsin A; negative for CK20). 44 cases were diagnosed using positivity with TTF-1 only. The remaining cases were diagnosed as primary lung carcinoma based on clinical and radiologic correlation (absence of other organ involvement). The patient demographic data (including age, nationality, gender) if available, and histologic diagnosis were recorded. This study was approved by the Institutional Scientific Review Board and Ethics Committee of St. Luke's Medical Center-Quezon City. Since this was a retrospective analysis, the ISRB and the IERC waived the need for an informed consent.

## Inclusion criteria

All Filipino patients diagnosed as Non-small cell Lung Carcinoma from St. Luke's Medical Center-Global City and other hospitals from the Philippines.

## Exclusion criteria

Formalin-fixed paraffin embedded (FFPE) samples from patients diagnosed with NSCLC containing less than 5% viable tumor cells were excluded from this study. Patients with incomplete histopathologic report were also excluded from this study.

## Collection of patient samples

The patient's FFPE blocks and H&E slides, together with histopathological report, were sent to the Cellular Immunology Section of the Institute of Pathology-St. Luke's Medical Center. The hematoxylin and eosin stained slides were viewed under the microscope to confirm that the tumor cells constitute more than 5% of the tissue mass. Macrodissection was performed on cases with less than 50% tumor cells in the tissue block/slide. A total of 626 tissue samples (444 biopsies, 108 pleural/ascitic fluids, 74 excision/resection) were assessed for EGFR mutation.

## DNA extraction

DNA was isolated from the FFPE samples after deparaffinization and extraction of 3-5mm thick paraffin sections in xylene. The Cobas Roche DNA Sample Preparation Kit (Roche Diagnostics, USA) and the Cobas cfDNA Sample Preparation Kit (Roche Diagnostics, USA) were used for manual sample preparations, which were based on nucleic acid binding to glass fibers.

## Mutation analysis by PCR and Sanger sequencing

After the extraction of DNA, the target DNA was then amplified and detected on the Cobas Z 480 analyzer (Roche Diagnostics, USA) using the amplification and detection reagents provided in the Cobas Roche EGFR Mutation Test v1 kit (Roche Diagnostics, USA). A mutant control and negative control were included in each run to confirm the validity of the run.

The Cobas Roche EGFR Test is designed to detect the following mutations:

- Exon 18: G719X (G719A, G719C, and G719S)
- Exon 19: deletions and complex mutations
- Exon 20: S768I, T790M, and insertions
- Exon 21: L858R and L861Q

## Statistical analysis

The Fischer's exact test was performed to reveal any significant correlation between the mutation status and gender, gender and specific mutation type.

## RESULTS

626 Filipino patients diagnosed with lung cancer were tested for common EGFR mutation subtypes by real time PCR using TaqMan primer probes for point mutations in exons 18, 20 and 21 and in frame deletion in exon 19. As shown in Table 1, of the 626 patients tested, 52% (n=325) were males and 48% (n=301) were females. The median age was 64 years (with a range of 13-94 years old). The overall EGFR mutational analysis result was positive in 49.4% (n=309), negative (wild type) in 50% (n=313) and invalid in 0.6% (n=4). Invalid results were secondary to DNA degradation, resulting to failure of PCR amplification or testing. The presence of EGFR mutation was significantly higher in females (60.2%) as compared with males (39.8%) (two

Table 1. Patient demographics (n=626)	
Variables	
Age	13-94
Median Age	64
Gender	
Male	325
Female	301
Histopathology	
Adenocarcinoma	575
Squamous cell	26
Adenosquamous	3
NSCLC, NOS	20
Adenocarcinoma with neuroendocrine features	2

tailed p test,  $p < 0.0001$ ) (Table 2). As to histologic classification with EGFR mutation, there were 575 cases of adenocarcinoma, 26 cases of squamous cell carcinoma, 20 cases of NSCLC NOS, 3 cases of adenosquamous cell carcinoma and 2 cases of adenocarcinoma with neuroendocrine features. EGFR mutations were identified in 49.9% of adenocarcinoma (n=287), 3.5% of squamous cell carcinoma (n=9), 50% of NSCLC NOS (n=10), 66.7% of adenosquamous cell carcinoma (n=2), and 50% of adenocarcinoma with neuroendocrine features (n=1) (Table 1).

In our study, there were 26 patients less than 40 years old (male n=11, female n=15), 221 patients between 40-60 years old (male n=114, female n=107) and 379 patients older than 60 years old (male n=200, female n=179). Although not statistically significant, the EGFR mutation rate was higher in patients with age >60 (Roche Diagnostics, USA) years as compared to < 60 years (61.4% vs. 38.5%) ( $p = 0.0661$ ). With respect to gender, mutation rate in females was higher in older individuals (>60 years) as compared to 40-60 years (66% vs. 32%; n=123 vs. 59), which was statistically significant ( $p = 0.022789$ ). In males, there was no statistical significance as to age group between 40-60 years and >60 years (54% vs. 41%; n=67 vs. 51;  $p$  value = 0.05313) (Tables 3a and 3b).

Among the 309 EGFR mutated cases, 54.7% (n=168) have deletions in exon 19, 27.4% (n=84) with point mutations in exon 21 L858R, 7.2% (n=22) with exon 20 insertion, 1.3% (n=4) with point mutation in exon 18 G719X, 0.7% (n=2) with point mutation in exon 18 L861Q and 0.3% (n=1) with point mutation in exon 20 T790M. Dual mutations in exons 19, 20 and 21 were found in 8.4% (n=26) of the cases. Dual mutations involving exon 19 in combination with others was seen in 15 (4.9%) patients; exon 21 L858R point mutations in combination

with others was seen in 11 (3.6%) patients. (Figure 1). Out of the 26 cases of squamous cell carcinoma, 9 cases (34.6%) showed EGFR mutation, which include: exon 19 deletion (55.6%, n=5/9), point mutations in exon 21 L858R (33.3%, n=3/9) and exon 20 insertion (11.1%, n=1/9). Exon 19 deletion is found to be the most common mutation among both males and females. But, the prevalence of mutations in exon 19 deletion ( $p = 0.0069$ ) and exon 21 L858R ( $p < 0.0001$ ) were significantly higher in females as compared with males (Table 4 and Figure 2). Between different age groups (<40 years, 40-60 years and >60 years), we did not find any statistical significance in terms of difference in the prevalence of EGFR mutation ( $p$  value=0.4815) (Figure 3).

### DISCUSSION

Because EGFR mutation status has been shown to have a significant prognostic and predictive role in the management of NSCLC, many countries have recommended an accurate EGFR mutational analysis before initiation of treatment to determine appropriate treatment with EGFR inhibitors. Several studies suggested that clinical response to TKIs with exon 19 del

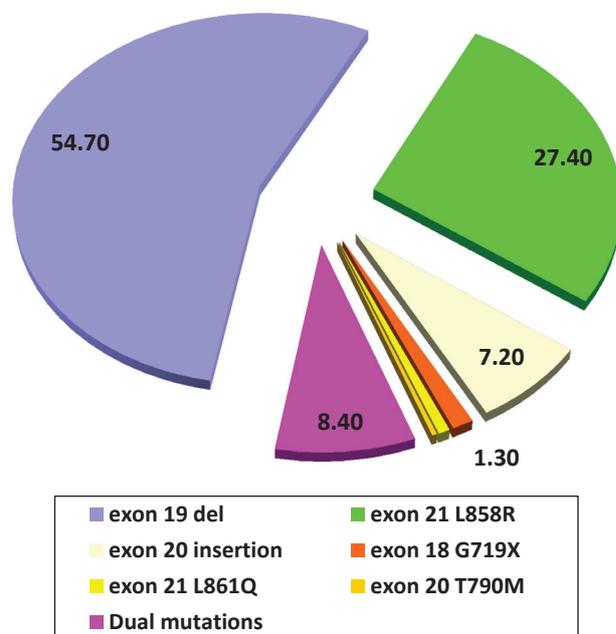


Figure 1. EGFR mutation status among NSCLC Filipino patients.

Table 2. Frequency and overall mutation rates in NSCLC				
	EGFR Positive	EGFR Negative	Percentage of EGFR Mutation	Significance
Male (n=325)	123	202	39.8%	< 0.0001
Female (n=301)	186	115	60.2%	

Table 3a. EGFR mutation status with respect to age and gender				
Age	Male (n=123)	Female (n=186)	Total	Significance
< 40 years	5	4	9	0.0661
40-60 years	51	59	110	
> 60 years	67	123	190	

Table 3b. EGFR mutation status with respect to age and gender						
Age	Male (n=123/325)		Significance	Female (n=186/301)		Significance
	EGFR positive	EGFR negative		EGFR positive	EGFR negative	
< 40 years	5	6	p=0.05313	4	11	p= 0.022789
40-60 years	51	63		59	48	
> 60 years	67	133		123	56	

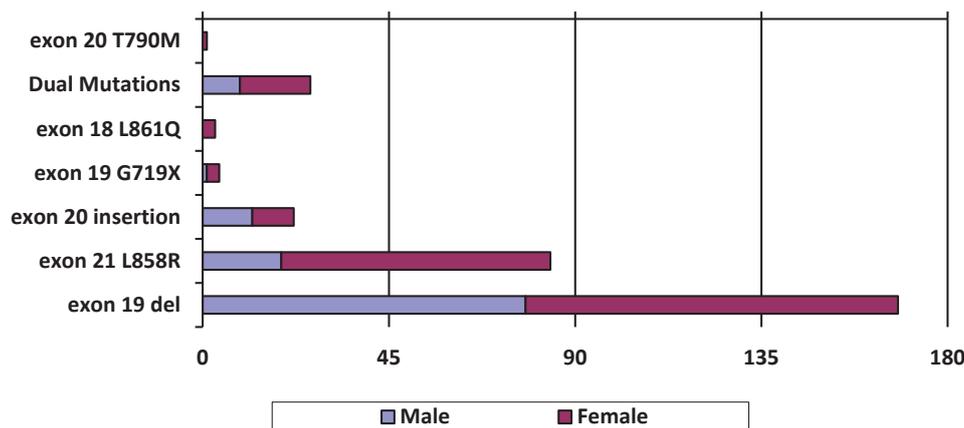


Figure 2. EGFR mutation status with respect to gender.

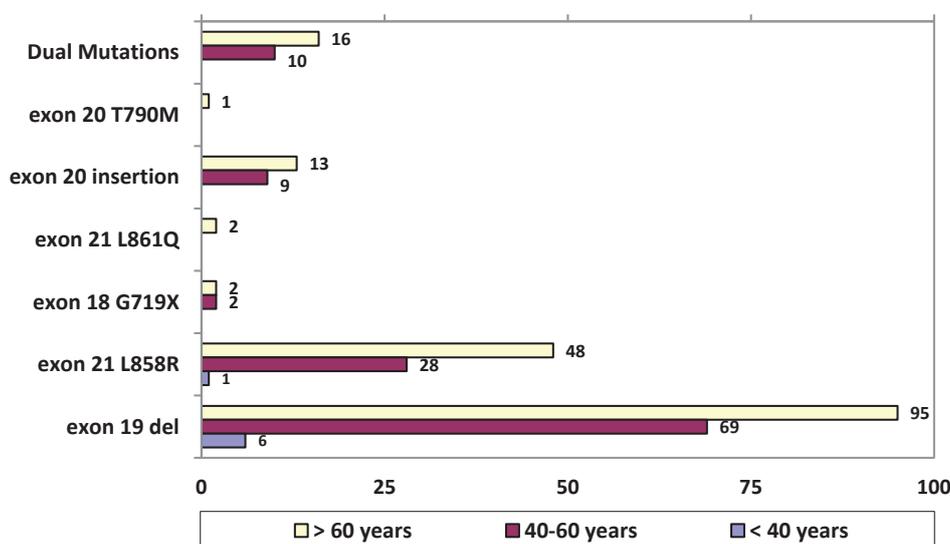


Figure 3. Specific EGFR mutation with respect to age group.

mutations might be more sensitive than with exon 21 L858R. In one study by Jiang-Yong Yu et. al, of the 453 patients with mutations in Exon 19 del and Exon 21 L858R, the response rate of TKIs in patients with Exon 19 del was significantly higher than that with exon 21 L858R mutations (55.2% vs. 43.7%).<sup>13</sup> For rare genotypes, recent studies showed that these could be targetable if appropriate TKI are selected. For example, mutations with G719X, Del18, E709K, insertions in exon 19, S768I or L861Q

have moderate sensitivities to gefitinib or erlotinib with response rate of 30%– 50%.<sup>9</sup> In up to 60%–80% of patients treated with TKIs, the tumor regresses dramatically, but after a median time of 9-12 months, all patients develop acquired resistance to the targeted therapy. A secondary mutation, *T790M*, in the exon 20 of the EGFR gene is the most frequent cause of acquired resistance, which is found in 50%–60% of relapsed cases. Among other causes of acquired resistance are: target-independent mechanisms such as MET amplification (4%), Human EGFR type 2 (*HER2*) amplification (8%–13%), *PIK3CA* mutation (2%), *BRAF* mutation (1%), histological transformation from NSCLC to SCLC (6%), or epithelial–mesenchymal transition (1%-2%). Unknown mechanism of acquired resistance is noted in 18% of cases.<sup>14-16</sup>

Table 4. EGFR (Exon 18-21) mutation types			
	Female N=186	Male N= 121	Significance
Exon 19 deletion	90	78	0.0069
Exon 21 L858R	65	19	0.0001
Exon 20 insertion	10	12	
Exon 18 G719X	1	3	
Exon 21 L861Q	2	0	
Exon 20 T790M	1	0	
Dual mutations			
Exon 19 deletion + Exon 20 insertion	5	2	
Exon 19 deletion + Exon 20 T790M	2	0	
Exon 19 deletion + Exon 18 G719X	2	0	
Exon 19 deletion + Exon 21 L858R	1	1	
Exon 19 deletion + Exon 21 L861Q	2	0	
Exon 21 L858R + Exon 20 insertion	3	5	
Exon 21 L858R + Exon T790M	2	0	
Exon 21 L858R + Exon 20 S768I	0	1	

In the Philippines, the incidence of EGFR mutations is sparsely explored. In the PIONEER study, the authors have demonstrated that approximately half (51.4%) of the patients with NSCLC from seven regions of Asia harbored EGFR mutations. As with regards to Asian regions, Vietnam (64.2%) has the highest incidence while India has the lowest incidence (22.2%). Frequency of EGFR mutations was significantly higher among women (61.1%) than men (44%) (Table 5).<sup>17,18</sup> In previous study from the Philippines, the EGFR mutation rate was also reported to be 42%.<sup>19</sup>

**Table 5.** Prospective, molecular epidemiology study of EGFR mutations in Asian patients with advanced non-small-cell lung cancer of adenocarcinoma histology (PIONEER)(adapted<sup>17</sup>)

	Number of Patients	EGFR Mutation Rates
Country/Region		
China	741	372 (50.2%)
Vietnam	120	77 (64.2%)
Taiwan	174	108 (62.1%)
Philippines	65	34 (52.3%)
Thailand	117	63 (53.8%)
Hong Kong	161	76 (47.2%)
India	72	16 (22.2%)
Gender		
Male	822	362 (44%)
Female	626	384 (61.1%)

In this study, we report the largest EGFR mutation profiling data among Filipino patients with NSCLC from a tertiary private hospital, which showed an overall 49.4% EGFR mutation, mostly were adenocarcinoma. This prevalence is similar to those reported in Asia.<sup>17</sup> Consistent with the literature, we found a significantly higher incidence of EGFR mutation among women than men (62% vs 39.8%). Among patients with squamous cell lung carcinomas, there have been few studies within East Asians with conflicting results: two study from China reported EGFR mutation rate of 21% (3/14) and 13.3% (4/30) compared to a Korean based study with a mutation rate of 7.3% (3/41).<sup>20-22</sup> We found EGFR mutation rate of 35% (9/26) in small cohort of 26 patients with squamous cell carcinoma. In which 4 of these cases were confirmed by immunohistochemical staining as histologically squamous cell carcinoma. Of these positive specimens, 6 came from FNAB/CT-guided biopsy of lung, 2 from bronchial biopsy and 1 lobectomy specimen. We cannot entirely rule out an adenocarcinoma component of Adenosquamous carcinoma in the cases where no immunohistochemical staining was performed. Nevertheless, rare occurrence of EGFR mutation among cases diagnosed as SQCC histologically warrants inclusion of these cases for EGFR mutation screening in routine practice.

With regards to individual mutation types, the most common mutation in these studies detected was deletion in exon 19 (54.7%) followed by L858R point mutation in exon 21 (27.4%), similar to that described in IPASS study. The IPASS study showed 53.6% had exon 19 deletions and 42.5% had a mutation at exon 21 (L858R).<sup>23</sup> The Pioneer study showed the following mutation rates: 24.6% for exon 19 deletion and 22.8% for L858R point mutation.

We found dual mutations in exons 19, 20 and 21 in 8.4% of the cases. In the Pioneer study, dual mutations were also seen; exon 19 deletion in combination with others comprising 24.3% [352 of 1450] and L858R point mutation in exon 21 alone in combination with others were 22.9% [332 of 1450].<sup>17</sup> But in the IPASS study, patients that were dually mutated were 4.2% (11/261).<sup>23</sup> The variability of the incidence of dual mutations identified in these studies may be secondary to different diagnostic sensitivities of the different molecular platforms utilized.

Major limitations of this study are the following: correlation of EGFR mutation patterns with other clinical characteristics (e.g. smoking history, patient’s family history, grading and staging of NSCLC), complete information on targeted treatment received, response to targeted therapy and overall survival data in the Filipino population. Since the cases were collected from 2015-2016, survival data of these patients were far from maturity.

