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OPEN ACCESS

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PHILIPPINE JOURNAL OF PATHOLOGY

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.

Warm Greetings!

Welcome to the December 2021 issue of the Philippine Journal of Pathology. Congratulations to the editorial team of the PJP and the PSP Board of Governors for their untiring efforts in coming up with this issue in spite of the COVID-19 pandemic.

The PJP editorial staff, the Board of Governors, and the PSP members worked harder to adapt and cope with this pandemic as limitation in the mobility, implementation of on and off lockdowns and the strict adherence to the health protocols are being implemented by our government. These have some toll in coming up with quality scholarly work but the PJP editorial staff and the PSP Board of Governors and its members continue to come up with this succeeding issue of the PJP. Their implacable support, commitment, and dedication made all this possible.

We are happy that you are joining us as readers and hope that you will also support us by submitting your scientific study and research paper for publication in PJP.

Let us hope for many more issues. More power to the Philippine Society of Pathologists, Inc. and the Philippine Journal of Pathology.

Wishing you all a Merry Christmas and a Prosperous 2022.

Roberto D. Padua Jr., MD, FPSP, MHA
President, Philippine Society of Pathologists, Inc.

Healthy Pilipinas

Sa Paskong darating
Santa Claus nyo'y ako rin
'Pagkat kayong lahat
ay naging masunurin
Dadalhan ko kayo
ng **mansanas** at **ubas**
May **kendi** at **tsokolate**,
peras, **kastanyas** na marami

Go lang sa Cravings! Pero wag mag-over eat!

Wag busugin ang sarili sa mga sweets!

Ligtas Christmas sa Healthy Pilipinas

Bakuna | Airflow | Mask | Hugas | Iwas

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Five Years of PJP

Quietly, while the world rages with the pandemic of our lifetime, the Philippine Journal of Pathology (PJP) celebrated its fifth year of publication. I find it appropriate to pause for a while and ask "what have we achieved over the past five years?"

First, we were able to professionalize the operations of the journal, through editorial guidelines at par with international standards of ethical publication, operating procedures that standardized the process of submission, peer review, and publication, and a virtual office and secretariat hired by the Society to man the day-to-day tasks of the journal.

Second, we have attained a sense of sustainability, and this is through the financial support of the Philippine Society of Pathologists (PSP). We are fortunate, for the leadership of the Society over the last five years have seen and continues to see the academic value of the journal. The Society's support extended beyond finances, as, over time, we have been able to engage more pathologists to share their time and expertise to peer review for the journal. These are invaluable inputs to our pursuit of academic excellence and scholarship, as peer review is the backbone of our publication.

Third, efforts to improve visibility, and accessibility are starting to pay off. CrossRef digital object identifiers ensured link permanence, improved searchability, and directed traffic to our journal website. We have been included in the ASEAN Citation Index. Our articles from 2016 to present are now searchable in the web. Some of our articles have also been cited by other researchers. This can only be possible if our articles can be "found" in the depths of the world wide web. Try looking for an article published in PJP in Google Scholar and you will find it. Moreover, our decision to go open access has translated to views and downloads of our manuscripts. Our statistics are modest, but over time, I predict that we will see better numbers in terms of views and citations. The dream of having our own "impact factor" may not be too farfetched after all.

Fourth, by publishing continuously and regularly for the last five years, we have somehow uplifted the research and publication awareness of our pathologists. Again, this is not an easy thing to do, from the conception and completion of the research, to the analysis and manuscript writing, and finally to hurdling the peer review and coming back with acceptable revisions. At the end of it, I am happy to see our younger pathologists proudly including publication in our journal as some form of academic accomplishment and sharing them in social media.

Fifth, as a Society, we have successfully relaunched an online journal management platform that made issues predictable, that is to say, regularly came out as scheduled every six months. This is no easy feat. One of the greatest challenges for local journals, based from shared experiences with other editors, is regularity and frequency. This is the reason why some local medical journals fail to come out with issues after some time and would need to be resuscitated and resurrected after several years in hiatus.

As a form of "paying it forward," I was offered to be an editorial consultant for the rebirth of the Scientific Proceedings of the Lung Center of the Philippines. Guided by the experiences I have gained from an international publication, which we used to re-establish the PJP, we guided them in turn. In a few months working with their dedicated and motivated editorial team, the journal was successfully relaunched this December. By providing this assistance, we are also advocating the Society's commitment to ethical standards of publication. It makes me proud when non-pathologists are impressed by the PJP and use it to improve their own publication.

Let me end this by thanking our authors, our reviewers, our readers, and the Society. For 2022 and beyond, we not only hope to continue, but we shall also aim to be included in the Directory of Open Access Journals, and WHO Western Pacific Region Index Medicus. We hope to increase the number of our articles and contribute to improvements in the practice of pathology in the country.

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

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Clinical Laboratory Regulation in the Philippines – Are we doing it right?

I write this article from my perspective of 34 years of practice in the Philippines having arrived in 1987 from my training in the USA. In my six years of training first in Anatomic and Clinical Pathology then in Immunopathology fellowship, I have witnessed the workings of laboratory regulation and accreditation in that country and was in culture shock when I started my practice here.

Laboratory automation was well underway in the Western hemisphere during my training. Hematology and Chemistry at the forefront of this technological revolution. But other disciplines were starting to catch up. Locally, few tertiary labs were starting to automate. I was, of course, excited to begin revolutionizing the practice of Clinical Pathology.

I had not counted on the backward state of laboratory regulation locally. Under the Department of Health regulatory office, Bureau of Research and Laboratories (BRL), inspection visits to the tertiary labs that I was connected with (one private and one government hospital lab), the inspectors were engineers who came with a list of requirements that included asking for Benedict's reagent even though we were already using automated chemistry methods. This stopped only ten years ago, thankfully when the BRL was renamed Bureau of Health Facilities Services (BHFS), now the Health Facilities Services Regulatory Bureau (HFSRB).

The other issue is that there tends to be over regulation. Elsewhere, the emphasis is on accurate laboratory test results which is achieved through good quality assurance programs and performing satisfactorily on semi-annual proficiency testing. On-site inspections are geared towards these goals as well as the right physical set-up necessary to achieve these results. Our local regulatory agency tends to aim at including training programs which normally are the province of professional societies.

An example is HIV testing. When it was first proposed, you have to apply for a separate HIV testing license apart from the license to operate a clinical laboratory. To get the license, a medical technologist must have to attend and pass an HIV Proficiency training course which includes counselling patients. The course was being given by Research Institute of Tropical Medicine (RITM) on a limited basis, thus, only few medical technologists were licensed, severely limiting the number of labs that can perform HIV testing.

I had argued against these regulations. The HIV antibody (and subsequently the HIV antigen test) test was a routine serologic procedure that can be done on a manual or automated method either by EIA or ECLIA. To run these tests, one needs only be conversant with the requirements for running the samples like any other serologic examination. Secondly, the ruling ignores the role of the pathologist who is responsible for the test result ultimately. Third, the medical technologist is not the best person to be counselling patients. It is the attending physician who orders the test. Putting the burden of counselling on the med tech is a disservice to the patient and takes the med tech away from his/her main job, which is to run the tests.

We now have a situation where the regulatory agency prescribes training which is often unavailable but labs are expected to comply. The offshoot is that the few medical technologists who had the training became highly sought after to be able to put up HIV testing in clinical labs, leading to a black market where these techs offer their licenses for a fee.

The requirement for pre- and post-test counselling actually deterred patients from testing since it attaches a stigma to the disease which was what the regulation was supposed to remove. In other countries, one can simply walk into a lab and ask for the HIV test without any additional requirements. Some even offer anonymous testing.

All these factors: limited testing, counselling requirements and the attendant stigma attached may have led to the explosive increase in HIV cases we are seeing now in the Philippines.

Fast forward to today and not much has changed. With the SARS-CoV-2 pandemic, molecular laboratories for RT-PCR testing of the virus had to be set up quickly to enable adequate testing. Regulations governing the physical set up described a sample laboratory design with instructions on one way flow. It was not meant to be the template but when we submitted our design following the instructions, it was not allowed. No matter how we modified it, it all boiled down to just follow the sample design, no matter if the space configuration did not allow for it. That to and fro took all of one month, precious time wasted at the height of the pandemic.

Training of pathologists was fortunately delegated to the Philippine Society of Pathologists Inc. (PSPI), rightly so since professional organizations should be conducting training and proficiency testing, as is being done in most countries.

Training of medical technologists was given to the RITM and the University of the Philippines (UP), both government institutions engaged in SARS-CoV-2 testing exclusively in the early part of 2020. Unfortunately, both were much too busy with the routine testing, and in fact were overwhelmed by the volume of tests requested that medical technologist training was done on a limited basis, further exacerbating the shortage of available testing facilities. There are a number of private institutions already doing molecular testing that could have been requested to take part in medical technologist training but were largely ignored. Even today, formal training by these two institutions is required of analysts though training courses are still very limited. Yet, it is a mandatory requirement for renewal of licenses to operate. This puts the laboratories in a bind. They can't get their staff trained due to the mandated institutions not offering the courses yet the regulatory agency rigidly requires it. Recently, the RITM has updated their training calendar and established a mechanism for recognizing additional training providers to catch up with the backlog of required trainings.

The result is that no additional shifts to accommodate more testing can be opened by the laboratories. They are limited to the staff that have been previously trained. In the early days of the pandemic, the HFSRB allowed labs with previously trained staff to train their new recruits. That is no longer allowed. It speaks of yet again an inability to trust the pathologist and med tech staff to competently train their own new techs, something that is being done in all sections of the clinical laboratory. The RT-PCR technique has been elevated to an esoteric technique that requires formal training, like what was done with HIV testing earlier.

Now we are witnessing an effort by DOH to formulate new regulations for other molecular testing in all its forms: infectious disease, cancer and genetic. Yet we have been practicing molecular pathology since the early 1990s. It is no longer exotic and is actually becoming part and parcel of clinical laboratory testing, much like ELISA and ECLIA were once deemed so advanced as to merit special training for HIV.

Many molecular tests are now very easy to perform. Cartridge-based molecular testing can even be done at point of care. Even Anatomic pathology practitioners do in-situ hybridization routinely for breast and other cancers. An offshoot of cancer molecular testing is that once identified, the protein that the gene codes for can also be targeted with immunohistochemistry.

Molecular testing is the future of clinical laboratory testing. If misguided regulations are put in effect, that may adversely affect progress of laboratory testing in the Philippines. We hope the DOH learns the lessons of the past so as not to repeat it in the future.

The regulatory agencies being burdened with many tasks and being short of technical staff should delegate tasks that can be done by others. It should coordinate with RITM and other training providers to see if the assigned training tasks can be done properly and in due time. It should also tap private entities that are as capable as these agencies in trainings

There is hope in the establishment of the Office for Health Laboratories (OHL) which is headed by a pathologist. Since regulation will still be with the HFSRB, there should be regular meetings with the OHL, the PSPI and Philippine Association of Medical Technologists (PAMET) for updated implementation of regulatory policies based on current clinical laboratory progress in technology and knowledge.

In addition, the DOH should further strengthen its collaborative work with PSPI and PAMET in ensuring a smooth flow of licensing and compliance. The PSP and PAMET should be tapped for its members to assist the DOH inspection teams which will improve the process by better communication with the laboratory staff during inspection.

Currently, a Technical Working Group on Molecular Laboratory testing is working on the future provisions of future regulations. We wish it success for the seamless adaptation of molecular testing by local laboratories.

Dr. Raymundo Lo is a respected pathologist of Philippine Children's Medical Center, an immunopathologist of St. Luke's Medical Center, Quezon City, a member of the Philippine Society of Pathologists, Inc., a columnist of the Manila Bulletin, an editorial board member of the Philippine Journal of Pathology, and a member of the COVID-19 Laboratory Experts Panel (CLEP) of the Department of Health.

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Healthy Pilipinas

Celebrate a fun Christmas! at Umiwas sa 3Cs!

Closed Areas

Crowded Settings

Close Contact

Para sure, 'wag kalimutan mag

Wash o'Clock

at do the hand washing steps to the tune of Jingle Bells!

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PHILIPPINE SOCIETY OF PATHOLOGISTS, INC.

PSP Statement on the Use of RT-PCR Kits for Detection of SARS-CoV-2 Variants of Concern

The **Philippine Society of Pathologists Inc.**, whose members are at the forefront of SARS-CoV-2 testing responses to the pandemic, views with concern the ongoing surge in cases which are being attributed to the spread of the Delta variant of concern (VOC).

The PSP wishes to assist in the identification of VOC particularly with regards to correlation with clinical findings when we are allowed to screen for VOC alongside with regular SARS-CoV-2 testing. We need to understand especially those breakthrough cases that become critically ill if these are due to variant infection.

However, the current situation is unclear due to the limitations of the Philippine Genome Center (PGC) to pursue more widespread testing for VOC due to many reasons. Hence, sampling of cases for genomic testing to detect VOC is very limited and may be subject to sampling errors. This is not to cast aspersions or allegations against the PGC which has more than enough work to do as it stands.

The knowledge gained with this approach can better assist our clinicians in the way they will manage cases and the LGUs in conducting more active surveillance. It will also enable us to better understand if the VOC have an effect on vaccine efficacy.

There are now several RT-PCR variant detection kits on the market which may enable us to detect VOC while doing regular SARS-CoV-2 testing. These kits, though labeled as "screens" show good concordance with sequencing data and therefore should be of use in our VOC testing. These novel COVID-19 technologies do not require additional inputs in terms of equipment, personnel or space and can be used in currently licensed molecular labs performing SARS-CoV-2 testing. They will greatly assist the PGC in terms of VOC detection given the opportunity to be used.

Hence, we are humbly requesting that these VOC SARS-CoV-2 kits be fast tracked for Emergency Use Approval by the FDA based on certification/approval of other countries for its use as a supplement to the testing being done for VOC. Once given EUA, there should be guidelines as well for the use of VOC RT-PCR as part of the national testing strategy.

As always, the Philippine Society of Pathologists Inc. stands ready to assist in whatever way it can to manage, control and prevent further spread of the SARS-CoV-2 virus in the country.

For the Philippine Society of Pathologists, Inc.:

Roberto D. Padua Jr., MD, FPSP, MHA
President, Philippine Society of Pathologists, Inc.

August 19, 2021

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Healthy Pilipinas

Physical Shopping sa Mall during Holiday Rush!

Add to cart muna para iwas exposure!

Doble ingat sa 3Cs!

Closed Areas

Crowded Settings

Close Contact

Ligtas Christmas sa Healthy Pilipinas

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OPEN ACCESS – ORIGINAL ARTICLE

Immunohistochemical Expression of MDM2 and p16 in Adipocytic Neoplasms Measuring Ten Centimeters or More in Diameter Among Filipino Patients In a Public Tertiary Hospital From 2017 to 2019*

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ABSTRACT

Introduction. A size of more than 10 cm suggests that a soft tissue tumor might be malignant. Pertinent ancillary diagnostic testing, such as immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH), may be done to confirm the diagnosis. Several studies have shown that size may be a useful criterion in determining which tumors are candidates for further molecular testing. MDM2 and p16 are IHC markers for atypical lipomatous tumor/well-differentiated liposarcoma (ALT/WDLPS).

Objectives. The primary objective of this study is to determine the proportion of tumors signed out as "lipomas" from 2017 to 2019, and measuring at least 10 cm, that express MDM2 and p16 on IHC and warrant revision as ALT/WDLPS.

Methodology. This is a descriptive, retrospective cohort study in which all lipomas from 2017 to 2019 that measured at least 10 cm were included. The size, age of the patient, and location of each tumor were documented. The slides of all eligible cases were reviewed and immunohistochemically stained for MDM2 and p16. For each case, the intensity and immunoreactivity of each stain were assessed using a modified, four-tier scoring system. Fisher's exact test was used to determine if a significant number of tumors expressed MDM2 or p16.

Results. Thirty (30) cases satisfied the inclusion and exclusion criteria. The average size of these tumors is 15.10 cm. There is no sex predilection. The most common location of these tumors is the extremities. None of the tumors expressed MDM2, and only one case was p16-positive. The case positive for p16 also showed cytologic atypia and variability in cell size, resulting in the revision of its diagnosis from lipoma to atypical lipomatous tumor. The rate of diagnosis revision after slide review and IHC studies is 3.33%.

Conclusion. None of the adipocytic tumors that measured at least 10 cm in diameter and were signed out as lipomas was MDM2 positive, and only one case was p16-positive. Thus, morphology remains the cornerstone in the diagnosis of adipocytic tumors. Careful microscopic evaluation is necessary to establish the diagnosis of malignancy in these tumors. Ancillary tests should only be considered in cases where the pathologic features are equivocal.

Key words: Neoplasms, Adipose Tissue; Lipoma; Liposarcoma; Extremities

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**This study was presented in the 26th Annual Residents' Research Forum of the University of the Philippines-Philippine General Hospital, Department of Laboratories, held on March 4, 2021.*

INTRODUCTION

Soft tissue tumors are neoplasms of fat, muscle, peripheral nerves, blood vessels, fibrous tissues, and also tumors with uncertain histogenesis. It is estimated that the annual incidence of benign soft tissue neoplasms is as high as 3000 cases per one million population, in contrast to soft tissue sarcomas, which are reported to have an incidence of about 50 cases per one million population. In a majority of cases, the etiology of most benign and malignant soft tissue tumors is unknown. Although specific genetic abnormalities are found in certain entities, suggesting a familial basis, most tumors appear to arise *de novo*.[1]

Lipomas are among the usual specimens encountered by pathologists in their daily practice. This tumor is the most common soft tissue neoplasm in adults. It usually arises in the subcutaneous tissue of the trunk and extremities. Several morphologic variants of lipomas exist. Some of

these variants have characteristic clinical features. For instance, most lipomas are painless, but angiolipoma is painful, with pain correlating with the degree of vascularity.[2] Spindle cell lipoma and pleomorphic lipoma are commonly found in the subcutis of the upper back, posterior neck, and shoulder region of middle-aged males.[3] Other variants may mimic certain sarcomas, such as chondroid lipoma, which may mimic chondrosarcoma.[2] Conventional lipomas are soft, mostly painless, and easily cured by excision. The prognosis is excellent.[4]

Atypical lipomatous tumors/well-differentiated liposarcomas (ALT/WDLPS) are among the most common malignant soft tissue tumors in adults. Three main subtypes of ALT are recognized: adipocytic (lipoma-like), sclerosing, and inflammatory. These patterns may be seen simultaneously in one lesion. Lipoma-like ALT is the most common subtype. The presence of substantial variation in cell size and nuclear atypia in fat cells or stromal cells should separate lipoma-like ALT from lipoma. In some cases, the atypia may be so focal that thorough sampling of the tumor should be considered and ancillary testing may be warranted.[5]

ALT/WDLPS are locally aggressive tumors that are more common in the 5th to 7th decade of life. They are usually encountered in the proximal lower extremities and in the retroperitoneum. They may recur locally if inadequately excised. The metastatic potential of ALT/WDLPS is nearly zero; however, this increases if the tumor undergoes dedifferentiation.[2,4]

Lipomas do not undergo malignant transformation; however, lipoma-like ALT/WDLPS resembles the former grossly and histologically.[2,6] Oftentimes, histologic criteria are sufficient to diagnose an atypical lipomatous tumor. Problematic cases include tumors measuring more than 10 cm in greatest dimension, lesions with equivocal atypia, recurrent lipomas, "lipomas" located in the retroperitoneum and deep abdominal or pelvic viscera, and cases with worrisome clinical or radiologic features.[7]

The World Health Organization (WHO) states that "all superficial soft tissue lesions measuring >5 cm, and all deep-seated lesions, are statistically likely to be sarcoma."[1] Johnson et al., recommends that all soft tissue tumors be considered malignant until proven otherwise if they have any of the following clinical features: increase in size, size more than 5 cm deep-seated, or painful. A size of less than 5 cm is said to be the best indicator of a benign lump.[8] These recommendations are reiterated by several guidelines on the diagnosis and management of soft tissue tumors.[9-11] Of these, size is the only feature that can be independently determined by the pathologist during gross examination.

Subcutaneous lipomas may occasionally grow beyond 5 cm.[8,12,13] Lipomas growing larger than 10 cm are rare.[14] The number of ALT/WDLPS is significantly higher in cases where the tumor size is >10 cm; therefore, adequate sampling is necessary to rule out ALT/WDLPS.[7,14-18] The College of American Pathologists (CAP) recommends submitting one section per centimeter of maximum dimension for histologic evaluation, but this guideline is not always strictly followed.[19]

Ancillary testing is sometimes necessary to confirm the diagnosis of ALT/WDLPS. Detection of *MDM2* amplification via fluorescent *in situ* hybridization (FISH) is the gold standard in differentiating lipomas from ALT/WDLPS.[15,16,18] Immunohistochemistry (IHC) is used as an alternative test if FISH is not available. MDM2, p16, and CDK4 are three IHC markers that may be used to support the diagnosis of ALT/WDLPS. p16 has the highest sensitivity (96.8%) of the three markers.[20] One hundred percent of ALTs express at least two of these markers.[21]

No definite consensus guidelines exist on when to do FISH or IHCs. Several authors have advocated testing large tumors, i.e.at least 10 cm, deep-seated lesions, and those with equivocal atypia, especially if the sample is limited, e.g., core biopsies.[15-18,22] In some Western centers, a size of at least 10 cm triggers testing for *MDM2* amplification. In one study, for adipocytic tumors that underwent ancillary molecular testing because of a size more than 10 cm, 68 out of 187 tumors (36%) proved to be ALT/WDLPS.[7] In the Philippine General Hospital (PGH), the diagnosis of lipoma relies mainly on gross and microscopic examination. The possibility of an ALT/WDLPS being signed out as a lipoma should therefore be considered in tumors with worrisome clinical features.

This study aims to evaluate the immunohistochemical expression of MDM2 and p16—the two stains for ALT/WDLPS that are currently offered by the Department of Laboratories—among tumors with a size of at least 10 cm that were signed out as "lipomas" in PGH from 2017 to 2019. Specific objectives of this study are: to determine the basic demographic information of eligible cases; to compare the characteristics (size, male:female ratio, age, and location) of eligible cases with those with a final diagnosis of malignant adipocytic tumors from the same time period; to determine the number of lipomas measuring at least 10 cm that express MDM2 or p16 via IHC; to determine if there is a significant difference between the immunohistochemical expression of MDM2 and p16 in adipocytic tumors; and to determine the degree of concordance between the original diagnoses and the diagnoses after slide review and IHC studies.

METHODOLOGY

This study is a descriptive, retrospective cohort study that involves surgical pathology cases signed out as "lipoma" from 2017 to 2019. Prior to the implementation of the study, ethical clearance was secured from the University of the Philippines – Manila Research Ethics Board.

Inclusion and Exclusion Criteria

This study included all adipocytic tumors that were submitted to the Department of Laboratories from 2017 to 2019 with a final diagnosis of "lipoma" or its variants. Furthermore, these tumors fulfilled all of the following conditions: the surgical procedure done to the specimen was at least an excision or resection; the tumor size was at least 10 cm, based on the gross description of the pathology resident and consultant in charge of the case; and no prior ancillary studies, either IHC or FISH, were done on the specimen.

Any of the following criteria excluded a specimen from this study: lipomas from patients with recurrent tumors, at least one of which was eventually signed out as liposarcoma, whether ancillary studies were done or not; lipomas diagnosed on core needle, incision, or wedge biopsies; and cases for which the microscopic slides and/or paraffin blocks could not be retrieved (e.g., slide reviews, missing blocks), or were not fit for further evaluation or testing (e.g., damaged paraffin blocks, minimal residual tissue within paraffin block that was not sufficient for IHC).

Data Collection Procedures

The surgical pathology reports of all soft tissue tumors with a definite histopathologic diagnosis of "lipoma" or one of its variants from 2017 to 2019 were reviewed. All cases that satisfied the inclusion and exclusion criteria were assigned unique code numbers. Data collected from the surgical pathology reports included the age and sex of the patient, location of the tumor as specified by the attending physician, size of the tumor based on the gross description, and the number of sections taken. The hematoxylin and eosin slides of these cases, as well as their corresponding paraffin blocks, were retrieved with the assistance of the staff of the Surgical Pathology Division.

Slide Review

To maintain anonymity, the microscopic slides were given new study-specific code numbers. All slides were evaluated by the principal investigator for the quality of their staining. All slides with poor staining quality were restained with the assistance of the Surgical Pathology Division staff. The investigators evaluated the microscopic slides of all cases for the two features of ALT/WDLPS: 1) presence of focal atypia in either the adipocytes or stromal cells, and 2) heterogeneity of cell size. Suitable paraffin blocks, based on the initial histopathologic evaluation, were submitted for further IHC testing.

Immunohistochemistry Studies

Antibodies against MDM2 (Bio-SB mouse monoclonal antibody BSB-64) and p16 (DB Biotech mouse monoclonal antibody clone R15-A) were used to stain the chosen paraffin blocks. Immunohistochemical staining of the slides were performed as per the manufacturer's protocols, as follows:

MDM2

Formalin-fixed paraffin-embedded tissues were cut and fixed on positively charged slides, followed by air-drying for 2 hours at 58°C. The tissues were deparaffinized, dehydrated, and rehydrated. Tissues were subjected to heat-induced epitope retrieval (HIER) using a suitable retrieval solution. Tissues were heated using water bath method. After heat treatment, slides were transferred in ImmunoDNA Retriever EDTA to room temperature. Automated staining methods were performed according to the instrument manufacturer's instructions. Between each step of IHC staining, slides were washed with ImmunoDNA washer solution. Slides were mounted for observation.

p16

Formalin-fixed paraffin-embedded tissues were deparaffinized, dehydrated, and rehydrated. Endogenous peroxidase was blocked by incubating the tissue in 3% hydrogen peroxide for 10 minutes. The slides were immersed in Tris-EDTA buffer at pH 9.0 and incubated at 95-97°C in a water bath for 25 minutes. The slides were allowed to cool for 15 minutes. The slides were stained with p16 using automated staining methods. The slides were then mounted for observation.

Interpretation of Immunohistochemical Expression of MDM2 and p16

The IHC slides were reviewed independently by the principal and co-investigators. MDM2 and p16 are nuclear stains; therefore, inconsistent staining patterns, e.g., cytoplasmic or membranous only, were considered as negative results. Nonspecific staining patterns were also documented but were still considered negative.

Each tumor was assessed using a modified version of the method by Thway et al. This method consists of a four-tier scoring system based on the intensity of reaction and immunoreactivity.[20] After quantification, a positive result was given if 1) there was at least moderate intensity for tumors with at least 11% of cells stained (patchy to diffuse), or 2) there was strong staining if only 1% to 10% of cells are stained (focal). Tumors with weakly staining nuclei and a focal pattern of staining were considered negative.[23] Table 1 presents a summary of this method.

In cases where the immunostains gave a positive result, the case was independently reviewed by the investigators and referred to a bone and soft tissue pathologist for concurrence.

Data Analysis

Microsoft Excel was used to tabulate the data on patient demographics (age, sex, and location of the tumor) and tumor characteristics (presence of atypia, cell size heterogeneity, and expression of MDM2 and p16). An independent statistician was consulted for data analysis. Fisher's exact test was used to determine if there was a significant number of tumors that expressed MDM2 or p16.

RESULTS

Demographics of Patients with Adipocytic Tumors in a Tertiary Hospital from 2017 to 2019

A total of 938 resected adipocytic tumors were submitted to the PGH Department of Laboratories for pathologic evaluation, including IHC studies, from 2017 to 2019. Of the 904 adipocytic tumors that were diagnosed as benign (96.38%), 36 cases satisfied the inclusion and exclusion criteria. The other 868 tumors were either benign, measured less than 10 cm, already diagnosed as malignant, or sampled using incision or core biopsy procedures. Of the 36 cases that satisfied the inclusion and exclusion criteria, the paraffin blocks of six cases, all from 2017, were not retrievable even after a diligent search by the Surgical Pathology staff; therefore, only 30 cases were included in the study. These were processed for IHC studies with MDM2 and p16.

The categories for tumor location follow the recommended anatomic primary site distribution of the AJCC Cancer Staging Manual, 8th edition.[24] The most common location

Table 1. Four-tier system for assessment of staining patterns*

Intensity	Reactivity	%	Interpretation (Intensity + Reactivity)	
Absent	Absent	0	No staining	Negative
Weak	Focal	1-10	Weak Intensity, Focal Immunoreactivity	
Moderate	Patchy	11-50	Moderate Intensity, Patchy to Diffuse Immunoreactivity	Positive
Strong	Diffuse	>50	Strong Intensity, **Any** Reactivity (Focal, Patchy, Diffuse)	

*The system is based on the 1) intensity and 2) reactivity of the stains. This system applies to both MDM2 and p16. Interpretation is done on atypical stromal or adipocytic cells with nuclear staining. If cells other than stromal or adipocytic cells show some degree of staining, the interpretation is *nonspecific*, which is equivalent to *negative*.

Table 2. A comparison between the eligible cases and the liposarcomas that were evaluated by the Department of Laboratories from 2017 to 2019

Characteristics	Lipomas Measuring at least 10 cm*	Malignant Adipocytic Tumors**
Number (N)	30	34
Average Size	15.10 cm (Median: 14 cm; Range: 10 to 40 cm)	18.70 cm (Median: 19 cm; Range: 4 to 31 cm)
Number of Sections taken for Microscopic Evaluation***	9 sections per 10 cm	8 to 9 sections per 10 cm
Male to Female Ratio	1:1	1:1.3
Mean Age (in years)	40	54
Most common location	Extremities	Extremities
Other locations	Trunk wall, head and neck	Retroperitoneum, abdominal and thoracic visceral organs, trunk wall, head and neck
Most common diagnosis	Lipoma****	ALT/WDLPS (14), Myxoid Liposarcoma (9), DDL (7)

* This column includes only the cases that had available paraffin blocks for further testing.
** All malignant adipocytic tumors from 2017 to 2019 were included for comparison.
*** This refers to the average number of tissue sections taken by the pathology resident during specimen grossing, as indicated in the surgical pathology report.
**** Part of the inclusion criteria is to have a diagnosis of lipoma.

is the extremities (13; 43.33%), followed by the trunk wall (12; 40%) and the head and neck region (5; 16.67%). None of the tumors have a visceral or retroperitoneal location (i.e., abdominal, pelvic, and retroperitoneal organs).

