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

Welcome to the June 2022 issue of the Philippine Journal of Pathology. I commend the editorial staff for their unending efforts and aspiration to be able to regularly publish quality and peer-reviewed articles.

The Board of Governors of the Philippine Society of Pathologists would like to encourage our junior and regular members to come up with scholarly research and fascinating case reports and series. Let us show our neighboring Asian countries and the world that the Filipinos are capable of generating quality research material.

Let us help sustain this journal by consistently submitting articles for peer-review and eventual publication in our very own platform. The Philippine Society of Pathologists will always support the endeavor of the PJP editorial team in achieving its goal of serving as the official platform for publication of articles related to Pathology and Laboratory Medicine.

Join us in proving the world that the Filipinos can deliver high-caliber and timely publication.

Mabuhay tayong lahat!

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

The Distance Between Two Points

I think there is a great deal of misconception as to what an editor *really* does in a journal. Structural edits, that is, copyediting – which includes correction of spelling and grammar, syntax and punctuation, checking abbreviations and acronyms, citations and references, style and consistency – forms but part of the overall process of "editing." Some people seem to think that this is only what an editor does (and this is already a lot of work, by itself). What happens between 2 points – manuscript submission and article publication – that, is the work of the editor, and it is by no means, easy.

When a manuscript is submitted it goes through a process that will lead either to its acceptance or rejection. This involves an initial screening as to whether the manuscript is acceptable within the scope of the journal, as to compliance with the editorial requirements. For all articles, the requirements include the author's/authors' certification that those listed have qualified as authors based on authorship criteria set forth by the International Committee of Medical Journal Editors (ICMJE), have declared that their work is original, unpublished, and not under consideration for publication elsewhere, have complied with the copyright transfer agreement of the journal, and have provided disclosure as to potential conflicts of interest. For original articles, the journal requires submission of a copy of the approval of the relevant ethics and technical review board; for case reports, informed consent of the subject to be featured in the article should be submitted. Those articles that have passed screening shall undergo peer review (i.e., sent to appropriate peer reviewers representing technical expertise or authority on the subject) and can follow either of the following fates: acceptance with no revisions required, acceptance with minor revisions, acceptance with major revisions, or rejection (i.e., declined for publication). Within this process, there may be a back-and-forth communication between the author and the reviewer through the editor, during which clarifications or requests for further information can be sought, authors can submit corrections based on the reviewers' recommendations and provide

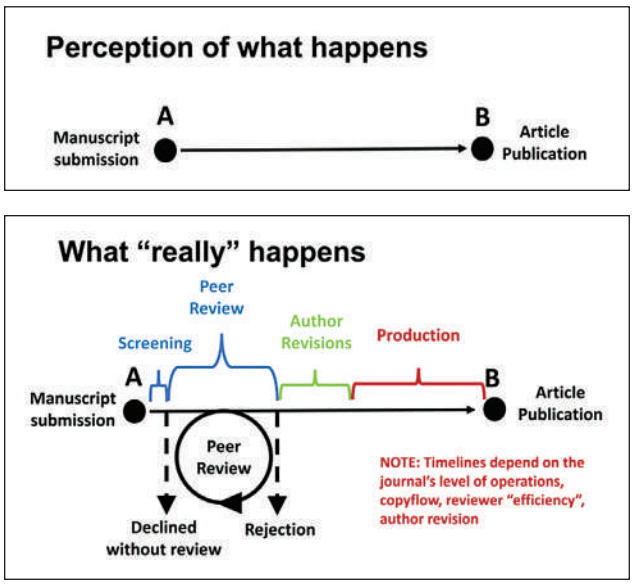

Figure 1. What happens to each article?

a point-by-point response to each comment, and final decision by the Editor-in-Chief. It does not stop there. Accepted articles go into production, in which copyediting and layout occur, with the necessary author approvals obtained on the final article that will be made public.

The Editor-in-Chief assumes responsibility for the articles published in the journal and this accountability continues so long as an article remains published. Policies are in place should there be a need for retraction or correction, but the main published article stays albeit marked as "retracted" or indicating that it has been "corrected."

The distance between manuscript submission and article publication is one that is not as straightforward as it may seem to other people. To be fair, many people contribute to each and every article and the Editor is in charge of overseeing this process. It requires passion, patience, and perseverance, objectivity, and resolve. Moreover, it requires time: that irreversible, irreplaceable resource that we all have very little of, and which I hope we are not wasting through this continued effort for a better practice.

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

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Anatomic Pathology Practice in the Philippines

The practice of Anatomic Pathology in the Philippines had been rather simple since the start in the early 1950s. The pathologist does a gross examination and description of the specimen submitted, takes sections for processing and reads and interprets the stained slide; thereafter issuing a Surgical Pathology report or in the case of autopsies, a Final Anatomic Diagnosis.

In the early 90s, immunohistochemistry (IHC) was introduced and had since become more accessible to most if not all pathologists. Molecular methods were introduced later at the advent of the 21st century. In-situ hybridization was initially used for her-2 neu in breast cancer cases but is now applied to other tumors.

The practice has largely been left to its own devices by regulatory authorities as the focus was on the upliftment of clinical laboratories and rightly so. In contrast to Clinical Pathology, which up until recently was more focused on supervision, quality assurance and continuing improvement by the Clinical Pathologist, Anatomic Pathologists actually render clinical diagnoses based on the gross and microscopic properties of the specimen and in correlation with clinical findings. The tissue processing is a necessary step to produce a quality microscopic slide but is largely a mechanical process, mediated by the histotechnologist who processes, cuts and stains the tissue section. It is similar to the Radiologist's practice where the images are sent to him/her for interpretation, again based on knowledge gained during residency training and specialization. Nowadays though, Radiologists rarely give unequivocal diagnoses but rather offer several considerations. In contrast, Anatomic Pathologists state unequivocal diagnoses in the large majority of cases. Thus, he/she is the ultimate arbiter of the patients' conditions.

Now comes disconcerting news on the new regulations concerning Anatomic Pathology in which all tertiary labs/hospitals are required to have histopathology sections. On the surface, this appears to be a progressive step. We all want the ideal situation. However, it appears this is based on an odd concept that the surgical pathology slides have to be produced in the same hospital/lab where the pathologist practices, or else the reading of a slide produced elsewhere is fraudulent.

This is probably an extension of the doctrine in Clinical Pathology where labs that outsource tests are required to send out the original results from the referral lab and not transfer the results to their own forms and letterheads. There is no issue about the requirement because the outsourced tests are purely the product of the referral lab which must be credited for the result, or investigated if the result is erroneous. However, in practically all instances, Anatomic Pathologists are not hospital/lab employees but are rather on a contractual basis or are free agents. Their rendering of diagnoses is subject to possible litigation when misdiagnosis is alleged but in which the hospital/lab carries no obligation or responsibility. The Anatomic Pathologist carries the ultimate responsibility for his/her diagnosis, from the initial gross examination all the way to interpreting the microscopic section, which may not necessarily come from the same lab he/she practices in.

In fact, this odd theory falls apart when you consider that many cases are referred from other institutions for second, third or fourth opinions. So, does it make the Surgical Pathologist who renders a second opinion liable for fraud just because he/she read slides from another hospital? If so, then current best practices will no longer be observed since the original diagnosis will be the one and only diagnosis. This will have to assume that we pathologists are perfect, which we are not, being prone to error as much as the next person. Which is why we have quality procedures in place in most progressive hospitals of requiring a second pathologist's opinion before releasing a diagnosis of malignancy. By the way, does this circumscription not constitute an infringement on the right to practice of pathologists, being free agents?

Assuming these issues are ironed out and the requirement is put in place, we have to contend with several issues that will impact its implementation. First is the issue of maintaining viability of the histopathology section, which requires a hefty financial investment in equipment and in manpower, not to mention the provision of additional space which most labs are hard up to provide. Many hospitals have cut down on the number of beds in operation because of nursing staff shortages. Some hospitals that are built to have 400 beds can only manage to open 100 beds. This number is only the possible occupancy but oftentimes, censuses are lower, thus the probability that there will be enough surgical pathology specimens to process in its histopathology lab is low. Will the hospital now raise its charges for surgical pathology specimens to levels that will maintain its histopathology operation? Will Philhealth or private insurance companies reimburse at those levels? Probably not.

What may happen is the hospital or laboratory will have to downgrade to secondary status if it cannot comply with the histopathology section requirement. It will mean further shrinkage of the hospital's operations and scope, which may eventually lead to its closure since Philhealth reimbursement is tied to the hospital category.

The next problem is staffing. For the longest time, histotechnology has not been properly taught or practiced. There is a dearth of good histotechnologists in the country since those who have been trained have gone to other countries in search of better opportunities.

We already have a shortage of medical technologists for the clinical lab. On top of this, we have even less histotechnologists who are properly trained. Staffing a histopathology section will definitely be a challenge.

Thus, the Surgical Pathologist has to contend with poor quality sections increasing the possibility of diagnostic error. It is vital that we have the highest quality microscopic sections to come up with the correct diagnosis.

In summary, the requirement for tertiary hospitals/ labs to have a histopathology section is fraught with many implications. There are professional issues of infringement on the right to practice one's profession and the privilege of patients to avail of a second opinion. It will create a crisis in smaller hospitals that cannot afford to invest in histopathology equipment or hire more techs, possibly causing them to downgrade their category with subsequent loss of income. It will be problematic for hospitals to hire histotechs or train them since there are no training opportunities for histopathology. Surgical pathology slide quality will be an issue if they are forced to hire untrained staff, leading to possible increase in diagnostic errors by pathologists.

All these do not augur well for our patients or health care system. We must find a way to address the issues before enforcing the requirement.

Raymundo W. Lo, MD, FPSP
Anatomic and Clinical Pathologist,
St. Luke's Medical Center,
Quezon City, Philippines

The author has been practicing Anatomic and Clinical Pathology in the Philippines since 1988. He was responsible for popularizing the use of immunohistochemistry through his lectures at the Philippine Society of Pathologists Inc. annual conventions and establishing the first full scale IHC section at St. Luke's Medical Center in the 1990s which has the most extensive menu of markers in the country as well as setting up in-situ hybridization testing using fluorescence (2008) and chromogenic (2002) methods. As the Liborio Gomez Memorial Lecturer in 2007, he emphasized the need for good quality surgical pathology microscopic slides and followed up with the founding of HistoSolutions Inc. in 2015 to provide quality routine H&E and IHC sections at reasonable prices to benefit both pathologists and the general public nationwide. He has co-authored the book, "Basic Histopathologic Techniques" in 2014.

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Position Paper on the Conduct of the Autopsy during and after the COVID Pandemic

With the widespread availability of COVID-19 vaccines, pathologists – both in-training residents and consultants – are attempting to return to their pre-pandemic workload and learning to live with COVID-19. One of the most important jobs of the pathologist is the performance of the autopsy, and residents, as future pathologists, must also be trained to be adept at performing an autopsy. Fear of COVID-19 should not be a deterrent nor an excuse for not performing autopsies when the benefits of performing an autopsy outweighs its risks. In this light, the following updated recommendations are made:

1. Negative pressure facility

Although a negative pressure autopsy chamber is ideal for handling COVID-positive or suspect patients, it is not necessary for non-COVID patients. COVID-19 tests performed prior to the autopsy and interpreted in light of the varying performance characteristics of the test method used is an important step in triaging a case for autopsy.

According to the US Center for Disease Control, aerosol production during the autopsy should be minimized. Although there are rare studies as to which procedures during the autopsy produce aerosol, it is generally agreed that using an oscillating saw during removal of the brain is aerosol-producing and should be avoided. Collection of naso-pharyngeal swabs in a dead person is expected to not produce aerosols as the coughing reflex cannot be elicited anymore.

2. COVID-19 testing prior to autopsy

As COVID-19 continues to cause significant infection, it needs to be ruled-out as a/contributing to the cause of death. As such, it is highly recommended that rapid antigen tests and/or RT-PCR be performed prior to the autopsy. The institution may decide which test to use based on the performance characteristics of the tests available to them and the turnaround times.

Rapid antigen tests have the advantage of being fast, easy to perform and can be performed in a point-of-care setting. Rapid antigen tests also correlate well with the contagiousness of a person. They are quickly replacing RT-PCR as a requirement for travel, hospital admission and the like.

RT-PCR remains the test of choice for COVID-19 as it detects COVID earlier than rapid antigen. However, the RT-PCR may remain positive for days, even weeks, after a person ceases to be contagious.

As with any laboratory test, caveats exist – a negative test does not totally exclude COVID-19 nor does a positive test denote a contagious or infectious state.

3. Proper PPE

Complete PPE in the form of disposable aprons/gowns, gloves, N95 masks, shields and shoe covers are necessary when performing COVID-19 positive/suspect cases.

This document is a supplement to DOH memorandum 2021-0425.

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OPEN ACCESS – ORIGINAL ARTICLE

Prevalence and Clinico-Pathologic Features of ALK Rearrangement Among Adult Filipinos with Non-Small Cell Lung Cancer in a Private Tertiary Care Hospital

Steffanie Charlyne Tamayo,[1] Rebecca Nagtalon,[1] Joanmarie Balolong-Garcia,[2] Yancel Donna Mascardo,[2] Jose Jasper Andal,[1] Daphne Ang,[1] Marcelo Severino Imasa,[2] Rex Michael Santiago[1]

[1]*Institute of Pathology, St. Luke's Medical Center, Quezon City, Philippines*
[2]*Section of Medical Oncology, St. Luke's Medical Center, Quezon City, Philippines*

ABSTRACT

Introduction. With advancements in the understanding of lung cancer biology, targeted therapy has become the rule rather than the exception. Patients with ALK rearrangements are amenable to therapy with Alectinib and other ALK inhibitors, which has been associated with better patient outcomes. While ALK rearrangement should be routinely tested in non-squamous non-small cell lung cancer (NSCLC), the cost and availability of this test is a prohibitive factor, particularly in the Philippine setting.

Objectives. This study aimed (1) to determine the prevalence of ALK-rearranged NSCLC among adult Filipino lung cancer patients in St. Luke's Medical Center (SLMC) from 2016 to 2018 and (2) to determine the clinico-pathologic features of adult Filipinos with ALK-rearranged NSCLC.

Methodology. This is a retrospective cross-sectional descriptive study wherein the prevalence of ALK-rearranged NSCLC, detected using fluorescence in-situ hybridization (FISH) or immunohistochemistry (IHC), was determined. Clinical data of patients for whom ALK testing was performed were collected. Hematoxylin and Eosin (H&E) slides were retrieved and reviewed for the presence of certain morphologic features. Patients whose H&E slides cannot be retrieved were excluded from the study.

Results. ALK rearrangement was seen in 7.8% (8/103) of tumors submitted for ALK testing. Patients with ALK-rearranged tumors were generally young, light smokers, and presented with advanced clinical stage. Clear cell features and solid pattern were noted in one case and three cases, respectively. However, due to small sample size, further statistical analysis could not be performed to analyze the association of these features with the presence of ALK rearrangement.

Conclusion. Despite a small sample size, the prevalence and clinical profile of ALK-rearranged NSCLC in our institution are congruent with those previously described in Western populations. The association of clinical profile and morphologic features with the presence of ALK rearrangement can be further explored in future studies.

Key words: lung neoplasms, carcinoma, non-small cell lung, anaplastic lymphoma kinase, fluorescence in situ hybridization

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Corresponding author: Steffanie Charlyne A. Tamayo, MD
E-mail: scatamayo1031@gmail.com
ORCiD: https://orcid.org/0000-0003-2935-4374

INTRODUCTION

As with other malignancies, the stepwise accumulation of oncogenic mutations has been implicated in the pathogenesis of lung cancer. Among the many genetic abnormalities seen in tumor cells, driver mutations are the ones essential for tumor cell survival, a phenomenon called oncogene addiction. Inactivation of these driver mutations are the basis of rational targeted therapy. Prototypical oncogenes in non-small cell lung cancer (NSCLC) include Epidermal Growth Factor Receptor (EGFR), Kirsten Rat Sarcoma Virus (KRAS) and Anaplastic Lymphoma Kinase (ALK). With advancements in the understanding of the biology of lung cancer, these oncogenes have become the object of targeted therapy.

ALK is a receptor tyrosine kinase whose coding gene, which spans 29 exons, is found at chromosome 2p23. The ALK

chromosome encodes a 1,620 amino acid which undergoes post-translational N-linked glycosylation. Normally, ALK is activated by dimerization; this results in auto-phosphorylation of three tyrosine residues. The normal physiologic function of ALK is largely unknown; however, it has been shown to initiate several signal transduction pathways, including the sonic hedgehog pathway, mammalian target of rapamycin, and phosphoinositide 3- kinase/ protein kinase B pathway.[1]

ALK mutations in cancer were first identified by Morris et al., in anaplastic non-Hodgkin's lymphoma (ALCL) where a t (2;5) (p23; q35) mutation resulted in a constitutively active ALK kinase.[1] Other malignancies, such as NSCLC, basal cell carcinoma, breast cancer, and colorectal carcinoma, have since been demonstrated to harbor ALK mutations. ALK activation occurs via three different mechanisms: (1) fusion protein formation, (2) ALK over-expression, and (3) activating ALK point mutations.[1] In NSCLC, the most common gene rearrangement is that with Echinoderm Microtubule-associated protein-like 4 (EML4), with the EML4-ALK mutation first being described in NSCLC in 2007. The EML4-ALK fusion is a product of an inversion in the short arm of chromosome 2 which leads to the fusion of the N-terminal domain of EML4 with the intracellular kinase domain of ALK.[1] This results into constitutive tyrosine kinase activity. Aside from EML4, numerous novel fusion partners have also been described as a result of next generation sequencing, including Trafficking from ER to Golgi regulator (TFG), Kinesin Family member 5B (KIF5B), Kinesin Light Chain 1 (KLC1) and Striatin (STRN).[2]

ALK-rearranged NSCLC are usually seen in younger patients who are never or are former/ light smokers. The most common histology is that of an adenocarcinoma with a solid or acinar pattern with focal signet ring cell features.[3,4]

Crizotinib, an orally available aminopyridine-derived small molecule ATP competitive inhibitor, was historically the first ALK inhibitor used clinically in the treatment of NSCLC. It induces a G1/S phase cell cycle checkpoint and apoptosis in ALK-rearranged tumor cells. It has been shown to be superior to standard chemotherapy in patients with previously treated, advanced, ALK-rearranged NSCLC, with noted improvements in response rates and global quality of life.[5] However, next-generation ALK inhibitors with greater systemic and central nervous system penetration and efficacy, such as Alectinib, Brigatinib, and Lorlatinib, are now the first-line treatment options. Among these, Alectinib is preferred due to longer-term follow-up of clinical trials with this agent.[6] Note that prior to targeted therapy, the presence of ALK rearrangements was not a favorable prognostic factor in NSCLC.[1] However, since the Federal Food and Drug Administration (FDA) approval of Crizotinib in 2011, ALK fusion detection is now considered standard of care in lung adenocarcinoma.[7]

Testing for ALK fusion has been facilitated by the commercial availability of a fluorescence in situ hybridization (FISH) assay that uses a dual-labeled "break-apart" probe. FISH is able to accurately and reliably detect all ALK rearrangements regardless of the fusion partner and is thus considered the gold standard for ALK fusion testing.[1] However, it is costly, requires expertise and experience to interpret properly, and often has a lengthy turnaround time. It also requires the presence of a minimum of 50 tumor cells to circumvent false negative results. Other common methods for ALK fusion testing include immunohistochemistry (IHC) and polymerase chain reaction (PCR). IHC has the following advantages: low cost, relative ease of implementation, ease of interpretation, and short turnaround time. While clinical testing for ALK gene rearrangements initially used FISH, the sensitivity and specificity of IHC versus FISH has been found to range from 81% to 100% and current guidelines consider IHC testing (ALK D5F3) as an equivalent alternative to FISH for ALK testing.[8] The high degree of concordance between FISH and IHC has been demonstrated in small biopsy and cytology specimens.[9,10] Currently, there is no recommended minimum number of cells in assessing ALK D5F3 IHC.[11] IHC can therefore be used as an initial standalone test.[12,13] PCR, though specific, sensitive, and less expensive than FISH, misses rare or novel translocations and can have contamination issues. Reverse transcriptase PCR (RT-PCR) is not recommended as an alternative to FISH for detecting ALK rearrangements in NSCLC.[12]

While it is currently part of international guidelines that ALK testing be performed in all patients with non-squamous NSCLC, the cost and availability of this test is a prohibitive factor, particularly in the Philippine setting. As such, this study was undertaken (1) to determine the prevalence of ALK-rearranged non-small cell lung cancer (NSCLC) among adult Filipino lung cancer patients in St. Luke's Medical Center (SLMC) as identified using FISH or IHC done at SLMC and (2) to determine the clinico-pathologic features of these adult Filipinos with ALK-rearranged NSCLC.

METHODOLOGY

This is a retrospective cross-sectional descriptive study wherein the prevalence of ALK-rearranged NSCLC cases identified using FISH analysis (Vysis ALK Break Apart FISH probe kit) or IHC (VENTANA anti-ALK D5F3 rabbit monoclonal primary antibody clone) performed at SLMC from 2016 to 2018 was determined.

A database search of the institution's laboratory information system was performed using ALK IHC and FISH test requisitions from 2016 to 2018. Available clinical data (age, sex, smoking history, and clinical staging) were then collated from the institution's electronic and clinic records. Hematoxylin and Eosin (H&E) slides of the specimens submitted for ALK testing were retrieved and jointly evaluated by two of the authors (one of which is a pulmonary pathology specialist) for the following: histologic subtype, morphological pattern, highest nuclear grade, and presence of signet ring or clear cell features, cribriform pattern, calcification, and necrosis. Specimens for whom the H&E slides cannot be retrieved were excluded from the study. Reports of ALK tests performed on the included cases were then retrieved from the electronic records and tabulated. The study's methodological flowchart is depicted in Figure 1.

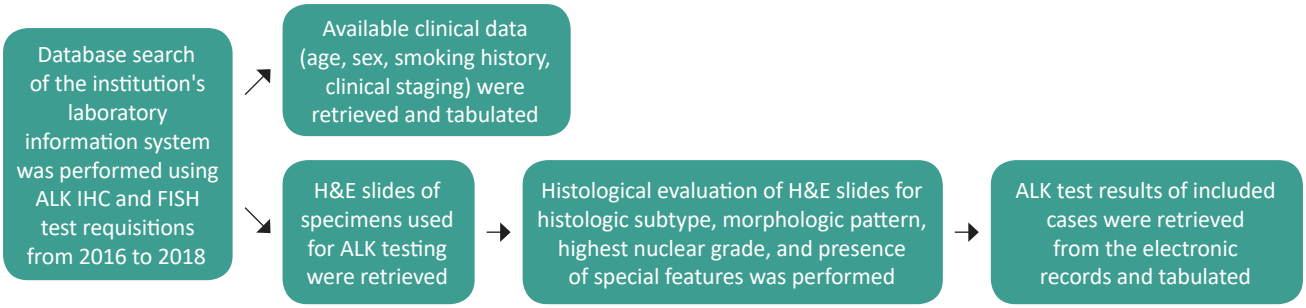

Figure 1. Methodological flowchart.

ALK, anaplastic lymphoma kinase. H&E, hematoxylin and eosin.

The following operational definitions were used in the study:

- Never smoker – patient who has never smoked or who has smoked less than 100 cigarettes in his or her lifetime
- Light smoker – patient with smoking history less than or equal to ten pack-years[7]
- Heavy smoker – patient with smoking history greater than ten pack-years
- ALK-rearranged – positive for ALK rearrangement
  - Identified using FISH by the presence of broken apart signals, two or more signal diameters apart, in > 50% of at least 50 tumor cells, or in an average of at least 15% of 100 tumor cells
  - Identified using IHC by the presence of strong granular cytoplasmic staining in any percentage of tumor cells
- ALK wild-type – negative for ALK rearrangement
- No special features – NSCLC cases without signet ring cells, clear cells, calcification, necrosis, or cribriform pattern
- Histologic grade cannot be assessed – NSCLC cases where the absence of a definitive morphologic pattern (lepidic, acinar, solid, papillary, and/ or micropapillary) precludes assessment of histologic grade

Table 1. Clinical features of ALK-rearranged and ALK wild type non-small cell lung carcinoma, retrieved from the institution's electronic and clinic records

	ALK-rearranged (N = 8) N (%)	ALK wild type (N = 91) N (%)
Sex		
Male	5 (62.5%)	54 (59.3%)
Female	3 (37.5%)	37 (40.7%)
Age		
Mean (years)	47.3	65.9
Median (years)	40	66
Range (years)	31-70	32-90
Smoking history		
Non-smoker	2 (25%)	21 (23.1%)
Smoker	3 (37.5%)	24 (26.4%)
Pack-years, mean	10.5	21.9
Pack-years, range	0.5-30	2.25-60
Unknown	3 (37.5%)	46 (50.5%)
Clinical Stage		
IA	0 (0%)	0 (0%)
IB	0 (0%)	1 (1.1%)
II	1 (12.5%)	0 (0%)
IIIA	0 (0%)	1 (1.1%)
IIIB	0 (0%)	0 (0%)
IV	4 (50%)	19 (20.9%)
Unknown	3 (37.5%)	70 (76.9%)

RESULTS

Although ALK testing by FISH and IHC are available in our institution, all cases covered by the study period were only submitted for testing by IHC. ALK rearrangement was seen in 7.8% (8/103) of tumors submitted for testing from 2016 – 2018. In the same period, the prevalence of ALK wild type NSCLC was 88.3% (91/103) while indeterminate results were seen in 3.9% (4/103) of cases due to paucity of tumor cells. No equivocal results were noted. The prevalence and clinical features of ALK-rearranged and ALK wild type cases are summarized in Table 1.

Patients with ALK-rearranged tumors had an age range of 31 to 70 years and mean and median ages of 47.3 and 40 years, respectively. Fifty percent (4/8) of these patients were clinically stage IV at the time of ALK testing. Of those with available smoking history, 40% (2/5) were non-smokers while among smokers, 66.7% (2/3) were light smokers. In contrast, ALK wild type tumors affected older patients, with an age range of 32 to 90 years and mean and median ages of 65.9 and 66 years, respectively. Nearly 21% (19/91) of patients with ALK wild type tumors were clinically stage IV at the time of ALK testing. Of those with available smoking history, 46.7% (21/45) were non-smokers while among smokers, 70.8% (17/24) were heavy smokers. Predominance of male sex was noted in both ALK-rearranged and ALK wild type tumors.

The morphologic features of ALK-rearranged and ALK wild type cases are summarized in Table 2. ALK testing in our institution was performed mostly on cytology specimens. Adenocarcinoma was the most common histologic subtype for both ALK-rearranged and ALK wild type tumors.

Among tumors whose morphological pattern can be definitively assessed, solid pattern was noted in 37.5% of ALK-rearranged tumors compared to 16.5% in ALK wild type tumors. Signet ring cell features, calcification, and cribriform pattern were only noted in ALK wild type tumors. Clear cell features and necrosis were seen in both ALK-rearranged and ALK wild type tumors. However, due to small sample size, further statistical analysis could not be performed to analyze the association

Table 2. Morphologic features of ALK-rearranged and ALK wild type non-small cell lung carcinoma

	ALK-rearranged (N = 8)	ALK wild type (N = 91)
Histologic subtype		
Adenocarcinoma	5 (62.5%)	76 (83.5%)
Adenosquamous carcinoma	0 (0%)	1 (1.1%)
Mucinous carcinoma	1 (12.5%)	0 (0%)
Non-small cell lung carcinoma	2 (25%)	9 (9.9%)
Pleomorphic carcinoma	0 (0%)	1 (1.1%)
Others	0 (0%)	4 (4.4%)
Morphological pattern[a]		
Lepidic	0 (0%)	2 (2.2%)
Acinar	1 (12.5%)	11 (12.1%)
Solid	3 (37.5%)	15 (16.5%)
Papillary	0 (0%)	8 (8.8%)
Micropapillary	2 (25%)	18 (19.8%)
Cannot be assessed	4 (50%)	64 (70.3%)
Histopathologic grade		
Grade 1	0 (0%)	0 (0%)
Grade 2	0 (0%)	1 (1.1%)
Grade 3	4 (50%)	26 (28.6%)
Cannot be assessed	4 (50%)	64 (70.3%)
Presence of histologic features[a]		
Signet ring	0 (0%)	3 (3.3%)
Clear cell	1 (12.5%)	9 (9.9%)
Calcification	0 (0%)	2 (2.2%)
Necrosis	2 (25%)	17 (18.7%)
Cribriform	0 (0%)	5 (5.5%)
No special features	6 (75%)	58 (63.7%)
Specimen type		
Core Biopsy	1 (12.5%)	24 (26.4%)
Cytology	3 (37.5%)	49 (53.8%)
Resection	4 (50%)	18 (19.8%)

[a] Multiple morphological patterns and histologic features were seen in some cases.

of these features with the presence of ALK rearrangement. A photomicrograph of the only ALK-rearranged tumor with clear cell features is depicted in Figure 2.

DISCUSSION

Though the sample size for this study is not large enough to perform statistical testing, the results are similar to those previously published in Western journals.[3-5] The prevalence of ALK rearrangement in our institution was not markedly different from previously published rates of ALK rearrangement in NSCLC, which range from 1.5-6.7% in unselected populations and 5% in Asian populations.[2,6] This relative rarity of ALK rearrangement compared to other molecular alterations in NSCLC makes accrual of a large cohort for epidemiologic studies less feasible.

Young age, history of never smoking or light smoking, and high stage disease were previously determined to be statistically different between ALK-rearranged and ALK wild type NSCLC in Western populations; these patterns are also seen in our study. However, it is important to note that these clinical features by themselves are not deemed sufficient to predict the presence of ALK rearrangement with a high degree of certainty.

