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Greetings!

Welcome to the June 2021 issue of the Philippine Journal of Pathology. My congratulations to the very hardworking editorial team of PJP who remained steadfast in their determination to come up with this issue and to the unwavering support of the Board of Governors of the Philippine Society of Pathologists, Inc.

As the Philippine Journal of Pathology continues to publish the contributions of our members of their scholarly work, they help us improve our diagnostic ingenuity. Researches and case reports help us in our shrewdness in handling our daily work issues in the Pathology Laboratory. We learned to adapt and innovate in this time of COVID-19 pandemic to meet the current demand for a safe working environment and coming up with quality laboratory test results released in a timely manner thus help in improving patient outcomes.

Let us look forward to the many more issues of the PJP and hope that you can join us as one of its contributors. God speed to the Philippine Society of Pathologists, Inc. and the Philippine Journal of Pathology.

Wishing you all a safe and healthy days to come.

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

Life, Death, Transitions

I am sharing with you the first issue of the Philippine Journal of Pathology for 2021, our tenth issue since we breathed life into our society publication in 2016. We have two case reports of gynecologic tumors, and an autopsy case of a disseminated hemato-lymphoid malignancy. Four original articles include a molecular pathology study for lung cancer, a real-world observational study on pooled testing among asymptomatic individuals, a cost effectiveness study on diagnostics for leukemia in the Philippines, and a 5-year soft tissue tumor review. On top of these, we are featuring a new section – Opinion, and we have on board, Dr. Raymundo Lo, to regularly provide his take on issues relevant to our profession and practice. This issue also comes alive with the inclusion of the entries and winners of the PSP 69th Annual Convention Photomicroscopy Contest sponsored by Omnibus Biomedical Systems.

Much has happened and is happening in this ongoing battle with COVID-19, the evolution of the national response, from surveillance to diagnostics to treatment, and now to immunization, the worrisome evolution and spread of variants of concern, the latest of which is the delta variant from South Asia, from the New Normal to the Next Normal and hopefully to the Better Normal.

On a personal note, however, no other event is more impactful to me than the loss of a mentor and friend, a beloved pathologist, and our adviser for the PJP, Dr. Jose Ma. C. Avila (Figure 1). It was he who helped us initiate efforts to revive our journal, and I can clearly remember him asking me in his straight-forward booming voice, "do you even have anything to publish?" We dedicate this issue to him to celebrate his life and his transition to the next.

Figure 1. Dr. Jose Ma. C. Avila†, PJP Editorial Adviser.

Early on, we knew that the government reference laboratory and its handful of sub-national laboratories, cannot provide the scale of testing needed for the pandemic without the help of the other government laboratories and those in the private sector. The last time I saw Dr. Joey face to face was when we were visiting Makati Medical Center to activate their molecular laboratory for COVID-19 testing. The last time I was able to talk to him was around the third quarter of 2020, when they were running short of viral transport media. He ended the call by encouraging me to endure, as by then my office faced so many logistic challenges and political pressures due to the weaknesses of the national laboratory response. His words, "you are doing a great job, under the circumstances," meant a lot and kept me afloat, among other things.

Two years ago, I wrote a management case on the national reference laboratory surge capacity response to a massive nationwide measles outbreak in the Philippines.[1] This was part of the requirements for my public management development course at the Development Academy of the Philippines, was subsequently presented to a panel, and was eventually published in our journal. Surge is defined as "a sizable increase in demand for resources compared with a baseline demand"[2] further described as "sudden, unanticipated escalations…caused by exceptional events."[3] On the other hand, surge capacity, is the ability to "manage a sudden or rapidly progressive influx…within the currently available resources at a given point in time,"[3] and further as the "ability to effectively and rapidly expand capacity"[3] in recognition of the fact that surge capacity relates not only to sufficiency of currently available resources but even the ability to effectively and rapidly expand capacity.

Kelen and McCarthy combined surge and surge capacity to introduce the concept of **surge response capability** (Figure 2) – which is the "ability of the surge capacity (i.e., resources that are available and can be made available) to accommodate the surge (i.e., demand for resources).[2]

Thus, when surge capacity exceeds surge, the surge response capability is greater than 1 (the operating unit can respond effectively); however, when surge exceeds surge capacity, the surge response capability is less than 1 (the operating unit's systems will be challenged and may even fail to respond). The last equation attempts to dissect the determining factors for surge capacity (system [integrity], space [size, quality], staff [numbers, skill], supplies [volume, quality]) and surge (event [type, scale, duration], influx, resource demand [consumption, degradation]).

It is very glaring in the relationships described that planning exists as a common, major, and independent

$$\text{Surge Response Capability} = \text{Planning} * \frac{\text{Maximized Available Resources}}{\text{Resource Demand}}$$

$$= \text{Planning} * \frac{\text{Surge Capacity}}{\text{Surge}}$$

$$= \text{Planning} * \frac{\text{System}_{(\text{integrity})} * \text{Space}_{(\text{space} * \text{quality})} * \text{Staff}_{(\text{numbers} * \text{skill})} * \text{Supplies}_{(\text{volume} * \text{quality})}}{\text{Event}_{(\text{type} * \text{scale} * \text{duration})} * \text{Influx} * \text{Resource Demand}_{(\text{consumption} * \text{degradation})}}$$

Figure 2. Functional relationship of surge response capability to surge capacity and surge *(Adapted from Kelen and McCarthy, 2006).*[2]

determinant of surge response capability. And yet the outbreaks of the past were not enough to stir us to really get down to business and plan for national health security. Dr. Lo, in his opinion piece, underscores the importance of learning our lessons and planning for the next pandemic, mentioning the pending bill on the creation of the Philippine Centers for Disease Control. Unfortunately, with the way discussions are going among lawmakers, the establishment of the CDC means the abolition of the very Institute that has stood its ground against this pandemic from Day 1, amid responding to other emerging and re-emerging threats. We can only hope that the powers-that-be can go beyond merging and dissolution of bureaus and offices, to purposively develop two things: a strong organization that can truly and capably function as *the* national public health institute, and a similarly strong network of laboratories that can be engaged readily as part of the country's surge capacity.

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

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Lessons from the Pandemic

We have seen the events of the pandemic unfold from our unique perspective as pathologists. Early on, we stood helpless as the virus ravaged our cities and towns with ferocity while we could hardly do anything. We were woefully unprepared to cope with the testing. Very few labs were capable of doing RT-PCR testing, exposing our unpreparedness in molecular pathology.

Now, we are empowered with the existence of over two hundred molecular labs that can be harnessed to combat the pandemic. Our once sleepy lives have been jolted into action by the urgency of identifying virus infections. Furthermore, the rampant spread of the virus has given rise to many variants, thus requiring much more testing than we are currently doing.

Almost as soon as the pandemic began last year, the Philippine Society of Pathologists Inc. (PSPI) produced position papers urging the use of pooled testing to expand testing capacity as a solution to the scarcity of reagents, PPE, and manpower, as well as a way to overcome cost constraints. We launched a study for proof of concept, resulting in the Department of Health's finally giving its imprimatur for its use on a national basis.[1] We held trainings for pooled testing to empower many labs to perform it, in the hope that it would further expand testing capacity. This, however, has remained constricted due to cost issues.[2]

Surveillance testing is very important, because asymptomatic persons are driving the spread in our population. If we do not identify and isolate these spreaders, we will continue to experience outbreaks and possibly more surges such as occurred early in the second quarter of 2021. Once again, the PSPI has proposed pooled testing as a means to curb localized outbreaks from which major surges may arise.[2] Unfortunately, the authorities seem to have turned a deaf ear to our call despite its potential benefits: It would mean an easing of lockdowns and other economic restrictions if it were to be implemented on a widespread basis. It would control the cycle of surges and lockdowns as are happening now in various areas in the country.

Now, to the issues still vexing our pandemic response. The fragmented reactions of local governments are a main concern. The devolution of health care was fine in previous situations, but the public health emergency of a pandemic demands a more cohesive approach on a national level. This will have to be solved by our lawmakers.

As in many emergencies, quick thinking with reflex actions based on previous experience and practice will solve many problems. Mental agility, however, has not been a manifest virtue of our government agencies. This cannot be the norm in pandemics. We need to plan ahead while still grappling with the SARS-CoV-2 pandemic, which we must presume is but a harbinger of things to come.

The proposed Philippine Centers for Disease Control (PCDC) will be a step in the right direction. Pathologists would be assigned as laboratory directors, while the rest can perform other functions in research, test development, and quality assurance. Hence, we can plan a more functional response if and when outbreaks, epidemics, and pandemics occur. It is we pathologists who are more acquainted with laboratory techniques and testing, so it is but right to have one at the helm. Make no mistake about it, we *will* have more pandemics. It is only a matter of time, now measured not in centuries as previously happened but more in decades or years.

The deficiencies we saw last year are the ones we should be addressing now. Capacity building for gene sequencing is fundamental to identifying new, emerging, and re-emerging viral and other infections capable of developing into pandemics. We cannot just rely on foreign agencies to identify a pathogen that has sprung in our midst.

The supply constraints we experienced are lessons to draw from so that we can respond more adequately and plan for a more secure supply chain not wholly dependent on foreign sources. We should develop our own molecular reagents, which should be validated and can be applied to these new pathogens in as quick a time period as possible.

Having identified and sequenced a new pathogen, we should be capable of developing our own testing kits with the various components we should have stocked up on previously. This—combined with the many molecular laboratories we now have and the validated pooled testing technique our Society has pioneered in—will prevent the huge backlog of testing we saw in the early months of last year.[2]

There will be questions as to the feasibility or viability of this scenario. Some may ask: What will this agency be doing while awaiting the next pandemic? Will the equipment become outdated, and reagents expire?

Well, no. We should not just be waiting for the next pandemic. We can cut our teeth, so to speak, on proactively solving public health concerns rather than just reacting as our health system is currently doing.

This agency should be monitoring diseases of concern like TB, HIV, polio, measles, and other viral infections plaguing our country now. Using the lessons learned from this pandemic, we can track possible outbreaks with routine periodic testing of sewage for polio virus, and even for SARS-CoV-2. What better way for us to do this than with the economics of scale that pooled testing provides?[2] The same technique can be used for other respiratory diseases that routinely visit the country.

Another object lesson is the impracticality of concentrating testing expertise in one or two institutions. This creates bottlenecks in combatting epidemics/pandemics, as we saw last year. We should continue training laboratory personnel and managers, both public and private, in testing techniques that matter in public health emergencies. Let us regionalize our efforts in public health surveillance by capacitating major regional centers for disease control.

Lack of training has been another issue in our response to this pandemic. We have been remiss in the training of medical technologists for molecular testing, again because of centralization. Let us have more capacity building for training as well. With the continuous exodus of our health workers, including medical technologists, we have no recourse but to keep training them as soon as they enter the workforce.

Planning should include embracing new technology as it arises. Updating our diagnostic armamentarium should be top priority now and in the future. Faster, more efficient, and accurate equipment makes for a more agile response to a rapidly developing infectious-disease scenario. Needless to say, this requires making our government leaders aware of its importance and for them to regularly allot the correct fiscal budget on a regular and not just a one-time basis.

Research should play a major role in public health matters. How else will we know the insidious-creeping incidence of infectious diseases without top-notch medical sleuthing? These centers for disease control should also make budget provisions for research and reagent formulations.

In summary, we should learn from our mistakes committed during this pandemic, which has already caught us flat-footed. Let us start our capacity building by investing in our public health laboratories, equipping them properly, staffing them with trained competent people, continuously monitoring endemic and emerging diseases, and insuring supply-chain continuity in the face of rapidly emerging infectious diseases. It is a war we cannot afford to lose.

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Dr. Raymundo Lo is a respected pathologist and 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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OPEN ACCESS – ORIGINAL ARTICLE

Programmed Death Ligand 1 (PD-L1) Expression and its Association with Clinicopathologic Profile in Patients with Non-Small Cell Lung Cancer in a Philippine Tertiary Medical Center

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ABSTRACT

Introduction. The current management of advanced non-small cell lung cancer (NSCLC) includes the characterization of Programmed Death Ligand-1 (PD-L1) expression for potential immune checkpoint inhibitor treatment. There is currently no available data regarding the patterns of PD-L1 expression in NSCLC, as well as their association with clinicopathologic profile in Filipino patients.

Methodology. Clinicopathologic characteristics of 187 consecutive NSCLC clinical samples with PD-L1 testing using the clone 22C3 pharmaDx kit were collected. The presence of stromal tumor-infiltrating lymphocytes (TILs) were assessed in hematoxylin and eosin-stained slides. PD-L1 expression on tumor cells (TC) and stromal TILs were evaluated.

Results. Of the 187 cases, there were 112 males and 75 females. The mean age at diagnosis was 66.4 years old (32-92 years old). It is composed of 131 cases of adenocarcinoma, 15 squamous cell carcinoma, 4 adenosquamous carcinoma, 32 non-small cell carcinoma, not otherwise specified, 3 poorly differentiated malignancy, 1 large cell carcinoma, and 1 mucinous carcinoma. Specimen types included 17 pleural fluid cell blocks, 60 tumor cell block samples, and 110 tissue biopsies. Tumor cell PD-L1 expression was identified in 59.1% of the 110 tissue biopsies. PD-L1 TPS for histologic specimens are as follows: TPS ≥50%, TPS 1-49%, and TPS <1% were observed in 23.6%, 35.5%, and 40.9% in our lung cancer cohort, respectively. Of the 77 cytology specimens, 50.6% presented with TC PD-L1 expression. TPS for this subgroup include: 49.4% with no PD-L1 expression, 35.1% with low PD-L1 expression, and 15.6% showing high PD-L1 expression. PD-L1 expression on TC did not correlate with age, sex, or histology for both specimen type subgroups. Stromal tumor-infiltrating lymphocytes were noted in 74.5% of tissue biopsies. Tumor cell block samples did not demonstrate stromal TILs. For tissue biopsies, female gender and TPS 1-49% were more likely to have <50% PD-L1 expression on TILs.

Conclusion. Overall TC PD-L1 expression was observed in more than half (55.6%) of NSCLC patients in our cohort. The prognostic value of PD-L1 and clinical response to immune checkpoint inhibitors in the Filipino population needs to be further investigated.

Key words: non-small cell lung cancer, lung cancer, PD-L1, Philippines

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INTRODUCTION

Worldwide and local statistics show that lung cancer remains to be the most frequently diagnosed of the cancer types, and also the most common cause of cancer mortality in both sexes.[1,2] In addition to traditional platinum-based chemotherapy, use of targeted and immune therapies have increased in the past years and yielded favorable outcomes. Molecular diagnostic tests including PD-L1 immunohistochemistry are utilized to identify possible targets of treatment.[3,4]

The World Health Organization has classified LC based on biology, treatment and prognosis into: 1) non-small cell lung cancer (NSCLC) and 2) small cell lung cancer. NSCLC is the more common (80%) of the variants, and it is further subdivided into two major types: 1) nonsquamous (including adenocarcinoma, large cell carcinoma and other cell types) and 2) squamous cell carcinoma. Majority of patients with NSCLC are diagnosed during the advanced

course of the disease, with about a third to half of patients diagnosed using cytology specimens alone.[3-5]

T cells play a vital role in tumor cell recognition and eradication. However, one of the major mechanisms by which a variety of solid tumors (melanoma and carcinoma of the lung, pancreas, breast, colon, stomach, ovary, kidney, and urothelium) evade the host immune response is through "adaptive immune resistance" by overexpression of Programmed Death Ligand-1 (PD-L1). In normal tissue, the Programmed Death-1 (PD-1)/ PD-L1 pathway controls the immune response. PD-1 (also known as CD279) is a surface receptor expressed on activated T cells, B cells, natural killer cells, activated monocytes and dendritic cells and is usually expressed in high numbers by tumor-inflitrating lymphocytes (TILs). PD-L1 (also known as B7-H1 or CD274) and Programmed Death Ligand-2 (PD-L2) are the two identified ligands of PD-1. Of the two ligands, PD-L1, which is expressed by a variety of cell types (placenta, endothelial cells, pancreatic islet cells, muscle, hepatocytes, epithelium, mesenchymal stem cells, B cells, T cells, dendritic cells, macrophages and mast cells), mainly moderates the immunosuppressive effects of the pathway. Contact of PD-L1 and its receptor PD-1 leads to apoptosis and suppresses T cell function, differentiation and survival. Hence, increased tumor cell (TC) expression of PD-L1 results in T cell tolerance by downregulation of the host immune response and avoidance of immune recognition which facilitates cancer proliferation.[6-14] The prognostic impact of PD-L1 expression in lung cancer is quite contradicting; however, several studies demonstrated that PD-L1 expression in NSCLC is associated with a poorer relapse-free and overall survival, the association being clearer in stage I tumors.[9,15-17]

Lung cancer treatment include immune checkpoint mechanisms involving the PD1-PD-L1 axis. PD-L1 expression on tumor cells has been previously reported to predict response to PD-1/PD-L1 inhibitors. Patterns of PD-L1 expression of lung cancer patients in many Asian (China, Japan and Korea) as well as some Western (Australia, Germany, Italy and USA) countries have been reported.[15] In a large clinical trial with 1143 lung cancer patients, prevalence and pattern of PD-L1 expression are as follows: 23.2% of patients with tumor proportion score (TPS) ≥50, 37.6% with TPS 1-49% and 39.2% had TPS <1%.[18] Data on the PD-L1 expression status of patients with lung cancer in the Philippines are not available at present, and since PD-L1 expression studies among ethnic groups are still relatively limited, further epidemiologic studies will be beneficial.