In summary, western population shows a mutation rate of 20%<sup>11,12</sup> and Asian population show a heterogeneous mutation rate of

22–64%.<sup>17-19</sup> EGFR mutation rate of 49.4% among Filipino population in our cohort is similar that of East Asian patients. EGFR mutations were significantly higher in females than males. Although EGFR mutation is common among younger patients, interestingly, we found mutation rate among females to be higher among older individuals (>60 years). This study warrants validation in a larger prospective study.

**CONCLUSION**

The incidence of EGFR mutations in NSCLC varies across different ethnicity. In previous reports, the frequency of EGFR mutations among the Asian population are approximately 30% (with a range of 22.2% to 64.2%) compared with the western population (20%). In the Philippines, the incidence of EGFR mutations is sparsely explored. Here we report the largest EGFR mutation profiling data among Filipinos with NSCLC in a private tertiary care setting, with a frequency of 49.4%. This prevalence is almost similar to those reported in Asia. EGFR is differentially mutated among NSCLC patients with different gender, as women have significantly higher incidence than men. With regards to individual mutation types, the most common mutations detected were deletion in exon 19 followed by L858R point mutation in Exon 21. Also, rare occurrence of EGFR mutation among cases diagnosed as squamous cell carcinoma histologically warrants inclusion of these cases for EGFR mutation screening.

Hence, this study establishes relevance of routine EGFR mutation diagnostics for NSCLC patients in the clinical setting and emphasizes effectiveness for adoption of EGFR inhibitors as a treatment among Filipino population. Further studies to correlate EGFR mutation patterns with other clinical characteristics (patient’s family history, smoking history, grading and staging of NSCLC), response to targeted therapy and overall survival in the Filipino population is warranted.

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All authors certified fulfillment of ICMJE authorship criteria.

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## Dedifferentiated Chordoma in a 53-year-old Female

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### ABSTRACT

Dedifferentiated Chordomas are rare variants of a malignant bone tumor arising from remnants of the embryonal notochord. Most cases are reported from chordomas that have recurred after surgical resection and/or radiation. Dedifferentiated Chordomas have an overall poorer prognosis compared with conventional chordomas due to their highly aggressive behavior and high metastatic potential. We report a case of a Dedifferentiated Chordoma from the sacrum in a 53-year-old female with no known prior surgery or radiation treatment. The associated clinical and radiologic features are discussed along with a review of the reported cases in the English literature. The diagnostic pitfalls and approach for Chordomas as well as the current and developing treatment modalities are also reviewed.

*Key words: chordoma, dedifferentiated chordoma, bone tumor, bone malignancy*

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### INTRODUCTION

Chordomas are low to intermediate grade, malignant tumors that arise from remnants of the embryonal notochord. These tumors are locally aggressive and have a propensity to recur and metastasize.<sup>1</sup> They are rare, with an incidence rate of less than 0.1 per 100,000 per year. According to most sources, chordomas account for approximately 5% of all malignant primary bone tumors.<sup>2</sup> Chordomas are most common in adults greater than 30 years of age, with a peak in incidence in the 6th decade. It is rare in patients less than 20 years old. Males are twice more commonly affected than females.<sup>1</sup>

There are three (3) recognized histologic variants: conventional, chondroid, and dedifferentiated. Another occasionally noted variant is the sarcomatous type. The axial spine is the most commonly-involved site. 60% of tumors are in the sacral spine, 25% occur in the spheno-occipital area, 10% are cervical, and 5% are thoracolumbar. Extra-axial sites are exceedingly rare. Surgery is considered the mainstay of treatment.<sup>1</sup>

The dedifferentiated variant of chordoma was first described by Meis et al., (1987).<sup>3</sup> It is characterized histologically as a tumor containing areas of conventional chordoma with a high-grade sarcomatous component, these areas being admixed and sharply demarcated.<sup>2</sup> There have been 16 reported cases in the English literature since 1970 that fulfill these specific criteria.<sup>4</sup> The dedifferentiated chordoma is discriminated from a sarcomatous chordoma wherein a transitional component between the conventional and sarcomatous elements exists in the latter. In terms of behavior and prognosis, however, there is no difference between the two.<sup>4</sup>

### CASE

A 53-year-old Filipino female presented with occasional back pain two (2) years prior to surgery. The pain was managed with intake of pain relievers. The patient did not report urinary incontinence, stool retention, tenesmus, sciatic-like pain or paresis of the lower extremities. The patient's personal and family medical histories were non-contributory. This continued until



five (5) months prior to surgery when the back pain was noted to have worsened and was no longer relieved by pain medications. Physical examination revealed a non-tender, movable mass on the left gluteal area. Abdominal ultrasound revealed a pelvic mass, initially considered to be an ovarian neoplasm. Exploratory laparotomy was done, revealing an unremarkable uterus and bilateral adnexa. Intraoperatively, a presacral mass was noted.

One (1) month prior to surgery, Magnetic Resonance Imaging (MRI) of the pelvic area was performed. A large, mixed intensity, lobulated, soft tissue presacral mass measuring 12.9 x 14.1 x 11.9 cm was noted. A chordoma was considered radiographically and clinically. The patient then underwent excision. Intraoperatively, the presacral mass was noted to have extended to the left gluteal area.

The primary mass measured 8 x 7.5 x 6 cm. The mass has cream-tan to tan, solid cut surfaces with foci of hemorrhage and necrosis. It is surrounded by a 0.2 cm-thick fibrous tissue. It is clearly-delineated from the surrounding muscle. Microscopic examination revealed the two (2) histologic components for the diagnosis of dedifferentiated chordoma. It consists of areas compatible with a conventional chordoma (Figures 1 and 2) and areas with sarcomatous differentiation (Figure 3). No transitional areas were seen between these components.

In addition, immunohistochemistry with Cytokeratin (CK), Epithelial Membrane Antigen (EMA) and S100 (Figures 4, 5 and 6) revealed moderate to strong staining in the tumor cells.

As of this writing, the patient has undergone radiotherapy of the sacral area and has remained tumor-free seven (7) months since the operation.

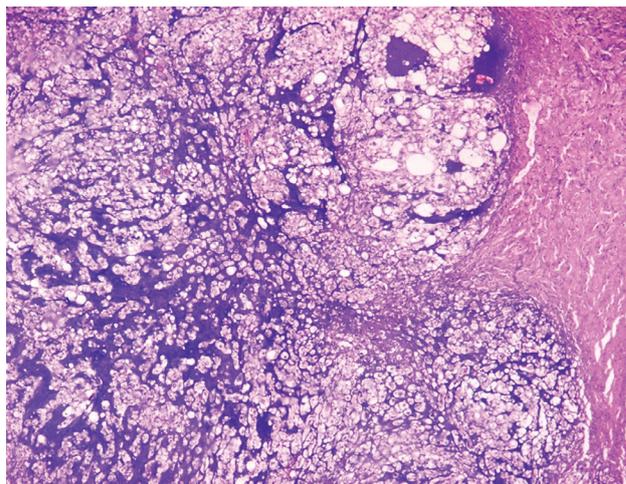
## DISCUSSION

Grossly, chordomas present typically as a lobulated tumor ranging from 5 to 15 cm in size. In most cases it is associated with extension beyond the bone and into the surrounding soft tissues.<sup>5</sup> Among the three (3) tumor variants, the dedifferentiated chordoma accounts for less than 5% of all chordomas.<sup>4</sup>

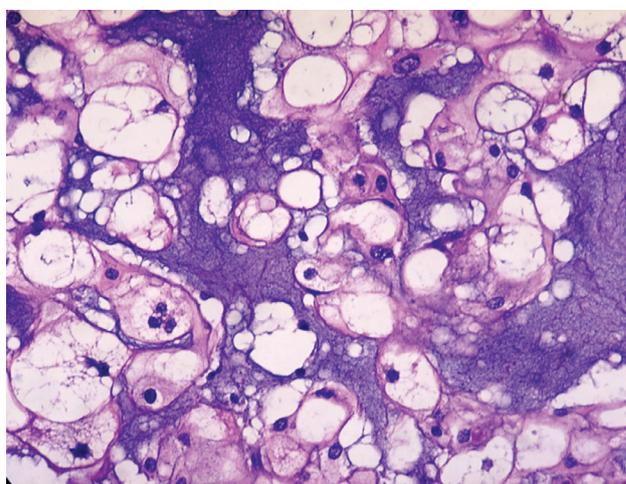
Patients' symptoms largely depend on location but are most commonly neurologic. Pain is the usual initial presenting symptom. Obstructive symptoms, such as constipation, may also occur.<sup>6</sup>

Radiographically, chordomas are commonly located centrally, are solitary and lytic, and contain large areas of geographic destruction of tissues with scattered calcified areas. The tumors are hypo or isointense with T1 MRI, whereas they are hyperintense with T2 MRI.<sup>6</sup> Imaging cannot distinguish between the various subtypes.<sup>4</sup>

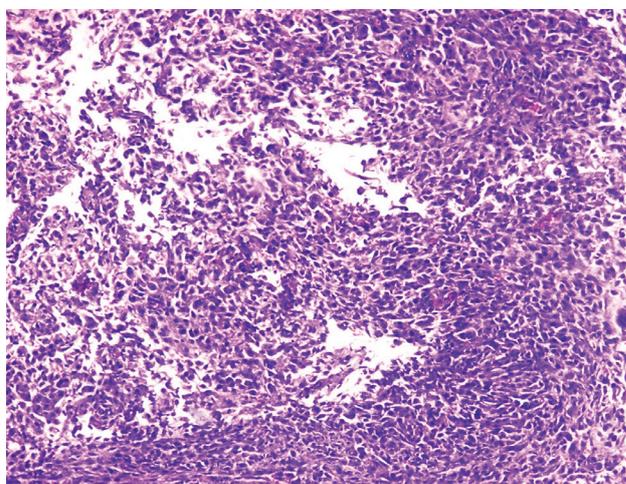
Microscopically, the presence of physaliphorous cells characterizes a chordoma. Physaliphorous cells have abundant pale, vacuolated or bubbly cytoplasm with small, hyperchromatic nuclei. These cells are admixed in a myxoid to chondromyxoid matrix and the tumor is divided into lobules by fibrous septa.<sup>1</sup> Immunohistochemistry and other special techniques are not routinely required for arriving at the diagnosis in the presence of these associated histomorphologic features.<sup>7</sup> If these classic features are variably present, however, immunohistochemistry is



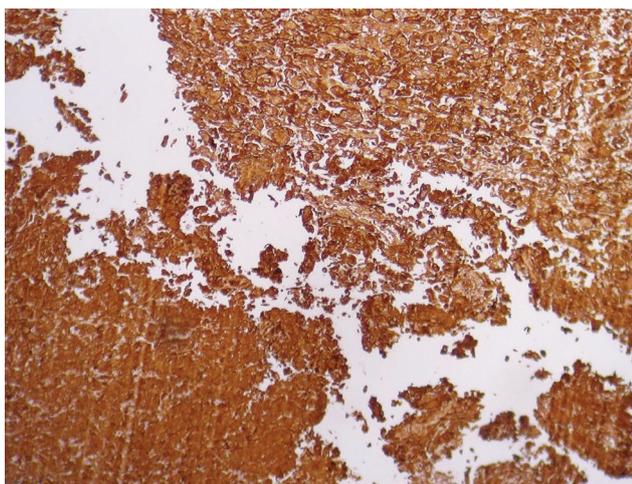
**Figure 1.** The tumor cells are infiltrative and are arranged in sheets, nests and chords. These areas are divided into lobules by fibrous septa (H&E, 20X).



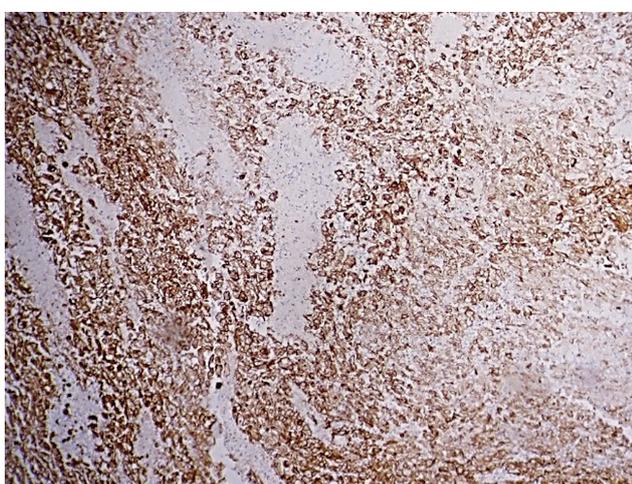
**Figure 2.** Cells with abundant pale, vacuolated/bubbly cytoplasm with small, hyperchromatic nuclei (physaliferous cells) seen alongside cartilage-like areas. There is mild to moderate nuclear atypia and rare mitoses (H&E, 40X).



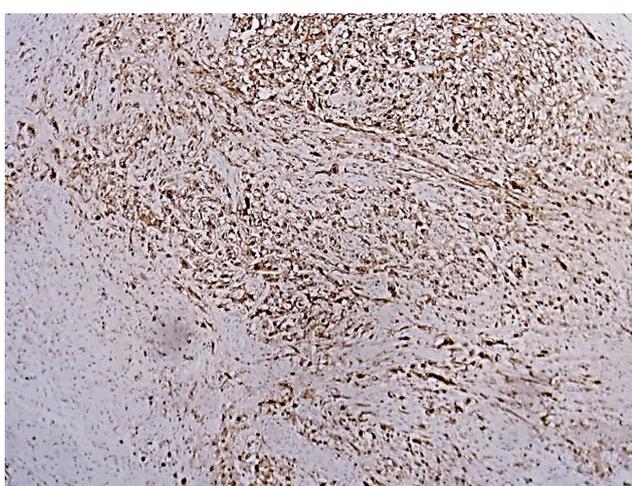
**Figure 3.** Dedifferentiated area with frank and pleomorphic, sarcomatous morphology (H&E, 20X).



**Figure 4.** Strong cytoplasmic and membranous staining with Cytokeratin in the tumor cells, including the sarcomatous areas (Cytokeratin, 20X).



**Figure 5.** Moderate membranous staining with Epithelial Membrane Antigen (EMA, 20X).



**Figure 6.** Moderate cytoplasmic and membranous staining with S100 (S100, 20X).

**Table 1.** Useful immunohistochemistry markers in ruling out differential diagnoses for chordoma

Entity	CK	EMA	S100	Brachyury
Chordoma	+	+	+	+
Chondrosarcoma	-	-	+	-
Renal Cell Carcinoma	+	+	Variable to negative	-

warranted to rule out the closest histologic mimickers. Among the closest differentials are chondrosarcoma and metastatic renal cell carcinoma. Ruling out these differentials is important as they differ significantly from chordoma in terms of management and prognosis.

Positivity for CK, EMA, and S100 are traditionally used to support the diagnosis of a chordoma.<sup>8</sup> This panel is generally sufficient to rule out the closest differential diagnoses (Table 1).

Immunoreactivity for these markers may be lost in the dedifferentiated areas. Should these prove insufficient, testing for Brachyury may be performed. It is the most sensitive and specific marker for tumors of notochordal origin, and it is completely absent in non-notochordal tumors. A study by Jambhekar et al., (2010) showed that Brachyury stained 90% of chordomas.

The mainstay of treatment for chordomas is surgical excision. Radiotherapy may be used in tumors near the skull base, and chemotherapy currently has no widely-accepted role.<sup>9</sup> Molecularly-targeted therapy has shown to be promising. A study by Casali et al., (2004) used the tyrosine-kinase inhibitor Imatinib mesylate for managing chordomas post-operatively.<sup>10</sup> The median disease-free interval was 32 weeks. This, among other treatments, are still under evaluation.

Tumor recurrence is high, generally associated with inadequate resection. The five (5)-year recurrence rate is 48%, while the 10-year rate is 67%.<sup>1</sup> The five (5) and 10-year survival rates are 45-77% and 28-50%, respectively. Metastatic disease is found in up to 43%<sup>6</sup> of cases either at the time of presentation or after excision.

**SUMMARY AND CONCLUSION**

Dedifferentiated chordoma is a rare and aggressive primary bone tumor. Surgery is the mainstay of treatment. No adjuvant treatment modalities have so far proven effective. It has high rates of recurrence and metastasis and carries an overall poor prognosis.

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**AUTHOR DISCLOSURE**

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## Platelet-Derived Growth Factor Receptor-Alpha D842v Mutation in a Spindle Cell Type Gastrointestinal Stromal Tumor\*

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### ABSTRACT

Molecular genotyping of gastrointestinal stromal tumors is not yet available in the Philippines. We report a case of a 75-year-old male with a gastric submucosal mass, who underwent gastroscopic/laparoscopic wedge resection. Histopathology and subsequent immunohistochemical staining with CD117 (CKIT) and DOG1 revealed diagnosis of gastrointestinal stromal tumor, spindle cell variant. On genotyping, the tumor harbored PDGFRA D842V mutation, a subtype resistant to Imatinib treatment.

*Key words:* gastrointestinal stromal tumor (GIST), platelet-derived growth factor receptor-alpha (PDGFRA)

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### INTRODUCTION

Molecular advancements in pathology have established various mutations in c-KIT and platelet-derived growth factor receptor-alpha (PDGFRA) genes, which affect the prognosis and therapy of GIST, specifically their response to tyrosine kinase inhibitors (Imatinib mesylate). Although majority of GIST have mutations in c-KIT gene, mutations in PDGFRA are seen in 5 to 7% of GISTs.<sup>1</sup>

Of the different PDGFR- $\alpha$  exons involved, a point mutation in exon of PDGFRA D842V has been reported to be notoriously resistant to Imatinib.<sup>2</sup> In this case report, a mutational analysis is performed on a diagnosed GIST and the presence of a PDGFRA D842V mutant is identified. The importance of molecular subtyping of GISTs, as recommended by the National Comprehensive Cancer Network (NCCN) and European Society for Medical Oncology (ESMO), will also be discussed.