Early studies also suggested the presence of unique histomorphologic features in ALK-rearranged tumors. These studies evaluated the possible association of the presence of signet ring cells, clear cells, hepatoid cytology, extracellular mucin, calcification, necrosis, and cribriform

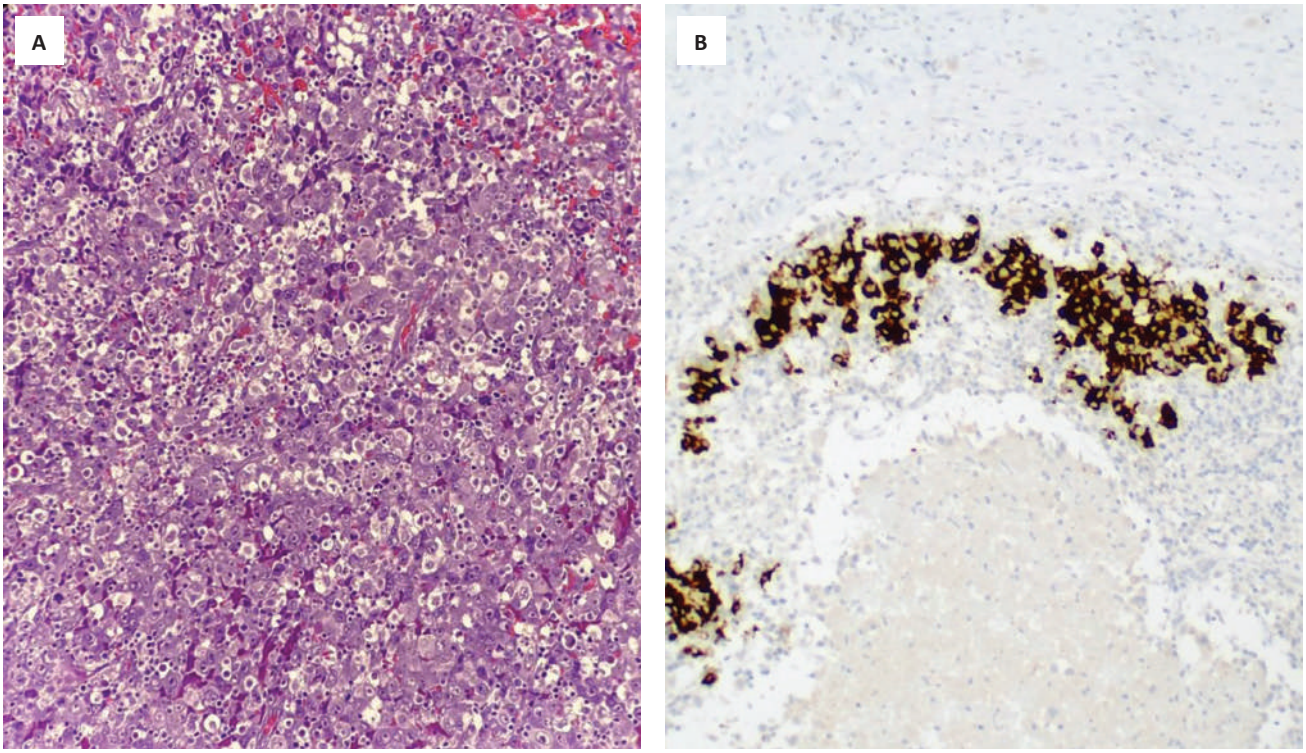

Figure 2. (A) Non-small cell lung carcinoma with clear cell features and solid pattern (H&E, 20x). **(B)** The same tumor demonstrating positivity for ALK on immunohistochemistry (ALK IHC, 20x).

pattern with ALK rearrangement. Of these, solid pattern and the presence of signet ring cells were consistently identified as an associated feature.[3,4] In the study by Rodig et al., a solid pattern of growth and the presence of signet ring cells comprising at least 10% of a tumor were noted in 56% of ALK-rearranged cases, compared to only 5% of ALK wild type cases. Nishino et al., likewise noted that a solid-predominant pattern and signet ring cells were more common in ALK-rearranged primary and metastatic lung tumors. Hepatoid morphology and the presence of psammoma bodies/ calcifications were also noted to be more common in primary, but not metastatic, lung tumors.[4] In specimens obtained by minimally invasive procedures (e.g., endobronchial and transthoracic biopsies, core biopsies, and cell blocks), the presence of signet ring cells was the only significant morphologic feature in ALK-rearranged tumors. In our study, although three of the eight ALK-rearranged tumors showed a solid pattern of growth, none of these showed signet ring cell features. Given our small number of ALK-rearranged cases, we could not conclude whether this difference is due to the small sample size, or whether other factors, such as regional differences and the type of specimen (predominantly cytology samples), could have played a role in the discrepancy. Further studies with a larger sample size are needed for a more thorough evaluation of the association between morphologic features and ALK rearrangements.

There has been a push for correlation with clinical profiles and establishment of a scoring system to efficiently triage tumors for ALK rearrangement studies in financially constrained settings. Nishino et al., have proposed one such scoring system, with a sensitivity of 89% and specificity of 75% in their study which included 226 primary lung tumors. Upon validation with a new cohort of 78 lung adenocarcinoma cases, their scoring system was noted to predict ALK rearrangement with a sensitivity of 88%, specificity of 45%, positive predictive value of 49%, and negative predictive value of 87%. However, the authors have recognized that this scoring system will not detect a minority of ALK-rearranged lung tumors and overall do not recommend morphologic analysis alone to screen for ALK rearrangements, since all of these patients are expected to benefit greatly from targeted therapy. In the Philippine setting, where the financial capabilities of the patient are always taken into consideration, it may be worthwhile to further explore morphologic screening for ALK-rearranged tumors in future studies. Until then, the lack of demonstrable correlation between ALK rearrangement and clinico-pathologic features supports the recommendation to test all primary lung adenocarcinomas and non-small cell lung carcinomas/ squamous cell carcinomas from never smokers.

CONCLUSION

Despite a small sample size, the prevalence and clinical profile of ALK-rearranged NSCLC in our institution are congruent with those previously described in Western populations. The association of clinical profile and morphologic features with the presence of ALK rearrangement can be further explored in future studies.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

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Application of the Milan System of Reporting Salivary Gland Cytopathology: A Retrospective Cytohistological Study in a Tertiary Medical Center

Carolyn Marie Legaspi, Elizabeth Ann Alcazaren, Jose Carnate Jr.

Department of Laboratory Medicine and Pathology, The Medical City, Pasig City, Philippines

ABSTRACT

Background. A fine needle aspiration biopsy has been established as a safe, minimally invasive procedure in evaluation of salivary gland lesions. The complex overlapping cytomorphology of these lesions are challenging for pathologists, hence the introduction of an evidence-based system, the Milan System of Reporting Salivary Gland Cytopathology, to improve overall patient care. The study was taken up to re-classify salivary gland lesions from previous FNA biopsies in order to determine sensitivity, specificity, positive and negative predictive values of FNA, and evaluate the risk of malignancy of the various categories of the Milan system.

Methodology. This was a 6-year retrospective descriptive study in a tertiary medical center. All salivary gland FNA cases were reviewed by two pathologists, and re-classified into the six categories of the Milan System. The number of false positive, false negative, true positive and true negative cases were obtained by comparing with the final histopathology diagnosis, and the risk of malignancy per category were calculated.

Results. A total of 76 cases were reviewed and the overall average of the two readers diagnostic accuracy were 85.02% (95% CI: 84.50-85.60%), sensitivity and specificity were 80.77% (95% CI: 79.90-81.60%) and 86.19% (95% CI: 85.70-86.70%), respectively; positive and negative predictive values were 62.16% (95% CI: 60.70-63.60%) and 94.17% (95% CI: 94.00-94.40%), respectively.

Conclusion. The Milan System category with the highest risk of malignancy was Malignant (Category VI - 100%). FNAB is still a reliable tool for clinicians, and use of the Milan System of Reporting Salivary Gland Cytopathology is beneficial in increasing efficacy of communication among clinicians to improve patient care.

Key words: cytopathology, fine needle aspiration biopsy, Milan System, salivary gland

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Corresponding author: Carolyn Marie D. Legaspi, MD
E-mail: carolynmarie.legaspi@gmail.com
ORCiD: https://orcid.org/0000-0002-9072-1308

INTRODUCTION

Salivary gland tumors comprise about 3% to 6.5% of all head and neck tumors.[1,2] To diagnose the nature of these lesions, a fine needle aspiration biopsy (FNAB) is usually performed. This procedure is widely accepted by clinicians, and is considered as an effective and minimally invasive procedure, with a reported sensitivity and specificity of 86 to 100% and 90 to 100%.[3]

The interpretation of the FNAB sample is a challenge to pathologists, as many salivary gland lesions have diverse cytomorphology, with benign and malignant tumors having significant morphologic overlap.[4] The accuracy of FNAB is dependent on multiple factors such as biopsy technique, adequacy and quality of the prepared smears, lesion morphology, and experience of the reading cytopathologist.[1] These aforementioned factors contribute to the complexity of the final FNA reading, which then affects the subsequent treatment and overall prognosis of the patient.[5]

In order to address the challenges of salivary gland FNA samples, the American Society of Cytopathology and the International Academy of Cytology began to work on a

uniform reporting system for salivary gland cytopathology in 2015, with the goal of increasing the overall effectiveness of FNAB.[3] This culminated in the publishing of the book, The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC) in 2018. It is an evidence-based system, which contains six categories that have corresponding risk of malignancy (ROM) and suggested clinical management strategies.[3] The six-tier classification system of Milan provides a standardization of terms, which pathologists can use to facilitate better communication with clinicians and improve overall patient care.[3]

This study was undertaken to retrospectively re-classify salivary gland lesions from previous FNA biopsies in order to determine sensitivity, specificity, positive and negative predictive values of FNA, and evaluate the risk of malignancy of the various categories of the Milan system.

METHODOLOGY

Sampling

This was a 6-year retrospective study performed in a tertiary institution. Clearance for the study was obtained from the Institutional Review Board. All cases of fine needle aspiration biopsy of the salivary gland from the year 2014 to 2020, with available surgical follow-up were included in the study. Those cases which lacked either an FNAB or histopathology result within the institution were excluded from the study.

Materials and methods

The demographic data, previous cytology, and histopathology results of patients were obtained by electronic records review. The corresponding slides for cytology cases were retrieved and reviewed by two board certified anatomic pathologists, one with subspeciality in cytology and another in head and neck pathology. Both readers were blinded to official cytology and histopathology results. Cases were randomly arranged for each reader. The Milan System for Reporting Salivary Gland Cytopathology was used in the re-evaluation of the cytological features of each case. Cases were then re-classified into the six categories.

In our institution, salivary gland lesions are aspirated by clinicians, fellows and residents trained in performing aspiration procedures, with or without image guidance. A gauge 22 or 23 needle is commonly used, and aspirates are placed on glass slides which are first air-dried then fixed in 95% ethanol. All smears are then processed in the histopathology section of the laboratory by staining with Papanicolaou stain.

The Milan System categories II, III and IVA were combined into a negative group, while categories IVB, V and VI were combined into a positive group for statistical analysis. This grouping was modeled after the study performed by Hafez et al., which stated that these groupings were chosen as they have similar overall patient management.[1]

Demographic data for each case, including patient's age, sex, and location of lesion were determined by frequency and percentage. Cytological cases were subclassified into true positives, true negatives, false positives (interpreted in cytology as positive, but was either benign or non-neoplastic on final histopathology), and false negatives (interpreted in cytology as negative, but was malignant on histopathology). The sensitivity, specificity, positive predictive value, negative predictive value and risk of malignancy (ROM) were computed first for each reader, and then averaged to obtain the overall values. The final histopathologic diagnosis was considered as the gold standard.

RESULTS

A total of 76 FNAB cases were included in the study. The site of involvement, and distribution of cases by location and age is shown in Table 1. Males (67.11%) were more commonly affected than females (32.89%), and occurred mostly between the ages of 21 to 40 years old (38.16%). The most commonly affected site was the parotid gland (78.95%), followed by the submandibular gland (17.11%).

Of the 76 cases reviewed, the most common cytologic diagnosis was benign neoplasm, Category IVA (46.05%, n=35/76), and were composed of pleomorphic adenoma (82.86%, n = 29/35), Warthin tumor (14.29%, n=5/35), and oncocytoma (2.86%, n=1/35). The second most common cytologic diagnosis was non-diagnostic (17.10%–26.32%, n=13-20/76), and were due to paucicellular smears, hemorrhagic smears, or the lack of lesional cells in a clinically defined mass.

Correlation with histopathology results showed two false positive cases, one which was reported as suspicious for acinic cell carcinoma oncocytic variant, was an oncocytoma on final histopathology; and another case which was reported as suspicious for adenoid cystic carcinoma, turned out to be a pleomorphic adenoma. There was one false negative case, which was read as pleomorphic adenoma on cytology, but turned out to be a low grade mucoepidermoid carcinoma. Two cases had mis-subtyping and were both called Warthin tumor on cytology but turned out to be an oncocytoma on final histopathology. Among the non-diagnostic cases, one was chronic sialadenitis on final histopathology, one was atypical lymphoid proliferation, three were lymphoepithelial cysts, one lipoma, one chordoma, one infarcted pleomorphic adenoma, three Warthin tumors, and two malignant cases (lymphoepithelial-like carcinoma, and carcinoma with adenosquamous and oncocytic features). Table 2 summarizes all discordant cyto-histological cases.

Table 1. Distribution of cases by age, sex, and site of involvement

Parameter	Number of cases (Total N = 76)
Sex	
Male	51 (67.11%)
Female	25 (32.89%)
Age (years)	
<20	2 (2.63%)
21 to 40	29 (38.16%)
41 to 60	26 (34.21%)
61 to 80	19 (25.00%)
Gland involvement	
Parotid gland	60 (78.95%)
Submandibular gland	13 (17.11%)
Unspecified	3 (3.95%)

Table 2. Cyto-histologic correlation of discordant cases

Milan System diagnostic category	Cytologic diagnosis	Histopathologic diagnosis (N)
I. Non-diagnostic	Non-diagnostic smears	Atypical lymphoid proliferation (1)
		Benign lymphoepithelial cyst (3)
		Chronic sialadenitis (1)
		Lipoma (1)
		Warthin tumor (1)
	Hemorrhagic smears	Warthin tumor (2)
		Chondroma (1)
		Infarcted pleomorphic adenoma (1)
		Lymphoepithelioma-like carcinoma (1)
		Carcinoma with adenosquamous and oncocytic features (1)
II. Non-neoplastic	Sialadenitis	Warthin tumor (2)
	Reactive lymphadenitis	Pleomorphic adenoma (1)
III. AUS	Oncocytic neoplasm, paucicellular smears	Granulomatous lymphadenitis with caseation necrosis consistent with tuberculous lymphadenitis; Unremarkable submandibular gland (1)
	Sparse atypical cells	High grade mucoepidermoid carcinoma (1)
IVA. Neoplasm, benign	Pleomorphic adenoma	Low grade mucoepidermoid carcinoma (1)
IVB. SUMP	-	-
V. Suspicious for malignancy	Suspicious for malignancy, consider acinic cell carcinoma, oncocytic variant	Oncocytoma (1)
	Suspicious for adenoid cystic carcinoma	Pleomorphic adenoma (1)
VI. Malignant	-	-

Table 3. Recategorization of cases according to the Milan System with computed risk of malignancy

Milan System diagnostic category	No. of cases	Reader 1 (ROM)		No. of cases	Reader 2 (ROM)	
I. Non-diagnostic	13	3/13	(23.08%)	20	2/20	(10.00%)
II. Non-neoplastic	8	0/8	(0%)	4	0/4	(0%)
III. AUS	3	1/3	(33.33%)	1	1/1	(100%)
IVA. Neoplasm, benign	35	0/35	(0%)	35	1/35	(2.86%)
IVB. SUMP	10	2/10	(20.00%)	6	1/6	(16.67%)
V. Suspicious for malignancy	4	4/4	(100%)	4	2/4	(50.00%)
VI. Malignant	3	3/3	(100%)	6	6/6	(100%)

The recategorization of cytological cases along with the ROM per category is shown in Table 3. Concordance and discordance between cytologic and histopathologic diagnosis was calculated for all cases, excluding the non-diagnostic category. Concordance was found at 95.24% (n=60/63) and 96.43% (n=54/56) for reader 1 and 2, respectively, and discordance was found at 4.76% (n=3/63), and 3.57% (n=2/56), for each reader, respectively.

For reader 1, the sensitivity, specificity, positive predictive value, and negative predictive values were 69.20% (95% Confidence Interval [CI]: 38.60–90.90%), 87.30% (95% CI: 76.50–94.40%), 52.90% (95% CI: 27.80–77.00%), and 93.20% (95% CI: 83.50–98.10%) respectively. For reader 2, the sensitivity, specificity, positive predictive value and negative predictive value were 69.20% (95% CI: 38.60–90.90%), 88.90% (95% CI: 78.40–95.40%), 56.20% (95% CI: 29.90–80.20%), and 93.30 (95% CI = 83.80–98.20%), respectively. The diagnostic accuracy was 84.21% (95% CI: 84.00–85.8%) and 85.83% (95% CI: 84.00–85.8%) for each reader, respectively.

Upon exclusion of the non-diagnostic category from analysis, the re-computed values are as follows: For reader 1, the sensitivity, specificity, positive predictive value and negative predictive value were 76.92% (95% CI: 46.19–94.96%), 84.00% (95% CI: 70.89–92.83%), 55.56% (95% CI: 38.27–71.60%), and 93.33% (95% CI: 73.84–97.44%). While for reader 2, the sensitivity, specificity, positive predictive value and negative predictive value were 84.62% (95% CI: 54.55–98.08%), 88.37% (95% CI: 74.92–96.11%), 68.75% (95% CI: 48.31–83.81%), and 95.00% (95% CI: 84.09–98.56%). The diagnostic accuracy was 82.54% (95% CI: 70.90–90.95%) and 87.50% (95% CI: 75.93–94.82%) for each reader, respectively.

For the overall findings, the average of the two readers were taken and the values are as follows: sensitivity and specificity were 80.77% (95% CI: 79.90-81.60%) and 86.19% (95% CI: 85.70-86.70%), respectively; while positive and negative predictive values were 62.16% (95% CI: 60.70-63.60%) and 94.17% (95% CI: 94.00-94.40%), respectively. The summary of all these values is seen in Table 4.

DISCUSSION

The MSRSGC is a relatively new classification system, which is evidence based and provides risk stratification by reporting ROM per category, with suggested clinical management.[3] The reported ROM per category can be found in Table 1. The ROM computed in the present study appears to be at par with the reported ROM in MSRSGC and other similar studies (Table 5).

When the non-diagnostic category was included in the analysis, the sensitivity of each reader (69.20%) was found to be lower than that reported in MSRSGC (86-100%), and in a meta-analysis of 92 studies (96.9%); while the specificity for each reader was at par (87.30 and 88.90%)

Table 4. Summary of computed statistics in comparison to other similar studies

Parameter	Present Study (Reader 1)		Present Study (Reader 2)		Present Study Average (Excluded ND cases)	Santiago et al[7]	Amita et al[8]	Hafez et al[1]	Farahani et al[6]	MSRSGC[3]
	Included ND Cases	Excluded ND Cases	Included ND Cases	Excluded ND Cases						
Sensitivity	69.20%	76.92%	69.20%	84.62%	80.77%	46%	89.4%	84.6%	96.9%	86-100%
Specificity	87.30%	84.00%	88.90%	88.37%	86.19%	100%	100%	88.2%	95.3%	90-100%
PPV	52.90%	55.56%	56.20%	68.75%	62.16%	90%	100%	78.6%	-	-
NPV	93.20%	93.33%	93.30%	95.00%	94.17%	91%	95.74%	91.8%	-	-

ND – Non-diagnostic; PPV – Positive Predictive value; NPV – Negative predictive value

Table 5. Comparison of ROM across several studies

Author	Category I	Category II	Category III	Category IVA	Category IVB	Category V	Category VI
MSRSGC[3]	25% (0 to 67%)	10% (0 to 20%)	20% (10 to 35%)	<5% (0 to 13%)	35% (0 to 100%)	60% (0 to 100%)	90% (57 to 100%)
Present Study							
Reader 1	23.08%	0%	33%	0%	20%	100%	100%
Reader 2	10%	0%	100%	2.86%	16.67%	50%	100%
Hafez 2019[1]	33.30%	11.8%	37.50%	2.10%	44.40%	60%	100%
Amita 2018[8]	-	6.25%	100%	0%	25%	100%	100%
Viswanthan 2018[9]	6.70%	7.10%	5%	38.90%	34.20%	92.60%	92.30%

with those reported in MSRSGC (90-100%) and the meta-analysis (95.3%).[3,6] Upon re-computation of these statistics to exclude those non-diagnostic cases, the sensitivity per reader increased (76.92% and 84.62%, respectively). In comparison, our values are similar those reported in a local study performed by Santiago et al., in 2016, which focused on parotid gland FNAB.[7] With a similar sample size of 76 cases, their findings were a sensitivity of 46% and specificity of 100%. In their study, the low sensitivity was due to a high false negative rate of 53.85% (n = 7/13).[7] This was attributed to the misdiagnosis of malignant salivary gland tumors as benign.[7] However it can be noted that for our study, the non-diagnostic cases contributed to the low sensitivity, which took up to 26.32% (n = 20/76) of the cases reviewed, as re-computation showed an increase in the sensitivity for each reader (Table 4). In our study the false negative rate is 30.77% (n = 4/13), much lower than the one presented in Santiago et. al.[7] In the present study, one case was read as pleomorphic adenoma by one reader, and the final outcome was a low-grade mucoepidermoid carcinoma. The other three cases were non-diagnostic; two cases with a final histopathology report of malignancy (Lymphoepithelioma-like carcinoma, Carcinoma with adenosquamous and oncocytic features); and one case with atypical lymphoid proliferation.

Among the non-diagnostic cases in our study, up to 20 (17.11–26.32%) were predominantly due to pauci-cellularity, or hemorrhagic smears. The non-diagnostic rate in other studies range from 5 to 10%, though some studies have a reported non-diagnostic rate of 4.3% up to 12%.[5,8,9] Some factors which may have contributed to the high number of non-diagnostic cases in our study may be poorly prepared slides, three of which contained obscuring blood, while others were due to the overt lack of lesional cells, and the fading of stains from storage, which rendered the slides more difficult to interpret. This is supported by the findings in similar studies which state that aspiration technique, presence of artifacts or obscuring elements, inherent lesion characteristics, and experience of the performer are among several factors that can contribute to the final diagnosis.[5,8,9]

Some studies suggest the use of rapid on-site evaluation (ROSE) to decrease the number of false negative cases.[5,10,11] It has been found that ROSE can be used to determine the adequacy of a sample and findings of atypia or malignancy during the procedure can be useful to facilitate early clinical decision making.[10,11] One of the disadvantages of ROSE may be the need for a proficient cytopathologist, or an expert on salivary gland tumors during the procedure.[5] However the current MSRSGC does not mention ROSE, instead they suggest to use the adequacy guidelines similar to that found in the Bethesda system for Reporting Thyroid Gland Cytopathology, or to count at least 60 lesional cells.[3] It is recommended to keep the non-diagnostic rate at 10% or below, in order to avoid high false negative rates.[3]

For the non-neoplastic cases in the study, the ROM for both readers were 0%, much lower compared to those reported in other studies (Table 5). Nearly all cases (62.5%, n = 5/8) classified under this category turned out to be chronic sialadenitis, with three cases being benign neoplasms (Warthin tumor and Pleomorphic adenoma) on final histopathology. Review of the cases revealed hypocellular smears containing mostly benign acinar cells with a predominantly chronic inflammatory infiltrate in the background (Figure 1). A Warthin tumor may be misdiagnosed as chronic sialadenitis, since both lesions contain a lymphoid background. This finding was similarly reported in a study by Amita et al.[8]

A total of three cases were classified under the AUS category, which turned out to be granulomatous lymphadenitis with caseation necrosis, chronic sialadenitis, and high grade mucoepidermoid carcinoma. The ROM for this category varies widely across studies from as low as 5% up to 100%, and also showed variation between the two readers (Table 5).[8,9] The variation in the ROM for this category reflects its heterogeneity. It includes lesions which may show reactive atypia or may represent poorly sampled neoplasms (Figure 2).[3] The MSRSGC has recommended that AUS be used in less than 10% of cases, and recommends a repeat FNAB for some lesions or surgery for more worrisome lesions.[3] It is suggested that careful assessment of smears, and paying attention to

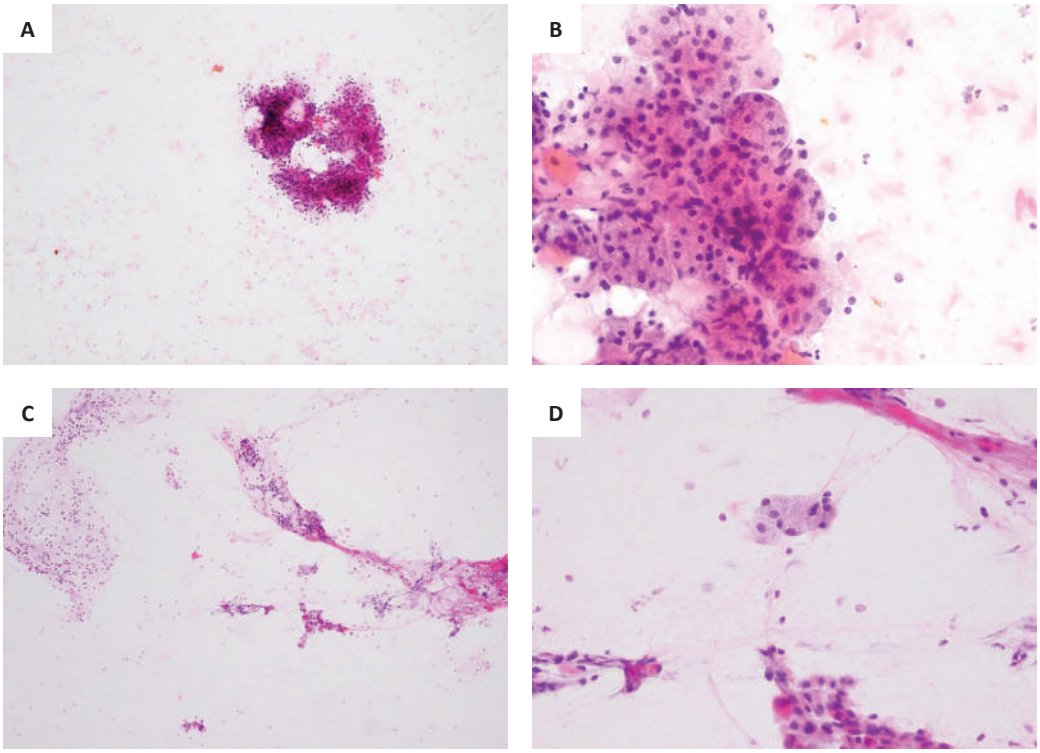

Figure 1. ***Non-neoplastic smears.*** Upper row: **(A and B)** This smear contained few groups of normal appearing acinar cells [**(A)** Papanicolaou, 100x and **(B)** 400x]. Lower row: **(C and D)** Smear containing rare acinar cells and background inflammatory cells [**(C)** Papanicolaou, 100x and **(D)** 400x].

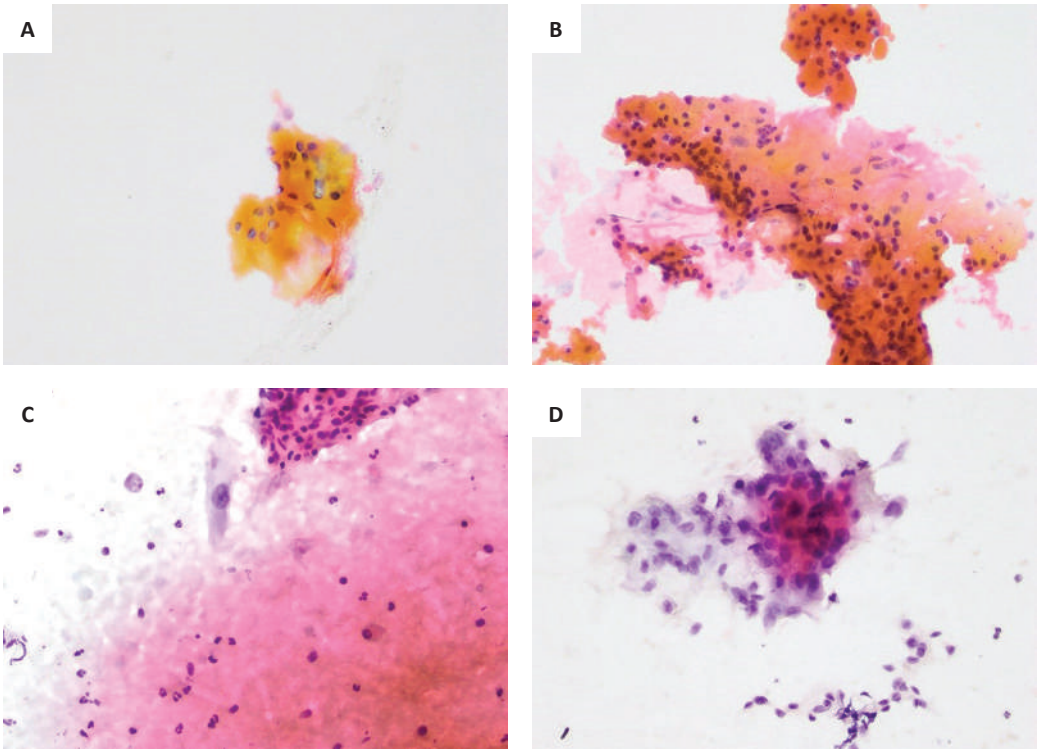

Figure 2. ***Atypia of undetermined significance.*** Upper row: **(A and B)** Cells shown were described to have oncocytoid features with mild nuclear atypia (Papanicolaou, 400x.) Lower row: Rare large atypical cells seen singly **(C)** or in groups **(D)** are shown, with enlarged nuclei and irregular nuclear borders (Papanicolaou, 400x).