PD-L1 expression, as evaluated by immunohistochemistry (IHC), is a predictive biomarker for response to PD-1/PD-L1 monoclonal antibodies (mAbs) or immune checkpoint inhibitors/blockers (ICIs or ICBs). Pharmacologic action of the mAbs is based on PD-1 or PD-L1 inhibition. The goal of treatment is to restore the host immune responses in order to recognize and eliminate tumor cells. Nivolumab and Pembrolizumab inhibit PD-1 receptors, while Atezolizumab and Durvalumab inhibit PD-L1.[3] Each monoclonal antibody is paired with their own PD-L1 antibody: Nivolumab (Bristol-Myers Squibb) with 28-8 rabbit antibody; Pembrolizumab (Merck & Co., Inc.) with 22C3 mouse antibody; Atezolizumab (Genentech) with SP142 rabbit antibody; Durvalumab (AstraZeneca) with SP263 rabbit antibody; and Avelumab (EMD Serono, Inc. and Pfizer) with 73-10 rabbit antibody.[19] Studies have shown a good concordance in TC scoring among the five different assays except for the SP142 clone, which showed lower rates of PD-L1 expression on tumor cells.[20-24] Although these assays were validated using resection or tissue specimens, and pre-analytic variability in cytology specimens is greater than surgically resected specimens, it is suggested that cell block material is as good as their histologic counterpart in PD-L1 biomarker analysis.[15,19,24] Moreover, data by Stoy et al., showed 91% success rate in PD-L1 IHC using cell blocks obtained using bronchoscopy.[25]

Pembrolizumab has been approved by the U.S. Food and Drug Administration (FDA) with the PD-L1 IHC 22C3 pharmDx as "companion diagnostic assay" in which only the patients who tested positive for the marker can be treated with the mAb. Initial approval involved stratification of PD-L1 expression in high (≥50%) and low (1-49%). Monotherapy with Pembrolizumab is preferred as the first line therapy option for metastatic NSCLC with PD-L1 Tumor Proportion Score (TPS) ≥50%, and as second line treatment in metastatic NSCLC patients with PD-L1 TPS ≥1%, provided that these tumors lack *EGFR* mutations, *ALK* translocations, *ROS1*, *METex14* skipping, *RET* or *BRAF* variants. Pembrolizumab had a better safety profile; and treatment resulted in longer overall survival and progression free survival in patients with previously untreated and previously treated, PD-L1 positive, advanced NSCLC as compared with traditional platinum-based chemotherapy. The National Comprehensive Cancer Network (NCCN) NSCLC panel also recommends the combination of Pembrolizumab plus chemotherapy as a first line treatment option in patients with metastatic NSCLC and negative for the specific molecular variants or mutations previously mentioned.[3,11,25-29] Nivolumab was FDA-approved as second line treatment for metastatic NSCLC with the PD-L1 IHC 28-8 only as a "complementary diagnostic assay," which only guides the therapy but is not required for patients to receive the drug.[3,11,19,26] The US FDA initially approved Atezolizumab in combination with traditional chemotherapeutic drugs, as another first-line treatment option for metastatic NSCLC patients with no *EGFR* or *ALK* aberrations, regardless of histology or level of PD-L1 expression.[30,31] Months later, Atezolizumab monotherapy has been FDA-approved as another first line therapy intervention for patients with metastatic NSCLC with PD-L1 TPS ≥50% or PD-L1 stained tumor-infiltrating immune cells (IC) covering ≥10% of the tumor area, and wild-type with respect to *EGFR* mutations and *ALK* translocations. The Ventana SP-142 PD-L1 assay (Ventana Medical Systems, Inc.) was simultaneously approved as a companion diagnostic device in selecting patients eligible for treatment with Atezolizumab. Among the mAbs, however, therapeutic options that include Pembrolizumab and Atezolizumab are preferred, and have received a category 1 recommendation from the NCCN NSCLC panel based on tolerability and experience with these regimens.[3] Despite all these advances in therapy, data on the prognostic effect of PD-L1 expression on tumor cells are conflicting.[16,17,32] In fact, an Egyptian study concluded

that the evaluation of both PD-L1 staining on tumor cells and CD8 TILs density, instead of PD-L1 alone, have a more relevant impact on prognosis.[6]

Lastly, interaction between tumor cells and TILs are described by the concept of immunoediting which involves an elimination phase, equilibrium phase, and escape phase. The innate and adaptive immune cells of the host identify and destroy the tumor cells during the elimination phase. In the equilibrium phase, cancer cells that survive the first phase enter the state of dormancy and escape immune surveillance. Once these tumor cells induce an immunosuppressive state, the host immune response fails to restrict their growth (escape phase) and this results in a clinically apparent disease. The presence of TILs, specifically CD8+ lymphocytes, in the tumor microenvironment has been shown by various studies to be associated with positive clinical outcome in many solid tumors including NSCLC and is also associated with increased responsiveness to PD-1 inhibition in NSCLC. The presence of TILs has also been proven to significantly affect the prognostic yield of TNM classification in both colorectal and breast cancer. PD-L1 expression on TILs is postulated to be driven by adaptive mechanisms wherein inflammation-mediated release of cytokines, particularly interferon-γ (IFN-γ), by activated TILs lead to increase in their PD-L1 expression. PD-L1 expression on TILs has been associated with favorable patient outcome or survival.[6,17,33-35,39]

The study aims to determine the prevalence and pattern of PD-L1 expression on tumor cells of patients with NSCLC in a tertiary medical center in the Philippines, and identify their correlation with the presence of TILs as well as with PD-L1 expression on TILs. It also intends to identify the clinicopathologic features of lung cancer patients associated with PD-L1 expression on both tumor cells and TILs.

Operational definitions

PD-L1 expression on TILs (with 50% as cutoff):
0 – No membrane and/or cytoplasmic staining (at any intensity) using PD-L1 IHC on all mononuclear inflammatory cells within tumor nests and adjacent supporting stroma

less than 50% – membrane and/or cytoplasmic staining (at any intensity) of less than 50% of mononuclear inflammatory cells within tumor nests and adjacent supporting stroma

50% or more – membrane and/or cytoplasmic staining (at any intensity) of 50% or more of mononuclear inflammatory cells within tumor nests and adjacent supporting stroma

METHODOLOGY

Study design, samples and data gathering

This cross sectional study includes 187 consecutive LC clinical samples with PD-L1 testing using the clone 22C3 pharmaDx kit in St. Luke's Medical Center, Global City (SLMC-GC) from January 2017 to June 2018. It includes biopsy and cytology specimens from the lungs and pleura from patients with biopsy-confirmed NSCLC who submitted their tissue samples for PD-L1 testing in SLMC-GC. Only samples with at least 100 viable tumor cells were included in the study since this is the minimum quantity of cells deemed acceptable for PD-L1 IHC testing. Small cell lung cancer specimens were excluded from the study. Data on clinicopathologic features including age, sex, and tumor characteristics (specimen, biopsy type and histologic subtype) were collected from the histopathology reports in the hospital database. The samples were categorized as primary or metastatic NSCLC based on histomorphologic features and/or clinical data or immunohistochemistry results, if available. Institutional review board approval of the research protocol for the study was obtained through the St. Luke's Medical Center Research and Biotechnology group.

Immunohistochemistry and assessment of stromal tumor-infiltrating lymphocytes

Both hematoxylin and eosin (H&E) slides and PD-L1 slides were prepared from 4-μm thick tissue sections of the formalin-fixed, paraffin-embedded (FFPE) tissue blocks of samples from NSCLC patients that were submitted for PD-L1 testing. The PD-L1 IHC 22C3 pharmDx assay employs the Monoclonal Mouse Anti-PD-L1 Clone 22C3 and is visualized utilizing the EnVision FLEX visualization system on the Dako Autostainer Link 48 system. The PD-L1 IHC staining were performed in batches, and the assay

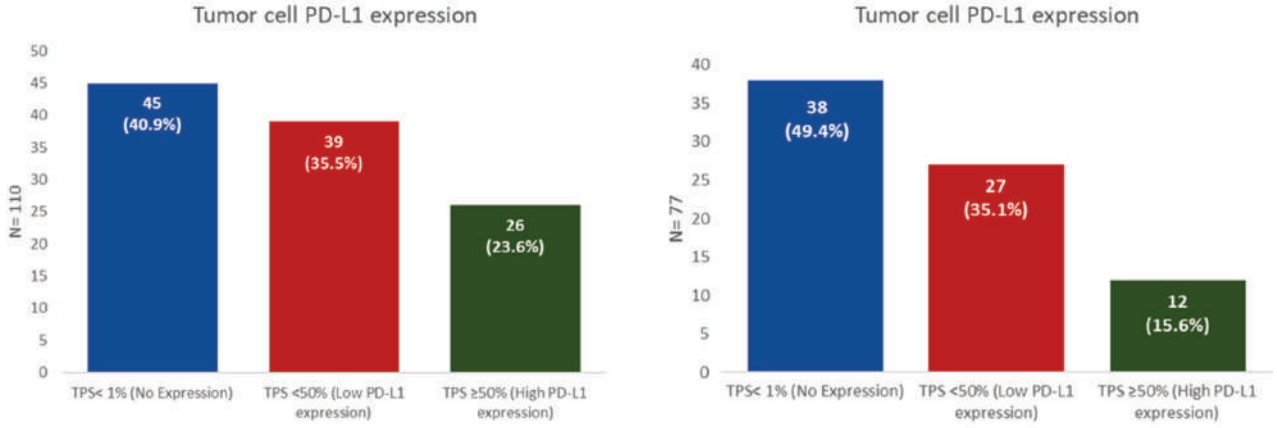

Figure 1. (A) Prevalence of low, high, and no PD-L1 expression on tumor cells in biopsy/resection specimens; **(B)** Prevalence of low, high, and no PD-L1 expression on tumor cells in cytology specimens.

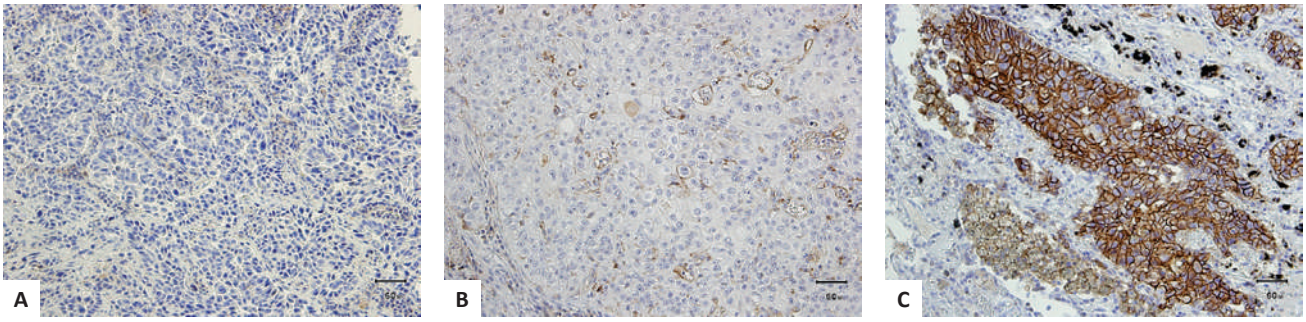

Figure 2. Immunohistochemistry images (10x). **(A)** TPS <1% (no PD-L1 expression); **(B)** TPS 1-49% (low PD-L1 expression); and **(C)** TPS ≥50% (high PD-L1 expression).

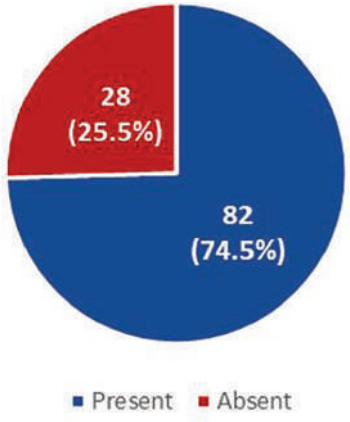

Figure 3. Prevalence of tumor-infiltrating lymphocytes in biopsy/resection specimens.

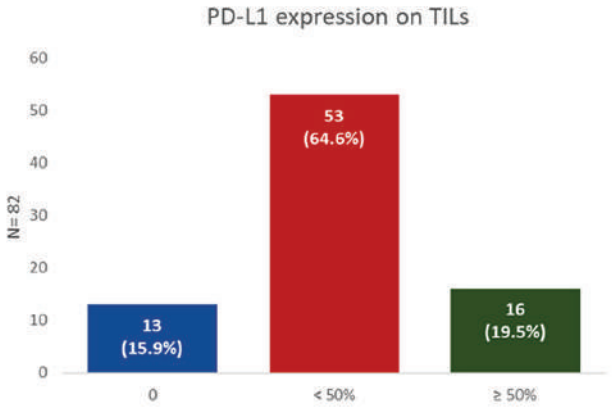

Figure 4. Prevalence of PD-L1 expression on tumor-infiltrating lymphocytes in biopsy/resection samples.

was used according to the manufacturer's instructions. Manufacturer- provided positive and negative cell line slides were included per batch run. Additional positive and negative controls (primary antibody was replaced by the buffer) using tonsil and skeletal muscle tissues were also included for every test slide. The cases underwent routine assessment of PD-L1 expression by tumor proportion scoring by two board-certified anatomic pathologists who have been previously trained in PD-L1 interpretation. Slides were filed after release of the official PD-L1 report which included the percentage of PD-L1 expression on tumor cells and presence or absence of tumor-infiltrating lymphocytes.

The above-mentioned pathologists independently reviewed the H&E and PD-L1 slides for the presence of stromal TILs, using the method previously described for breast cancers.[36] PD-L1 expression of stromal TILs was scored thereafter.

PD-L1 positive TC are viable tumor cells that exhibit membranous staining at any level. As per standard recommendation, PD-L1 expression on TC is determined by the tumor proportion score (TPS), which is the percentage of viable tumor cells displaying partial or complete membranous staining at any intensity. PD-L1 expression on TC was classified into: TPS <1% (No PD-L1 expression; Figure 2A), TPS 1 to 49% (Low PD-L1 expression; Figure 2B) and TPS ≥50% (High PD-L1 expression; Figure 2C). On the other hand, as there is no standardized method for assessment of PD-L1 expression on TILs yet, we arbitrarily assigned 50% as the cutoff, and PD-L1 expression on TILs were subdivided into: 0 (Figure 5A), less than 50% (Figure 5B) and 50% or more (Figure 5C). In cases of disagreement, the slides were reviewed by the two pathologists (JLA and DCA) to reach a consensus. There is substantial agreement between the two pathologists with a Kappa coefficient of 0.663, p value of less than 0.001, and 95% CI (0.543, 0.753). This is based on the interpretation of Kappa values by Landis and Koch scale, with Kappa value <0.0 having poor agreement, and 1 having almost perfect agreement.[40]

Data Analysis

An interobserver reliability analysis using the Cohen's Kappa (κ) was performed to determine the reproducibility of scoring for PD-L1 expression on TILs by the participating pathologists. Interpretation was based on the methodology by Landis and Koch.[40] Descriptive statistics were presented as frequencies, proportions, and tables. Numerical data were described using mean, standard deviation, median, and minimum and maximum values. Relationship between the clinicopathologic characteristics and PD-L1 expression was assessed using logistic regression analysis. Statistical analyses were performed using IBM SPSS Statistics version 25 (IBM, Armonk, New York). A p-value of <0.05 was considered statistically significant.