On the other hand, out of the 938 cases, 34 cases were signed out as malignant adipocytic tumors, comprising 3.62% of all tumors. Sixteen of these cases were established as liposarcomas through further IHC studies. The most common type of liposarcoma is ALT/WDLPS (14 cases; 41.18%). The most common location for malignant adipocytic neoplasms is the extremities (15 cases; 44.12%), followed by the retroperitoneum (8 cases; 23.53%), abdomen and thoracic visceral organs (5; 14.71%) trunk wall (4; 11.77%), and the head and neck region (2; 5.88%).

Table 2 shows the comparison between the eligible cases and the malignant adipocytic tumors from 2017 to 2019.

Pathologic Findings of Adipocytic Tumors Measuring at Least 10 Centimeters

The thirty lipomas that qualified for this study had an average size of 15.10 cm (95% CI: 13 to 17.2), ranging from 10 cm to 40 cm, and a median size of 14 cm. All adipocytic tumors were signed out as lipomas or one of its variants; one was described as having fat necrosis, while another tumor was signed out as osteolipoma. The osteolipoma case was also the largest of the eligible tumors, measuring 40 cm in gross tumor dimension based on the gross description.

On microscopic examination, eight lipomas presented with cellular atypia, including the lipoma with fat necrosis and osteolipoma. Five of these tumors were located in the extremities, including the osteolipoma, which was located in the right thigh. Except for the osteolipoma, which had a moderate degree of stromal cell atypia, the atypia seen in these tumors were at most mild, i.e., minimal variation in nuclear size and absence of hyperchromasia. Most of the tumors did not present with variability in cell size, except for the lipoma with fat necrosis, which showed mild variability, and the osteolipoma, which had moderate variation in cell size.

The collected data for all 30 cases included in this study are summarized in Table 3.

Expression of MDM2 and p16 in Adipocytic Tumors

None of the lipomas were positive for MDM2 via IHC using the modified method by Thway et al. Four lipomas presented with weak, focal staining with MDM2, but these were not sufficient to be interpreted as positive. Of these four lipomas, three also presented with mild to moderate cellular atypia, as described, including the osteolipoma. Similarly, all but one of the specimens did not present with positive p16 immunostaining. Because most of the IHC results for MDM2 and p16 were negative, Fisher's exact test could not be performed to determine if there is any significant difference between the expression of the two stains.

However, a significant number of cases (n=19; p-value=0.035; α=0.05) presented with nonspecific staining for p16, in which nuclear and cytoplasmic staining was observed in cells or tissues other than the atypical stromal cells. These include the cytoplasm and membranes of benign adipocytes, inflammatory cells, endothelial cells, and areas of fat necrosis. Figure 1 shows representative photomicrographs of the various staining patterns observed with p16 immunostain.

The one case that presented with strong, diffuse nuclear positivity with p16 in the atypical stromal cells is the osteolipoma case. The results for this case were interpreted as p16-positive. After consultation with other pathologists, including a bone and soft tissue subspecialist, the diagnosis was revised to ALT/WDLPS. Therefore, the rate of diagnosis revision after p16 IHC and case review is 3.33% (1/30). Figure 2 shows representative photomicrographs of the aforementioned case.

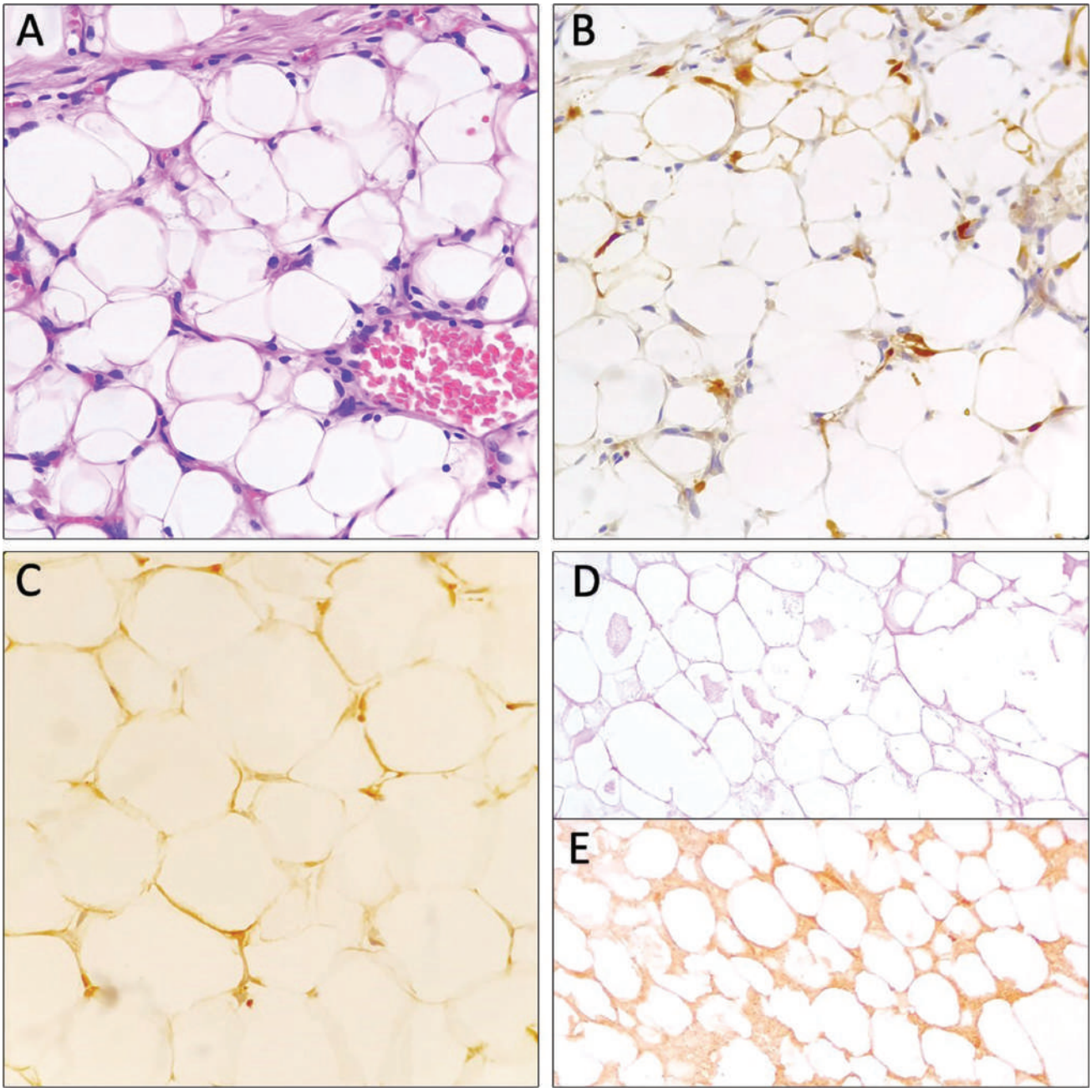

Figure 1. Representative photomicrographs of the different lipoma cases reviewed. **(A)** Lipomas are composed of mature adipocytes with or without the presence of other mesenchymal derivatives, such as fibrous tissues and blood vessels. The nuclei of the adipocytes are small and pushed to the periphery. In this photomicrograph, the adipocytes are roughly the same size, while the prominent nuclei belong to endothelial cells (H&E, 400x). **(B)** to **(E)** p16 may present with non-specific staining patterns. **(B)** The nuclei of endothelial cells are nonspecifically stained with p16 (HRP, 400x); **(C)** Occasionally, the peripheral cytoplasm and cellular membranes of adipocytes show moderate staining with p16 (HRP, 400x); **(D)** Fat necrosis is characterized by the lack of nuclear staining, adipocyte dropout, and cytoplasmic vacuolization (H&E, 100x); **(E)** p16 shows diffuse, moderate cytoplasmic staining in areas of fat necrosis (HRP, 100x).

Table 3. Tumor Characteristics, Pathologic Features, and Immunohistochemistry Profiles*

Study number	Age	Sex	Size	Sections	Adequacy of Sections (%)	Original Diagnosis	Specific Location	General Location	Atypia	Variability of Cell Size
MCM-01	31	F	14.5	5	34.48	Lipoma	Back	Trunk	N	N
MCM-02	44	F	12.3	16	130.08	Lipoma	Shoulder, right	Extremity	N	N
MCM-03	27	F	10	8	80.00	Lipoma	Upper back	Trunk	N	N
MCM-04	68	M	10.5	8	76.19	Lipoma	Shoulder, left	Extremity	N	N
MCM-05	60	M	13	10	76.92	Lipoma	Back	Trunk	N	N
MCM-06	41	M	11.5	20	173.91	Lipoma	Neck, right	Head and neck	Y (mild)	N
MCM-07	43	M	13	13	100.00	Lipoma	Shoulder, right	Extremity	N	N
MCM-08	25	M	14	8	57.14	Lipoma	Upper back	Trunk	N	N
MCM-09	44	M	12	8	66.67	Lipoma	Axilla, left	Extremity	N	N
MCM-10	40	F	20	20	100.00	Lipoma with fat necrosis	Lower back	Trunk	Y (mild)	Y (mild)
MCM-11	54	F	12	10	83.33	Lipoma	Back	Trunk	N	N
MCM-12	40	F	13	9	69.23	Lipoma	Back	Trunk	N	N
MCM-13	43	F	14.5	15	103.45	Lipoma	Gluteal area	Extremity	N	N
MCM-14	53	F	16.2	19	117.28	Lipoma	Flank, right	Trunk	N	N
MCM-15	72	M	14.5	17	117.24	Lipoma	Trunk	Trunk	N	N
MCM-16	44	F	27.5	28	101.82	Lipoma	Thigh, left	Extremity	Y (mild)	N
MCM-17	19	F	14	13	92.86	Lipoma	Lower abdomen	Trunk	N	N
MCM-18	79	F	40	6	15.00	Osteolipoma	Thigh, right	Extremity	Y (moderate)	Y (moderate)
MCM-19	10	M	20	25	125.00	Lipoma	Shoulder, right	Extremity	N	N
MCM-20	61	F	12	10	83.33	Lipoma	Arm, right	Extremity	Y (mild)	N
MCM-21	58	F	11	11	100.00	Lipoma	Back	Trunk	N	N
MCM-22	46	M	15	16	106.67	Lipoma	Thigh, left	Extremity	Y (mild)	N
MCM-23	34	M	13	16	123.08	Lipoma	Inguinal area, left	Extremity	N	N
MCM-24	3	M	17.5	16	91.43	Lipoma	Neck, anterior	Head and neck	Y (mild)	N
MCM-25	4	F	15	16	106.67	Lipoma	Chest wall	Trunk	N	N
MCM-26	66	F	11	12	109.09	Lipoma	Supraclavicular area	Head and neck	N	N
MCM-27	47	M	16	11	68.75	Lipoma	Occipital area	Head and neck	N	N
MCM-28	12	M	15	15	100.00	Lipoma	Arm, left	Extremity	Y (mild)	N
MCM-29	2	M	15	12	80.00	Lipoma	Thigh, left	Extremity	N	N
MCM-30	38	M	10	1	10.00	Lipoma	Occipital area	Head and neck	N	N

*This summary table presents the tumor characteristics, pathologic features, and staining patterns with MDM2 and p16 of the cases that were included in the study.

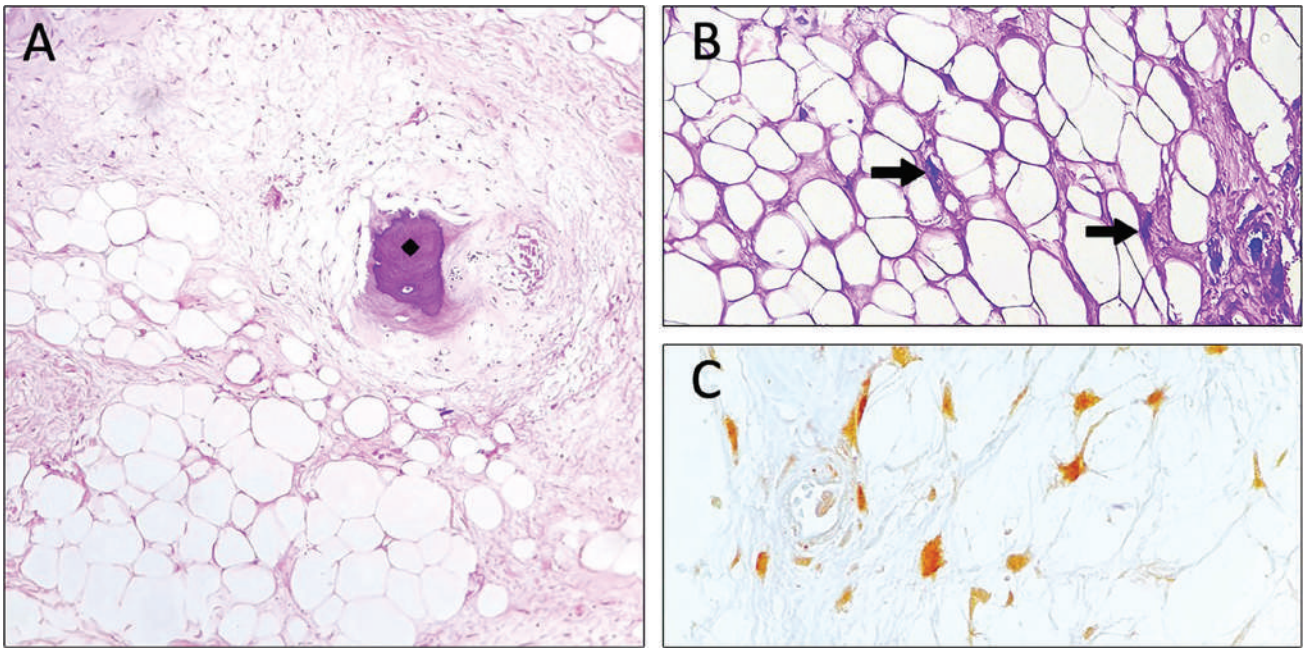

Figure 2. Atypical lipomatous tumor with osseous metaplasia. **(A)** This tumor from the thigh of a 70-year-old female was initially signed out as an osteolipoma due to the presence of mature lamellar bone (◆) admixed with lipomatous areas (H&E, 40x); **(B)** On slide review, variability in cell size was noted, as well as atypical stromal and adipocytic cells (→; H&E, 100x); **(C)** Immunostaining with p16 showed strong, diffuse, nuclear staining in the atypical stromal cells (HRP, 400x).

MDM2			p16			Revised Diagnosis
Intensity	Immunoreactivity	Interpretation	Intensity	Immunoreactivity	Interpretation	
Absent	Absent	Negative	Moderate	Diffuse	Non-specific	No Revision
Absent	Absent	Negative	Absent	Absent	Negative	No Revision
Absent	Absent	Negative	Absent	Absent	Negative	No Revision
Absent	Absent	Negative	Absent	Absent	Negative	No Revision
Absent	Absent	Negative	Absent	Absent	Negative	No Revision
Absent	Absent	Negative	Absent	Absent	Negative	No Revision
Absent	Absent	Negative	Absent	Absent	Negative	No Revision
Absent	Absent	Negative	Absent	Absent	Negative	No Revision
Absent	Absent	Negative	Weak	Patchy	Non-specific	No Revision
Absent	Absent	Negative	Moderate-Strong	Patchy-Diffuse	Non-specific	No Revision
Absent	Absent	Negative	Weak-Moderate	Diffuse	Non-specific	No Revision
Absent	Absent	Negative	Weak-Moderate	Patchy	Non-specific	No Revision
Absent	Absent	Negative	Weak-Moderate	Patchy	Non-specific	No Revision
Absent	Absent	Negative	Weak	Patchy	Non-specific	No Revision
Absent	Absent	Negative	Absent	Absent	Negative	No Revision
Absent	Absent	Negative	Weak	Focal	Non-specific	No Revision
Absent	Absent	Negative	Moderate	Focal	Non-specific	No Revision
Absent	Absent	Negative	Moderate	Focal	Non-specific	No Revision
Weak	Focal	Negative	Strong	Diffuse	Positive	Atypical Lipomatous Tumor
Absent	Absent	Negative	Absent	Absent	Negative	No Revision
Weak	Focal	Negative	Weak-Moderate	Patchy	Non-specific	No Revision
Absent	Absent	Negative	Moderate-Strong	Focal	Non-specific	No Revision
Absent	Absent	Negative	Weak-Moderate	Patchy	Non-specific	No Revision
Weak	Focal	Negative	Weak-Moderate	Patchy	Non-specific	No Revision
Weak	Focal	Negative	Weak-Moderate	Focal	Non-specific	No Revision
Absent	Absent	Negative	Weak	Patchy	Non-specific	No Revision
Absent	Absent	Negative	Weak	Patchy	Non-specific	No Revision
Absent	Absent	Negative	Weak-Moderate	Patchy	Non-specific	No Revision
Absent	Absent	Negative	Moderate	Patchy	Non-specific	No Revision
Absent	Absent	Negative	Absent	Absent	Negative	No Revision
Absent	Absent	Negative	Absent	Absent	Negative	No Revision

DISCUSSION

Size as Basis for the Immunohistochemical Evaluation of Adipocytic Tumors

Current practices in the pathologic evaluation of soft tissue tumors make use of size primarily for staging purposes.[24] ALTs are suspected in the setting of a large, slow-growing tumor, especially when located in the proximal extremities or the trunk. ALT/WDLPS in the retroperitoneum or thoracoabdominal cavity may go unnoticed until it reaches a size of 20 cm.[5] All lipomatous tumors with a retroperitoneal or visceral location evaluated in our institution were given a diagnosis of ALT/WDLPS.

The size cut-off for suspecting an ALT/WDLPS varies, ranging from 5 cm to 15 cm.[5,7,15,17,22,25] Ten centimeters was chosen as the cut-off for this study because most of the previous studies reviewed utilized this size. This is also the cut-off used by Clay et al., and Thway et al., to prompt recommendation for further tests for *MDM2* amplification, by FISH or surrogate IHC markers.[15,22] The location of the tumor also affects the cut-off for size. For masses located in the deep soft tissues and the retro-peritoneum, most authors agree that 10 cm is a reasonable cut-off for suspecting ALT/WDLPS.[26,27]

In this study, there is a difference in the average size between the eligible lipomas and the diagnosed cases of liposarcomas (15.10 cm vs. 18.70 cm); however, this study demonstrates that size should not be used as the sole basis for reflex testing with MDM2 and p16 IHC. Most of the eligible cases, even though they measure more than 10 cm, demonstrated no or minimal atypia and cell size heterogeneity. The lack of atypia correlates well with the absence of staining with MDM2 in all cases and the nonspecific or absent staining with p16 in 29 out of 30 cases. Therefore, in the clinical setting of a large mass located in the proximal extremities, correlation with cellular atypia and cell size heterogeneity on microscopic evaluation is of utmost importance and remains the foundation in diagnosing lipoma-like ALT/WDLPS. In one study involving 405 extremity-based tumors, a cut-off of 15 cm was recommended for doing ancillary tests for tumors without diagnostic cytologic atypia.[28] In relation to the results of this study, this cut-off might be more suitable as a basis for further molecular testing of tumors with neither cytologic atypia nor heterogeneity in cell size.

Nonetheless, a large size remains a vital clue to the diagnosis of ALT/WDLPS. The sole case that had its diagnosis revised from osteolipoma to atypical lipomatous tumor measured 40 cm, which was an outlier via the interquartile method (IQR=3; upper bound=19.50 cm). The tumor also showed strong, diffuse, nuclear positivity for p16 and weak, focal, nuclear staining for MDM2. A review of the pathology report of the case revealed that only six sections were taken. Cases such as this will benefit from the CAP recommendation of taking one section per centimeter, allowing a more thorough microscopic evaluation for cellular atypia and heterogeneity of cell size, which are both present in the case.

One other caveat for this case is that an atypical spindle cell/pleomorphic lipomatous tumor (ASPLT) should

be included in its differential diagnoses. ASPLT and ALT have overlapping features, including a persistently enlarging mass, predilection for the thigh, presence of atypical spindle cells and adipocytes, heterologous differentiation, and positivity for p16. However, ASPLT does not demonstrate *MDM2* amplification, either by FISH or IHC. The case demonstrated focal, weak staining with MDM2. Nevertheless, along with the revised diagnosis, a recommendation was made to perform *MDM2* FISH to fully rule out an ASPLT. Differentiating ASPLT from ALT is important because the risk of recurrence for ASPLT is low (10-15%) even if the lesion incompletely excised, and there is no documented risk for metastasis or dedifferentiation.[29,30]

The Limited Utility of Immunohistochemistry Studies Using MDM2

The characteristic cytogenetic aberration seen in ALT/WDLPS is a supernumerary ring and/or giant marker chromosome. Although lipomas and ALT/WDLPS affect the same chromosomal region, lipomas are characterized by translocations of 12q13-15. In ALT/WDLPS, there is amplification of the genes located in 12q13-15.[31] Among the genes amplified in the 12q13-15 region are the oncogenes *MDM2* and *CDK4*. The gene product of *MDM2* is an inhibitor of p53, which is important in cell cycle arrest, senescence, and apoptosis. On the other hand, the gene product of *CDK4* mediates the progression of the cell cycle through the G1 phase, eventually leading to cellular proliferation. In the presence of increased CDK4 expression, p16 is upregulated to perform its inhibitory function.[32]

Initial studies on the sensitivity and specificity of MDM2 showed promising results for the diagnosis of ALT/WDLPS and DDL, with a sensitivity and specificity of 97% and 92%, respectively.[31] Succeeding studies comparing the performance of MDM2 IHC with *MDM2* FISH showed that the latter is superior in detecting ALT/WDLPS. As such, FISH for *MDM2* amplification remains the *prima facie* evidence for well-differentiated and dedifferentiated liposarcomas, with a sensitivity and specificity of 92-94% and 96-100%, respectively.[33] The cost of FISH may deter patients from availing it; therefore, IHC with known ALT markers may still be considered as an alternative test to establish the diagnosis of ALT/WDLPS.

The results of the study showed that all tumors had a negative result for MDM2 IHC; however, the absence of staining does not entirely preclude the diagnosis of ALT. Of note, four of these tumors showed weak, focal staining with MDM2. Three of these tumors showed mild to moderate cytologic atypia. One tumor also showed a positive result for p16, with its diagnosis ultimately being revised from osteolipoma to ALT. The latter finding suggests that weak, focal staining with MDM2 might be demonstrated in some ALT cases; however, in line with the results of previous studies, it might be more prudent to use at least two IHCs markers like CDK4 and p16 in order to clinch the diagnosis.[31,34] Although CDK4 is not available locally, this IHC has a reported sensitivity and specificity of 86% and 89%, which are less than those of p16 but higher than those of MDM2.[20]

The results of the study also suggest that in general, large lipomas with minimal or no atypia and without cell size variability might benefit less from further testing with IHCs. FISH remains an option should there be a strong suspicion for a malignant lesion on clinical grounds, especially in cases with limited material for ancillary tests, e.g., core needle biopsies.[16]

Nonspecific Staining with p16: A Potential Pitfall

Several studies have indicated that p16 can be used as another marker to differentiate ALT/WDLPS from deep-seated lipomas and lipomas with equivocal atypia.[18,20,35,36] One study even showed that p16 is more sensitive than MDM2 and CDK4. The combination of p16 and CDK4 is more sensitive than the combination of either with MDM2.[20] Only one case showed a definite positive result for p16 in this study, as previously discussed. This case, which had its diagnosis revised from osteolipoma to ALT/WDLPS, showed strong and diffuse staining for p16. This staining pattern is consistent with the recommended criteria by several authors for a positive p16 interpretation.[20,35]

A significant number of lipomas (19/30; $p=0.035$) exhibited nonspecific staining with p16, which is characterized as staining of any intensity and any localization, i.e., nuclear, membranous, or cytoplasmic in non-atypical stromal cells. Nonspecific staining was observed in endothelial cells, cell membrane and cytoplasm of mature adipocytes, inflammatory cells, and areas of fat necrosis. This is in line with the findings of previous studies, in which they noted that lipomas with secondary changes have a propensity to stain nonspecifically with p16.[35,37,38] Therefore, when using p16 for the diagnosis of ALT/WDLPS, careful interpretation is warranted in order to avoid misinterpretation of nonspecific staining patterns.

Because of nonspecific staining, it is imperative to do p16 along with another ALT marker. Using a panel of markers is also useful in the setting of DDLs, because other malignancies may be positive for p16. These tumors include leiomyosarcoma, undifferentiated pleomorphic sarcoma, desmoid tumors, endometrial stromal sarcomas, sarcomatoid carcinomas, and gastrointestinal stromal tumors.[36]

Evaluation of Adipocytic Tumors: Surgical Pathology Practice Recommendations for a Tertiary Government Institution

Confirming the diagnosis of an ALT/WDLPS is beneficial for patients. In the setting of a lipoma-like ALT, the patient may benefit from closer surveillance and appropriate surgical intervention, e.g., marginal excision. The latter is particularly important because marginal resection is indicated for ALTs to avoid recurrence and dedifferentiation, and to reduce the morbidity associated with wide resection.[39] Even in DDLs with no well-differentiated component, the results of IHC or FISH can help identify the possible lineage of a tumor. This is important in determining if other treatment modalities, such as chemotherapy and radiotherapy, should be employed. Based on the preceding discussion, the following guidelines are recommended to maximize the pathologic evaluation of adipocytic tumors:

1. Size should not be used solely as a trigger for reflex IHC testing, especially in the absence of cytologic atypia and cell size heterogeneity.
2. One section per centimeter should be taken during the gross examination of lipomatous tumors, especially for extremity-based masses with a size of at least 15 cm, or those located in suspicious anatomic primary sites such as the retroperitoneum.
3. For lipoma-like ALT/WDLPS, careful evaluation for stromal atypia and heterogeneity of cell size should be done before considering ancillary tests. If the atypia is equivocal, FISH for *MDM2* amplification is preferred over IHCs.
4. When evaluating the immunohistochemical expression of MDM2, a negative IHC result does not entirely rule out a malignancy.
5. For immunostaining with p16, careful evaluation must be done to exclude the possibility of nonspecific staining.
6. If, for socioeconomic reasons, *MDM2* FISH is not an option, and ALT/WDLPS is a strong consideration, a panel of at least two liposarcoma markers should be requested to overcome their respective limitations.

CONCLUSION

None of the adipocytic tumors that measured at least 10 cm in diameter and were signed out as lipomas was MDM2 positive, and only one case was p16-positive. In contrast to the malignant adipocytic tumors, these lipomas are smaller, have no sex predilection, and are not seen in the retroperitoneum and thoracoabdominal viscera. Expression of p16 and MDM2 on IHC was mostly negative, precluding the determination of any significant difference between the immunohistochemical expression of MDM2 and p16. The diagnosis rendered based on morphological evaluation alone remained unchanged in the vast majority of cases even after immunohistochemical studies. The rate of diagnosis revision after slide review and IHC studies is 3.33%, indicating a high degree of concordance between the original and reviewed diagnoses.

As this study suggests, size alone should not automatically trigger further testing with either IHC or FISH. Morphology remains the cornerstone in the pathologic diagnosis of adipocytic tumors. A thorough gross examination should be done for larger tumors, ensuring a sufficient number of sections for careful microscopic examination. Correlation with clinical features, such as tumor location, is also helpful to establish the diagnosis. Only in cases where the histopathologic features are equivocal should ancillary tests be considered. *MDM2* FISH is preferred over IHCs; however, a panel composed of MDM2 and p16 may be considered if FISH is not available or accessible.

RECOMMENDATIONS

To fully evaluate IHCs as an acceptable alternative to FISH, the following recommendations are made:

1. Further studies with a larger sample size, which includes both benign and malignant adipocytic tumors, may be done to better assess the correlation between MDM2 and p16 expression;
2. A prospective study on how the immunohistochemical expression of liposarcoma markers correlate with other clinical features (e.g., pain, rapid growth) may be pursued;
3. Studies using p16 on established cases of liposarcomas, whether ALT or DDL, may be done to determine its utility as an alternative marker to FISH; and
4. The sensitivity and specificity of IHC studies with MDM2 and p16 vis-à-vis FISH for established cases of liposarcomas in the local setting may be explored.

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STATEMENT OF AUTHORSHIP

All authors fulfilled the ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

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OPEN ACCESS – ORIGINAL ARTICLE

The Diagnostic Accuracy of Hematologic Parameters, Neutrophil-Lymphocyte Ratio and Platelet-Lymphocyte Ratio, in Malignant and Benign Epithelial Neoplasms of the Ovary in Philippine General Hospital Service Patients

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ABSTRACT

Background and Objectives. Early detection of ovarian neoplasms confer a better outcome and prognosis for patients. Although newer diagnostic modalities have been recently developed, the availability and accessibility of complete blood count parameters specifically, neutrophil-lymphocyte ratio (NLR) and platelet-lymphocyte ratio (PLR) make it a convenient and cost-effective marker to aid as a pre-operative predictor of epithelial ovarian neoplasms. We aim to determine the significance and relationship of preoperative NLR and PLR in predicting a diagnosis of malignant surface epithelial ovarian tumor.

Methodology. We gathered surgical pathology reports and complete blood count parameters of service patients with benign and malignant surface epithelial ovarian neoplasms. Diagnostic accuracy of NLR and PLR was determined by using receiver operating curve (ROC) plots. Optimal cutoff points were set using the Youden index.