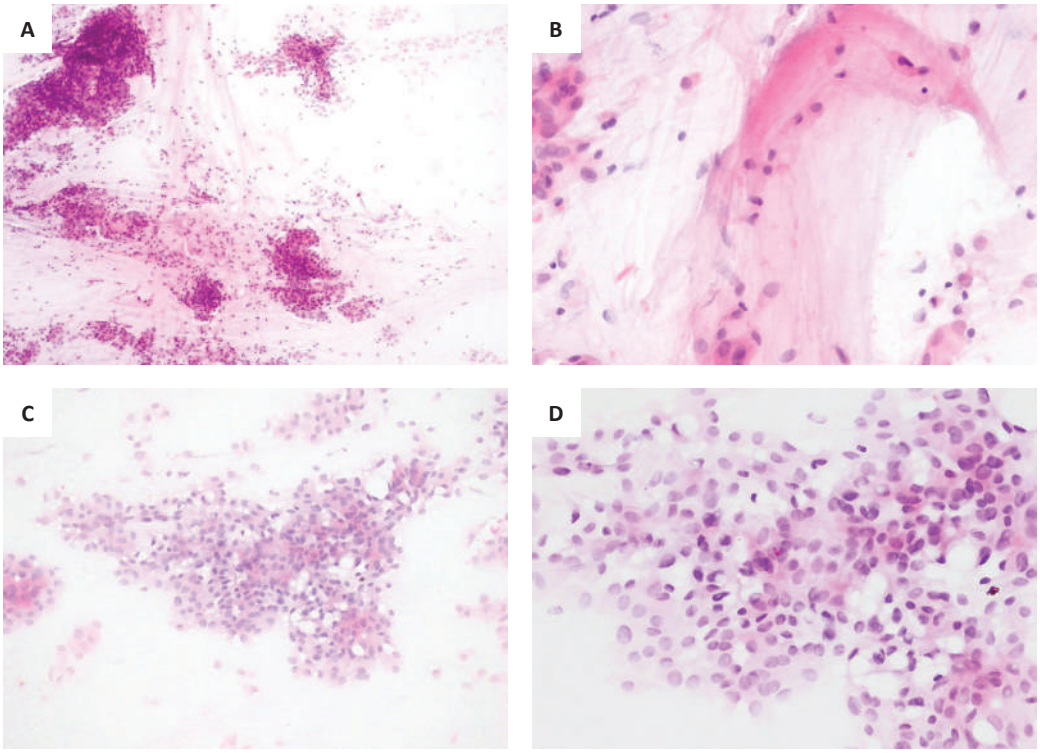

Figure 3. ***Non-neoplastic smears.*** Smears of **(A)** and **(B)** show groups of bland appearing, plasmacytoid cells within an eosinophilic fibrillary-like stromal background [**(A)** Papanicolaou, 100x and **(B)** 400x]. **(C)** The cells are fairly uniform, without ball-like clustering (Papanicolaou, 100x). **(D)** There is mild to no nuclear atypia with fine chromatin, and vacuole-like spaces are seen in the cytoplasm (Papanicolaou, 400x).

detail may aid in reducing the use of this category, thus lowering the variation in ROM.[1] Some features to take note of, besides cellular atypia, would be the presence or absence of mucin in the background, heterogeneity of the cell population and the degree of atypia in lymphoid populations.[1,3] One study further investigated the ROM of AUS category, by subclassifying AUS further into 6 groups which were: reactive and reparative atypia; squamous, oncocytic, or metaplastic changes; low cellularity; specimens with preparation artifacts, mucinous cystic lesions; and lymph node or lymphoid lesions.[12] This study found that further subtyping of the AUS category showed differences in ROM, and highest ROM (100%) was noted in the specimens with preparation artifacts hampering the distinction between non-neoplastic and neoplastic lesions.[12] They therefore suggest that subtyping AUS cases may be beneficial to guide clinical management.[12]

The category of benign neoplasms composed the bulk of the present study (46.05%, n = 35/76), and the most common entities were pleomorphic adenoma and warthin tumor. This is similar to other studies which also reported pleomorphic adenoma as the most commonly aspirated benign lesion.[5–7,13] The ROM obtained in the present study for this category is also at par with similar studies (Table 5). In the present study, one case of low grade mucoepidermoid carcinoma was called a pleomorphic adenoma in cytology (Figure 3). It is stated that there is much difficulty in distinguishing benign from low grade lesions, due to their overlapping cytomorphologic features.[13] Review of the smears showed increased cellularity, though individual cells had an overall bland appearance, with minimal atypia and poor staining. Careful examination of the smears showed some cells with rare cytoplasmic inclusions. These factors in addition to possible misinterpretation of background stroma, may lead to an erroneous diagnosis.[5,13] In addition, it is recommended to have a smear stained with Giemsa or Diff Quick, as these stains better highlight the appearance of background stroma.[3]

In the present study, up to ten cases were classified under SUMP category. The ROM for this category was also comparable to other similar studies (Table 5). Two cases of SUMP turned out to be adenoid cystic carcinoma, another two were basal cell adenoma, and the remaining cases were cellular pleomorphic adenoma. This category is used when a diagnosis for a definitive entity cannot be made, and malignancy cannot be excluded.[3] The high cellularity of smears, predominantly basaloid population of cells, and matrix poor background are among the following factors which contribute to this diagnosis and is similarly found in other studies (Figure 4).[1,8,13]

In the present study, four cases were classified under suspicious malignancy, which comprised 5.26% of all reviewed cases. Two of the four cases were falsely positive, and final histopathology showed an oncocytoma, and another was pleomorphic adenoma (Table 2). The increased cellularity, along with presence of cellular atypia, obscuring blood and quality of stains were among some factors attributed to the misdiagnosis (Figure 5). This finding is similar to one reported study wherein smears were suspicious for a mucoepidermoid

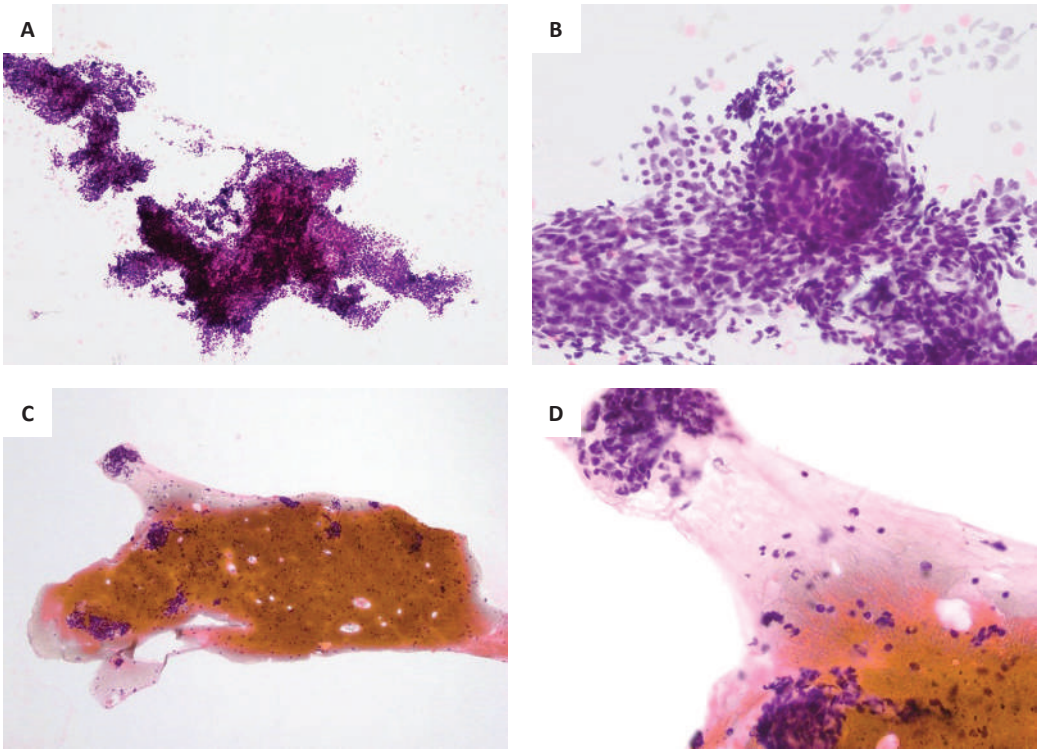

Figure 4. ***Salivary gland neoplasm of uncertain malignant potential.*** Upper row: **(A)** and **(B)** showing a cellular smear composed of sheets of basaloid appearing cells with mild nuclear atypia. There is lack of any distinct matrix in the background [**(A)** Papanicolaou, 100x and **(B)** 400x)]. Lower row: The thick preparation of **(C)** and **(D)** slightly obscure nuclear features of this sample, though the basaloid character of the cells can still be appreciated [**(C)** Papanicolaou, 100x and **(D)** 400x)].

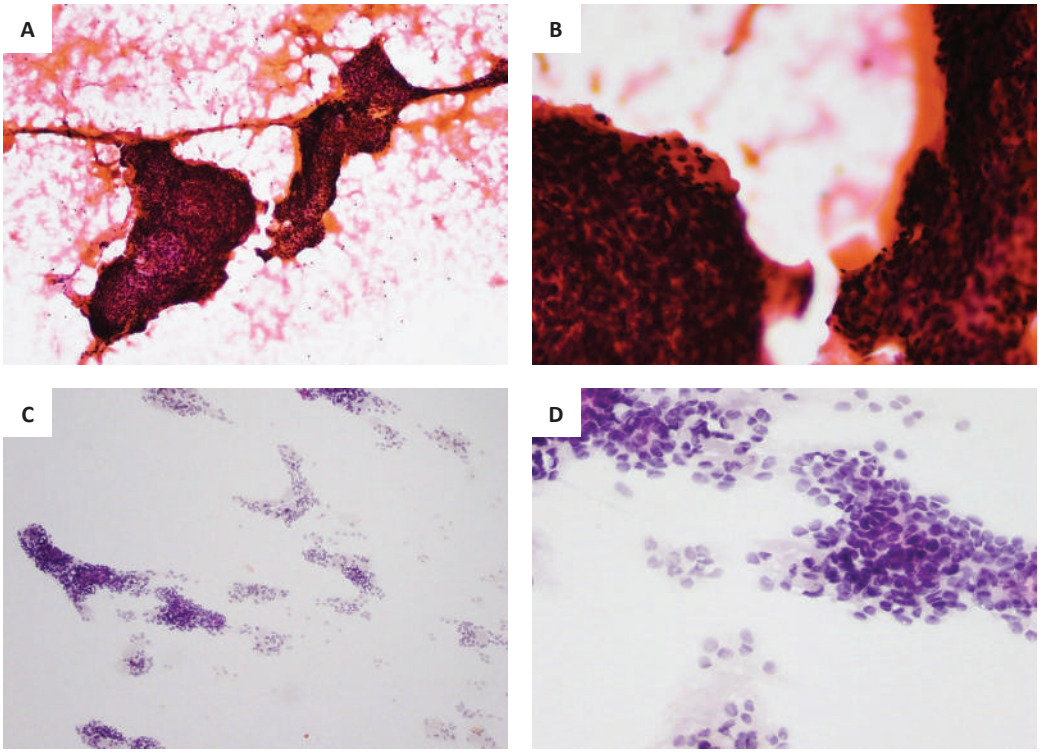

Figure 5. ***Suspicious for malignancy.*** Upper row: Smears of **(A)** and **(B)** show large groups of cells obscured by blood. Overall cellularity is increased and show atypical features with overlapping enlarged, hyperchromatic nuclei with variable eosinophilic cytoplasm [**(A)** Papanicolaou, 100x and **(B)** 400x]. Lower row: Smears of **(C)** and **(D)** show basaloid cells with atypical nuclear features of hyperchromatic nuclei, irregular nuclear membranes, and scant cytoplasm [**(C)** Papanicolaou, 100x and **(D)** 400x].

carcinoma, but turned out to be a pleomorphic adenoma on histopathology.[13] As for the malignant category, all cases had cytohistologic correlation, and the ROM was at par with that reported in the MSRSGC (Table 5).

CONCLUSION

The current study finds that the sensitivity is lower than that reported by MSRSGC.[3,6] This may be due to the discordant cases which were predominantly non diagnostic, with poor cellularity or poor quality of smears. This highlights the importance of pre-analytical factors in rendering the final diagnosis. ROSE may be recommended to decrease the number of non-diagnostic samples and facilitate clinical management. Lesion morphology is still a challenge, however the overall ROM of the present study is found to be comparable to that reported in MSRSGC and other similar studies, which is shown in Table 5.[1,3,8,9] The slight variation in ROM, especially for AUS category, may be attributed to the heterogeneity of included samples and experience of the reading pathologists. Using a tiered classification system like the MSRSGC can facilitate standardization of reporting and improve clinical decision making. The overall findings of the study suggest that FNAB is still a reliable tool for clinicians in the diagnosis of salivary gland tumors, and that application of MSRSGC in the local setting can be beneficial in reducing misdiagnosis and facilitate better patient care.

Some limitations of the current study include the limited sample size, retrospective design, and the faded quality of stored smears. It is recognized that the entities described in this study may not represent those seen in other institutions. Furthermore, the lack of Giemsa-stained smear preparations may have contributed to the misdiagnosis of some cases. It is thus recommended to consider including this stain as part of the routine processing procedure for future salivary gland samples. The interobserver variability and concordance rates between or among observers was not determined in this study. The determination of an over-all recategorization of cases for final cytologic diagnosis among readers was not performed in this study. Further investigation of the Milan System to determine concordance among pathologists, or investigation using a prospective study design may also be undertaken.

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STATEMENT OF AUTHORSHIP

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AUTHOR DISCLOSURE

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OPEN ACCESS – ORIGINAL ARTICLE

Preliminary Study on Prevalence of P16-Positive Squamous Cell Carcinoma of the Oral Cavity, Oropharynx and Larynx in Rizal Medical Center and its Histomorphologic Correlation

Jorel Renly Gamboa and Thomas Jeff Lim Jr.

Department of Pathology and Laboratory Medicine, Rizal Medical Center, Pasig City, Philippines

ABSTRACT

Background. A considerably large portion of the cases of cancer, particularly squamous cell carcinoma (SCC) involving the head and the neck may be due to consumption of tobacco and alcohol. However, its increase in occurrence at specific sites of the head and neck may indicate the possibility of other etiological factors. One of which is infection by certain high-risk human papillomavirus (HPV). P16 immunochemistry serves as a very good surrogate marker of active HPV in these tumors. The detection of HPV-related head and neck cancers have relevance in clinical practice because of its prognostic implications.

Objectives. The general objective of this study is to determine the prevalence of p16-positive SCC in the oral cavity, oropharynx, and larynx in Rizal Medical Center from January 2019 to December 2019. The specific objective is to compare the Hematoxylin and Eosin (H&E) stain histomorphology (keratinization and mitotic activity) of p16-positive versus p16-negative specimens.

Methodology. This is a cross-sectional study which included all routine histopathology specimens coming from the oral cavity, oropharynx and larynx in Rizal Medical Center for the year 2019 with a diagnosis of squamous cell carcinoma. The tissue specimens considered were those that measured at least 1 cm in diameter, or aggregate diameter if tissue is fragmented. The slides of all eligible cases were reviewed and immunohistochemically stained for p16. The p16 IHC slides were read as either positive or negative, while the mitotic activity and keratinization were observed in the H&E-stained slides. The interpretation of the diplomate pathologists for each of the slides were documented and corresponding statistical analyses were performed.

Results. P16 IHC showed twenty-one (88%) p16 negative cases and three (12%) p16 positive cases. In terms of mitosis, ten cases have mitosis falling within the 1-10 per high power field (HPF) range (42%), six within 11-20 per HPF (25%), and 8 cases have ≥21 mitosis per HPF (33%). In terms of keratinization, three cases are non-keratinizing (12%) and twenty-one cases are keratinizing (88%). There is significant difference in the keratinization histology ($p<0.05$) of the p16-positive versus the P16-negative cases. On the other hand, no significant difference in the mitotic activity ($p>0.05$) was noted.

Conclusion. There is a low prevalence of HPV-related SCC of the oral cavity, oropharynx, and larynx in Rizal Medical Center. The histomorphologic findings confirm that keratinization, significantly predicts HPV status in oropharyngeal SCC. Mitotic activity may not be reliable in predicting the HPV status or p16 IHC reactivity of a case. Keratinization in oropharyngeal SCCs may provide valuable information in certain instances, particularly when HPV testing is not immediately available, although the combined tumor morphology and p16 IHC is more ideal.

Key words: papillomavirus infections, squamous cell carcinoma of head and neck, mouth, larynx

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Corresponding author: Jorel Renly S. Gamboa, MD
E-mail: info@rmc.doh.gov.ph
ORCiD: https://orcid.org/0000-0002-1501-3301

INTRODUCTION

A considerably large portion of the cases of cancer involving the head and the neck is by higher-than-normal consumption of tobacco and alcohol. However, the noticeably increased occurrence of the said phenomenon at specific sites indicates the possibility that other etiological factors are involved. In certain localities, it has been reported that infection by certain high-risk types of high-risk human papillomavirus (HPV) are implicated in cases of head and neck cancers – the most notorious of which is oropharyngeal cancer. Evidence suggested by current studies identify HPV16 as being associated in cancers of the tonsils, the base of the tongue, and other sites in the oropharynx.[1] This is not to say that HPV16

is solely responsible for the development of these cancers since several risk factors have also been found to protract their prevalence and worsen their effects such as changing sexual behaviors, involvement in oral sex, high turnover rates in terms of sexual partners, and involvement in sexual intercourse with someone of the same sex.[2,3]

In the United States, HPV-associated cancer rates increased for oropharyngeal cancer from year 1999 to 2015.[3] In the Philippines, Bruni et al., mentioned that there is an increased incidence of oropharyngeal cancer in the year 2018. The annual number of new cancer cases for males is 311 and 118 for females.[3] Currently, there is no available local data on the prevalence of p16 positivity for head and neck squamous cell carcinoma (SCC).

Because of the prognostic implications of HPV-related oropharyngeal SCC, histomorphology and p16 immunohistochemistry (IHC) may help physicians in their clinical judgement and treatment approaches. P16 IHC may be utilized as an alternative means to indicate the presence of high-risk HPV[4] and it offers a more cost-effective, more manageable and convenient alternative to HPV-specific testing.

The detection of HPV in oropharyngeal SCC has relevance in clinical practice because of its prognostic implications. Several studies in the past have indicated better prognostic outlooks and improved disease-free survival for patients diagnosed with HPV-positive tumors in the oral cavity. Some medical researchers opined that the significantly better clinical outlook for HPV-positive tumors is attributed to their radiosensitivity and focality. In fact, the National Comprehensive Cancer Network (NCCN) guidelines have prescribed separate treatment algorithms for p16-positive and p16-negative oropharyngeal SCCs.[5] The actual prevalence of HPV in oral SCC must also be elucidated because it has implications to public health. For instance, said statistics will provide an input for health agencies to determine whether or not there is a need to allocate resources for HPV vaccination or prevention. The determination of morphologic features such as keratinization status and mitotic index as predictors of HPV status may be of value in resource-limited settings when p16 IHC is not readily available.

OBJECTIVES

General objective

To determine the prevalence of p16-positive SCC in the oral cavity, oropharynx, and larynx in Rizal Medical Center from January 2019 to December 2019.

Specific objective

To compare the Hematoxylin and Eosin (H&E) stain histomorphology (keratinization and mitotic activity) of p16-positive versus p16-negative specimens.

REVIEW OF RELATED LITERATURE

Head and neck SCC usually manifest in the larynx, oropharynx, nasopharynx, hypopharynx, paranasal sinuses, oral cavity, and salivary glands.[6] They have been listed as among the commonly occurring cancers in the world with estimated figures of around 600,000 new cases and approximately 320,000 deaths yearly, indicating an increasing trend from the figures obtained in the previous years.[7] Large scale epidemiological researches have shown that head and neck cancers are more common among men than women. Moreover, about 90% of head and neck cancers are SCC, rendering said histologic type as the most commonly occurring.[6] The presence of HPV in these tumors as identified in several studies suggests the etiological implication of HPV in tumorigenesis. Initially, HPV was believed to account for at least 23% of oropharyngeal cancer.[8] In more recent studies, however, it has been shown that at least 70% of oropharyngeal cancer incidence in the US in the last three decades may be casually linked to HPV.[9] These may be conferred by the fact that unlike cervical cancer which affects only women, oropharyngeal cancer affects both men and women.

Worldwide data on the prevalence and type distribution of HPV in head and neck SCC have been systemically reviewed and subjected to meta-analysis with results showing HPV DNA to be less prevalent in the in the oral cavity (24.2%) and in the larynx/hypopharynx (22.1%) than in the oropharynx (45.8%). HPV16 is implicated in 82.2% of all HPV DNA-positive cases, thereby indexing it as genotype most frequently found in head and neck SCC.[10]

The relative prevalence of HPV-associated oropharyngeal SCC varies among geographic regions, the highest (29%-93%) cases having been observed from economically developed countries.[11] HPV has been identified as the primary and essential etiologic agent of cervical cancer due to its susceptibility of being transmitted through sexual intercourse. In like manner, HPV-positive oropharyngeal SCC has also been associated with sexual behavior.[12]

In the Philippines, a 2013 study estimated that the annual incidence (per 100,000 population) of cancer in the oral cavity is 2.4, while laryngeal and oropharyngeal cancers had an annual incidence of 1.5 and 1.3, respectively.[7] In terms of incidence among women, cancers of the lip/oral cavity rank 7th, cancers in the larynx rank 10th, and that in the pharynx ranks 13th.[7] Excessive alcohol and tobacco consumption have been listed as the main risk factors in head and neck SCC. In response, campaigns against alcohol and tobacco use in several western countries over the recent decades have significantly reduced the incidence of cancers in the oral cavity and the larynx. Unfortunately, said measures seem to have failed where oropharyngeal cancer is concerned.[11,13] In fact, it has been observed that there remains to be a steady increase in cases of SCC in the oropharynx and this is especially true in countries with more developed economies.

HPV types are either low-risk or high-risk. Carcinomas are usually implicated with high-risk serotypes of HPV, 16 and 18.[14] In cases of carcinogenesis involving HPV, molecular activity is characterized by the papillomaviral DNA integrating with the host DNA. HPV E6 binds with the gene product p53, which is a tumor-suppressor, and inactivates it. At the same time, the HPV E7 does the same thing with the retinoblastoma tumor-suppressor protein (pRb), effectively deactivating the tumor-suppressing function of the retinoblastoma gene which results to cell

cycle progression.[15] The inactivation of the retinoblastoma protein by E7 causes a feedback loop that increases the activity of the P16 cyclin-dependent kinase which upregulates cell cycle. This marked upregulation of P16 has been observed in cases of head and neck SCC caused by high-risk HPV because this molecular activity leads to a paradoxical overexpression of the tumor-suppressor protein p16, which is consistently overexpressed in the nuclei and cytoplasm of tumors with transcriptionally active high-risk HPV.[16] P16 IHC thus serves as a very good surrogate marker of active HPV in these tumors.

The prognostic value of p16 positivity in head and neck SCC has been documented by several medical researchers. In particular, studies that looked into the better clinical outcome for patients with p16-positive oropharyngeal SCC corroborate that patients with HPV-related tumors in the head and neck region have better clinical outlooks than HPV-negative patients.[17-19] The importance of determining pl6-positivity for better prognosis holds true for patients treated with radiotherapy alone as well as for those who received treatment that combined systemic treatment and radiotherapy.

The prognosis for HPV-related SCC shows markedly better outlooks than for HPV-negative carcinomas notwithstanding the tendency of HPV-related SCCs to metastasize to neck lymph nodes early in the course of disease. In fact, risk of death is 30-50% lower for HPV-related oropharyngeal SCC than for HPV-negative SCC. However, patients with history of excessive tobacco use and/or are currently (heavy) smokers may not benefit from the aforesaid improved prognosis. HPV-related oropharyngeal SCC responds better to both primary chemoradiation and surgical treatment. This may be conferred by the fact that these tumors have lower mutation rates and are less genetically complex than HPV-negative cancers. There are now definitive prospective studies showing that the prognosis of HPV-related OPSCC patients has improved such that the head and neck oncology community is essentially united in the concept that all new patients should be tested for high-risk HPV.[20]

According to Chernock (2012), most HPV-unrelated tumors have keratinizing characteristics while most HPV-related SCCs in the oropharynx appear usually as non-keratinizing. Microscopically, HPV-related non-keratinizing tumors have the characteristic of aggregating into large nests with borders that push against each other due to the absence of adequate stromal response. These tumors tend to undergo frequent mitoses and is characterized by an accumulation of dead cells in a central location, otherwise known as central comedonecrosis. The shape of the tumor cells varies; some are ovoid while other are spindle-shaped. The cell borders are often indistinct. These tumor cells are also characterized by hyperchromatic nuclei with no distinct nucleoli. There is either very minimal squamous maturation or none at all. Alternately, HPV-related tumors have also been described as basaloid, basal-like, poorly-differentiated, or non-keratinizing.[21] According to Bishop et al. (2015, 2017), HPV-SCCs are characterized by elevated mitotic rates and frequent tumor necrosis. In contrast, non-HPV-related keratinizing SCCs are typically composed of infiltrative nests with prominent stromal desmoplasia. The tumor cells are polygonal with distinct cell borders and have more abundant, eosinophilic cytoplasm. Squamous maturation is diffuse.[22,23]

METHODOLOGY

This is a cross-sectional study that involves routine histopathology specimens coming from the oral cavity, oropharynx and larynx in Rizal Medical Center for the year 2019 diagnosed as SCC. This study was reviewed and granted approval by the Institutional Review Board (IRB) of Rizal Medical Center.

Inclusion and exclusion criteria

This study involves routine histopathology specimens coming from the oral cavity, oropharynx and larynx in Rizal Medical Center for the year 2019 diagnosed as SCC. The tissue specimens must fulfill the following conditions: the surgical procedure done to the specimen was at least an incision, wedge, excision or resection; for incision or wedge biopsies, the tissue on the slide must be at least 1 cm; and no prior IHC was done on the specimen. Any of the following criteria excluded a specimen from this study: other carcinomas of the sites of interest aside from SCC, tissue size less than 1 cm in diameter or aggregate diameter; and cases for which the microscopic slides and/or paraffin blocks could not be retrieved (slide reviews, missing blocks). The researchers focused on tissue specimens with greater than or equal to 1 cm aggregate/greatest tissue area on the slide to ensure adequate tissue area for study and better assessment of mitotic activity and keratinization.

Study sample

A total of twenty-four (24) tissue specimens from the oral cavity, oropharynx and larynx, with a diagnosis of SCC obtained as per inclusion and exclusion criteria, were included in this study.

P16 IHC staining

A specific slide and block was chosen for p16 IHC staining and sent to Providence Hospital Laboratory for processing. Heat-Induced Epitope Retrieval (HIER) was used as the antigen retrieval method. Slides were stained by mouse monoclonal p16 IHC stain (Clone name: E6H4™) by CINtec®, using the Ventana Benchmark XT machine.

Hematoxylin & Eosin stain and Immunohistochemical stain interpretation

The labels of slides stained with H&E and p16 IHC were covered and were instead assigned with numbers one to twenty-four (1 to 24). Three (3) board certified anatomic pathologists were blinded and asked to interpret the stained slides. The p16 IHC slides were read as either positive or negative, while the mitotic activity and keratinization were observed in the H&E-stained slides. The interpretation of the pathologists for each of the slides were tallied and recorded. Concordance of interpretation is achieved when two of the three pathologists have the same reading for p16 IHC, keratinization, and mitotic index. The microscope used was the Olympus CX23 light microscope.

P16 IHC

AJCC criteria for p16 immunopositivity is diffuse (≥75%) tumor expression, with at least moderate staining intensity.

Overexpression of p16 is usually localized to tumor cell nuclei and cytoplasm, so that p16 staining that is localized only to the cytoplasm is deemed non-specific and, thus, not diagnostic (negative)[24] (Figures 1 and 2).

Keratinization

Keratinizing SCCs appear pink on low power due to abundant keratinizing cytoplasm, keratin pearls, and prominent intercellular bridges. They are characterized by their growth in irregular cords and nests with associated stromal desmoplasia. In contrast, features indicative of non-keratinizing SCC include growth in circumscribed sheets and nests of cells without associated desmoplasia; a blue appearance on low power due to high nuclear-cytoplasmic ratio; tumor infiltrating lymphocytes and lack of keratin; plentiful tumor necrosis; and lack of intercellular bridges[23] (Figures 3 and 4).

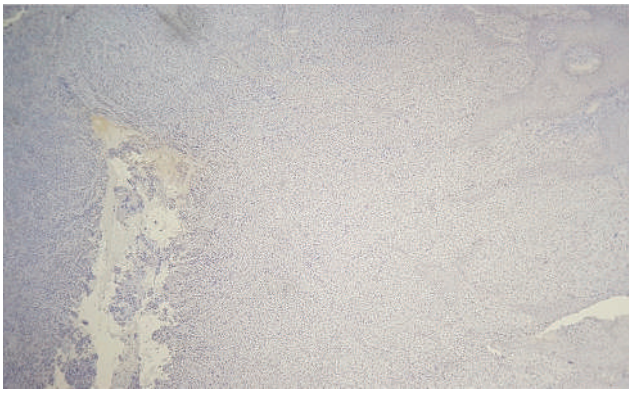

Figure 1. P16 negative IHC staining (P16 IHC, 40x).

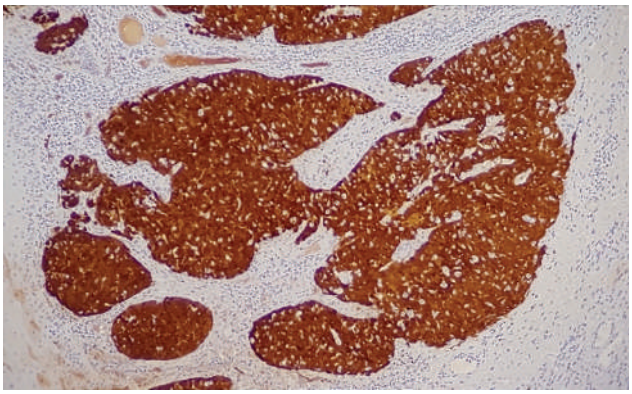

Figure 2. P16 positive IHC staining. (P16 IHC, 100x).

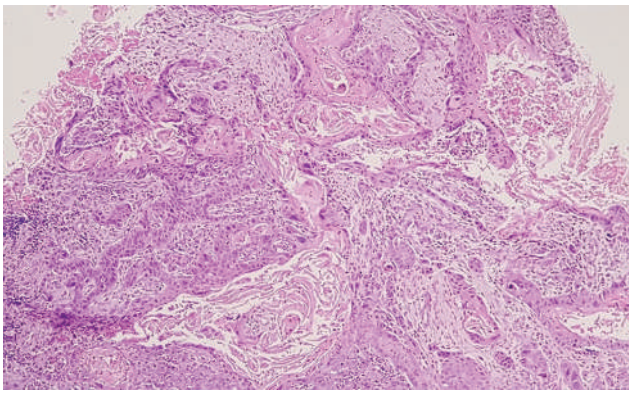

Figure 3. Keratinizing SCC (H&E, 100x).

Mitosis

Mitotically active cells were counted in a total of 10 high power fields (HPF) (Figure 5).

Data handling and analysis

Obtained data was encoded in MS Excel and summarized using descriptive statistics. Categorical data were presented as frequencies and percentages. The Shapiro-Wilk Test was used to test the normality of the dataset in the three variables, namely, IHC staining, mitosis activity, and keratinization. Shapiro-Wilk Test of normality provides that the dataset is normal only if the obtained *Sig.*-value is greater than 0.05 ($p>0.05$). Shapiro-Wilk Test results for IHC staining, mitosis activity, and keratinization all yielded a *Sig*-value of 0.000 which did not satisfy normal distribution. Hence, the use of nonparametric tests in treating all three datasets.

Test for significant differences between p16 IHC staining and the variables mitotic activity and keratinization were determined using Mann-Whitney U test at 0.05 significance level. For easy and effective visualization, stacked or clustered bar charts was created. Interrater agreement was assessed by computing Fleis Multirater Kappa coefficient (K). The results are as follows: mitosis, K=0.250, keratinization, K=0.343, and for p16 Prevalence, K=0.379 which all signify fair strength of agreement using the K value interpretation by Altman.

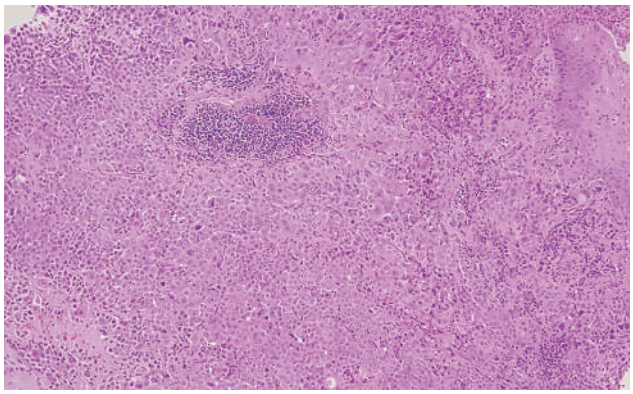

Figure 4. Non-keratinizing SCC (H&E, 100x).