RESULTS

Overall

A total of 187 subjects were included in the study, the clinicopathologic characteristics of whom were summarized

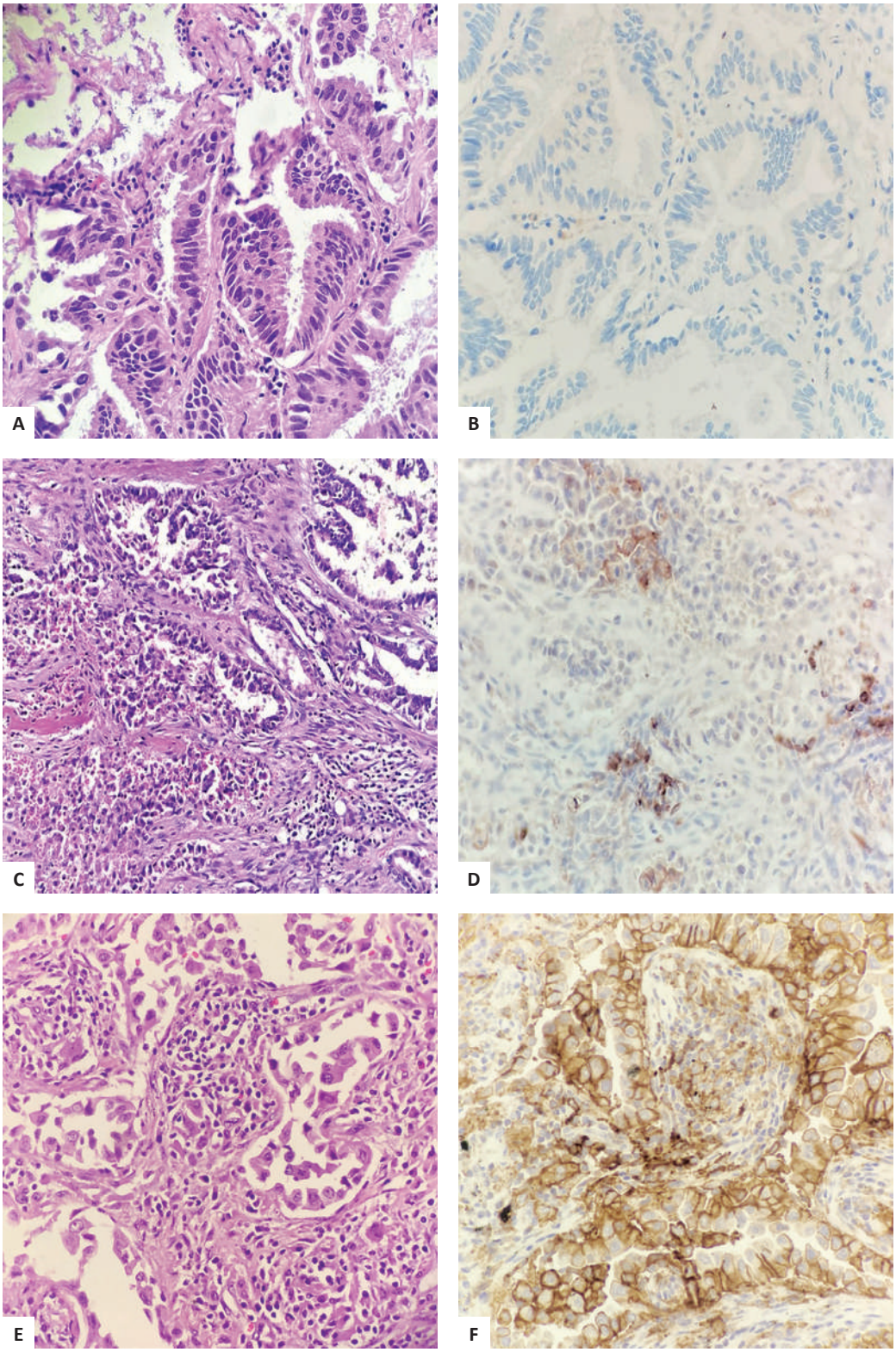

Figure 5. Representative H&E and IHC images (10x). **(A, B)** No PD-L1 expression on TILs; **(C, D)** <50% PD-L1 expression on TILs; and **(E, F)** ≥50% PD-L1 expression on TILs.

in Table 1. The mean age at presentation was 66.4 years old (± 10.7), with a minimum age of 32 years and a maximum age of 92. One hundred twelve (60.0%) were males and 75 (40.0%) were females. Majority (86.6%) of the specimen were obtained from the lungs and 25 (13.4%) were from pleural tissue or effusion. One hundred ten (58.8%) biopsy/resection and 77 (41.2%) cell block samples were evaluated in this study. Sixty (77.92%) of cell block samples were from the lungs, while 17 (22.08%) were from pleural effusion. The most common histopathologic diagnosis was adenocarcinoma (70.1%) followed by NSCLC (17.1%), and squamous cell carcinoma (8.0%). The overall prevalence of tumor cell PD-L1 expression in NSCLC patients in this study was 55.6% (104).

Tumor biopsy/resection specimens

The clinicopathologic profile of the 110 patients with biopsy/resection specimen were displayed in Table 2. The mean age was 65.48 years old (±10.66). Patients were 32 to 92 years of age. Forty-five (40.9%) were females and 65 (59.1%) males. The most common histopathologic diagnosis was adenocarcinoma (70%) followed by NSCLC, NOS (16.4%), and squamous cell carcinoma (10.9%). The prevalence of tumor cell PD-L1 expression in lung cancer patients with biopsy/resection samples in this study was 59.1%. Figure 1A shows that among the 110 subjects with biopsy/resection specimens, 26 (23.6%) had high PD-L1 expression (Figure 2C), while 39 (35.5%) had low PD-L1 expression (Figure 2B).

As displayed in Figure 3, tumor-infiltrating lymphocytes were noted in 82 (74.5%) biopsy/resection samples, and PD-L1 expression on TILs was noted in 69 (84.1%) of these patients. Figure 4 exhibits that 53 (64.6%) of 82 subjects with biopsy/resection specimen demonstrated less than 50% PD-L1 expression (Figures 5C and 5D), 16 (19.5%) showed ≥ 50% PD-L1 expression (Figures 5E and 5F), and the remaining 13 (15.9%) did not express PD-L1 (Figures 5A and 5B).

Table 3 shows that in biopsy/resection samples, tumor cell PD-L1 expression was noted in 28 (37.8%) of the 45 females and 37 (56.9%) of 65 males. Sixty-one (59.2%) of the 103 specimens from the lungs, and 4 (57.1%) of 7 pleural tissue specimens had PD-L1 expression on tumor cells. Tumor cell PD-L1 expression were noted in 45 (58.4%) of 77 biopsy/resection specimens diagnosed as adenocarcinoma, 12 (66.7%) of 18 cases diagnosed as NSCLC, NOS, 6 (50.0%) of 12 cases diagnosed as squamous cell carcinoma, and all cases with the diagnosis of adenosquamous carcinoma and poorly differentiated malignancy. Fifty-one (62.2%) biopsy/resection samples with tumor-infiltrating lymphocytes showed PD-L1 expression on tumor cells. There is no statistically significant association between the histopathologic features and PD-L1 expression on tumor cells in lung cancer patients with biopsy/resection specimens.

Tumor-infiltrating lymphocytes were noted in 33 (73.3%) of 45 females and 49 (75.4%) of 65 males with biopsy/resection samples. Seventy-seven (74.8%) 103 lung specimens and 5 (71.4%) of 7 pleural tissue specimens demonstrated tumor-infiltrating lymphocytes. TILs were noted in all biopsy/resection specimens diagnosed with squamous cell carcinoma, adenosquamous carcinoma, poorly differentiated malignancy, and large cell carcinoma. TILs were also noted in 55 (72.4%) adenocarcinoma cases, and in 12 (66.7%) of biopsy/resection samples with a diagnosis of NSCLC, NOS. No TILs were demonstrated in the sample diagnosed with mucinous carcinoma.

PD-L1 expression on TILs was displayed by 31 (90.0%) of 33 females and 39 (79.6%) of 49 males. Sixty-five (84.4%) of 77 lung resection specimens and 4 (80.0%) of 5 pleural tissue specimens exhibited PD-L1 expression on TILs. Majority (84.1%) of the 82 biopsy/resection samples showed tumor-infiltrating lymphocyte PD-L1 expression. All cases of large cell carcinoma and poorly differentiated malignancy demonstrated tumor-infiltrating lymphocyte PD-L1 expression. PD-L1 expression on TILs was also noted in 91.7% of biopsy/resection samples diagnosed with squamous cell carcinoma, 83.6% of adenocarcinoma cases, and 83.3% of NSCLC, NOS cases.

Logistic regression analysis showed no association between tumor location and tumor histology, and PD-L1 expression on tumor-infiltrating lymphocytes. There is an association between sex and PD-L1 expression on TILs, wherein females are 8.25 times more likely to have <50.0%

Table 1. Clinicopathologic characteristics of the lung cancer cohort (overall)

Age (years)		
Mean	66.4 ± 10.7	
Median	67.0	
Minimum	32.0	
Maximum	92.0	
	Frequency	**Percent**
Sex (n=187)		
Female	75	40.0
Male	112	60.0
Tumor Location (n=187)		
Lung	162	86.6
Pleural tissue/pleural fluid	25	13.4
Type of Biopsy (n=187)		
Biopsy/resection	110	58.8
Cell Block	77	41.2
Tumor Histology (n=187)		
Adenocarcinoma	131	70.1
Adenosquamous carcinoma	4	2.1
Squamous cell carcinoma	15	8.0
NSCLC, NOS	32	17.1
Poorly differentiated malignancy	3	1.6
Large cell carcinoma	1	0.5
Mucinous carcinoma	1	0.5
Total Tumor Cell PD-L1 Expression (n=187)		
Present	104	55.6
Absent	83	44.4

Table 2. Clinicopathologic characteristics of lung cancer patients with lung or pleural biopsy/lung resection and pleural fluid/lung cytology specimens

	Biopsy/resection		**Cytology**	
Age (years)				
Mean	65.48 ± 10.66		67.65 ± 10.70	
Median	66.5		68.0	
Minimum	32.0		36.0	
Maximum	92.0		90.0	
	Frequency	**Percent**	**Frequency**	**Percent**
Sex				
Female	45	40.9	30	39.0
Male	65	59.1	47	61.0
Tumor Location				
Lung	103	93.6	59	76.6
Pleural tissue/pleural fluid	7	6.4	18	23.4
Tumor Histology				
Adenocarcinoma	77	70.0	54	70.1
Adenosquamous carcinoma	1	0.9	3	3.9
Squamous cell carcinoma	12	10.9	3	3.9
NSCLC, NOS	18	16.4	14	18.2
Poorly differentiated malignancy	1	0.9	2	2.6
Large cell carcinoma	1	0.9	0	0.0
Mucinous carcinoma	0	0	1	1.3
Total Tumor Cell PD-L1 Expression				
Present	65	59.1	39	50.6
Absent	45	40.9	38	49.4

Table 3. Clinicopathologic characteristics and total tumor cell PD-L1 expression of lung cancer patients with biopsy/ resection specimens

Characteristics	Total Tumor Cell PD-L1 Expression		Total
	Presence n (%)	Absence n (%)	
Sex			
Female	28 (37.8)	17 (62.2)	45
Male	37 (56.9)	28 (43.1)	65
Tumor Location			
Lung	61 (59.2)	42 (40.8)	103
Pleural tissue	4 (57.1)	3 (42.9)	7
Tumor Histology			
Adenocarcinoma	45 (8.4)	32 (41.6)	77
Adenosquamous carcinoma	1 (100.0)	0	1
Squamous cell carcinoma	6 (50.0)	6 (50.0)	12
NSCLC	12 (66.7)	6 (33.3)	18
Poorly differentiated malignancy	1 (100.0)	0	1
Large cell carcinoma	0	1 (100.0)	1
Mucinous carcinoma	0	0	0
Tumor-Infiltrating Lymphocytes			
Presence	51 (62.2)	31 (37.8)	82
Absence	14 (50.0)	14 (50.0)	28

Table 4. Clinicopathologic characteristics and total tumor cell PD-L1 expression of lung cancer patients with cytology specimens

Characteristics	Total Tumor Cell PD-L1 Expression		Total
	Presence n (%)	Absence n (%)	
Sex			
Female	14 (46.7)	16 (53.3)	30
Male	25 (53.2)	22 (46.8)	47
Tumor Location			
Lung	29 (49.2)	30 (50.8)	59
Pleural tissue	10 (55.6)	8 (44.4)	18
Tumor Histology			
Adenocarcinoma	27 (50.0)	27 (50.0)	54
Adenosquamous carcinoma	2 (66.7)	1 (33.3)	3
Squamous cell carcinoma	3 (100.0)	0	3
NSCLC	6 (42.9)	8 (57.1)	14
Poorly differentiated malignancy	1 (50.0)	1 (50.0)	2
Large cell carcinoma	0	0	0
Mucinous carcinoma	0	1 (100.0)	1
Tumor-Infiltrating Lymphocytes			
Presence	0	0	0
Absence	0	0	0

TIL PD-L1 expression than males. An association was also noted between the percent PD-L1 expression on TILs and PD-L1 expression on tumor cells. Biopsy/resection samples with low tumor cell PD-L1 expression were 12.4 times more likely to have <50.0% PD-L1 expression than samples with high tumor cell PD-L1 expression.

Cytology specimens

A total of 77 subjects with cell block specimens were included in this study, the clinicopathologic features of whom were listed in Table 2. The mean age is 67.65 years old (± 10.70) with a range of 36 to 90 years old. 30 (39.0%) were females and 47 (61.0%) males. The most common histopathologic diagnosis was adenocarcinoma (70.1%) followed by NSCLC, NOS (18.2%), and squamous cell carcinoma and adenosquamous carcinoma (3.9%). In this study, the prevalence of tumor cell PD-L1 expression for lung cancer patients with cytology specimen was 50.6%. Figure 1B shows that of the 77 cytology samples evaluated, 12 (15.6%) had high PD-L1 expression and 27 (35.1%) had low PD-L1 expression.

Tumor-infiltrating lymphocytes were not appreciated in lung cancer patients with cell block samples.

In patients with cell block samples (Table 4), tumor cell PD-L1 expression was noted in 14 (46.7%) of the 30 females and 25 (53.2%) of 47 males. Twenty-nine (49.2%) of the 59 specimens from the lungs, and 10 (55.6%) of 18 pleural fluid specimens had PD-L1 expression on tumor cells. Tumor cell PD-L1 expression were noted in all 3 cytology samples with a diagnosis of squamous cell carcinoma, 27 (50.0%) of 54 adenocarcinoma cases, 6 (42.9%) of 14 NSCLC, NOS cases, 2 (66.7%) of 3 adenosquamous carcinoma cases, and 1 (50%) of 2 cases diagnosed with poorly differentiated malignancy. PD-L1 expression was not demonstrated by the cytology specimen with a diagnosis of mucinous carcinoma. There is no association between clinicopathologic characteristics and PD-L1 expression on tumor cells among the subjects.

DISCUSSION

Over the last few years, various novel therapeutic options, including immune checkpoint inhibitors (ICIs), were introduced for NSCLC. Current NCCN guidelines incorporate immune checkpoint inhibitors, either as monotherapy or in combination with traditional chemotherapeutic drugs, for the treatment of NSCLC. Immune checkpoint blockade leads to reactivation of immune response against tumor cells.[13] The NCCN NSCLC panel also recommends molecular diagnostic tests for *EGFR* mutations, *ALK* translocation, *ROS1* fusion, *BRAF* variants and PD-L1 immunohistochemistry for advanced and metastatic cases. ICIs target the PD-1/ PD-L1 pathway, a dominant pathway that contributes to immune evasion by certain tumors. Assessment of PD-L1 protein expression by IHC is the best predictive marker for response to immunotherapy with ICIs.[3,16]

Several IHC assays using different antibody clones have been developed to assess for PD-L1 protein expression for eventual targeted therapy. These assays follow different staining protocols, and scoring algorithms and cutoffs are not standardized. Except for the antibody clone SP142 in which scoring is based on either TC PD-L1 expression or the fraction of tumor area of the tumor occupied by PD-L1 expressing tumor-infiltrating IC, scoring of PD-L1 expression for most assays involve the evaluation of TC PD-L1 expression alone.[21,22,41] Among the ICIs available in the market, regimens which include Pembrolizumab and Atezolizumab received a category 1 recommendation for use in NSCLC treatment by the NCCN.[3] PD-L1 IHC 22C3 pharmDx has been approved by the U.S. FDA as "companion diagnostic assay" for patient selection for Pembrolizumab therapy. The drug is recommended for patients with TC PD-L1 expression of at least 1%.[3,11,26-30] This recent recommendation and subsequent change in scoring of TC PD-L1 expression.[42] occurred along the course of this study. Across studies, the proportion of PD-L1 expressing tumor cells in lung cancer is wide-ranging. This might be attributed to the difference in

sample size, subject population, and antibody panel used, tumor heterogeneity and decay of antigenicity in archived specimens.[7,15,18,26]

Our study aimed to determine the prevalence of PD-L1 expression in NSCLC in a tertiary hospital in the Philippines and determine their association with pathologic features. The overall prevalence of PD-L1 expression on tumor cells in NSCLC in our study population is 55.6%. Tumor cell PD-L1 expression was appreciated in 59.1% of histologic specimens. Of these, 40.9% of samples had no TC PD-L1 expression, 35.5% displayed low TC PD-L1 expression and 23.6% showed high TC PD-L1 expression. On the other hand, 50.6% of cytology samples demonstrated PD-L1 expression on tumor cells. Among these cases, 49.4%, 35.1% and 15.6% showed no, low and high PD-L1 expression, respectively. As in most previous studies,[7,16-17,33] we found no significant association between clinicopathologic characteristics and PD-L1 expression on the tumor cells in NSCLC in both specimen types.