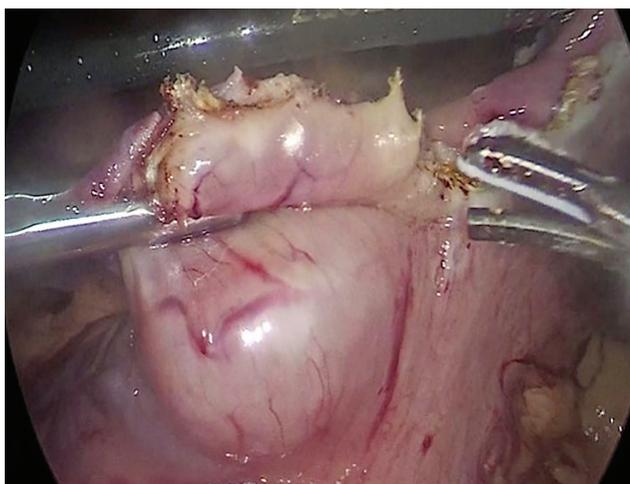
### CASE

A 75-year-old male complained of 6-month history of epigastric pain accompanied by generalized body weakness, early satiety and vomiting of previously ingested food. Endoscopy was done which showed a submucosal mass along the anterior body of the stomach. A subsequent whole abdominal CT scan with contrast was done which showed a soft tissue mass along the anterior mid-body of the stomach, measuring 3 x 2.4 cm. The patient underwent laparoscopic wedge resection of gastric mass with intraoperative gastroscopy/laparoscopic and endoscopic cooperative surgery (LECS) and the specimen was sent for histopathologic examination (Figure 1).

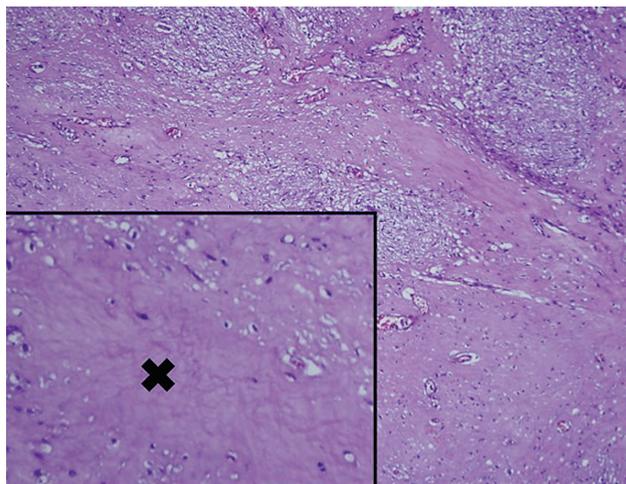
### Histopathology

Grossly, a light brown to brown, soft to rubbery, irregular tissue fragment was noted measuring 4.8 x 3.5 x 2.3 cm. Sectioning revealed a 2.5 x 2.3 x 2.1 cm. light brown, well-circumscribed mass underneath the mucosa. It has focal areas of hemorrhage.

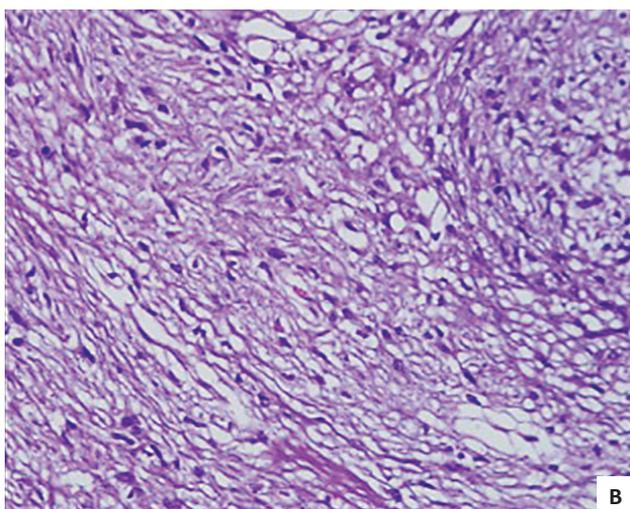
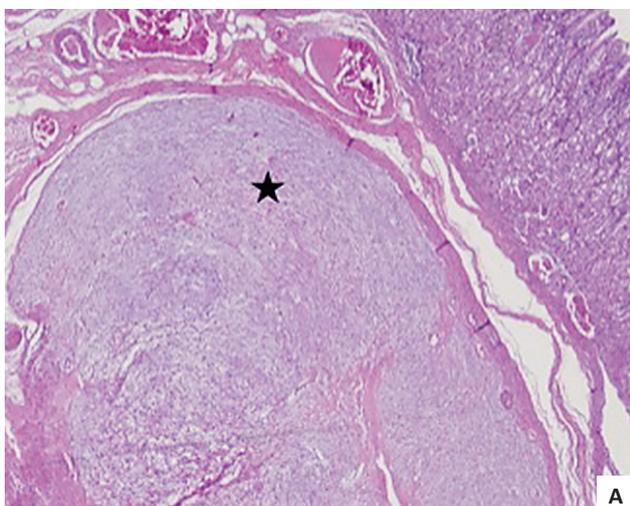




**Figure 1.** A soft tissue mass along the anterior mid-body of the stomach, measuring 3 x 2.4 cm.



**Figure 3.** Gastric mass with areas of necrosis (X).



**Figure 2.** Microscopic sections of gastric mass. (A) Gastric mass at 10X magnification. (B) Gastric mass at 40X magnification.

Microscopy showed nests and sheets of spindle cells infiltrating the muscularis propria. The cells have elongated nuclei, with fine chromatin, inconspicuous nucleoli and moderate amount of pale eosinophilic and fibrillary cytoplasm (Figure 2). Mitotic count

was 0 to 1 per 50 high power fields. However, areas of necrosis were seen in the specimen (Figure 3). The immunohistochemical stains done showed diffusely positive CD117 and DOG1, and negative for Desmin and S100, supporting the diagnosis of gastrointestinal stromal tumor (Figure 4). Based on the National Comprehensive Cancer Network (NCCN), it was stratified as having very low risk for progressive disease.

**Molecular Testing**

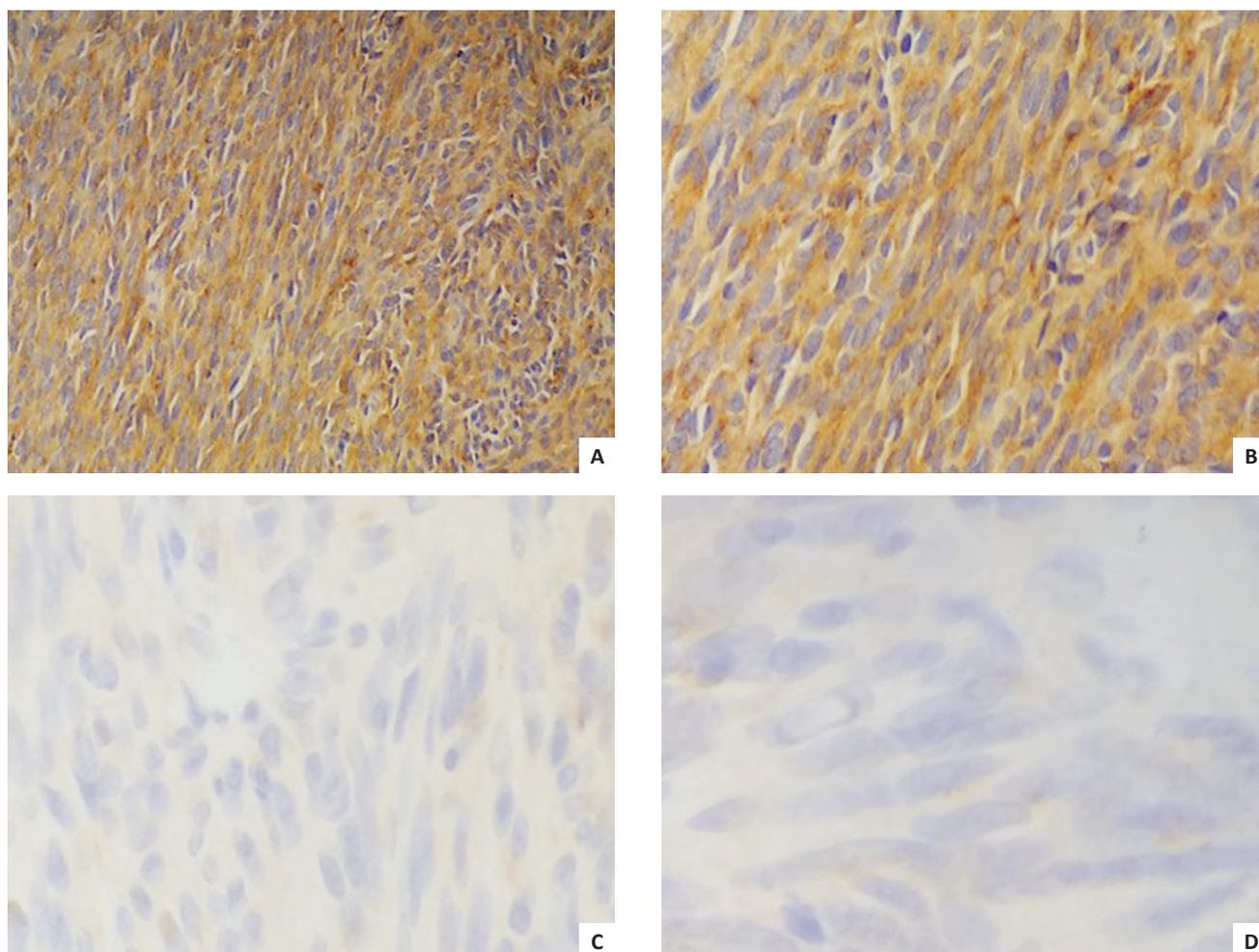
The tumor block was submitted for mutational analysis by Next Generation Sequencing carried out using Illumina MiSeq (San Diego, California). Briefly, the TruSight Tumor 15 (TST) assay panel screens for hotspot regions in 15 genes (AKT1, BRAF, EGFR, ERBB2, FOXL2, GNA11, GNAQ, KIT, KRAS, MET, NRAS, PDGFRA, PICK2CA, RET and TP53), across 250 amplicons.

DNA was extracted from 6 x 4 mm sections of the tumor block using Qiagen FFPE kit and DNA was quantitated using qubit. An extension and ligation-based amplification library preparation assay specific for each of the two strands of DNA was used for all targets and the index sequence was incorporated into tailed universal PCR primers. The resulting libraries were then sequenced using Illumina MiSeq with a minimum read depth of at least 1000x coverage for all amplicons. Demultiplexing and read alignment was then performed using the reference genome UCSC HG19 human build. The Illumina somatic variant caller was used for variant calling.

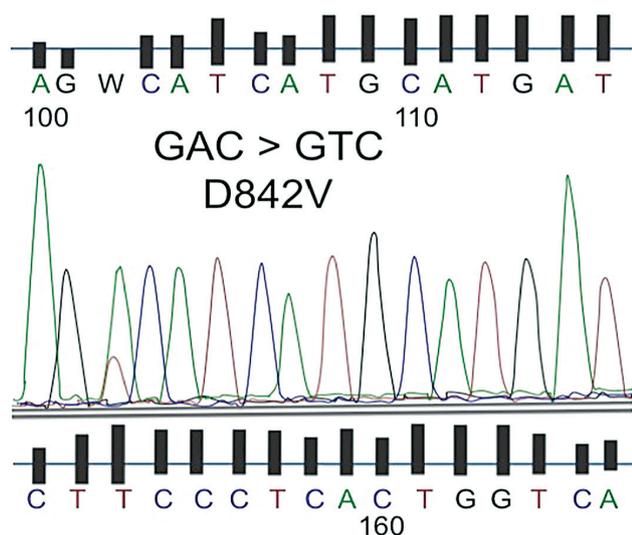
Sanger sequencing was performed to confirm the positive result (Figure 5). PDGFR-α exon 18 was amplified using the following primers: forward primer 5’CAGTACACAGATGGCTTGATC3’ and reverse primer 3’TGAAGGAGGATGAGCCTGAC5’.

**DISCUSSION**

Gastrointestinal stromal tumors are mesenchymal tumors commonly arising in the stomach (60%), followed by jejunum and ileum (30%), duodenum (5%) and colorectal (<5%).<sup>3</sup> In the absence of mutational testing, diagnosis of GIST relies heavily on immunohistochemical staining with CD117 and DOG1, of which the patient tested positive. These immunohistochemical stains can detect most GISTs except for a few (3% to 5%) that harbor PDGFRA mutation.<sup>4</sup>



**Figure 4.** Immunohistochemical stains of the gastric mass. The tumor cells are positive in (A) DOG1 and (B) CD117; and negative in (C) S100 and (D) Desmin.



**Figure 5.** Sanger sequencing result of PDGFRA exon 18 showing D842V mutation.

Gastrointestinal stromal tumors have activating kit (CD117)-positive or platelet-derived growth factor receptor alpha (PDGFRA) mutations resulting in the constitutive activation of protein tyrosine kinase signaling.<sup>5-7</sup> The most common

KIT mutations are detected in exon 11 (66-71%), exon 9 (10-13%), exon 13,14,17 (1% each). PDGFRA mutations (8%) are described in exon 18 (5-6%), exon 12 (1%) and exon 14 (1%).<sup>4</sup> Of importance in this case is the PDGFRA mutation at exon 18, with the missense mutation leading to an amino acid change from aspartic acid to valine (D842V). Tumors with PDGFRA D842V usually have an epithelioid morphology, indolent course and remain localized with low risk of recurrence.<sup>8-9</sup>

However, GISTs harboring this mutation are usually resistant to Imatinib,<sup>1</sup> Interestingly, our case, although PDGFRA positive, histomorphology exhibited spindle cell features. While the tumor size and mitotic count favor a very low risk of progression, the presence of tumor necrosis may indicate a worrisome feature that may warrant close follow up. In a study done by Liang et al, 2007, included in the clinicopathologic parameters that indicate worse prognosis are advanced clinical stage, tumor diameter, mitotic index, coagulative necrosis and risk grade.<sup>10</sup>

Some reports show aggressive behavior of PDGFRA mutants.<sup>11</sup> It is believed that the resistance of PDGFRA-mutant GISTs correlates selectively with the substitution mutations that affect codon D842V of the activation loop, which is associated with a conformational shift of the adenosine triphosphate (ATP)-binding pocket from an “open” or active conformation to a “closed” or inactive conformation.<sup>12</sup>

In a study of 18 participants with PDGFRA mutation who were treated with first line Imatinib, the authors found a significantly different objective response rate between patients with the D842V mutation and those with non-D842V mutations (0% [0/5] vs. 71% [5/7],  $p=0.03$ ). They also found a significantly poorer progression free survival between D842V mutations than those with non-D842V mutant GISTs: median 3.8 months vs. 29.5 months ( $p<0.001$ ).<sup>13</sup>

## CONCLUSION

This case of PDGFRA D842V mutant GIST, histologically classified as spindle cell type with very low risk of progressive disease, raises possibilities that spindle cell type GISTs resistant to standard treatment are present within our population. Hence, mutational profiling of gastrointestinal stromal tumors should be incorporated to routine clinical practice. These patients should be encouraged to participate in clinical trials for investigational drugs with potential activity against this mutation.<sup>12</sup>

For this case, surgery still remains as the primary mode of treatment.<sup>11</sup> With the negative surgical margins, tumor size of 2.5 cm. and mitotic count of 0 to 1 per 50 high power fields, NCCN recommends observation and surveillance. To address this, the patient should be followed up every 3 to 6 months, with recommendations of imaging modalities.

## ETHICAL CONSIDERATION

All efforts to secure patient's consent have been exhausted. The patient's anonymity is ensured. No other identifiers were included.

## STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

## AUTHOR DISCLOSURE

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## Malignant Glomus Tumor of the Heart in a 64-Year-Old Male Presenting with Stroke

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### ABSTRACT

Glomus tumor is a soft tissue neoplasm usually observed as a solitary, or sometimes multicentric painful mass, that rarely occurs extracutaneously. We describe a rare case of malignant glomus tumor of the heart in a 64-year-old male diagnosed with a left ventricular mass. Echocardiography and color flow Doppler revealed a large echogenic mobile structure in the left ventricular cavity that was surgically resected. The histopathologic diagnosis was malignant cardiac glomus tumor. We describe the histopathology, differential diagnosis and clinical presentation of this extremely rare primary cardiac tumor.