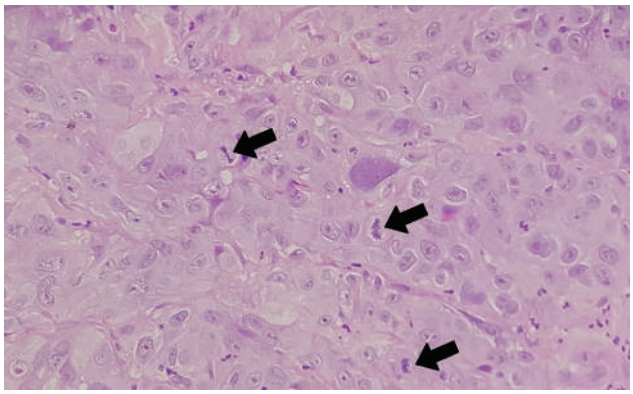

Figure 5. Mitotically active cells *(black arrows)* (H&E, 400x).

RESULTS

Out of the twenty-four slides submitted for p16 IHC stain, three (12%) turned out to be p16 positive, and 21 (88%) were p16 negative. All of the three p16 positive cases were from subjects within the age bracket 60-73 years. Two of the positive cases were from male subjects and one from a female. The sources of the p16 positive specimens were the following: oropharyngeal mass, alveolar ridge mass, and tonsillar mass.

On the other hand, p16 negative cases had an age bracket of forty-eight to eighty-two years (48-82), with thirteen (13) males and eight (8) females. Tissues of the p16 negative cases were from the tongue, larynx, alveolar ridge and oropharyngeal areas (Table 1).

The H&E slides were then evaluated for mitotic activity and keratinization. Of the 24 cases, ten cases have mitosis falling within the 1-10 per high power field (HPF) range (42%), six within 11-20 per HPF (25%), and 8 cases have ≥21 mitosis per HPF (33%). In terms of keratinization, three cases (12%) are non-keratinizing and twenty-one cases (88%) are keratinizing (Table 2).

The three p16 positive cases fell under different mitotic count ranges. In the p16 negative cases, there were nine (38%) that fell under the mitotic range of 1-10, five (21%) fell under 11-20, seven (29%) fell under ≥21/HPF (Table 3).

For the p16 positive cases, two have non-keratinizing histology (75%) and one has a keratinizing histology (8%). For p16 negative group there were eighteen (75%) cases that are keratinizing and three (13%) that are non-keratinizing (Table 4).

The p16 positive group had a higher mitotic activity by 0.77 than the p16 negative group as reflected in the mean ranks (positive=13.17, negative=12.40) (Table 4). Mann-Whitney U test was utilized to compare the difference of these 2 groups in terms of mitotic activity given that the distribution of observations for the aforesaid variables were not approximately normally distributed. Results show that the slight difference in the mean ranks between p16 positive and p16 negative is not significant (U= 29.5, p=0.852) at 0.05 significance level.

Regarding keratinization, the p16 positive group and the p16 negative group had a mean rank difference of 6.29 which was found to be significant (U=15.000, p=0.041) at 0.05 significance level.

Table 1. Demographics and stratification of P16 positive and P16 negative cases

	p16-positive (n=3)	p16-negative (n=21)
Age	60 - 73	48 - 82
Gender	Male – 2 Female – 1	Male – 13 Female – 8
Specimen sources	Oropharynx – 1 Alveolar ridge – 1 Tonsil – 1	Tongue – 10 Larynx – 9 Oropharynx – 1 Alveolar ridge – 1

Table 2. Profile in terms of p16 IHC stain, mitosis, and keratinization

p16 IHC stain	n (%)
Negative	21 (88%)
Positive	3 (12%)
Mitosis / high power field	
1-10	10 (42%)
11-20	6 (25%)
≥21	8 (33%)
Keratinization	
Non-keratinizing	3 (12%)
Keratinizing	21 (88%)

Table 3. Mitotic activity of p16 negative and p16 positive cases

p16 IHC staining	Mitosis / high power field n (%)		
	1 - 10	11 - 20	≥ 21
Negative	9 (38%)	5 (21%)	7 (29%)
Positive	1 (4%)	1 (4%)	1 (4%)

Table 4. Keratinization of p16 negative and p16 positive cases

p16 IHC staining	Keratinization n (%)	
	Keratinizing	Non-keratinizing
Negative	18 (75%)	3 (13%)
Positive	1 (4%)	2 (8%)

DISCUSSION

In this study, three out of the twenty-four included cases turned out to be p16 positive, and belonging to the older population in the 60-73 years age bracket. This is in contrast to other western studies which show higher HPV-association in younger individuals. The prevalence of HPV-related cancers in the Philippines and Southeast Asia in general, is not well established. Most publications regarding HPV-associated oropharyngeal SCC are from western countries and are noted to have a high incidence and cases are still rising. This indicates the need for better diagnostics regarding HPV-related cases of the oral cavity, oropharynx and larynx because in our country to establish a more accurate epidemiological data in the Philippines.

Specimens from the tongue and larynx turned out to be p16 negative. This result is in agreement with data from other studies showing that high-risk HPV infection is present in low percentage (about 5%) in the oral cavity, larynx and hypopharynx.[25,26] In terms of keratinization, the p16 positive cases tended to have non-keratinizing histology while those that are p16 negative are likely to have keratinizing features. This is consistent with the data seen in some studies.[21,27] In terms of mitosis, results show that the slight difference in the mean ranks between p16 positive and p16 negative is not significant at α-0.05. This result may indicate that HPV-related SCCs in the p16 positive cases does not necessarily imply higher mitotic activity. This also means that lower mitotic activity does not necessarily confer the absence of HPV-association. This is in contrary to the claim in one study by Stevens and Bishop (2017), that HPV-related SCCs have high mitotic rates.[23]

CONCLUSION

There is a low prevalence of HPV-related SCC of the oral cavity, oropharynx, and larynx among patients in Rizal Medical Center. This study also highlights the need to address issues on the lack of available local data and clear-cut diagnostic protocols or consensus given that there are prognostic and treatment implications. The findings in this research confirm that tumor histomorphology, specifically the keratinization, significantly predicts HPV status in oropharyngeal SCC. Mitotic activity may not be a reliable marker in predicting the HPV status or p16 IHC reactivity of a case. Keratinization in oropharyngeal SCCs may provide valuable information in certain instances, particularly when HPV testing is not immediately available, although the combined tumor morphology and p16 IHC is more ideal.

RECOMMENDATIONS

Further studies with larger sample size may strengthen the prevalence of p16-positive oropharyngeal SCCs in Rizal Medical Center. This may also help in better assessing histomorphologic characteristics like mitotic activity. Larger sample sizes may determine whether mitotic activity could predict p16 IHC result or HPV status of oropharyngeal SCCs. Studies of more histomorphologic features like desmoplasia, nuclear-cytoplasmic ratio, tumor-infiltrating lymphocytes and tumor necrosis are recommended to better compare p16 positive and p16 negative oropharyngeal SCCs in H&E-stained slides. Given its low occurrence in the oral cavity and larynx, the researcher recommends focusing more on p16/HPV studies of tissues coming from the oropharynx and tonsils. Additional pathologists are also recommended in assessing histomorphologic features for a higher and reliable measurement of agreement and lower inter-observer subjectivity and variability. Further diagnostic studies involving PCR-based techniques and DNA testing are also recommended for an optimum diagnosis and to give clinicians substantial evidence for the optimal approach to these kinds of tumors.

ETHICAL CONSIDERATIONS

This paper has been reviewed and granted approval by the Rizal Medical Center Institutional Review Board. Consent forms from patients were not needed for this study because no personal data of the patients were disclosed in any way. The slides for the collected specimens were covered and labelled with assigned numbers to assure anonymity. All physical documents, if any, that may have contained confidential information were disposed using a paper shredder immediately after the study results were obtained.

STATEMENT OF AUTHORSHIP

Both authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

Both authors declared no conflict of interest.

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OPEN ACCESS – ORIGINAL ARTICLE

Determination of Rates of Malignancy on Archival Salivary Gland Fine-Needle Aspiration Biopsy after Application of the Milan System for Reporting Salivary Gland Cytopathology in the Philippine General Hospital: A 1-Year Retrospective Study*

Nikko Paolo Cablao,[1] Pauleen de Grano,[2] Agustina Abelardo,[1] Jose Ma. Avila,[+] Dahlia Teresa Argamosa[1]

[1]*Department of Laboratories, Philippine General Hospital*
[2]*Department of Otorhinolaryngology, Philippine General Hospital*

ABSTRACT

Background. The Milan System for Reporting Salivary Gland Cytopathology (MSRGC) aims to increase the overall effectiveness of salivary gland FNAB by defining six general diagnostic categories with corresponding Rates of Malignancies (ROM). This study aims to use this system to categorize salivary gland FNAB in the Philippine General Hospital and stratify ROM per category.

Methodology. In this study a total of 326 cases have been collected and reviewed, of which 154 (47.2%) had either surgical or clinical follow-up. The cases were assigned a Milan category by 3 cytopathologists blinded from the original diagnoses and from each other's readings.

Results. The overall sensitivity, specificity, PPV, and NPV in detecting neoplasm is at 71.6%, 90.9%, 88.3%, and 76.9%, respectively. On the other hand, the sensitivity, specificity, PPV, and NPV in detecting malignancy is at 52%, 92.9%, 59.1%, and 90.7%, respectively. The computed ROM is as follows: Category I 7.89%, Category II 9.43%, Category III 20%, Category IVa 10.53%, Category IVb 60%, Category V 75%, and Category VI 100%.

Conclusion. The overall diagnostic utility of salivary gland FNAB, as well as the computed ROM per diagnostic category are comparable to internationally published literature. This study also validates the MSRSGC as a valuable tool in stratifying ROM in salivary gland lesions.

Key words: cytopathology, fine needle aspiration biopsy, FNAB, Milan System, salivary gland, rates of malignancy

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Corresponding author: Nikko Paolo R. Cablao, MD
E-mail: nikkopaolocablaomd@gmail.com
ORCiD: https://orcid.org/0000-0002-9095-0848

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INTRODUCTION

Fine-needle aspiration biopsy (FNAB) is an accepted first-line investigation for palpable head and neck masses, and allows separation of inflammatory from neoplastic, and benign from malignant lesions.[1]

The diagnostic role of FNAB in the evaluation of salivary gland lesions has been well established by generating cost-effective care and appropriate management strategies.[2] The reported overall sensitivity and specificity of salivary gland FNAB range from 86-100% and 90-100%, respectively as reported in most series.[3] The ability of salivary gland FNAB to render a specific diagnosis is limited by sampling, lack of architectural details, and cytomorphologic overlap between different salivary gland lesions.[4] This challenge was further magnified by the lack of a uniform reporting system that resulted in reduced clarity of communication between cytopathologists and clinicians.[5]

This has led to the American Society of Cytopathology (ASC) and the International Academy of Cytology (IAC) to organize an international taskforce composed of cytopathologists, surgical pathologists, and head and neck surgeons with the proposal of a tiered classification system consisting of a limited number of diagnostic categories with clear definitions; each diagnostic category associated with an implied Rate of Malignancy (ROM). This unified effort

was then called "The Milan System for Reporting Salivary Gland Cytopathology (MSRSGC)" in September 2015.[6]

To date, few studies have been published which tackled the role and impact of the MSRSGC in the diagnosis and management of salivary gland lesions; and with the assimilation of data from other institutions, the MSRSGC is expected to evolve and reflect the current knowledge of salivary gland FNAB.[7] With that said, the main objective of this study is to examine the effect of applying the MSRSGC to salivary gland aspirates and calculate the ROM associated with each category in Philippine General Hospital (PGH).

METHODOLOGY

This research utilized a retrospective cross-sectional study design. All of the salivary gland FNAB cases from both the PGH and the University of the Philippines-Pathology Research Laboratory (UP-PRL) for the year 2018 were reviewed.

Sampling

All service and pay salivary gland FNAB cases from the PGH and the UP-PRL done during the year 2018 were included. The FNAB done should be from indicated anatomic locations of major and minor salivary glands; which include the buccal mucosa, labial mucosa lingual mucosa, soft and hard palate, and floor of mouth.[8] FNAB cases of proven cases of salivary gland neoplasms who already underwent definitive surgery prior to the said cytologic biopsy (i.e., recurrences), and those with a history of malignancy (i.e., metastasis) were excluded in this study.

Materials and methods

FNABs that are done at the UP-PRL utilized a 25-gauge needle attached to 10 cc syringe, assisted by an aspirator gun/syringe holder. At least 1 air-dried slide smear and 1 alcohol-fixed slide smears are rendered from the aspirate. The air-dried slide is prepared with Diff-Quik staining while the alcohol-fixed slide is prepared with Papanicolaou staining. In some cases, cell block is prepared from cystic aspirates. Rapid on-site evaluation is performed to evaluate for adequacy of material.

FNABs done at the PGH Outpatient Department (OPD) are sent to either the UP-PRL or the OPD laboratory for processing. FNABs done at the wards are sent to the PGH central laboratory for processing. The gauge of the needle used, utilization of a syringe holder, and actual technique in aspiration are uncertain for cases not performed at the UP-PRL.

Data collection

The FNAB results from the UP-PRL and PGH were reviewed by an independent data extractor (IDE), and salivary gland FNAB cases for the year 2018 were retrieved. Salivary gland FNAB include those cytologic studies done for lesions indicated as having been obtained from the pre-auricular, post-auricular, submandibular, submental, maxillary, and floor of mouth areas. For cases with more than one FNAB performed on the same lesion but on separate occasions, the latest FNAB was selected and the earlier one was not used.

These FNAB cases were examined for presence of definitive surgical follow-up by the IDE through searching the OpenMRS using available patient identifiers. For cases with diagnoses that fell under the non-diagnostic, non-neoplastic/ inflammatory category with no available histopathologic follow-up, a clinical follow-up via chart review was done by the ORL co-investigator to check for medical management and outcome of the biopsied lesion. All the gathered data were recorded in standardized Case Data Forms. The definitive follow-up of the cases, whether surgical or clinical, were then classified as either "Non-Neoplastic," "Benign," or "Malignant," based on the retrieved histopathology report. Cases with no definitive follow-up available were classified under "Non-Diagnostic."

Classification using the MSRSGC

The slides of all the included cases, each accompanied by a standardized Cytopathologist Milan Classification Form, were sent separately to the three cytopathologists for slide review and independent blinded classification using the MSRSGC. The final Milan category for a particular case was based on the agreement of at least two of the three cytopathologists. FNAB cases in which the three cytopathologists have differing classifications were grouped together and were not assigned a final Milan category.

Data analysis

All necessary information were entered into an electronic spreadsheet via MS Excel 2018. Descriptive statistics were done, and the number of cases per Milan category were tallied together with their corresponding definitive outcomes. The sensitivity, specificity, PPV, NPV of salivary gland FNAB in (i) differentiating neoplastic from non-neoplastic lesions, and (ii) detecting malignancy were computed with 95% CI. Only FNAB cases with definitive outcomes were included in these computations.

In the calculation for presence or absence of neoplasm and malignancy, SG-FNAB cases classified as Category III (AUS) and IVb (SUMP) were grouped under positive for neoplasm and malignancy, respectively. This is based on the finding by Wang et al., in 2017 of a high percentage of Category III and IVb cases with malignant histopathologic follow-up.[9] Lastly, the ROM and OROM per Milan category were computed with 95% CI.

RESULTS

A total of 326 cases were identified for the year 2018. Majority of these cases were from the UP-PRL composed of 271 cases (83%), while the remainder come from PGH OPD and PGH Central Laboratory, with 29 (9%) and 26 (8%) cases, respectively. The age of the patients ranged from 1 to 87 (Mean = 40); 139 (42.6%) of which were male and 187 (57.4%) were female. Of the 326 lesions, 167 (51.2%) were from the location of the parotid gland, 93 (28.5%) were from the submandibular area, 33 (10.1%) were from the submental/sublingual area, and 33 (10.2%) were from areas where minor salivary glands are present (e.g., lip, oral cavity, maxilla, zygomatic area). Among those with slides (n = 272), the mean number of slides per case was 2 (68%) with a range of 1-8 slides per case. 93 cases (34.2%) had both Diff Quik and Papanicolaou-stained slides, none had Diff Quik slides only, 179 cases (65.8%) had Papanicolaou-

stained slides only, and 26 cases (9.6%) had cell block preparations. Based on the MSRSGC categorization, 102 cases (31.3%) were grouped as Category I, 107 cases (32.8%) as Category II, 8 cases (2.5%) as Category III, 75 cases (23%%) as Category IVa, 16 cases (4.9%) as Category IVb, 8 cases (2.5%) as Category V, and 4 cases (1.2%) as Category VI. 6 cases (1.8%) were not assigned to any of the above categories and grouped under "Unclassified" because there was no consensus between the three cytopathologists for these cases. Definitive follow-up, whether surgical or clinical, was available for 154 cases (47.2%): 95 cases (24.48%) turned out to be non-neoplastic while 91 cases (23.45%) were neoplastic. Benign histopathologic follow-up comprises 57 cases (14.69%) while 34 cases (8.76%) were malignant. There were 202 cases (52.06%) with no available definitive surgical or clinical follow-up.

As shown in Table 1, out of the 102 cases under Category I, 64 (62.7%) had no surgical and/or clinical follow-up thus classified under cases with no definitive diagnosis; while 25 (25%) turned out to be non-neoplastic, 10 (9.8%) turned out to be benign, and 3 (2.94%) turned out to be malignant. There were 107 FNAB cases under Category II; 54 (50.5%) had no definitive diagnosis, 45 (42.1%) turned out to be non-neoplastic, 3 (2.8%) were benign, and 5 (4.67%) were malignant. Out of the 8 cases under Category III, 3 (37.5%) had no definitive diagnosis, 4 (50%) were non-neoplastic, none was benign, and 1 (12.5%) turned out to be malignant. Of the 75 cases under category IVa, 37 cases (49.3%) had no definitive diagnosis, 1 case (1.3%) turned out to be non-neoplastic, 33 cases (44%) were benign, and 4 cases (5.3%) were malignant. There were 16 cases under Category IVb; 6 (37.5%) of which had no definitive diagnosis, 1 (6.25%) was non-neoplastic, 3 (18.75%) were benign, and 6 (37.5%) turned out to be malignant. Out of the 8 cases in Category V, 4 (50%) had no definitive diagnosis, 1 (12.5%) was non-neoplastic, none were benign, and 3 (37.5%) were malignant. Lastly, of the 4 cases under Category VI, only 1 (25%) had no definitive diagnosis while the remaining 3 cases (75%) were malignant.

The diagnostic utility of FNAB in detecting both salivary gland neoplasm and malignancy are shown in Table 2. The sensitivity, specificity, PPV, and NPV of FNAB in detecting salivary gland neoplasm are as follows: 71.62%, 90.91%, 88.33%, 76.92%, respectively. On the other hand, the sensitivity, specificity, PPV, and NPV of FNAB in detecting salivary gland malignancy are as follows: 52%, 92.86%, 59.09%, and 90.7%,respectively. The OROM and ROM per Milan category is summarized in Table 3. The calculated OROM for each Milan category are as follows: Category I (2.94%), Category II (4.67%), Category III (12.5%), Category IVa (5.33%), Category IVb (37.5%), Category V (37.5%), and Category VI (75%). The cumulative OROM across all categories is at 8.59%. On the other hand, the calculated ROM for each Milan category are as follows: Category I (7.89%), Category II (9.43%), Category III (20%), Category IVa (10.53%), Category IVb (60%), Category V (75%), and Category VI (100%). The cumulative ROM across all categories is at 18.18%.

Unclassified cases (n = 6) were not included in the computation of OROM, ROM, as well as in computing for sensitivity, specificity, PPV, and NPV. Out of the 6 cases, 3 cases (50%) were lost to follow-up, while the remaining 3 cases (50%) turned out to be malignant on definitive biopsy or surgery. Among those with available follow-up data, one is a case of a 42-year-old female with right submandibular mass initially diagnosed on FNAB as "atypical cells present favor non-small cell carcinoma," which turned out to be Diffuse Large B-Cell Lymphoma (DLBCL) after tissue biopsy and further investigation with immunohistochemistry studies. Another case is that of a 56-year-old female with a mass on the floor of mouth initially diagnosed on FNAB as "rare epithelial cells suggestive of a neoplastic process," but turned out to be Adenoid Cystic Carcinoma on definitive surgery. The last case is that of a 63-year-old female with a left buccal mass initially signed out on FNAB as "atypical cells present," but on definitive surgery turned out to be Sebaceous Carcinoma. Each case is composed of 2 Papanicolaou-stained slides only.

Table 1. Distribution of definitive follow-up per Milan category

Milan category	Total cases	Non-diagnostic	Non-neoplastic	Neoplastic	
I	102	64 (62.7%)	25 (25%)	13 (12.75%)	
				Benign 10 (9.8%)	Malignant 3 (2.94%)
II	107	54 (50.5%)	45 (42.1%)	8 (7.48%)	
				Benign 3 (2.8%)	Malignant 5 (4.67%)
III	8	3 (37.5%)	4 (50%)	1 (12.5%)	
				Benign 0	Malignant 1 (12.5%)
IVa	75	37 (49.3%)	1 (1.3%)	37 (49.3%)	
				Benign 33 (44%)	Malignant 4 (5.3%)
IVb	16	6 (37.5%)	1 (6.25%)	9 (56.25%)	
				Benign 3 (18.75%)	Malignant 6 (37.5%)
V	8	4 (50%)	1 (12.5%)	3 (37.5%)	
				Benign 0	Malignant 3 (37.5%)
VI	4	1 (25%)	0	3 (75%)	
				Benign 0	Malignant 3 (75%)

Table 2. Diagnostic utility of FNAB in detecting salivary gland neoplasm and malignancy

	Detecting neoplasm (%)	95% CI	Detecting malignancy (%)	95% CI
Sensitivity	71.6	59.9 – 81.5	52.00	31.3 – 72.2
Specificity	90.9	82.2 – 96.3	92.86	86.9 – 96.7
PPV	88.3	78.6 – 94.0	59.09	41.0 – 75.1
NPV	76.9	69.7 – 82.8	90.70	86.6 – 93.6

Table 3. Computed OROM and ROM per Milan category

Milan category	Malignant on follow-up (n)	Total FNAB (n)	FNAB with follow-up (n)	OROM (%)	ROM (%)	95% CI
I	3	102	38	2.94	7.89	(1.6 - 21.4)
II	5	107	53	4.67	9.43	(3.1 – 20.6)
III	1	8	5	12.50	20.00	(0.5 – 71.6)
IVa	4	75	38	5.33	10.53	(2.95 – 24.8)
IVb	6	16	10	37.50	60.00	(26.2 – 87.8)
V	3	8	4	37.50	75.00	(19.4 – 99.4)
VI	3	4	3	75.00	100.00	(29.2 – 100)
Total	28	326	154	8.59	18.18	

Table 4. False negative cases in detection of malignancy

	Case control #	Location	Initial FNAB	Milan category	Definitive diagnosis
1	179	Infraauricular mass	Scant atypical squamous epithelium	I	Trichilemmal carcinoma
2	309	Preauricular mass	Hemorrhagic cyst fluid only	I	Adenoid cystic carcinoma
3	400	Submandibular mass	Hemorrhagic aspirate	I	Langerhans cell histiocytosis
4	84	Infraauricular mass	Atypical cells present suspicious for malignancy	II	Non-Hodgkin diffuse large B-cell lymphoma
5	174	Preauricular mass	Scattered salivary acinar cells in an acute on chronic inflammatory background	II	Non-Hodgkin lymphoma
6	244	Preauricular mass	Acute inflammatory pattern	II	Squamous cell carcinoma.
7	326	Submandibular mass	Polymorphous lymphocytic population suggestive of a reactive process. Recommend solid tissue biopsy	II	Atypical round cell proliferation, consider Non-Hodgkin lymphoma
8	340	Submandibular mass	Benign cyst contents	II	Mucoepidermoid carcinoma
9	49	Infraauricular mass	Cell findings consistent with benign mixed tumor	IVa	Non-invasive adenocarcinoma arising from a BMT
10	183	Parotid mass	Consistent with malignant epithelial neoplasm, cannot rule out a possible salivary gland or thyroid origin	IVa	Adenocarcinoma
11	246	Parotid mass	Basaloid neoplasm with fibromyxoid stroma, cannot rule out an adenoid cystic carcinoma	IVa	Adenoid cystic carcinoma
12	399	Parotid mass	Benign mixed tumor	IVa	Salivary duct carcinoma ex-pleomorphic adenoma

Note: The Milan Category was assigned after these cases were independently and blindly reviewed by 3 cytopathologists without knowledge of the actual initial FNAB diagnosis.

DISCUSSION

The overall follow-up rate of salivary gland FNAB in this study is at 47.2% (154 out of 326 cases). These include cases that were found out to be non-neoplastic, benign, or malignant based on definitive histopathology or clinical follow-up. This value is comparable[10,11] and even higher[5,12,13] compared to other studies. Majority of those with no follow-up came from the Category I group at 62.7% followed by the Category II group at 50.5%. A possible reason for this is that inflammatory conditions are the most common pathology affecting the salivary glands.[14] In addition, when the non-diagnostic cohort is excluded in both the initial FNAB and the definitive outcome, the most common lesion affecting salivary glands belong to the non-neoplastic category. Some may have resolved spontaneously thus causing the patient to no longer seek follow-up. Interestingly, a significantly high percentage of cases under Category IVa (49.3%) and Category V (50%) also have poor follow-up for reasons that are yet unclear. A plausible explanation is that some of these patients might have been referred or voluntarily transferred to a nearer and more accessible health facility for definitive management.

In this study, more than half (51.23%) of the lesions sampled were said to have been taken from the parotid gland. This is followed by lesions taken from the sub-mandibular gland at 28.5%. These findings are consistent with published data; majority of salivary gland lesions arise from the parotid gland.[15-17] All cases had alcohol-fixed Papanicolaou-stained smears while only 93 cases (34.2%) had the complimentary air-dried Diff Quik-stained smears. This means that only 34.2% of the cases followed the recommendation of the MSRSGC wherein a combination of air-dried and alcohol-fixed smears should be the mainstay in evaluating salivary gland FNAB. The inherent qualities of the matrix material, cytoplasmic features, and the nature of a proteinaceous or mucinous background is better appreciated using air-dried Diff Quik preparations. On the other hand. Alcohol-fixed Papanicolaou slides can be useful for the assessment of nuclear qualities and degree of cytologic atypia.[3]

The sensitivity of FNAB in detecting salivary gland neoplasm is higher (71.62%) as compared to that in detecting salivary gland malignancy (52%). This means that 21 out of the 74 FNAB cases reported to be neoplastic on definitive follow-up had been initially classified as non-neoplastic. The false negative rate for detecting neoplasm is computed at 28.4%. Out of these 21 cases, 13 (62%) were initially grouped under Milan Category I (Non-Diagnostic) on FNAB. These non-diagnostic smears were reported as either hemorrhagic, acellular smears, or as smears consisting of cyst fluid only. The even lower sensitivity of FNAB in detecting salivary gland malignancies (52%) seen in this study indicate a higher false negative rate of 48% for detecting malignancies. In our study, 12 cases had been initially classified as non-malignant on FNAB but turned out otherwise on definitive follow-up. These are tabulated in Table 4.

Published data have shown that sensitivity in detecting neoplasm and malignancy range from 50%[10] to 95%.[18] In a local study by Santiago et al., a similarly low sensitivity for diagnosis of malignancy at 46% was noted.[19] False negative results are often caused by inadequate sampling with insufficient cellularity of the aspirate[10] and heterogeneity in the performance and level of experience among clinicians and pathologists.[20] This scenario is true in the PGH as not all FNABs are done by pathologists; some are performed by clinicians and medical interns at the OPD (8.9%) or at the bedside in the wards (8%). Moreover, some FNABs from the PRL were also performed by clinicians and were just sent for staining and interpretation. However, data on the number of these PRL cases that were sent from clinicians are beyond the scope of this study. Low sensitivity and high-performance heterogeneity show the greatest room for improvement in salivary gland FNAB.[4]

On the contrary, the results for specificity in this study means that there is high true negative rate, and that FNAB can be used as a tool to confirm a high clinical suspicion that is indicative of a neoplasm or malignancy. The specificity of FNAB in detecting salivary gland neoplasm and malignancy is at 90.9% and 92.9%, respectively. These are comparable to published values in international studies.[10-12,15-17, 21, 22-26]

The MSRSGC emphasized risk stratification rather than specific diagnoses, providing an ROM for each category, with corresponding recommended management that would guide clinicians for better patient care.[7] The total

Table 5. Comparison of computed ROM with select internationally published data

Authors	Country	Sample size*	I	II	III	IVa	IVb	V	VI
Faquin and Rossi (MSRSGC)	Italy	—	25 (0-67%)	10 (0-20%)	20 (10-35%)	<5 (0-13%)	35 (0-100%)	60 (0-100%)	90 (57-100%)
Cablao et al.	Philippines	154	7.9	9.4	20.0	10.5	60.0	75.0	100.0
Wei et al.	USA	4514**	25.0	10.2	12.5	3.4	37.5	58.6	91.9
Liang et al.	USA	110	50.0	60.0	12.5	3.2	72.7	100.0	100.0
Viswanathan et al.	India	373	6.7	7.1	38.9	5.0	34.2	92.9	92.3
Kala et al.	India	172	25.0	5.0	20.0	4.4	33.3	85.7	97.5
Thiryayi et al.	UK	138	8.5	1.6	0.0	1.9	26.7	100.0	100.0
Choy et al.	Singapore	376	14.5	26.7	29.3	2.7	19.1	87.5	100.0

*Only those with histopathologic and clinical follow-up
**From 29 reviewed studies worldwide

OROM, which is the number of malignant cases divided by the total number of FNABs across all diagnostic categories estimates the rate at which a certain salivary gland lesion is malignant prior to doing a biopsy. In our study the calculated total OROM is at 8.59%, meaning there is an 8.59% chance that any particular salivary gland lesion from a patient who presents to the clinic could be malignant. This aspect was not explored in previous studies.

What is more important, however, in the diagnostic point of view, as is suggested in the MSRSGC, is the ROM per diagnostic category. Table 5 summarizes the computed ROMs per Milan diagnostic category of some selected and available published studies. The estimated ROMs reported by Faquin and Rossi, the proponents of the MSRSGC, lifted from available literature is also presented.

The computed ROM for Category I (7.9%) is lower compared to the estimates of Faquin and Rossi published in the MSRSGC (25%). However, they also reported that ROM values for this category may range from 0% to 67%.[3] The result from this study is comparable to the findings of Viswanathan et al., (6.7%)[24] and Thiryayi et al., (8.5%).[27] There may be an overestimation in the other studies wherein certain non-diagnostic cases were still taken into the equation even though there were succeeding FNABs with diagnostic findings on follow-up. In the present study, non-diagnostic cases that had another diagnostic FNAB on follow-up were not counted in the computation for ROM. In the present study, majority of the definitive diagnoses in Category I was classified under non-neoplastic (65.8%), followed by benign neoplasm (26.3%).