Lung cancers as well as some other solid tumors exhibiting TILs, particularly CD8+ lymphocytes, in the tumor stroma are found to have better survival, and are associated with increased responsiveness to ICI. In addition, past studies have observed that NSCLC tumors demonstrating PD-L1 expression on TILs have better prognosis as compared to tumors with no PD-L1 expression on stromal TILs.[6,17,33-35,41] Therefore, information on the presence of TILs as well as their PD-L1 expression status adds up to the prognostic value of the test. Evaluation for the presence of TILs based on morphology in H&E-stained slides following the criteria established for breast cancer[37] was also done in the study.

Zhao et al., demonstrated that the median positive rate of PD-L1 expression on tumor-infiltrating immune cells is 36.37%. The group also described the different mechanisms implicated in PD-L1 expression in tumor cells and tumor-infiltrating immune cells.[34]

In our study, 82 (74.5%) of 110 biopsy/resection samples displayed stromal TILs. PD-L1 expression on TILs was noted in 84.1% of these 82 cases. TILs in 53 (64.6%) specimens showed less than 50% PD-L1 expression, and 16 (19.5%) samples exhibited ≥50% PD-L1 expression. A few studies discovered a correlation between tumor cell PD-L1 expression and the presence of stromal tumor-infiltrating lymphocytes.[30,37] As with PD-L1 expression in tumor cells, PD-L1-expression in tumor-infiltrating lymphocytes is also assumed to weaken the host immune response against tumor cells.[9]

Except for the finding that female subjects with biopsy/resection specimen are more likely to have less than 50% PD-L1 expression on TILs, there was no association between TILs and PD-L1 expression on TILs, and clinicopathologic features in both specimen type subgroups. The interpretation might be limited by the lack of clinical data i.e., smoking history and clinical stage upon procedure. We also found that histologic samples with low TC PD-L1 expression were more likely to have <50% PD-L1 expression on TILs. Further investigation should be carried out to explore the association between low PD-L1 expression on TILs and gender, as well as its correlation with low PD-L1 expression in tumor cells. Phases 1 and 2 of the Blueprint PD-L1 immunohistochemistry comparability project concluded that variability of PD-L1 staining in immune cells is greater than that of TC staining. Also, in contrast with the strong reliability in PD-L1 scoring of TCs among pathologists, evaluation of PD-L1 expression on ICs has poor reliability.[23,24] He et al., concluded that PD-1, instead of PD-L1 expression, was correlated with TILs.[17,32]

The present study is limited by its retrospective approach, lack of clinical data (smoking history, staging and clinical response) and lack of information regarding driver mutation status, the substantial number of cytology and biopsy specimens, and the non-uniformity of the pre-analytic variables involving the samples submitted for testing. In actuality, since it is a less invasive method of specimen collection, at least a third of lung malignancies are diagnosed based on cytology alone. However attractive it may seem, greater pre-analytic variability involving preparation techniques, fixatives, preservatives and stains, affects cytology specimens. Nonetheless, although they provide a limited tumor material and some tumors may display heterogeneity, good agreement has been observed between cytologic and histologic specimens.[5,19] With sufficient tumor sample, PD-L1 testing will greatly impact patient management and assist the oncologists in their treatment decisions for patients with lung cancer. This is the first local study to describe PD-L1 expression on both TC and TILs in NSCLC and its association with histopathologic features. The prognostic value of PD-L1 expression and clinical response to ICIs in the Filipino population remains to be explored.

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STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

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OPEN ACCESS – ORIGINAL ARTICLE

Analysis of Results of SARS-CoV-2 RT-PCR Testing and Pooling Strategies for Screening of Asymptomatic Individuals – The Philippine Children's Medical Center Experience

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ABSTRACT

Background. The availability of reverse transcription-polymerase chain reaction (RT-PCR) in detecting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is limited by the scarcity of resources prompting the use of pooling strategies. Evaluated in this study is the Philippine Children's Medical Center's (PCMC) experience in pooled testing done in asymptomatic population.

Objectives. Review the pooled SARS-CoV-2 RT-PCR results and case investigation forms (CIF) in asymptomatic population. Determine the incidence of SARS-CoV-2 in asymptomatic population and compare all the individual and pooled tests results. Determine the number of saved test kits and identify clustering in the community.

Methodology. This is a retrospective study that reviewed the pooled and individual SARS-CoV-2 RT-PCR results using Allsheng Auto-Pure 32a extraction kit, Sansure Biotech PCR machine and Maccura Sars-CoV-2 test kits. The pooling protocol used by the institution followed the recommendation by Lo et al., in the study entitled "An Evaluation of Pooling Strategies for RT-qPCR testing for SARS-CoV-2 infection."

Results. There are 1828 samples which resulted to 165 negative, 68 indeterminate, and 137 positive pools. There are 157, 135, and 68 pools containing 5 individual samples that were classified as negative, positive and indeterminate pools, respectively. Additionally, the negative pools contained 8 pools with 3 individual samples and the positive pools contained 2 pools with 2 individual samples. Deconvolution of the positive and indeterminate pools resulted to 227 and 74 positive individuals, respectively. In this review, the laboratory saved 24% of the test kits and shorten the overall turnaround time by 23 hours.

Conclusions and Recommendations. The incidence of SARS-CoV-2 in the population is higher compared to the prevalence of infection in the country. Pooled testing conserved test kits and congruence of pooled and individual ORF Ct-values was observed. An in-depth study including other genes is recommended and assessment of pooling in other population may be pursued.

Key words: reverse transcription-polymerase chain reaction, severe acute respiratory syndrome coronavirus 2, 2019 coronavirus disease

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INTRODUCTION

Statement of the Problem

Coronavirus disease of 2019 (COVID-19) is caused by SARS-CoV-2, and was declared a pandemic in March 2020 by the World Health Organization (WHO).[1] The gold standard for the detection of SARS-CoV-2 is by nucleic acid amplification testing (NAAT) with the use of RT-PCR. In the Philippines, mass testing is limited by the expense of SARS-CoV-2 tests and the number of accredited testing laboratories. These test limitations compelled the Department of Health (DOH) to make guidelines on testing for COVID-19 that are geared towards symptomatic and high-risk individuals, those with travel history, and health care providers.[2] However, despite these guidelines, the need for mass testing is apparent to survey the population.

On August 17, 2020, the Philippine Society of Pathologists Inc. (PSP) announced that pooled testing can be used as a strategy to minimize the number of test kits utilized

and decongest the backlog in accredited testing centers. The society further recommends that pools of five are the most cost-effective strategy to use given the prevalence of infection in the country.[3] On November 23, 2020, The DOH released a guideline on the conduct of COVID-19 pooled testing.[4]

Pooled testing is a strategy wherein samples are grouped into a particular number of pools. If the pooled sample result is negative, all the samples in that pool are presumed negative. Otherwise, if pooled sample is positive or indeterminate, all the specimens in the pools are tested individually.[5]

Majority of the literature available regarding pooled testing are done theoretically or in a small scale. In this study, the application of pooling in a community setting involving asymptomatic individuals was evaluated.

Significance of the Study

Due to the emerging numbers of SARS-CoV-2 positive asymptomatic individuals, mass testing became essential in the battle against COVID-19. As a strategy to facilitate mass testing, the PSP recommended pooling strategies to make tests available to the general population. Reviewed in this study were samples submitted for SARS-CoV-2 RT-PCR pooled testing from asymptomatic population in a local government unit (LGU). More so, the study aims to review the turnaround time and cost-effectivity of pooled testing in the asymptomatic population.

Review of Literature

The first case of COVID-19 was reported in Wuhan City, China in December 2019. Clinical symptoms of COVID-19 in patients vary but they often present with acute respiratory illness. In early January 2019, SARS-CoV-2 was identified as the causative agent of COVID-19.[6] SARS-CoV-2 is a β-coronavirus characterized by an enveloped non-segmented positive-sense RNA virus from subgenus sarbecovirus and subfamily Orthocoronavirinae.[7] The most common mode of transmission by SARS-CoV-2 is thru droplet expelled during talking, sneezing or coughing, however other modes like contact surface spread and aerosol transmission were reported. It is evident that a high risk of transmission is associated with prolonged exposure to an infected person, especially if these individuals are symptomatic, while exposure to asymptomatic contacts are less likely to result in transmission.[8] Despite limited literatures on asymptomatic infections of COVID-19 cases, a systemic review by Gao et.al showed that there are significant proportion of SARS-CoV-2 RT-PCR positive asymptomatic cases at 1.6% in China, 30.8% in Japan, 51.7% in Diamond Princess, 10.7% in Korea, 56.5 % in Washington and 15.8% in Wuhan Children.[9]

The detection of SARS-CoV-2 was made possible by identifying the complete genomic structure of Wuhan-Hu-1 coronavirus (WHCV), a strain of SARS-CoV-2 isolated from a COVID-19 worker in the Wuhan seafood market. It was shown that the genome of WHCV contains variable number of ORF. Majority of the viral RNA are in the first ORF that translates two polyproteins, pp1a and pp1ab and encodes 16 non-structural proteins (NSP), while the remaining ORFs encode accessory and structural proteins. The other essential structural proteins encoded by the virus are spike (S) glycoprotein, small envelope (E) protein, matrix (M) protein, and nucleocapsid (N) protein.[1] There are several emerging tests for SARS-CoV-2, but the current gold standard is by NAAT which includes RT-PCR. RT-PCR is a laboratory test that converts short strands of ribonucleic acid (RNA) to deoxyribonucleic acid (DNA). The newly formed DNA strands undergo amplification until a measurable amount is detected known as the Ct-value.[6] In a review of eighteen literatures done by Rao et.al., it was concluded that the Ct-value is significantly associated with the viral load. The review further concluded that lower Ct-value is associated with a meager clinical outcome and may be useful in predicting the clinical course and prognosis of the patient.[10]

Due to the rapid spread of the virus and increase in the demand for tests, limited numbers of test kits and overflowing backlogs of testing laboratories have become a major hindrance in the fight against this pandemic. Several testing strategies are being studied to address the challenges of testing, and one of the options proposed is pooled testing. Pooled testing is a testing scheme that is directed towards minimizing the number of tests conducted using pooled subsets. If a pool of n sample tests are negative, all the samples are considered negative while positive and indeterminate pools will be tested in individually.[11]

As of April 23, 2021, there are 144,358,956 confirmed cases of COVID-19 with 3,066,113 mortality as per the World Health Organization.[12] In the Philippines there are 971,049 COVID-19 confirmed cases and 16,370 of whom died.[13] Despite the guidelines set to conserve testing, it is now evident that large-scale population testing which can be achieved by pooling is necessary to survey asymptomatic population, to trace asymptomatic COVID-19 carriers that are difficult to identify and isolate, to assure that healthcare workers are not contagious, to screen and protect high risk population, to accurately estimate the spread of infection, to assess the effectiveness of quarantine measures and social distancing, and to allow the safe return to work.[14]

Summary of Literature on Pooled Testing in Asymptomatic Population

Das et al., conducted a surveillance study on SARS-CoV-2 RT-PCR using pooled testing involving 7,000 asymptomatic individuals. These samples were grouped into 700 pools and only eight pools were positive. All positive pools yielded only one positive specimen each after subpooling. An incidence of 0.11% positivity was taken from the pooled asymptomatic cases and the experiment was able to save 6,220 test kits.[15]

A pooling study conducted by Lohse et.al involves 1191 samples from asymptomatic population. In this study the prevalence of SARS-CoV-2 using RT-PCR pooling strategy is 1.93% and the researchers were able to save 924 test kits.[16]

In a pooled testing study done in Kenyan Coast, there are 1500 samples submitted, wherein 250 pools are taken (6 samples in each pool). There are 75 positive pools, and individual runs of the positive pools resulted in 112 positive samples (7.5% from the original 1500 samples). It

was further noted that positive pools with lower Ct-values contain multiple positive individuals. Moreover, pooled samples with multiple positive individuals had an average decrease of Ct-value by 1.59 in comparison to pooled samples with single positive individual.[17]

A pooled study involving hospitalized patients that have low risk for SARS-CoV-2 was done using Xpert Xpress SARS-CoV-2 test which involved 530 samples pooled in groups of 3. This resulted in 4 positive pools and a total of 179 pooled samples with a positivity rate of 0.8%.[18]

OBJECTIVE OF THE STUDY

General Objectives

To analyze the pooled SARS-CoV-2 RT-PCR test results in conjunction with the data seen in the CIF of nasopharyngeal and oropharyngeal (NP/OP) samples submitted by a LGU at PCMC on September 2, 2020 to September 8, 2020.

Specific Objectives

- Determine the incidence of asymptomatic individuals with SARS-CoV-2 that are detected by RT-PCR using pooling strategies.
- Determine how pooled sample results affect individual testing.
- Determine if pooled testing can conserve test kits in a large-scale population.
- Determine if clustering of SARS-CoV-2 positive asymptomatic individuals can be detected by pooling strategies.

Operational Definition of Terms and Variables

RT-PCR – A laboratory technique that combines reverse transcription of RNA into DNA with amplification of specific DNA targets using polymerase chain reaction.

Pooled testing – Testing scheme done wherein individual samples are pooled into a certain number. If the pooled sample is negative, all individual samples are considered negative. Otherwise, if pooled sample is positive or indeterminate, subpooling or individual testing of specimens will be done.

Ct-value – Number of cycles required for fluorescent signal to cross the threshold for detection of gene amplification. This is inversely proportional to the amount of target nucleic acid in the sample.

METHODOLOGY

Research Design

This is a descriptive study that retrospectively reviewed the NP/OP specimens and CIF submitted at PCMC from a LGU on September 2, 2020 to September 8, 2020 for SARS-CoV-2 RT-PCR pooled testing. The pooling method and interpretation used was patterned after the prior study of Lo et al.[3]

Subject and Sample Size Computation

The minimum sample size of the study is 1429, this was computed using Epi Info version 7.2.2.6. The sample size of the study was estimated using single population proportion formula with the following assumptions: 99% confidence interval, 1.0% margin of error, and 2.2% frequency of PCR-confirmed asymptomatic SARS-CoV-2 cases in the Philippines. The population of the study was limited to asymptomatic individuals aged 19 years old and above. These samples were taken from different barangays, agencies and includes tricycle drivers and market vendors in a LGU.

Description of the Study Procedure

A review of the SARS-CoV-2 RT-PCR test results using Macurra's SARS-CoV-2 Fluorescent PCR kit was done. The Macurra's SARS-CoV-2 Fluorescent PCR kit is a qualitative assay that allows the detection of SARS-CoV-2 nucleic acids, namely, ORF, E, and N gene. The Macurra's SARS-CoV-2 Fluorescent PCR kit is approved by the National Medical Products Administration, Certification Experts and the Food and Drug Administration of the Philippines.

The pooled samples were reviewed to assure that the following classifications were followed: negative, positive, and indeterminate pools (N gene only, N and/or E genes and late amplification of the ORF gene). Descriptive analysis of the individuals called as negative and positive under the pooling classification were done.

Testing Procedure

The pooling strategy used was patterned after the study of Lo et al. In the study, pools of 5 was the recommended method and has a sensitivity of 83% and an estimated specificity of 100%. From the submitted NP/OP samples, an aliquot of 50 uL were taken and pooled into groups of 5 which amounted to 250 uL (pools that had 2 and 3 individual samples amounted to 100 to 150 uL, respectively). The pooled samples were placed in a cryogenic vial and then mixed using a vortex mixer. After mixing, 50 uL aliquots from the pooled samples were taken and underwent SARS-CoV-2 RT-PCR testing. All the specimens in the negative pools were considered negative. All the specimens in the pools that were classified as positive and indeterminate were tested individually.

The individual and pooled samples were tested using Macurra SARS-CoV-2 Fluorescent PCR kit, Allsheng Auto-Pure 32A extraction kit, and Sansure Biotech Ma-600 RT-PCR machine. All procedures were performed in strict compliance with the biosafety guidelines and using the manufacturers' instructions. The results were then recorded and encoded in Microsoft Excel files.