*Key words:* cardiac tumor, left ventricular mass, glomus tumor, malignant cardiac glomangioma, embolic stroke

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### INTRODUCTION

Glomus tumor is a mesenchymal neoplasm involving less than 2% of soft tissue tumors.<sup>1</sup> They usually present as painful solitary or multicentric masses, most commonly located subungually and also occurs in the dermis or subcutis of the upper or lower extremity.<sup>2</sup> They are reported to also primarily arise in unusual locations such as the respiratory tract,<sup>3</sup> mediastinum,<sup>4</sup> stomach,<sup>5</sup> vulva,<sup>6</sup> penis,<sup>7</sup> and eye.<sup>8</sup>

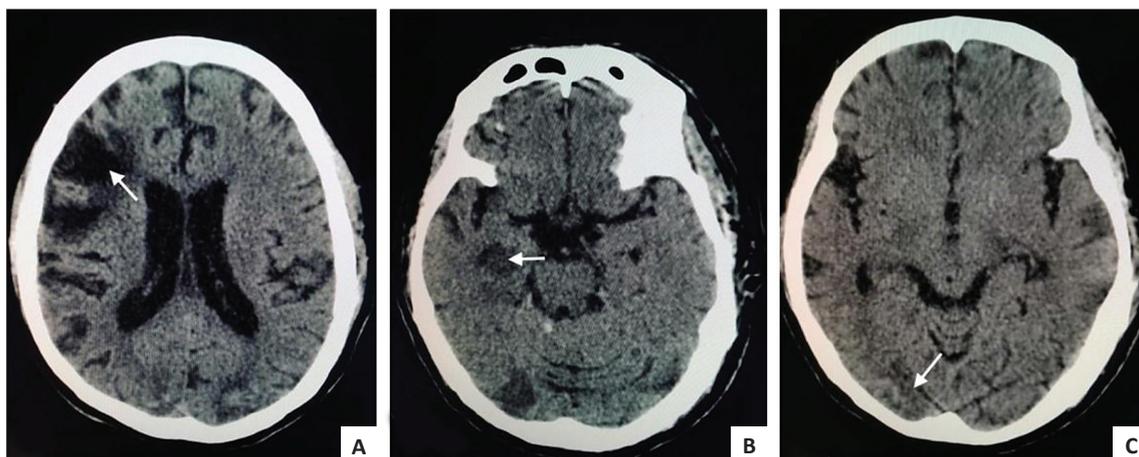
### CASE

The patient is a 64-year-old male who presented with right-sided body weakness but without episodes of chest pain, dyspnea, orthopnea or palpitations. Consultation was done and he was advised surgical intervention, hence admitted in our institution. During his hospital stay, computed tomography (CT) scan of the head was done revealing finding of acute infarct in the right thalamus and right hippocampus, and old hemorrhagic infarct in the right frontal lobe and bilateral occipital lobes (Figure 1).

Echocardiography and color flow Doppler reveal a large echogenic mobile structure seen in the left ventricular cavity, which appears to have a stalk attached to the anterior interventricular septum and anterolateral wall. This measures 3.4 x 2.4 cm at its widest dimension with an estimated area of 6.8 cm. Excision of the mass was done. The specimen was sent for histopathologic examination. The procedure was uneventful and patient was eventually discharged improved.

On gross examination, the specimen consists of several fragmented, tan-brown to gray, irregular, rubbery to friable tissues aggregately measuring 3 x 2 x 1.5 cm (Figure 2). Microscopic sections revealed soft tissue fragments composed of lesional cells in sheets surrounding endothelial-cell-lined vascular channels and supported by fibrous stroma. The individual lesional cells exhibit round to ovoid, hyperchromatic to vesicular nuclei, some with prominent nucleoli and lightly eosinophilic cytoplasm. Few mitotic figures are appreciated. Immunohistochemical studies show the lesional cells with diffuse cytoplasmic reactivity against vimentin and focal reactivity against smooth muscle actin and pancytokeratin (AE1/3). S-100, desmin, calretinin and chromogranin are all negative. The Ki-67 proliferation fraction is





**Figure 1.** (A) CT scan of the head showing encephalomalacic changes in the right frontal lobe. (B) Acute infarct involving the right hippocampus. (C) Hemorrhagic infarct in the occipital lobe.



**Figure 2.** Gross features of the neoplasm showing several fragments of tan-brown, friable to rubbery tissue.

between 20-25% (Figures 3). These findings, in the context of the light microscopic findings provided further evidence to support the above interpretations.

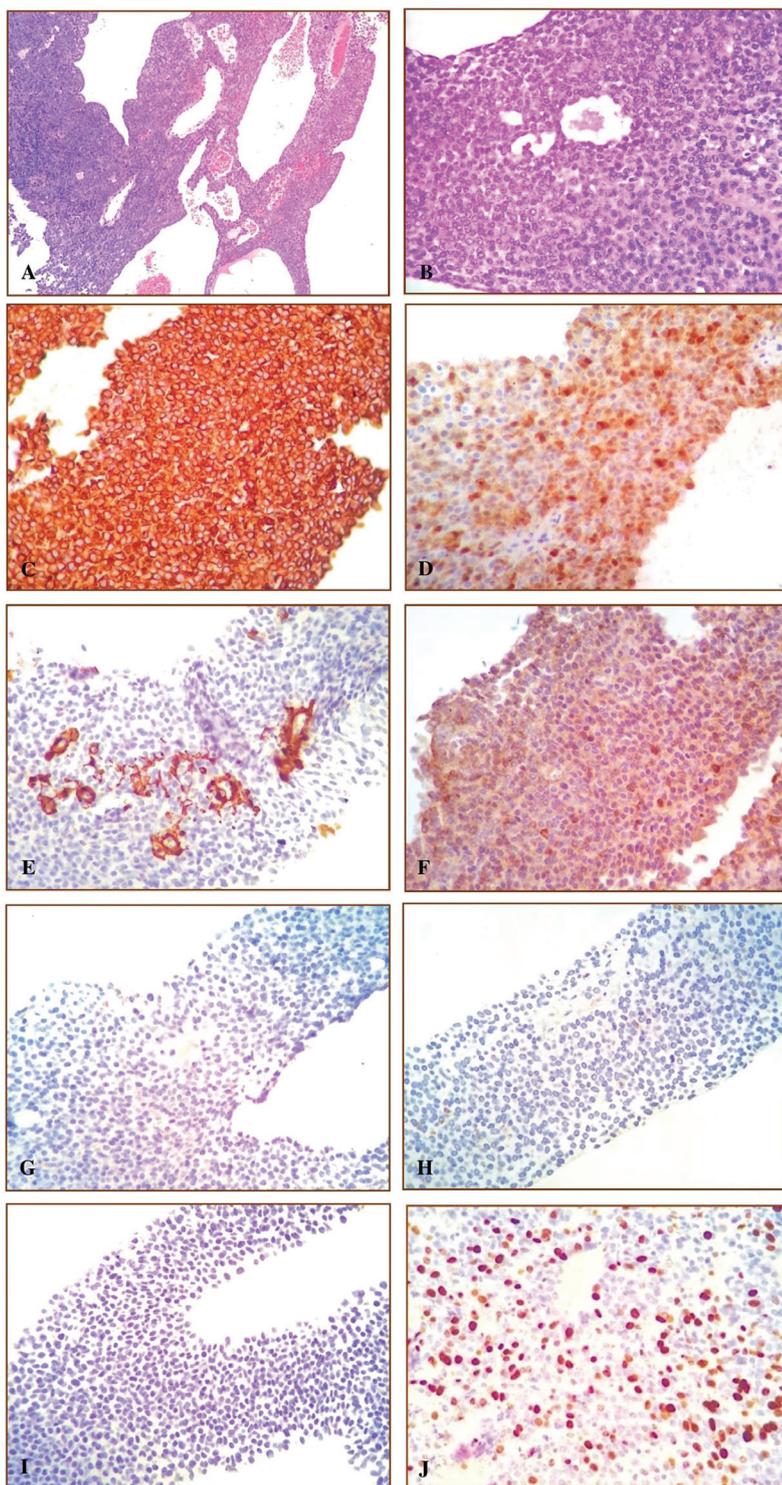
## DISCUSSION

Glomus tumors are rare neoplasms comprising of less than 2% of all soft tissue tumors.<sup>1</sup> These arise from a neuromyoarterial structure, the glomus body,<sup>3</sup> a thermoregulator usually found in dermal and precoccygeal areas,<sup>1</sup> whose main function is to regulate skin circulation.<sup>3</sup> These may also be localized subungually involving the finger tip pulp, which accounts to the common presentation of the tumor as subungual lesion of the fingers,<sup>3</sup> but may also involve areas of the deep dermis in the distal extremities including the palm, wrist, forearm, and foot.<sup>1</sup> Most glomus tumors are solitary, but may appear as multicentric.<sup>2</sup> They often occur in children to adolescent age with a predilection toward males. Multicentric tumors, however, are more prevalent in females.<sup>7</sup> They may appear asymptomatic<sup>7</sup> but typically presents with a characteristic clinical triad of pain, pinpoint tenderness and cold hypersensitivity.<sup>4</sup> Glomus tumors have also been reported in rare locations such as the respiratory tract,<sup>3</sup> mediastinum,<sup>4</sup> stomach,<sup>5</sup> vulva,<sup>6</sup> penis<sup>7</sup> and eye.<sup>8</sup> We describe a highly unusual case of a

glomus tumor of the heart. To our knowledge, this is the fourth glomus tumor case to be reported originating from the heart, and a first in this institution.<sup>9-11</sup>

Typical glomus tumors are well-circumscribed lesions composed of capillary-sized vessels enclosed by cuffs of glomus cells.<sup>7</sup> These are subdivided into three categories; solid glomus tumor, glomangioma, or glomangiomyoma, which is dependent upon the composition of vascular structures, smooth muscle cells, glomus cells and their varying proportions.<sup>3</sup> Characteristic cytomorphologic features are small, uniform, and round cells with centrally located, round nuclei, rimmed by an amphophilic to lightly eosinophilic cytoplasm. The solid glomus tumor is the most common involving approximately 75% of cases, glomangiomas being the second most common involving approximately 20%, and lastly glomangiomyomas accounting only <5% of cases.<sup>3</sup> Atypical glomus tumors are classified by tumor location, size, nuclear atypia, and mitotic figures. This can be categorized into four groups; malignant glomus tumor, glomus tumor of uncertain malignant potential, symplastic glomus tumor, and glomangiomas.<sup>4</sup> Malignant glomus tumors account to less than 20 cases<sup>2</sup> and shows features of severe atypia, increased mitotic activity (>5/50 hpf) or presence of atypical mitosis. Deeply located glomus tumors, located in the visceral organs, including the heart, falls under malignant glomus tumor or glomus tumor of uncertain malignant potential.<sup>4</sup> Folpe et al., proposed a reclassification of malignant glomus tumor to include deep location and a size of more than 2 cm in addition to increased mitotic activity and nuclear atypia in its criteria.<sup>12</sup> Glomus tumor of uncertain malignant potential may only have one of the following: superficial location with increased mitotic activity (>5/50 hpf), or a size of >2 cm only, or deep location.<sup>4,12</sup> Tumors with severe nuclear atypia but lacks criteria for malignant glomus tumor are classified under symplastic glomus tumor. Tumors with diffuse growth resembling angiomatosis with prominent glomus component but lacks criteria for malignant glomus tumor or glomus tumor of uncertain malignant potential are classified under glomangiomas.<sup>4</sup>

Approximately 10% of glomus tumors exist as multiple variants with hereditary familial tendency. The gene responsible for its multiple inherited variant is interrelated to chromosome 1p21–22.3 mutation, transmitted in an autosomal dominant mode with incomplete penetrance and 10% occurrence.<sup>7</sup> Neurofibromatosis, a condition arising secondary to tumor suppressor gene NF1 mutations, is associated with glomus tumors. Germ line



**Figure 3.** (A) Microscopic sections reveal a lesion composed of neoplastic cells in sheets surrounding endothelial-cell-lined vascular channels supported by a vascular stroma (H&E 100X). (B) The individual neoplastic cells exhibit polygonal, hyperchromatic to vesicular nuclei, prominent nucleoli and lightly eosinophilic cytoplasm (H&E 400X). (C) The lesional cells show diffuse cytoplasmic reactivity against Vimentin (400X). (D) and focal reactivity against S100 (400X). (E) The lesional cells show focal reactivity against SMA, prominent on the blood vessels, (400X). (F) and focal reactivity against pancytokeratin (400X). (G) The lesional cells show non-reactivity against Calretinin, (400X). (H) and non-reactivity against Chromogranin (400X). (I) The lesional cells show non-reactivity against Desmin, (400X). (J) and a proliferation fraction of 20-25% by Ki-67 (400X).

mutations in NF1 were found to be present among patients with glomus tumors. On the other hand, somatic NF1 mutations were classified in glomus cells that are alpha-SMA-positive.<sup>13</sup>

The clinical presentation involved in cardiac tumors is usually divided into cardiac symptoms (dyspnea, palpitations, angina, arrhythmias, and cyanosis), systemic manifestations (fever, cachexia, arthralgia, Raynaud's phenomenon, rash, and anemia), and embolic manifestations.<sup>14</sup> Our patient suffered from two episodes of stroke a year apart. On CT, acute infarct involving the right thalamus, and right hippocampus is seen. Old hemorrhagic infarcts involve the left frontal lobe and bilateral occipital

lobes. According to Dias et al, primary cardiac tumors should be considered as a possible cause for embolic manifestations. In his study, cardiac myxoma was the most common cause at 72.6%, followed by fibromas at 6.9%, thrombi at 6.4%, and lastly sarcomas at 6.4%.<sup>14</sup> Surgical resection of cardiac tumors remains the treatment of choice in symptomatic cases.<sup>15</sup> Metastasis is rare but tumor recurrence has been reported.<sup>11</sup>

A differential diagnosis of cardiac glomus tumor include neuroendocrine tumor, mesothelial/monocytic incidental cardiac excrescences (MICE) or lesion of aggregated monocytes and mesothelial cells (LAMM), and smooth muscle neoplasms.

Neuroendocrine tumors, particularly carcinoid tumors, may be confused with glomus tumors, as they are histomorphologically similar; however, the immunohistochemical profile of carcinoid tumors show reactivity to neuroendocrine markers such as chromogranin, as well as with pancytokeratin.<sup>3</sup> Our case is focally reactive against pancytokeratin but nonreactive against chromogranin. MICE or LAMM are composed of cells with round to oval nuclei rimmed by pink cytoplasm, with prominent nuclear grooves, and occasional nucleoli. These cells are reactive against calretinin, and pancytokeratin. Our case is nonreactive to calretinin.<sup>16</sup> Smooth muscle neoplasms are composed mainly of spindle cells arranged in fascicles. Immunohistochemically, they are reactive against smooth muscle markers,<sup>3</sup> such as desmin and smooth muscle actin, which are both nonreactive in our case.

## CONCLUSION

In summary, we report a rare case of a malignant glomus tumor of the heart. This tumor fulfills the criteria for malignancy due to its deep seated location, size (>2cm) and proliferation fraction of 20-25%. Clinically, as with any cardiac masses, this may present with a variety of cardiac-related symptoms and may be causes for embolism and stroke. Surgical excision is an effective treatment in symptomatic cases of malignant glomus tumor of the heart. These tumors rarely metastasize and generally follow a benign clinical course despite its classification. However, recurrence has been reported. We further find that although these neoplasms histologically resemble neuroendocrine tumors, MICE/LAMM, and smooth muscle neoplasms, proper histomorphologic analysis and corresponding immunohistochemical tests may be of use in the differentiation of these lesions. To the best of the authors' knowledge this is the first case of malignant glomus tumor of the heart in this institution.

## STATEMENT OF AUTHORSHIP

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## AUTHOR DISCLOSURE

The authors declared no conflict of interest.

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## Aberrant Diffuse Expression of p63 in Prostate Adenocarcinoma

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### ABSTRACT

We report a rare case of prostatic adenocarcinoma with diffuse aberrant p63 expression in the luminal cells. p63-positive prostatic adenocarcinoma often has distinctive morphology and immunoprofile, but may be confused with benign mimickers of prostate cancer. It is suggested that this tumor variant is molecularly distinct from usual type prostatic adenocarcinoma. Despite sometimes exhibiting seemingly unfavorable Gleason patterns, a less aggressive biologic behavior is often observed. Literature regarding molecular profile, morphologic characteristics, grading, and prognosis of this entity is reviewed.

*Key words: aberrant, AMACR/HMWCK/p63 cocktail, needle biopsy, prostatic adenocarcinoma, p63*

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### INTRODUCTION

In prostate biopsies, the diagnosis of prostatic adenocarcinoma (PCa) is often challenging, especially when the morphologic features are insufficient to establish a definite diagnosis. In evaluating these equivocal cases, either high-molecular weight cytokeratin (HMWCK) or p63 preferably or a combination of these two with AMACR/p504S/RACEMASE, either in a double or triple cocktail, is commonly used as an adjunctive tool in distinguishing PCa from benign mimickers. The absence of immunostaining for basal cells with the use of p63 and HMWCK, which are nuclear and cytoplasmic antibodies, respectively confirms PCa.<sup>1</sup> In addition, this diagnosis is supported by positive immunostaining for  $\alpha$ -methylacyl-CoA racemase (AMACR), a luminal marker that is significantly (but not exclusively) upregulated in PCa.<sup>2</sup>

Recently, cases of an extremely rare variant of PCa with aberrant p63 expression (p63-PCa) have been described. These are characterized by distinct nuclear p63 expression in a non-basal distribution and lack of staining for HMWCK. In 2008, Osunkoya et al reported a series of 21 cases of p63-PCa on needle biopsy and radical prostatectomy specimens.<sup>3</sup> Since then, a few cases of p63-PCa have been reported and a review of the corresponding radical prostatectomy specimens of the 21 needle biopsies with p63-pCa have been published.<sup>4-7</sup> Thus far, no such case has been documented in the country. Herein, we present an additional case of p63-PCa and review the existing literature regarding its molecular profile, histomorphologic characteristics, grading and prognostic implications.