There is agreement between results of this study for the ROM of Categories II and III with that published in the MSRSGC. The ROM for Category II (9.4%) is comparable to that published in the MSRSGC (10%)[3], as well as in studies by Wei et al., (10.2%)[18] and Viswanathan et al., (7.1%).[24] On the other hand, comparable ROMs have also been observed in this study (20%) with that published in the MSRSGC (20%)[3] and with the study by Kala et al., (20%).[28] Also, the percentage of cases under Category III at 2.5% is well within the recommended desirable number of <10% of all salivary gland FNAB samples in an institution.[3] However, one caveat in this diagnostic category, according to the MSRSGC is that the ROM is not yet well defined due to the lack of literature pertaining to salivary gland aspirates classified as AUS.

As for Category IVa (Benign), the present study's ROM (10.5%) is higher compared to those published in literature. 4 out of 38 cases initially classified under Category IVa up turned out to be malignant on definitive follow-up. In the MSRSGC, they reported a mean ROM of <5% for this category. However, they also cited that the ROM for benign neoplasms on FNAB may range from 0% to 13%.

This study's computed ROM for Category IVb (60%) is also higher compared to that reported in the MSRSGC (35%).[3] However, it should be noted that in the same literature, they also cited an ROM at a range of 0 to 100% for this category. Liang et al., reported a higher ROM for this category (72.7%).[29] In a study by Hang et al., in 2018, wherein Category IVb cases were further explored and subtyped based on predominant cytomorphology, they found varying values for ROM within the same diagnostic category. For those with a predominant oncocytic or squamoid component, the ROM reached as high as 61% which is comparable with that in our study. Other subgroups were those with basaloid cytomorphology (ROM = 40%) and myoepithelial cytomorphology (ROM = 18.8%).[9]

Results for Category V (Suspicious for Malignancy) and Category V (Malignant) are slightly higher at 75% and 100%, respectively, when compared to values estimated by the MSRSGC. Faquin and Rossi estimated the ROM of Category V to range from 0% to 100%, while that of Category VI to range from 57% to 100%.[3] Also, in general values derived in this study are comparable with other international studies, which range from 58.6% to 100% for Category V and 91.9% to 100% for Category VI.

Lastly, it is worth looking into the possible reasons behind the 6 unclassified cases in this study. In all cases, at least one cytopathologist assigned a category of AUS (Category III). These cases are often associated with pre-analytical factors such as technique in aspiration and smearing, air drying artifacts, obscuring background, or the inherent characteristics of the lesion resulting in scant numbers of well-preserved cells.[3] It can be noted that in 3 of these cases, a note on the limited number and quality of cells had been made. Currently, adequacy criteria for salivary gland FNAB are not well established.[13] None of these cases had repeat FNABs done. Another thing that is common among all unclassified cases is that each case has only 2 Papanicolaou-stained slide smears. A combination of air-dried Diff Quik-stained smears and alcohol-fixed Papanicolaou-stained smears is the mainstay of salivary gland FNAB.[3] The lack of air-dried Diff Quik slides limits evaluation of matrix material, cytoplasmic features, and nature of proteinaceous or mucinous background in these cases. The lack of radiologic and clinical data (e.g., size of mass, duration of symptoms, rate of growth, associated pain/paresthesia, accompanying infection or fever) provided to

the cytopathologists during the study also played a major role in the difficulties that arose in classifying cases. As emphasized in the literature, FNAB forms an integral part together with clinical examination and radiologic investigation in the assessment of salivary gland lesions.[11]

Also, worth looking into is the ROM in this subgroup. 3 out of 6 (50%) had definitive follow-up and all of them turned out to be malignant. In this light, difficult cases should always be examined more closely with the proper clinical and radiologic data. Moreover, the really challenging cases should have the concurrence of at least one cytopathologist, and a repeat FNAB should be suggested whenever possible.

LIMITATIONS

The study is limited by the lack or inadequacy of clinical and radiologic information for some FNAB cases. There is likewise a lack of uniformity in the use of both air-dried and alcohol-fixed slide smears in the evaluation of salivary gland FNAB. Another limitation is the heterogeneity in the performance of FNAB. Procedures done at the UP-PRL use the prescribed 25-gauge needle attached to a 10 cc syringe with an aspirator. Rapid on-site evaluation is performed for specimen adequacy at the UP-PRL. In the clinics, however, the gauge of the needle, size of the syringe, and utilization of an aspirator, are unknown. Moreover, there is no rapid on-site adequacy evaluation. These factors restrict the diagnostic potential of FNAB in detecting neoplasm and malignancy of the salivary glands.

CONCLUSION

The low sensitivity in detecting neoplasm (71.6%) and malignancy (52%) indicate that the FNAB should not be indiscriminately used as a screening tool in evaluation salivary gland lesions. However, FNAB proves to be an excellent tool in confirming a clinical suspicion of neoplasm or malignancy, as evidenced by its high specificity in detecting neoplasm (90.9%) and malignancy (92.86%). As a whole, the sensitivity, specificity, PPV, and NPV of FNAB; and the ROM per diagnostic category computed in the study are comparable to that in published literature. To our best knowledge, this is the first Philippine study which looked into the ROM of salivary gland FNAB using the diagnostic categories recommended by the Milan system. More so, this study validates the MSRSGC as a valuable tool in stratifying ROM in salivary gland lesions to better guide clinical management.

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OPEN ACCESS – CASE REPORT

The Utility of Immunohistochemistry in Diagnosing Tubulocystic Renal Cell Carcinoma with Papillary Morphology

Allison Kaye Pagarigan and Erland del Rosario

Department of Pathology and Laboratory Medicine, National Kidney and Transplant Institute, Quezon City, Philippines

ABSTRACT

Tubulocystic renal cell carcinoma (TC-RCC) is a recently recognized, rare but distinct malignant entity. Pathologists have endeavored to completely define its histomorphologic, immunohistochemical and molecular features. Recounted is a case where the diagnosis of TC-RCC was confounded by presence of papillary morphology. Immunohistochemical expression of alpha-methyl acyl-CoA-racemase and vimentin with corresponding negativity for CK7 and CD10, following distinctive gross and microscopic findings, confirmed a diagnosis of TC-RCC. This report demonstrates the strategic value of performing immunohistochemistry studies to establish a diagnosis of TC-RCC especially when unusual histologic features are encountered. Immunohistochemistry continues to be the most practical approach to diagnosis as molecular testing methods, such as next generation sequencing, remain unfeasible in the local setting. Cautious prognostication is required as accounts of recurrence and metastasis continue to emerge.

Key words: renal cell carcinoma, histology, immunohistochemistry, diagnosis, surgical pathology

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Corresponding author: Allison Kaye L. Pagarigan, MD
E-mail: nkti.pathology3@gmail.com
ORCiD: https://orcid.org/0000-0001-6374-1015

INTRODUCTION

Tubulocystic renal cell carcinoma (TC-RCC) of the kidney is a rare entity with only about a hundred cases reported in literature.[1] Though recognized to have distinct macroscopic, microscopic and immunohistochemical features in the 2016 World Health Organization Classification of Tumors,[2] recent studies have challenged the presence of papillary architecture as an acceptable morphologic variation of the disease. Immunohistochemistry studies have emerged as a reliable, affordable and readily available means of confirming a diagnosis of TC-RCC when tumor morphology deviates from its classic histologic description and are further augmented by molecular and cytogenetic testing. This report aims to demonstrate the diagnostic utility of immunohistochemistry studies in the case of a young adult male with tubulocystic renal cell carcinoma exhibiting classic and papillary morphology with later occurrence of pulmonary and skeletal metastases.

CASE PRESENTATION

A previously healthy 27-year-old male presented with a one-year history of intermittent and progressive right flank pain. Sudden onset hematuria prompted consult and subsequent work-up. Urinalysis revealed red, turbid urine with significant elevations in leukocyte, erythrocyte and bacterial counts with presence of ghost cells. Serum creatinine (1.0 mg/dL) and estimated glomerular filtration rate (102.69 mL/min/1.73 m^2) were within normal limits. Triple phase computed tomography (CT) scan of the whole abdomen identified a globularly enlarged right kidney (11.7 x 7.2 x 7.2 cm) with a heterogeneously enhancing, endophytic, mid- to inferior pole mass measuring 5.3 x 5.3 x 6.5 cm with involvement of the infundibulo-calyceal system. The radiographic impression was transitional cell carcinoma (Figure 1).

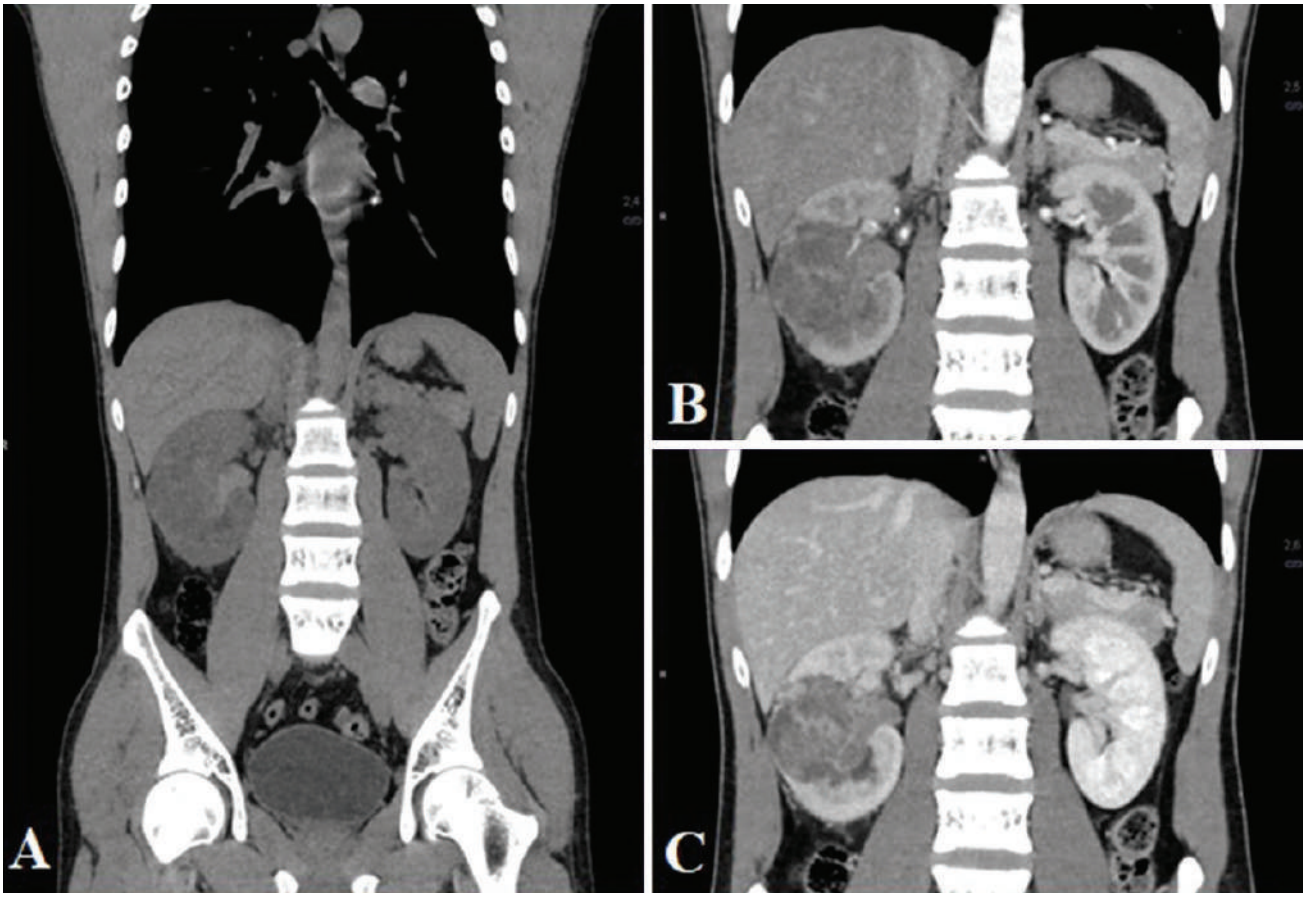

Figure 1. Triple-phase CT scan **(A)** plain, **(B)** arterial phase, **(C)** venous phase showing a heterogeneously enhancing, endophytic renal mass involving the infundibulo-calyceal system.

Longitudinal anti-hilar sectioning of the nephrectomy specimen revealed a 6 x 6 x 5 cm, firm, well-circumscribed, multicystic, cream-white to tan-yellow, mid- to inferior pole mass, bearing resemblance to a sponge. Cyst linings appeared smooth with sizes ranging from less than 1 mm to approximately 9 mm in diameter. Serous, straw-colored fluid cyst contents were expressed on sectioning. Gross evidence of previous intralesional hemorrhage was not observed. No solid areas were noted and no stones were retrieved. Renal sinus involvement was grossly observed but tumor was limited to the kidney without involvement of the pelvicalyceal system, Gerota's fascia, renal vein nor ureter (Figure 2).

Microscopic sections disclosed a tumor composed of varisized cystic structures lined by a single layer of cuboidal cells with hobnailed appearance. Some cysts displayed a proliferation of this lining epithelium forming papillary configurations. Neoplastic cells were characterized by enlarged, moderately pleomorphic, vesicular nuclei with prominent to inclusion-like eosinophilic nucleoli and abundant amounts of cytoplasm with occasional vacuolization and clearing (WHO/ISUP histologic grade 3) (Figure 3). Lymphovascular space invasion was not identified. Minimal necrosis was noted. A pathologic stage classification of pT3a was assigned.

Immunohistochemistry studies revealed diffuse expression of vimentin and alpha-methyl acyl-CoA racemase

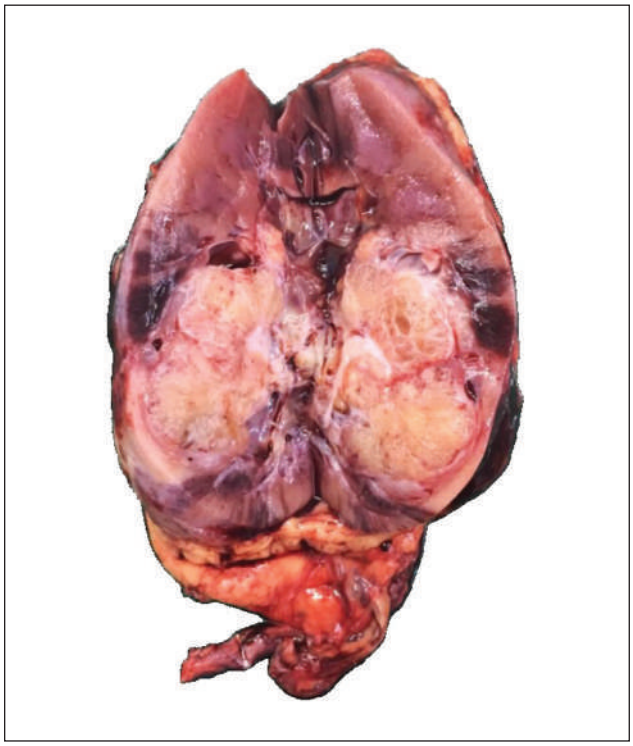

Figure 2. Bivalved nephrectomy specimen revealing a mid- to inferior pole mass with sponge-like or "bubble wrap" appearance. No solid areas are identified.

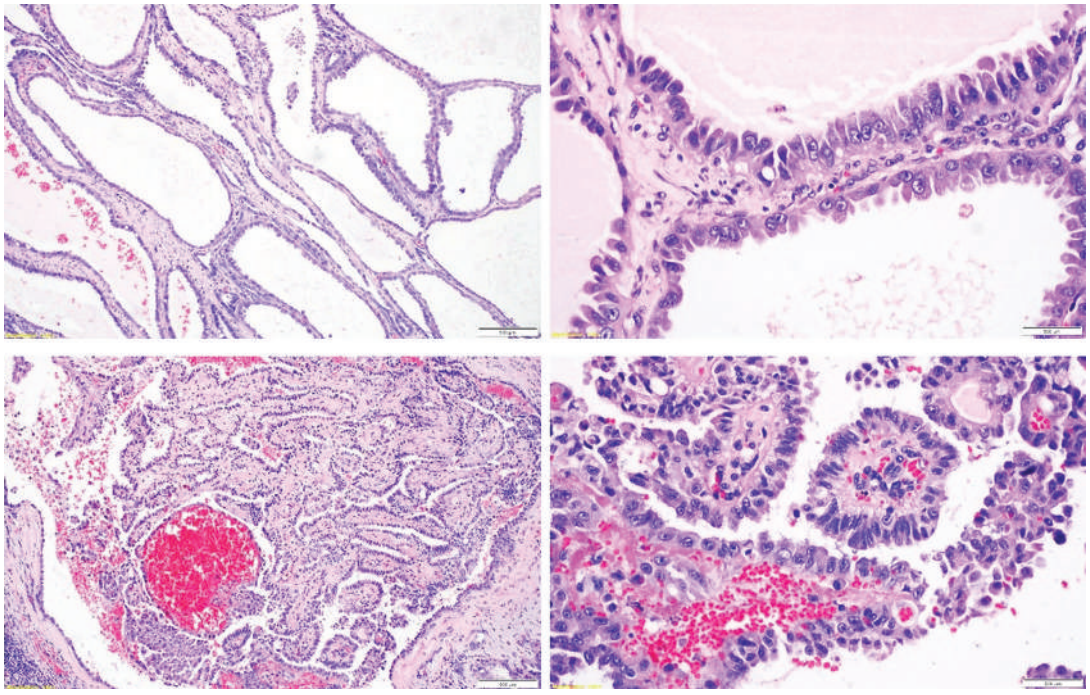

Figure 3. Microscopic tumor sections exhibiting tubulocystic and papillary morphology lined by hobnailed cells with World Health Organization/International Society of Urologic Pathology (WHO/ISUP) grade 3 nuclei (H&E, 100x and 400x).

(AMACR). CD10 was negative in neoplastic cells with uninvolved glomeruli and scattered inflammatory cells serving as the internal control. Cytokeratin 7 (CK7) was likewise negative in tumor cells but was expressed in entrapped benign renal tubules. Papillary formations did not express CK7 (Figure 4). A final immunomorphologic diagnosis of tubulocystic renal cell carcinoma was rendered.

Fifteen months post-operatively, multiple pulmonary nodules and a lytic lesion in the manubrium were visualized on chest CT. Five months following detection of the pulmonary nodules, the patient suffered from a pathologic fracture of the right femoral neck. Bone scintigraphy displayed increased tracer uptake in the clavicles, pelvis, vertebrae and right femur signifying high probability of osseous metastases. Biopsy of the most accessible pulmonary nodule revealed metastatic TC-RCC with morphology and immunohistochemical expression being consistent with the previously diagnosed renal mass. In addition, Napsin-A was performed to assess for a primary lung adenocarcinoma which was subsequently ruled out by its lack of expression in tumor cells (Figure 5). A multidisciplinary approach to management was initiated.

DISCUSSION

Tubulocystic renal cell carcinoma is a rare malignant yet indolent entity, constituting less than 1% of all renal cell carcinomas with only about a hundred cases reported in literature to date. It exhibits a strong male predilection and wide age distribution. Abdominal pain and hematuria are presenting symptoms, but the vast majority of tumors are discovered incidentally. Grossly, there is involvement of the renal cortex or corticomedullary junction by a solitary, well-circumscribed mass composed of multiple small to intermediate-sized cysts creating a spongy or "bubble-wrap" cut surface. Microscopically, the tumor is composed of varisized tubules lined by a single layer of flattened, cuboidal to columnar, hobnail epithelium exhibiting WHO/ISUP grade 3 nucleoli and abundant eosinophilic, oncocytoma-like cytoplasm.[2] The diagnosis is largely based on the presence of classic histological features however, immunohistochemical markers may aid in diagnosis. TC-RCC is consistently positive for AMACR, vimentin, parvalbumin and cytokeratins 8, 18 and 19. Variable positivity for CD10, CK7 (focal weak expression), carbonic anhydrase IX, PAX2 and cytokeratin 34BE12 have been reported.[1,3-5]

There have been accounts of TC-RCC occurring in association with other neoplasms, most commonly papillary renal cell carcinoma (PRCC).[3] It is observed that TC-RCC may bear pathologic similarities with PRCC but gene expression profiling data indicates that TC-RCC has a unique molecular signature.[4] Driven by the contradictory results of cytogenetic approaches in several studies that supported or refuted the presence of aberrations in chromosomes 7 and 17 in TC-RCC, Lawrie et al., conducted the largest molecular study on TC-RCC employing miRNA expression analysis and targeted next generation sequencing and discovered a high prevalence of ABL1 and PDGFRA gene mutations only rarely expressed in other renal cell carcinoma types including PRCC.[6] In addition, losses in chromosome 9 and the Y chromosome detected via next generation sequencing and fluorescence in situ hybridization have been reported.[7] FISH analysis using chromosome enumeration probes on the patient's specimen revealed gains in both chromosome 7 and 17 – expected findings given the close molecular relationship of TC-RCC and PRCC.

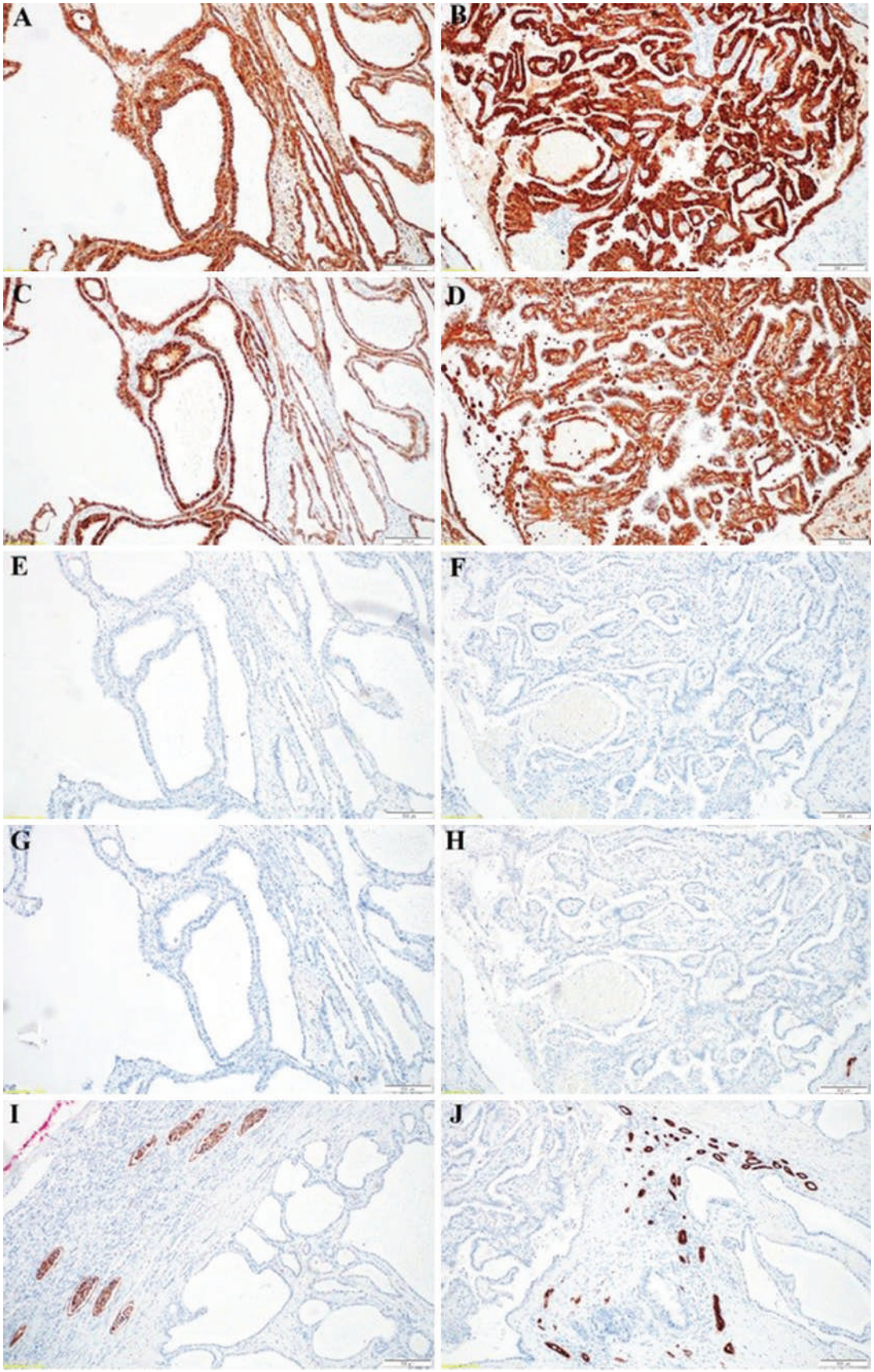

Figure 4. Immunohistochemistry. **(A and B)** Vimentin, positive diffuse strong cytoplasmic expression. **(C and D)** Alpha-methyl acyl-CoA racemase (AMACR), positive diffuse strong cytoplasmic granular expression. **(E and F)** CD10, negative expression in tubulocystic and papillary areas. **(G and H)** Cytokeratin 7 (CK7), negative expression in tubulocystic and papillary areas. **(I)** CD10 internal control, non-neoplastic glomeruli. **(J)** CK7 internal control, non-neoplastic renal tubules entrapped between papillary and tubulocystic areas.

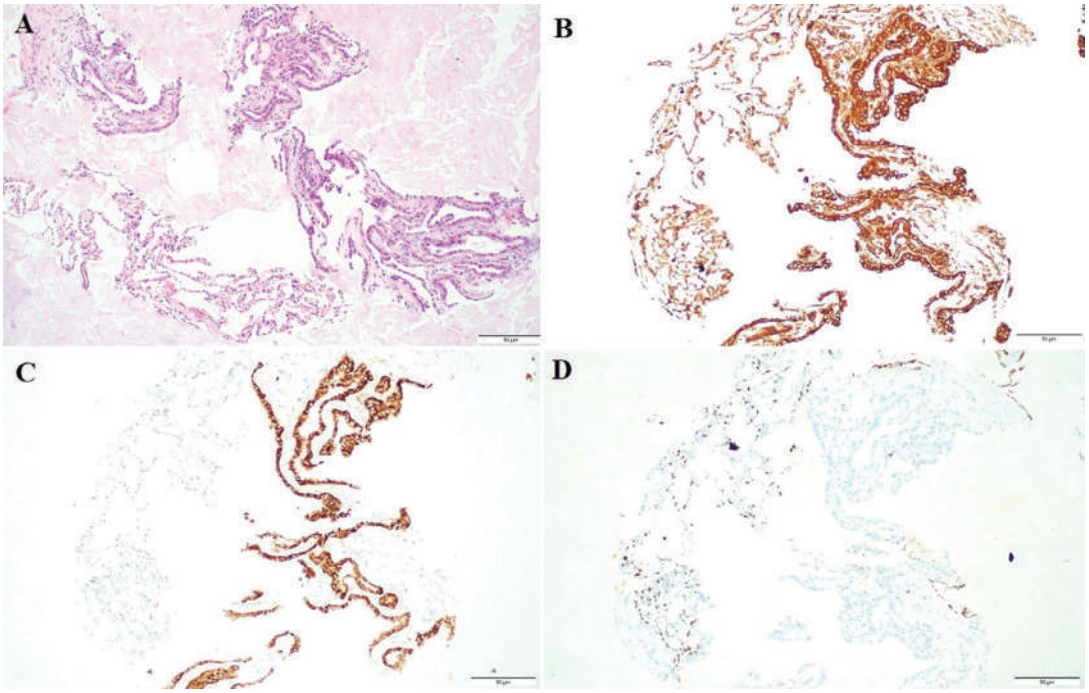

Figure 5. Pulmonary metastasis, cell block and immunohistochemistry. **(A)** Metastatic tubulocystic renal cell carcinoma (TC-RCC) (right) with non-neoplastic pulmonary alveoli (left) (H&E, 100x). **(B)** Vimentin, positive diffuse strong cytoplasmic expression in metastatic neoplastic cells and pulmonary alveoli. **(C)** AMACR, positive diffuse strong cytoplasmic granular expression in metastatic neoplastic cells; negative expression in pulmonary alveoli. **(D)** Napsin-A, negative expression in neoplastic cells with patchy cytoplasmic expression in scattered pneumocytes serving as internal control.

As TC-RCC is a diagnosis primarily based on histology, pathologists have sought to refine the morphologic criteria applicable to this disease. Although papillary components are deemed acceptable in current tumor classification texts,[2] a study of nine TC-RCC cases by Sarungbam et al., recommended that TC-RCC be diagnosed using strict morphologic criteria and only when presenting in "pure" form, that is, without variable architectural patterns such as papillary or poorly differentiated foci.[7] The considerable presence of papillary morphology became a diagnostic dilemma for the case at hand. Pending more extensive molecular analysis, the highly characteristic spongy gross appearance with distinct lack of solid areas, cytologic features such as diffuse cellular hob nailing with presence of high-grade nuclei, and immunohistochemical expression of AMACR and vimentin with absence of reactivity for CK7 and CD10, all favored a profile of TC-RCC over the main differential of PRCC.

Other considered differentials with tubulocystic patterns and hobnailed cells are easily distinguished from TC-RCC by clinical, macroscopic and histopathologic criteria. Multilocular cystic renal neoplasm of low malignant potential shows cysts lined by neoplastic cells with abundant clear cytoplasm and WHO/ISUP grade 1 to 2 nuclei. Fumarate hydratase deficient RCC distinctly occurs with cutaneous and uterine leiomyomas in 85% of cases. Identification of perinuclear halos and AMACR negativity aids in diagnosis of this tumor. Collecting duct carcinoma grossly appears solid and necrotic and is associated with a desmoplastic stromal reaction and high-grade behavior[2] (Table 1).

Although majority of TC-RCCs behave indolently, there still exist reports of tumor recurrence and distant metastasis.[8-10] Given the evolving body of knowledge on TC-RCC, an integrative approach to management becomes imperative to providing optimal care.

CONCLUSION

Tubulocystic renal cell carcinoma presents with unique histopathologic features and specific genetic aberrations. Immunohistochemistry serves as a valuable tool in establishing a diagnosis of TC-RCC amidst morphologic mimics. As the biologic behavior of TC-RCC remains to be established, due caution must be exercised in its prognostication. Further studies are necessary to better define the diagnostic criteria for this new subtype of renal tubular epithelial malignancies and to provide greater insight into its clinical outcomes.

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Acknowledgments are extended to the National Kidney and Transplant Institute's Department of Pathology and Laboratory Medicine and Department of Urology.

ETHICAL CONSIDERATION

Patient consent was obtained before submission of the manuscript.