Results Interpretation

Interpretation of individual results was patterned after Maccura SARS-CoV-2 Fluorescent PCR Kit. As quality control, every run must have a valid control (ORF Ct-value </ 32, internal control (IC) Ct-value </ 38, E Ct-value </32 and N Ct-value </32), otherwise samples are invalid and should be retested. Target genes are positive if the ORF, E, and N genes Ct-values are </ 38, </37, </38, respectively with a valid IC. Target genes are negative if there are no Ct-values or the ORF, E, N genes, and IC CT-values are >38, >37, >38, and </38, respectively. Based on these Ct-values, samples are interpreted as follow: 1. A positive ORF gene with presence of any positive N and/or E genes is positive, 2. A positive ORF gene with no N and/or E genes must be repeated, if still positive, this will be interpreted

as SARS-CoV-2 positive, 3. Positive N and/or E genes with no ORF gene will be correlated with other medical findings, and if results are inconsistent with the clinical presentation, additional testing is suggested to confirm the results, otherwise result will be released as negative.

The interpretation of pools was patterned after Lo et al., and the Centers for Disease Control and Prevention (CDC).[3,5] Pools that show no gene amplification with valid controls are interpreted as negative. Pools that showed a positive ORF gene with or without N and E genes are interpreted as positive. Pools that show any gene amplification other than ORF, any unusual or non-sigmoid amplification, and late or low amplification are interpreted as indeterminate.

Data Collection and Outcomes

All the data and CIF submitted from the LGU to PCMC last September 2, 2020 to September 8, 2020 for RT-PCR and pooling study were evaluated. The corresponding data that fit the inclusion criteria were reviewed to determine if sample pools were classified appropriately.

The results of pooled and individual tests in conjunction with the data in the CIF were tabulated and described statistically to determine the incidence, viral load, practicality, and cost-effectiveness of pooled testing in the community.

Ethical Consideration

An approval from the Institutional Review Board at PCMC was done prior to the commencement of the study. Confidentiality of the individuals included in this study was the highest priority. This research complied with the Data Privacy Act (2012) and National Ethical Guidelines for Health and Health-Related Research. The study's research assistant, gathered the raw data from the CIF and assigned the corresponding numerical value to all the samples to ensure patient's privacy. The research assistant tabulated the numerical values (age, gender, and demographic location) and removed any personal information and accession numbers. All the data taken from the study were stored in a flash drive and shall be kept for 5 years. Materials and data that were obtained from the study will be a property of PCMC and the researchers and not by the LGU. Should the LGU require any information from the study, they must do so in formal writing.

Data Processing and Analysis

The pertinent data were collated and statistically evaluated using Microsoft Excel. The demographic profile of included subjects were taken from the CIF and analyzed. The incidence of positive cases were calculated in asymptomatic individuals and were further evaluated as per pool and individual test results. The number of test kits used in the performed pooled testing strategy were compared to the number of projected test kits used should conventional RT-PCR had been done.

RESULTS

A total of 1828 NP/OP samples were evaluated from which there were 165 negative, 137 positive, and 68 indeterminate pools. The negative pools had 157 pools with 5 individual samples and 8 pools with 3 individual samples. The positive pools had 135 pools containing 5 individual samples and 2 pools containing 2 individual samples. All 68 indeterminate pools had 5 individual samples. All 809 samples within the negative pools were considered negative. The 137 positive pools contained 679 individual samples wherein 227 (33%) were positive and the indeterminate pools contained 340 samples wherein 74 (21.8%) individual samples were positive.

Table 1 shows the distribution of positive individuals in the deconvoluted pools. In the positive pools, there are 63 pools containing at least 1 positive sample, 52 pools containing 2 positive samples, 16 pools containing 3 positive samples and 3 pools containing 4 positive samples. The indeterminate pools were further subclassified into N gene, N and E genes, and late amplification. In pools with N gene amplification, there are 27 pools containing at least 1 positive sample, 9 pools containing 2 positive samples and 2 pools containing 3 positive samples. In pools with N and E genes amplification, there are 10 pools containing at least 1 positive sample and 3 pools containing 2 positive samples. In the pools with late amplification there are 7 pools with at least one positive sample.

Among the positive individual samples in the positive pools, 13 (6%) had an ORF Ct-values of <20, 25 (11%) had an ORF Ct-values between 20-25 and 189 (83%) had an ORF Ct-values of >25. In the indeterminate pools, all the 68 positive individual samples had an ORF Ct values of >25. (Table 2)

Table 1. Distribution of the positive individuals in the deconvoluted pools

Number of positive samples per pool	Positive Pools	Indeterminate Pools		
		N	N and E	Late amplification
1	63	27	10	7
2	52	9	3	0
3	16	2	0	0
4	3	0	0	0
5	0	0	0	0

Table 2. Results of pooled samples and individual samples that underwent SARS-CoV-2 RT-PCR. The ORF Ct-value of individual samples were subclassified into <20, 20-25 and >25

Pooled Samples	Individual Samples Interpretation	Number	ORF (Ct-values)	Number	Ct-values positive %
Negative: 165					
Positive: 137					
	Positive	227	<20	13	6%
			20-25	25	11%
			> 25	189	83%
	Negative	452			
Indeterminate: 68					
N gene: 45	Positive	51	<20	0	0%
			20-25	0	0%
			> 25	51	100%
	Negative	174			
N and E genes: 14	Positive	16	<20	0	0%
			20-25	0	0%
			> 25	16	100%
	Negative	54			
Late Amplification: 9	Positive	7	<20	0	0%
			20-25	0	0%
			> 25	7	100%
	Negative	38			

Table 3. Positivity rate of barangays and agencies tested for SARS-CoV-2 RT-PCR

Community Tested	Positive Tested	Samples Tested	Positivity Rate
Barangays			
Barangay 1	16	123	13%
Barangay 2	9	90	10%
Barangay 3	20	54	37%
Barangay 4	0	11	0%
Barangay 5	15	46	48%
Barangay 6	4	6	67%
Agencies			
Agency 1	210	1334	16%
Agency 2	6	25	24%
Agency 3	5	17	29%
Agency 4	4	32	13%
Agency 5	0	2	0%
Agency 6	6	52	12%

Table 4. Number of test kits save in pooled testing in comparison to conventional testing

Number of Test Kits		
With Pooling		**Conventional Testing**
Pools of 5	370	
Individual Testing	1019	1828
Total Runs	1389	
Total Saved Tests	**439**	
Percentage of Saved Tests	**24%**	

Included in this study are 1596 (87%) males and 232 (13%) females with an average age of 34 years old taken from different barangays and agencies. Table 3 shows the samples tested and positivity rates for the respective barangays and agencies. In contrast to the 16.5% positivity rate in the population, Barangays 3,5,6 had a positivity rate at 37%, 48%, and 67%, respectively, while Agencies 3 and 2 had a positivity rate of 29% and 24%, respectively. Also included in the population are the market vendors and tricycle drivers which had positivity rates of 16.19% and 15.74% respectively.

Table 4 shows the number of test kits used with pooled testing in contrast to conventional testing. In comparison to the 1828 test kits that would have been used had conventional testing been done, pooled testing saved 24% of test kits since only 1389 test kits were used. More so, the overall turnaround time was shortened by 23 hours.

DISCUSSION

Evaluated are 1828 samples from asymptomatic individuals in a LGU taken on September 2, 2020 to September 8, 2020. The individual samples were grouped into 370 pools (165 negative, 137 positive, and 68 indeterminate pools). Among the 679 individuals that constitute the positive pools, 227 turned out to be positive by individual testing giving a 33% positivity rate. Among the 340 individuals that constitute the indeterminate pools, 74 turned out to be positive by individual testing giving a 21.7% positivity rate. Individual testing of the indeterminate pools is necessary since the dilution of samples showed loss of sensitivity in the pooled samples; hence testing of indeterminate pools may reduce the loss of sensitivity in pooling and allows the detection of samples with low viral load.[3,5,11] Also noted are small number of positive pools (1.5%) that did not yield any positive sample when retested individually, this may be attributed to sample carry over, technical and procedural errors.

Review of the individual specimens' ORF Ct-values showed that 6%, 11% and 83% of the positive pools had an ORF Ct-values of <20, 20-25 and >25, respectively, while all the positive individuals in the indeterminate pools had an ORF Ct-values of >25. Further analysis of the samples showed that the positive individuals in the positive pools had an average ORF Ct-value of 30.23. On the other hand, the pools with N gene only, N and E genes, and late amplification had an average ORF Ct-values of 34.93, 34.95 and 36.52, respectively. These data showed that despite the dilution effect of pooling, PCMC's testing laboratory was still able to obtain congruence in the Ct-values of the pooled and individual samples. Although an indirect measure of viral load, the assessment of Ct-values is necessary since it can be a relative measure of viral copies if the samples are tested in a standard condition.[3,19] This was supported in a multicenter study done in Bahrain, wherein it was said that symptomatic presentation was significantly associated with lower Ct-values.[20] Furthermore, a study done by Faíco-Filho et al., showed that individuals with severe COVID-19 had a median Ct-value of 21.5, while mild and moderate illnesses had Ct-values of 22 and 27, respectively.[21]

In major universities in the United States, pooled surveillance testing to detect person with asymptomatic infection was used. The residents in these universities were tested twice weekly, off-campus undergraduates were tested once or twice a week and graduate students were tested every week. Using pooling, they were able to test 10,265 students from which 84 samples were positive and 51% of the positive students were asymptomatic. All the positive students were quarantined, and testing frequency were increased in areas with infection. Due to the test compliance achieved in this study, pooled surveillance testing was made available to the faculty, staff and student athletes.[22] Observed in this study are barangays and agencies with higher positivity rates than the population's positivity of 16.5%. Unfortunately, the sample groups in this study were not proportionally grouped and were limited by the retrospective nature of the study, hence the difficulty to survey infection in the population. In a position paper by the PSP, the utility of pooled testing to contain viral transmission in the community was acknowledged. They proposed that communities exposed to a confirmed SARS-CoV-2 positive individual must be assessed for development of symptoms, 5 days after exposure from the index case. All exposed symptomatic members of the community must be tested individually, while all the asymptomatic members of the community shall be subjected to pooled RT-PCR.[23]

In comparison to conventional testing, the use of pooling strategies conserved around 24% of tests kits, a saving of php 1,570,303. Despite the substantial savings in pooled testing, the laboratory must be cautious since test savings will decline as the positivity/prevalence rates increase. To avoid this, the testing laboratory may pool the family members, work sectors, and communities together.[3] In general, pooled testing shortens the laboratory's turnaround time,

however part of the limitation of pooling is the increase in the turnaround time of positive individuals. In this population, the turnaround time of all the samples is 73 hours, while the projected turnaround time of individual RT-PCR tests of 1828 samples is 96 hours if pooled testing was not done. Also, with the use of pooled testing, less personnel and resources were used in the laboratory.

CONCLUSION

Pooled testing is a good strategy to catch SARS-CoV-2 infection in asymptomatic individuals. It is cost-effective, efficient and may be used to mass test the general population, LGUs and provinces where resources are sparse. Pooled testing done in the population showed high pick-up rate in those with low ORF Ct-value an indirect measure of the relative amount of viral load.

RECOMMENDATION

Pooled testing is cost-effective and beneficial in the screening of asymptomatic population. It may serve as a useful tool to assess the SARS-CoV-2 infectivity in the community. When conducting pooled testing, the pool size must be adjusted in concordance with the population's prevalence. It is recommended to conduct a study in both symptomatic and asymptomatic individuals with comparison of Ct-values and disease severity. Despite the congruence of ORF Ct-values in pooled and individual tests, an in-depth study with emphasis on the indeterminate pools is recommended. A study which uses pooled testing that are limited to high-risk population like tricycle drivers and market vendors may be done. A prospective study that focuses on disease surveillance and population demographics is warranted.

LIMITATION

Due to the retrospective nature of the study, the data in the community and workplace sectors are not proportional, hence it is difficult to calculate the incidence of infection for the sectors. Since this is a descriptive study, testing of the negative pools was not performed, in line with this, there is a minute risk of not catching the weak positive individuals due to sample dilution. Although there is an overall decrease in the turnaround time, samples of the positive individual will have to be tested twice, increasing its turnaround time. Additional training and technical skills are needed by the medical technologists and pathologists to perform pooled testing.

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STATEMENT OF AUTHORSHIP

All authors fulfilled the ICMJE authorship criteria.

AUTHOR DISCLOSURE

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APPENDICES

Appendix 1. Data Collection Form

Pooled Sample	Genes	Ct-value	Result	Individual Samples	Genes	Ct-value	Result
Pooled Sample No. 1	Control			Individual Sample 1	Control		
	ORF1a/b				ORF1a/b		
	E				E		
	N				N		
				Individual Sample 2	Control		
					ORF1a/b		
					E		
					N		
				Individual Sample 3	Control		
					ORF1a/b		
					E		
					N		
				Individual Sample 4	Control		
					ORF1a/b		
					E		
					N		
				Individual Sample 5	Control		
					ORF1a/b		
					E		
					N		

Appendix 2. Dummy Tables

Table 1. Pooled samples interpretation

Pooled Samples Interpretation	Number	Percentage
Negative		
Positive		
Indeterminate		
Total Number of Pools		

Table 2. Individual samples interpretation

Pooled Samples	Individual Samples Interpretation	Number	ORF1a/b (Ct-values)	Number	Ct-values positive %
Negative:					
Positive:					
	Positive		<20		
			20-25		
			> 25		
	Negative				
Indeterminate:					
N gene:	Positive		<20		
			20-25		
			> 25		
	Negative				
N and E genes:	Positive		<20		
			20-25		
			> 25		
	Negative				
Late Amplification:	Positive		<20		
			20-25		
			> 25		
	Negative				

Table 3. Individual sample results summary

Pooled Samples	Negative Result	Positive Result	Total Samples	Positivity Rate
Negative Pools				
Positive Pools				
Indeterminate Pools				
Total				

Table 4. Test kits used in pooled testing vs conventional testing

Number of Test Kits		
With Pooling		Conventional Testing
Pools of 5		
Individual Testing		
Total Runs		
Total Saved Tests		
Percentage of Saved Tests		

OPEN ACCESS – ORIGINAL ARTICLE

Cost-Effectiveness of Limited Screening Panel for Acute Lymphoblastic Leukemia Diagnosis in a Resource-Limited Setting

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ABSTRACT

Background. Flow cytometry is an invaluable tool in the diagnostic evaluation of acute leukemia and post therapy monitoring; however, majority of Filipino population cannot afford the cost. The use of a minimal screening panel which is both cost-effective and provides an accurate diagnosis of acute lymphoblastic leukemia is seen as an alternative.

Objectives. We aim to determine the cost-effectiveness and accuracy of using a minimal screening panel for the diagnosis of acute lymphoblastic leukemia (ALL).

Methodology. We selected a limited panel of 9 antibodies comprising of CD45/CD19/CD20/CD10/HLA-DR/CD34/cCD3/cCD79a/cTdt and retrospectively reviewed newly diagnosed cases of B-cell and T-cell ALL from September 2016 to December 2019 using this panel.

Results. Out of 719 bone marrow aspirates submitted for basic leukemia flow cytometric analysis we identified 268 ALL cases (239 B-ALL and 29 T-ALL).

In all cases, a diagnosis was established using the limited panel. Compared to the current cost of our comprehensive panel (₱ 9,903.60). This limited panel cost ₱ 3,062.29, that offers a 69.08% savings per test, which translated to a ₱1.2 million savings a year (for an average of 180 annual cases).

Conclusion. We underscore the utility of a limited panel for the diagnosis of ALL. Although this panel remains to be assessed with a larger validation cohort, its application in resource-limited developing countries is diagnostically useful and cost-effective.

Recommendation. The use of a limited panel of 9 antibodies is recommended as a screening panel for patients who are highly suspected of having ALL both clinically and initial bone marrow smear assessment.

Key words: limited screening panel, acute lymphoblastic leukemia, pediatric population, Filipino

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INTRODUCTION

The diagnosis of acute lymphoblastic leukemia (ALL) entails the integration of cell morphology, immunophenotype and genetics/cytogenetic studies.[1,2] Cellular morphology is the first step in the diagnosis of ALL, but given that there are no morphologic criteria to distinguish whether the blasts are of the B- or T-cell lineage, other ancillary tests were sought.