### CASE

We report a case of a 62-year-old male who presented with urinary tract infection. Work-up revealed an enlarged prostate gland weighing 25 grams by ultrasonography; and an elevated prostate-specific antigen (PSA) of 13 ng/mL. He underwent a transrectal ultrasound-guided (TRUS) prostate biopsy reported as benign prostatic hyperplasia with high-grade prostatic intraepithelial neoplasia in another institution; hence, he was advised PSA monitoring. A month after the procedure, his PSA decreased to 3.7 ng/mL. However, the patient was lost to follow-up. On consult few months later, PSA was noted to have increased to 6.08 ng/mL. He underwent another TRUS biopsy (now assessed



by the primary author), which revealed a small focus of atypical glands present suspicious for prostatic adenocarcinoma, in one core. These few well-formed, individual, atypical glands were seen seemingly infiltrating in between benign acini. The glands of interest show rigid lumina and multilayered neoplastic cells which have enlarged hyperchromatic nuclei with rare prominent nucleoli and amphophilic cytoplasm. Immunohistochemical analysis for p63 (antibody clone 7JUL, Biocare Medical®), high molecular weight cytokeratin (HMWCK, antibody clone 34□E12, Leica Bond®), and  $\alpha$ -methyl acyl coenzyme-A racemase (AMACR, antibody clone 13H4, Dako®) in a PIN4 cocktail was performed in accordance with the recommendation by International Society of Urological Pathology (ISUP).<sup>8</sup> The atypical glands showed granular luminal and cytoplasmic positivity for AMACR; and no HMWCK-expressing basal cells were identified among these glands. Surprisingly, some of their nuclei were strongly positive for p63 (Figure 1). The final diagnosis was prostatic adenocarcinoma, involving 5% of one core, with diffuse aberrant staining for p63. A Gleason score of 3+3=6 would be designated if this was a classical acinar prostatic adenocarcinoma.

## DISCUSSION

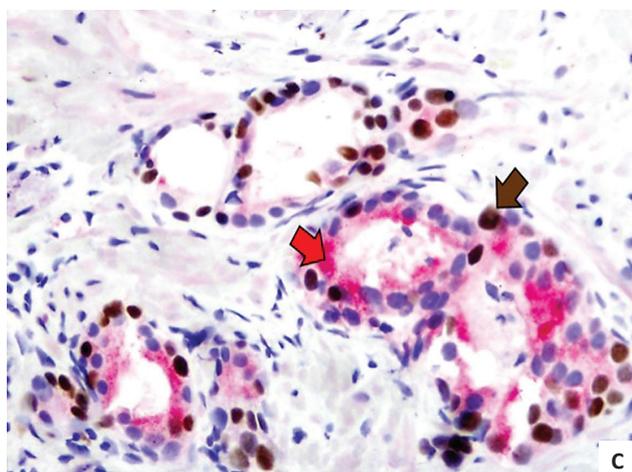
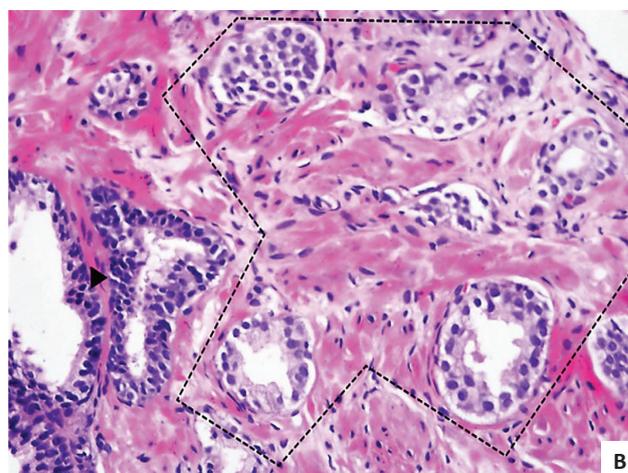
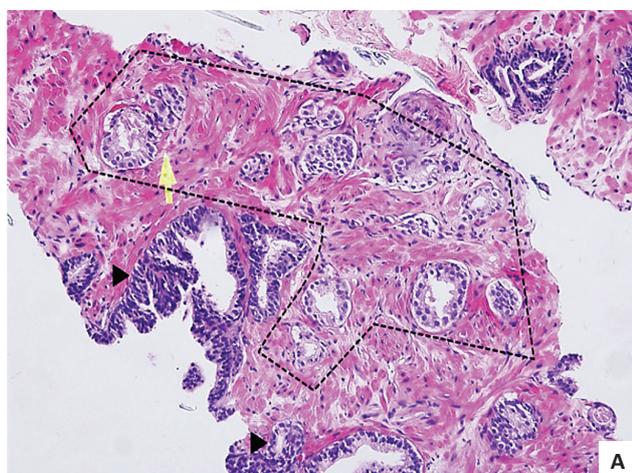
### The role of p63 in prostate development and tumorigenesis

The significance of p63 expression in p63-pCa remains relatively unknown. The transcription factor p63 is encoded by the TP63 locus, which is a member of the TP53 and TP73 family. It plays

a critical role in the formation and maintenance of the prostate stem cells that subsequently differentiate into the basal and secretory cells of the mature prostate epithelium.<sup>9</sup> Within normal prostate epithelium, p63 is selectively expressed in the nuclei of basal cells and is consistently absent in the luminal secretory and neuroendocrine cells.<sup>10</sup>

The vast majority of prostate cancers show loss of p63. The role of TP63 in the development of prostate cancer remains controversial. The debate on whether TP63 is a tumor suppressor gene or an oncogene is mostly due to its structural complexity. It contains 16 different exons coding multiple mRNA isoforms that share a common core DNA binding domain but exhibit varying 5' and 3' ends. Alternate promoters generate two different N-terminal variants: isoforms with an acidic transactivation domain, which are known as TA isoforms; and isoforms that lack this amino-terminal domain known as  $\Delta$ N isoforms. Alternative splicing at the 3' end produces three different C-terminal variants, termed  $\alpha$ ,  $\beta$ , and  $\gamma$ .<sup>11</sup> The predominant isoform in normal prostate and p63-expressing prostatic adenocarcinoma is  $\Delta$ Np63.<sup>12,13</sup>

Studies on  $\Delta$ Np63 expression in p63-PCa, which is demonstrated by  $\Delta$ Np63-specific polyclonal antibody (p40), show that most of the aberrant p63-positive tumors have diffuse positivity for p40 in 96% of cases (23/24). All conventional PCa were negative for p40 in the tumor cells.<sup>14</sup> Since  $\Delta$ Np63 acts as an oncogene, the persistence of p63 expression may serve to drive tumorigenesis and allow maintenance of cancer stem cells in p63-PCa.



**Figure 1.** Prostate core biopsy from the study case. (A) Atypical glands (within dashed lines) with rigid luminal borders are seen infiltrating between benign acini (arrowhead) (H&E 100x). (B) On higher magnification, the cells have enlarged hyperchromatic nuclei with prominent nucleoli and moderate amount of amphophilic cytoplasm (H&E 400x). (C) Immunohistochemical staining for PIN4 decorates secretory luminal cells with nuclear and cytoplasmic immunoreactivity for p63 (brown arrow) and AMACR (red arrow), respectively, and lack of basal staining for HMWCK (PIN4 400x).

TP63 mutational analysis and mRNA quantification in human prostate cancer specimens by Takahashi et al detected no TP63 mutation. However, there is downregulation of p63 expression compared to normal in 39% of cases, and upregulation in 34% of cases.<sup>15</sup> These findings have been challenged since the prostate cancer cell lines examined were generally p63-negative; and contamination with normal basal cells may have confounded the study. To improve purity of the prostate cancer cells used, a similar study was conducted using laser capture microdissection, RT-PCR and gene sequencing for mutational analysis of TP63 in primary tumors, 20 metastases, 28 tumor xenografts, and 7 prostate cancer cell lines. Results showed that the pattern of TP63 mRNA expression in normal prostate tissue is retained in primary prostate cancers, although the levels of expression were markedly reduced.<sup>12</sup> Because similar levels of TP63 mRNA for all isoforms were detected even after laser capture microdissection, prostate cancers undeniably express TP63 mRNA. A potentially functional TP63 mutation was identified in only one prostate tumor. Since majority of the prostate cancer cell lines and patient tumors examined did not contain TP63 mutations, it is suggested that somatic mutations are not the cause of downregulation of p63 expression in majority of prostate cancers. Further, if TP63 is functioning as prostate cancer gene it likely functions as a tumor suppressor.<sup>12</sup> These findings support that it is indeed possible to have prostate cancers with positive p63 immunostaining since the p63 protein is still expressed, albeit in reduced amounts; and may even be upregulated in some cases. The molecular mechanisms underlying the absence of immunostaining of p63 in the vast majority of prostate cancer specimens are yet to be elucidated.

### Molecular profile and immunophenotype of p63-PCa

Extensive genomic analyses of prostate cancer have identified copy number alterations, epigenetic perturbations, and chromosomal rearrangements associated with prostate carcinogenesis. Only a few studies have investigated the molecular distinction between p63-PCa and usual-type prostatic adenocarcinomas. Fusions between the androgen-regulated genes, most commonly the androgen-regulated gene transmembrane protease, serine 2 (TMPRSS2) and v-ets erythroblastosis virus E26 oncogene homolog (ERG), occur in approximately 50% of prostate cancers.<sup>16</sup> These rearrangements are highly specific for PCa or high-grade prostatic intraepithelial neoplasia.<sup>17</sup> In a study by Baydar et al and Wu et al, fluorescence in-situ hybridization on one p63-pCa case and ERG immunohistochemistry on two p63-pCa cases, respectively, were performed; and they found that it lacked TMPRSS2-ERG translocation.<sup>4,5</sup>

Tan et al., collected 37 p63-PCa tumors on radical prostatectomy and biopsy to characterize p63-PCa based on common molecular changes seen in usual-type prostatic carcinomas, including ERG gene rearrangements, serine protease inhibitor Kazal-type 1 (SPINK1) expression, phosphatase and tensin homolog (PTEN) loss and glutathione S-transferase pi 1 (GSTP1) methylation. Results showed that p63-expressing tumors lacked ERG rearrangements by fluorescence in situ hybridization (0/14) and ERG protein expression (0/37). The lack of ERG protein expression in p63-PCa was highly statistically significant compared to unselected cohorts of usual-type adenocarcinomas at their institution where 49.3% (534/1083) are positive for ERG protein.<sup>13</sup>

A recent study, which employed a bioinformatics approach termed Cancer Outlier Profile Analysis, has suggested that the lack of TMPRSS2-ETS family gene rearrangements in

usual-type prostatic adenocarcinoma may be associated with other characteristic molecular changes, such as SPINK1 overexpression.<sup>17</sup> Inactivation of PTEN, a key tumor suppressor gene that is commonly lost in prostate cancer, is strongly associated with ERG fusion-positive tumors.<sup>18</sup> Conversely, in p63-pCa, no tumor expressed SPINK1 or showed PTEN protein loss (0/19).<sup>13</sup>

Hypermethylation of the CpG island at the promoter of GSTP1 has been described as one of the earliest and most commonly found genome alterations arising during prostate carcinogenesis, present in >90% of prostate cancer cases but not in normal prostate tissues. In approximately 95% of usual-type prostatic adenocarcinomas (88/91) cytidine nucleotides in GSTP1 promoter sequences of GSTP1 are hypermethylated, resulting in transcriptional silencing of the gene.<sup>19</sup> In contrast, 74% (14/19) of p63-expressing tumors expressed GSTP1 protein, at least focally, and 33% (2/6) entirely lacked GSTP1 CpG island hypermethylation by bisulfite sequencing.<sup>13</sup> Based on these evidences, it appears that p63-positive PCas may represent a molecularly distinct subtype of PCa.

Usual-type prostatic adenocarcinoma exhibit a luminal cell immunophenotype as these tumors lack basal markers, such as p63 and HMWCK, and diffusely express low molecular weight cytokeratins and markers of androgen axis signaling. A study on p63-positive tumors on radical prostatectomy and biopsy evaluated subsets based on their expression of basal and luminal cell markers. Despite p63 positivity, basal cytokeratins such as CK14 and CK15 were negative in all cases (0/8) and CK5/6 was weakly and focally positive in 36% (4/11) of cases. In contrast, these tumors uniformly expressed luminal-type cytokeratin proteins, such as CK18 (13/13) and CK8 (8/8), and markers of androgen axis signaling commonly observed in luminal cells, including androgen receptor (10/11) and NKX3.1 (8/8). These findings demonstrate that p63-pCa have mixed luminal/basal immunoprofile.<sup>13</sup>

### Histomorphologic characteristics of p63-PCa

In a study by Osunkoya et al.,<sup>3</sup> 90.5% of cases showed a distinctive morphology composed predominantly of glands, nests, and cords with atrophic cytoplasm, hyperchromatic nuclei, and visible nucleoli. In approximately 16% of cases, usual-type prostatic adenocarcinoma and high-grade prostatic intraepithelial neoplasia were present.<sup>3</sup> Giannico et al., investigated the morphologic features of p63-pCa in 21 radical prostatectomy specimens. In 18 cases (85.7%), p63-PCa showed a distinctive morphology consisting of atrophic, poorly formed glands, with multilayered and often spindle-shaped, and basaloid nuclei with prominent nucleoli. In a minority of cases, p63-pCa resembled usual-type acinar atrophic adenocarcinoma or was lined by columnar cells. Similar to usual PCa, the glands exhibit an infiltrative architecture and nuclear atypia including hyperchromatic nuclei and few prominent nucleoli.<sup>7</sup> The current case shows a minute focus of atypical glands (less than a millimeter in dimension) demonstrating the same infiltrative architecture we commonly associate with classical prostatic adenocarcinoma. This was the first feature that alerted our attention that these could be neoplastic glands. However, a departure from the commonly seen atrophic appearance of these glands is that the tumor cells possess more cytoplasmic volume.

Gleason grading was 3+5=8 (38%) and 3+3=6 (28.5%) in majority of the cases. p63-PCa often coexisted with usual-

type acinar prostate carcinoma in 85.7% of cases; but these were usually present in separate nodules. Overall, p63-pCa comprised 65% of the total cancer volume.<sup>7</sup> In two other case reports of p63-pCa diagnosed by transrectal ultrasound-guided prostate biopsies, the atypical prostatic glands exhibited an infiltrative pattern. The cells have mildly enlarged nuclei and rare prominent nucleoli. Both cases were graded as Gleason score 3+3 =6.<sup>4,5</sup>

Aberrant p63 expression is a potential diagnostic pitfall because the immunohistochemical profile may be mistaken as that of benign or atypical glands when either only p63 or basal cell cocktails (p63/HMWCK) are used. Giannico et al., found that the acini in p63-pCa appear frequently atrophic, with a high nuclear-cytoplasmic ratio and a basaloid appearance. This distinctive morphology warrants consideration of basal cell proliferations, such as basal cell hyperplasia and basal cell carcinoma.<sup>7</sup> However, the diagnosis of p63-pCa over basal cell proliferation is favored by the lack of HMWCK expression, and positivity of AMACR and PSA.<sup>5</sup>

### Prognostic implications

Based on available studies, p63-pCa portends a more favorable prognosis than usual-type PCa. It has been proposed that the loss of p63 is associated with higher Gleason scores, an increased likelihood of metastasis, and worse prognosis in mouse metastasis models and in human clinical samples.<sup>20</sup> This is in concurrence with studies by Osunkoya et al., Baydar et al., and Giannico et al., which showed organ-confinement of p63-pCa in 100% (8 of 8), 100% (1 of 1), and 76% (16 of 21) of radical prostatectomy cases, respectively.<sup>3,4,7</sup> There were no lymph node metastases in all 12 of 21 cases with lymph node dissection.<sup>7</sup>

Although majority of cases had an overall Gleason score of  $\geq 8$  in the study of Giannico et al.,<sup>7</sup> mean Ki-67 expression was low (<5%) in all p63-PCa cases with similar expression in the coexisting acinar-type carcinoma. Low Ki-67 (6.25%) was also observed in another study.<sup>3</sup> Due to the discordance between the Gleason score and biologic behavior of p63-pCa, the use of the Gleason grading system may potentially lead to overtreatment. This raises the question on whether these tumors should be assigned a Gleason score.

It is important to make a distinction between aberrant nuclear and cytoplasmic expression of p63. A prospective study among 298 men, who were diagnosed with prostate cancer with predominantly cytoplasmic staining for p63-positive tumor cells, revealed an increase in prostate cancer-specific mortality with increasing expression of cytoplasmic p63 (tertiles). The shift in p63 localization may alter p63 stability, leading to disrupted cell cycle arrest and apoptosis.<sup>21</sup> In regard to the role of p63 in prostate development and tumorigenesis, another protein, aldehyde dehydrogenase 1A (ALDH1), has been reported to be associated with aberrant cytoplasmic p63 expression. In this study 18 out of 45 prostate cancer patients have high expression of cytoplasmic p63. They also reported that higher level of cytoplasmic p63 expression is correlated with higher proliferation by using Ki-67 staining.<sup>22</sup> This is in contrast to the low Ki-67 expression observed in aberrant nuclear p63 expression.

Currently, the prognostic significance of p63-pCa is still not well established. Additional studies are warranted to fully understand the biologic behavior of p63-pCa.

## CONCLUSION

In conclusion, despite representing a rare variant of prostatic adenocarcinoma, recognition of aberrant diffuse p63 expression is critical because its confusing immunohistochemical staining pattern may be misinterpreted as simply benign or atypical especially when the lesion is focal and minute; and when p63/HMWCK basal cell cocktails are used. Pathologists should maintain a high index of suspicion for malignancy when infiltrative architecture and nuclear atypia are observed. In contrast to classic type prostatic adenocarcinomas, p63-PCa exhibit mixed luminal/basal immunophenotype; uniformly lack ERG gene rearrangement, SPINK1 expression, and PTEN loss; and frequently express GSTP1. Despite having unfavorable Gleason patterns, most of these tumors are found to be organ-confined on radical prostatectomy. With the discordance between their Gleason scores and biologic behavior, Gleason grading of these tumors remains debatable.