Results. We have included 351 cases of ovarian surface epithelial neoplasms, 209 of which were benign and 142 of which were malignant. The ROC curve for PLR had an area under curve (AUC) of 0.6629 [0.6043, 0.7215]. The optimal cut-off point of was set at 195.99 with the maximal Youden index of 0.295 [9.193, 0.396]. The corresponding sensitivity of this test to determine malignancy at this point was 56.5% [47.8, 64.6] while the specificity was at 73.2% [66.7, 79.1]. The ROC curve for NLR had an AUC of 0.6616 [0.6051, 0.7180]. The optimal cut-off point of was set at 2.60 with the maximal Youden index of 0.316 [0.219, 0.413]. The corresponding sensitivity of this test to determine malignancy at this point was 76.1% [68.2, 82.8] while the specificity was at 55.5% [48.5, 62.4].

Conclusion. The utility of CBC parameters such as PLR and NLR are cost-effective tools which may have some diagnostic value but, they cannot be used as a stand-alone predictor of malignancy and must be correlated with other clinical, laboratory and radiologic studies.

Key words: ovarian neoplasms, blood cell count, lymphocytes, neutrophils, blood platelets

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INTRODUCTION

According to the Philippine Cancer Society, ovarian cancer ranks as the 10th most common site of cancer in both sexes combined and 5th most common cancer in women. Approximately 90% of these ovarian malignancies are surface epithelial tumors. Among them are mucinous, serous, endometrioid, clear cell and Brenner carcinomas.[1] These neoplasms primarily present as a mass that is usually discovered at a much later time in the course of illness, with approximately 66% having metastasis outside of the pelvis at the time of diagnosis. This late detection contributes to a more severe morbidity and poorer outcome.[2-4] Although early detection is still the best approach to ovarian neoplasms, this may be clinically challenging because patients may present with non-specific signs and symptoms in the early stages of disease.

Histopathologic diagnosis remains definitive of tumor nature. The preoperative impression guides surgical technique and intraoperative staging. For equivocal cases,

intraoperative consultation (frozen section) may be done. Clinical data and history may give suspicion towards a benign or malignant diagnosis such as age, family history and sonographic findings among others. Tumor markers have been utilized and no marker has been used as frequently as CA-125.[4] However, like all tumor markers, CA-125 is not wholly sensitive and specific, with variable results depending on menopausal status. Tumors outside the gynecologic tract and other inflammatory states may give elevated CA-125 values.[5]

The search for improved diagnostic modalities for ovarian neoplasms continues. Studies have demonstrated the importance of the inflammatory response in the development of cancer and its progression. Inflammatory cells play a key role in the tumorigenesis. Complete blood count (CBC) parameters and their relationship with cancers have been studied over the years. This affordable and accessible test is included in all admitting and preoperative work ups, making it a convenient marker, specifically, platelet-lymphocyte ratio (PLR) and neutrophil-lymphocyte ratio (NLR). The theory behind the use of NLR is backed up by the pathophysiology of systemic inflammation. Inflammation increases the risk and progression of malignancies and is known to play an important role in tumorigenesis, among them include initiation, promotion, malignant conversion, invasion, and metastasis.[6] The rationale on the use of PLR, on the other hand, is due to previous studies indicating that in tumor and host tissues, increase production of thrombopoietic cytokines, mainly interleukin 6, leads to paraneoplastic thrombocytosis which eventually results in tumor growth and progression.[7] The degree of immune response inherent in solid tumors, represents the body's overall retortion to the tumor such that progression is associated with systemic inflammation.[8-9] Conversely, chronic inflammation is one of the contributors to oncogenesis.[10] It has also been demonstrated that there is a significant rise in inflammatory markers in different cancers. CBC parameters in inflammation have been the subject of recent studies about cancers in various organ system, even in prognostication.[11-14] Of particular interest is the recent hypothesis that a microenvironment and subsequent remodeling and transformation of the epithelial cells by proinflammatory cytokines initiate the development of epithelial ovarian cancers.[15] Since chronic inflammation plays a significant role in the pathogenesis of ovarian cancers, systemic inflammatory response markers such as neutrophil-lymphocyte and platelet-lymphocyte ratio have been evaluated and advocated because of its simplicity, accessibility and cost-effectiveness.

The relationship between neutrophils and oncogenesis has been the subject of recent studies in that neutrophilia, tumor-infiltrating neutrophils and elevated NLR has been associated with poor clinical outcomes, most notably in renal cell carcinoma, melanoma, colorectal cancer, hepatocellular carcinoma, cholangiocarcinoma, glioblastoma, gastrointestinal stromal tumors, gastric, esophageal, lung, ovarian and head and neck cancer.[14] In a study comparing neutrophil and NLR in patients with ovarian carcinoma, NLR was shown to have a significantly higher value in predicting tumor recurrence and disease-specific survival.[16] Another study done in South Korea compared NLR among patients with epithelial ovarian cancers versus patients with benign epithelial ovarian tumors and healthy controls. They have found out that the preoperative NLR was significantly higher in subjects with ovarian cancer than those with a benign tumor. They have also utilized their finding using Cox multivariate analysis, to demonstrate that NLR positivity (above the 2.60 cut-off), age and late stage (Stage III or IV) are independent poor prognostic factors with NLR being having the highest predictive power.[17] With regards to using NLR as a prognostic factor for the stage of disease, progression-free survival (PFS) and overall survival (OS), several studies have found a relationship between advance stage disease, decreased overall survival and even adverse surgical and platinum-based therapy resistance with increasing NLR.[18-20] Like NLR, the use of PLR as a prognostic factor for malignant epithelial tumors shows that increased PLR levels are associated with advanced-stage disease, poor response to chemotherapy, and poor surgical outcome.[17,19]

General Objective

To determine the significance and relationship of preoperative NLR and PLR in predicting a diagnosis of malignant surface epithelial ovarian tumor.

Specific Objectives

1. To compare patient factors such as age, size of tumor and CBC parameters in the benign and malignant ovarian epithelial tumors.
2. To compare the values of NLR of post-operatively diagnosed benign ovarian epithelial tumors versus the values of NLR of post-operatively diagnosed malignant ovarian epithelial tumors.
3. To correlate and compare the values of PLR of post-operatively diagnosed benign ovarian epithelial tumors versus the values of PLR of post-operatively diagnosed malignant ovarian epithelial tumors.
4. To determine the sensitivity and specificity of PLR and NLR at generated cut-off points from the Receiver Operating Characteristics (ROC) curve.
5. To analyze the difference in PLR and NLR between patients with malignant ovarian epithelial tumors and those with benign ovarian epithelial tumors and to determine whether this difference can be of clinical utility to the surgeons.

METHODOLOGY

Data Collection

The Philippine General Hospital, a governmental tertiary and national university hospital, receives referrals for subspecialty care. Service patients, mostly those diagnosed with adnexal tumors, commonly ovarian, are referred to the Gynecologic Oncology out-patient for surgical intervention. Surgically removed tissues from these patients are sent for routine histopathologic evaluation; sometimes for intraoperative consultation (frozen section) depending on the case. The section of Surgical Pathology under the Department of Laboratories receives the specimens and assigns them to the pathology resident for gross examination and initial histopathologic evaluation. Afterwards, the case is shown to a consultant pathologist wherein the final diagnosis will be rendered.

The study was cross-sectional by design and involves patients diagnosed with epithelial ovarian neoplasms in Philippine General Hospital service patients for the year 2015 and 2016. The histopathologic reports indicating the patient's diagnosis are obtained from the Section of Surgical Pathology organ file records.

The study included 351 cases of women, ages 11 to 83 years old, assessed preoperatively with an adnexal mass, surgically managed by Gynecologic Oncology physicians, and diagnosed with an epithelial ovarian neoplasm by the consultant pathologist. The study population is further divided into benign (209 patients) and malignant (142 patients) groups. Patients with a benign ovarian tumor served as the control group (group I), while those with a malignant diagnosis belong to the disease group (group II). Patients with a borderline diagnosis fell under group I because it is considered that their clinical course behaves more similarly to a benign condition rather than a malignancy. We also excluded patients with other primary or concurrent neoplastic malignant lesions of the female genital tract (e.g., uterine or cervical) or elsewhere (e.g., colonic tumors). In the event of a benign tumor in one ovary and a diagnosis of malignancy in the other, we have included this patient and her data in malignancy group. We also recorded additional patient information in the surgical pathology report such as age, size of tumor and laterality.

Simultaneously, patient CBC records were retrieved in the Laboratory Information System (LIS). The CBC parameters recorded includes red blood cell (RBC) and white blood cell (WBC) count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet count and the differential counts of WBC (lymphocyte, neutrophil, monocyte, eosinophil and basophil). Patients without CBC records have been excluded from the study as well as patients with platelet counts values written as "clumps." The date of the surgery was determined, and we have only used CBC results that have been done preoperatively and would not exceed a month before the surgery.

Statistical Analysis

CBC parameters were organized via Microsoft Excel. The neutrophil and lymphocyte counts were computed in absolute values from their percentages based on the WBC count. The NLR was obtained by dividing the neutrophil count with the lymphocyte count. Likewise, the PLR was obtained by dividing the platelet count with the lymphocyte count. Mann-Whitney U test was done to determine whether there is a significant difference in the variables (age, ovarian size, CBC parameters, NLR and PLR) between the benign and malignant groups.

To determine the diagnostic accuracy of the PLR and NLR in determining malignancy, their values were plotted in the Receiver Operating Characteristics (ROC) curve. The ROC curve plots the true positive rate (TPR) against the false positive rate (FPR) and corresponding Area Under the Curve (AUC) has been determined. The cut point with maximal Youden index was reported as the optimal cut-off point. Youden index is a statistic defined as the sum of the sensitivity and specificity minus one, with a maximum value equal to one, which denotes a perfect test, and a minimum value equal to zero, which denotes the test has no diagnostic value. It is used when sensitivity and specificity are diagnostically equally important or desirable. The data and ROC curve were tested and generated using Stata version 17.1.

Ethical Considerations

Ethical review and approval were sought and was done through the University of the Philippines Manila Research Ethics Board (UPMREB). All surgical pathology reports and CBC results were deidentified and given a numerical code to ensure anonymity of the patients.

Limitations of the Study

The sample population included service patients for the year 2015 and 2016. This excluded patients from the pay services where histopathology reports were not arranged in the organ filing system. Also, other patient information outside of the histopathology report and CBC were not included in the study. Therefore, any comorbidities and medical conditions were unknown to us.

RESULTS

Characteristics of Benign and Malignant Groups

We have included in this study 351 cases of ovarian surface epithelial neoplasms. We have used the data of 209 benign and 142 malignant cases. We have 194 diagnosed cases of epithelial ovarian neoplasms in 2015 and 202 cases in 2016. There was an increase in cases from 2015 to 2016 in the benign and malignant groups by 11% and 22%, respectively. Figure 1 shows the distribution of benign and malignant patients according to year.

Mucinous tumors, specifically mucinous adenoma and mucinous adenocarcinoma, was the most common diagnosis comprising 47% (increased to 70%, including the borderline mucinous tumors) and 40% of the benign and malignant groups, respectively. This was followed by the serous tumors, serous adenoma and serous adenocarcinoma comprising 29% (increased to 30% including

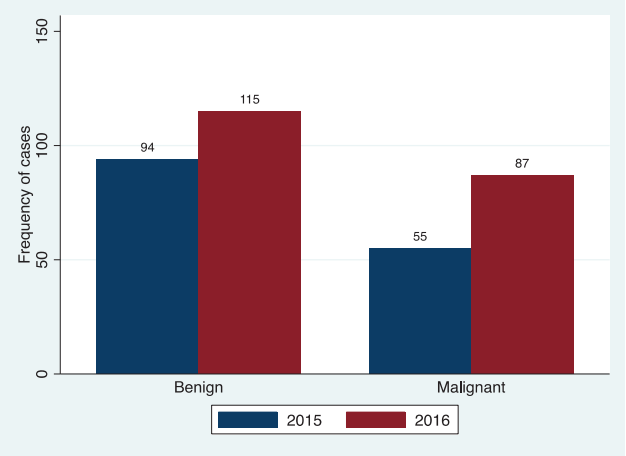

Figure 1. Distribution of benign and malignant cases in 2015 and 2016.

the borderline serous tumors) and 28% of the benign and malignant groups, respectively. The distribution of the benign and malignant diagnoses is shown on Figure 2.

Age, size of the mass and CBC parameters between the benign and malignant group were compared based on the median, interquartile range and p-value using Mann-Whitney U test. The summary of these findings is shown on Tables 1 to 3. There was significantly higher median/mean rank of the following variables among the malignant ovarian tumor group than the benign ovarian tumor group: age, RDW, platelet count, WBC count, absolute neutrophil count, PLR and NLR. On the other hand, there was significantly lower median/mean rank of the following variables among the malignant ovarian tumor group than the benign ovarian tumor group: hemoglobin, MCV, MCH, MCHC, absolute lymphocyte count and lymphocyte differential count. For the following variables, there was no sufficient evidence to conclude that they have significant median/mean rank differences between the benign and malignant ovarian tumor groups: tumor size, RBC count, hematocrit, monocyte differential count and basophil differential count.

The ROC Curve of PLR and NLR

The ROC curve for PLR had an AUC of 0.6629 [0.6043, 0.7215]. The optimal cut-off point was set at 195.99 with the maximal Youden index of 0.295 [9.193, 0.396]. The corresponding sensitivity of this test to determine malignancy at this point was 56.5% [47.8, 64.6], while the specificity was at 73.2% [66.7, 79.1]. Figure 3 shows the PLR values on the ROC curve; plotted as the x-axis being the true positive rate (sensitivity) and y-axis as the false positive rate (1-specificity).

The ROC curve for NLR had an AUC of 0.6616 [0.6051, 0.7180]. The optimal cut-off point was set at 2.60 with the maximal Youden index of 0.316 [0.219, 0.413]. The corresponding sensitivity of this test to determine malignancy at this point was 76.1% [68.2, 82.8], while the specificity was at 55.5% [48.5, 62.4]. Figure 4 shows the NLR values on the ROC curve; plotted as the x-axis being the true positive rate (sensitivity) and y-axis as the false positive rate (1-specificity).

Figure 5 shows a comparison between the ROC curves of PLR and NLR, it showed the seemingly inverse relationship the two variables had with each other, that while NLR had greater sensitivity, PLR had greater specificity. Table 4 summarizes the ROC curve analysis.

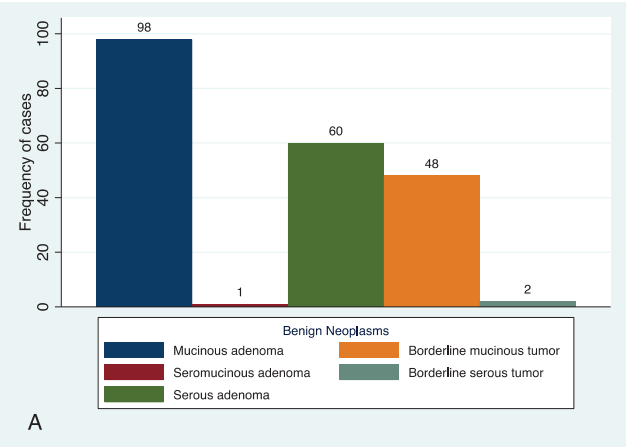

Figure 2. Distribution of **(A)** benign ovarian tumors and **(B)** malignant ovarian tumors according to specific diagnosis.

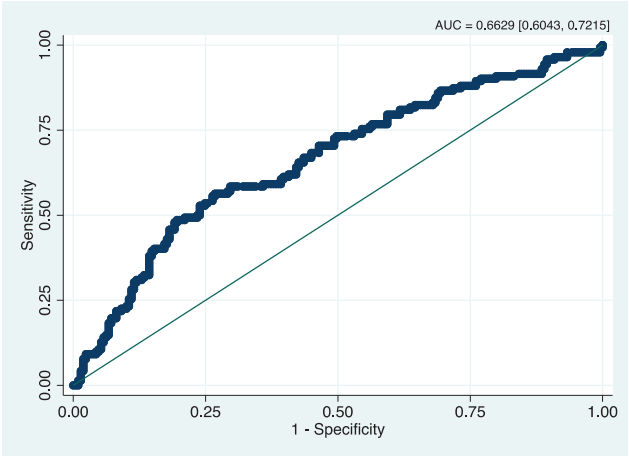

Figure 3. ROC curve for PLR with an AUC of 0.6629 [0.6043, 0.7215]. The optimal cut-point is 1.99 based on the Youden index of 0.295 [0.193, 0.396]. This cut-point has sensitivity of 56.3% [47.8, 64.6] and specificity of 73.2% [66.7, 79.1].

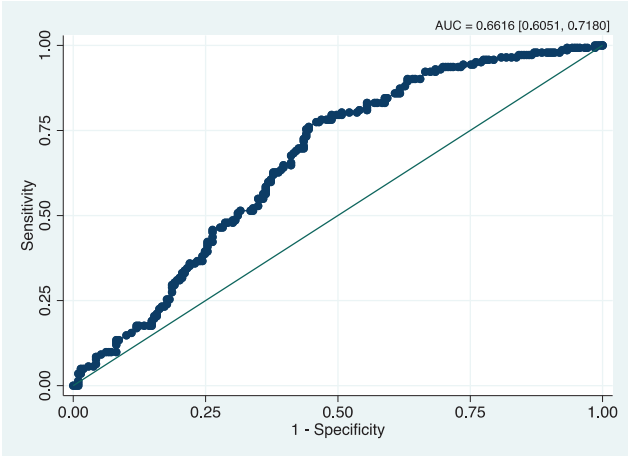

Figure 4. ROC for NLR with an AUC of 0.6616 [0.6051, 0.7180]. The optimal cut-point is 2.60 based on the Youden index of 0.316 [0.219, 0.413]. This cut-point has sensitivity of 76.1% [68.2, 82.8] and specificity of 55.5% [48.5, 62.4].

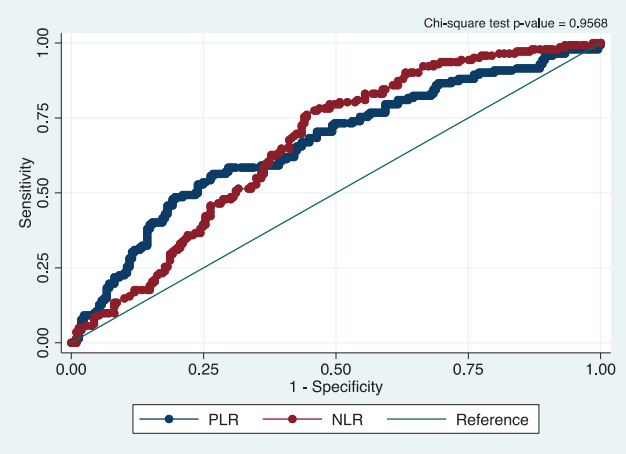

Figure 5. ROC curves of PLR and NLR have no sufficient evidence of significant differences in AUC (p-value = 0.9568).

DISCUSSION

ROC Curve Analysis

Our findings do not stray from data in other studies as demonstrated in a systematic review by Prodromidou et al., that included 18 studies, using PLR and NLR to detect and assess prognosis (in progression-free survival and overall survival). This study mentions that majority of the studies had relatively similar cut-off values denoting applicability of these biomarkers. However, actual efficacy remains relatively small ranging between 55% to 80%.[18]

Ozaksit et al., evaluated PLR as a diagnostic test to differentiate adnexal tumors between neoplastic and non-neoplastic in adolescent patients. PLR was used in conjunction with the size of the mass, CA-125 and mean platelet volume. With a PLR cut-off value at 140, they found that the sensitivity and specificity of PLR in diagnosing neoplastic tumors is 65.7% and 57.6%, respectively.[21] Further studies by Polat et al., Yildrim et al. and Bakakak et al. also used ROC analysis to differentiate malignant tumors from the benign tumors with their cut-off points set at 2.47, 3.35 and 3.47 respectively.[22-24] Their NLR sensitivity ranged from 55% to 68.8% while specificity ranged from 54.1% to 81%. The above studies also found that PLR was a significant predictor of malignancy. Their PLR cut-off values were at 144.3, 572.9 and 161.13 respectively. Yildrim et al., set their cut-off point at 100% sensitivity in exchange for a poor 0.38% specificity. The NLR sensitivity of Polat et. al and Bakakak et al., were 54% and 66.7%, respectively and the specificity was at 59% and 77.9%, respectively. Compared to the three reported studies, we report a higher NLR sensitivity. Although we are uncertain as to the exact reason for this striking difference, we can hypothesize that patients who sought consult at our institution may have already been in the higher stage of their disease at the time of admission and surgery.

Clinical Use of PLR and NLR

We reiterate that CBC and its parameters were never done to diagnose malignancy outright but is only meant to give the clinician a very initial suspicion of malignant condition. Being the most common first line diagnostic test, like most screening methods, increasing sensitivity is better. Among the two, NLR has a higher sensitivity at the set cut-off point. Values above a particular cut-off point indicate the level of sensitivity and specificity at which the diagnosis would most likely be a malignancy. Conversely, when we maximize sensitivity to 95%, the cut-off for NLR would be about 1.64-1.65. Consequently, our specificity would drop to 22-24% but clinicians may be interested at this point with detection of any possibility of a malignancy. If we were to give PLR the same 95% sensitivity, the cut-off would be along 85-86 and in exchange for a decreased specificity of 10-11%. Surely, a highly sensitive test casts a wider net and thus leads to an overdiagnosis of malignancy. But we intended that this data be interpreted in a way that PLR

Table 1. Clinicopathologic characteristics of the post-operatively diagnosed ovarian epithelial tumor patients in the Philippine General Hospital in the years 2015-2016

Variable	Benign		Malignant		*p*-value*
	Median	IQR	Median	IQR	
Age, years	38.5	27	49	16	<0.0001
Ovarian tumor size, cm	16	11	16	9	0.5669
Complete blood count					
RBC count, 10^6/uL	4.35	0.67	4.33	0.65	0.4286
Hemoglobin, g/dL	124	19	116.5	19	<0.0001
Hematocrit, %	38	5	37	5	0.0559
Mean corpuscular volume, fL	86.8	6.4	85.6	8.1	0.0484
Mean corpuscular hemoglobin, pg	28.7	2.8	27.6	3.4	<0.0001
Mean corpuscular hemoglobin concentration, g/dL	328	19	317	25	<0.0001
Red cell distribution width, %	13.4	1.5	14.55	2.9	<0.0001
Platelet count, 10^9/uL	295	109	364.5	193	<0.0001
WBC count, 10^3/uL	8.62	4.37	10.325	5.67	0.0001
Absolute neutrophil count, 10^3/uL	5.26	4.26	7.63	5.31	<0.0001
Absolute lymphocyte count, 10^3/uL	2	0.96	1.84	0.97	0.0099
Differential count					
Neutrophil, %	63	20	73	16	<0.0001
Lymphocyte, %	26	17	18.5	14	<0.0001
Monocyte, %	5	2	5	2	0.2747
Eosinophil, %	2	3	2	2	0.0273
Basophil, %	0	1	0	1	0.8913
Platelet – lymphocyte ratio	143.16	95.23	206.7	167.91	<0.0001
Neutrophil – lymphocyte ratio	2.41	2.98	3.82	4.18	<0.0001

*Mann Whitney U test

Table 2. Comparison of the Median and Interquartile Range Between Group I (Benign) Service Patients in the Philippine General Hospital for the year 2015 and 2016

Variable	Borderline Mucinous		Borderline Serous		Mucinous Adenoma		Serous Adenoma	
	Median	IQR	Median	IQR	Median	IQR	Median	IQR
Age (years)	43	25.5	53	22	35	26	39	27
Size (cm)	20.5	7.25	16.5	1	16	10	9.5	10.5
RBC (x10^6 cells/uL)	4.275	0.71	4.445	0.89	4.36	0.61	4.39	0.705
WBC (x10^3 cells/uL)	9.425	4.615	10.89	6.46	7.59	3.99	9.19	5.445
Hemoglobin (g/dL)	118.5	20.5	125	16	124	16	125	20
HCT (%)	0.365	0.05	0.38	0.06	0.38	0.05	0.38	0.05
MCV (fL)	85.25	8.9	86.35	4.7	87.65	5.6	86.15	5.95
MCH (pg)	28.2	3.3	28.25	1.9	28.8	2.7	28.9	2.5
MCHC (g/dL)	322	24.5	327	4	328	16	330.5	14.5
RDW (%)	13.95	1.95	13.3	0	13.25	1.5	13.5	1.9
PLT (x10^9 cells/uL)	327	122	329	104	290.5	98	287.5	103
Neutrophl	0.685	0.185	0.605	0.05	0.59	0.21	0.675	0.23
Lymphocyte	0.21	0.155	0.28	0.02	0.28	0.17	0.245	0.195
Monocyte	0.05	0.02	0.05	0.02	0.06	0.03	0.05	0.02
Eosinophil	0.02	0.03	0.065	0.05	0.03	0.04	0.02	0.025
Basophil	0.01	0.01	0	0	0	0.01	0	0.01
PLR	180.425	156.55	113.485	40.89	135.355	78.31	137.71	111.23
NLR	3.285	3.675	2.165	0.33	2.075	2.25	2.75	4.88

Table 3. Comparison of the Median and Interquartile Range Between Group II (Malignant) Service Patients in the Philippine General Hospital for the year 2015 and 2016

Variable	Mucinous Adenocarcinoma		Serous Adenocarcinoma		Clear Cell Carcinoma		Endometrioid Carcinoma	
	Median	IQR	Median	IQR	Median	IQR	Median	IQR
Age (years)	49	18	44.5	18.5	51	15	52	7
Size (cm)	20	6	11	5.5	17	6	13	8
RBC (x10^6 cells/uL)	4.36	0.67	4.31	0.645	4.28	0.83	4.05	0.69
WBC (x10^9 cells/uL)	9.06	4.26	10.33	5.885	10.61	6.83	12.51	8.17
Hemoglobin (g/dL)	120	20	118.5	19	107	32	112	21
HCT (%)	0.37	0.06	0.365	0.055	0.35	0.08	0.36	0.05
MCV (fL)	86.5	5.8	85.45	9.65	83.8	8.2	85	10.4
MCH (pg)	27.8	2.6	27.65	4.4	26.6	4.1	27.5	2.3
MCHC (g/dL)	317	20	318	30.5	304	25	319	22
RDW (%)	14.2	1.9	14.85	3.7	14.6	1.8	15	2.9
PLT (x10^9 cells/uL)	352	156	362.5	171	432	281	399	210
Neutrophil	0.7	0.14	0.735	0.17	0.77	0.15	0.78	0.15
Lymphocyte	0.22	0.11	0.185	0.145	0.13	0.15	0.15	0.12
Monocyte	0.06	0.03	0.06	0.01	0.04	0.02	0.04	0.02
Eosinophil	0.02	0.03	0.015	0.02	0.01	0.02	0.01	0.02
Basophil	0	0.01	0.01	0.01	0	0.01	0	0.01
PLR	198.53	145.83	181.335	172.02	260.43	201.94	224.09	109.87
NLR	3.23	2.87	3.82	4.02	5.92	9.58	5.2	4.31

Table 4. Summary of ROC Analysis: Cut-off point, Sensitivity, Specificity and AUC

	AUC	Cut-off point	Youden Index	Sensitivity	Specificity
PLR	0.6629 [0.6043, 0.7215]	195.99	0.295 [9.193, 0.396]	56.5% [47.8, 64.6]	73.2% [66.7, 79.1]
NLR	0.6616 [0.6051, 0.7180]	2.60	0.316 [0.219, 0.413]	76.1% [68.2, 82.8]	55.5% [48.5, 62.4]

and NLR would only alert the clinician and stress the need for ordering more specific tests.

An AUC of 0.66 for both PLR and NLR is useful for as a screening tool and because they are not intended to be used in isolation. In practice, risk of malignancy indices (RMI) comprised of CA-125, menopausal status and ultrasonographic findings are used to improve diagnostic performance.[25] It is not uncommon that studies include other tumor markers, specifically CA-125, to increase diagnostic accuracy. They have seen that this further increased diagnostic ability, particularly in the early stages of disease.[23] A study evaluated the use of NLR, as well as tumor markers CA-125 and CA-19-9, in predicting a benign and borderline versus malignant mucinous ovarian tumor for patients intraoperatively diagnosed as a borderline mucinous tumor. The WBC count, neutrophil and NLR appear to be significantly higher in the malignant group. Comparing the definitive diagnosis of the frozen section, they found out that CA-19-9 and NLR has the highest sensitivity in diagnosing a malignant mucinous tumor with an 81% and 78% sensitivity, respectively.[25] Pairing PLR and NLR values to these RMI achieves greater diagnostic accuracy.[26,27] The added convenience from the readily accessible PLR and NLR makes it a good and flexible screening tool. Furthermore, intraoperative histopathologic consultation or frozen section may still be done and will always be an option for tumors that remain equivocal despite all these factors.

CONCLUSION AND RECOMMENDATIONS

Early detection of ovarian malignancies is a deterrent to a fatal course and poor prognosis. Hence, any novel biomarkers have gained much attention in research. The utility of CBC parameters such as PLR and NLR have some diagnostic value but at present, they cannot be entirely independent of other clinical, laboratory and radiologic signs indicative of malignancy. The CBC parameters are indeed important cost-effective tools but may still be affected by unaccounted variables and pathologies outside our ovarian neoplasms. Studying PLR and NLR further, albeit in a more specific and controlled sample such as in menopausal women or those with early-stage disease may help limit and determine the exact value of PLR and NLR. We recommend further evaluation of NLR and PLR against other currently used diagnostic modalities such as CA-125, International Federation of Gynecology and Obstetrics (FIGO) staging and imaging studies to compare its acceptability as a prognostic marker. Studies regarding determination of cut-off values may also be done to determine an acceptable level of sensitivity and specificity for NLR and PLR.