Flow cytometry is a crucial tool in the rapid diagnosis and accurate classification of leukemia.[3] It employs physical characterization including cell size, granularity and DNA content. These parameters are measured simultaneously as the suspension pass through a measuring device. Highly specific monoclonal antibodies are used to recognize surface, cytoplasmic and nuclear antigens present in leukocytes and these are labeled with the use of fluorochromes, the most widely used of which are FITC, phycoerythrin and allophycocyanin.[4-6]

Flow cytometric evaluation in addition to its diagnostic use can be utilized to assess relapse and or residual disease following therapy. The use of appropriate antibody panels

will aid in the identification of cell type, cell lineage, the stage of maturation and clonality.[4]

An extensive panel of antibodies is used in order to make a definitive diagnosis of acute leukemia. The antibody panel primarily include surface markers (i.e., CD45, CD34, CD117, HLA-DR, CD4, CD8, CD19, CD10, CD20, CD33, CD13, CD56, CD14, CD64, CD11c, CD41a, glycophorin A, anti-kappa and anti-lambda) and cytoplasmic markers (i.e., IgG2a, IgG1, cCD3, cCd79a, cMPO and cTdT).[7-10] However, the use of this panel is expensive and majority of Filipino population cannot afford the cost. In this setting, screening with the use of a limited number of antibody can help in reducing the financial burden of flow cytometry.[9,11-13]

METHODOLOGY

This study utilized B-cell and T-cell acute lymphoblastic leukemia diagnosed by flow cytometric evaluation coupled with cellular morphology. Bone marrow aspirate in heparinized tubes or peripheral blood in EDTA tubes were subjected to three-colored flow cytometry. For the diagnosis, a basic leukemia panel was used in all cases (Appendix A). The panel comprised of 23 antibodies including surface markers (CD45, CD4, CD8, CD34, CD117, HLA-DR, CD13, CD3, CD33, CD19, CD10, CD20, CD5, CD56, CD14, anti-kappa and anti-lambda) and cytoplasmic markers (IgG2a, IgG1, cCD3, cCD79a, cMPO and cTdT).

A limited panel of nine (9) antibodies (CD45, CD19, CD10, CD20, HLA-DR, CD34, cCD3, cCD79a and cTdT) were selected.[4,12,14] Using this panel, we retrospectively reviewed all newly-diagnosed pediatric (0-18 years old) cases of B-cell and T-cell acute lymphoblastic leukemia from September 2016 to December 2019.

The study is limited to patients from our institution and cases sent from other institutions for diagnosis were not included in the study population. Relapse and residual B-cell and T-cell lymphoblastic leukemia cases were also excluded from the study.

The computed minimum sample size for the study was 56. The sample size for the study was estimated using single population proportion formula with the following assumptions: 100% sensitivity, 100% specificity and 55% prevalence ALL based on the results of the study done by Artaiz et al.[15] The sample size was calculated using sample size estimation formula for diagnostic studies.

The data were tabulated and descriptive statistics were presented as frequencies and tables. The sensitivity, specificity and predictive values were calculated for the minimal panel compared with the basic leukemia panel.

RESULTS

A total of 719 bone marrow aspirate were submitted and subjected to a comprehensive flow cytometric analysis. Of this, 268 were ALL cases; 239 (89.2%) of which were B-lymphoblastic leukemia (B-ALL) and 29 (10.8%) were T-lymphoblastic leukemia (T-ALL). There were 59 cases by which ALL was the clinical consideration, however no abnormal blast population was noted on flow cytometry.

The commonly expressed B-cell antigens in B lymphoblastic leukemia were CD79a (97.4%), CD10 and CD19 (96.7%), cTDT (94.98%), HLA-DR (90.3%) and CD34 (85.4%). The other markers that yield positivity were: CD20 (44%), CD13 (34.3%), CD33 (17.57%) and CD45 (5%). A diagnosis of B-ALL was established with the use of the limited antibody panel in 100% of cases (239/239). This was based on the positivity of cCD79a and other B cell markers (CD10, CD19 and CD20) and immature markers namely CD34 and cTdt (Table 1).

All 29 T-ALL cases expressed cCD3 and CD5 (100%). Surface CD3 was expressed in 89.7 % of cases. Other markers that yield positivity were: cTdT (79.3%), CD8 (68.96%), CD4 (31%), CD34 (24.1 %), CD13 (17.2%) and CD33 (10.34%). Cytoplasmic CD79a was negative in all cases. A diagnosis of T ALL established with the use of the limited antibody panel in 100% of cases (29/29) (Table 2).

From this data, the sensitivity and specificity of the limited screening panel was at 100%. The positive and negative predictive values were both 100%.

The current cost of our basic leukemia panel is ₱ 9,903.60, compared to the limited panel which cost ₱ 3,062.29. This offers a 69.08% savings per test, which translates to a ₱ 1.2 million savings per year (for an average of the 180 annual cases) (Table 4) (Appendix B).

DISCUSSION

Immunophenotyping was used as a means of identifying and quantifying a single cell population which can be accomplished by staining the population of interest with two or more antibodies simultaneously.[6] There has always been a need of thorough and careful selection of marker

Table 1. Antigen expression of B-ALL cases

	B-Acute Lymphoblastic Leukemia (n= 239)						
	cTdT	CD34	cCD79a	CD10	CD19	CD20	HLA-DR
Positive	227 (94.98%)	204 (85.4%)	233 (97.5%)	231 (96.7%)	231 (96.7%)	106 (44.3%)	216 (90.3%)
Negative	12 (5.0%)	5 (14.8%)	6 (2.5%)	8 (3.3%)	8 (3.3%)	133 (55.6%)	23 (9.7%)

Table 2. Antigen expression of T-ALL cases

	T-Acute Lymphoblastic Leukemia (n= 29)									
	cTdT	CD34	cCD3	CD3	CD5	CD4	CD8	CD13	CD33	cCD79a
Positive	23 (79.3%)	7 (24.1%)	29 (100%)	25 (89.7%)	29 (100%)	9 (31%)	20 (68.96%)	5 (17.2%)	3 (10.3%)	NIL
Negative	7 (26.9%)	19 (73.1%)	NIL	1 (3.8%)	NIL	20 (68.96%)	9 (31.%)	24 (82.8%)	26 (89.7%)	29 (100%)

Table 3. 2x2 table for the computation of sensitivity, specificity and predictive values of the limited screening panel

Limited Panel Flow	Basic Leukemia Panel		Total
	Positive	Negative	
Positive	268	0	268
Negative	0	59	59
Total	268	59	327

Table 4. Cost of the basic leukemia panel compared with the limited screening panel

Costing	Basic Leukemia Panel	Limited Screening Panel
Cost Per Test	9,903.06	3,062.29
Annual Cost	1,782,550.80	551,212.20
Annual Savings		1,231,338.60

combinations based on their specificity in the identification of lineage, stage of maturation and aberrant phenotype expression. The use of appropriate monoclonal antibody clones and fluorochrome combinations must also be considered. The use of these markers in combination is more pertinent than that of individual markers, for they provide a unique immunophenotype enabling the identification of the cell population in question.[9,11,16,17]

The antibody panel for the limited flow cytometry were lineage-specific B-cell and T-cell markers which include cytoplasmic CD79a and cytoplasmic CD3, respectively. Cellular maturity was assessed by the presence or absence of the following markers: CD45, CD34, cytoplasmic TdT, CD10 and CD19.[1,8,9,18]

As opposed to the other antigens which are anchored on the cell membrane, TdT is found in the nucleus. Hence, TdT staining is performed intracellularly after rendering both the cell membrane and nuclear membrane permeable.[6,19]

Degree of lineage maturation of the population of interest was evaluated by CD45 and CD34, since these markers are considered the most efficient in defining immaturity. CD45 enabled one to differentiate hematopoietic cells by their pattern of intensity which can be correlated with both cell lineage and maturity. For this reason, CD45 was a major marker in the identification of blast population based on its dim expression and the exclusion of normal hematopoietic cells. Of the 268 diagnosed ALL cases, 5% of this expressed dim positivity to CD45. To further refine and confirm the gating of blasts, CD34 was used.[11,16,20] In this study, B-ALL and T-ALL expressed CD34 in 85.4% and 24.1% respectively.

Cytoplasmic CD79a expression appears early during B-cell commitment. This occurs after the expression of Tdt and prior to the acquisition of CD19. In conjunction with cytoplasmic CD79a, virtually all cases of B-ALL express CD19. CD19 is deemed a sensitive B-cell marker but has a low specificity prompting the need for cytoplasmic CD79a to improve lineage assignment.[11,16] HLA-DR is also a helpful marker in the detection of acute leukemia and may be the most sensitive marker for B-ALL.[13] 97.5% of all B-ALL cases expressed CD79a and the remaining 2.5% of cases did not, hence the use of another B-cell marker (e.g., CD19 or CD20) was warranted. According to Swerdlow et al., CD79a has been noted to be positive in a number of T-ALL cases, hence these individual markers are not specific. However, combined expression of these markers will strongly support the diagnosis of B-ALL.[1]

CD10 often expressed in B-ALL can represent a population of both immature cells and normal B cell development. This type of maker is needed in the comparison with and differentiation from normal B-cell development patterns.[11]

In order to differentiate B-ALL from T-ALL, the incorporation of cytoplasmic CD3 is warranted. Lineage specific cytoplasmic CD3 is constantly expressed at high levels in T-ALL, which made the gating of blasts easier.[1,16] The interpretation of cytoplasmic CD3 should be coupled with surface CD3 for the reason that most of the T-ALL cases express cCD3 with negative smCD3.[16] All 29 cases of T-ALL expressed cCD3 and none expressed CD79a.

In a study by Singh et al., a minimal panel of eight antibodies were proposed (CD45/CD34/CD19/MPO/cytoCD3/CD64/CD117/CD79a) and a diagnostic yield of 97.5% was achieved. Their study was based on a 200 population, by which only 5/200 required an additional set of antibodies to properly classify the leukemic process.[4] Our study had a sensitivity and specificity of 100%, which meant that all 268 ALL cases were duly diagnosed by the use of the proposed limited screening panel.

In 2018, the World Bank said that amidst the good economic performance of the country, poverty remains high and the pace of poverty reduction has been slow.[21,22] The additional expense of healthcare ancillary procedures adds to the financial burden of the average Filipino.[22] The use of the limited screening panel cuts the cost of flow cytometry by 69.08%, hence easing the financial burden.

CONCLUSION

We underscore the utility of a limited panel for the diagnosis of ALL. Although this panel remains to be assessed with a larger validation cohort, its application in resource-limited developing countries is diagnostically useful and cost-effective.

RECOMMENDATION

The use of a limited panel of 9 antibodies is recommended as a screening panel for patients who are highly suspected of having ALL both clinically and by initial bone marrow smear assessment. A study on limited screening panel that will extend to cases of acute myeloid leukemia is also proposed.

STATEMENT OF AUTHORSHIP

All authors fulfilled the ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

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APPENDICES

Appendix A

Table 1. Basic leukemia panel composition

	Surface		
	FITC	**PE**	**PerCP**
1			CD45
2	CD4	CD8	CD45
3	CD34	CD117	CD45
4	HLA-DR	CD13	CD45
5	CD3	CD33	CD45
6	CD19	CD10	CD45
7	CD20	CD5	CD45
8	CD56	CD14	CD45
9	Anti-kappa	Anti-lambda	
	Cytoplasmic		
10	IgG2a	IgG1	CD45
11	cCD3	cCD79a	CD45
12	cMPO	cTdT	CD45

Appendix B

Table 2. Costing for basic leukemia panel

Tube #	Product Description	Test per Vial	SRP	Price per Tube
1	CD45 PerCP	200	32,602.00	163.01
2	CD4/CD8	100	56,010.64	280.05
2	CD45 PerCP	200	32,602.00	163.01
3	CD34 FITC	200	56,010.64	280.05
3	CD117 PE	100	35,265.96	352.66
3	CD45 PerCP	200	32,602.00	163.01
4	HLA-DR FITC	200	45,638.30	228.19
4	CD13 PE	200	62,234.04	311.17
4	CD45 PerCP	200	32,602.00	163.01
5	CD3 FITC	200	37,340.43	186.70
5	CD33 PE	200	20,317.00	101.59
5	CD45 PerCP	200	32,602.00	163.01
6	CD19 FITC	200	37,340.43	186.70
6	CD10 PE	200	47,712.77	238.56
6	CD45 PerCP	200	32,602.00	163.01
7	CD20 FITC	200	37,340.43	186.70
7	CD5 PE	200	47,712.77	238.56
7	CD45 PerCP	200	32,602.00	163.01
8	CD56 FITC	100	31,117.02	311.17
8	CD14 PE	200	33,191.49	165.96
8	CD45 PerCP	200	32,602.00	163.01
9	Anti-kappa/Anti-lambda	100	56,010.64	560.11
9	CD45 PerCP	200	32,602.00	163.01
10	Simultest IgG2a/IgG1	100	51,861.70	518.62
10	CD45 PerCP	200	32,602.00	163.01
11	cCD3 FITC	200	37,340.43	186.70
11	CD79a PE	100	40,265.96	402.66
11	CD45 PerCP	200	32,602.00	163.01
12	MPO FITC	100	33,191.49	331.91
12	TdT PE	100	37,340.43	373.40
12	CD45 PerCP	200	32,602.00	163.01
	FLUIDICS			2,505.48
			Total	**9,903.06**

OPEN ACCESS – ORIGINAL ARTICLE

A Five-Year Review of Soft Tissue Tumors with Intermediate Malignant Potential and Soft Tissue Sarcomas in a Tertiary Hospital: University of the Philippines – Philippine General Hospital*

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ABSTRACT

Objective. Describe the epidemiology of Soft Tissue Tumors with Intermediate Malignant Potential (STTI) and Soft Tissue Sarcomas (STS) diagnosed in Philippine General Hospital, Department of Laboratories, Section of Surgical Pathology, from years 2014 to 2018.

Methodology. We utilized a descriptive, retrospective, cross-sectional study design and involved all newly-diagnosed cases of STTI and STS that fit the specified set of inclusion and exclusion criteria.

Results. Out of 1896 cases of probable STTI and STS on initial screening, 680 cases (36%) were included in the study. Of the 1216 excluded cases, 815 (43%) needed ancillary diagnostic workup for definitive classification. Sarcoma, Not Otherwise Specified (n=149; 21.9%; 95% CI [18.80, 25.02]) was the most common diagnosis, followed by gastrointestinal stromal tumor (n=91; 13.4%; 95% CI [10.82, 15.94]) and leiomyosarcoma (n=62; 9.1%; 95% CI [6.95%, 11.28%]). Median age was 47 years, with a slight female predominance (n=371; 55%; 0.83 male to female ratio). The extremities (n=244, 36%) were the most frequent site.

Conclusion. The significant amount of cases excluded in the study may account for the differences of distribution. Despite the increased immunohistochemistry tests available, there is still an apparent inaccessibility to ancillary diagnostic methods that are necessary in the diagnosis of STTI and STS.