## ETHICAL CONSIDERATION

All efforts to secure patient's consent have been exhausted. The patient's anonymity is ensured. No other identifiers were included.

## STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

## AUTHOR DISCLOSURE

The authors declared no conflict of interest.

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## Histopathological Detection of *Mycobacterium Tuberculosis* and *Mycobacterium Leprae* using a Modified Acid-Fast Technique

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### ABSTRACT

**Introduction.** *Mycobacterium tuberculosis* and *Mycobacterium leprae* are acid-fast organisms with lipid-rich cell walls that resist decolorization with acidified alcohol after application of a dye with heat. The Ziehl-Neelsen and Fite Faraco staining technique, which are diagnostic tools for identification of acid-fast bacilli (AFB) found in histopathologic samples, are based on this principle. A modification of the Ziehl-Neelsen technique is described as an alternative rapid and reliable method of diagnosis for prompt detection and treatment.

**Methodology.** One hundred and seven (107) archived tissue specimens from autopsy and dermatology cases interpreted as positive for *M. tuberculosis* and *M. leprae* were stained using the proposed modified acid-fast (MAF) technique compared with Fite Faraco (FF) staining method as reference standard. Each specimen was read by two independent evaluators.

**Results.** The degree of diagnostic agreement of the MAF with FF was calculated. For autopsy (n=16) and dermatology (n=91) samples, the Cohen's kappas are 0.765 (substantial) and 0.397 (fair), respectively. Overall, the Cohen's kappa is 0.458 (moderate).

**Conclusion.** The proposed modified Acid-Fast staining method may be considered as an alternative to the conventional Ziehl-Neelsen method and the Fite Faraco method in identifying positive acid-fast bacilli in tissue samples taken from clinical cases of *M. tuberculosis* and *M. leprae*.

**Key words:** acid-fast bacilli, *Mycobacterium tuberculosis*, *Mycobacterium leprae*, tuberculosis, leprosy, Ziehl-Neelsen, Fite Faraco

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### INTRODUCTION

Tuberculosis and leprosy remain among the world's top infectious diseases.<sup>1-3</sup> It has been estimated that about one-third of the world's population is infected with tuberculosis. It has killed nearly two million people each year and is the second leading cause of death worldwide among communicable diseases.<sup>4</sup> In the Philippines, it is the sixth leading cause of death and illness. In 2011, the World Health Organization (WHO) estimated 260,000 incident cases in the country, and that 28,000 people inflicted with the disease, die in a year.<sup>5</sup>

Leprosy on the other hand, has a global registered prevalence of 176,176 cases at the end of the year 2015. In the same year, the number of new cases reported was 211,973. The number of new cases indicates the intensity of the continued transmission of the disease.<sup>6</sup> In 2010, the Philippines had 2,041 new cases detected and 2,873 prevalent cases, while the Western Pacific Region registered 5,055 and 8,386 cases, respectively.<sup>7</sup>

Laboratory diagnosis of *Mycobacterium leprae* is generally made by microscopic and histopathological examination of slit skin smears. The Fite Faraco technique is the oldest method used to detect *mycobacterium leprae* in tissue specimens. This technique, however, has been shown to have low sensitivity ranging from 40% to 70%,<sup>8</sup> is more complex, and takes two and a half hours of staining time from the principal investigator's clinical experience.



*Mycobacterium tuberculosis* is diagnosed using the Ziehl-Neelsen Stain/Acid-Fast Stain which differentiates acid-fast from non-acid-fast bacilli. Although microbiological culture remains as the gold standard for this type of Mycobacterial infection, it takes 1 hour and 15 minutes, and has a limited sensitivity and specificity.<sup>9</sup> Histopathology remains an important method in diagnosis of the disease.

This study aims to determine the diagnostic agreement of a Modified Acid-fast (MAF) staining technique in the histopathological diagnosis of acid-fast bacilli (AFB) in tissue specimens, compared with the Fite Faraco (FF) staining method.

## METHODOLOGY

The study was conducted at the Research Institute for Tropical Medicine (RITM). One hundred and seven (107) formalin-fixed paraffin-embedded archived tissue specimens from previous autopsy and dermatology cases from 2003–2016 were used. Out of the 107 specimens, 16 were from excision during autopsy from different organs of patients who expired from *M. tuberculosis*, and 91 were obtained by punch biopsy from skin lesions of patients seen at the Institute's dermatology clinic with impression of *M. leprae*.

The tissue samples were processed by standard paraffin wax techniques. Each tissue specimen was cut into three sections, 3-4 micra ( $\mu$ ) in thickness. A 3- $\mu$  tissue thickness was utilized for big tissue specimens, such as autopsy and biopsy specimens, while a 4- $\mu$  tissue thickness was utilized for small tissue specimens. One tissue section was utilized for the Fite Faraco Stain, and another section for the Modified Acid-fast Stain. The third section was intended to be used as a back-up sample, in the event that the tissue is sloughed off and the process is to be repeated.

### Fite Faraco Stain

The requirements for the FF stain were the following solutions: (1) xylene-peanut oil solution, which is 1 part peanut oil (local brand) and 2 parts xylene (Merck), (2) carbol fuchsin solution: 2.5 ml melted phenol crystal (BDH), 5.0 ml absolute alcohol (Univar), 0.5 gm basic fuchsin (Merck), and 50 ml distilled water, (3) 1% hydrochloric acid solution: 100 ml 70% alcohol (Univar) and 1 ml concentrated hydrochloric acid (Merck), (4) methylene blue solution (Stock): 1.4 g methylene blue (Merck) and 100 ml 95% alcohol (Univar), (5) methylene blue solution (working): 10 ml methylene blue stock Solution and 90 ml distilled water.

The tissue sections were first deparaffinized through two changes of xylene-peanut oil solution for 12 minutes each and hydrated with distilled water. After draining, the excess oil was blotted to capacity. Thereafter, it was stained in carbolfuchsin stain for 1 hour at 25-35°C room temperature, and washed in tap water for 3 minutes. The slides were then differentiated individually with 1% hydrochloric acid for one minute until sections were faint pink by visual inspection. It was again washed in tap water for 3 minutes, before counterstaining with working methylene blue solution until light blue color is achieved. Excess methylene blue was rinsed off using tap water. Slides were then dipped in xylene before mounting with a resinous mounting medium. The specimen slides were screened for acid-fast bacilli under the microscope. The acid-fast bacilli stain bright red, while nuclei and the background stain blue.

### Modified Acid-fast Stain

The solutions needed for the modified Ziehl-Neelsen stain (Z-N) were the following: (1) carbol fuchsin solution: 2.5 ml melted

phenol crystals (BDH), 5.0 ml 100% alcohol (Univar), 0.5g basic fuchsin (Merck), 50 ml distilled water, (2) 0.3% acid alcohol (Univar), (3) methylene blue Solution (Stock): 1.4g methylene blue (Merck), 100 ml 95% alcohol (Univar), (4) Methylene Blue Solution (Working): 10 ml methylene blue Stock Solution, 90 ml distilled water.

The tissue sections were first deparaffinized, each dipped 3-4 times in descending grade of ethanol, and hydrated with distilled water. The tissue section was immersed in carbol fuchsin solution in a Coplin staining jar and heated for 30 minutes at 63 degrees Celsius in a constant temperature oven. The stain was washed off with running water before decolorizing with 0.3% acid alcohol, 2-4 dips until a faint pink color was achieved, depending on the thickness of the tissue. It was again washed in running water, with excess stain drained off, before counterstaining with methylene blue. Counterstaining was done in 5-6 dips, depending on the stain uptake of the tissue. The tissue section was washed again with tap water and rinsed in distilled water to remove mineral deposits, contaminants and other impurities. It was then dehydrated in 95% ethanol for 2-3 dips. Slides were then dipped in two changes of xylene for 5 minutes each before mounting with a resinous mounting medium. The acid-fast bacilli stain red to bright red, while non-acid-fast organisms are expected to stain blue.

### Slide Interpretation

The samples from each specimen were randomly numbered so that the examiners were blinded to the sample identities, thereby ruling out bias. Before the start of the sample reading, inter-rater reliability was measured for the three independent evaluators using 20 pairs of randomly selected slides, separate from the actual slides in the study. The actual inter-rater reliability was 75.7%, using the intra-class coefficient reliability function of SPSS.

Each of the tissue specimen was read by two independent evaluators under oil immersion microscopy. Bacterial load was determined through quantitative microscopy of the slides under 100X oil immersion lens. The entire area of the section was examined at 100X magnification carefully. The sample is considered positive when one or more acid-fast bacteria is detected in at least one area of the tissue sample. If the bacilli were seen as purple or violet, the staining procedure was repeated with a back-up slide. If there is a difference in the reading of the two evaluators, a third evaluator read the specimen.

### Data Analysis

The degree of agreement of the MAF staining method, compared with FF Staining method as the reference standard, was evaluated using Cohen's kappa. This statistical method is useful for analyzing the agreement between two methods applied to the same sample, especially if one is considered the reference standard and the other is an alternative method.<sup>10,11</sup>

### Ethical Considerations

The study was reviewed by the RITM Institutional Research Board. The samples used in the study were de-identified, so as to protect the anonymity of patients. Only the researchers were able to access the samples in the laboratory.

## RESULTS

Table 1 shows the crosstabulation of the results of the readings using the MAF compared to the FF method as the reference

**Table 1.** Cross tabulation of results of the MAF staining method in relation with the Fite Faraco staining method (n=107)

Modified Acid Fast Staining	Fite Faraco Staining								
	All samples (n=98)			Autopsy samples (n=13)			Dermatology samples (n=85)		
	+	-	Total	+	-	Total	+	-	Total
+	87	2	89	13	1	14	74	1	75
-	11	7	18	0	2	2	11	5	16
Total	98	9	107	13	3	16	85	6	91

**Table 2.** Diagnostic accuracy of MAF staining method using Fite Faraco staining method as reference standard

	All samples	Autopsy samples	Dermatology samples
Cohen's kappa	0.458 (moderate)	0.765 (substantial)	0.397 (fair)
p-value	0.000	0.002	0.000

standard, including a separate cross tabulation for autopsy and dermatology samples. Table 2 summarizes the measures and interpretation of diagnostic agreement<sup>11</sup> for the entire sample, and separately for the two different sample groups.

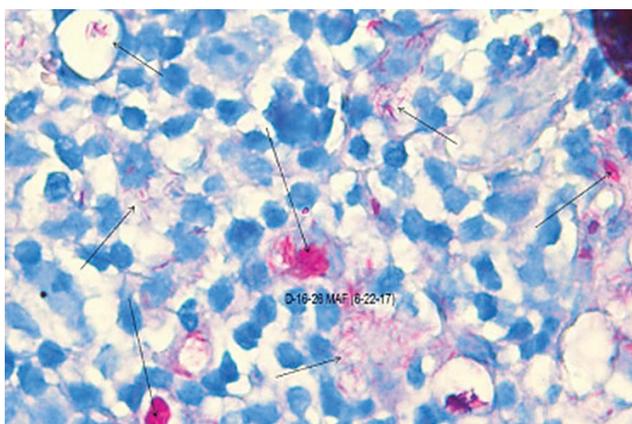
Results showed that the MAF staining method showed moderate overall diagnostic accuracy, compared with the Fite Faraco method. The autopsy and dermatology samples were separated to examine the high number of false negative results in the over-all analysis. All the 11 false negative results were from the dermatology samples. As a result, the MAF staining method for autopsy samples had substantial diagnostic accuracy, compared with dermatology samples.

Figures 1 to 4 illustrate the staining results for the dermatology and autopsy samples using the Modified Acid-fast stain and the Fite Faraco stain.

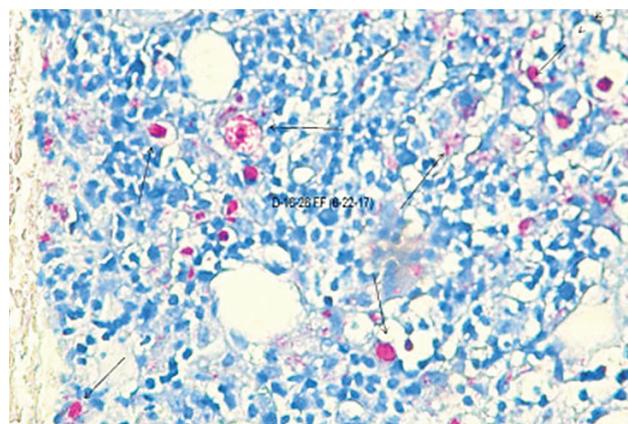
After establishing the diagnostic accuracy of the MAF staining method in randomly selected histopathology samples, the two staining methods were performed on 10 autopsy negative controls and 15 dermatology negative controls to identify false positive results that can be attributed to inappropriate reaction of the staining procedure. These negative controls were collected from samples that did not have a *M. tuberculosis* or *M. leprae* clinical diagnosis. None of these negative samples were found to be positive using the MAF and Fite Faraco staining methods.

**DISCUSSION**

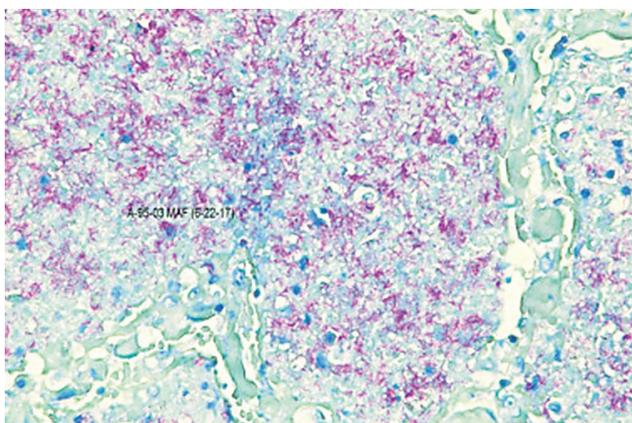
The MAF staining method is a modification of the Ziehl-Neelsen (Z-N) method which the primary investigator developed during her experience in the Histopathology Laboratory of RITM. This modified method involved samples that are processed



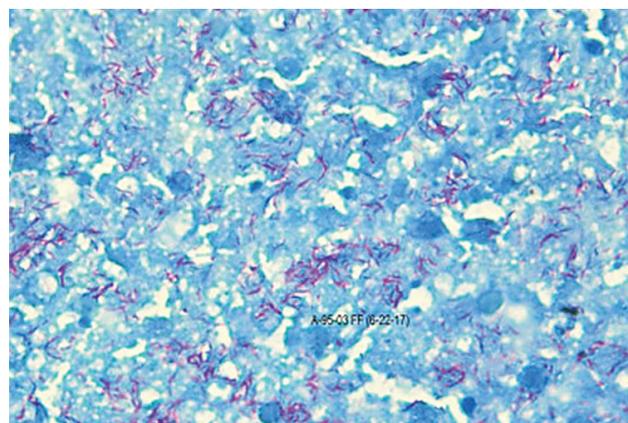
**Figure 1.** Dermatology sample stained using the Modified Acid-fast procedure under oil immersion (100X).



**Figure 2.** Dermatology sample stained using the Fite Faraco procedure under oil immersion (100X).



**Figure 3.** Autopsy sample stained using the Modified Acid-fast procedure under oil immersion (100X).



**Figure 4.** Autopsy sample stained using the Fite Faraco procedure under oil immersion (100X).

for 30 minutes at 63o Celsius in a constant temperature oven, instead of 1 hour under ambient temperature. Instead of using 2% Sulfuric Acid as the decolorizing agent for one minute, 0.3% Hydrochloric Acid was used for 5 seconds. Lastly, counterstain and dehydration times were done for only five seconds, which are shorter by 40 seconds compared to the conventional method.

Some samples positive for FF were negative in the MAF. This may be due either to the thickness of the cut or low bacterial load (i.e., paucibacillary specimens). Initially, there were 11 cases cut at 3µ which were positive for FF but negative in the MAF. These tissues were recut at a thicker 6µ using the microtome. Thereafter, only 3 positive for FF were negative in the MAF, supporting the earlier hypothesis.

These findings can be related to the MAF being similar to the Z-N method, which is known to have high sensitivity and positive predictive value, but with lower specificity.<sup>12-14</sup> The main goal of the Z-N method is to differentiate an AFB from a non-AFB, though not all *Mycobacterium* species can be detected well with this method. *M. tuberculosis* and *M. ulcerans* are strongly acid-fast which Z-N technique can be best used. FF, on the other hand, is more appropriate for *M. leprae*, which is weakly acid-fast.<sup>9,15-17</sup> The Z-N method also requires a high bacterial load (5000 – 10000 AFB/ml) for detection, which may result to false negative results. Deparaffinization with peanut oil and xylene mixture with the Fite Faraco method protects the waxy coat of the bacilli which prevents shrinkage and disappearance during the process.<sup>16,18</sup> Finally, the acid-fast property of *Mycobacteria* can also be affected by the age of the colonies, exposure to ultraviolet light and the heating process involved, and the medium where the bacteria were cultured.<sup>19</sup>

The shortened dyeing time with the carbol fuchsin from 1 hour to 30 minutes showed positive results because, regardless of the time, once stained, these microorganisms are resistant to destaining and cannot be decolorized easily with acid-alcohol solutions. Moreover, the added heat in the procedure enhanced the penetration of the carbol fuchsin dye through the bacterial cell wall and into the cytoplasm.<sup>9,15,20,21</sup>

Using hydrochloric acid instead of sulfuric acid as a decolorizing agent also showed to be effective. The Revised National Tuberculosis Programme recommends the use of sulfuric acid as the decolorizing agent as it easily removes background material even from thick smears making identification of AFB easier.<sup>22-24</sup> On the other hand, the World Health Organization recommends the use of hydrochloric acid in alcohol to provide clean smears and enhance smear positivity, instead of sulfuric acid which was reported to produce unclean smears that can lower smear positivity for AFB.<sup>22,24</sup> Various studies comparing the two agents showed that smears using hydrochloric acid as decolorizing agent have higher sensitivity and specificity compared with those using sulfuric acid.<sup>22-24</sup> In other researches, hydrochloric acid is as good as sulfuric acid as a decolorizing agent.<sup>23,25</sup> In addition, hydrochloric acid is more economical, less costly, easier and safer to dilute and use.<sup>24-26</sup>

A shorter period for counterstaining compared with the conventional Z-N method was also observed to be effective with the prevention of masking and turning the bacilli purple.<sup>27</sup>

Several limitations were faced in this study, such as the use of archived tissues from past cases. An appropriate gold standard

testing (e.g. PCR) was not done with the samples, which precluded the conduct of tests for sensitivity and specificity of the modified acid-fast staining procedure. Future studies should utilize slide blocks cut at 6µ using the microtome to limit false negative results and compare the results of the modified acid-fast staining procedure with PCR of the specimen.