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All authors fulfilled the ICMJE authorship criteria.

AUTHOR DISCLOSURE

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OPEN ACCESS – ORIGINAL ARTICLE

Intraobserver and Interobserver Agreement in the Scoring of PD-L1 (SP142) and Tumor-Infiltrating Lymphocytes in Triple Negative Breast Cancers*

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ABSTRACT

Objectives. Known for their poor outcomes, triple negative breast cancers (TNBCs) have been investigated for immune checkpoint inhibitors that target Programmed death ligand 1 (PD-L1). In the recent decade, tumor-infiltrating lymphocytes (TILs) have also become potential biomarkers. The aim of the study is to determine the reproducibility of PD-L1 scoring system for TNBC (SP142 clone) and TILs interpretation in the local setting through intra- and interobserver agreement.

Methodology. Forty-three primary resection specimens TNBC were evaluated on two occasions with PD-L1 (Roche VENTANA SP142 assay) and TILs by two breast pathologists and one general pathologist on physical glass slides. PD-L1 expression was determined by at least 1% positivity among immune cells within the tumoral area and contiguous peritumoral stroma while TILs was assessed based on International Immuno-Oncology Biomarker Working Group on Breast Cancer. Kappa statistic for PD-L1 and TILs categories while intraclass correlation coefficient (ICC) were assessed, with cutoffs of 0.80 and 0.70, respectively.

Results. The overall interrater kappa statistic for PD-L1 on the first and second rounds were weak at 0.506 (95% CI: 0.334-0.679) and minimal at 0.314 (95% CI: 0.142-0.487), respectively. Intraobserver kappa statistic for PD-L1 were varied across the three readers while interobserver kappa values for PD-L1 showed none (0.181) to moderate (0.789) agreement. The TILs intraobserver reliability showed poor to good agreement, with the highest ICC of 0.889 (95% CI: 0.805-0.938).

Conclusion. This study demonstrated variable intra and interobserver agreement for both TILs and PD-L1 expression. Although it is desirable to have strong to almost perfect agreement, the kappa and ICC values suggest additional room for improvement. In light of the repercussions in management of patients who will undergo immune checkpoint inhibitor therapy, regular training sessions, concurrences of equivocal results, and possible use of digital pathology as a medium in interpreting TILs and PD-L1 stains to achieve consistent results.

Key words: breast cancer, triple negative breast cancer, immunohistochemistry, PD-L1, TILs

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INTRODUCTION

Breast cancer still remains as the number one reported cancer. In 2020, there are 27,163 cases of newly diagnosed breast cancer (17.7%) in the Philippines.[1] Triple negative breast cancers (TNBC), by definition, lack the expression for estrogen receptor (ER), progesterone receptor (PR) and HER2/neu using immunohistochemistry (IHC) stains. TNBCs are known for their poor outcomes and are unresponsive to conventional hormonal therapy.[2] Tumor-infiltrating lymphocytes (TILs) were reported to have prognostic value in TNBC and are used as a clinical biomarker for the prognostication of TNBC. Higher TILs were regarded as good prognostic indicators for patients who receive atezolizumab monotherapy.[3]

Programmed death ligand 1 (PD-L1) found on the surface of some inflammatory cells has a role in the downregulation of the immune pathway leading to reduced production of cytokines, increasing tumorigenesis and tumor aggressiveness.[4] In addition, PD-L1 positivity was seen to be associated with increased stromal TILs and improved survival. Both PD-L1 interpretation and TILs require

necessary experience and training prior to becoming the standard of histopathological reports of breast carcinoma.[5]

Reporting of TILs have been encouraged as part of the histopathological examination of breast carcinomas, spearheaded by the International Immuno-Oncology Biomarker Working Group on Breast Cancer and mentioned in the 2019 WHO Classification of Breast Tumors.[6,7] However, its implementation as part of routine practice requires validation, which includes testing its reproducibility. To date, there are limited studies on intraobserver and interobserver variability in the local setting. PD-L1 is a biomarker for TNBC, and it would be a good measure to evaluate its precision and applicability among Filipino pathologists. It can also be used as a measure of service quality within the laboratory by looking into the consistency and accuracy of rendered results. Hence, this study aimed to determine the reliability of PD-L1 and TILs scoring.

METHODOLOGY

Case Selection

This study was approved by The Medical City Institutional Review Board. All TNBC patient specimens from 2017-2019 were identified using the laboratory information system (Technidata) of the Section of Anatomic Pathology, Department of Laboratory Medicine and Pathology. The specimen selection criteria were as follows: resection specimens confirmed to have TNBC by IHC stains from 2017 to 2019. Patients with equivocal or positive HER2 IHC stains, DCIS-only by histopathologic diagnosis were excluded. Out of 100 TNBC from 2017-2019, 43 resection specimens were included in the study. None of the specimens were metastatic TNBC. Clinicopathologic information such as age, sex, tumor size, tumor focality, histologic type, Nottingham histologic grade, nuclear grade, pathologic stage, lymph node metastasis, lymphovascular invasion, presence or absence of DCIS, previous neoadjuvant chemotherapy was obtained from medical records and the laboratory information system.

Readers

Three pathologists were identified as part of the study. Their expertise varied from three to more than 30 years of experience in anatomic pathology. Two out of three pathologists had prior clinical observership in breast pathology. In terms of PD-L1 interpretation, one had prior experience with PD-L1 SP263 clone, but none had PD-L1 any previous sign-out with the SP142 clone. All pathologists underwent training using the Roche Tissue Diagnostics Pathology Education Portal for PD-L1 (SP142) TNBC Interpretation Education and were subsequently certified. On the other hand, no online training for TILs scoring was available as of this writing. The readers familiarized with the TILs working group consensus guidelines and used reference images from their website.

PD-L1 Scoring and TIL Evaluation

The IHC stains were performed on paraffin embedded tissues fixed in 10% neutral buffered formalin for 6-72 hours. All chosen tissues were stained with Roche VENTANA PD-L1 (SP142) Assay antibody detection kit using BenchMark ULTRA System (Ventana Medical Systems, Inc.) by one proficient registered medical technologist. Pre-analytic factors such as positive and negative controls, adequacy of specimen (at least 50 viable invasive tumor cells) and tissue processing were considered prior to scoring PD-L1.[8] The hematoxylin and eosin (H&E) stained slides and previous ER, PR and HER2 IHC stains were evaluated alongside the PD-L1 stained slides. Positivity for PD-L1 expression was determined by a cut-off of 1% among immune cells with dark brown punctate, linear, or circumferential staining within the tumoral area and contiguous peritumoral stroma. Conversely, a score below 1% is considered negative.[8] TIL scoring was performed on the H&E stained slide based on the recommendations of International Immuno-Oncology Biomarker Working Group on Breast Cancer. Evaluation of the stromal area within the defined tumor borders for mononuclear infiltrates were done using low-power magnification (10X). Percentage of stromal lymphocytes were reported as averages of the entire area evaluated. TILs were reported as a continuous variable, as recommended by the TILs working group.[6] For this study, interpretations were categorized arbitrarily as follows: low (0-10%), intermediate (11-50%), high (>50%), following reproducibility studies on TILs.[9,10] Interpretation was performed on two separate occasions following at least a two-week washout period. The sequence of control numbers was randomized, and the pathologists were blinded to the results of their previous interpretations.

Analysis

Descriptive statistics of the demographic, histopathological variables of the case selection were determined using frequency and percentage for categorical variables and mean and standard deviation for continuous data. PD-L1 expression and categorical TILs group were also reported in frequencies, by each reader. Intra- and interobserver agreement for interpretation of PD-L1 was determined using Cohen's kappa while overall agreement was determined using Fleiss's Kappa. Interpretation of kappa for PD-L1 was based on the proposed ranges of McHugh: 0.00-0.20 as none, 0.21-0.39 as minimal, 0.40-0.59 as weak, 0.60-0.79 as moderate, 0.80-0.90 as strong, and above 0.90 as almost perfect agreement. This is more stringent than Cohen's original suggestion for interpreting kappa, with larger ranges. As the value of kappa decreases, the percent reliability of data decreases due to increasing disagreement among readers. Hence, interpretation of kappa must be done in the appropriate clinical and laboratory context.[11] An acceptable kappa for PD-L1 would be 0.80 and above with McHugh's kappa interpretation to ensure quality results in the clinical laboratory setting.

On the other hand, intra- and interobserver agreement for TILs reported as categorical data were determined using weighted kappa. For TILs reported as continuous data, the Intraclass Correlation Coefficient (ICC) as single measures was used to determine the agreement among three readers. While there are no standard values for interpreting ICC, an ICC of 0.7 is considered acceptable among three readers with a 95% CI. Interpretation of the ICC, as recommended by Koo and Li, were as follows: less than 0.50 as poor, 0.50-0.75 as moderate, 0.75-0.90 as good and greater than 0.90 as excellent reliability.[12] Statistical Package for the Social Sciences (SPSS) version 20 was used in the analysis.

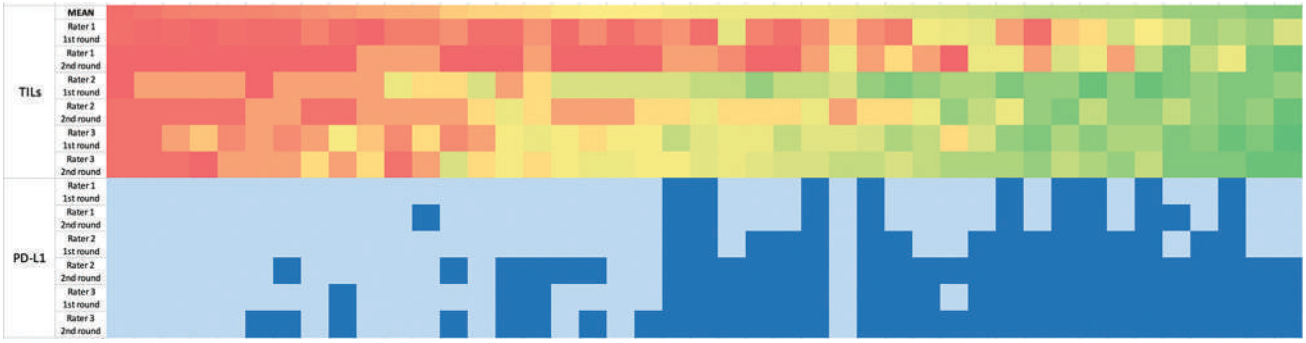

Figure 1. Heat map of the individual interpretations of TILs and PD-L1 arranged according to mean on the topmost row.

RESULTS

Among 1039 samples tested for ER, PR and HER2 from 2017-2019, 100 specimens (9.62%) were TNBCs and only 43 were resection specimens that were included in the study. Core biopsies were excluded The most frequent tumors were invasive breast carcinoma of no special type (IDCA) including medullary pattern (67.4%), followed by invasive lobular carcinoma (16.3%) and metaplastic carcinoma (7.0%), and other entities (16.3%). There were nine cases that had neoadjuvant chemotherapy (Table 1).

Table 1. Baseline clinical characteristics of triple negative breast cancer and PD-L1 expression in a tertiary hospital

Clinical characteristics	n	%
Age, year mean (SD)	50.9 (±11.73)	11.7%
20-39 years	7	16.3%
40-59 years	28	65.1%
60 years and above	8	18.6%
Sex (Female)	43	100.0%
Tumor size, cm mean (SD)	4.1	3.5%
Tumor focality		
Single	35	81.4%
Multiple	8	18.6%
Histologic type		
Invasive breast carcinoma of no special type	29	67.4%
Invasive lobular carcinoma and related entities	7	16.3%
Metaplastic carcinoma and other subtypes	3	7.0%
Microinvasive carcinoma	3	7.0%
Mucinous carcinoma	1	2.3%
Nottingham Histologic Grade		
I	6	15.0%
II	24	60.0%
III	10	25.0%
Nuclear grade		
1	4	9.3%
2	24	55.8%
3	15	34.9%
Lymph node metastasis	16	37.2%
Lymphovascular invasion	20	46.5%
Presence of DCIS	23	53.5%
Necrosis in DCIS	16	37.2%
Neoadjuvant therapy	9	20.9%

Individual scores for both PD-L1 expression and TILs are visually represented in a heat map, arranged by the mean TILs score from the three readers (Figure 1). Table 2 shows the reported frequencies of PD-L1 expression and TILs by each reader. Most of the TILs were rated as low, most especially by the first reader. PD-L1 disagreements occurred in nine and eight out of 43 cases in the first and second rounds, respectively, while TILs disagreements on the categorical level occurred in 28 and 25 out of the 43 cases in the first and second rounds, respectively.

The overall kappa statistic for PD-L1 on the first and second rounds were weak at 0.506 (95% CI: 0.334-0.679) and minimal at 0.314 (95% CI: 0.142-0.487), respectively. Intraobserver kappa statistic for PD-L1 were varied across the three readers: 0.482 (weak), 0.707 (moderate) and 0.870 (strong) (Table 3). Interobserver kappa values for PD-L1 showed a wide range of values from none to moderate agreement, with the lowest values belonging to reader 1 vs reader 3 ($k = 0.181$) on the second round, and reader 1 vs reader 2 ($k = 0.230$) on the second round. Moderate agreement was observed between reader 2 and reader 3 on both occasions ($k = 0.639$, $k = 0.789$) (Table 4).

In terms of categorical TILs, the overall ICC was weak at 0.494 (95% CI: 0.149-0.720) and moderate at 0.667 (95% CI: 0.391-0.821) on the first and second rounds, respectively. The intraobserver reliability showed moderate to good agreement, with the highest ICC of 0.889 (95% CI: 0.805-0.938) in terms of quantitative TILs and highest weighted kappa of 0.723 (95% CI: 0.551-0.895) for categorical TILs, both belonging to reader 3 (Table 3). ICCs between each reader were tabulated in Table 4. ICCs generally showed poor to good agreement (0.281 to 0.825). Good agreement was noted to be between readers 2 and 3 on both occasions. Of special note is the medullary pattern of IDCA (5/43 cases), wherein the TILs scoring ranged from low to high (0-80%) and the PD-L1 was rated positive on 21 out of 30 occasions (for both rounds).

Table 2. Frequency of PD-L1 and TILs interpretation among three readers

	Reader 1		Reader 2		Reader 3	
	Round 1 %	Round 2 %	Round 1 %	Round 2 %	Round 1 %	Round 2 %
PD-L1 ≥1%	20.9	25.6	37.2	34.9	44.2	30.2
PD-L1 <1%	79.1	74.4	62.8	65.1	55.8	69.8
TILs – Low (0-10%)	74.4	76.7	32.6	60.5	34.9	32.6
TILs – Intermediate (11-50%)	20.9	16.3	32.6	20.9	46.5	51.2
TILs – High (>50%)	4.7	7.0	34.8	18.6	18.6	16.3

Table 3. Intraobserver agreement of PD-L1 and TILs

	PD-L1		TILs			
	Kappa (95% CI)	*p*-value	Kappa (95% CI)	*p*-value	ICC (95% CI)	*p*-value
Reader 1	0.870 (0.696-1.000)	<0.001	0.418 (0.134-0.701)	<0.001	0.666 (0.462-0.804)	<0.001
Reader 2	0.482 (0.269-0.695)	<0.001	0.449 (0.269-0.629)	<0.001	0.629 (0.212-0.820)	<0.001
Reader 3	0.707 (0.500-0.915)	<0.001	0.723 (0.551-0.895)	<0.001	0.889 (0.805-0.938)	<0.001

Table 4. Interobserver agreement of PD-L1 and TILs

	PD-L1		TILs			
1st Round	Kappa (95% CI)	*p*-value	Kappa (95% CI)	*p*-value	ICC (95% CI)	*p*-value
1 vs 2	0.618 (0.378-0.857)	<0.001	0.225 (0.090-0.360)	0.003	0.281 (-0.093-0.587)	<0.001
2 vs 3	0.639 (0.428-0.849)	<0.001	0.690 (0.522-0.858)	<0.001	0.812 (0.615-0.904)	<0.001
1 vs 3	0.347 (0.145-0.549)	0.003	0.228 (0.059-0.397)	0.007	0.417 (-0.040-0.698)	<0.001
2nd Round	Kappa (95% CI)	*p*-value	Kappa (95% CI)	*p*-value	ICC (95% CI)	*p*-value
1 vs 2	0.230 (0.037-0.423)	0.037	0.379 (0.170-0.588)	<0.001	0.612 (0.299 - 0.789)	<0.001
2 vs 3	0.789 (0.593-0.984)	<0.001	0.500 (0.328-0.671)	<0.001	0.825 (0.626 - 0.913)	<0.001
1 vs 3	0.181 (0.005-0.358)	0.077	0.289 (0.117-0.461)	<0.001	0.565 (-0.022 - 0.814)	<0.001

DISCUSSION

The prevalence of TNBCs in our institution is consistent with epidemiologic data of TNBCs, ranging from 10-20% of all invasive breast carcinomas.[13] The potential of PD-L1 as a biomarker in patients with TNBC was investigated on the IMpassion130 trial, as well as other studies, where overall survival benefit was suggested with the inclusion of an immune checkpoint inhibitor plus paclitaxel in patients with PD-L1 expression.[14,15] Other anti-PD-L1 antibodies have been studied, namely durvalumab and avelumab, with similar molecular mechanisms.[16] However, overall survival benefit of PD-L1 expression in a meta-analysis showed that breast cancer patients with increased PD-L1 expression led to poorer outcomes. A higher PD-L1 expression has been associated with lymph node metastasis, higher histologic grade and negative ER. All of which are linked to lower overall survival.[17] In spite of this, immune checkpoint inhibitors may still have overall benefit given the limited data available to date. Moreover, TIL scoring is prognostic for TNBC in the context of PD-L1 expression. It is an emerging prognostic marker that has been recommended by the International Immuno-Oncology Biomarker Working Group on Breast Cancer and published in the 2019 WHO Classification of Breast Tumours.[6] CD20+ TILs and PD-L1+ TILs were seen as independent prognostic factors for both TNBC and inflammatory breast cancer.[18] TILs have been the target of interest for oncologists because of its potential as a biomarker, a predictive marker and a marker for targeted therapies.[19–21]

Eligibility for PD-L1 (SP142) immune checkpoint inhibitors requires a positivity of ≥1%, a very low cutoff for positivity. PD-L1 may stain both tumor cells and tumor-infiltrating immune cells, which can be a source of confusion. The pattern of staining expected among immune cells should be dark brown, punctate, sometimes circumferential staining, especially for macrophages or dendritic cells. Immune cells may be stain singly or as aggregates. On the other hand, tumor cells can exhibit moderate to strong linear or circumferential stain. It is therefore required for the pathologist to compare the H&E staining to allow distinction between tumor and immune cells.[8]

The laboratory must ensure that the issued result is validated and precise. In the case of PD-L1 expression, an agreement of less than 0.80 raises concerns on reproducibility. PD-L1 scoring has been subjected to various scrutiny because of its variability in interpretation. Intra- and interobserver agreement in this study showed varied results, with modest agreement. Studies on interobserver variability of PD-L1 expression on TNBCs are mixed, with one showing high intraobserver and interobserver agreement while some show low reproducibility of SP142 assays.[5,22] Among antibody clones, the SP142 antibody showed the lowest interobserver agreement, but had substantial intraobserver agreement (k = 0.798 and 0.861), with the authors concluding that it is a reproducible interpretation.[23] On the other hand, reproducibility of the PD-L1 scoring was put into question in a study consisting of 19 pathologists across 14 different institutions. As the number of observers increased, the overall percent agreement decreased. The implications of this study is considerable in that some patients may or may not receive the proper treatment given the differences in scoring.[21,22] Disagreements in PD-L1 expression in terms of categorical TILs showed no specific predilection, as illustrated by the heatmap in Figure 1. Hence, differences in interpreting PD-L1 were not necessarily affected by the amount stromal TILs present in the specimen.

Standard protocols for TIL scoring were proposed by the TILs Working Group using H&E-stained slides with no need for ancillary procedures. No designated cutoffs on agreement have been proposed to date, and acceptable intra- and interpersonal agreement would depend on the institution or clinical use.[6] Similar studies on TILs concordance have been published. In one study, an ICC cutoff of 0.70 for concordance of TIL assessment was deemed acceptable. Though not statistically significant, the authors concluded that TILs interpretation was reproducible but required further refinement and is yet to be implemented in the clinical setting. Specimen factors which may lead to incorrect reading of TILs include apoptosis, individual cell necrosis and stromal fibroblasts.[10] Other factors were presence of reactive plasma cells mimicking tumor cells, plasmacytoid tumor cells mimicking infiltrating plasma cells, and specimens

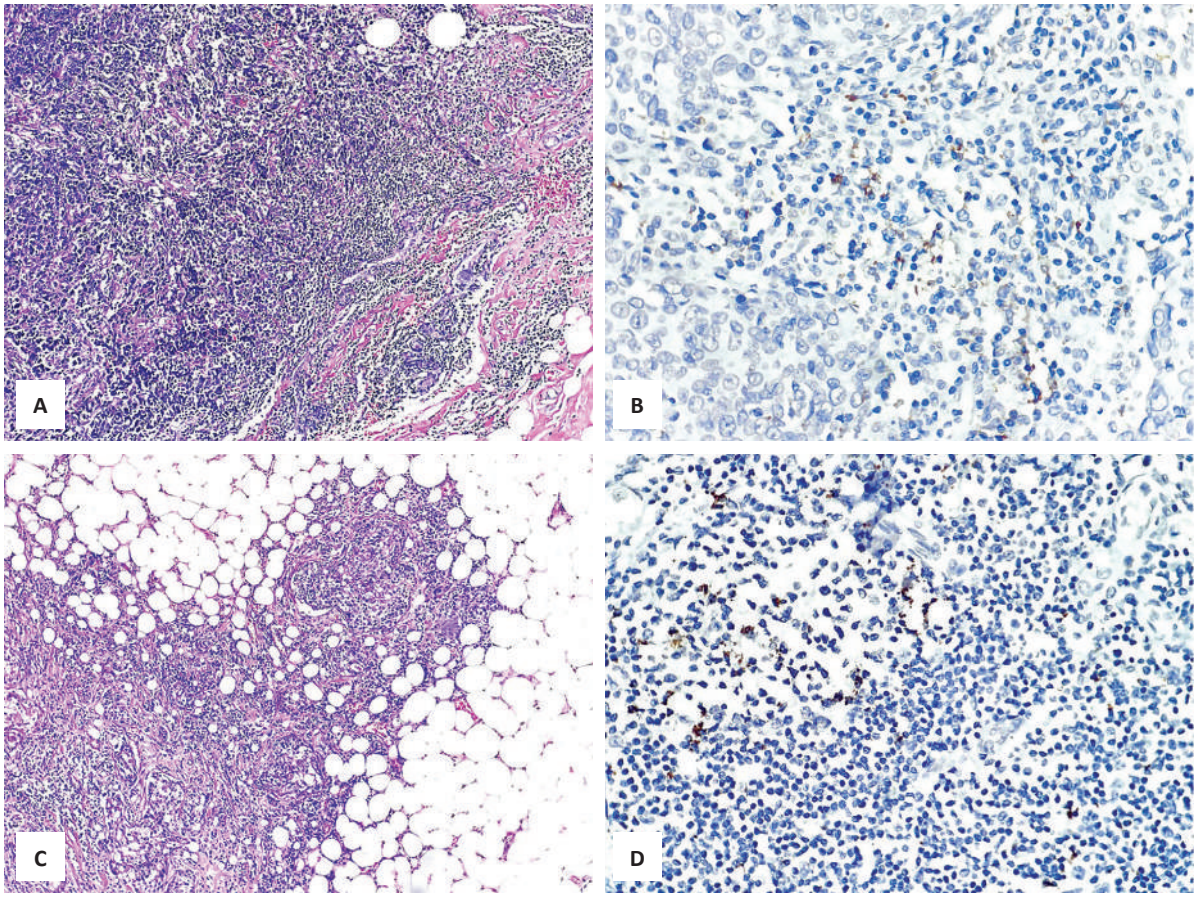

Figure 2. (A and C) Examples of cases with discordant TILs (H&E, 10x) with **(B and C)** their corresponding discordant PD-L1 (SP142) stains (H&E, 40x). Both are IDCA by histologic subtype, with **(A)** showing medullary pattern.

with heavy immune infiltrates.[9] The kappa statistic must be interpreted in the proper clinical context, especially in the laboratory setting where high degree of precision is imperative. The results of the study showed only modest results, compared to the studies which produced good, if not substantial, reliability.[9,10,24] The wide range of intra- and interobserver agreement among readers brings into question the reproducibility of TILs in the local setting. Though standard protocols for TILs and PD-L1 evaluation were provided by the TILs Working Group and Roche Tissue Diagnostics, respectively, a standardized training for evaluation is warranted to improve the reproducibility of results.

Regardless of whether Filipino pathologists are ready to adapt this approach to breast specimens, breast pathology continues to move forward with the advancement of new treatment options. In fact, integrating PD-L1 and TIL scoring into routine practice would provide a comprehensive "immuno-oncological marker" for TNBC patients. It is of utmost importance to ensure its validity and reproducibility by optimizing the workflow from patient selection to tissue processing and standardized evaluation involving multiple disciplines.[21]

This study demonstrated variable intra and inter-observer agreement for both TILs and PD-L1 expression. Although it is desirable to have strong to almost perfect agreement, the kappa and ICC values suggest additional room for improvement. The reasons for which may be due to the new adaptation of the SP142 assay in the institution and the limited experience of the pathologists in interpreting PD-L1 (SP142) immunostains despite taking the recommended course material. The variability of the results can also be due to the differences in pathologists' experience in signing out PD-L1 assays. The first reader has prior experience, albeit with a different clone (SP263) for non-small cell lung carcinoma, with a case load of more than 20 cases in the past two years while the second and third readers were naïve to PD-L1 interpretation. It is suggested that thresholds of each pathologist be realigned by reviewing discordant results to improve reliability. Methodology can be improved with further training and perhaps the use of digital pathology with standardized images for comparison.[6] To improve reproducibility, whole slide images with the aid of computer-based image analysis and automated quantification were recommended.[21] Further studies on PD-L1 expression may be done to investigate variables such as tissue samples, type of tumor, tumor heterogeneity which may affect consistency in interpretation. In light of the repercussions in management of patients who will undergo immune checkpoint inhibitor therapy, the following are suggested to achieve consistent results: regular training sessions for scoring, concurrences of equivocal results, and possible use of digital pathology as a medium in interpreting TILs and PD-L1 stains.

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STATEMENT OF AUTHORSHIP

All authors fulfilled the ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

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EDITORIAL COMMENT

In August 27, 2021, Roche announced their decision to voluntarily withdraw the US accelerated approval for Tecentriq® (atezolizumab) in combination with chemotherapy (Abraxane®, albumin-bound paclitaxel) for the treatment of adults with unresectable locally advanced/metastatic triple-negative breast cancer whose tumors express PD-L1. The drug has other approved indications for other types of cancer. Ventana PD-L1 (SP142) assay is an approved companion diagnostic device for selecting TNBC candidates for atezolizumab treatment. Our peer reviewer pointed out the effect of the withdrawal of the drug from the market as a treatment for TNBC to the significance of the SP142 companion diagnostic assay. Philippine Journal of Pathology, however, believes that the paper still deserves to be published. It retains its value as an academic paper for Filipino pathologists, pathologists in general, to learn much from in terms of mutation detecting assays for candidate screening, cancer prognostication, treatment monitoring, et cetera. In a post-review communication, the authors state that "*though atezolizumab was withdrawn from the market, the SP-142 study demonstrates variable thresholds of pathologists and how differences in experience can affect interpretation. Addressing these gaps in these diagnostic tools may help in terms of quality improvement in the practice of pathology.*" Further, they add that "*the SP-142 assay may not (no longer) be used for TNBCs but still has utility for other malignancies, such as NSCLC (non-small cell lung carcinoma), though with different cut-offs and cells of interest. This exposes the need for training of pathologists and adjustments of thresholds in interpreting PD-L1 expression, especially since these biomarkers are new to the country with limited accessibility. This applies to other clones and brands currently available in the market and those in future development.*"

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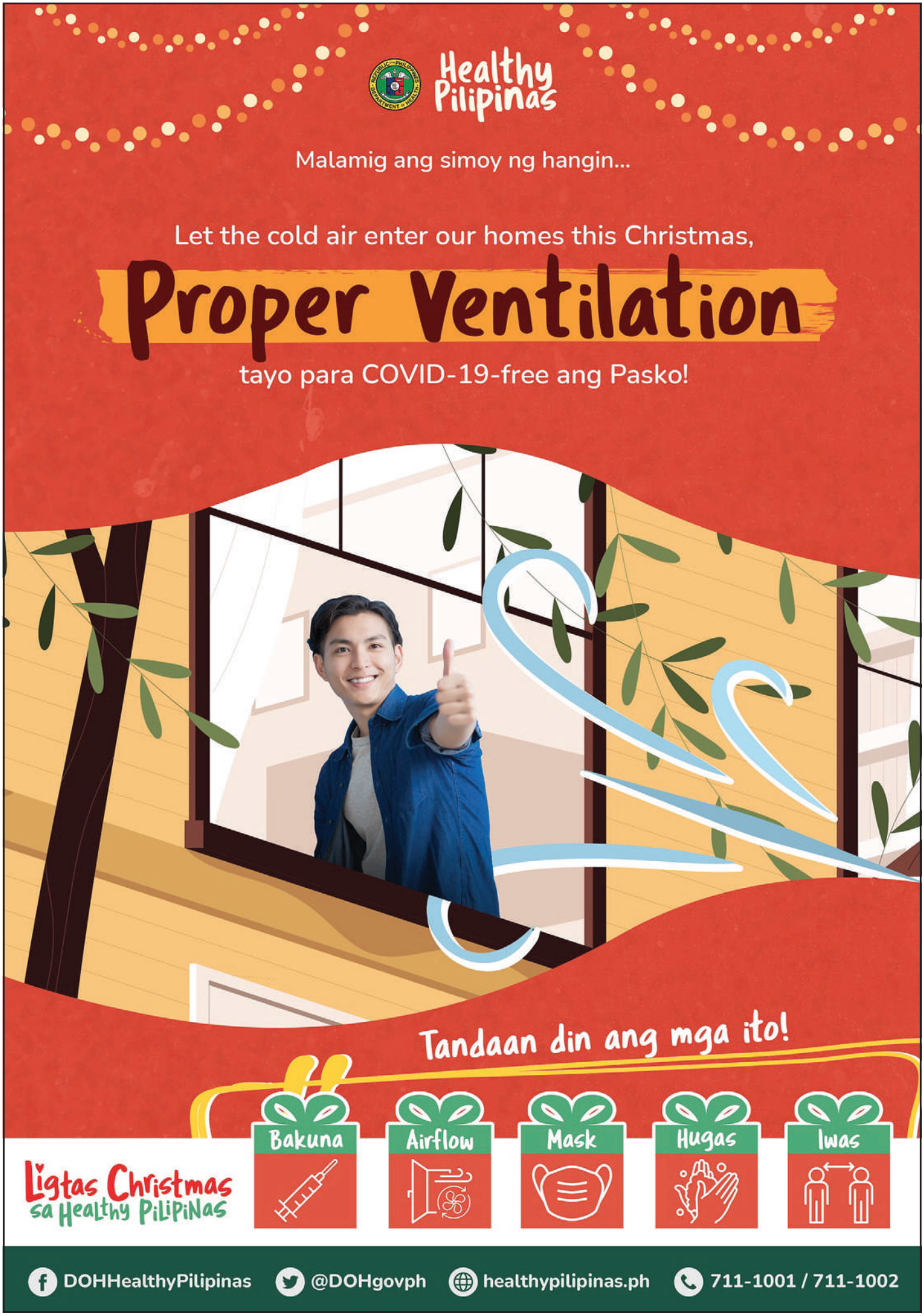

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OPEN ACCESS – CASE REPORT

A Case of Dentinogenic Ghost Cell Tumor

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ABSTRACT

Among the ghost cell lesions, Dentinogenic Ghost Cell Tumors (DGCT) are among the rarest. We report a case of a 45-year-old Filipino man, who presented with a right mandibular mass. Microscopic examination showed a solid neoplasm composed of islands of odontogenic epithelium with areas showing aberrant keratinization forming ghost cells and dentinoid material. We also discuss the pertinent differential diagnosis of ghost cell-containing odontogenic tumors. We report this case due to its rarity, its morphological resemblance to ameloblastoma, and its potential for malignant transformation.