Key words: soft tissue neoplasms, sarcoma, Philippines

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INTRODUCTION

Soft tissue is defined as nonepithelial extraskeletal tissue of the body, principally derived from the mesoderm, with some contribution from the neuroectoderm. It is represented by the voluntary and involuntary muscles, fat, fibrous tissue and vessels supporting these tissues. Components of the peripheral nervous system are included as well, since tumors from nerves present as soft tissue masses with similar differential diagnoses and therapeutic measures. It does not include the reticuloendothelial system, glia, and supporting tissue of various parenchymal organs.[1,2]

Tumors arising from soft tissues are mainly classified according to their line of differentiation or adult tissue they resemble. They are further divided into benign and malignant forms. Benign tumors resemble their normal tissue counterparts, have a limited capacity for autonomous growth, exhibit little tendency to invade and have a low rate of local recurrence following conservative therapy. Malignant tumors, or STS, in contrast, are locally aggressive, capable of invasive growth, recurrence, and distant metastasis, and require radical surgery to ensure total removal. Unfortunately, this dichotomous differentiation is not perfect as the term sarcoma does not correlate well with likelihood or rapidity of metastasis. Due to this, STS are sometimes qualified by a statement regarding degree of differentiation or the histologic grade. Tumors of intermediate or borderline malignancy

are characterized by frequent recurrence but rare metastasizing potential.[1,3,4]

Benign soft tissue tumors outnumber malignant tumors by a factor of 50, according to Goldblum et al. STS are relatively rare compared to other neoplasms and reported incidences vary from less than 1 to 1.5% of all cancers, with an annual incidence of 50 per million population. The incidence and distribution of STS appear to be similar in different regions of the world. This is also noted by WHO wherein no significant geographical difference in sarcoma incidence was found. Despite this, it has been reported that a relationship exists between patient age, sex, tumor histologic type and tumor site.[1,3]

According to the SEER database, the incidence of STS varies with age. In children younger than 10 years of age, the annual incidence was 0.9/100,000 children. It was found to be higher in adults over the age of 70 years, with an incidence of 18.2/100,000 adults. The WHO reports the median age at diagnosis to be at 65 years.[2]

In general, STS are found to be more common in males, but gender and age-related incidences vary among the histologic types. There is also no proven racial variation.[1,2]

STS can occur anywhere, but most arise from the large muscles of the extremities, the chest wall, the mediastinum, and the retroperitoneum. Seventy-five percent are located in the extremities, most commonly in the thigh. Ten percent each are found in the trunk wall and retroperitoneum.[3,5]

The diversity of soft tissue tumors is emphasized in the 2020 WHO Classification of Tumors of Soft Tissue, wherein they are listed according to their line of differentiation. Tumors are classified under undifferentiated/unclassified sarcomas if no line of differentiation is identified using presently available technology and is a diagnosis of exclusion. They account for 20% of all STS and occur in all ages with no observed sexual predilection.[3]

According to the SEER Cancer Statistics Review (2012-2016), sarcoma, not otherwise specified (22.3%) was found to be the most common STS diagnosis reported. This is followed by liposarcoma (16.6%), leiomyosarcoma (12.8%), miscellaneous other sarcomas (8.1%), fibrosarcoma (7.9%), synovial sarcoma (4.6%), dermatofibrosarcoma (4.3%), malignant fibrous histiocytoma (4.2%), hemangiosarcoma (4.1%), and giant cell and extraskeletal bone sarcomas (3.2%).[2]

A systematic review on STS in the Asia-Pacific Region was done by Ngan et al., in 2013. Thirty-five published articles were included, 29 of which were from Australia, Korea and Taiwan. No study from Indonesia, New Zealand and the Philippines met the inclusion criteria. Pleomorphic sarcoma and liposarcoma were found to be the most common histologic type reported (23/32 studies). The mean or median age of patients with STS was found to be 40 years or older (27/30 studies), while the minimum age was younger than 18 years (14/30 studies). They found most sarcomas to be located in the extremities, consistent with reported literature worldwide.[6]

In a study by Ngelangel and Wang on Cancer and the Philippine Cancer Control Program in 2002, STS were reported to be 4.2 per million among children aged 0-14 years old. Rhabdomyosarcoma and fibrosarcoma were the only specific entities cited with incidence rates of 2.3 and 0.8 per million, respectively. However, this study was limited to Rizal province and four cities in Metro Manila, namely, Quezon, Manila, Caloocan and Pasay. These incidence rates were based on data gathered in the said areas during the years 1983 to 1992.[7]

OBJECTIVES

General Objective

- Describe the epidemiology of STTI and STS diagnosed in Philippine General Hospital (PGH), Department of Laboratories, Section of Surgical Pathology, from January 1, 2014 to December 31, 2018.

Specific Objectives

- Enumerate all cases of STTI and STS diagnosed in the hospital.
- Determine the distribution of STTI and STS diagnosed in the hospital, according to age, sex, tumor classification, tumor histologic type and tumor site.
- Compare the epidemiology of STTI and STS diagnosed in the hospital with published literature.

METHODOLOGY

Study Design

This is a descriptive, retrospective, cross-sectional study design.

Study Population

The study involved all cases of diagnosed STTI and STS, in accordance with the inclusion criteria below. It enumerated all cases that fulfilled the set criteria within the time period specified.

Inclusion Criteria

1. All newly-diagnosed inpatient and outpatient cases rendered with a definite diagnosis of STTI and STS (inclusive of soft tissue tumors with intermediate and malignant potential listed in the ***WHO Classification of Tumors of Soft Tissue*, 2020**) from January 1, 2014 to December 31, 2018, in the PGH, Department of Laboratories, Section of Surgical Pathology, confirmed using histomorphologic assessment, with or without ancillary immunohistochemistry and/or molecular testing.

Exclusion Criteria

1. Cases rendered with a definite diagnosis of a benign soft tissue tumor, as listed in the ***WHO Classification of Tumors of Soft Tissue*, 2020**.
2. Cases of soft tissue tumors whose biologic behavior cannot be classified as benign, intermediate, or malignant due to limited information in the final diagnosis.
3. Cases with incomplete data on age, sex, tumor site and histologic diagnosis.
4. Cases of recurrent or persistent STTI and STS that are status post treatment (e.g., chemotherapy, radiotherapy, etc.).

Data Collection and Processing

Data were obtained from surgical pathology reports of all patients that have been diagnosed with STTI and STS from January 1, 2014 to December 31, 2018. Only data on the patients' age, sex, tumor site and histologic diagnoses were obtained from the surgical pathology reports.

A research assistant was hired to assist in the data collection, with permission requested from the Chair of the Department of Laboratories, as well as from the Head and Supervisor of the Section of Surgical Pathology. The principal investigator trained the research assistant regarding the data collection procedure, with emphasis on patient privacy and confidentiality.

Retrieval of anonymized data from surgical pathology reports into data collection forms (Appendix A) were facilitated by an authorized laboratory technologist who subscribed, sworn and signed the Confidentiality and Non-Disclosure Undertaking of the hospital. The data collection forms were then given to the research assistant for encoding into the master spreadsheet (Appendix B).

Data collection was done within office hours and within the Section of Surgical Pathology, to ensure that records remain in the section and minimize risk of breach of patient confidentiality. A brief diagrammatic workflow is provided in Appendix C.

Intervention

Not applicable.

Outcome

Epidemiology of STTI and STS in PGH.

Analysis

Data were collected and tabulated using Microsoft Excel 2019. Microsoft Excel 2019 and Stata version 16 were used to analyze the data which were entered according to a coding manual (Appendix D).

Descriptive statistics were computed for all demographic variables available using Microsoft Excel 2019 and Stata software. Summary statistics (i.e., mean, range, median) were used for quantitative variables like age while for categorical data, data were summarized using frequencies and proportions. Tables and graphs were utilized to display findings more clearly. Data processing and analysis were carried out using Microsoft Excel 2019 and Stata software.

Ethical Considerations

Prior to commencement of the study, an institutional ethical approval coursed through the PGH Expanded Hospital Research Office was sought. The study was conducted only upon approval from the University of the Philippines Manila Research Ethics Board (UPMREB).

The patient demographic and clinical information needed in the study were retrieved from the surgical pathology reports filed at the Department of Laboratories, Section of Surgical Pathology. Patients names were not obtained. There was no patient-investigator interaction and only surgical pathology records were accessed for review.

The principal investigator solely funded this research.

Waiver of consent

A waiver of consent was requested from UPMREB since there are no risks to participants and the method of data collection ensured that none of the participants were identifiable and anonymity was ensured (NEGHHR 2017 provision 16.2.3 and 17.1). The waiver did not adversely affect the rights and welfare of the participants (NEGHHR 2017 17.2) and the research was not practicably carried out without the waiver (NEGHHR 2017 17.3). A plan for data collection was discussed earlier in the protocol.

RESULTS AND DISCUSSION

A total of 680 cases diagnosed as STTI and STS were identified and included in the study after extensive review of all surgical pathology reports signed out at PGH, Department of Laboratories from January 1, 2014 to December 31, 2018. This comprised 36% of the 1896 cases of probable STTI and STS gathered on initial screening (Figure 1). Steps were taken to ensure that each diagnosis belonged to only one patient. Cases of recurrent or persistent STTI and STS that underwent

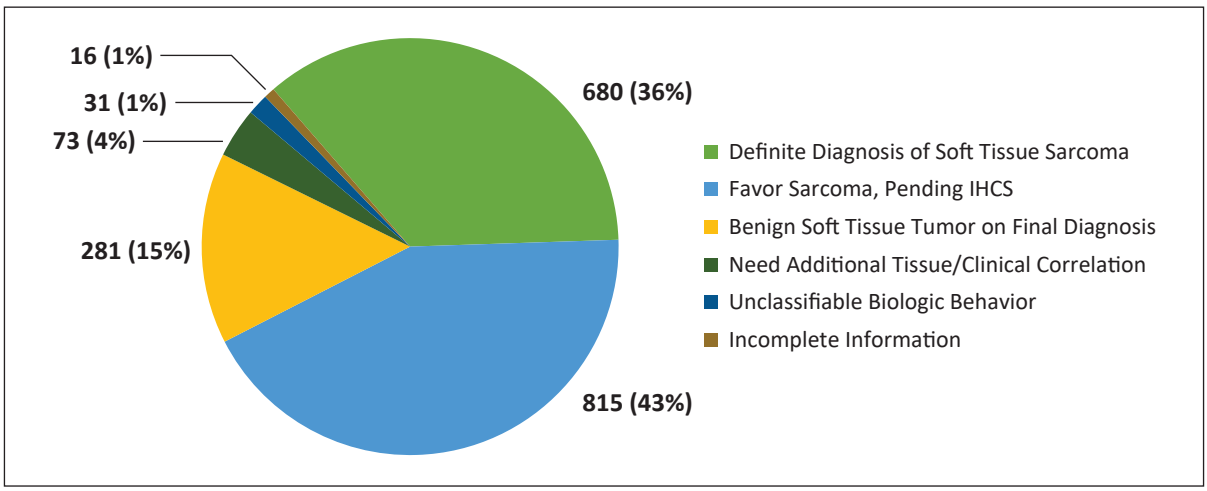

Figure 1. Distribution of cases of probable STTI and STS on initial screening.

treatment (e.g., chemotherapy, radiotherapy, etc.) were counted as one, together with the initial diagnosis prior to treatment, so as to prevent duplication of counts.

A total of 1216 out of 1896 cases (64%) of probable STTI and STS were excluded upon initial screening. Majority (n=815 out of 1216, 67%) of the inconclusive cases were either attributed to total lack of, or incomplete, immunohistochemical (IHC) workup (Table 1). This is a significant finding given the setting of a tertiary referral hospital, highlighting the need for improvement of availability and accessibility of necessary ancillary diagnostic tests, including, but not limited to immunohistochemistry methods and cytogenetic studies.

Figure 2 shows an increasing trend in the number of STTI and STS diagnosed in PGH from January 1, 2014 to December 31, 2018.

Figure 3 shows the age and sex distribution of STTI and STS in PGH from 2014-2018, stratified according to age. The 2020 WHO Classification of Tumors of Soft Tissue reports the general distribution of sarcomas to have a slight male predominance. Results of the study show that there is a slight, albeit insignificant, female predominance (n=371; 55%; 95% CI [50.82, 58.30]; 0.83 male to female ratio; p value=0.9997), over males (n=309; 45%; 95% CI [41.70, 49.18]) in the sarcomas diagnosed in PGH.

As with other malignancies, sarcomas are increasingly common with older age, with a reported median age of 65 years.[8] Results showed that sarcomas were most common among the older adults, 45-64 years of age (n=240, 35%), however, with a much younger median age of 47 years. This finding is still consistent to the reported "mean or median age" of "40 years or older" (n=27 studies) in the systematic review of STS in the Asia-Pacific Region by Ngan et al., in 2013.[6]

The order of distribution of sarcomas according to age and tumor classification is shown in Figure 4. Sarcomas have been reported to have varied age-related incidences. Of special note is embryonal rhabdomyosarcoma, which is known to occur almost exclusively in children, and synovial sarcoma, which mostly occurs in young adults. Results of the study revealed that of the 11 cases of embryonal rhabdomyosarcoma NOS in the years 2014-2018, ten of these were found to be in patients less than 18 years old (range = 3 to 22 years old), with a median age of 10 years. Another 12 cases of sarcomas were

Table 1. Summary of excluded cases

Reason for exclusion	Number of cases (%)
Cases favoring the diagnosis of STTI/STS, pending recommended immunohistochemical studies to exclude other tumors.	815 (67)
Cases rendered with a final diagnosis of a benign soft tissue tumor.	281 (23)
Cases with STTI/STS in the differential diagnosis but with recommendation for additional tissue biopsy, excision, or clinical correlation for definite classification.	73 (6)
Cases of probable STTI/STS whose biologic behavior cannot be classified as benign, intermediate, or malignant due to limited information in the final diagnosis.	31 (3)
Cases of probable STTI/STS with incomplete data on age, sex, tumor site and histologic diagnosis.	16 (1)
Total	**1216**

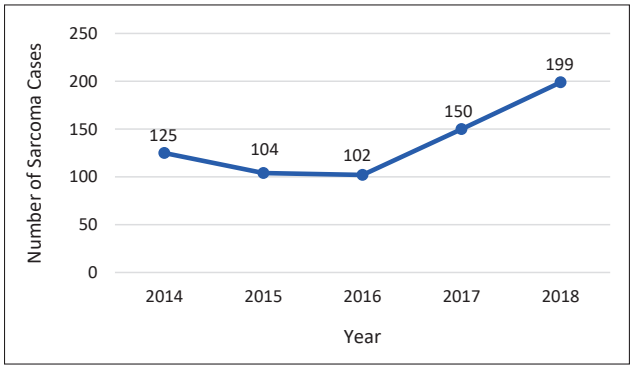

Figure 2. STTI and STS diagnosed in PGH from January 1, 2014 to December 31, 2018.

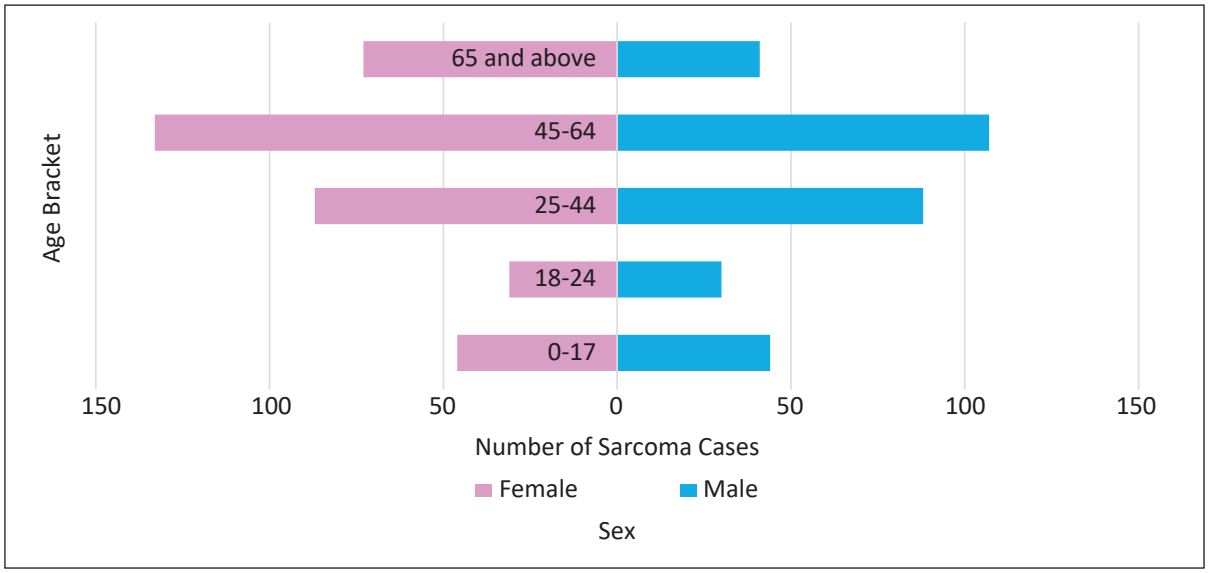

Figure 3. Age and sex distribution of STTI and STS diagnosed in PGH from January 1, 2014 to December 31, 2018.