Table 1. Differential diagnosis for tubulocystic renal cell carcinoma[2-5]

Differential diagnosis	Morphologic findings	Immunohistochemical expression	Molecular features
Tubulocystic RCC	varisized tubules lined by a single layer of flattened, cuboidal to columnar, hobnail epithelium exhibiting WHO/ISUP grade 3 nucleoli and abundant eosinophilic cytoplasm	Positive Expression: PAX8 AMACR RCC marker Vimentin Parvalbumin CK8, CK18 and CK19 Variable Expression: CD10 CK7 CA IX PAX2 Cytokeratin 34βE12 Negative Expression: p63	ABL1 and PDGFRA gene mutations[6] Aberrations in chromosomes 7 and 17 and loss in chromosome 9 and Y chromosome have been reported[7]
Papillary RCC	papillary/ tubulopapillary structures with delicate fibrovascular cores often containing foamy macrophages and psammoma bodies, lined by a single or pseudostratified layer of neoplastic cells with high WHO/ISUP nuclear grade and abundant eosinophilic cytoplasm; necrosis and hemorrhage	Positive Expression: PAX8 PAX2 AMACR RCC marker Vimentin CD10 CK7 Cytokeratin AE1/AE3 CAM 5.2 EMA Negative Expression: Cytokeratin 34βE12 p63	Gains (trisomy / tetrasomy) in chromosome 7 and 17 and loss of Y chromosome are classic findings among other various reported mutations
Multilocular Cystic Renal Neoplasm of Low Malignant Potential	cyst walls are lined by a single layer of tumor cells with abundant clear cytoplasm and WHO/ISUP grade 1 nuclei; fibrous septa also contain clusters of tumor cells	Positive Expression: PAX8 CA IX	Chromosome 3p deletion and VHL gene mutations
Fumarate Hydratase-deficient RCC	papillary structures lined by large cells with large nuclei, inclusion-like eosinophilic nucleoli and abundant eosinophilic cytoplasm; solid, tubular and tubulocystic variants have been noted	Positive Expression: S-(2-succino)cysteine Negative Expression: FH	Germline mutations in fumarate hydratase gene at 1q42.3-q43
Collecting Duct Carcinoma	morphologic criteria include medullary involvement, predominant tubular (tubulopapillary or tubulocystic) morphology, stromal desmoplasia, high-grade cytology, infiltrative growth pattern and absence of other RCC types or urothelial carcinoma	Positive Expression: PAX8 CK7 CK19 Cytokeratin 34βE12 Vimentin Variable Expression: AMACR Negative Expression: SMARC B1 CD10 RCC marker	Various chromosomal losses HER2/neu amplification and SMARCB1 mutations have been reported

AMACR, alpha-methyl acyl-CoA racemase; CA IX, carbonic anhydrase IX; CK, cytokeratin; EMA, epithelial membrane antigen; FH, fumarate hydratase; PAX2, paired box 2 transcription factor; PAX8, paired box 8 transcription factor; PDGFRA, platelet-derived growth factor receptor alpha; RCC, renal cell carcinoma; SMARC, switching defective/sucrose nonfermenting (SWI/SNF) related, matrix associated, actin dependent regulators of chromatin; VHL, Von Hippel-Lindau gene; WHO/ISUP, World Health Organization / International Society of Urologic Pathology

STATEMENT OF AUTHORSHIP

Both authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

Both authors declared no conflict of interest.

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OPEN ACCESS – CASE REPORT

Glomangiopericytoma: A Rare Sinonasal Neoplasm

Karen Damian[1] and Rachel Alegata[2]

[1]*Department of Laboratories, University of the Philippines – Philippine General Hospital*
[2]*La Vina General Hospital, Inc., Poblacion, Valencia, Bukidnon*

ABSTRACT

Glomangiopericytoma is a rare neoplasm of the nasal and paranasal sinuses comprising less than 1% of all tumors of the said region. We report of a 59-year-old hypertensive male who presented with epistaxis. CT scan findings showed a mass in the right nasal cavity with extension into the ethmoid and sphenoid sinuses. Histopathologic diagnosis was glomangiopericytoma confirmed with immunohistochemistry studies. Prognosis is favorable with complete resection of tumor and long-term monitoring.

Key words: glomangiopericytoma, paranasal sinus neoplasms, intranasal neoplasms, sinonasal hemangiopericytoma-like tumor

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Corresponding author: Karen B. Damian, MD, FPSP
E-mail: karenbdamian@gmail.com
ORCiD: https://orcid.org/0000-0003-3634-3788

INTRODUCTION

Glomangiopericytoma is a rare vascular tumor of the nasal cavity and paranasal sinuses. It comprises less than 0.5% of all sinonasal neoplasms with a characteristic and prominent perivascular growth pattern.[1] Gross appearance is similar to the more common nasal inflammatory polyps. Immunomorphologic features will differentiate this tumor from other intranasal neoplasms and soft tissue hemangiopericytomas arising from other sites.[2]

CASE

We report a case of a 59-year-old male, known hypertensive and diabetic, who had a history of on and off epistaxis several months prior to admission. Successive epistaxis led to a consult with an otorhinolaryngologist who noted a right intranasal mass. CT scan of the paranasal sinuses revealed a polypoid soft tissue mass in the right posterior nasal cavity extending into the posterior ethmoid sinus superiorly and into the right side of the sphenoid sinus posteriorly. The mass measured 2.6 x 2.5 x 1.5 cm and exhibited fairly homogenous contrast enhancement. There was no lytic nor sclerotic changes observed in the adjacent osseous structures. The right superior and middle turbinates were obscured by the said mass (Figure 1). An uneventful nasal endoscopy was eventually done to control the bleeding. Resection of the tumor was done several weeks later with no reported complications.

Histopathology of the mass reveals a cellular tumor composed of variedly sized blood vessels, some showing branching, staghorn appearance. The overlying respiratory epithelium was unremarkable and uninvolved. The tumor cells were composed of spindle shaped cells with ovoid nucleus, eosinophilic cytoplasm and inconspicuous nucleoli (Figure 2). Immunohistochemistry studies revealed diffuse positivity with smooth muscle actin and negative staining with CD45, pan-cytokeratin, CD31, CD34 and S-100 (Figure 3). Based on the abovementioned features, this case was signed out as glomangiopericytoma.

DISCUSSION

Glomangiopericytoma is a rare mesenchymal tumor arising almost exclusively from the nasal cavity or paranasal sinuses. It comprises less than 0.5% of all sinonasal tumors.[1-3] There is a slight female preponderance with a female to male ratio of 3:1. Most patients experience nasal obstruction and epistaxis,[3] similar to that experienced by our patient. Etiology is still not clear; however, predisposing factors include trauma, corticosteroid use, hypertension and pregnancy. Our patient denied previous trauma and corticosteroid use but has a history of hypertension. Hemangiopericytomas were first described by Stout and Murray in 1942 as a soft tissue tumor with a distinct branching proliferation of vascular channels and perivascular hyalinization of small blood vessels.[4] Over the years, the concept of hemangiopericytomas evolved as these tumors were noted to occur in the nasal and paranasal sinuses in 5% of cases. These nasal and sinonasal hemangiopericytomas also behaved indolently compared to its soft tissue counterparts, had distinct morphologic features and were noted to show true pericytic differentiation. As such, in 1976, Compangno and Hyam termed these lesions as hemangiopericytoma-like intranasal tumors.[4,5] In 2005, the World Health Organization (WHO) proposed the term glomangiopericytoma to reflect the findings of several studies that show this tumor's similarity and close relationship with glomus tumors.[5] Glomangiopericytomas are indolent tumors with overall excellent survival (>90% 5-year survival) when complete excision is achieved. Recurrences are usually a result of inadequate resection. The lesions appear fleshy pink to red, hemorrhagic and polypoid on gross examination. Histologically, the characteristic appearance is that of cells arranged in various architecture, separated by vascular channels in staghorn or "antler-like" configuration. Mitosis is rare, necrosis and hemorrhage are uncommon and nuclear pleomorphism is mild to absent.[4,5] A malignant glomangiopericytoma should be suspected in the presence of large tumor size (>5 cm), bony invasion, pleomorphic nuclei, increased mitotic activity (>4/10 high power fields) and increased proliferation activity (>10% Ki-67 proliferation index).[6] Other benign spindle cell neoplasms may be confused with glomangiopericytomas and some close differential diagnosis include: angiofibroma, vascular leiomyoma, lobular capillary hemangioma and solitary fibrous tumors. Angiofibromas are seen almost exclusively in adolescent males. Histologically, they present with large, ectatic vessels, abundant stromal collagen and bland stellate shaped cells. This tumor will stain positive with androgen receptor 22 and β-catenin. Sinonasal leiomyomas are uncommon and can present with staghorn-type vascularity with ovoid nuclei, and prominent fascicular growth pattern. Strong positivity with desmin can help differentiate this tumor from glomangiopericytoma. Lobular capillary hemangioma can also present grossly as an intranasal mass. This tumor is also vascular with a central feeder vessel and gives rise to smaller, slit-like vascular channels. Immunohistochemistry studies show positivity with CD31 and CD34, highlighting the tumor cells' endothelial differentiation. Sinonasal solitary fibrous tumors are also uncommon tumors with variable vascularity and haphazardly arranged cells. Absence of perivascular hyalinization and presence of coarse collagen bundles distinguish this from glomangiopericytoma. The tumor cells of solitary fibrous

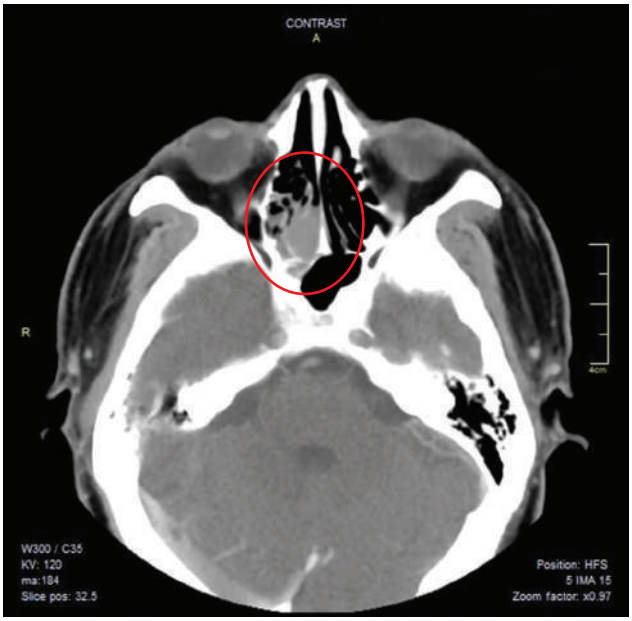

Figure 1. CT scan showed a 2.6 x 2.5 x 1.5 cm polypoid soft tissue mass in the right posterior nasal cavity extending into the posterior ethmoid sinus and into the right side of the sphenoid sinus.

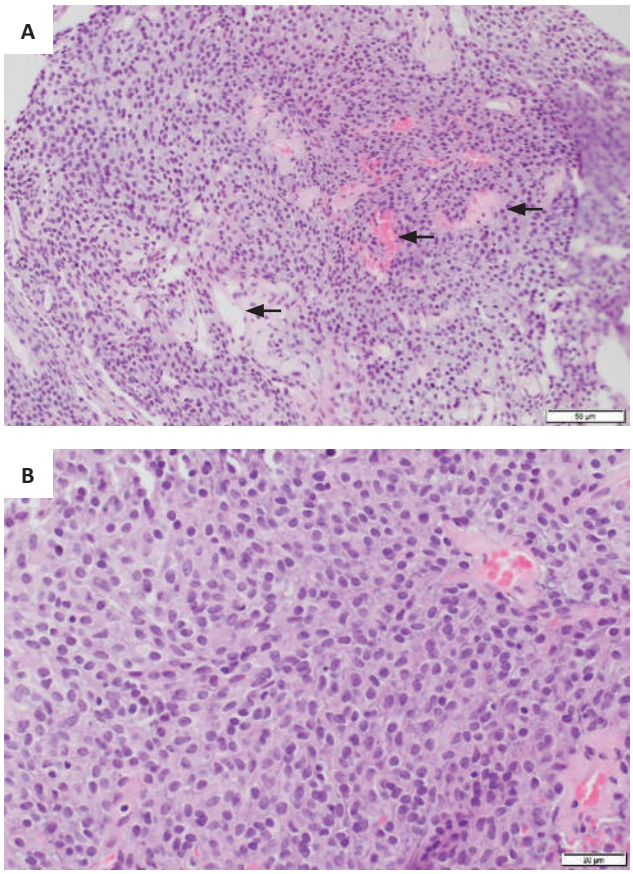

Figure 2. (A) Cellular tumor composed of variedly sized blood vessels (H&E, 20x). **(B)** The tumor cells composed of spindle shaped cells with ovoid nucleus, eosinophilic cytoplasm and inconspicuous nucleoli (H&E, 40x).

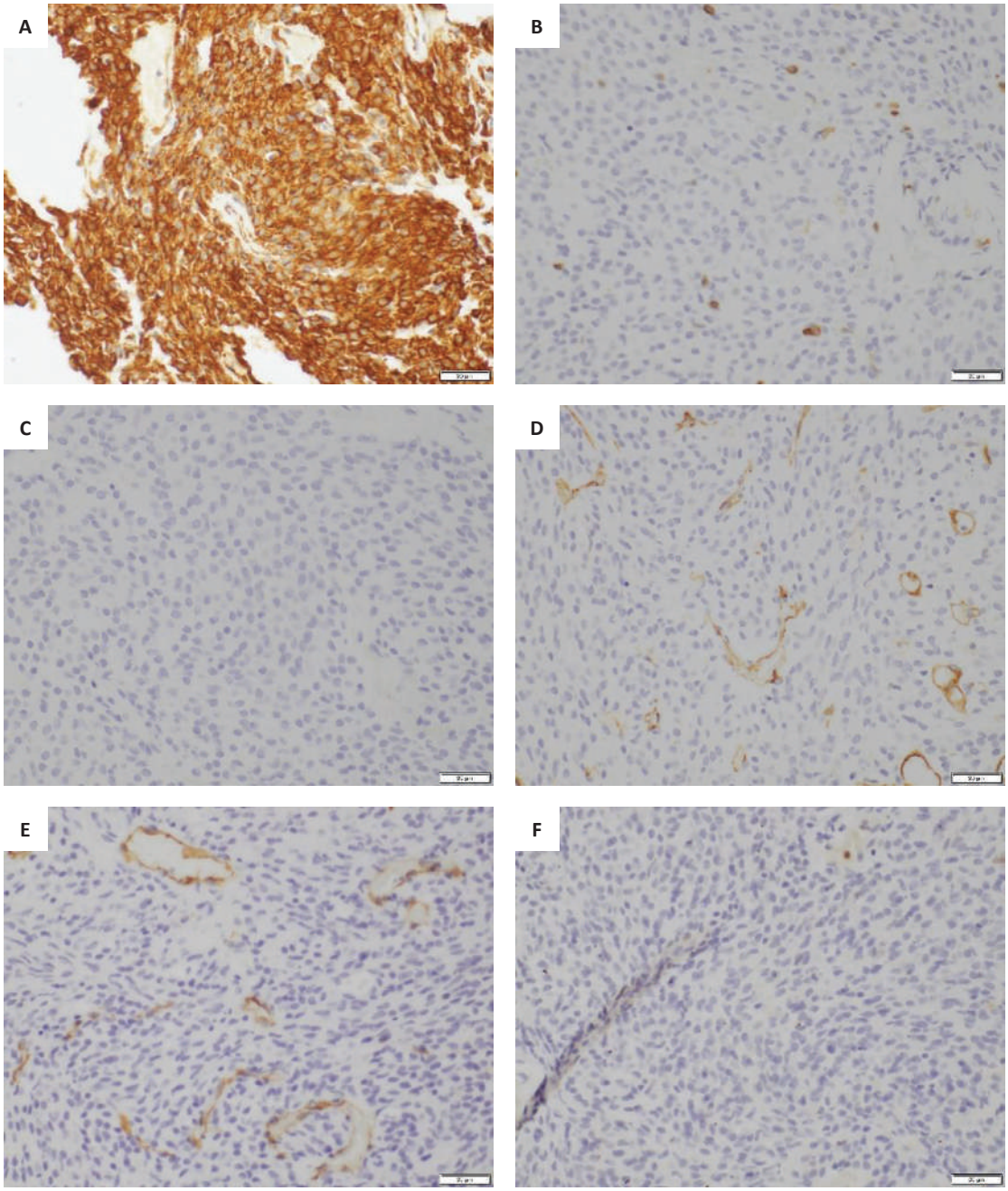

Figure 1. Immunohistochemistry profile of the tumor. **(A)** Smooth muscle actin positive tumor cells (HRP, 40x); **(B)** CD45 negative (HRP, 40x); **(C)** Pan-cytokeratin negative (HRP, 40x); **(D)** CD31 negative (HRP, 40x); **(E)** CD34 negative (HRP, 40x); **(F)** S-100 negative (HRP, 40x).

tumor stain positively with CD34 and variably with smooth muscle actin.[4,6] Immunohistochemistry studies can aid in the differentiation of glomangiopericytoma from the abovementioned benign tumors. Glomangiopericytomas show diffuse reactivity with smooth muscle actin and vimentin and negativity with CD45, CD31, desmin, S-100, STAT-6 and NSE. The tumorigenesis and molecular genetics of glomangiopericytoma are not well-established, however, studies have shown that mutation activation of β-catenin with the associated cyclin D1 overexpression are central events in the pathogenesis of glomangiopericytoma.[7,8] β-catenin is a cadherin-associated membrane protein that is involved in the regulation of cell-to-cell adhesion, a terminal component of the Wnt-signaling pathway. Accumulation of β-catenin results in nuclear translocation, with the nuclear expression of β-catenin demonstrated to up-regulate cyclin D1, leading to its oncogenic activation.[8] Several studies have shown the utility of β-catenin and cyclin D1 in the diagnosis of glomangiopericytoma. Most studies are in agreement that show strong nuclear expression with β-catenin in virtually all glomangiopericytoma.[8-10] Similarly, cyclin D1 was also noted to exhibit nuclear positivity in most (70-100%) of the tumor cells.[8,9] Complete surgical resection is the standard treatment in glomangiopericytoma with radiotherapy reserved only for cases that are inoperable or metastatic.[11] Lymphatic and hematogenous spread of malignant glomangiopericytoma have been reported in 5% of cases and were seen to involve distant organs such

as the lungs, liver and bone.[5] Our patient eventually underwent complete resection with no untoward post-surgery events reported. However, at present, he is already lost to follow up. The patient could have benefitted from a regular follow-up since recurrence may occur even with a long disease-free interval.[5] Recurrence has been reported in 15.1% of cases and is most commonly due to inadequate resection. The WHO in the 2017 Classification of Tumors, describes this tumor as an indolent tumor with the prognosis being favorable with an excellent survival rate.[6,12]

CONCLUSION

Glomangiopericytoma is a rare, indolent neoplasm of the sinonasal region. Surgery remains the treatment of choice and is curative when completely resected. Reporting of cases will help in increasing knowledge, aid in establishment of diagnosis and create definitive guidelines for the treatment for glomangiopericytoma.

ETHICAL CONSIDERATION

The authors have tried to reach out to the patient through his cell phone number and email address, but they have not received any response from him. They also tried to contact him through his attending physician, but the patient has already been lost to follow up. They have also alerted the hospital's radiology and laboratory departments in case the patient goes for a subsequent examination but up until the present, the patient has not returned to the hospital. The authors have exercised due diligence in trying to reach the patient for his consent, but they have not been successful in doing so.

STATEMENT OF AUTHORSHIP

Both authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

Both authors declared no conflict of interest.

FUNDING SOURCE

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OPEN ACCESS – CASE REPORT

Brain Metastasis of Papillary Ovarian Adenocarcinoma

Sonia Chhabra, Niti Dalal, Sunita Singh

Department of Pathology, Pt. B. D. Sharma Post Graduate Institute of Medical Sciences (PGIMS), Rohtak, Haryana, India

ABSTRACT

Brain metastasis from epithelial ovarian cancer is a rare diagnostic entity with a reported incidence of 1- 2%. Serous epithelial ovarian cancer is usually associated with a poor prognosis and is the most common malignancy metastasizing to the brain. The median time from primary diagnosis to development of cerebral lesions is directly correlated with the initial tumour grade and stage. The median survival after diagnosis of brain metastases is 6 months. It is suggested that brain imaging studies should be included in the follow up of patients after treatment for ovarian carcinoma. We report a case of brain metastasis of ovarian adenocarcinoma 2 years post-surgery and six cycles of chemotherapy.

Key words: brain, metastases, ovarian, adenocarcinoma

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Corresponding author: Corresponding author: Niti Dalal, MD
E-mail: drnitidalal@gmail.com
ORCiD: https://orcid.org/0000-0003-0862-0426

INTRODUCTION

A 46-year-old female was brought to the emergency department due to loss of consciousness. The patient had a history of right-sided weakness associated with headache 1½ months prior. Her headache was intermittent in nature and located at the frontal region. There were 2-3 episodes of generalized seizures and 3-4 episodes of projectile vomiting for the last 20 days. Two years prior, the patient had been diagnosed with high grade serous epithelial ovarian carcinoma stage III having CA-125 levels of 800 IU/ml. The patient underwent total abdominal hysterectomy, bilateral salphingo-oophorectomy and omentectomy. The final histopathology report showed high grade serous epithelial ovarian carcinoma. Post-surgery, the patient underwent 6 cycles of chemotherapy and was on regular follow up. The follow up was uneventful for 1 ½ years. Her last serum CA-125 level was 10.6 1U/ml before being lost to follow up.

Following this, non-contrast computed tomography (CT) scan of the brain was advised which showed a ring enhancing lesion at the left frontal region measuring 3.5 x 3.2 cm. The lesion was at the grey-white mater junction with necrotic areas surrounded by hypodense areas. There was midline shift of 1.1 cm to the left with no evidence of infarction or intracranial bleeding. Radiologically, a probable diagnosis of metastasis was made. Magnetic resonance imaging (MRI) showed left frontal space-occupying lesion. Serum CA-125 levels were elevated (110.8 IU/ml). Left frontoparietal craniotomy was performed. A biopsy sample was submitted with a clinical impression of abscess. Gross examination showed multiple grey brown to grey white, soft, friable tissue fragments measuring 5 x 4 x 2 cm in total. Microscopic examination revealed pleomorphic cells with prominent nucleoli arranged in papillary architecture and clusters invading normal brain parenchyma. There were areas of hemorrhage and necrosis. On immunohistochemical staining, these tumour cells were positive for cytokeratin, epithelial membrane antigen (EMA), and CA-125, and negative for glial fibrillary acidic protein (GFAP). A final diagnosis of metastatic deposits from papillary adenocarcinoma of ovary was made.

DISCUSSION

Brain metastases from epithelial ovarian carcinoma is a rare diagnostic entity with a reported incidence of 1-2% and is associated with poor prognosis.[1] Epithelial ovarian carcinoma most commonly progresses to intraperitoneal dissemination, followed by metastasis to the pleural cavity, liver, and lung.[2] Brain metastasis are more common with primary tumours of the lung, breast, renal, colorectal carcinomas and melanoma. A study done by Piura and Piura suggested that out of all gynaecological cancers, the incidence of brain metastasis of ovarian malignancy is 1.2% which is twice the incidence associated with cervical or endometrial cancer.[3] The most common histologic subtype of ovarian carcinoma associated with brain metastasis is the serous type, followed by mixed epithelial, endometrioid adenocarcinoma, mucinous, undifferentiated, and clear cell type.[4]

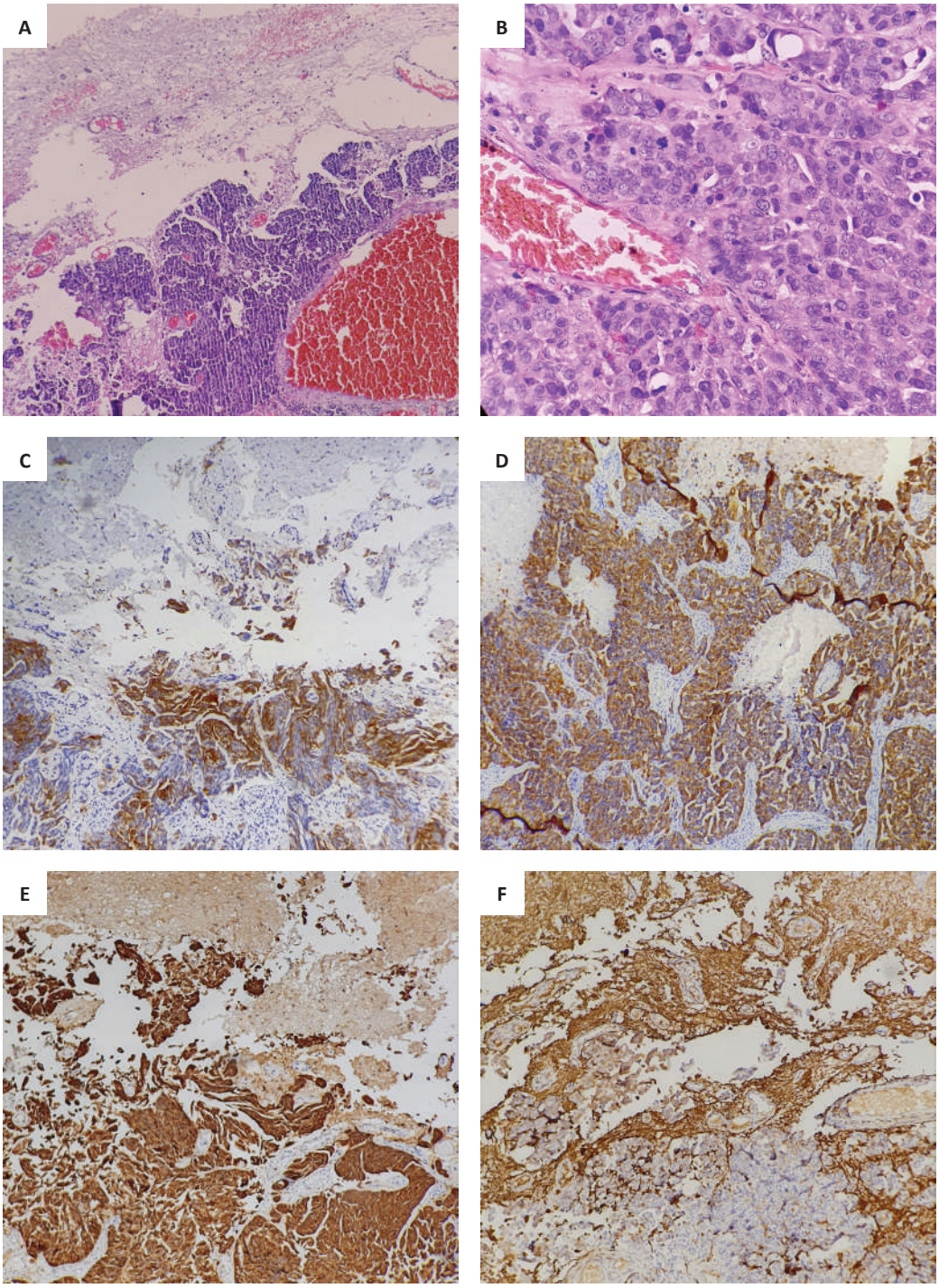

Figure 1. **(A)** Infiltration of papillary adenocarcinoma of ovary into brain parenchyma (H&E, 100x); **(B)** High power view of papillary adenocarcinoma of ovary (H&E, 400x); **(C)** CA-125 positivity in metastasis of ovarian carcinoma (IHC, 100x); **(D)** Cytokeratin positivity in metastatic of ovarian carcinoma (IHC, 100x); **(E)** EMA positivity in metastatic of ovarian carcinoma (IHC, 100x); **(F)** GFAP positivity in normal brain parenchyma and negativity in metastatic of ovarian carcinoma (IHC, 100x).

Some studies have demonstrated the correlation between germline mutations of BReast CAncer gene 1 (BRCA-1) mutations and incidence of brain metastases in ovarian carcinoma. BRCA-1 and BRCA-2 gene mutations, which are detected in 10% of ovarian carcinoma, are associated with aggressive behaviour and metastatic disease.[4]

In the brain, the cerebrum is the most common site for metastases, followed by the cerebellum, and leptomeninges.[3] The frontal lobe is the most commonly involved area.[4] Symptoms of brain metastases include headaches, nausea, vomiting, confusion, dizziness, decreased mental status, general or extremity weakness, urinary incontinence, gait disturbance, ataxia, visual disturbance including diplopia, photophobia, speech impairment, syncope, and seizures. Contrast enhanced MRI brain and CT scan together, are the most accurate imaging modality. Metastasis on CT scan appears as a heterogeneous, contrast enhancing lesion.

The multimodal treatment approach includes surgical resection of the brain metastases, whole brain radiotherapy, and chemotherapy. In the case of single brain metastasis, surgery should be considered if the site is approachable and the tumour is producing mass effects. In the case of multiple metastases, multimodality treatment approach is advised.[3] The reported median time from primary diagnosis to development of cerebral lesions ranged from 11 to 46 months and directly correlated with the initial tumour grade and stage.[1,4] Patients with poorly differentiated ovarian carcinoma (grade 3) had a median time interval of 1.5 years between diagnosis and brain metastasis. Patients with well- to moderately differentiated ovarian carcinoma (grades 1 and 2) had a median time interval of 4.73 years. The median survival after diagnosis of brain metastases is 6 months, however, multimodal treatment approach improves the outcome of the patient.[1] A combination of surgery, radiotherapy and chemotherapy has a median survival time of 20 months; 17 months for surgery and radiotherapy; 9.1 months for radiotherapy and chemotherapy; 4.5 months for radiotherapy only; 7.5 months for chemotherapy only; and 18 months for stereotactic radiosurgery (SRS) or gamma-knife radiosurgery (GKRS). Out of all these, SRS and GKRS yielded better survival results.[1]

Serum CA-125 is done as a part of routine follow-up for ovarian cancer patients, however, it cannot be relied upon to detect CNS relapse. It is advisable to include brain imaging studies in the follow up of patients after treatment for ovarian carcinoma.[1,2]

The patient started chemotherapy but was lost to follow up after 1 month. There was no other information available regarding further treatment.

CONCLUSION

Ovarian cancers rarely metastasize to the brain and is associated with poor prognosis. A careful clinical examination and proper therapeutic approach, including chemotherapy and radiotherapy, may lead to prolonged survival.

ETHICAL CONSIDERATION

Patient consent was obtained before submission of the manuscript.

STATEMENT OF AUTHORSHIP

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AUTHOR DISCLOSURE

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OPEN ACCESS – CASE REPORT

Ectopic Schistosomiasis Presenting as Ruptured Appendicitis with Periappendiceal Abscess Formation: An Alternative Pathogenetic Perspective

Marcel Joshua del Fierro, Yvette Tan, Larissa Lara Torno

Department of Internal Medicine, Metro Davao Medical and Research Center, Inc., Davao City, Philippines

ABSTRACT

Schistosomiasis is still a public health burden in the Philippines. Chronic infection with *Schistosoma japonicum*, the only species endemic in the Philippines, clinically manifests itself in a wide variety of pathologies usually correlated with the anatomical site of adult worm activity and deposition of eggs. One of the documented ectopic sites for Schistosoma ova is the appendix. A rare sequela of this is acute appendicitis and an even rarer consequence is progression to appendiceal rupture leading to acute peritonitis. We present a case of a 27-year-old Filipino residing in Davao City but born in Agusan Province who initially complained of right lower quadrant abdominal pain but presented at the emergency room with generalized abdominal tenderness with signs of peritoneal irritation. Exploratory laparotomy with an infraumbilical incision revealed ruptured appendicitis with periappendiceal abscess formation and appendectomy was subsequently done. Schistosoma infection of the appendix was subsequently established by histopathological analysis. Furthermore, features observed suggest an atypical pathogenetic process contrary to the putative pathogenesis of most cases of acute appendicitis.