Key words: dentinogenic ghost cell tumor, odontogenic cyst, ghost cells, odontogenic tumors

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INTRODUCTION

Dentinogenic ghost cell tumors are a rare form of ghost cell lesion, accounting for less than 3% of cases. It is described as a locally invasive tumor and was considered as a solid variant of calcifying odontogenic cyst. Here we present a case of a 45-year-old, Filipino male, with a history of right mandibular mass.

DISCUSSION

A 45-year-old Filipino male presented with a three month history of right mandibular mass. Examination revealed that it was non-tender, non-erythematous, with no pain on salivation, and no difficulty eating or swallowing. A CT scan revealed an expansile hypodense lesion involving the mandibular ramus and body (Figure 1). The clinical impression was that of an ameloblastoma. The patient underwent a marginal mandibulectomy and the specimen was sent for histopathologic evaluation.

The specimen consisted of two irregularly-shaped soft to gritty tissues which measured 2.6 x 1.5 x 1.0 and 4.5 x 3.0 x 2.0 cm. Microscopic examination showed a solid neoplasm composed of islands of odontogenic epithelium with areas showing aberrant keratinization forming ghost cells and dentinoid material (Figure 2). The odontogenic epithelium resembles ameloblastoma, with columnar to cuboidal cells, hyperchromatic nuclei, peripheral palisading and reverse nuclear polarity (Figure 3). The presence of aberrant keratinization with calcification forming abundant ghost cells (Figure 4), and dentinoid material surrounding epithelial cells were noted (Figure 5). Based on these features, a diagnosis of dentinogenic ghost cell tumor (DGCT) was rendered.

The rarest among the ghost cell lesions, DGCT accounts for less than 3% of cases.[1] It can involve a wide age range, with a peak incidence at 40-60 years, with a male predilection.[1,2] Most commonly, it occurs as an intraosseous lesion, affecting the posterior maxilla and mandible.[1,2] The size of the mass varies between one to more than ten centimeters in diameter, but is frequently asymptomatic.[2] Etiogenesis remains unclear, but is attributed to cell rests

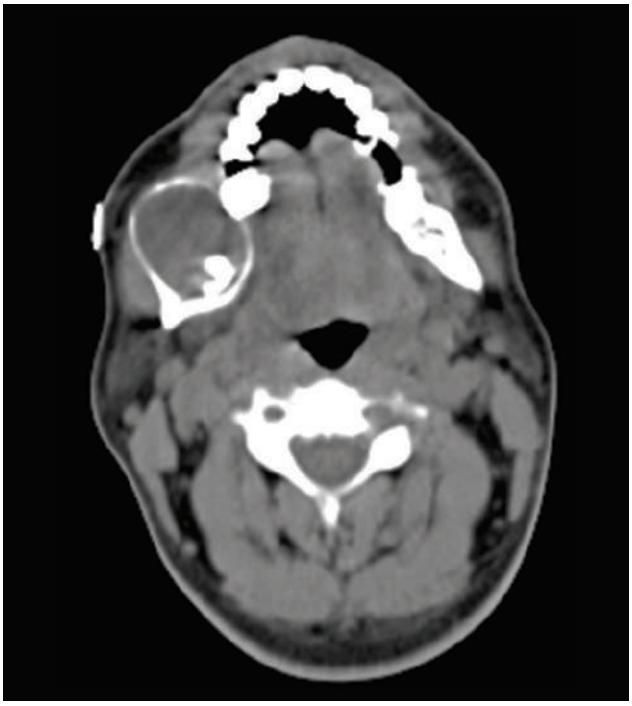

Figure 1. CT scan of the mandible shows a 4.9 x 3.5 x 4.4 cm expansile, predominantly hypodense lesion in the right hemi-mandible, involving the mandibular ramus and body associated with cortical thinning.

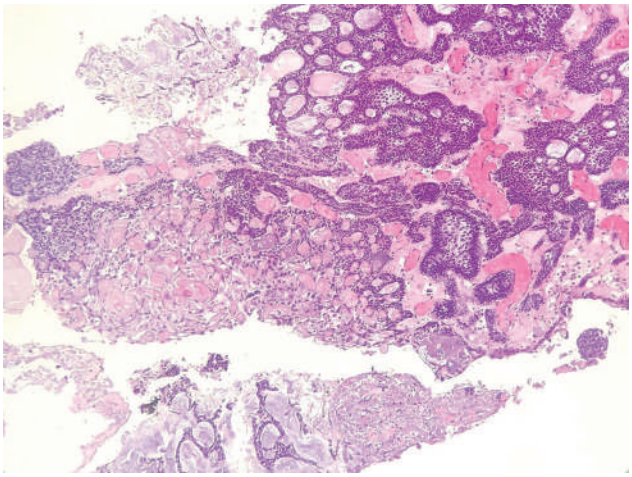

Figure 2. Islands of odontogenic epithelium with areas showing aberrant keratinization forming ghost cells and dentinoid material (H&E, 100x).

of Serres or the surface epithelium.[2] Molecular findings reveal that mutations in ß-catenin, a transcriptional activator of the Wnt pathway, are associated with tumors with ghost cells[3]. Mutations in ß-catenin found in DGCTs have been shown to correlate with ß-catenin immunohistochemistry, however these are not utilized in the diagnosis of DCGTs, the diagnosis being largely made on the basis of morphology.[3,4]

Described as a locally invasive neoplasm, DGCTs are characterized by sheets and rounded islands of odontogenic epithelial cells, which resemble ameloblastoma, seen in a mature connective tissue.[1,2] The resemblance includes

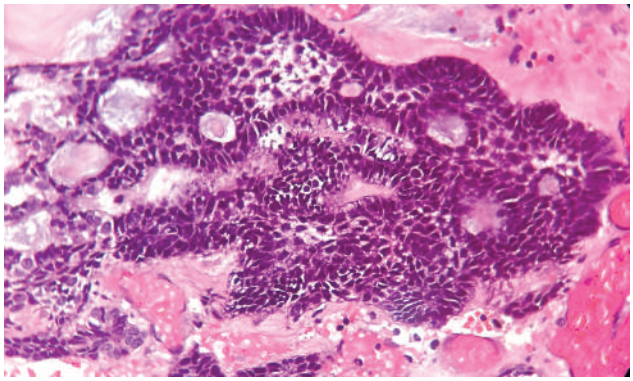

Figure 3. Odontogenic epithelium with peripheral palisading and reverse polarity. Some microcystic spaces are present (H&E, 400x).

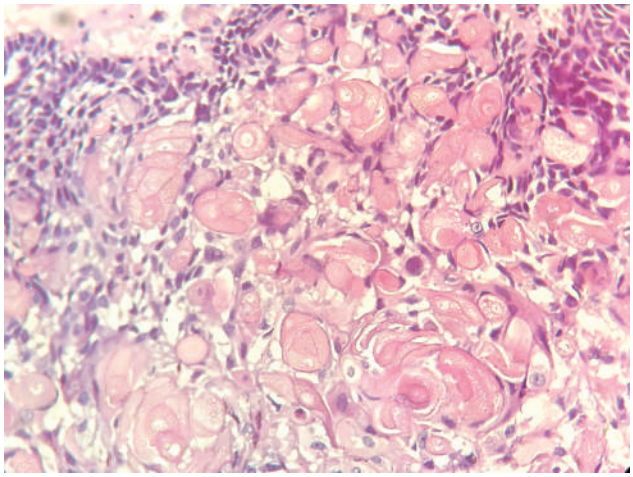

Figure 4. Aberrant keratinization with calcification forming abundant ghost cells (H&E, 400x).

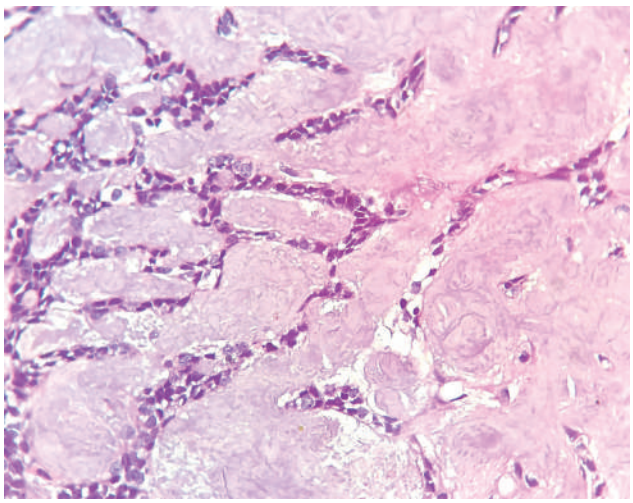

Figure 5. Dentinoid material forming around epithelial cells (H&E, 400x).

characteristic peripheral palisading, reverse nuclear polarization, and presence of a stellate reticulum within the cell nests. In addition, the other characteristic features of DGCTs are the presence of ghost cells and dentinoid material.[1] While the exact origin of the ghost

cells is unclear, it should be noted that they are not pathognomonic, and may also be found in other neoplasms such as ameloblastoma.[2] However, the proportion of ghost cells in DGCTs is much higher (>1-2%) than that in ameloblastoma, making it together with the presence of dentinoid features necessary to the diagnosis of DGCT.[1]

Pertinent differential diagnoses of DGCTs, other than ameloblastoma, include calcifying odontogenic cyst, and ghost cell odontogenic carcinoma. As the name suggests, calcifying odontogenic cysts are unicystic compared to DGCTs, which are solid tumors.[1] On the other hand, ghost cell odontogenic carcinoma may arise from a DGCT or from a calcifying odontogenic cyst precursor, but is differentiated by cytologic evidence of malignancy.[1] This includes mitosis, pleomorphism, hyperchromasia, necrosis and an infiltrative growth pattern.[1]

DGCTs can be treated surgically through enucleation or surgical resection.[2] However due to the small number of reported cases, there is no consensus for an optimal treatment.[1] Long term follow up is recommended due to a recurrence rate of up to 73% in conservatively treated cases.[1] Cases of malignant transformation have also been reported.[1]

CONCLUSION

DGCT is a rare tumor that shares morphological features with other ghost cell-containing odontogenic tumors and with ameloblastoma with which it may be confused. Also, owing to its rarity, there is currently no consensus on an optimal treatment with long term follow up being recommended due to observations of malignant transformation. Reporting these cases may help further elucidate the long-term behavior and most appropriate management of the entity.

ETHICAL CONSIDERATION

Ethics clearance was obtained for the case. The authors ensured that all identifying marks have been removed.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

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OPEN ACCESS – CASE REPORT

Corticosteroid-associated Changes Resembling a Demyelinating Brain Lesion in Diffuse Large B-cell Lymphoma (DLBCL): A Case Report

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ABSTRACT

Biopsies of suspected lymphoma cases with history of pre-biopsy corticosteroid therapy present several diagnostic issues, such as the inability to demonstrate the neoplastic hematolymphoid cells, the similarity of post-corticosteroid changes with inflammatory demyelinating lesions, and the possibility of a demyelinating lesion preceding a central nervous system lymphoma. This report presents the case of a 51-year-old immunocompetent male with a solitary callosal mass, with immunomorphologic features suggestive of a demyelinating lesion on initial biopsy, and upon re-biopsy after three months revealed a diffuse large B-cell lymphoma. Awareness of these issues in post-corticosteroid stereotactic biopsy specimens, together with adequate clinical and radiologic data, is important for proper diagnosis and further therapeutic guidance.

Key words: corticosteroids, demyelination, diffuse large B cell, lymphoma

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INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is a neoplasm of medium to large B lymphoid cells, commonly presenting among elderly males in the seventh decade. Primary central nervous system lymphoma (PCNSL) accounts for <1% of all non-Hodgkin lymphomas and 2.4-3% of all brain tumors. The most common locations are the frontal lobe, periventricular brain parenchyma, and the posterior fossa. Corpus callosum involvement is rare and is reported in only 5% of cases.[1] Secondary involvement of the central nervous system (CNS) by systemic lymphoma has an estimated incidence of 2-5%,[2,3] and leptomeningeal is more common than parenchymal metastasis.[4]

The gold standard for diagnosis is histopathologic examination of stereotactic biopsy specimens. However, in cases wherein corticosteroid treatment was initiated prior to biopsy, typical microscopic characteristics of DLBCL may not be seen. Features may be non-specific or show features of demyelinating lesions. Furthermore, demyelinating disease may confound the diagnosis of DLBCL because of their similar radiologic characteristics and response to corticosteroids.

This report discusses a case of a callosal mass that was initially suggestive on histopathology to exhibit demyelinating disease, but on second biopsy of the mass three months after, was diagnosed as DLBCL.

CASE

A 51-year-old male presented with a six-month history of intermittent bifrontal headache and progressive memory lapses. On admission, the patient was given two doses of Dexamethasone 4 mg, with slight improvement of symptoms. Brain MRI with contrast revealed an enhancing

well-defined mass at the corpus callosum, extending to the occipital forceps, with mass effect. The mass was T1W-hypointense and T2W-intermediate/mildly hyperintense, and radiologic considerations included a CNS lymphoma.

Stereotactic biopsy of the lesion was performed seven months after symptom onset. This was one week from the administration of the last corticosteroid dose. Immunohistomorphologic evaluation revealed a lesion composed of many macrophages and reactive astrocytes, as seen in Figure 1. Few scattered parenchymal and perivascular inflammatory cells were also present, mostly composed of T-cells, and few B-cells highlighted by CD20 and PAX5. These cells, although arranged around vessels, had small nuclei and inconspicuous nucleoli. The lesion also demonstrated a low proliferation index. The case was signed out as a macrophage-rich lesion with chronic inflammation, astrogliosis, and axonopathy, with considerations primarily favoring a tumefactive demyelinating disease. With the history of presurgical corticosteroid therapy, and the clinical and radiologic suspicion of a CNS lymphoma, included in the report was a suggestion for a re-biopsy at least three weeks after the last steroid administration. On admission, further workup revealed a firm mass on the left submandibular area. Aspiration was performed, and results were consistent with cyst contents.

In the interim, the patient continued to have memory lapses, and developed new-onset left-sided weakness and disorientation. Subsequent imaging studies performed in another institution revealed interval increase in the size of the mass with significant vasogenic edema. A second stereotactic biopsy of the lesion was done ten

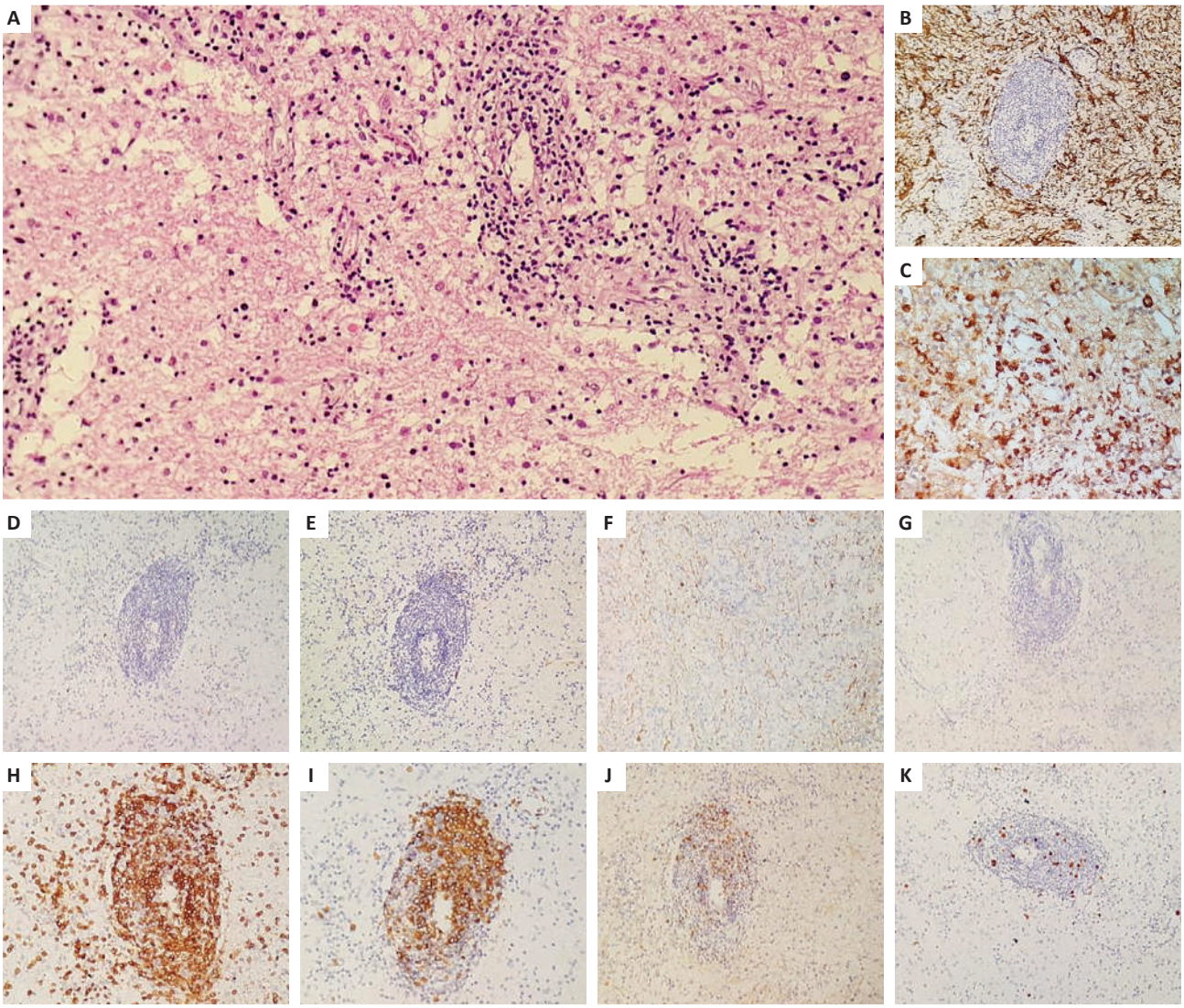

Figure 1. ***Morphology of the first biopsy.*** **(A)** Neural tissue with scattered inflammatory cells, some of which surround vessels (H&E, x200). **(B)** Immunohistochemistry for glial fibrillary acidic protein (GFAP) demonstrates astrogliosis, as it highlights the reactive astrocytes (x200). **(C)** CD68 highlights numerous parenchymal and perivascular macrophages (x200). **(D)** p16 and **(E)** p53 staining are positive in few scattered reactive astrocytes, expected in wild-type genotypes (x200). **(F)** Neurofilament highlights relatively intact axonal processes. **(G)** Immunohistochemistry for IDH R132H expression is negative (x200). **(H)** CD3 is positive in perivascular and scattered parenchymal T-cells, **(I)** CD20 and **(J)** PAX5 highlight few small parenchymal and perivascular B-cells, and **(K)** Ki-67 is positive in 3-5% of perivascular cells, and in less than 1% of glial cells (x200).

months from the symptom onset, corresponding to three months from the last corticosteroid dose. The biopsy now showed sheets of enlarged atypical cells with round to oval, hyperchromatic nuclei, and scant cytoplasm, as seen in Figure 2. Immunohistochemistry studies with CD20 revealed diffuse strong positivity in the enlarged cells, supporting a B-cell neoplasm. Further studies with Cyclin D1 and Bcl-2 ruled out other considerations such as a mantle cell lymphoma and marginal zone lymphoma, and studies with CD10, MUM1, Bcl-6, c-MYC confirmed the diagnosis of a diffuse large B-cell lymphoma, non-germinal center B-cell subtype.

Further workup included serologic tests for HIV, HAV, HBV, and HCV – all of which were negative. A positron emission tomography-computed tomography (PET-CT) was also done, which revealed uptake in one cervical lymph node, correlating to the previously biopsied left submandibular area. Post- operative period was uneventful.

DISCUSSION

PCNSL is a rare extranodal form of lymphoma, accounting for 1% of non-Hodgkin lymphomas and 3- 5% of primary brain tumors in immunocompetent patients, with a peak incidence at the fifth to seventh decade of life, and a median age of 56 years. It is more common among males, with a male-to-female ratio of 3:2. The most common locations include the posterior fossa (13%), frontal lobe (8%), parietal lobe (7%), occipital lobe (3%), basal ganglia and periventricular brain parenchyma (10%), and corpus callosum (5%).[1] Secondary CNS involvement by systemic lymphomas is more common than PCNSL; reported incidence is at around 5% but range from 2-27%. This wide variation is attributed to patient inclusion criteria, different histologic subtypes, and different methods for assessing CNS involvement.[2] DLBCL is the most common systemic lymphoma to involve the CNS, at an estimated incidence of 2- 5%.[3] Leptomeningeal (33-100%) is more

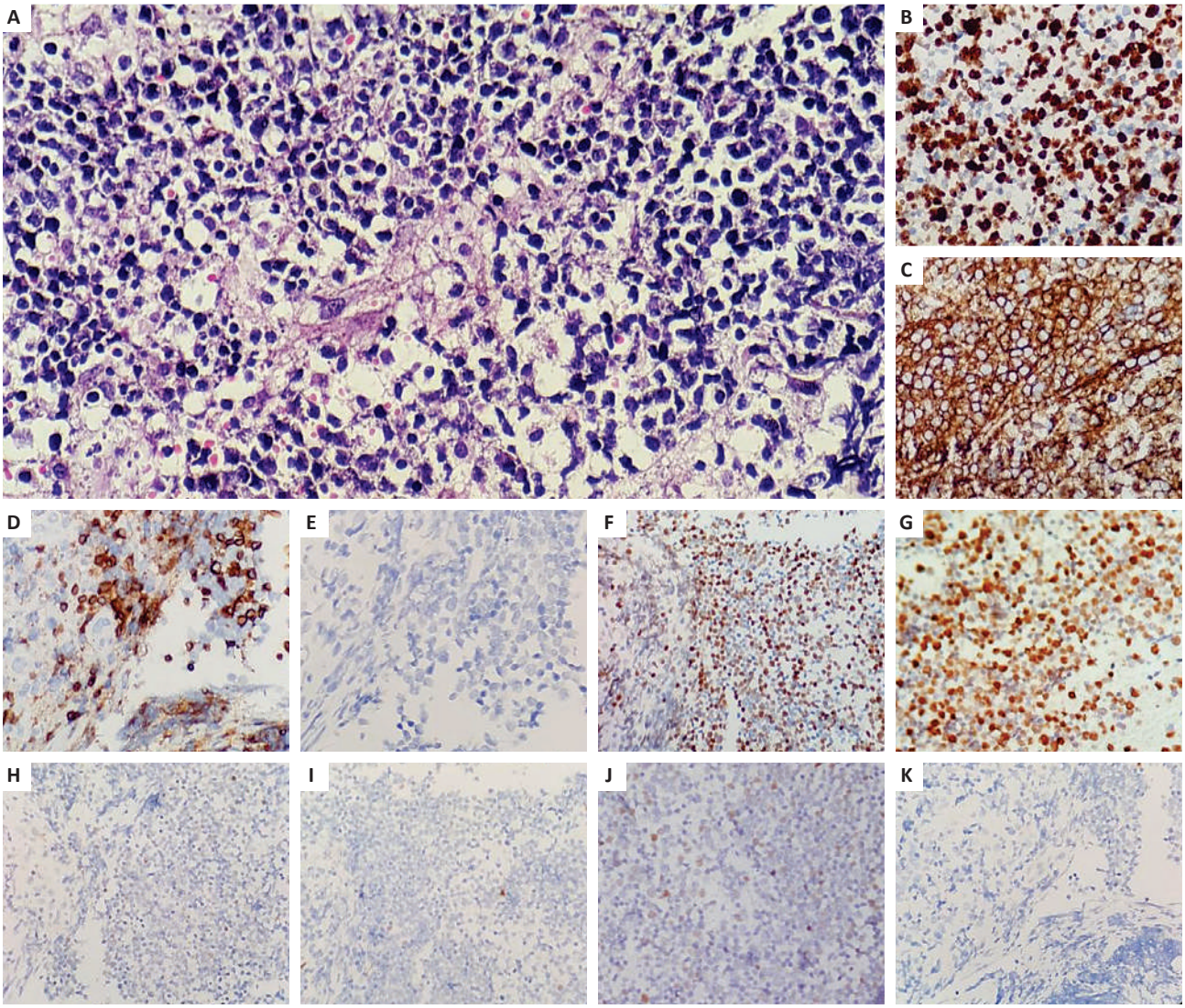

Figure 2. ***Morphology of the second biopsy.*** **(A)** Microsections show sheets of medium to large lymphoid cells with hyperchromatic nuclei, irregular nuclear borders, occasionally prominent nucleoli, and scant cytoplasm (H&E, x400). **(B)** Ki-67 shows a high proliferation index (greater than 90%), and **(C)** CD20 highlights the neoplastic B-cells (x200); **(D)** Immunohistochemistry for CD3 is positive in scattered reactive T-cells, and **(E)** CD30 is negative (x200); **(F)** Bcl-6 and **(G)** MUM-1 are positive in approximately 70% of the cells; **(H)** Bcl-2, **(I)** Cyclin D1, **(J)** c-myc and **(K)** CD10 are negative in the cells of interest (x200).

common in cases of secondary CNS involvement, and occasionally may involve the superficial cortex.[4] Deep, periventricular involvement typically seen in PCNSL is rare in secondary lymphomas.[2] CNS involvement of systemic lymphomas usually manifests within two years of diagnosis of initial diagnosis, with a median time of less than one year.[3] Patients would present with cognitive dysfunction, psychomotor slowing, and focal neurologic symptoms.[1]

MRI is a sensitive technique for detecting DLBCL, which will demonstrate hypointense T1-weighted and isointense to hyperintense T2-weighted images, with densely enhancing postcontrast images, and perilesional edema. Although these findings were consistent with the radiologic profile of the patient's callosal mass, stereotactic biopsy is still necessary, as it is considered the gold standard for diagnosis.[2,3]

On microscopy, the tumor is typically highly cellular, containing large areas of geographic necrosis centrally, and perivascular cuffing and splitting of the argyrophilic fiber network peripherally. Clusters or individual tumor cells infiltrate the surrounding tissue diffusely, accompanied by a prominent astrocytic and microglial activation and a reactive inflammatory infiltrate consisting of mature T and B cells and foamy histiocytes. The tumor cells have atypical medium- to large-sized round, oval, irregular, or pleomorphic nuclei with distinct nucleoli, expressing mature B-cell markers (PAX5, CD19, CD20, CD22, and CD79a) without plasma cell markers (CD38 and CD138). Most cases also express BCL6 and IRF4/MUM1. CD10 is a marker that is more common in systemic DLBCL than in primary CNS lymphoma.[5] Distinction of secondary CNS involvement of systemic DLBCL cannot be distinguished morphologically from PCNSL. Molecular genetics such as comparative IG gene analysis of both tumors is necessary to differentiate between these entities.[3]

In some cases of CNS neoplasms, corticosteroid therapy is initiated to lower increased intracranial pressure. However, steroids induce apoptosis in malignant and even non-malignant lymphocytes, which can lead to rapid disappearance of the lesion on imaging and within biopsy specimens.[3] The diagnosis of CNS lymphoma, then, becomes challenging when biopsies are taken post-corticosteroid therapy. The microscopic picture can show nonspecific reactive and chronic inflammatory lesions consisting of T cells, macrophages, and reactive astrocytes. Reactive gliosis and microglial activation may be prominent. Necrosis may or may not be present. Foamy macrophages may also predominate, and their presence raises the suspicion of steroid-mitigated CNS lymphoma. In some cases, few neoplastic B cells may be present only in small numbers. Mature B-cells may be mingled, usually forming perivascular collections, or scattered individually throughout the parenchyma.[2,3]

Demyelinating lesions, primarily multiple sclerosis (MS), may also confound the diagnosis of DLBCL, because of their similar radiologic features on MRI and their responsiveness to steroid therapy.[2] MS plaques that appear on MRI as tumor-forming may be biopsied and misdiagnosed as glioma or lymphoma in as often as 18% of cases. MS plaques would typically appear on MRI as multiple, well- demarcated homogeneous small ovoid lesions with no mass effect. However, atypical radiologic features may confound the diagnosis, such as a solitary large lesion larger than 2 cm, associated mass effect, perilesional edema, and presence of ring enhancement. Active MS plaques are classically characterized by preferential loss of myelin relative to axons; prominent perivascular lymphocytic infiltrates composed mostly of T-cells, and to a lesser extent B-cells; large numbers of parenchymal and perivascular macrophages; reactive astrocytosis; and cerebral edema.[6] Although the diagnosis may be straightforward based on morphology, problematic patterns include specimens which only show a few lymphoma cells in a background of reactive brain tissue, and active demyelinating lesions with proliferating perivascular B cells.[2] The morphologic features of demyelinating lesions may even be indistinguishable from steroid-induced inflammatory reactions, in the absence of evidence for demyelination.[3] However, subtle histologic features that are more prominent in steroid-treated lymphomas than in primary demyelination include the presence of incomplete and inhomogeneous demyelination, extensive inflammation – particularly of CD3+ T lymphocytes,[7] and decreased axon density and preservation, and the presence of axonal spheroids.[8] In addition, other clinical and radiologic findings may raise the suspicion of PCNSL, such as: middle to older age with no prior clinical episodes or radiographic lesions suggestive of MS, lack of spinal cord involvement, and increased enhancement or lesion size over time[9] – features present in our case.