## CONCLUSION

The Modified Acid-fast staining method showed potential as an alternative to the Fite Faraco method in detecting AFB in tissues.

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## ETHICAL CONSIDERATION

Patient consent was obtained before submission of the manuscript.

## STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

## AUTHOR DISCLOSURE

The authors declared no conflict of interest.

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None.

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

Updated December 2017

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## 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](http://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](mailto: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.

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### C. History of the Recommendations

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

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

### A. Defining the Role of Authors and Contributors

#### 1. Why Authorship Matters

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

Because authorship does not communicate what contributions qualified an individual to be an author, some journals now request and publish information about the contributions of each person named as having participated in a submitted study, at least for original research. Editors are strongly encouraged to develop and implement a contributorship policy. Such policies remove much of the ambiguity surrounding contributions, but leave unresolved the question of the quantity and quality of contribution

that qualify an individual for authorship. The ICMJE has thus developed criteria for authorship that can be used by all journals, including those that distinguish authors from other contributors.

### 2. Who Is an Author?

The ICMJE recommends that authorship be based on the following 4 criteria:

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

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

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

The individuals who conduct the work are responsible for identifying who meets these criteria and ideally should do so when planning the work, making modifications as appropriate as the work progresses. 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 co-authors. The corresponding author should be available throughout the submission and peer-review process to respond to editorial queries in a timely way, and should be available after publication to respond to critiques of the work and cooperate with any requests from the journal for data or additional information should questions about the paper arise after publication. Although the corresponding author has primary responsibility for correspondence with the journal, the ICMJE recommends that editors send copies of all correspondence to all listed authors.

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

tific 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 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 non-profit, 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 their own commitments and those of their 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 ongoing.

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

## **C. Responsibilities in the Submission and Peer-Review Process**

### **1. Authors**

Authors should abide by all principles of authorship and declaration of conflicts of interest detailed in section IIA and B of this document.

### **a. Predatory or Pseudo-Journals**

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

## **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 post in a place that potential authors can easily access.

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

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

#### **d. Integrity**

Editorial decisions should be based on the relevance of a manuscript to the journal and on the manuscript's originality, quality, and contribution to evidence about important questions. Those decisions should not be influenced

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

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

### 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 in-

appropriate behavior that is incompatible with a position of trust.

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

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

### 2. Editorial Freedom

The ICMJE adopts the World Association of Medical Editors' definition of editorial freedom, 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

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

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

Nonessential identifying details should be omitted. Informed consent should be obtained if there is any doubt that anonymity can be maintained. For example, masking the eye region in photographs of patients is inadequate protection of anonymity. If identifying characteristics are de-identified, authors should provide assurance, and edi-

tors 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, Retractions, Republications, and Version Control

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

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

- The journal should publish a correction notice as soon as possible detailing changes from and citing the original publication; the correction should be on an electronic or numbered print page that is included in an electronic or a print Table of Contents to ensure proper indexing.
- The journal should also post a new article version with details of the changes from the original version and the date(s) on which the changes were made.
- The journal should archive all prior versions of the article. This archive can be either directly accessible to readers or can be made available to the reader on request.
- Previous electronic versions should prominently note that there are more recent versions of the article.
- The citation should be to the most recent version.

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

Errors serious enough to invalidate a paper's results and conclusions may require retraction. However, retraction with republication (also referred to as "replacement") can be considered in cases where honest error (e.g., a misclassification or miscalculation) leads to a major change in the direction or significance of the results, interpretations,

and conclusions. If the error is judged to be unintentional, the underlying science appears valid, and the changed version of the paper survives further review and editorial scrutiny, then retraction with republication of the changed paper, with an explanation, allows full correction of the scientific literature. In such cases, it is helpful to show the extent of the changes in supplementary material or in an appendix, for complete transparency.

## **B. Scientific Misconduct, Expressions of Concern, and Retraction**

Scientific misconduct 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 as the Committee on Publication Ethics (COPE) ([publicationethics.org/resources/flowcharts](http://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 retraction and original article should be linked in both directions and the retracted article should be clearly labelled as retracted in all its forms (abstract, full text, PDF). Ideally, the authors of the retraction should be the same as those of the article, but if they are unwilling or unable the editor may under certain circumstances accept retractions by other responsible persons, or the editor may be the sole author of the retraction or expression of concern. The text of the retraction should explain why the article is being retracted and include a complete citation reference to that article. Retracted articles should remain in the public domain and be clearly labelled as retracted.

The validity of previous work by the author of a fraudulent paper cannot be assumed. Editors may ask the author's institution to assure them of the validity of other work published in their journals, or they may retract it. If

this is not done, editors may choose to publish an announcement expressing concern that the validity of previously published work is uncertain.

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

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

## **C. Copyright**

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

## **D. Overlapping Publications**

### **1. Duplicate Submission**

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

### **2. Duplicate and Prior Publication**

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

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

When authors submit a manuscript reporting work that has already been reported in large part in a published article or is contained in or closely related to another paper

that has been submitted or accepted for publication elsewhere, the letter of submission should clearly say so and the authors should provide copies of the related material to help the editor decide how to handle the submission. See also Section IV.B.

This recommendation does not prevent a journal from considering a complete report that follows publication of a preliminary report, such as a letter to the editor, a preprint, or an abstract or poster displayed at a scientific meeting. 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 participants enrolled, key outcomes, and adverse events). The ICMJE encourages authors to include a statement with the registration that indicates that the results have not yet been published in a peer-reviewed journal, and to update the results registry with the full journal citation when the results are published.

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

Authors who attempt duplicate publication without such notification should expect at least prompt rejection of the submitted manuscript. If the editor was not aware of

the violations and the article has already been published, then the article might warrant retraction with or without the author's explanation or approval.

See COPE flowcharts for further guidance on handling duplicate publication.

### 3. *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 (e.g., Angelo M. Journal networking in nursing: a challenge to be shared. *Rev Esc Enferm USP*. 2011 Dec 45[6]:1281-2,1279-80,1283-4. Article in English, Portuguese, and Spanish. No abstract available. PMID 22241182).

### 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 (e.g., from a public database, or systematic reviews or meta-analyses of the same evidence), the manuscripts should be considered independently because they may differ in their analytic methods, conclusions, or both. If the data interpretation and conclusions are similar, it may be

reasonable although not mandatory for editors to give preference to the manuscript submitted first. Editors might consider publishing more than one manuscript that overlap in this way because different analytical approaches may be complementary and equally valid, but manuscripts based upon the same 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 and unique, persistent dataset identifier.

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

### i. Registration

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

Briefly, the ICMJE requires, and recommends that all medical journal editors require, registration of clinical trials in a public trials registry at or before the time of first patient enrollment as a condition of consideration for publication. Editors requesting inclusion of their journal on the ICMJE website list of publications that follow ICMJE guidance [[icmje.org/journals.html](http://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 relationship between a health-related intervention and a health outcome. Health-related interventions are those used to modify a biomedical or health-related outcome; examples include drugs, surgical procedures, devices, behavioral treatments, educational programs, dietary interventions, quality improvement interventions, and process-of-care changes. Health outcomes are any biomedical or health-related measures obtained in patients or participants, including pharmacokinetic measures and adverse events. The ICMJE does not define the timing of first participant enrollment, but best practice dictates registration by the time of first participant consent.

The ICMJE accepts publicly accessible registration in any registry that is a primary register of the WHO International Clinical Trials Registry Platform (ICTRP) ([www.who.int/ictrp/network/primary/en/index.html](http://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](http://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 those that have fields that contain uninformative information, or registrations that are not made publicly accessible such as phase I trials submitted to the EU-CTR. Although not a required item, the ICMJE encourages authors to include a statement that indicates that the results have not yet been published in a peer-reviewed journal, and to update the registration with the full journal citation when the results are published.

The purpose of clinical trial registration is to prevent selective publication and selective reporting of research outcomes, to prevent unnecessary duplication of research effort, to help patients and the public know what trials are planned or ongoing into which they might want to enroll, and to help give ethics review boards considering approval of new studies a view of similar work and data relevant to the research they are considering. Retrospective registration, for example at the time of manuscript submission, meets none of these purposes. Those purposes apply also to research with alternative designs, for example observational studies. For that reason, the ICMJE encourages registration of research with non-trial designs, but because the exposure

or intervention in non-trial research is not dictated by the researchers, the ICMJE does not require it.

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

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

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

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

## ii. Data Sharing

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

1. As of 1 July 2018 manuscripts submitted to ICMJE journals that report the results of clinical trials must contain a data sharing statement as described below.

2. Clinical trials that begin enrolling participants on or after 1 January 2019 must include a data sharing plan in the trial's registration. The ICMJE's policy regarding trial registration is explained at [www.icmje.org/recommendations/browse/publishing-and-editorial-issues/clinical-trial-registration.html](http://www.icmje.org/recommendations/browse/publishing-and-editorial-issues/clinical-trial-registration.html). If the data sharing plan changes after registration this should be reflected in the statement submitted and published with the manuscript, and updated in the registry record.

Data sharing statements must indicate the following: whether individual deidentified participant data (including data dictionaries) will be shared; what data in particular will be shared; whether additional, related documents will be available (e.g., study protocol, statistical analysis plan, etc.); when the data will become available and for how long; by what access criteria data will be shared (including with whom, for what types of analyses, and by what mechanism). Illustrative examples of data sharing statements that would meet these requirements are provided in the **Table**.

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

## IV. MANUSCRIPT PREPARATION AND SUBMISSION

### A. Preparing a Manuscript for Submission to a Medical Journal

#### 1. General Principles

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

Electronic formats have created opportunities for adding details or sections, layering information, 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](http://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/](http://www.stard-statement.org/)). Journals are encouraged to ask authors to follow these guidelines because they

Table. Examples of Data Sharing Statements That Fulfill These ICMJE Requirements\*

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

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

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/](http://www.equator-network.org/home/)) and the NLM's Research Reporting Guidelines and Initiatives ([www.nlm.nih.gov/services/research\\_report\\_guide.html](http://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 in-

cludes 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](http://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/](http://www.consort-statement.org/resources/downloads/extensions/consort-extension-for-abstracts-2008pdf/)). Funding sources should be listed separately after the abstract to facilitate proper display and indexing for search retrieval by MEDLINE.

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

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

#### **c. Introduction**

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

#### **d. Methods**

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

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

#### ***i. Selection and Description of Participants***

Clearly describe the selection of observational or experimental participants (healthy individuals or patients, including controls), including eligibility and exclusion criteria and a description of the source population. Because the relevance of such variables as age, sex, or ethnicity is not always known at the time of study design, researchers should aim for inclusion of representative populations into all study types and at a minimum provide descriptive data for these and other relevant demographic variables. Ensure correct use of the terms sex (when reporting biological factors) and gender (identity, psychosocial or cultural factors), and, unless inappropriate, report the sex and/or gender of study participants, the sex of animals or cells, and describe the methods used to determine sex and gender. If the study was done involving an exclusive population, for example in only one sex, authors should justify why, except in obvious cases (e.g., prostate cancer). Authors should define how they determined race or ethnicity and justify their relevance.

#### ***ii. Technical Information***

Specify the study's main and secondary objectives—usually identified as primary and secondary outcomes. Identify methods, equipment (give the manufacturer's name and address in parentheses), and procedures in sufficient detail to allow others to reproduce the results. Give references to established methods, including statistical methods (see below); provide references and brief descriptions for methods that have been published but are not well-known; describe new or substantially modified methods, give the reasons for using them, and evaluate their limitations. Identify precisely all drugs and chemicals used, including generic name(s), dose(s), and route(s) of administration. Identify appropriate scientific names and gene names.

#### ***iii. Statistics***

Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to judge its appropriateness for the study and to verify the reported results. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (such as confidence intervals). Avoid relying solely on statistical hypothesis testing, such as *P*

values, which fail to convey important information about effect size and precision of estimates. References for the design of the study and statistical methods should be to standard works when possible (with pages stated). Define statistical terms, abbreviations, and most symbols. Specify the statistical software package(s) and versions used. Distinguish prespecified from exploratory analyses, including subgroup analyses.

#### ***e. Results***

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

Give numeric results not only as derivatives (e.g., percentages) but also as the absolute numbers from which the derivatives were calculated, 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. Discuss the influence or association of variables, such as sex and/or gender, on your findings, where appropriate, and the limitations of the data. Do not repeat in detail data or other information given in other parts of the manuscript, such as in the Introduction or the Results section.

Link the conclusions with the goals of the study but avoid unqualified statements and conclusions not adequately supported by the data. In particular, distinguish between clinical and statistical significance, and avoid making statements on economic benefits and costs unless the

manuscript includes the appropriate economic data and analyses. Avoid claiming priority or alluding to work that has not been completed. State new hypotheses when warranted, but label them clearly.

## **g. References**

### **i. General Considerations**

Authors should provide direct references to original research sources whenever possible. References should not be used by authors, editors, or peer reviewers to promote self-interests. 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.

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

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

Some but not all journals check the accuracy of all reference citations; thus, citation errors sometimes appear in the published version of articles. To minimize such errors, references should be verified using either an electronic bibliographic source, such as PubMed, or print copies from original sources. Authors are responsible for checking that none of the references cite retracted articles except in the context of referring to the retraction. For articles published in journals indexed in MEDLINE, the ICMJE considers PubMed the authoritative source for information about retractions. Authors can identify retracted articles in MEDLINE by searching PubMed for “Retracted publication [pt]”, where the term “pt” in square brackets stands for publication type, or by going directly to the PubMed’s list of retracted publications ([www.ncbi.nlm.nih.gov/pubmed?term=retracted+publication+\[pt\]](http://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](http://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, Reporting, Editing, and Publication of Scholarly Work in Medical Journals: Sample References ([www.nlm.nih.gov/bsd/uniform\\_requirements.html](http://www.nlm.nih.gov/bsd/uniform_requirements.html)) webpage and detailed in the NLM’s Citing Medicine, 2<sup>nd</sup> edition ([www.ncbi.nlm.nih.gov/books/NBK7256/](http://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 radiological and other clinical and diagnostic images, as well as pictures of pathology specimens or photomicrographs, send high-resolution photographic image files. Before-and-after images should be taken with the same intensity, direction, and color of light. 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.

In the manuscript, legends for illustrations should be on a separate page, with Arabic numerals corresponding to the illustrations. When symbols, arrows, numbers, or letters are used to identify parts of the illustrations, identify and explain each one clearly in the legend.

#### **j. Units of Measurement**

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.

### **B. Sending the Manuscript to the Journal**

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

*A full statement to the editor about all submissions and previous reports that might be regarded as redundant publication of the same or very similar work.* Any such work should be referred to specifically and referenced in the new paper. Copies of such material should be included with the submitted paper to help the editor address the situation. See also Section III.D.2.

*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.

*A statement on authorship.* Journals that do not use contribution declarations for all authors may require that the submission letter includes a statement that the manuscript has been read and approved by all the authors, that the requirements for authorship as stated earlier in this document have been met, and that each author believes that the manuscript represents honest work if that information is not provided in another form. See also Section II.A.

*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.

Many journals provide a presubmission checklist to help the author ensure that all the components of the submission have been included. Some journals also require that authors complete checklists for reports of certain study types (e.g., the CONSORT checklist for reports of randomized controlled trials). Authors should look to see if the journal uses such checklists, and send them with the manuscript if they are requested.

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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## Instructions to Authors

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

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

### ARTICLE SECTIONS

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

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

#### Original articles

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

#### Reviews

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

#### Case Reports

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

#### Feature articles

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

#### Autopsy Vault

The PJP highly welcomes articles on autopsy protocols of cases. The article must include a summary presentation of the history, evaluation and work-up, clinical course of a case, followed by the autopsy procedure performed, gross and

microscopic findings, discussion, learning points and conclusion. The PJP recognizes the instructional and educational value of articles under this section. The abstract should be from 50 to 75 words and should not be structured. A manuscript for the Autopsy Vault should not exceed 25 typewritten pages (including tables, figures, illustrations and maximum of 30 references) or 6000 words.

#### Images in Pathology

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

#### Brief Communications

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

#### Editorials

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

#### Letters to the Editor

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

#### Special Announcements

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

### COVER LETTER

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

### PJP AUTHOR FORM

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

### GENERAL FORMATTING GUIDELINES

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

### SPECIFIC FORMATTING GUIDELINES

#### Title and Authors

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

### Abstract

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

### Keywords

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

### Text

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

### References

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

#### One to Six Authors

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

Mokdad AH, Bowman BA, Ford ES, Vinicor F, Marks JS, Koplan JP. The continuing epidemics of obesity and diabetes in the US. *JAMA*. 2001;286(10):1195-1200.