Key words: schistosomiasis, Schistosoma japonicum, acute appendicitis, periappendiceal abscess

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Corresponding author: Marcel Joshua L. del Fierro, MD
E-mail: mdelfierro@up.edu.ph
ORCiD: https://orcid.org/00000-0001-6530-4302

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INTRODUCTION

Schistosomiasis is still a public health problem, especially in endemic areas in the Philippines. The risk for *S. japonicum* infection spans approximately 12 million people living in 28 provinces located across 12 different geographical regions.[1] These regions have no distinct dry season and comprise predominantly rice-growing areas. Transmission continues to be high because climatic conditions and current rice farming methods maximize contact between freshwater snails and humans. Furthermore, a 2011 study done in Northern Samar showed that bovine and water buffaloes play a major role in the transmission of schistosomiasis with an infection prevalence of approximately 90% making control efforts even harder.[2]

Without intervention, *Schistosoma* typically survives in the host body for up to 5 years but there have been reports of chronic infections for up to 40 years.[3] Long-term infection or repeated reinfection with *S. japonicum* causes two types of morbidities: those with subtle clinical manifestations and those with end-organ complications. Subtle clinical manifestations are caused by elevated inflammatory cytokines induced by ova or the parasite itself. Chronic anemia, growth retardation, impaired cognitive abilities, and malnutrition have been documented in children with *S. japonicum* infection.[4,5] The end-organ complications are sequelae of granuloma formation with subsequent tissue injury and fibrosis. Several ectopic localizations for various species of Schistosoma have been documented before.[6,7] The first-ever known case of appendiceal schistosomiasis was reported by Turner in 1909 and has been described in many reports, especially in endemic areas but to our knowledge, there is only one other reported case that progressed to ruptured appendicitis and subsequent

periappendiceal abscess formation.[8,9] We present an unusual case of appendiceal Schistosomiasis wherein the patient had a ruptured appendix with localized periappendiceal abscess formation.

CASE

A 27-year-old Filipino female residing in Davao City (4 years) but originally from Agusan Province of the Philippines, came into our institution with a 2-day history of sudden onset of epigastric pain which later migrated to the right lower quadrant of the abdomen. There was no fever, anorexia, vomiting, or dysuria. She had no comorbidities or previous surgeries. The patient decided to undergo ultrasonography of the whole abdomen as an outpatient but yielded unremarkable results. An increase in the severity of the pain prompted consult in our institution.

At the emergency room, a physical examination of the abdomen revealed tenderness on all quadrants upon light palpation, with note of muscular guarding. Vital signs remained normal and the patient was afebrile. Other physical examination findings were unremarkable.

A pregnancy test done was negative. Complete blood count revealed only a slight increase in white blood cell count of 14.58 x 10^9/L with normal hemoglobin levels and no eosinophilia. There was no pyuria in the urinalysis and coagulation studies and serum creatinine was normal. However, blood chemistry revealed hypokalemia at 3.2 mmol/L.

The preliminary diagnosis of this case was acute abdomen probably from ruptured appendicitis. The patient was immediately started on intravenous Ampicillin-Sulbactam, intravenous correction of potassium, and prepared for surgical intervention. The team decided on exploratory laparotomy with an infraumbilical midline incision. Intraoperatively, the appendix measured 7 cm x 2 cm and was gangrenous with a perforation near the base and the antimesenteric area. Packets of pus were noted around the appendix, both at the left and right paracolic gutters and at the pelvic gutter. Excision of the appendix with lysis and suctioning of all packets of pus was done. Adequate peritoneal lavage was ensured. An appendix was sent to surgical pathology for microscopic examination.

Histopathological analysis of the appendix revealed dense neutrophilic infiltration in the mucosa, submucosa, muscularis propria, serosa, and mesoappendix (Figure 1). There is pus in the appendiceal lumen with large areas of necrosis. Infiltration of Schistosoma ova with granuloma formation in the submucosa and muscularis propria is noted (Figure 2). Praziquantel was started at 40 mg/k/day at 2 divided doses and sent home improved.

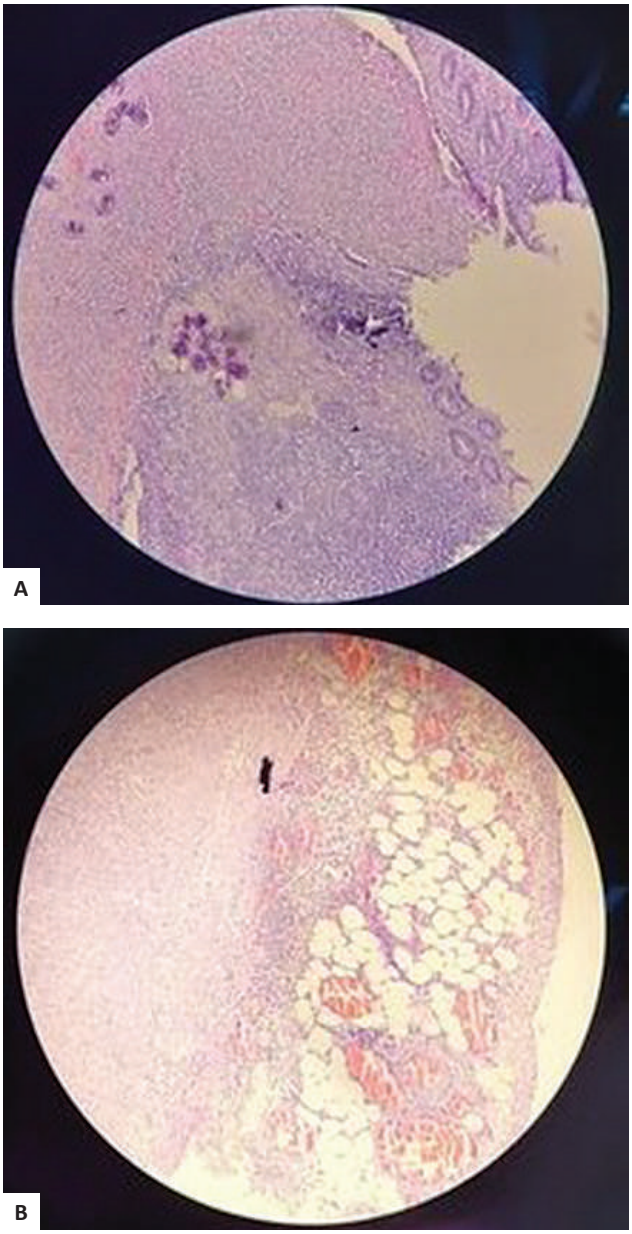

Figure 1. (A) Dense neutrophilic infiltration in the mucosa, submucosa, muscularis propria, serosa and **(B)** mesoappendix (H&E, 4x).

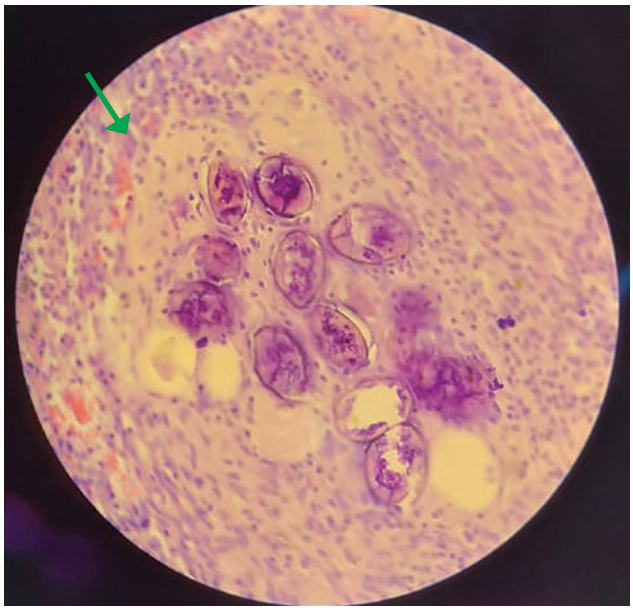

Figure 2. Schistosoma ova with granuloma formation *(green arrow)* found in submucosa layer (H&E, 40x).

DISCUSSION

In chronic schistosomiasis, clinical manifestations depend on the organ involved. These are classified into hepato-intestinal, hepatosplenic, pulmonary, cerebral, and ectopic forms.[5] Although rare, ectopic schistosomiasis has been reported in the heart, ovaries, uterus, fallopian tubes, ureters, urinary bladder, and the appendix.[6]

The preferred anatomic location for residence and egg deposition varies by species. *S. japonicum* in particular often resides in the inferior and superior mesenteric veins. After the eggs are deposited into the vascular lumen, digestive compounds are utilized by the schistosome so that around half of the ova can penetrate the blood vessel wall and enter the bowel including the appendix. The rest of the ova proceeds to the liver where they are filtered from the circulation.[10]

In our patient, *S. japonicum* ova were identified in the appendix making it the most obvious cause of appendicitis. However, the role of the Schistosoma ova in the pathogenesis of acute appendicitis and the subsequent rupture of the said organ in our patient remains unclear. Obstruction followed by infection from fecal contaminants is thought to be an important mechanism in the pathogenesis of acute appendicitis. However, using histopathological criteria, Satti et al., reported that there are 2 distinct histopathologic features of acute Schistosomal appendicitis: obstructive and granulomatous. Obstructive schistosomal appendicitis results from obstruction of the appendiceal lumen seen in chronic infection due to fibrosis around calcified eggs which increases the risk of other infections. On the other hand, granulomatous schistosomal appendicitis is caused by active granulomatous inflammation with eosinophilic necrosis around the ova. This was used as a marker for active schistosomal infection and is more congruent in the histopathological examination of the appendix in our patient.[11] In this case, there is the presence of histopathologic features that suggest that appendicular obstruction played no role in the pathogenesis of acute appendicitis and subsequent rupture. We hypothesize that the cause of appendicitis and subsequent rupture of the appendiceal wall, in this case, was caused by granulomatous tissue destruction. We contrast this to the only other report of ruptured appendiceal schistosomiasis with abscess formation by Al-Waheeb in 2008 wherein histopathological analysis of the sections from the appendix and omentum yielded no granulomatous response. Both cases led to rupture of the appendix and subsequent abscess formation but through different mechanisms.[9]

The gold standard for the diagnosis of schistosomiasis is the detection of parasite ova in fecal specimens. We performed direct microscopy on three separate stool samples to look for evidence of adult worm activity, but all yielded negative for parasites or ova. This is the reason why we had to rely on histopathologic analysis to clinch the diagnosis of schistosomiasis in this case. A possible explanation for this finding is that ova seen in the appendix of the patient may be old and adult worms that have already died out. However, this is contradicted by our histopathologic findings that suggest an ongoing infection. Nonetheless, we committed to an ongoing infection and decided to administer Praziquantel. Praziquantel is the recommended treatment for all species and all forms of schistosomiasis at 40 mg/kg, which is highly effective in approximately 91.7% of treated individuals.[12]

CONCLUSION

Putative pathogenesis of most cases of acute appendicitis involves luminal obstruction commonly by fecalith, lymphoid hyperplasia, fecal debris, true calculus, or tumor. In our case, we lack evidence of obstruction which suggests a possible existence of a non-obstructive form of appendicitis predominated by a granulomatous process.

Furthermore, in the absence of pathognomonic clinical preoperative and intraoperative findings, histopathologic diagnosis of patients with appendicitis is required for proper intervention.

Recent epidemiological and zoological studies seem to show that the national prevalence of schistosomiasis in the Philippines may have been initially underestimated leading to the relaxation of control measures. Schistosomiasis remains a public health burden and doctors practicing in endemic areas should be aware of the possibility of seeing atypical presentations of this parasitic disease.

ETHICAL CONSIDERATION

Patient consent was obtained before submission of the manuscript.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

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OPEN ACCESS – CASE REPORT

A Case Report on Carcinosarcoma of the Pancreas with a Concise Literature Review

Christine Santos and Rosalie Sabina Michiko Samonte

Department of Pathology and Laboratory Medicine, National Kidney and Transplant Institute, East Avenue, Diliman, Quezon City

ABSTRACT

Carcinosarcoma is a rare neoplasm that most commonly affects the uterus. In the pancreas, fewer than thirty cases are reported worldwide. We present a 47-year-old female with epigastric pain, and jaundice. Histopathology revealed a pancreatic head mass showing a biphasic tumor composed of seventy percent Pancreatic Ductal Adenocarcinoma, and thirty percent High Grade Sarcoma with immunohistochemistry using Pancytokeratin, Vimentin, Desmin, S-100, Smooth Muscle Actin, CD34, and Ki-67.

Key words: pancreatic carcinosarcoma, pancreas, carcinosarcoma, immunohistochemistry, surgical pathology, diagnosis

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Corresponding author: Christine D. Santos, MD
E-mail: cdsantos1102@gmail.com
ORCiD: https://orcid.org/0000-0002-5476-3578

INTRODUCTION

The most common pancreatic tumor is ductal adenocarcinoma. Undifferentiated carcinoma is one of its subtypes, and it has three distinct patterns: anaplastic undifferentiated carcinoma, sarcomatoid undifferentiated carcinoma, and carcinosarcoma. Carcinosarcoma is a biphasic tumor with epithelial and mesenchymal components. Each component should account for at least 30% of the tumor. Furthermore, the epithelial and mesenchymal components should be immunophenotypically distinct.[1] The incidence of carcinosarcoma in the pancreas is not well established. The *Surveillance, Epidemiology, and End Results (SEER) program* documented fewer than 30 cases worldwide.[2] In a 6-year institutional review at Zhejiang University in China, only 9 carcinosarcomas were identified among 1,824 cases of pancreatic ductal adenocarcinoma.[3] The purpose of this paper is to show the gross, histology, and immunohistochemistry profile of a case of pancreatic carcinosarcoma with multiple nodal metastases and to review the literature.

CASE

A 47-year-old female presented with epigastric pain, followed by jaundice, rash, acholic stools, and tea-colored urine. On blood examination, the patient had elevated liver enzymes and bilirubin levels. Imaging revealed the following findings: biliary obstruction due to a pancreatic head mass showing primary neoplasm features, mass effect on the duodenum and distal common bile duct, several cystic lesions at the pancreatic body and tail, and prominent lymph nodes (Figure 1). Whipple's procedure and superior mesenteric vein anastomosis were performed, and the patient was discharged stable after 8 days.

The gross examination of the pancreatic head exhibited an ill-defined, soft to firm, tan-yellow, solid mass which measured 8 x 7 x 5 cm. The areas near the anterior surface revealed a solid, tan-pink to cream-white, homogenous, smooth, and firm surface. The mass was located near the pancreatic parenchymal margin, posterior resection

margin, and anterior pancreatic surface, and it enfolded the common bile duct. An ulceration measuring 3 x 1 cm ran from the mass to the duodenum. The common hepatic duct, duodenojejunal resection margins, and ampulla of Vater were far from the mass. There were increased numbers of peripancreatic lymph nodes (Figure 2).

The morphology revealed a biphasic tumor with pancreatic ductal adenocarcinoma comprising 70% of the mass. These are composed of irregularly shaped, cystically-dilated, tubular, cribriform, and haphazardly arranged glands. The neoplastic cells had large, moderately pleomorphic, hyperchromatic to vesicular nuclei, coarse

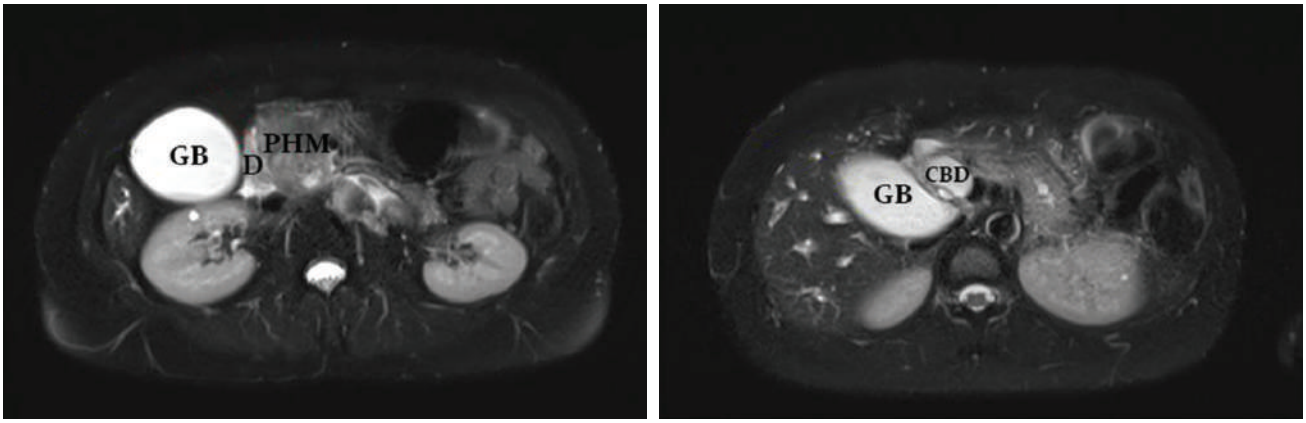

Figure 1. Magnetic Resonance Imaging of pancreatic carcinosarcoma cross-sectional view. CBD indicates common bile duct; D, duodenum; GB, gallbladder; PHM, pancreatic head mass.

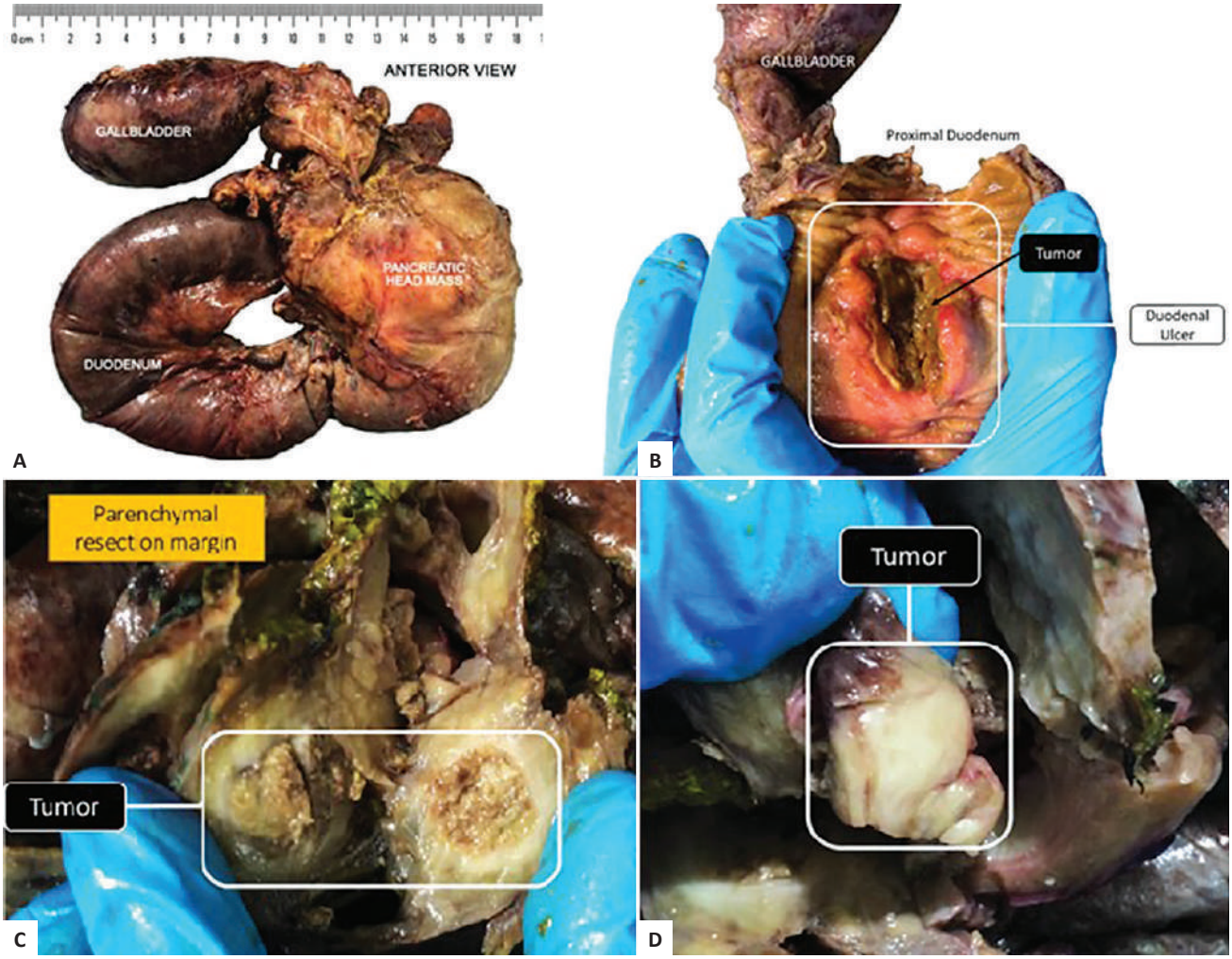

Figure 2. Gross appearance of pancreatic carcinosarcoma. **(A)** The entire pancreaticoduodenectomy specimen with a large pancreatic head mass attached to the duodenum. **(B)** Pancreatic carcinosarcoma is ulcerating the duodenum. **(C)** Cut surface of ill-defined and rough pancreatic carcinosarcoma. **(D)** Solid and homogenous cut surface near the anterior pancreatic surface.

chromatin, prominent nucleoli, and scant to ample eosinophilic cytoplasm. There is desmoplastic stroma and perineural invasion. The intermixed sarcomatous component accounted for 30% of the mass. These are composed of sheets of spindle-shaped cells characterized by large, pleomorphic, vesicular nuclei, coarse chromatin, inconspicuous nucleoli, and scant to abundant eosinophilic cytoplasm. The cells were arranged in a haphazard, herring-bone, and whirling pattern. There was a note of background basophilia, numerous mitotic figures (57 per 10 high power fields), and foci with necrosis and multinucleated giant cells (Figure 3).

The tumor invaded the duodenum, lymphatic vessels, and two peripancreatic lymph nodes. Intraductal papillary mucinous neoplasm with columnar epithelial lining was also evident.

Immunohistochemistry studies revealed a carcinomatous component with positive, strong Pancytokeratin expression and focal Vimentin expression. On the other hand, sarcomatous components expressed Vimentin while being negative for Pancytokeratin, and other mesenchymal markers, S-100, Smooth muscle actin (SMA), Desmin, and CD34. Ki-67 was high in both components (Figure 4 and Table 1). CD68 highlighted the multinucleated giant cells (GC). Hence, a diagnosis of Pancreatic Carcinosarcoma was made.

The management plan was implemented which included 12 cycles of gemcitabine and oxaliplatin. In the interim of chemotherapy, five months post-operatively, thickening and stranding of the mesentery, as well as multiple mesenteric nodularities, were seen on a triple-phase CT scan. Afterwards, eleven months post-operatively, the entire abdomen was filled with heterogeneous echoes with septations and loculations. After sixteen months, the following lesions were observed. A heterogeneously enhancing cystic mass in the pancreatic tail, as well as several calcified and non-calcified parenchymal nodules in both lungs, developed. The radiologic considerations were mesenteric carcinomatosis, tumor recurrence, and pulmonary metastases. However, no additional biopsies and cytology procedures were performed to confirm the diagnosis of metastasis. Instead, palliative care was selected as a treatment option.

Table 1. Immunohistochemistry studies done for the case

Immunohistochemical stain	Carcinomatous component	Sarcomatous component
Pancytokeratin	+	-
Vimentin	+ (Focal)	+
S-100	-	-
Desmin	-	-
SMA	-	-
CD34	-	-
Ki-67	50-60 %	60-70 %

DISCUSSION

Carcinosarcomas are rare biphasic tumors that frequently affect the female genital tract, but can occasionally occur

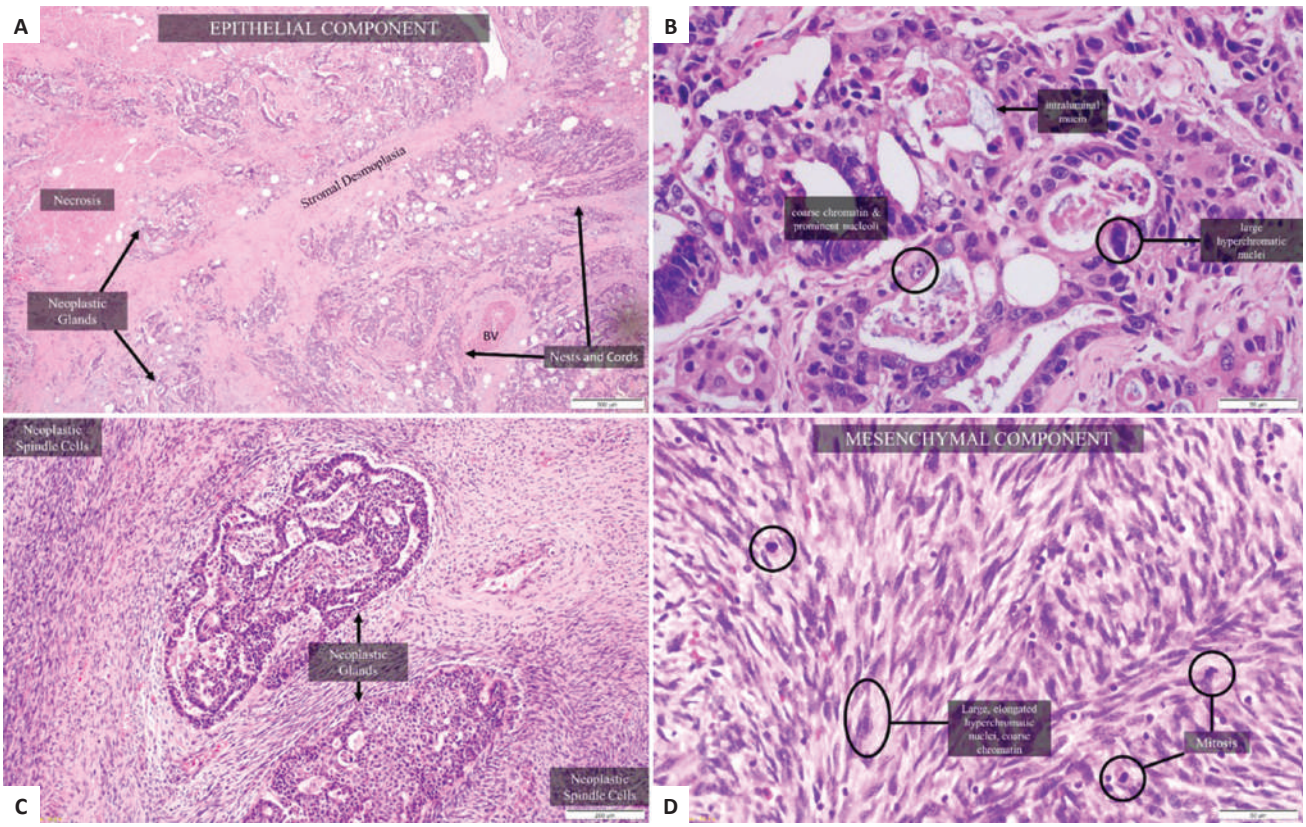

Figure 3. Hematoxylin and eosin-stained histologic sections of pancreatic carcinosarcoma. **(A)** Scanner view and **(B)** high power magnification of the carcinomatous component (CC) showing ductal adenocarcinoma (x40) and (x400). **(C)** Mosaic pattern of CC and sarcomatous component (SC) (x100). **(D)** High power magnification of SC showing neoplastic spindle cells with mitotic figures (x400).