Another entity that poses a diagnostic dilemma are contrast-enhancing "sentinel" demyelinating lesions that precede PCNSL. These lesions are radiologically and morphologically like MS and are followed by development of PCNSL within 12 months but may be up to two years.[1] Possible explanations for this include malignant transformation of a chronic inflammatory process, demyelination due to anti-myelin bodies secreted by lymphoma cells, and sampling error.[2] Another theory posed is that this reaction may represent the first immune response against the developing PCNSL.[3] These lesions regress spontaneously or upon corticosteroid treatment. There is no published report on the incidence of this entity; however, there are a few case reports that describe this phenomenon[9-11] and none describe this entity in the local literature. On microscopy, these lesions will show no neoplastic B cells, and instead would exhibit variable demyelination, T and B cells, plasma cells, macrophages, astrogliosis, and well-preserved axons.

The effects of corticosteroids on CNS lymphoma are temporary, and recurrence may occur once therapy is ceased. Because of this, prompt diagnosis may be delayed. In a study by Bruck et al., of 933 patients, pretreatment with corticosteroids of patients suspected with PCNSL prevented diagnosis in up to 50%.[12] Another study found that steroid use increased the risk of vanishing tumor among PCNSL cases by 14% per 100 mg dexamethasone equivalent, leading to delayed diagnosis.[13] These provide credence to the recommendations in current practice to withhold corticosteroid use in those suspected with CNS lymphomas prior to stereotactic biopsy. The rate of re-

biopsy among PCNSL patients with pre-biopsy corticosteroid treatment was at 12% in one retrospective study.[14]

The dose and timing of steroid administration prior to a non-diagnostic biopsy have wide ranges: In a Turkish study of PCNSL patients, the patients received 4 mg dexamethasone with 6-hour intervals, ranging from 2-30 days, with an interval between steroid use and biopsy of 0-2 days. 53% of these patients needed a second biopsy or PCR to establish the diagnosis of PCNSL.[15] In the study of vanishing tumor among PCNSL cases, the mean cumulative dose in dexamethasone equivalents was 555 + 704 mg prior to the nondiagnostic biopsy.[13] Finally, in the retrospective study mentioned previously, the cases had a median total dosage of steroids equivalent to 325 mg (range 25-6325 mg), taken at a median duration of 5 days (range 1-90 days), and with median time from treatment to biopsy of 0 days (range 0-180).[14] Our case received a total of 8 mg of dexamethasone, taken within two days, the last dose of which was given eight days prior to the biopsy. This total dose is below the median and mean total steroid doses reported in other studies that led to a non-diagnostic stereotactic biopsy, further showing the wide variability to responses in corticosteroids among CNS lymphoma patients.

The recommended duration of corticosteroid abstinence has not been established, mostly because of high individual variation of response. In line with this, even a repeat biopsy cannot guarantee a definitive diagnosis.[3] In previous studies, the interval between the first and second biopsy ranged from six to eleven months.[9,10] In a cohort study by Barrantes-Freer et al., the interval between the first and second biopsy for which PCNSL was histologically confirmed was 3 to 32 weeks.[7] In another study, the median time to recurrence after the first non-diagnostic biopsy was 53 days, but with a wide range from 8 to 648 days.[13] Our case was diagnosed three months after the last dose, which is consistent with the previous studies cited.

CONCLUSION

The diagnosis of CNS lymphoma becomes a challenge once corticosteroid therapy has been initiated, as it induces apoptosis of the neoplastic B cells and leads to rapid regression of the tumor on imaging and within biopsies. The morphologic features of post-steroid biopsies may not demonstrate the neoplastic B cells, and may only exhibit non-specific inflammatory and reactive processes indistinguishable from other demyelinating diseases such as MS. As such, corticosteroid therapy for cases of suspected CNS lymphoma should be withheld to prevent delay of diagnosis and to initiate the proper therapy regimen. However, it should be kept in mind that repeat biopsies even after withholding corticosteroid therapy may not guarantee a proper diagnosis of CNS lymphoma. Furthermore, demyelinating lesions like MS may precede PCNSL and further complicates the proper diagnosis of a CNS lymphoma.

ETHICAL CONSIDERATIONS

This case report was submitted to and acknowledged by the Institutional Review Board of The Medical City.

STATEMENT OF AUTHORSHIP

Both authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

Both authors declare no conflict of interest.

FUNDING SOURCE

None.

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EBV-positive Diffuse Large B-cell Lymphoma, NOS, in a Filipino Patient: Mimickers and Essential Ancillary Studies

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ABSTRACT

Epstein-Barr virus positive diffuse large B-cell lymphoma (EBV+ DLBCL) is prevalent among Asians but is underreported in the Philippine setting. We report the case of an 88-year-old male who presented with difficulty swallowing. CT scan showed an ill-defined soft tissue focus with calcifications in the supraglottic to hypopharyngeal region measuring approximately 2.6 x 1.7 x 1.5 cm, and multiple lymphadenopathies in the head and neck. Biopsy of the masses at the left tonsil, left arytenoid mucosa, pyriform sinus, and aryepiglottic fold showed large lymphoid cells with several Reed-Sternberg-like cells in a background of small lymphocytes, neutrophils, few eosinophils and histiocytes. A panel of immunohistochemical stains and EBER-ish were performed to differentiate among six entities that were morphologically similar to the patient's case, namely, classic Hodgkin lymphoma, T-cell/histiocyte-rich large B-cell lymphoma (THRLBCL), DLBCL, NOS, anaplastic variant, B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classic HL (gray zone lymphoma), and infectious mononucleosis (IM). The neoplastic cells expressed CD20, CD30, CD45, PAX5, CD10, MUM-1, BCL6, BCL2, and c-myc, while CD3, CD15 and ALK-1 were negative. The cells of interest also showed nuclear staining (30-40%) on Epstein-Barr virus encoding RNA in-situ hybridization (EBER-ish). The Ki-67 showed a proliferation index of 40-50%. Given the differences in prognosis and treatment among these diseases, judicious use of immunostains and EBER-ish is recommended for accurate diagnosis.

Key words: Immunohistochemistry, Philippines, DLBCL, Epstein-Barr virus, EBV+ DLBCL

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INTRODUCTION

Diffuse large B-cell lymphoma, of no special type (DLBCL, NOS) is a neoplasm composed of diffusely growing B lymphocytes with nuclei twice as large as that of normal lymphocytes. Histomorphology and immunohistochemical studies have allowed sub-classifications of DLBCL, aiding in both diagnosis and treatment.[1] Epstein-Barr virus positive DLBCL, NOS (EBV+ DLBCL) is one such classification which accounts for 5-15% of DLBCL patients among Asians.[2] From the time that it was initially described by Oyama et al in 2003, there have been multiple studies on its prevalence across Asia.[3] EBV+ DLBCL is further classified into two subtypes: a polymorphic subtype with surrounding reactive infiltrates, and a monomorphic subtype composed of sheets of large cells. These subtypes are important to note because of their close resemblance to other lymphoproliferative neoplasms.[1]

Despite the increased prevalence of EBV+ DLBCL in Asian countries, this entity remains underreported in the Philippine setting. An exhaustive search on Philippine e-journals, HERDIN Plus, and the Philippine Journal of Pathology yielded no reports of EBV+ DLBCL. This report aims to add to the body of knowledge on this disease in the local setting, and elucidate a method to differentiate among six morphologically similar entities.

CASE

This is a case of an 88-year-old Filipino, previously healthy male who presented with rapidly worsening

difficulty of swallowing solid food, which started two weeks prior to consultation. This symptom was associated with gradual weight loss of approximately 10 kg. There was no fever, night sweats, chest pain, or difficulty of breathing. Complete blood count showed the following result: hemoglobin of 80 g/L, hematocrit of 0.25, white blood cell count of 8.50 x 10^9/L, and platelet count of 287 x 10^9/L. Differential count was predominantly neutrophilic with no immature cells seen. Serum lactate dehydrogenase (LDH) was normal. Serum EBV level was not tested. CT scan showed an ill-defined soft tissue focus with calcifications in the supraglottic to hypopharyngeal region measuring approximately 2.6 x 1.7 x 1.5 cm, and prominent, enlarged, round to ovoid lymph nodes at the submental, left submandibular, right mid-jugulocarotid and left upper to lower jugulocarotid and posterior cervical spaces (Figure 1). An F-18 FDG PET scan done a month later revealed multiple FDG-avid (Lugano Score 5) lymphadenopathies scattered in the bilateral cervical, right axillary, mediastinal, right peribronchial, right subpleural, peritoneal, omental, mesenteric, retroperitoneal, pelvic and inguinal regions, with the largest lymphadenopathy measuring 3.2 x 2.8 cm in greatest dimensions (Figure 2). Laryngoscopy showed masses at the left tonsil, left arytenoid mucosa, pyriform sinus, and aryepiglottic fold which were submitted for histopathologic evaluation.

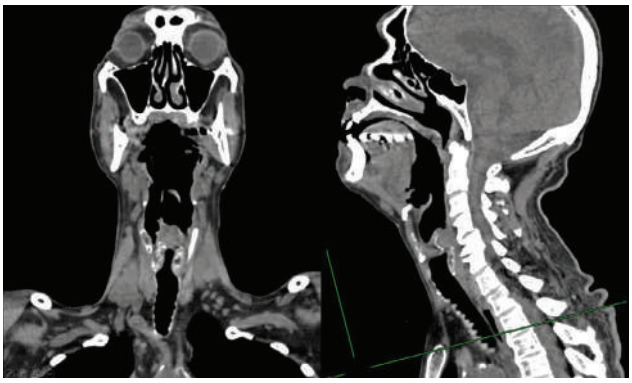

Figure 1. CT scan showing an ill-defined soft tissue focus along with enlarged, prominent cervical lymph nodes.

Microscopic examination showed proliferation of large lymphoid cells with several Reed-Sternberg-like cells in a background of small lymphocytes, neutrophils, few eosinophils and histiocytes. (Figure 3). Using formalin-fixed, paraffin-embedded tissue sections, immunohistochemical studies were performed on automated staining platforms according to the manufacturer's instructions. The antibodies used, their dilutions, and respective vendors were as follows: anti-CD3 (clone 2GV6; Ventana/Roche Tissue Diagnostics, Tucson, AZ, USA; 1:100); anti-CD10 (clone SP67; Ventana/Roche Tissue Diagnostics, Tucson, AZ, USA; 1:100); anti-CD15 (clone MMA; Ventana/Roche Tissue Diagnostics, Tucson, AZ, USA; 1:100); anti-CD20 (clone L26; Ventana/Roche Tissue Diagnostics, Tucson,

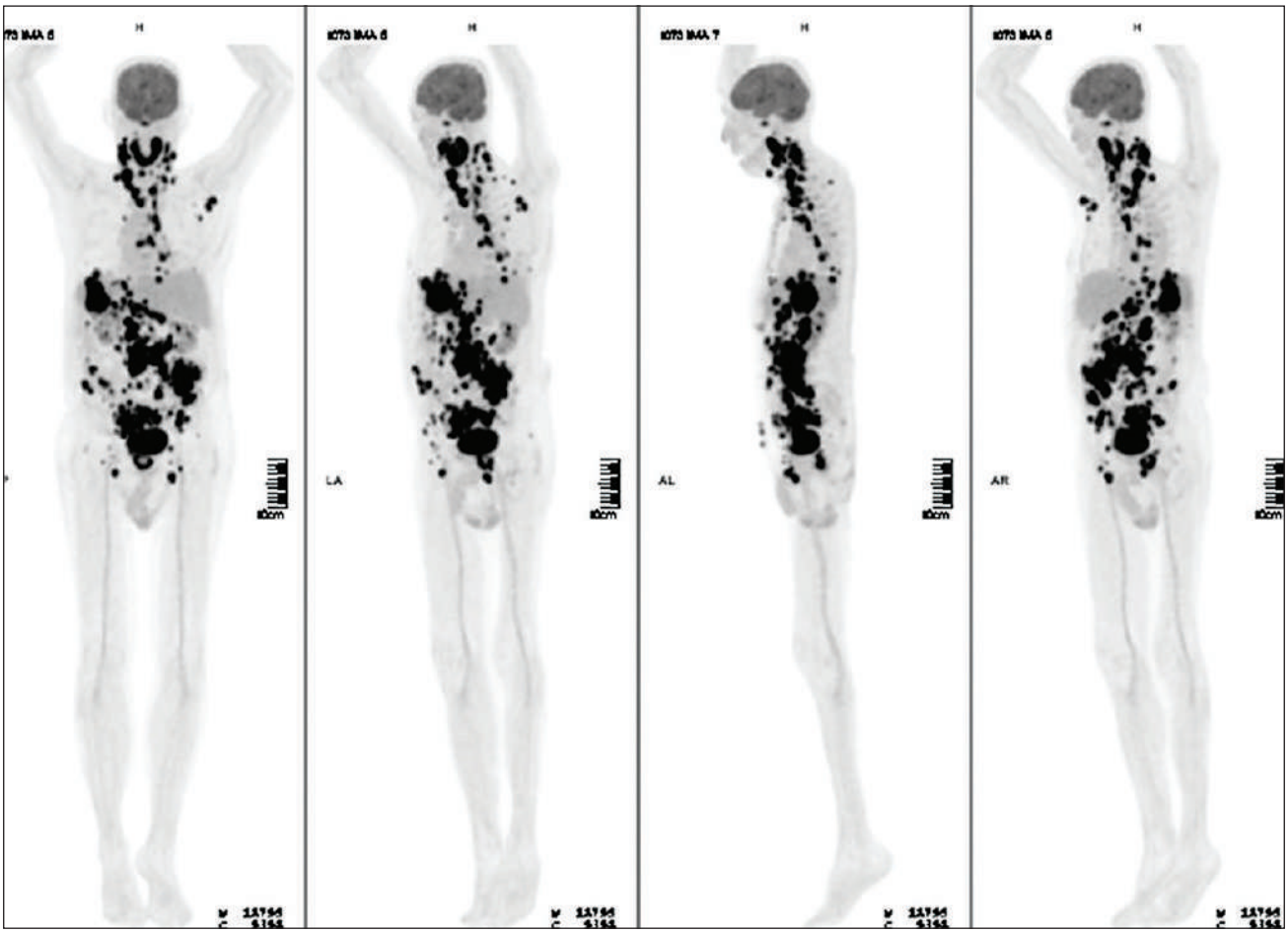

Figure 2. F-18 FDG PET scan showing multiple FDG-avid (Lugano Score 5) lymphadenopathies.

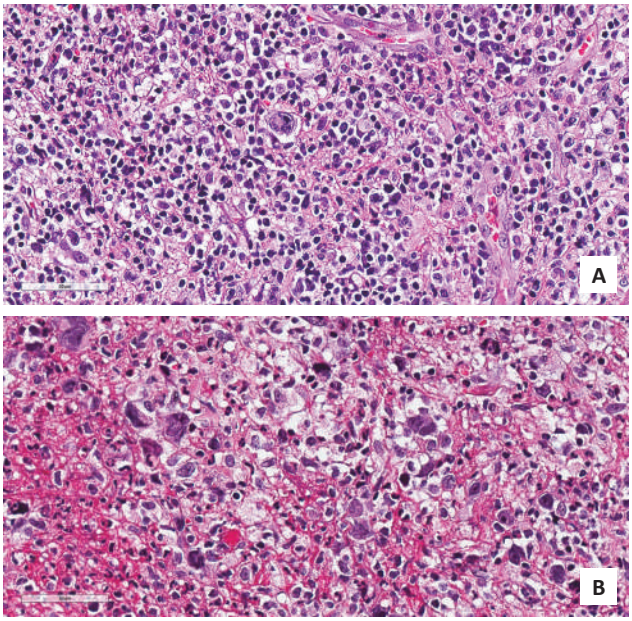

Figure 3. (A and B) The tumor is composed of sheets of large lymphoid cells surrounded by small lymphocytes, neutrophils, few eosinophils and histiocytes. There are also many scattered Reed-Sternberg-like cells showing irregularly shaped nuclei, prominent nucleoli, and scant basophilic cytoplasm (H&E, 40x).

AZ, USA; 1:100); anti-CD30 (clone Ber-H2; Ventana/Roche Tissue Diagnostics, Tucson, AZ, USA; 1:100); anti-CD45 (clone RP2/18; Ventana/Roche Tissue Diagnostics, Tucson, AZ, USA; 1:100); anti-PAX5 (clone SP34; Ventana/Roche Tissue Diagnostics, Tucson, AZ, USA; 1:100); anti-MUM-1 (clone MRQ-43; Ventana/Roche Tissue Diagnostics, Tucson, AZ, USA; 1:100); anti-BCL6 (clone GI191E/A8; Ventana/Roche Tissue Diagnostics, Tucson, AZ, USA; 1:100); anti-BCL2 (clone 124; Ventana/Roche Tissue Diagnostics, Tucson, AZ, USA; 1:100); anti-c-myc (clone 9E10; Ventana/Roche Tissue Diagnostics, Tucson, AZ, USA; 1:100); anti-ALK-1 (clone ALK01; Ventana/Roche Tissue Diagnostics, Tucson, AZ, USA; 1:100); and anti-Ki-67 (clone 30-9; Ventana/Roche Tissue Diagnostics, Tucson, AZ, USA; 1:100). Epstein-Barr encoding region in-situ hybridization (EBER-ish) was performed using EBER probe (Leica BONDMAX, Leica Biosystems, Buffalo Grove, IL, USA), using the built-in protocol from the manufacturer.

The large lymphoid cells as well as the Reed-Sternberg-like cells expressed CD45, CD20, PAX5, CD30 (30%), CD10 (60%), BCL6 (30%), MUM-1 (80%), BCL2 (20%), and c-myc (10%), while CD3, CD15 and ALK-1 were negative. The cells of interest also showed nuclear staining (30-40%) on Epstein-Barr encoding region in-situ hybridization (EBER-ish). The Ki-67 showed a proliferation index of 40-50% (Figure 4). Based on the morphology and the

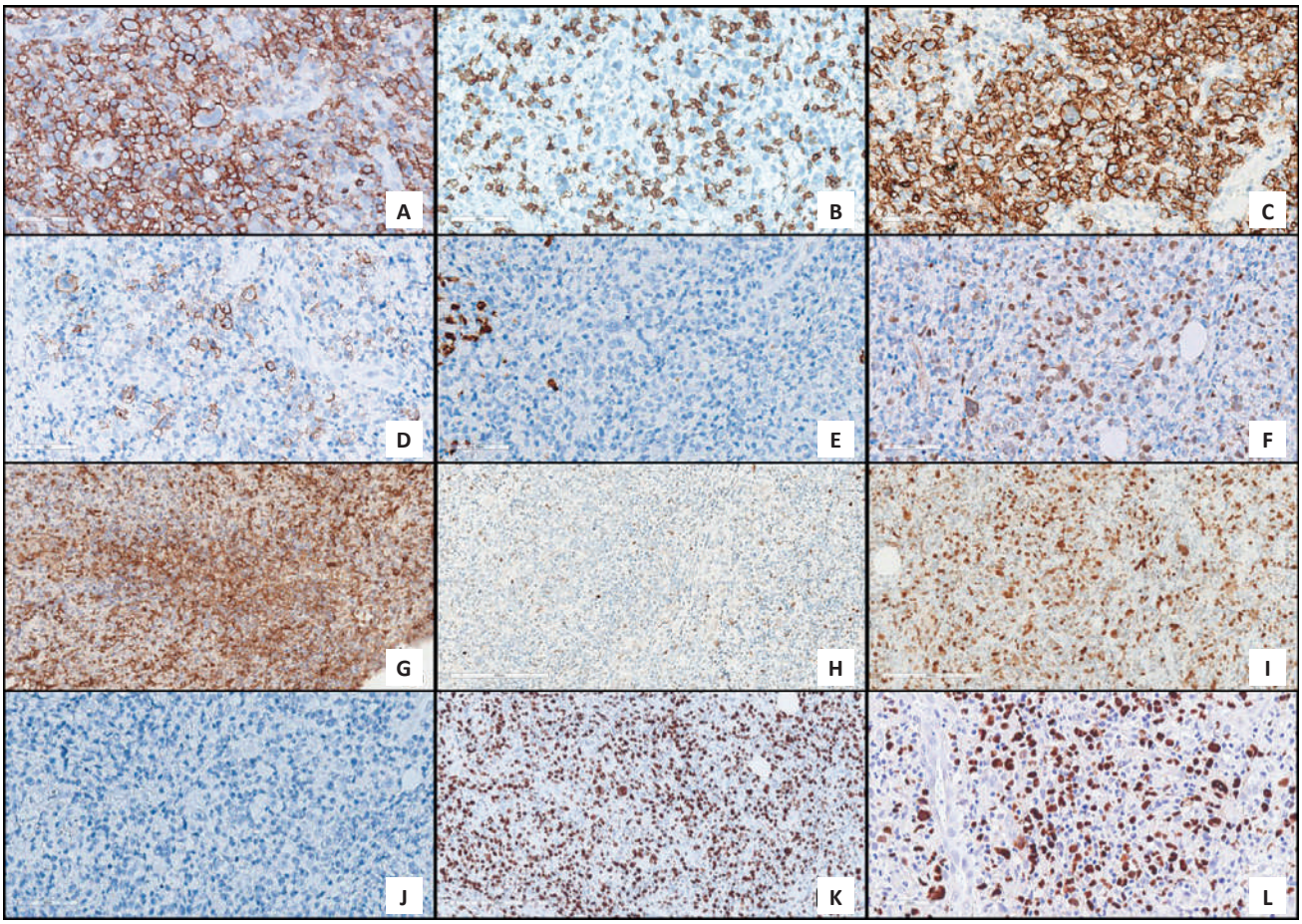

Figure 4. The large cells and Reed-Sternberg-like (RS-like) cells were positive for LCA **(A)**, CD20 **(C)**, and PAX5 **(F)** (40x). Some of the large cells and RS-like were positive for CD30 (30%) **(D)**. The tumor cells also expressed CD10 (30%) **(G)**, BCL6 (30%) **(H)** and MUM-1 (80%) **(I)** (20x). The immunostains for CD3 **(B)**, CD15 **(E)**, and ALK-1 **(J)** were negative (40x). Ki-67 shows a high (40-50%) proliferation index **(K)** (20x). Approximately 30-40% of the large cells showed nuclear staining on Epstein-Barr encoding region in-situ hybridization **(L)** (20x).

immunoprofile, this case was thus signed out as an EBV+ DLBCL, NOS.

DISCUSSION

EBV is one of the first oncogenic viruses to be identified. It is oncogenic partly due to its link with immunosuppression and chronic antigenic activation. Worldwide, EBV infection has a prevalence ranging between 80-95%. It is more commonly associated with nasopharyngeal carcinoma and Burkitt lymphoma, though it is not rarely linked with various other tumors.[4]

EBV+ DLBCL has a higher prevalence in Asia than in Europe and America (Table 1).[5-11] In the Philippines, there have been published studies documenting lymphomas in general, but no recent literature focusing on DLBCL, EBV positive or otherwise. A 2004 study of various cases of lymphoma at a tertiary hospital showed a greater prevalence of non-Hodgkin lymphoma (NHL) as compared to Hodgkin lymphoma (HL), involving a median age of 29 years and most often involving the neck. Most of the cases were Stage IIA on diagnosis.[12] One study done more than a decade ago reported few cases of EBV+ HL, but not EBV+ DLBCL.[13] It is postulated that genetic factors (e.g., HLA types) and EBV strains play a role in the regional differences in EBV prevalence.[4]

Various studies across many populations report the median age of DLBCL to be 70 years, with higher incidence in males and in developing countries. A similar trend is observed with EBV+ DLBCL.[2] There have been reports of this entity appearing over a wide range of ages, though it is less common and carries a better prognosis among younger patients; thus, the "elderly" designation in EBV+ DLBCL was removed in the 2017 edition of the WHO classification guidelines.[2,14-16] Immunosenescence, which comes with aging, might explain the higher prevalence of EBV+ DLBCL and related diseases among the elderly. It involves dysregulation of the T-cell response, loss of immunosurveillance, deficiencies in cytokine production, and anergic memory cells.[4]

Majority of EBV+ DLBCL patients present with extranodal masses (particularly in the tonsils, skin, lungs, and gastrointestinal tract) and lymphadenopathy. Around 60% of patients present with B symptoms, such as fever, night sweats, and weight loss.[2, 17] LDH levels are often increased.[2] EBV+ DLBCL predominantly presents with a mixed proliferation of large transformed cells, immunoblasts and Reed-Sternberg-like cells, in a background of plasma cells, plasmablasts, histiocytes, epithelioid cells and small lymphocytes. This polymorphic pattern, as is seen in this case, can resemble T-cell/histiocyte-rich large B-cell lymphoma (THRLBCL) or classic HL. Geographical areas of necrosis or angioinvasion may or may not be present. A monomorphic subtype has also been described, composed of sheets of large cells, which may be difficult to differentiate from EBV negative DLBCL without immunohistochemical studies.[2,18]

The similarity of morphologic features between EBV+ DLBCL and other lymphomas presents a diagnostic difficulty. Other considerations based on morphology and clinical presentation included T-cell/histiocyte-rich large B-cell lymphoma (THRLBCL), DLBCL, NOS, anaplastic variant, and B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classic HL (gray zone lymphoma). A benign entity, infectious mononucleosis (IM), was also considered. Various immunohistochemical studies were employed to identify the type of tumor from among its morphologically similar counterparts. A comparison of the immunoprofile of this case and the differential diagnoses is presented in Table 2.

On the initial panel, a B-cell origin was considered due to strong expression of CD20 in the large cells and RS-like cells, and negative CD3. The Ki-67 indicated a moderate to high proliferation index. Among the known B-cell lymphomas, these five entities were selected as differential diagnoses due to the common feature of large B cells and occasional RS-like cells, surrounded by different types of reactive cells, with moderate to high proliferation index. Four of these entities are also more prevalent among elderly males as in this case. Clinically, these entities present with nodal or extranodal masses, with or without lymphadenopathies, with gray zone lymphoma being the exception.