#### More than Six Authors

Rhynes VK, McDonald JC, Gelder FB, et al. Soluble HLA class I in the serum of transplant recipients. *Ann Surg*. 1993; 217 (5): 485-9.

#### Authors Representing a Group

Moher D, Schulz KF, Altman D; for the CONSORT Group. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomized trials. *JAMA*. 2001;285(15):1987-1991.

#### Book

Byrne, DW. Publishing your medical research paper: What they don't teach in medical school. Baltimore: Williams & Wilkins, 1998.

#### World Wide Web

Barry JM. The site of origin of the 1918 influenza pandemic and its public health implications. [Commentary]. *JTranslational Med*. January 20, 2004;2(3):1-4. <http://www.translational-medicine.com/content/2/1/3>. Accessed November 18, 2005.

### Tables

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

### Figures and Graphs

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

### Illustrations and Photographs

- Where appropriate, all illustrations/photographic images should be at least 800 x 600 dpi and submitted as image files (preferably as .png, .jpeg 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.
- All letterings for illustration should be of adequate size to be readable even after size reduction.
- Place explanatory notes and legends, as well as definitions of abbreviations used below the illustration/photograph.
- Up to a maximum of five (5) illustrations/ photographs are allowed.

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

### EDITORIAL PROCESS (Figure 1)

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

### EDITORIAL OFFICE CONTACT INFORMATION:

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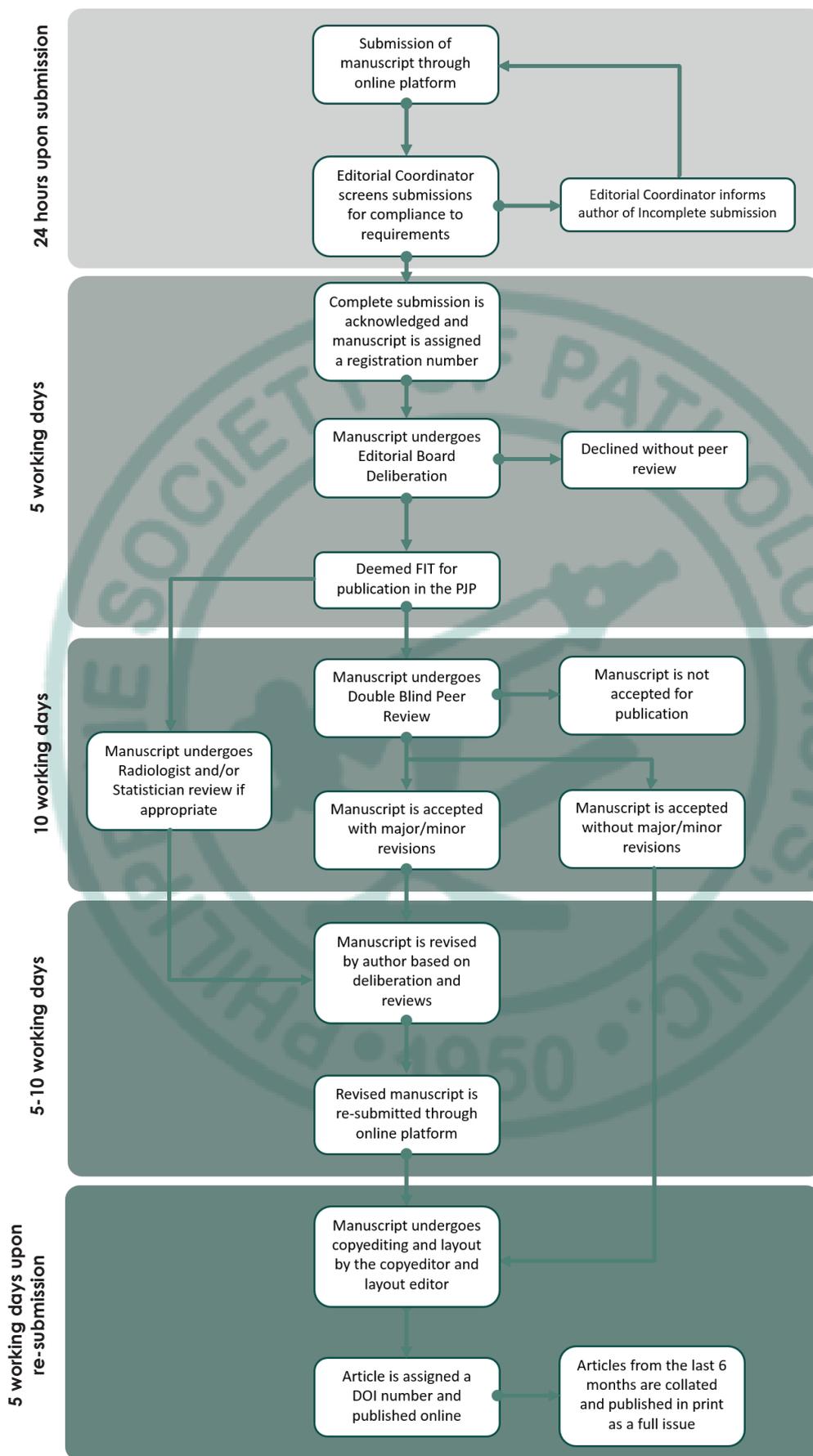


Figure 1. Editorial Process Flow.



**PJP AUTHOR FORM**

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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.

**AUTHOR DECLARATIONS**

- The undersigned author(s) of the manuscript hereby certify, that the submitted manuscript represents original, exclusive and unpublished material. It is not under simultaneous consideration for publication elsewhere. Furthermore, it will not be submitted for publication in another journal, until a decision is conveyed regarding its acceptability for publication in the PJP.
- The undersigned hereby certify, that the study on which the manuscript is based had conformed to ethical standards and/or had been reviewed by the appropriate ethics committee.
- The undersigned likewise hereby certify that the article had written/informed consent for publication from involved subjects (for case report/series only) and that in case the involved subject/s can no longer be contacted (i.e., retrospective studies, no contact information, et cetera), all means have been undertaken by the author(s) to obtain the consent.

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

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

AUTHOR NAME	RELATIONSHIP	MANUFACTURER/ SUPPLIER/ COMPANY

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

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

Author Name	Signature	Date (MM/DD/YYYY)



### PATIENT CONSENT FORM

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]  
above to appear in the Philippine Journal of Pathology (PJP) subject to its  
publication policies and ethical standards.

***I have seen and read the material to be submitted to the PJP and thoroughly understand the following:***

- The Information will be published in the PJP without my name. It is the obligation of the PJP to make all attempts, within its reasonable jurisdiction and authority, to ensure my anonymity.
- The Information may also be placed on the PJP website.
- The PJP shall not allow the Information to be used for advertising or packaging or to be used out of context (i.e., used to accompany an entirely different article or topic).
- I can withdraw my consent at any time before publication, but once the Information has already been sent to press, it is my understanding that it will not be possible to revoke the consent.

Signed: \_\_\_\_\_  
[signature over complete name]

Date: \_\_\_\_\_

***Witness:***

Signed: \_\_\_\_\_  
[signature over complete name]

Date: \_\_\_\_\_

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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
<b>DOMAIN 1: RESEARCH TEAM AND REFLEXIVITY</b>		
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
<b>DOMAIN 2: STUDY DESIGN</b>		
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?
<b>DOMAIN 3: ANALYSIS AND FINDINGS</b>		
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?

**EQUATOR** stands for Enhancing the QUALity and Transparency Of health Research. It is an international initiative that started in 2008 whose main objective is to improve the reliability and value of scholarly publication of health research through promotion of transparent, complete, and accurate reporting. The Network promotes standards, guidelines and checklists of reporting requirements for various types of studies, from clinical trials and observational studies to reviews and case reports.

The complete checklists and full guidelines are available at <http://equator-network.org>.



## 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	<input type="checkbox"/> Yes <input type="checkbox"/> 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.
<b>TITLE</b>			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	_____
<b>ABSTRACT</b>			
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.	_____
<b>INTRODUCTION</b>			
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).	_____
<b>METHODS</b>			
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.	_____
<b>RESULTS</b>			
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]).	_____
<b>DISCUSSION</b>			
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.	_____
<b>FUNDING</b>			
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](http://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
<b>TITLE</b>		
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
<b>INTRODUCTION</b>		
Background / rationale	2	Explain the scientific background and rationale for the investigation being reported
Objectives	3	State specific objectives, including any prespecified hypotheses
<b>METHODS</b>		
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) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —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 <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants (b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —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) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —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) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time <i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure <i>Cross-sectional study</i> —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
<b>DISCUSSION</b>		
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
<b>OTHER INFORMATION</b>		
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](http://www.strobe-statement.org).

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Section and Topic	No.	Item
<b>TITLE OR ABSTRACT</b>		
	1	Identification as a study of diagnostic accuracy using at least one measure of accuracy (such as sensitivity, specificity, predictive values, or AUC)
<b>ABSTRACT</b>		
	2	Structured summary of study design, methods, results, and conclusions (for specific guidance, see STARD for Abstracts)
<b>INTRODUCTION</b>		
	3	Scientific and clinical background, including the intended use and clinical role of the index test
	4	Study objectives and hypotheses
<b>METHODS</b>		
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)
Test Methods	9	Whether participants formed a consecutive, random or convenience series
	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
Analysis	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
	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
<b>RESULTS</b>		
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
<b>DISCUSSION</b>		
	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
<b>OTHER INFORMATION</b>		
	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.
<b>TITLE AND ABSTRACT</b>			
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.	_____
<b>INTRODUCTION</b>			
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.	_____
<b>METHODS</b>			
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	<i>Single study-based estimates:</i> Describe fully the design features of the single effectiveness study and why the single study was a sufficient source of clinical effectiveness data.	_____
	11b	<i>Synthesis-based estimates:</i> 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	<i>Single study-based economic evaluation:</i> 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	<i>Model-based economic evaluation:</i> 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.	_____
<b>RESULTS</b>			
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	<i>Single study-based economic evaluation:</i> 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	<i>Model-based economic evaluation:</i> 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.	_____
<b>DISCUSSION</b>			
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.	_____
<b>OTHER INFORMATION</b>			
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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Section / Topic	Item no.	Checklist item
<b>TITLE AND ABSTRACT</b>		
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.
<b>INTRODUCTION</b>		
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. 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.
Objectives	4	Clearly describe the primary and any secondary objectives of the study, or specific hypotheses being tested.
<b>METHODS</b>		
Ethical statement	5	Indicate 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).
Experimental procedures	7	A time-line diagram or flow chart can be useful to illustrate how complex study designs were carried out. 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.
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.
<b>RESULTS</b>		
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.
<b>DISCUSSION</b>		
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](http://www.nc3rs.org.uk/ARRIVE)

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## Revised Standards for Quality Improvement Reporting Excellence (SQUIRE 2.0)

No	Item	Guide questions / description
<b>TITLE AND ABSTRACT</b>		
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
<b>INTRODUCTION</b>		
<b>WHY DID YOU START?</b>		
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
<b>METHODS</b>		
<b>WHAT DID YOU DO?</b>		
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
<b>RESULTS</b>		
<b>WHAT DID YOU FIND?</b>		
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
<b>DISCUSSION</b>		
<b>WHAT DOES IT MEAN?</b>		
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 c. Efforts made to minimize and adjust for limitations
17	Conclusions	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
<b>OTHER INFORMATION</b>		
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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Section / Topic	Item no.	Description
<b>ADMINISTRATIVE INFORMATION</b>		
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)
<b>INTRODUCTION</b>		
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)
<b>METHODS: PARTICIPANTS, INTERVENTIONS, AND OUTCOMES</b>		
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
<b>METHODS: ASSIGNMENT OF INTERVENTIONS (FOR CONTROLLED TRIALS)</b>		
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.*

**EQUATOR** stands for Enhancing the QUALity and Transparency Of health Research. It is an international initiative that started in 2008 whose main objective is to improve the reliability and value of scholarly publication of health research through promotion of transparent, complete, and accurate reporting. The Network promotes standards, guidelines and checklists of reporting requirements for various types of studies, from clinical trials and observational studies to reviews and case reports.

The complete checklists and full guidelines are available at <http://equator-network.org>.



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

Section / Topic	Item no.	Checklist item	Reported on page no.
<b>TITLE AND ABSTRACT</b>			
	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)	_____
<b>INTRODUCTION</b>			
Background and objectives	2a	Scientific background and explanation of rationale	_____
	2b	Specific objectives or hypotheses	_____
<b>METHODS</b>			
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	_____
<b>RESULTS</b>			
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)	_____
<b>DISCUSSION</b>			
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	_____
<b>OTHER INFORMATION</b>			
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](http://www.consort-statement.org).

**EQUATOR** stands for Enhancing the QUALity and Transparency Of health Research. It is an international initiative that started in 2008 whose main objective is to improve the reliability and value of scholarly publication of health research through promotion of transparent, complete, and accurate reporting. The Network promotes standards, guidelines and checklists of reporting requirements for various types of studies, from clinical trials and observational studies to reviews and case reports.

The complete checklists and full guidelines are available at <http://equator-network.org>.

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- Indicate all the authors (complete names and affiliations); and
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<input checked="" type="checkbox"/>	<b>Author Form</b> <ul style="list-style-type: none"><li>• Ensure all authors have qualified as authors based on ICMJE authorship criteria;</li><li>• Ensure all authors have read and agreed to the Certification;</li><li>• Ensure all authors have read and provided disclosure of conflicts of interest; and</li><li>• Submit a scanned copy of the fully accomplished form.</li></ul>
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<input checked="" type="checkbox"/>	<b>Title Page</b> <ul style="list-style-type: none"><li>• Full names of the authors directly affiliated with the work (First name and Last name), highest educational attainment;</li><li>• Name and location of not more than 1 institutional affiliation per author; and</li><li>• If presented in a scientific forum or conference, provide a footnote indicating the name, location and date of presentation.</li></ul>
<input checked="" type="checkbox"/>	<b>Abstract</b> <ul style="list-style-type: none"><li>• Provide an abstract conforming with the format;</li><li>• Structured for Original Articles, Review Articles: Objective/s, Methodology, Results, Conclusion;</li><li>• Unstructured for Case Reports and Feature Articles; and</li><li>• Do not place cross references within the abstract.</li></ul>
<input checked="" type="checkbox"/>	<b>Keywords</b> Provide 3-6 keywords (listed in MeSH)
<input checked="" type="checkbox"/>	<b>Content</b> <ul style="list-style-type: none"><li>• Provide text/content in IMRAD format (Introduction, Methodology, Results and Discussion, Conclusion);</li><li>• 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;</li></ul>

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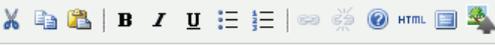
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5	<a href="#">PJP AUTHOR SUBMISSION CHECKLIST V.01.2015.DOCX</a>	Supplementary File	175KB	02-05

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Amado O. Tandoc III, MD, DPSP  
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1 - 1 of 1 Items

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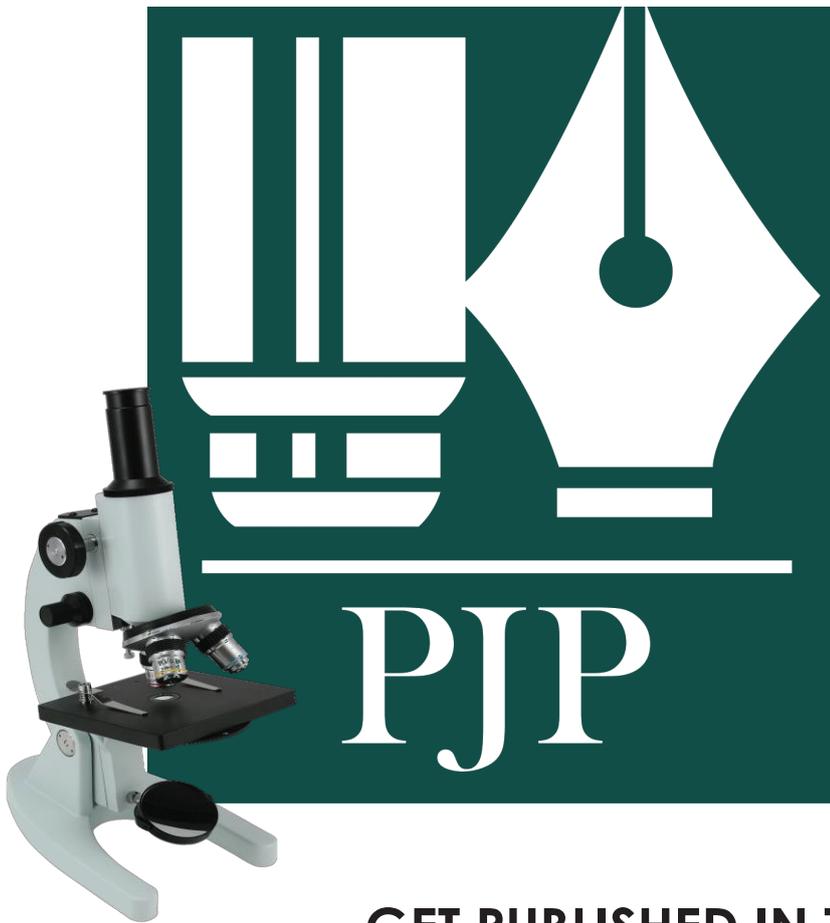
Thank you and kind regards,

Amado O. Tandoc III, MD, DPSP  
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