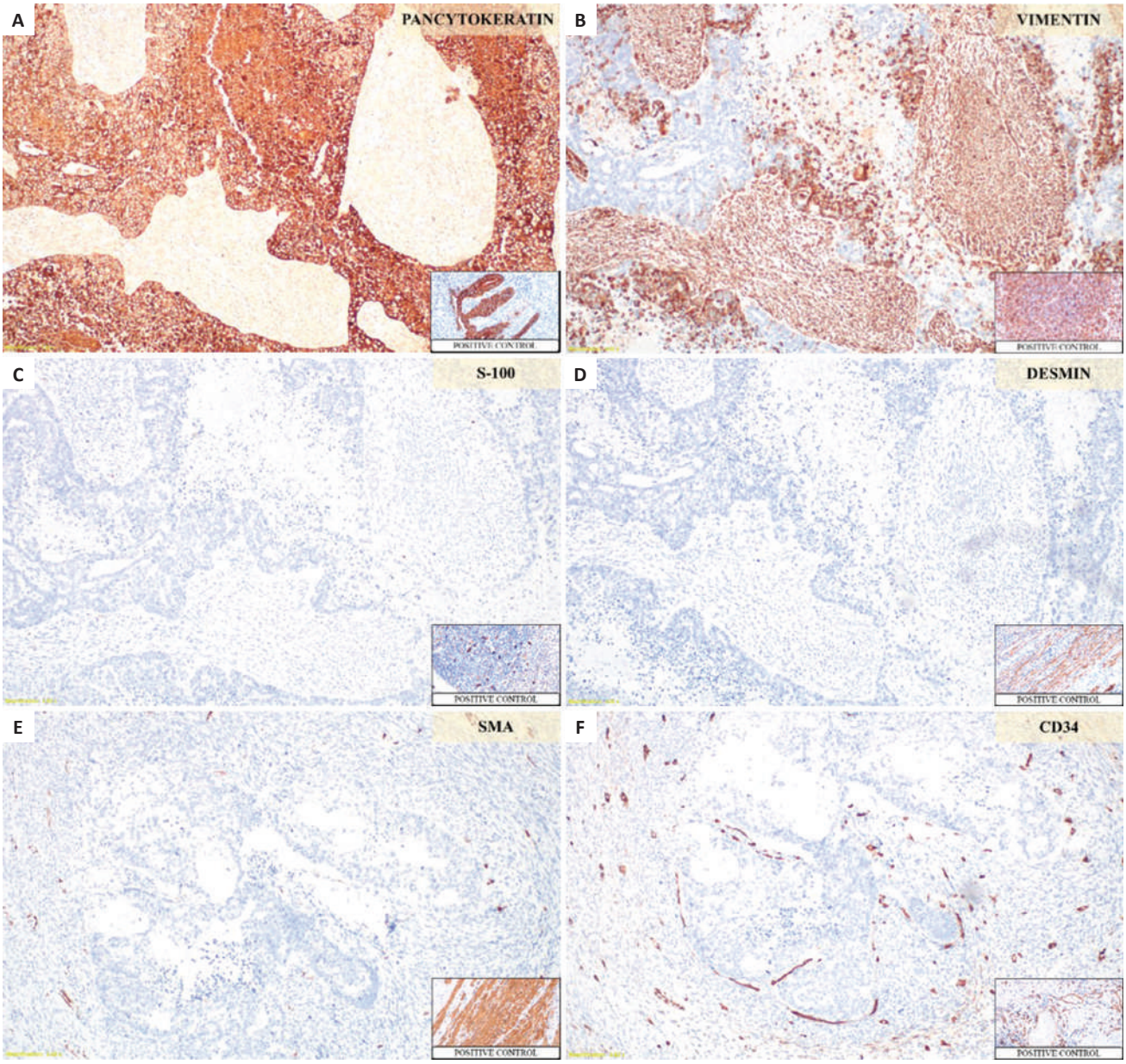

Figure 4. Immunohistochemical findings of pancreatic carcinosarcoma. **(A)** Cytokeratin highlights the carcinomatous component (CC) and negative staining of the sarcomatous component (SC) (x100). **(B)** Vimentin highlighting the SC and non-specific expression in the CC (x100). **(C)**, **(D)**, **(E)**, and **(F)** show negative staining on both CC and CC using S-100, Desmin, Smooth muscle actin (SMA), and CD34, respectively (x100).

in the prostate, urinary tract, head and neck, and gastrointestinal system, including the pancreas. The histogenesis of these tumors is still undefined. The following theories are considered: 1) Collision: wherein two independent malignancies are colliding in one organ, 2) Combination: both components are derived from an early mutation of a single multipotent cell, 3) Conversion: explaining that epithelial tumors undergo metaplastic transformation into mesenchymal tumors; and 4) Composition: describing that the mesenchymal component is a stromal response to the epithelial tumor.[4]

Genomic analysis of microdissected tissues showed consistent KRAS mutations on both carcinomatous and sarcomatous components, with single-nucleotide mutations, c.35G>A and c.35G>T at codon 12 and codon 34.[3-8] KRAS Q61H and TP53 Q100X mutations are also recently discovered to be present in both components.[3] In a case series by Ruess, molecular analysis of these tumors revealed that pancreatic carcinosarcomas are of monoclonal origin. KRAS mutation is a distinctive driver mutation in pancreatic ductal adenocarcinoma. Because of the similarities in KRAS mutations, it is postulated that pancreatic carcinosarcoma originated from pancreatic ductal adenocarcinoma. As explained by the authors, these findings may support the conversion theory of epithelial-mesenchymal transition (EMT). This would also justify WHO's classification of pancreatic carcinosarcoma as a subtype of pancreatic ductal adenocarcinoma.[4] Moreover, Nakano supported the idea that KRAS mutation in

codon 12 elicited the adenocarcinoma, and mutation in codon 34 induced the sarcomatous transformation.[8] Unfortunately, limited cases had molecular studies to verify the definite pathogenesis.

The majority of the cases documented worldwide had Ductal Adenocarcinoma (76%), few Mucinous Cystadenocarcinoma (13%), Adenosquamous Carcinoma (3%), Intraductal Papillary Mucinous Carcinoma with Invasive Adenocarcinoma (3%) and Adenocarcinoma with Squamous areas (3%) for the epithelial component. On the other hand, complex undifferentiated mesenchymal components are reported. They are described as spindled cells without explicit comment on their malignancy (27%), others are referred to be malignant but without definite diagnosis or differentiation (17%). One case is described as a High-Grade Spindle Cell with focal Chondrosarcoma and myogenic differentiation (3%). In several cases, a specific diagnosis is rendered: Malignant fibrous histiocytoma/ Undifferentiated Pleomorphic Sarcoma / Pleomorphic Spindle Cell Sarcoma (MFH/UPS/PSCS) (27%), MFH/UPS with Osteosarcoma (13%), Leiomyosarcoma (3%), Osteosarcoma (7%) and Embryonal Rhabdomyosarcoma (3%) (Table 2).[1-15]

Immunohistochemistry with Pancytokeratin and Vimentin demonstrated the distinction between the epithelial and mesenchymal components. The other epithelial markers with positive expression are CAM 5.2, Epithelial Membrane Antigen (EMA), Carcinoembryonic Antigen (CEA), Cytokeratin 7 (CK7), and Cytokeratin 19 (CK19). Contrary to most, the given case showed focal expression to Vimentin. Reported cases also exhibited focal expression to Smooth Muscle Actin (SMA) and Desmin in the mesenchymal component.[5] Ki-67 as a proliferation index ranged from 2-60% and 2-75% in the carcinomatous and sarcomatous components, respectively. High-grade sarcoma is identified given the necrosis, high mitoses, and nuclear atypia. Common involvement of the duodenum, peripancreatic soft tissues, lymph nodes, and liver metastasis were identified.[9]

Treatment options included surgery with or without the addition of chemotherapy. Although data on better prognosis with the benefit of chemotherapy is inadequate, analysis in a small population showed significance. Likewise, a locally advanced or metastatic pancreatic cancer warrants the use of adjunct systemic therapy. FOLFIRINOX is the recommended treatment regimen in the usual Pancreatic Ductal Adenocarcinoma, and Gemcitabine is an alternative therapy for patients with poor performance status and unable to tolerate toxic side effects.[16] Given the limited reported cases of Pancreatic Carcinosarcomas, no standard treatment has been established. The possible option is to offer Gemcitabine, as it may also target the sarcomatous component.[17] However, EMT was associated to Gemcitabine resistance. Wang provided molecular evidence of this association. The study demonstrated that Notch-2 and its ligand, Jagged-1, are upregulated in Gemcitabine-resistant cells linking it to the epithelial-mesenchymal transition phenotype.[18] The overall survival has a mean of 15 months and 14 days in 25 cases (Table 2).[1-15]

CONCLUSION

A case of pancreatic carcinosarcoma with extension to the duodenum and nodal metastases is presented. Pancreatic ductal adenocarcinoma and High-grade sarcoma are documented with immunohistochemical studies. Characterization of these tumors is essential as it influences treatment plan, behavior and prognosis.

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ETHICAL CONSIDERATION

Patient consent was obtained before the submission of the manuscript.

STATEMENT OF AUTHORSHIP

Both authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

Both authors declared no conflict of interest.

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None.

Table 2. Published case reports of carcinosarcoma

	Author	Age/Sex	Symptom	Localization and extent	Size (mm)	CC	IHC on CC									SC	IHC on SC									LN	KRAS and TP53 mutation	Teatment	Survival (months) Recurrence
							CK	Vimentin	S-100	Desmin	Myogenin	SMA	CD34	CD68	Ki-67		CK	Vimentin	S-100	Desmin	Myogenin	SMA	CD34	CD68	Ki-67				
1	***Our case***	47/F	Abdominal pain, jaundice	Head to duodenum	80	PDAC	+	+F	-	-	ND	-	-	+GC	50-60%	High Grade Sarcoma	-	+	-	-	ND	-	-	+GC	60-70%	2/35		S/p pylorus preserving whipples, end to end anastomosis of SMV S/p chemotherapy (GemOx) x 12 cycles	> 10 Peritoneal carcinomatosis, Stage IV
2	***Li, et al.*** [3]	60/M	Abdominal pain	Tail	75	PDAC	+	-	ND	ND	ND	ND	ND	ND	ND	Undifferentiated spindle cells (7/9); MFH and Osteosarcoma (2/9)	-	+	ND	ND	ND	ND	ND	ND	ND	0/19		Total pancreatectomy	2 Liver at 1 month
3		66/M	Painless jaundice	Head	40		+	-	ND	ND	ND	ND	ND	ND	ND		-	+	ND	ND	ND	ND	ND	ND	ND	0/27		Whipple, Gemcitabine plus Nab-paclitaxel	11 Liver at 3 months
4		69/M	Incidental finding	Head	25		+	-	ND	ND	ND	ND	ND	ND	ND		-	+	ND	ND	ND	ND	ND	ND	ND	3/33		Whipple, FOLFIRINOX	19 LN at 13 months
5		56/F	RUQ pain, jaundice	Head	100		+	-	ND	ND	ND	ND	ND	ND	ND		-	+	ND	ND	ND	ND	ND	ND	ND	5/30		Total pancreatectomy	39 Liver at 3 months
6		51/F	Epigastric pain, jaundice	Head	45		+	-	ND	ND	ND	ND	ND	ND	ND		-	+	ND	ND	ND	ND	ND	ND	ND	0/22		Whipple, Gemcitabine	17 Liver at 10 months
7		48/F	Epigastric pain	Tail	80		+	-	ND	ND	ND	ND	ND	ND	ND		-	+	ND	ND	ND	ND	ND	ND	ND	2/24		Total pancreatectomy	NA NA
8		67/F	Epigastric pain	Head	64		+	-	ND	ND	ND	ND	ND	ND	ND		-	+	ND	ND	ND	ND	ND	ND	ND	6/6		Whipple	4 Liver at 3 months
9		59/M	Abdominal pain	Head	53		+	-	ND	ND	ND	ND	ND	ND	ND		-	+	ND	ND	ND	ND	ND	ND	ND	3/21		Whipple	NA NA
10		49/M	Abdominal pain	Body	80		+	-	ND	ND	ND	ND	ND	ND	ND		-	+	ND	ND	ND	ND	ND	ND	ND	38/42		Distal pancreatectomy	NA NA
11	***Liu, et al.*** [10]	66/F	Abdominal pain, nausea, jaundice	Head	50	PDAC	+	-	-	-	ND	-	ND	ND	30%	Undifferentiated Sarcoma	-	+	-	-	ND	-	ND	ND	20%	NA		Cholecystectomy with bile ductjejunum, Roux-en-Y anastomosis, radioactive seed implantation	> 12 NA
12	***Still, et al.*** [2]	59/F	Abdominal pain, nausea and emesis	Head to duodenum, main pancreatic duct and intrapancreatic bile duct	25	Moderately Differentiated Adenocarcinoma	+ F	NA	NA	NA	NA	NA	NA	NA	NA	High Grade Spindle Cell with focal chondrosarcoma and myogenic differentiation	NA	NA	NA	+F	-	NA	NA	NA	NA	2/28		Pancreaticoduodenectomy, Neoadjuvant trial (6 cycles of FOLFIRINOX-Oxaliplatin, Irinotecan, Fluorouracil and Leucovorin), Chemotherapy with gemcitabine and paclitaxel	13 NA
13	***Salibay, et al.*** [11]	49/M	Abdominal pain	Body/tail	NA	Moderately Differentiated Adenocarcinoma	+	ND	ND	-	-	-	ND	ND	50%	High Grade Spindle Cell Sarcoma	-	ND	ND	+F	-	+F	ND	ND	50%	1/1		Total abdominal hysterectomy with right salpingo-oophorectomy and exploration of the pancreatic mass, Pancreatic biopsy, hepatodochal lymphadenectomy, gemcitabine and docetaxel with no response, followed by ifosfamide and Adriamycin with progression	10 NA
14	***Ruess, et al.*** [4]	73/F	Epigastric pain	Head to periadipose tissue	42	PDAC	+	-	-	ND	ND	ND	ND	ND	15%	Malignant mesenchymal component with undifferentiated spindle-shaped cells	+F	+	+F	ND	ND	ND	ND	ND	50-60%	0/17	exon 2 of KRAS gene with c.35G>A substitution leading to a p.G12D mutation on CC and SC	Pancreaticoduodenectomy	4 NA
15	***Jia, et al.*** [9]	44/F	Abdominal pain and jaundice	Head to peripadipose tissues	30	Moderately Differentiated Adenocarcinoma	ND	-	ND	ND	ND	ND	ND	ND	ND	Osteosarcoma	-	+	ND	ND	ND	ND	ND	ND	ND	3/18		Whipple, gemcitabine and raltitrexed	>31 NA
16	***Bai, et al.*** [5]	71/M	General symptoms of abdominal pain, jaundice, nausea, vominting, anemia, weight loss or incidental finding	Head	50	PDAC	+	-	ND	-	ND	-	ND	-	35 %	MFH/UPS + Osteosarcoma, focally	+F	+	ND	-	ND	ND	ND	-	35%	0/20	c.35G>A on both components	Pancreaticoduodenectomy, Splenectomy, Postoperative chemotherapy	11 Tumor recurrence or metastasis at 9 months
17		49/M		Head	50	PDAC	+	-	ND	-	ND	-	ND	-	15%	Osteosarcoma + MFH/ UPS	-	+	ND	-	ND	ND	ND	GC+	20%	0/1	c.35G>A on both components	Pancreaticoduodenectomy, Postoperative chemotherapy	39 NA
18		74/M		Head	80	PDAC	+	-	ND	-	-	-	ND	-	50%	PSCS	-	+	ND	-	-	-	ND	F+	60%	0/5	c.35G>T on both components	Pancreaticoduodenectomy	10 Tumor recurrence or metastasis at 9 months
19		38/M		Body/tail	160	MCAC	+	-	ND	-	ND	-	ND	-	25%	PSCS	-	+	ND	-	ND	ND	ND	GC+	20%	NA	KRAS c.35G>T on both components	Distal Pancreatectomy, Splenectomy, Gamma kinfe Radiosurgery, Postoperative chemotherapy	NA Tumor recurrence or metastasis at 26 months
20		67/M		Head	35	PDAC	+	-	ND	-	-	-	ND	ND	ND	ERMS	-	+	ND	+P	-	-	ND	ND	ND	NA		Pancreaticoduodenectomy, Postoperative chemotherapy	47 NA
21		60/F		Body/tail	75	MCAC	+	-	ND	-	ND	ND	ND	-	ND	MFH/UPS	-	+	ND	ND	ND	ND	ND	+GC	ND	NA		Pancreaticoduodenectomy	15 Tumor recurrence or metastasis at 12 months
22		75/F		Head	45	PDAC	+	-	ND	-	ND	-	ND	-	ND	PSCS	-	+	ND	-	ND	ND	ND	-	ND	0/10		Pancreaticoduodenectomy, Traditional Chinese medical herbal treatment	29 NA
23		59/M		Body/tail	55	PDAC	+	-	ND	-	ND	ND	ND	ND	20%	MFH/UPS	-	+	ND	ND	ND	ND	ND	ND	20%	NA	KRAS c.35G>A on both components	Pancreaticoduodenectomy, Splenectomy, Postoperative chemotherapy	17 NA
24	***Shi, et al.*** [12]	74/F	Incidental finding	Tail	50	MCAC	+	-	ND	ND	ND	ND	ND	ND	ND	Malignant spindle cells	-	+	ND	ND	ND	ND	ND	ND	ND	NA		Distal pancreatectomy, Splenectomy	NA NA
25	***Cicy, et al.*** [13]	50/M	Abdominal pain	Head	60	Adenocarcinoma	+	-	ND	ND	ND	ND	ND	ND	ND	PSCS	ND	+	-	-	ND	+	-	ND	Low	0/9		Whipple pancreaticoduodenectomy	> 47 days NA
26	***Oymaci, et al.*** [14]	48/M	Epigastric pain	Head to duodenum and periadipose tissue	35	PDAC	+	-	-	-	-	-	-	-	2%	High grade pleomorphic spindle cells	-	+	-	-	-	+F	-	-	2%	2/16		Extended pancreaticoduodenectomy	20 days NA
27	***Palaniappan, et al.*** [15]	46/M	Jaundice	Head to duodenum	30	Adenosquamous Carcinoma	+	-	-	-	ND	-	ND	-	High	Leiomyosarcoma	-	+	-	-	ND	+	ND	NS	High	0/5		Pancreatoduodenectomy, Gemcitabine	>28 NA
28	***Kim, et al.*** [6]	48/M	Incidental finding	Tail	70	MCAC	+	+ F	-	-	-	-	-	-	ND	MFH/UPS	-	+	-	-	-	-	-	-	ND	4/15	G to A transition at codon 12 of K-ras gene	Distal pancreatectomy with splenectomy and colonic segmental resection, Gemcitabine	4 Liver and peritoneum
29	***Okamura, et al.*** [7]	64/F	Incidental finding	Tail	35	IPMC with Invasive adenocarcinoma	+	ND	ND	ND	ND	ND	ND	+ GC	ND	Osteosarcoma with heterologous components	ND	+	ND	ND	ND	ND	ND	ND	ND	NA	KRAS (G35A mutation in exon 1) abd TP53 (T337A mutation in exon 4) in both components	Distal pancreatectomy, Gemcitabine	>12 NA
30	***Nakano, et al.*** [8]	82/F	Hypochondralgia, jaundice	Head to duodenum and transverse mesocolon	180	Adenocarcinoma with focal squamous areas	+	-	ND	-	-	-	ND	ND	ND	PSCS	+	+	-	-	ND	-	ND	ND	ND	NA	G to A transition at codon 12 and 34 on both components	Radical pancreatoduodenectomy with partial resection of the transverse colon	13 days NA

CC, Carcinomatous Component; CK, Cytokeratin; F, Focal; GC, Giant Cells; IHC, Immunohistochemical Stain; IPMC, Intraductal Papillary Mucinous Carcinoma; MCAC, Mucinous Cystadenocarcinoma; MFH, Malignant Fibrous Histiocytoma; MFH/UPS, Malignant Fibrous Histiocytoma / Undifferentiated Pleomorphic Sarcoma; NA, Not available; ND, Not determined; NS, Non-specific; PDAC, Pancreatic Ductal Adenocarcinoma; PSCS, Pleomorphic Spindle Cell Sarcoma; SC, Sarcomatous Component; SMA, Smooth Muscle Actin

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REGISTRATION FEES

2nd Announcement

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PHILIPPINE JOURNAL OF PATHOLOGY

Instructions to Authors

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

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

ARTICLE SECTIONS

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

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

Original articles

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

Reviews

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

Case Reports

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

Feature articles

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

Autopsy Vault

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

Images in Pathology

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

Brief Communications

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

Editorials

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

Letters to the Editor

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

Special Announcements

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

COVER LETTER

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

PJP AUTHOR FORM

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

GENERAL FORMATTING GUIDELINES

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

SPECIFIC FORMATTING GUIDELINES

Title and Authors

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

Abstract

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

Keywords

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

Text

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

References

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

One to Six Authors

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

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

More than Six Authors

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

Authors Representing a Group

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

Book

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

World Wide Web

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

Tables

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

Figures and Graphs

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

Illustrations and Photographs

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

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

EDITORIAL PROCESS (Figure 1)

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

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The Philippine Journal of Pathology
2nd Floor, Laboratory Research Division
Research Institute for Tropical Medicine
Filinvest Corporate City
Alabang, Muntinlupa City 1781
Editor-in-Chief: **Amado O. Tandoc III, MD, FPSP**
Telefax number: (+632) 88097120
E-mail: philippinepathologyjournal@gmail.com
Website: http://philippinejournalofpathology.org

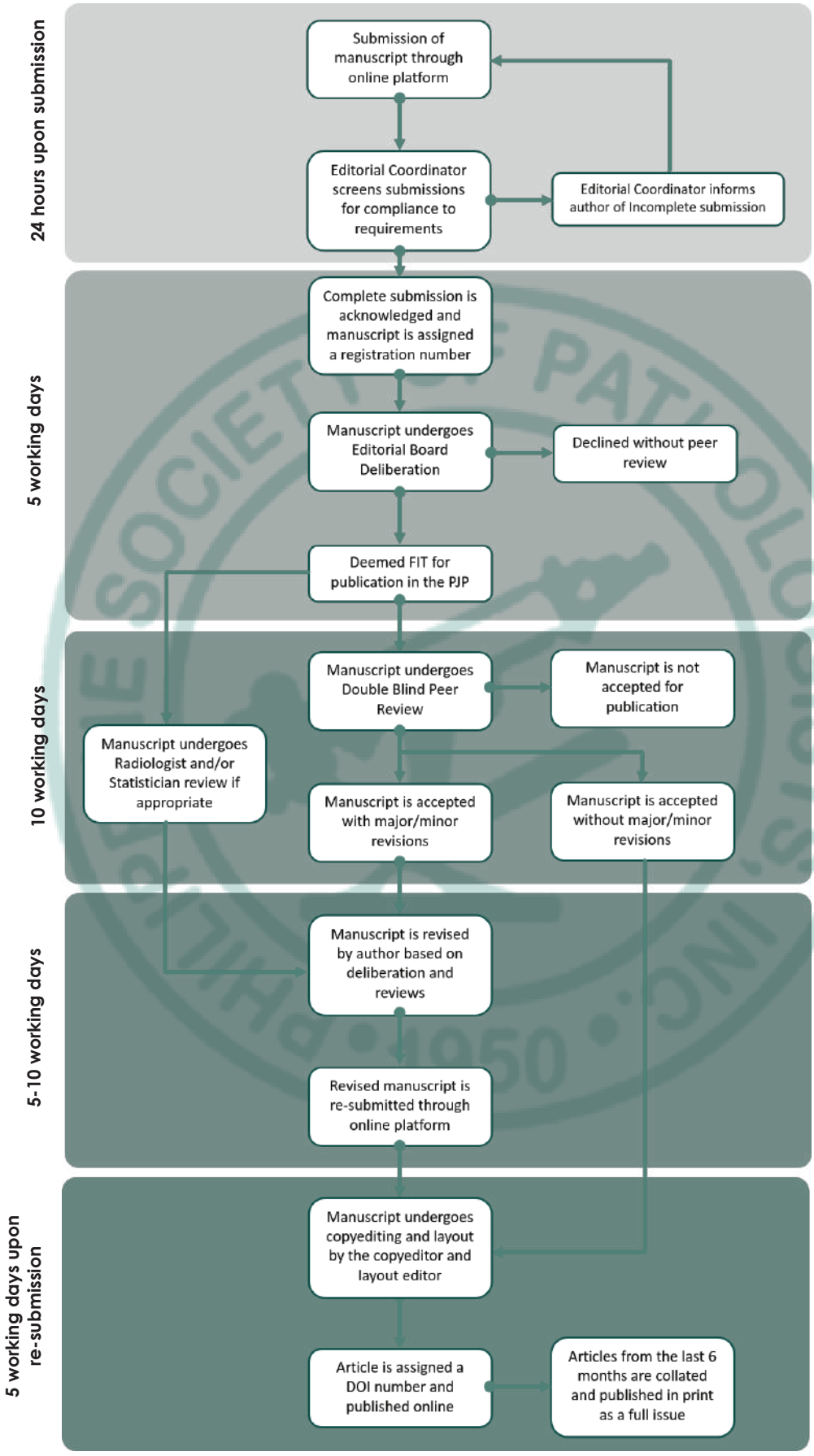

Figure 1. Editorial Process Flow.

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

COMPLETE TITLE OF MANUSCRIPT

_____

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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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AUTHOR DISCLOSURE OF CONFLICTS OF INTEREST

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

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

AUTHOR NAME	RELATIONSHIP	MANUFACTURER/ SUPPLIER/ COMPANY

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

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

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ICMJE INTERNATIONAL COMMITTEE of MEDICAL JOURNAL EDITORS

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

Updated May 2022

I. About the Recommendations

A. Purpose of the Recommendations

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

B. Who Should Use the Recommendations?

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

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

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

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

C. History of the Recommendations

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

II. Roles and Responsibilities of Authors, Contributors, Reviewers, Editors, Publishers, and Owners

A. Defining the Role of Authors and Contributors

1. Why Authorship Matters

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

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

2. Who Is an Author?

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

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

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

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

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

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

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

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

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

3. Non-Author Contributors

Contributors who meet fewer than all 4 of the above criteria for authorship should not be listed as authors, but they should be acknowledged. Examples of activities that alone (without other contributions) do not qualify a contributor for authorship are acquisition of funding; general supervision of a research group or general administrative support; and writing assistance, technical editing, language editing, and proofreading. Those whose contributions do not justify authorship may be acknowledged individually or together as a group under a single heading (e.g., "Clinical Investigators" or "Participating Investigators"), and their contributions should be specified (e.g., "served as scientific advisors," "critically reviewed the study proposal," "collected data," "provided and cared for study patients," "participated in writing or technical editing of the manuscript").

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

B. Disclosure of Financial and Non-Financial Relationships and Activities, and Conflicts of Interest

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

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

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

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

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

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

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

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 and disclose their relationships and activities when fulfilling their roles in the process of article review and publication.

a. Authors

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

b. Peer Reviewers

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

c. Editors and Journal Staff

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

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

2. Reporting Relationships and Activities

Articles should be published with statements or supporting documents, such as the ICMJE Disclosure Form, declaring:

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

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

C. Responsibilities in the Submission and Peer-Review Process

1. Authors

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

a. Predatory or Pseudo-Journals

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

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

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

2. Journals

a. Confidentiality

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

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

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

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

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

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

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

b. Timeliness

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

c. Peer Review

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

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

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

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

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

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

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

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

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

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

d. Integrity

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

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

e. Diversity and Inclusion

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

f. Journal Metrics

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

3. Peer Reviewers

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

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

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

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

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

D. Journal Owners and Editorial Freedom

1. Journal Owners

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

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

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

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

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

2. Editorial Freedom

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

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

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

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

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

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

E. Protection of Research Participants

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

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

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

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

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

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

A. Corrections, Retractions, Republications, and Version Control

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

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

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

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

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

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

B. Scientific Misconduct, Expressions of Concern, and Retraction

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

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

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

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

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

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

C. Copyright

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

D. Overlapping Publications

1. Duplicate Submission

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

2. Duplicate and Prior Publication

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

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

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

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

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

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

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

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

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

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

See COPE flowcharts for further guidance on handling duplicate publication.

3. Preprints

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

a. Choosing a Preprint Archive

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

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

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

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

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

c. Referencing Preprints in Submitted Manuscripts

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

4. Acceptable Secondary Publication

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

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

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

5. Manuscripts Based on the Same Database

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

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

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

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

E. Correspondence

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

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

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

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

F. Fees

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

G. Supplements, Theme Issues, and Special Series

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

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

H. Sponsorship or Partnership

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

I. Electronic Publishing

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

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

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

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

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

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

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

J. Advertising

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

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

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

K. Journals and the Media

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

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

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

- Editors can foster the orderly transmission of medical information from researchers, through peer-reviewed journals, to the public. This can be accomplished by an agreement with authors that they will not publicize their work while their manuscript is under consideration or awaiting publication and an agreement with the media that they will not release stories before publication of the original research in the journal, in return for which the journal will cooperate with them in preparing accurate stories by issuing, for example, a press release.
- Editors need to keep in mind that an embargo system works on the honor system—no formal enforcement or policing mechanism exists. The decision of a significant number of media outlets or biomedical journals not to respect the embargo system would lead to its rapid dissolution.
- Notwithstanding authors' belief in their work, very little medical research has such clear and urgently important clinical implications for the public's health that the news must be released before full publication in a journal. When such exceptional circumstances occur, the appropriate authorities responsible for public health should decide whether to disseminate information to physicians and the media in advance and should be responsible for this decision. If the author and the appropriate authorities wish to have a manuscript considered by a particular journal, the editor should be consulted before any public release. If editors acknowledge the need for immediate release, they should waive their policies limiting prepublication publicity.
- Policies designed to limit prepublication publicity should not apply to accounts in the media of presentations at scientific meetings or to the abstracts from

these meetings (see Duplicate Publication). Researchers who present their work at a scientific meeting should feel free to discuss their presentations with reporters but should be discouraged from offering more detail about their study than was presented in the talk, or should consider how giving such detail might diminish the priority journal editors assign to their work (see Duplicate Publication).
- When an article is close to being published, editors or journal staff should help the media prepare accurate reports by providing news releases, answering questions, supplying advance copies of the article, or referring reporters to appropriate experts. This assistance should be contingent on the media's cooperation in timing the release of a story to coincide with publication of the article.

L. Clinical Trials

1. Registration

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

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

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

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

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

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

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

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

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

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

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

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

2. Data Sharing

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

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

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

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

IV. Manuscript Preparation and Submission

A. Preparing a Manuscript for Submission to a Medical Journal

1. General Principles

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

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

2. Reporting Guidelines

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

3. Manuscript Sections

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

a. Title Page

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

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

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

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

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

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

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

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

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

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

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

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

b. Abstract

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

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

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

c. Introduction

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

d. Methods

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

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

i. Selection and Description of Participants

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

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

ii. Technical Information

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

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

iii. Statistics

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

e. Results

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

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

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

f. Discussion

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

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

g. References

i. General Considerations

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

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

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

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

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

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

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

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

ii. Style and Format

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

h. Tables

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

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

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

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

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

i. Illustrations (Figures)

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.

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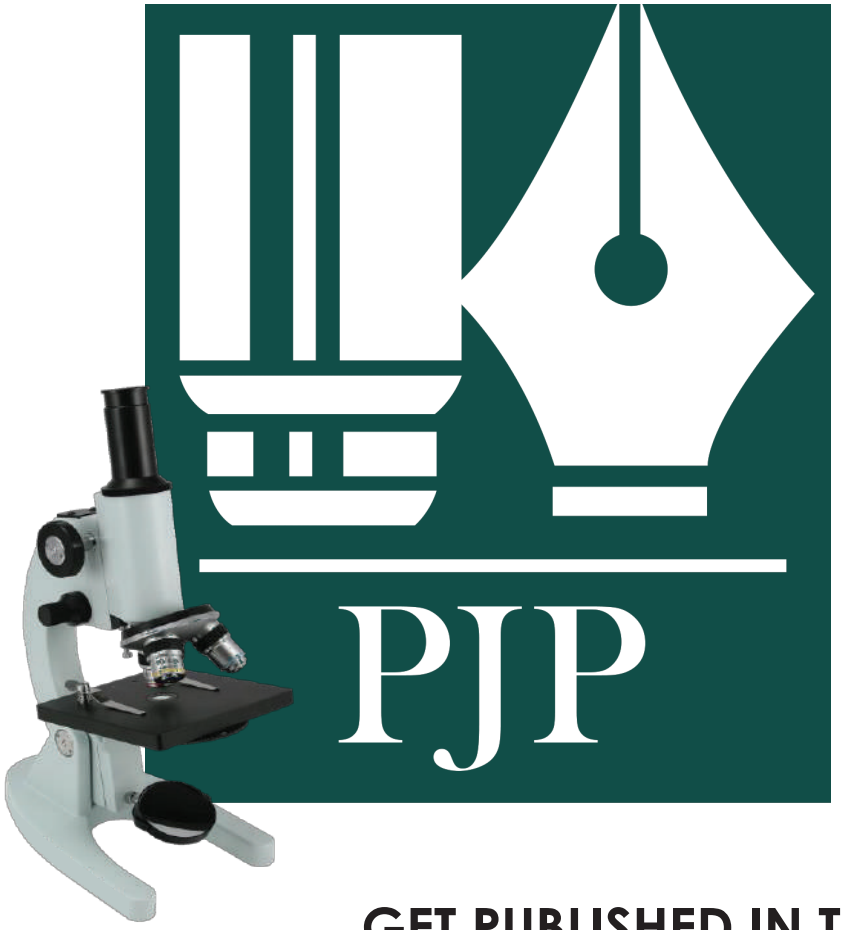

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