Morphologically, one of the leading considerations was classic Hodgkin lymphoma, particularly the mixed cellularity subtype. This is the most common subtype in developing countries. It is noted that mixed cellularity cHL resembles the polymorphic subtype of EBV+DLBCL. The strong positivity of PAX-5, and CD45 in this case, however, made this diagnosis less likely. In addition, cHL usually has a slower progression and is often nodal in presentation. THRLBCL is characterized by large B cells, surrounded by small T-cells and histiocytes. However, it is defined as having neoplastic cells occupying <10% of the cell population. EBER-ish is also mostly negative in

Table 1. Prevalence of EBV+ DLBCL among different countries

Country	Age groups studied	Sample	% prevalence	EBER cut-off
Japan [5]	55-84	18/260	6.9%	30%
China [6]	Elderly >50	25/141	18%	20%
	<50	10/74	14%	20%
	Elderly >50	19/147	13%	50%
	<50	7/77	9%	50%
Korea [7]	Elderly >50	35/376	9.3%	20%
	<50	13/195	6.7%	20%
Germany [8]	Elderly >50	4/169	2%	"majority"
Switzerland, Italy, Austria [9]	Elderly >50	8/258	3.1%	10-100%
Mexico [8]	Elderly >50	9/136	7%	"majority"
United States [10]	Elderly >60	5/95	5.3%	None; at least 80% of cells in tumors considered positive

Table 2. Comparison of immunohistochemical findings in the patient and differential diagnoses[2,17, 21, 22]

IHCs	Patient	Classic Hodgkin Lymphoma	T-cell/histiocyte-rich large B cell	B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classic HL	EBV+ DLBCL, NOS	DLBCL, NOS	Infectious mononucleosis
CD3	Negative	Negative, but (+) in 10% of cases	Negative in large neoplastic cells; (+) in T cells in background	Negative in large cells; (+) in T cells in background	Negative	Negative	(+) in surrounding small cells
CD20	(+)	Variably positive and weak in 20-40% of cases	(+) in large neoplastic cells	(+) strong in cases that resemble cHL (-) in cases that resemble DLBCL	(+)	(+); can be dim if with plasmacytoid differentiation	(+) in immunoblasts and germinal center B-cells
Ki-67	40-50%	High	High	High	High	40-90%	Moderate to high
PAX-5	(+) strong	Weakly positive; negative in 10% of cases Stronger in nodular lymphocyte-predominant HL	(+) strong in large neoplastic cells	(+) strong	(+) strong	(+) strong	(+) in immunoblasts
CD15	Negative	(+); Negative in 15-25%, usually in nodular lymphocyte-predominant	Negative	(+) in cases that resemble DLBCL (-) in cases that resemble cHL	Negative	Negative	Negative
ALK-1	Negative	Negative	Negative	Negative	Negative	Negative	Negative
CD30	(+) 30%	(+)	Negative	(+)	(+/-), usually weak and partial	(+) only 10-20% and anaplastic	(+) in immunoblasts
CD10	(+) 30%	Negative	(+)	Negative	Negative	(+) in 30-50%	Negative
BCL6	(+) 30%	(+)	(+)	(+) Variable	Negative	(+) in 60-90%	Negative
BCL2	(+) 20%	(+)	(+) variable	(+) Variable	(+) Infrequent	(+) Variable [criteria: 50% or more of cells]	Negative
c-myc	(+) 10%	(+) variable	(+) variable	(+) Variable	(+) Infrequent	(+) Variable [criteria: 40% or more of cells]	No data
EBER-ish	(+) 30-40%	often positive in mixed-cellular and lymphocyte depleted (75% of cases)	Rarely positive	Negative (positive in 15%)	(+); arbitrary cutoff of 20-40% of cells.	Negative	(+)
CD45	(+)	Negative (+) in nodular lymphocyte-predominant	(+) in large neoplastic cells	(+), focally	Negative	(+) Variable	(+) in immunoblasts
MUM-1	(+) 80%	(+)	(+)	(+)	(+)	(+) in 35-65% of cases if with plasmacytoid differentiation	(+)

this entity. Gray zone lymphoma has variable histologic features; the main criteria is that the immunohistochemical profiles are not consistent with what is expected from the morphology. There is a morphologic and immunoprofile overlap between cHL and DLBCL. In this case, the morphology suggests cHL, but immunohistochemical profile most closely matches DLBCL, NOS, which makes gray zone lymphoma a possible diagnosis. However, this disease mostly presents in young to middle aged patients, and often occurs in the mediastinum. EBER-ish is also frequently negative in gray zone lymphoma. It is known that EBV+ DLBCL is usually of non-germinal center B-cell origin, however, in a study done by Lu et al., at least 11.5% of EBV+ DLBCL cases were CD10 positive.[6]

A similar dilemma was discussed in a case report by Wang and colleagues, describing a tumor that was histologically similar to DLBCL, classic HL, and gray zone lymphoma. The study used other techniques not readily available in the Philippine setting, such as OCT2 and BOB1 immunostains; however, a definite diagnosis was still not established. It was proposed that EBV is associated with lymphomas that may not fit neatly into WHO classifications or IHC-driven categorizations.[19]

A non-neoplastic differential, though no less important, is infectious mononucleosis (IM). While it is observed more often in younger patients, it can present in older age and be mistaken for malignancy. IM presents clinically with fever, pharyngitis, and cervical to generalized lymphadenopathy in 50% of patients. Immunoblasts are increased, sometimes forming sheets, which can stain positive for CD30 and CD45, like in this patient. There can also be large, mono- to multinucleated cells with prominent nucleoli, which can be mistaken for Reed-Sternberg or Hodgkin cells. However, in IM, there is relatively preserved follicular architecture, and the background is polymorphous and composed mostly of small T-lymphocytes (CD3+ and CD8+).[20] BCL2 and BCL6 are also negative in IM.[21]

Ancillary studies to differentiate reactive from neoplastic hematolymphoid entities include flow cytometry to determine the clonality of populations through light chain restriction, morphologic assessment of light chain Kappa/Lambda mRNA ratio with CISH, and/or detection of gene rearrangements in BCL2, BCL6 and MYC with FISH.[21,22]

A clonal IGH and/or IGK gene rearrangement by Southern blot, polymerase chain reaction, or next-generation sequencing will also support the diagnosis of a B-cell neoplasm. However, molecular methods are only necessary if the histologic and immunohistochemical features are equivocal.[23]

There have also been some studies done, further detailing the genomic features of EBV+DLBCL and other lymphomas using next generation sequencing, gene expression profiling and other molecular methods. It may be useful in the future, once these technologies are more widely available. [24,25]

The prognosis and appropriate treatment of EBV+ DLBCL is markedly different between these closely related entities.[2,26] EBV positivity is associated with more advanced stage, extranodal involvement, and poorer response to treatment.[2,4]

Although lymphomas in general are chemosensitive, the exact chemotherapeutic regimen to be used will differ according to the specific type. HL generally involves adjacent nodes in the same anatomic site, and typically spreads to adjacent nodal areas; isolated deposits in distant nodes is rare.[27] Combination chemotherapy consisting of doxorubicin, vinblastine, bleomycin, and dacarbazine (ABVD) is typically given, and a scoring system is currently in use to determine patient's prognosis.[28] The International Prognostic Score (IPS) is the most commonly used risk stratification system for this disease, and is highly predictive of freedom from disease progression.[29] This includes clinical and laboratory parameters such as gender, serum albumin, hemoglobin, stage, age, white blood cell count, and lymphocyte count.[29] Initial treatment with chemotherapy, with or without radiotherapy, generally results in cure.

Gray zone lymphoma is a rare neoplasm that has a more aggressive course with poorer outcomes.[30] This typically presents in males, and is more often diagnosed at an advanced stage.[30] There are currently no standard management guidelines for gray zone lymphoma. However, a prospective study has found that these patients typically respond to a combination regimen consisting of dose-adjusted etoposide, doxorubicin, and cyclophosphamide with vincristine, prednisone, and rituximab (DA-EPOCH-R), with 62% of patients achieving continuous complete remission.[31]

Patients with NHL typically present with painless peripheral lymphadenopathy. This may or may not be accompanied by B symptoms. DLBCL is the most common histologic subtype of NHL, accounting for around 31% of this disease. Prognosis for this disease depends on histology and clinical parameters rather than stage, and a scoring system is currently being used to determine prognosis. The International Prognostic Index (IPI) identified age, serum LDH, performance status, stage, and extranodal involvement to be predictive of survival.[32] Combination therapy with cyclophosphamide, doxorubicin, vincristine, and prednisone, with or without rituximab (R-CHOP), is the first-line treatment.[33] The treatment strategies of EBV+ DLBCL currently follow that of other DLBCL, however, clinical trials involving activation of lytic viral genes that will render tumor cells susceptible to antiviral treatment are currently being studied. Other therapeutic methods under study include boosting the anti-viral immune response with vaccines or EBV-specific cytotoxic lymphocytes.[34]

Although the previously discussed entities involve lymphohematopoietic tissues, their biologic and clinical behaviors are distinct. It is therefore imperative that an accurate histopathologic diagnosis is made in order to determine the correct treatment strategy for these patients.

CONCLUSION

EBV+ DLBCL should be considered as a differential diagnosis in immunocompetent patients presenting with rapidly enlarging extranodal masses and multiple lymphadenopathies, especially if histologic examination of the mass reveals RS-like cells in a reactive background. It is also important to perform immunohistochemical studies and EBER-ish to attain a specific diagnosis. It is recommended that clinicians and pathologists test for and report EBV positivity in cases of lymphoma as this affects prognosis and treatment.

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ETHICAL CONSIDERATION

Patient consent was obtained before submission of the manuscript.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

All authors declared no conflict of interest.

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Gastric Glomus Tumor: A Case Report

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ABSTRACT

Glomus tumor is an uncommon mesenchymal neoplasm usually described in the distal extremities, and rarely involving visceral organs. We report the case of a 27-year-old Filipino female who presented with episodes of dizziness and weakness, associated with a low hemoglobin count. Further work-up showed a 5.5 cm submucosal gastric mass, which was demonstrated on microscopic and immunohistochemical studies to be a gastric glomus tumor (GGT). Although rare, GGTs should be part of the differential diagnoses of submucosal gastric masses.

Key words: glomus tumor, stomach neoplasms, immunohistochemistry

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INTRODUCTION

Gastric glomus tumor (GGTs) is a rare mesenchymal neoplasm mainly arising within the submucosa of the gastric antrum. It commonly presents with epigastric discomfort or upper gastrointestinal bleeding symptoms,[1] and diagnosis is based on histologic examination, supported with immunohistochemical studies.[2] Glomus tumors are more commonly described in the extremities, and rarely seen in other organs. This case is a 27-year-old Filipino female with a history of low hemoglobin levels and a submucosal gastric mass on imaging.

CASE

A 27-year-old Filipino female consulted at the emergency department for episodes of dizziness and weakness which started a week prior. Upon workup, her hemoglobin levels were low at 39 g/dL, prompting transfusion of three units of packed red blood cells. She was eventually discharged stable. An abdominal CT scan with contrast was performed on an outpatient basis, which revealed a 6.3 x 4.5 x 5.2 cm hypodense mass involving the wall and lumen of the gastric pyloantral region and the 1st and 2nd segments of the duodenum, with luminal narrowing, as shown in Figure 1. Considerations at this point were gastrointestinal stromal tumor, adenocarcinoma, lymphoma, and hemangioma, and the patient underwent surgical procedure. No preoperative biopsy was performed.

A partial gastrectomy specimen was received from the antrum, and further opening revealed a well-defined, 5.5 x 3.7 cm intramural mass, with a dark red, soft to hemorrhagic cut surface. It narrows the lumen but does not completely obstruct it. The overlying mucosa is not ulcerated, and the serosa was smooth on gross examination. Microscopic examination of the mass as shown in Figure 2 showed nests and cords of monomorphic small round cells with sharp borders, arranged around dilated sinusoidal blood vessels. The individual cells had uniform round nuclei with focal mild atypia, fine chromatin, conspicuous nucleoli, and fairly moderate amounts of eosinophilic cytoplasm. Mitotic count was at 1-2 per 50 high power fields. No areas of necrosis were seen. Lymphovascular

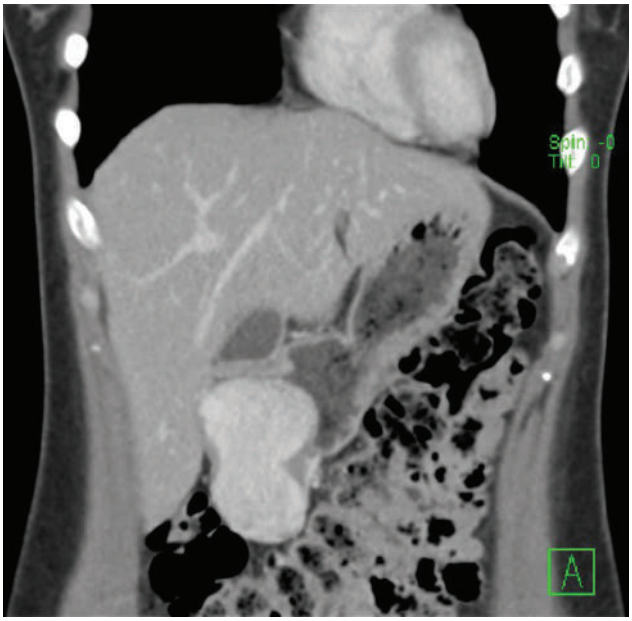

Figure 1. Coronal CT scan image of the enhancing, submucosal gastric mass.

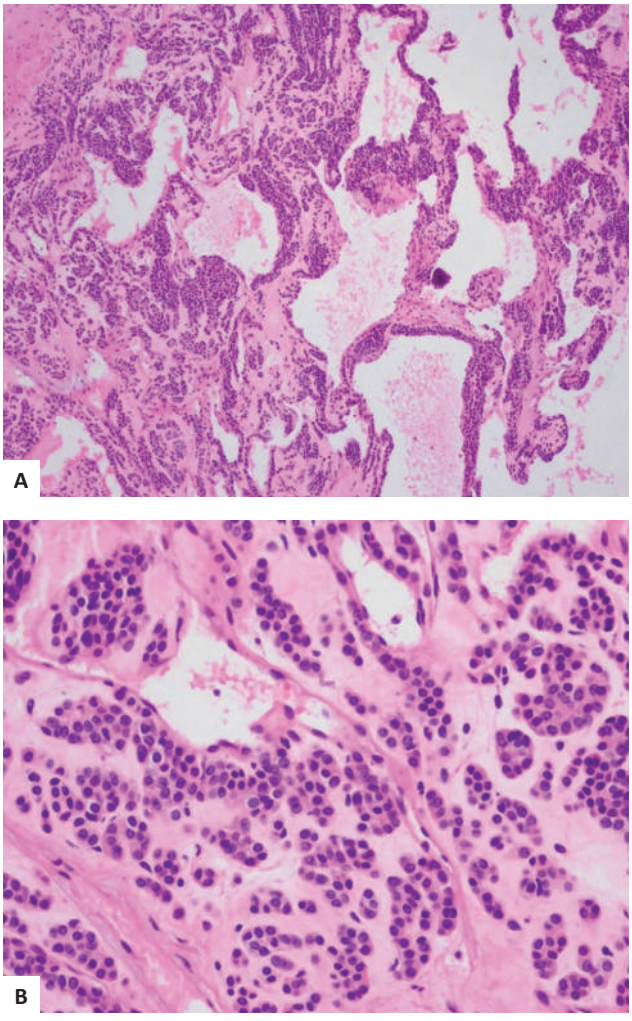

Figure 2. Microscopic images of the gastric tumor, which discloses nests of monotonous round cells arranged around dilated blood vessels **(A)** H&E, 100x, **(B)** H&E, 400x.

space invasion and focal visceral peritoneal involvement were identified. Immunohistochemical studies showed the tumor was positive for muscle-specific actin (MSA), smooth muscle actin (SMA), and vimentin, as shown in Figure 3. It was focally and weakly positive for synaptophysin and CD56, and negative for Cam 5.2, CK, LCA, CD34, chromogranin, c-kit, and Ki-67. Caldesmon was not available at the institution.

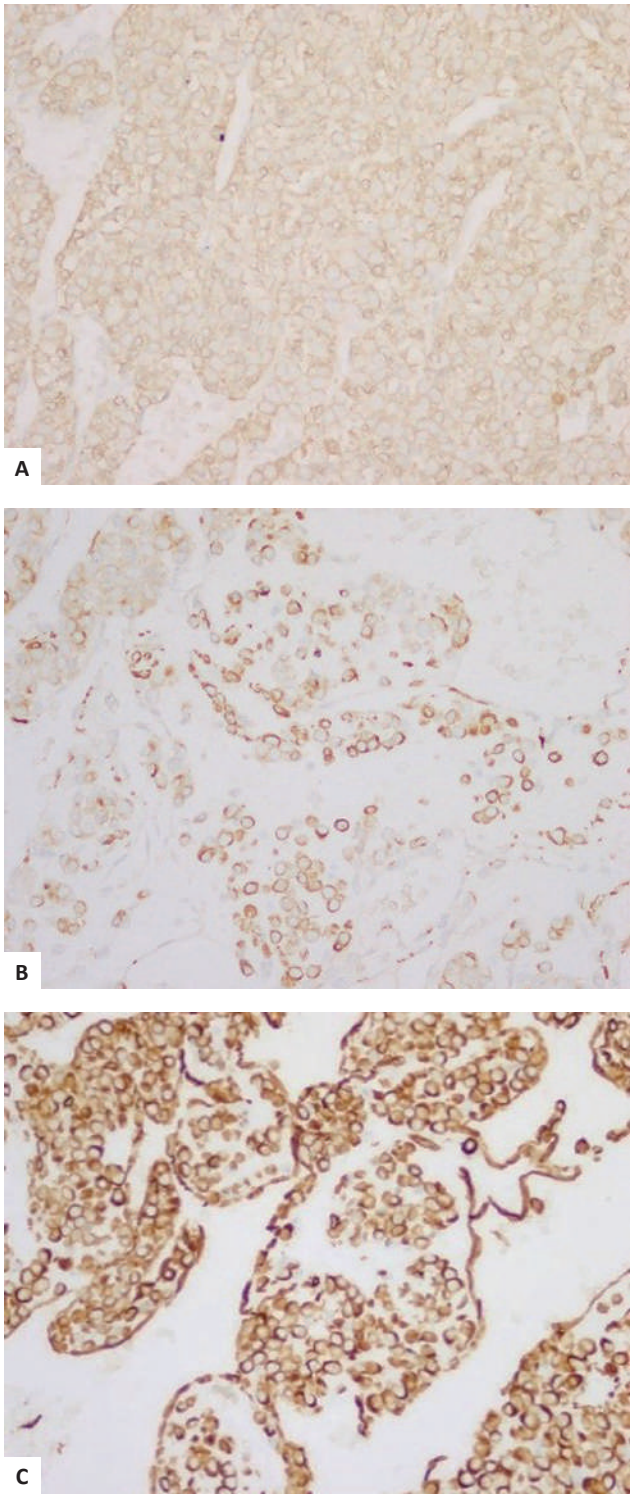

Figure 3. The tumor is positive for **(A)** SMA, **(B)** MSA, and **(C)** vimentin immunohistochemistry, 400x.

DISCUSSION

Glomus tumors are rare lesions that account for less than 2% of soft tissue tumors. They are of mesenchymal origin that resemble the perivascular modified smooth muscle cells of the normal glomus body. They mostly occur in the distal extremities, and rarely in other sites.[3] In the gastrointestinal tract, they almost always arise in the stomach, in particular the antrum.[1] They are much rarer than other mesenchymal tumors in the stomach, accounting for approximately 1% of gastric mesenchymal tumors.[2,4,5] In the stomach, glomus tumors have a strong female predominance, and may occur at any age, with a median age of 55 years (range 19-90 years). It usually presents with symptoms of upper gastrointestinal bleeding, epigastric pain, and on occasion, obstructive symptoms.[1] In our case, the patient's symptoms and low hemoglobin count was likely due to blood loss caused by the gastric glomus tumor.

Implicated in the pathogenesis of gastric glomus tumors are the NOTCH family of genes in sporadic cases, inactivating mutations in the glomulin gene (*GLMN*) in multiple familial glomus tumors, and *BRAF* mutations in a minority of cases.[1]

Gastric glomus tumors are often well-circumscribed and intramural in location[1], and imaging studies are usually unable to differentiate glomus tumors from other mesenchymal tumors in the stomach.[5,6] Hence, diagnosis relies on microscopic examination and immunohistochemical studies. Histologically, the tumor consists of a monotonous population of small round cells with distinct borders, central dark and round nuclei, and moderate amounts of eosinophilic to clear cytoplasm. The cells are in nests and clusters, arranged around dilated blood vessels. The stroma is focally myxoid or hyalinized.[1,2] Mitotic activity is usually low, but it may have focal nuclear atypia and vascular invasion, the latter of which is not associated with an adverse prognosis. It strongly expresses SMA in virtually all cases, and may also express caldesmon, collagen IV, laminin, and vimentin.[1,2] Although caldesmon was not performed in this case due to its unavailability in the institution, the authors felt the combined sensitivity of SMA and MSA was sufficient in confirming a smooth muscle lineage. A strong SMA expression is in fact a desirable diagnostic criterion of this tumor.[1] Synaptophysin may be expressed focally but are usually negative for other neuroendocrine markers. It is also negative for desmin, S100, keratin, CD34, KIT, and DOG1. GGTs are similar morphologically and immunohistochemically to glomus tumors of other sites.[1]

The morphologic differential diagnoses of GGT include neuroendocrine tumors, epithelioid gastrointestinal stromal tumor (GIST), paraganglioma, hemangiopericytoma, and lymphoma. Neuroendocrine tumors also consist of a uniform population of small round cells but have relatively coarse chromatin and scant cytoplasm. These also express keratins and multiple neuroendocrine markers, but SMA is negative.[5] Epithelioid GIST may also have cells that contain eosinophilic to clear cytoplasm, which can look like GGTs with epithelioid morphology. The stroma is not typically rich in vessels in GIST, however. GIST strongly expresses CD117, DOG-1, and CD34, and only focally express SMA. On the other hand, GGTs are consistently negative for CD117.[4,5] Paragangliomas, although less common in the stomach, may also mimic a glomus tumor. They are, however, strongly positive for synaptophysin and chromogranin, and negative for SMA.[5] Hemangiopericytomas, like GGT, also show dilated blood vessels, but the former has angulated and branching vessels. These tumors are negative for smooth muscle markers, and positive for CD34.[7] Lymphomas may also be difficult to distinguish morphologically from GGT, especially on frozen sections. Immunohistochemistry can easily resolve this;[7] GGTs are usually negative for CD20 and CD45.[5]

Most GGTs are benign, and definitive treatment is through complete excision of the tumor by wedge or segmental resection or partial gastrectomy. The criteria for malignancy in GGTs remain undefined due to insufficient large-scale studies. The usual variables used in determining malignancy for peripheral soft tissue tumors include a deep location and size >2 cm, presence of atypical mitotic figures, and presence of moderate to high nuclear grade and ≥5 mitoses/10 mm^2.[1] However, other studies propose this as unsuitable for GGTs, as most of these tumors grow larger than 2 cm,[2,4,5] and even tumors with low mitotic counts can occasionally metastasize, especially those of a larger size (> 5cm).[5,8]

SUMMARY AND CONCLUSION

GGTs are among the rarer neoplasms that may arise from the stomach. Symptoms and radiologic findings may be nonspecific, and diagnosis is made on the histologic examination, supported by immunohistochemical studies. They are often benign, and complete excision is usually the definitive treatment. Despite its rarity, they should be included in the differential diagnoses of patients presenting with upper gastrointestinal bleeding symptoms.

ETHICAL CONSIDERATIONS

This case report was submitted to and acknowledged by the Institutional Review Board of The Medical City.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declare no conflict of interest.

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OPEN ACCESS – ORIGINAL ARTICLE

Ovarian Carcinosarcoma with Yolk Sac Tumor in a Post-Menopausal Female: A Case Report and Review of Literature

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ABSTRACT

Yolk sac tumor is the second most common subtype of ovarian germ cell tumors and is rare in post-menopausal women. The few cases in literature have found that in this age group, yolk sac tumors more commonly present as a mixed component, combined with epithelial tumors. We report a case of a 60-year-old female who presented with an enlarging abdominopelvic mass. Imaging pointed to an ovarian new growth. Total abdominal hysterectomy with bilateral salpingo-oophorectomy revealed a tumor with three populations composed of carcinomatous, sarcomatous, and germ cell components, which was ultimately diagnosed as an ovarian carcinosarcoma with concurrent yolk sac tumor based on histo-morphology and immunohistochemical staining. This report also discusses the proposed pathogenesis, treatment, and prognosis of this uncommon entity.

Key words: carcinosarcoma, yolk sac tumor, ovary, postmenopausal

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INTRODUCTION

Ovarian yolk sac tumors (YSTs), formerly known as endodermal sinus tumors, are the third most common ovarian malignant germ cell tumors (GCTs), accounting for approximately 1% of ovarian malignancies and 14.5%–16.4% of all ovarian malignant GCTs.[1] They usually occur in the second to third decades of life and are rare in postmenopausal women. The majority of YSTs arising in old age tend to concurrently accompany variable types of somatic epithelial neoplasms. Rutgers et al. (1987) were the first to report a case of ovarian epithelial cancer associated with YST.[2] It was only until Nogales et al. in 1996 when a YST was reported to occur with malignant Müllerian mixed tumor (MMMT).[3] Since then, there have only been 5 reported cases in English literature in which YST was admixed with epithelial and spindle cell components in postmenopausal women (Table 1). We report a case of a 60-year-old post-menopausal female who presented with an enlarging abdominopelvic mass, ultimately diagnosed as a case of ovarian carcinosarcoma with concurrent YST based on histomorphology and immuno-histochemical staining.

Table 1. Mixed yolk sac tumors with epithelial component in post-menopausal women *(adopted from Roth et al., 2011)*[6]

Reference	No. of Cases	Histologic Diagnosis
Nogales et al. (1996)[3]	1	MMMT, YST
Garcia-Galvis et al. (2008)[4]	1	MMMT, YST
Fadare et al. (2019)[5]	3	C1 MMMT, YST / C2 MMMT, YST / C3 MMMT, YST
Total cases	5	

C, case; MMMT, malignant Müllerian mixed tumor; YST, yolk sac tumor

CASE

The case is a 60-year-old postmenopausal patient who presented with a two-month history of enlarging abdominal girth, especially at the right lower quadrant. Physical

examination upon admission revealed a palpable right hemi-abdominal mass up to the level of the umbilicus, measuring roughly 8 cm. in widest diameter, described as cystic, doughy to firm, non-tender, with limited mobility. Base serologic levels of human epididymis protein 4 (HE4), cancer antigen 125 (CA 125), and carbohydrate antigen 19-9 (CA 19-9) were determined**,** which showed elevated levels of HE4 and CA 125 at 502.2 pmol/L (normal range at ≤140 pmol/L) and 689.3 U/mL (normal range at 0.00 - 35.00 U/mL), as well as normal levels of CA 19-9 at <1.20 U/mL (normal level at 0.00 - 37.00 U/mL). Pelvic ultrasound showed a multiloculated solid cystic structure located posteroinferior to the uterus, described as having multiple papillations. A whole abdominal CT scan was also done, which showed a large thin-walled multiloculated heterogeneously enhancing, predominantly cystic mass in the abdomino-pelvic region with an irregular, heterogeneously enhancing soft tissue component located anterosuperiorly. Clinical diagnosis by the gynecologist and medical oncologist was of an ovarian new growth.

Total abdominal hysterectomy with bilateral salpingo-oophorectomy (TAHBSO) was done, as well as cyto-reductive surgery and hyperthermic intraperitoneal chemotherapy with Cisplatin. Intraoperatively, the pelvo-abdominal cystic mass was identified to be the right ovary. The right fallopian tube was stretched out and seen to envelop the right ovarian mass. There were multiple mesenteric implants, enlarged pelvic lymph nodes, and minimal perihepatic ascitic fluid.

The TAHBSO specimen was sent for histopathology. Gross examination revealed that the right ovary was converted into a yellow-tan to pink-tan, solid to cystic mass measuring 12.5 x 11 x 7.5 cm. Sectioning of the mass revealed solid to cystic cut surfaces with egress of chocolate brown fluid with some clots. The solid portions had cream tan to yellow, soft to friable cut surfaces with areas of hemorrhage and necrosis.

Microscopic examination of the ovarian mass disclosed a malignant neoplasm with heterogeneous architecture, primarily composed of a carcinomatous and a sarcomatous component. Architecturally, there was blending of the two components in some areas. The carcinomatous component comprised roughly 60% of the tumor and was composed of infiltrative nests and papillae of epithelial tumor cells with enlarged, hyperchromatic, pleomorphic nuclei, and scant to moderate amount of cytoplasm (Figure 1). The sarcomatous component comprised roughly 30% of the tumor and was composed of sheets of atypical spindle cells with pleomorphic, hyperchromatic nuclei (Figure 2). Brisk mitotic activity is also noted. No heterologous mesenchymal elements were seen in the microsections obtained.

Another histological component was seen in the solid portion of the mass, comprising roughly 10% of the tumor. It had areas with microcystic and reticular architecture in a myxoid stroma (Figure 3). Present in this area are structures arranged in tubulo-papillary pattern with a central vascular core, lined by cuboidal to columnar epithelial-like cells (Figure 3, inset). With the heterogeneity of the tumor showing both glandular and mesenchymal components, an initial histopathologic diagnosis of carcinosarcoma in the right ovary was considered. However, due to the presence of areas with microcystic and reticular architecture not commonly seen in carcinosarcoma, the case was referred to a subspecialist. Immunohistochemical stains were also requested.

Immunohistochemical staining results showed positive staining for Paired-box gene 8 (Pax-8) and Estrogen Receptor (ERA) in the areas showing glandular differentiation (Figure 4). The same tumor population was negative for Sal-like protein 4 (SALL4) and Glypican-3. These results favor the presence of a malignant epithelial component. On the other hand, while some of the sarcomatous component showed diffuse positivity for CK,

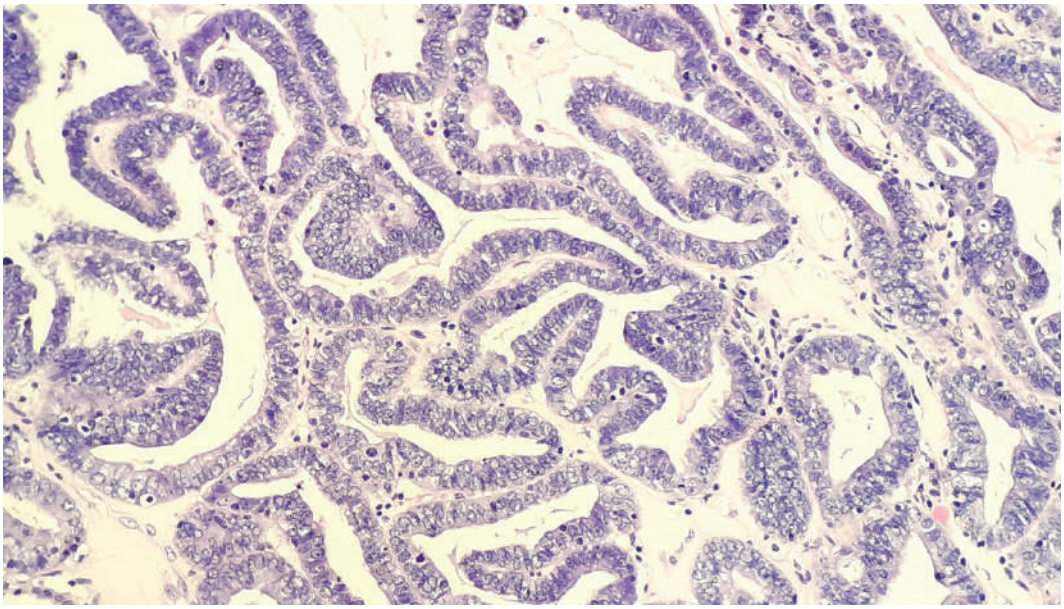

Figure 1. The carcinomatous component show glands and papillae lined with epithelial tumor cells with enlarged, hyperchromatic, pleomorphic nuclei and scant to moderate amount of cytoplasm (H&E, 40x).

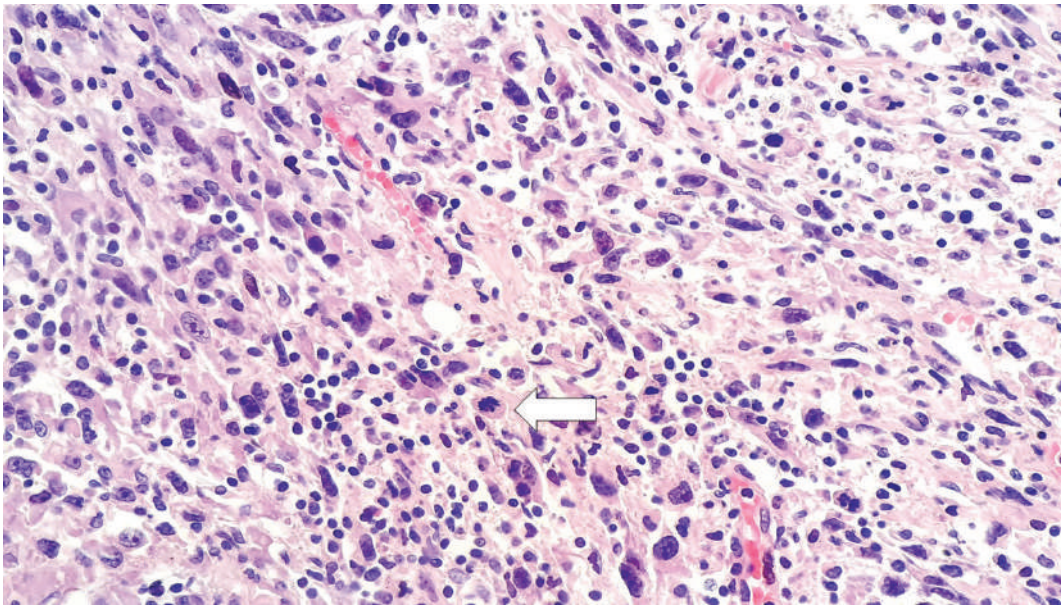

Figure 2. The sarcomatous component is composed of loose sheets of atypical spindle cells with pleomorphic, hyperchromatic nuclei. A mitotic figure can be seen (arrow) (H&E, 40x).

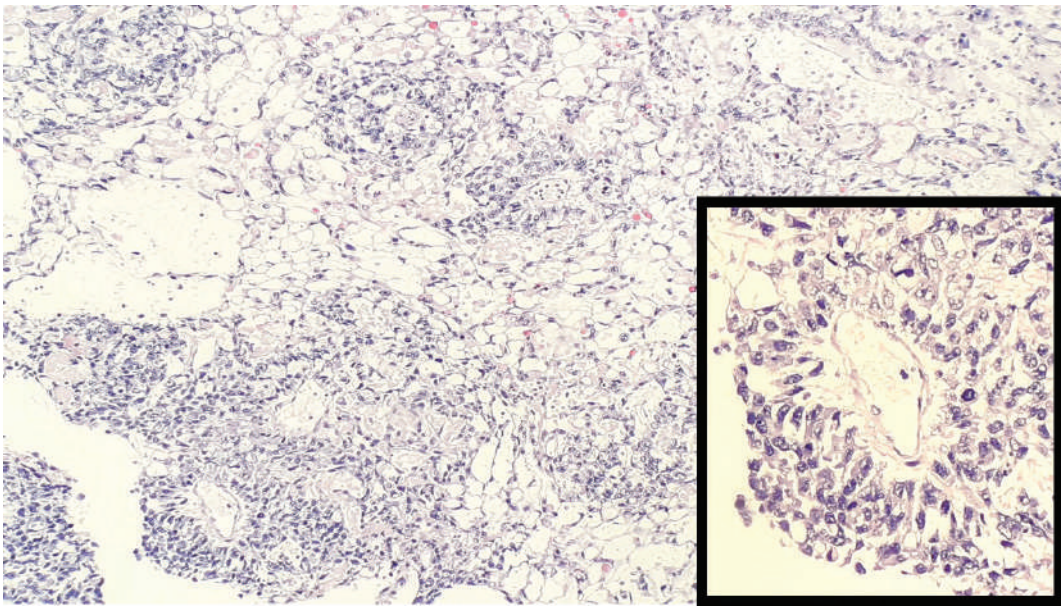

Figure 3. Yolk sac tumor component with microcystic and reticular architecture in a myxoid stroma. Inset. Schiller-Duval bodies with a central vascular core lined by cuboidal to columnar epithelial-like cells (H&E, 10x, Inset 40x).

the higher grade areas showed diffuse positivity for CD10 and Vimentin, as well as negativity for CK, supporting its mesenchymal nature (Figure 5). An additional stain for PAX8 was done which was also negative. Desmin also stained negative, supporting the absence of heterologous rhabdomyoblasts. Lastly, SALL4 and Glypican-3 were both found to be expressed in the reticular and myxoid areas where the Schiller-Duval bodies were noted (Figure 6). The positivity of these stains supports a YST component. Alpha-fetoprotein (AFP) was also done, which was negative in this case.

Thus, a diagnosis of carcinosarcoma with a synchronous YST was made, which was seen to extend to the uterine serosa. The fallopian tubes and left ovary were uninvolved. Pelvic lymph node dissection revealed metastasis to the mesenteric lymph node. Liver nodules were also seen and a portion of the liver was submitted, but was ultimately negative for tumor. A mesenteric implant seen intraoperatively was positive for tumor (2 cm in widest dimension). Peritoneal fluid was also sent for cytology which had atypical cells present; however, immunohistochemistry was not done to further elucidate the nature of these cells. The pathologic stage was determined as pT3bN1M0 based on AJCC (American Joint Committee on Cancer, 8th edition), and FIGO (International Federation of Gynecology and Obstetrics) System Stage IIIB.

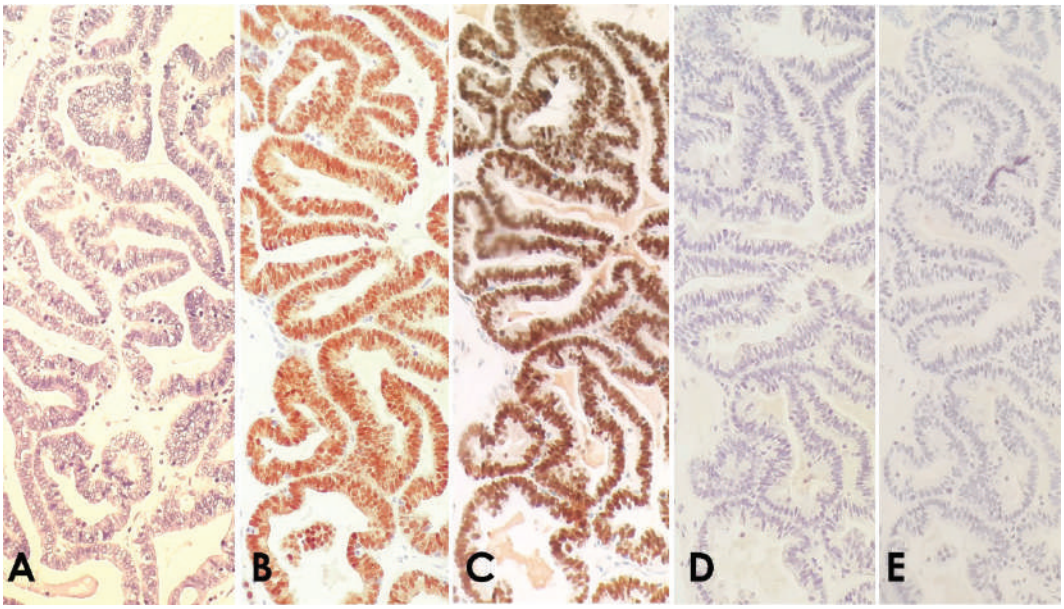

Figure 4. Carcinomatous component **(A)** H&E, 10x; **(B)** PAX8 with diffuse positive nuclear staining, 10x; **(C)** ERA with diffuse positive nuclear staining, 10x; **(D)** SALL4 negative, 10x; **(E)** Glypican negative, 10x.

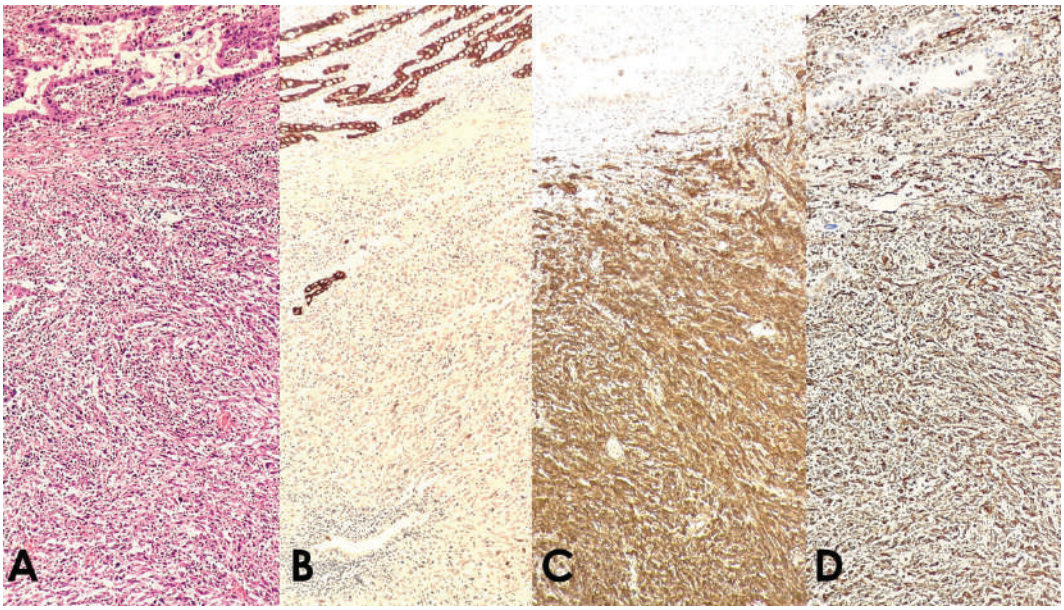

Figure 5. Sarcomatous component **(A)** H&E stain, 10x; **(B)** CK negative, 10x; **(C)** CD10 with diffuse positive cytoplasmic staining, 10x; **(D)** Vimentin, with diffuse positive cytoplasmic staining, 10x.

Post-operatively, the patient received 10 cycles of chemotherapy with Paclitaxel-Carboplatin. Serology markers (carcinoembryonic antigen [CEA], HE4, and Ca-125) were monitored every 1-3 months (Figure 7). Serum AFP was determined roughly one month post-operatively and found to be within normal range at <1.30 ng/mL (normal range at 0.00 - 8.00 ng/mL). As of writing, the patient is currently alive and undergoing serologic monitoring. A repeat whole abdominal CT scan 5 months after the surgery revealed no discrete enhancing mass lesion or abnormal fluid collection in the surgical bed, but with a slight increase in the size of the enlarged precaval lymph node; enlarged lymph nodes are also seen in the para-aortic region.

DISCUSSION

YSTs occur rarely in elderly women, and when they do, they are mostly combined with other epithelial tumor components,[6] unlike YSTs in younger women which present as pure or mixed with other germ cell components. As germ cells are not identified histologically in the ovaries of postmenopausal women, a direct origin of malignant neoplasms from germ cells is highly unlikely at that age, and the possibility of a collision tumor, in part derived from germ cells and in part from epithelium, is unlikely. Roth et al., proposed that in older women, the germ cell component arises through the transformation of the epithelial precursor neoplasm, referred to as "neometaplasia."[6]

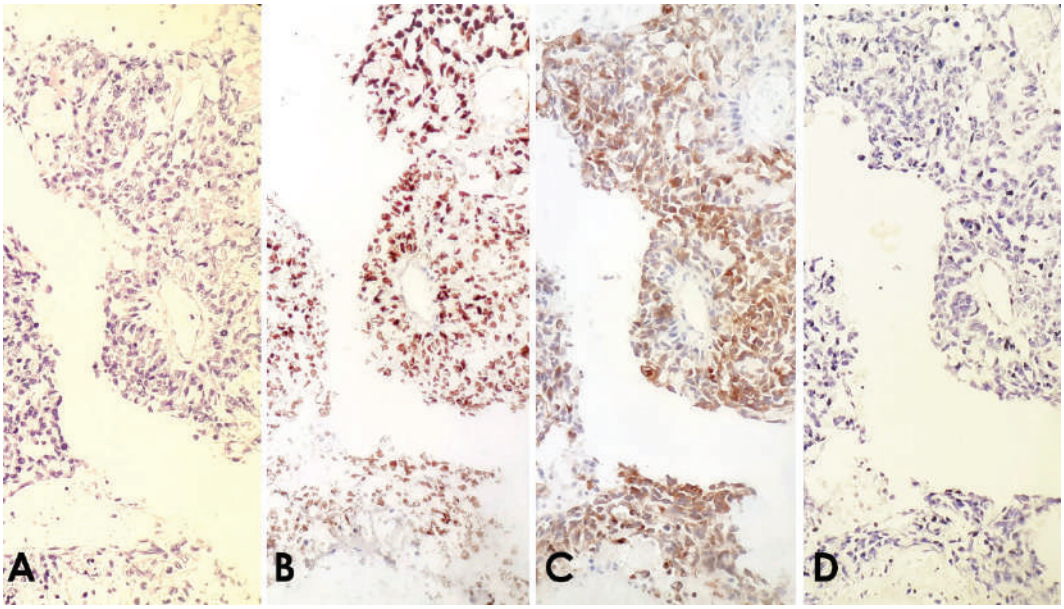

Figure 6. Yolk sac tumor component **(A)** H&E, 10x; **(B)** SALL4 with diffuse positive nuclear staining, 10x; **(C)** Glycipan-3 with diffuse positive nuclear staining, 10x; **(D)** AFP negative, 10x.

McNamee et al., proposed the term "somatically-derived YSTs" for such tumors which have undergone neometaplasia from epithelial precursors.[7] One point raised was how the YST components in their study were often diffusely positive with epithelial markers (EMA, BerEP4, CK7), which may reflect true epithelial differentiation in the YST component in keeping with the morphology of a glandular variant. In support of this shared origin between the different morphologic phenotypes, Ahn et al., were able to identify a tumor component with transitional histologic features between the YST, mucinous, and large-cell neuroendocrine tumor components in their specimen, so much so that the PAX8, glypican-3, and SALL4 immunoreactivity patterns were observed in these morphologically overlapping areas.[8]

The presence of spindle cells in the specimen infer the need to distinguish between a true carcinosarcoma from an endometrioid carcinoma with spindle cells, so called sarcomatoid carcinoma. The distinction between carcinosarcoma and sarcomatoid carcinomas lies on two things: first, the degree of atypia of the glandular and sarcomatous components, and second, whether or not the two components are spatially distinct from one another.[9] In our case, both epithelial and sarcomatous components were notably high grade with increased cellular pleomorphism and mitotic activity. In addition, although some areas in our case showed intermingling of the epithelial and sarcomatous components, carcinosarcomas in the ovary are noted to show two distinct components but are typically intermingled with one another. Diffuse

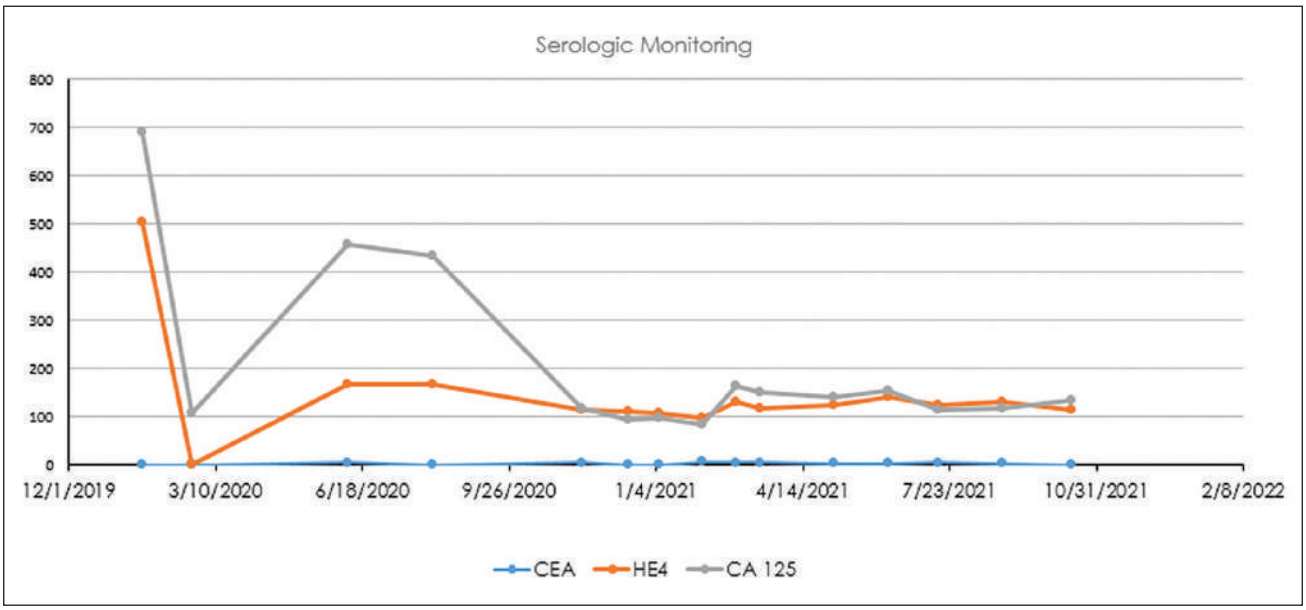

Figure 7. Serologic Markers. CEA, HE4, and CA-125 levels were monitored post-operatively, which showed a decreasing trend.

positivity with CD10 and vimentin exclusively in the spindle cell areas, as well as negativity for PAX8 and CK in the high-grade spindle cell areas, is consistent with the sarcomatous component of a carcinosarcoma.

The most common and distinctive patterns for YST in postmenopausal women include the reticular, or microcystic, and the endodermal, or pseudopapillary pattern.[10] Schiller-Duval bodies are most often associated with the latter and are diagnostic of YSTs. These were seen in our case. On the other hand, endometrioid carcinoma is the most common epithelial component in mixed YSTs in post-menopausal women[6,10] Endometriosis often coexists with these tumors as a precursor lesion.[10,11] Only five cases had carcinosarcoma as the epithelial component since its first mention in English literature. [3-5]

PAX8 was used to identify the epithelial component in our case. PAX8, a member of the mammalian paired box genes 1 to 9 that encodes a transcription factor involved in embryogenesis, is thought to play a regulatory role in cell fate decisions during the development of Müllerian organs.[12] As it is associated with Müllerian development, it is negative in those ovarian tumors not associated with Müllerian surface epithelium, including GCTs and sex cord stromal tumors. Other immunohistochemical stains that can be used for differentiating ovarian YST from endometrioid carcinoma include Epithelial membrane antigen (EMA), CK7, and BerEP4.[7,13] These stains are generally negative or only focally positive for YST, and are generally diffusely positive for Müllerian adenocarcinoma.

AFP is a stain that has conventionally been positive in YST. However, YSTs often express AFP only focally, as demonstrated in the study by Roma et al., so much so that positivity for AFP did not reliably establish the diagnosis of YST in their case.[12] Newer markers glypican-3 and SALL4 are useful in the identification of the YST component.[6] Positive cytoplasmic staining for glypican-3, an oncofetal protein expressed in fetal liver and malignant tumors of hepatocytic lineage, is more sensitive than AFP but not as specific.[11] On the other hand, although SALL4, a zinc finger transcription factor, is not specific for YSTs amongst germ cell neoplasms, it is a specific and sensitive marker for GCTs and its positive nuclear staining is useful in distinguishing YSTs from non-GCTs such as carcinomas.[7] In our case, the YST component was positive for glypican 3 and SALL4, and negative for AFP.

As the YST component arises by transformation of an epithelial ovarian neoplasm resulting in a different molecular pathway than those of GCTs in younger patients, it may be less sensitive to chemotherapy than those that arise de novo.[14,15] Because of its rarity, there are currently no systemic treatment guidelines available. As with pure GCTs, Bleomycin-Cisplatin-Etoposide (BEP) chemotherapy is a potential choice for treatment and may be effective not only in the germ cell but also in the epithelial component.[10] On the other hand, as what is recommended for carcinosarcomas,[14] adjuvant therapy with cisplatin or carboplatin-based chemotherapy, such as carboplatin and paclitaxel, has also been found to be effective.[6,13,16] The core of effectivity in both treatments rests on the platinum ingredient, which was found to be effective against both epithelial ovarian cancer and GCTs.[10] For our patient, she has undergone Cytoreductive Surgery with Hyperthermic Intraperitoneal Chemotherapy (Cisplatin). Post-operatively she has also received 10 cycles of chemotherapy with Paclitaxel-Carboplatin.

The biologic behavior and prognostic significance of a mixed YST and carcinosarcoma in the ovary of postmenopausal women are unclear due to the limited case numbers and insufficient clinical information. In general, YST in the postmenopausal population is associated with a distinct biologic behavior characterized by poor prognosis even with early-stage disease, whether or not an epithelial component is detected.[13] Recurrence occurs within 7 months despite systemic chemotherapy,[6] and mortality within 8 months to 2 years.[6,10,15] Some studies have suggested that the disease-free survival of patients with mixed YST-epithelial carcinoma follows the epithelial component. The median survival for such cases is estimated to be less than 18 months to less than 2 years.[16] As of writing, our patient is currently alive roughly 20 months after being diagnosed with the disease at FIGO Stage IIIB.

Elevated tumor markers have been shown to be an independent poor prognostic indicator.[10] Serum markers may normalize during chemotherapy, but this may reflect regression of only one component of the mixed lesion. Our case did not have available serum levels of AFP before surgery as YST in postmenopausal patients is very rare and its diagnosis was not suspected before surgery. Levels of CA-125 were elevated which may be attributed to the epithelial component in our case.

CONCLUSION

We report a case of mixed YST with carcinosarcoma in a postmenopausal female. Because of its rarity, the combination of histomorphology and immunohistochemical stains may aid in its identification. Though YST components may be less responsive to traditional germ-cell tumor chemotherapy because of its differentiation from epithelial components, adjuvant Platinum-based chemotherapy is useful as it targets both epithelial ovarian tumors and germ-cell tumors. Ovarian YSTs in postmenopausal women have a poor prognosis, and therefore more active treatment and post-operative monitoring are needed.

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ETHICAL CONSIDERATION

Patient consent was obtained before submission of the manuscript.

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All authors certified fulfillment of ICMJE authorship criteria.

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The authors declared no conflict of interest.

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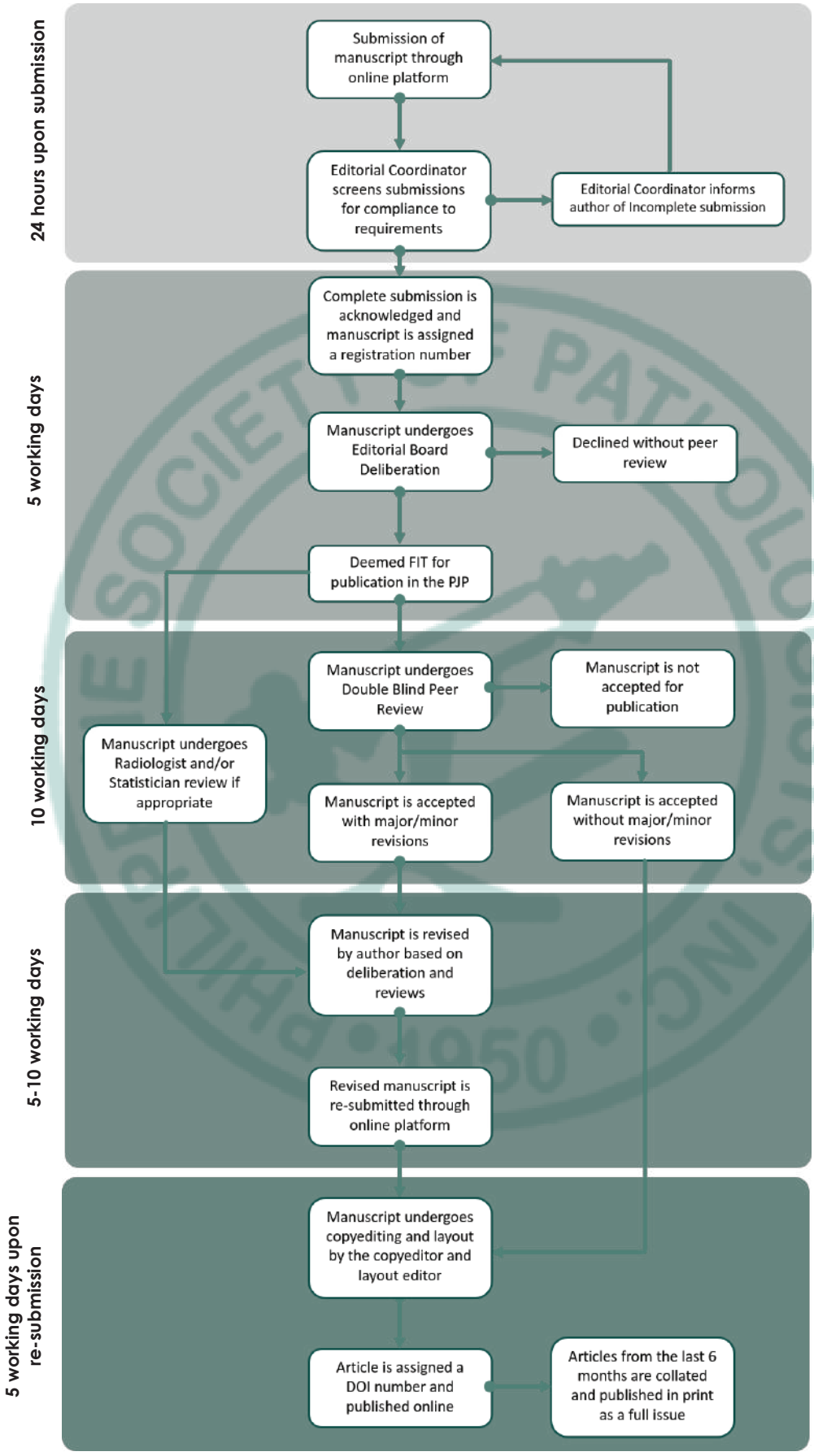

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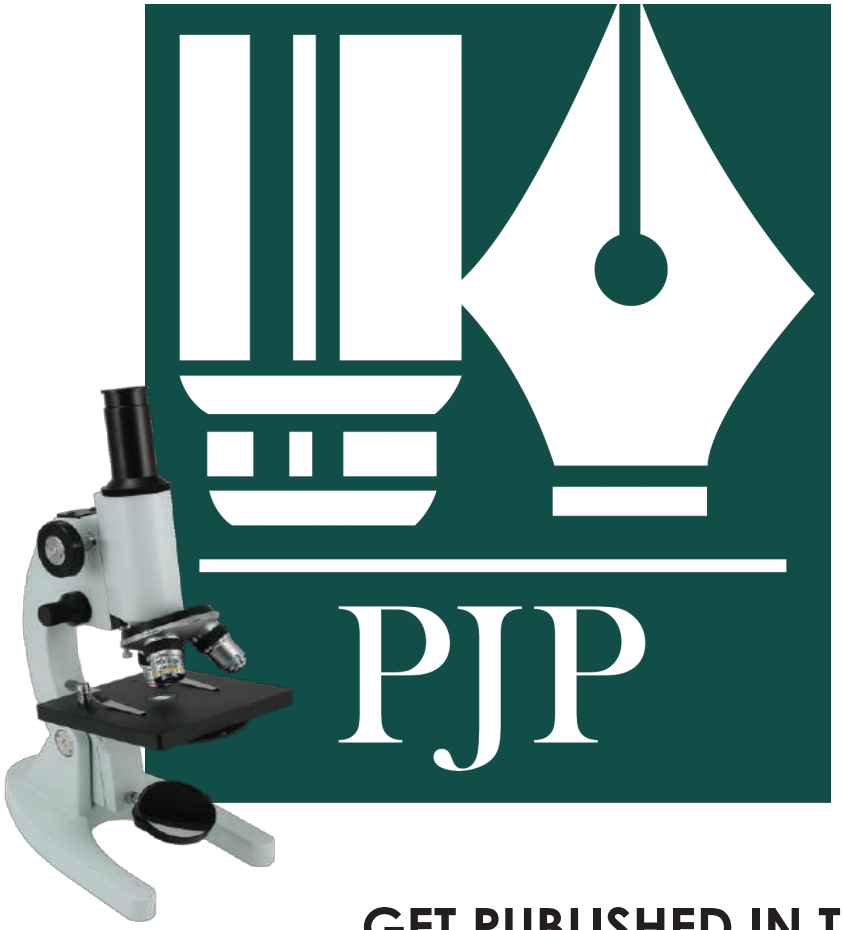

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