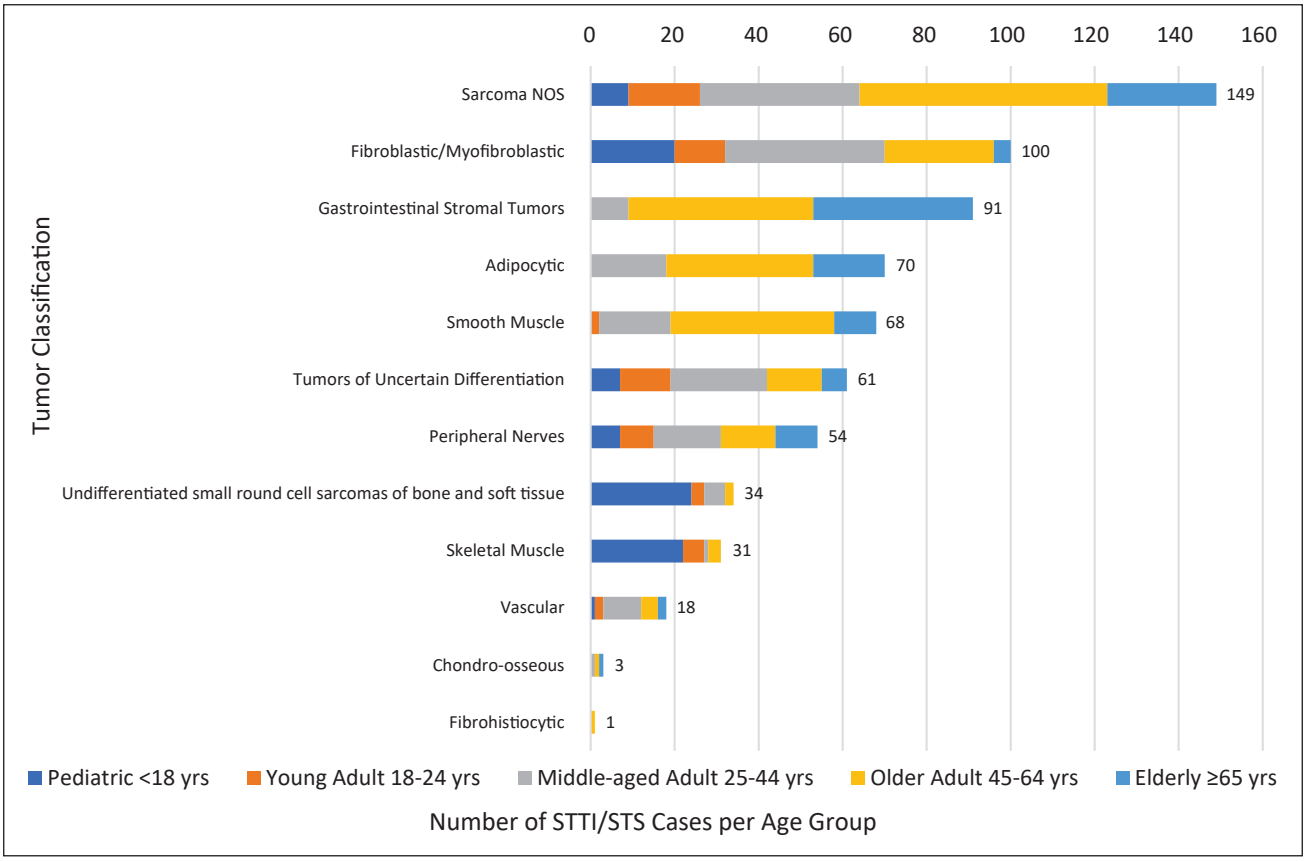

Figure 4. Distribution of STTI/STS diagnosed in PGH from January 1, 2014 to December 31, 2018 according to age and tumor classification.

diagnosed as "Rhabdomyosarcoma", without further subclassification (median age = 12.5). Among these, six cases were below 18 years old. Based on the epidemiology of rhabdomyosarcomas in this age group, some of these may in fact be of embryonal subtype.

Among the sarcomas with definite tumor classification, fibroblastic/myofibroblastic tumors were the most common (n=100; 14.7%; 95% CI [12.04%, 17.37%]). Majority of the cases were classified under Sarcoma, Not Otherwise Specified (Sarcoma NOS; n=149; 21.9%, 95% CI [18.80%, 25.02%]). This is an additional category, in addition to the diagnoses dictated by the 2020 WHO Classification of Soft Tissue Tumors, arbitrarily delegated in this study and adopted from the SEER database, for cases diagnosed as definite STTI and STS, pending more specific tumor classification. This fact remains to be a significant limiting factor in the diagnosis of STTI and STS and reiterates the need for more advanced techniques in the diagnosis of sarcomas received in our institution.[2,8]

The top ten most common STTI and STS histologic types enumerated in this study are listed in Table 2. The results of this study highlight both the similarities and differences between incidences of diagnosed STTI and STS in different populations. Both the SEER database and results of this study showed Sarcoma NOS (p=0.8055), leiomyosarcoma (p=0.0045), synovial sarcoma (p=1.0000) and dermatofibrosarcoma (p=0.01587) to be among the most commonly diagnosed sarcomas. Leiomyosarcoma and dermatofibroma were found to be significantly different in the two populations (p<0.05). Following

Table 2. Top ten most commonly diagnosed STTI and STS histologic types in PGH, from January 1, 2014 to December 31, 2018, and in SEER, 2012-2016

Results		SEER, 2012-2016	
STTI/STS Histologic Type	**Number of Cases (%)**	**Histologic Type**	**Percentage (%)**
Sarcoma, NOS	149 (21.9)	**Sarcomas, NOS**	22.3
Gastrointestinal stromal tumor	91 (13.4)	Liposarcomas	16.6
Leiomyosarcoma NOS	62 (9.1)	**Leiomyosarcomas**	12.8
Malignant peripheral nerve sheath tumor NOS	54 (7.9)	Miscellaneous other sarcomas	8.1
Ewing sarcoma	32 (4.7)	Fibrosarcomas	7.9
Synovial sarcoma NOS	31 (4.6)	**Synovial sarcomas**	4.6
Solitary fibrous tumor NOS	25 (3.7)	**Dermatofibrosarcomas**	4.3
Myxoid liposarcoma	22 (3.2)	Malignant fibrous histiocytoma	4.2
Fibromatosis	22 (3.2)	Hemangiosarcomas	4.1
Dermatofibrosarcoma protuberans NOS	20 (2.4)	Giant cell and extra-skeletal bone sarcomas	3.2

Sarcoma NOS, gastrointestinal stromal tumor (GIST) is the most common diagnosed STTI/STS in our institution, which was not included in the SEER 2012-2016 ten most common sarcomas. Malignant peripheral nerve sheath tumor (MPNST), Ewing sarcoma, solitary fibrous tumor, myxoid liposarcoma, and fibromatosis were not among the most common in the SEER database. [2]

WHO cites undifferentiated pleomorphic sarcoma (UPS, previously malignant fibrous histiocytoma and listed as such in the 2012-2016 SEER data), liposarcoma, leiomyosarcoma, myxofibrosarcoma, synovial sarcoma,

and MPNST to comprise approximately 65% of STS.[8] In the exhaustive systematic review of STS in the Asia-Pacific region by Ngan et al., pleomorphic sarcoma and liposarcoma were the predominant histologic types.[6]

Solitary fibrous tumor NOS and fibromatosis were among the top ten sarcomas reported in this study. Both tumors belong to the category of Intermediate Malignant Potential in the WHO Classification and were notably absent in the SEER 2012-2016 data, which only reported frankly malignant sarcomas.

Results showed that 36% (n=244) of the STTI and STS diagnosed in PGH were located in the extremities (Figure 5). This is followed by the trunk (n=128, 18.8%) and head and neck (n=126, 18.5%) in close proximity to each other. The data is congruent with the WHO database which also showed the extremities to be the common site, although twice more often at 75%. Ten percent each are found in the trunk wall and retroperitoneum.[8] The extremities were also the most common site of STTI and STS in the systematic review by Ngan et al.[6]

Tables 3 and 4 in the succeeding pages show the overall distribution of sarcomas diagnosed in PGH in the years 2014-2018 according to tumor classification, age, sex and tumor site, respectively. There were four STTI/STS diagnoses that couldn't be classified under the current WHO Classification due to need for further subtyping, namely, "Fibromatosis," "Hemangioendothelioma," "Rhabdomyosarcoma" and "Rhabdomyosarcoma, Embryonal-Alveolar." Separate counts were done for these entries. No case of Malignant Glomus Tumor, under Pericytic/Perivascular STS in the WHO tumor classification, has been diagnosed in the years 2014-2018.

The significant amount of cases excluded in the beginning of the study may account for the differences of distribution in the STTI and STS diagnosed in PGH. Unless these cases are pursued until a specific diagnosis is reached, the true incidence may never be known. Of course, even in the ideal setting, there will be tumors that remain elusive to classification, which may aptly be called true Sarcomas, not otherwise specified. In the United States SEER database for STS in the years 2002-2014, Sarcoma, NOS was the most common category at 14.8%.[2,9] In the latest WHO Classification, these tumors that failed to show any identifiable line of differentiation after analysis using presently available technology are classified under Undifferentiated Sarcoma, which comprises approximately 20% of all STS.[8]

Despite the apparent unmet need in diagnostic ancillary testing, the increased number of diagnosed STTI and STS cases through the years 2014 to 2018 remains to be promising. This may simply be due to an increase in number of patients catered by PGH. However, this may also be reflective of the successful efforts of the Division of Surgical Pathology in acquiring and providing immunohistochemical stains necessary in the diagnosis of STTI and STS. There may have been an increase in the number of tumors, initially diagnosed as nonspecific "Spindle Cell Neoplasms," subjected to immunohistochemical studies, and subsequently rendered with the definitive diagnosis of STTI or STS.

In the recent years, the hospital has been able to increase the number of diagnostic tests which cater specifically to the diagnosis of sarcomas. However, there is still plenty of room for improvement of molecular and cytogenetic testing in the classification of sarcomas. Furthermore, as majority of the patients in PGH belong to the lower socioeconomic strata of the country, other factors such as the accessibility of these more costly diagnostic tests to the catered population of the hospital cannot be discounted.

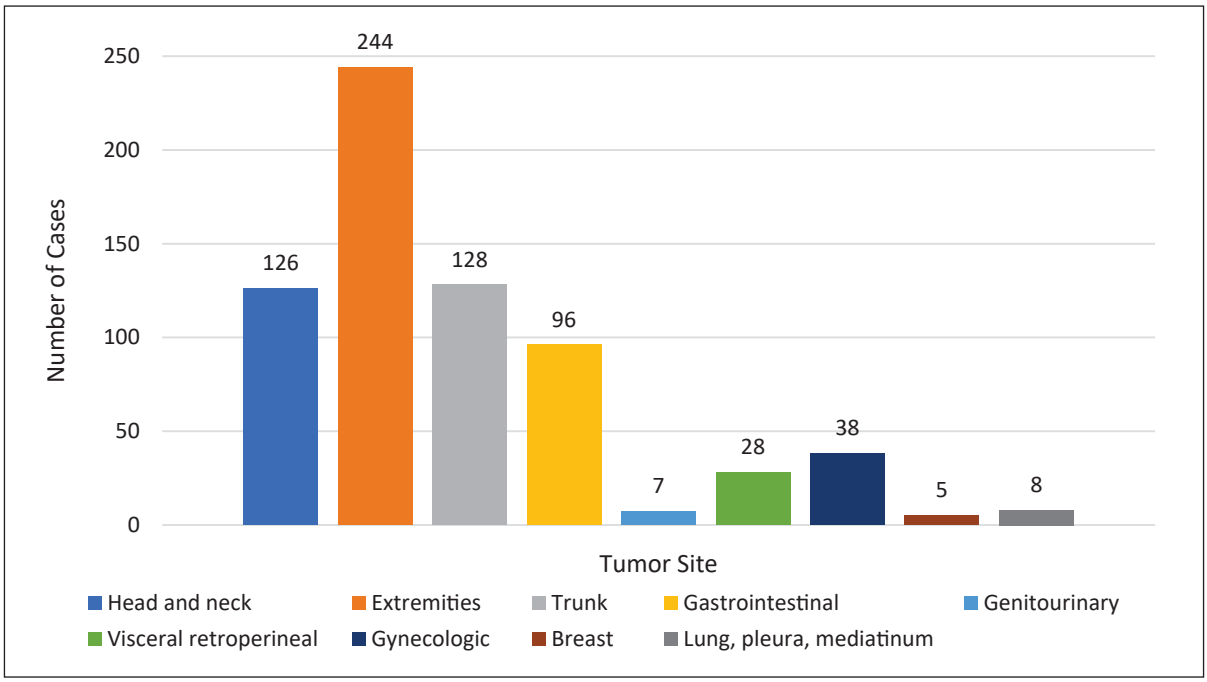

Figure 5. Distribution of STTI and STS diagnosed in PGH from January 1, 2014 to December 31, 2018 according to tumor site.

Table 3. Distribution of STTI and STS according to tumor classification, age and sex, Philippine General Hospital, 2014-2018

Tumor Classification and Histologic Type	Number	Proportion[a]	95% CI[c]	Median age[c] (years)	Age Group, *n*					Sex, *n*	
					Pediatric <18 years	Young adult 18-24 years	Middle-aged Adult 25-44 years	Older Adult 45-64 years	Elderly ≥65 years	Male	Female
Adipocytic	70	10.3%	8.01%, 12.58%	56	**0**	**0**	**18**	**35**	**17**	**40**	**30**
Atypical lipomatous tumor	16	2.4%	1.21%, 3.49%	51.5	0	0	4	10	2	11	5
Liposarcoma, well-differentiated, NOS	16	2.4%	1.21%, 3.49%	58	0	0	3	8	5	9	7
Lipoma-like liposarcoma	0	0.0%	NA	NA	0	0	0	0	0	0	0
Inflammatory liposarcoma	0	0.0%	NA	NA	0	0	0	0	0	0	0
Sclerosing liposarcoma	0	0.0%	NA	NA	0	0	0	0	0	0	0
Dedifferentiated liposarcoma	7	1.0%	0.27%, 1.79%	62	0	0	2	2	3	4	3
Myxoid liposarcoma	22	3.2%	1.91%, 4.57%	55.5	0	0	8	9	5	11	11
Pleomorphic liposarcoma	8	1.2%	0.37%, 1.99%	59	0	0	0	6	2	5	3
Epithelioid liposarcoma	1	0.1%	0.01%, 0.44%	NA	0	0	1	0	0	0	1
Myxoid pleomorphic liposarcoma	0	0.0%	NA	NA	0	0	0	0	0	0	0
Fibroblastic/Myofibroblastic	100	14.7%	12.04, 17.37	**38**	**20**	**12**	**38**	**26**	**4**	**33**	**67**
Fibromatosis[b]	22	3.2%	1.91%, 4.57%	26	9	1	8	4	0	7	15
Solitary fibrous tumor, benign	0	0.0%	NA	NA	0	0	0	0	0	0	0
Palmar/plantar-type fibromatosis	0	0.0%	NA	NA	0	0	0	0	0	0	0
Desmoid-type fibromatosis	4	0.6%	0.01%, 1.16%	31.5	2	0	2	0	0	2	2
Extra-abdominal desmoid	1	0.1%	0.01%, 0.44%	NA	1	0	0	0	0	0	1
Abdominal fibromatosis	1	0.1%	0.01%, 0.44%	NA	0	0	0	1	0	1	0
Lipofibromatosis	0	0.0%	NA	NA	0	0	0	0	0	0	0
Giant cell fibroblastoma	0	0.0%	NA	NA	0	0	0	0	0	0	0
Dermatofibrosarcoma protuberans NOS	20	2.9%	1.67%, 4.21%	41	0	3	12	5	0	8	12
Pigmented dermatofibrosarcoma protuberans	0	0.0%	NA	NA	0	0	0	0	0	0	0
Dermatofibrosarcoma protuberans, fibrosarcomatous	1	0.1%	0.01%, 0.44%	NA	0	0	0	0	1	0	1
Myxoid dermatofibrosarcoma protuberans	2	0.3%	0.01%, 0.70%	NA	0	0	0	2	0	1	1
Dermatofibrosarcoma protuberans with myoid differentiation	0	0.0%	NA	NA	0	0	0	0	0	0	0
Plaque-like dermatofibrosarcoma protuberans	0	0.0%	NA	NA	0	0	0	0	0	0	0
Solitary fibrous tumor NOS	25	3.7%	2.26%, 5.09%	44	4	4	10	7	0	8	17
Fat-forming (lipomatous) solitary fibrous tumour	0	0.0%	NA	NA	0	0	0	0	0	0	0
Giant cell-rich solitary fibrous tumour	0	0.0%	NA	NA	0	0	0	0	0	0	0
Inflammatory myofibroblastic tumour	7	1.0%	0.27%, 1.79%	18	3	1	0	3	0	2	5
Epithelioid inflammatory myofibroblastic sarcoma	0	0.0%	NA	NA	0	0	0	0	0	0	0
Myofibroblastic sarcoma	6	0.9%	0.18%, 1.59%	26	0	3	1	2	0	0	6
Superficial CD34-positive fibroblastic tumor	0	0.0%	NA	NA	0	0	0	0	0	0	0
Myxoinflammatory fibroblastic sarcoma	0	0.0%	NA	NA	0	0	0	0	0	0	0
Infantile fibrosarcoma	0	0.0%	NA	NA	0	0	0	0	0	0	0
Solitary fibrous tumor, malignant	2	0.3%	0.01%, 0.70%	NA	0	0	1	1	0	1	1
Fibrosarcoma NOS	4	0.6%	0.01%, 1.16%	37.5	1	0	3	0	0	1	3
Myxofibrosarcoma	3	0.4%	0.01%, 0.94%	65	0	0	0	1	2	0	3
Epithelioid myxofibrosarcoma	0	0.0%	NA	NA	0	0	0	0	0	0	0
Low-grade fibromyxoid sarcoma	1	0.1%	0.01%, 0.44%	NA	0	0	1	0	0	1	0
Sclerosing epithelioid fibrosarcoma	1	0.1%	0.01%, 0.44%	NA	0	0	0	0	1	1	0
Fibrohistiocytic	1	0.1%	0.01-0.44	NA	0	0	0	1	0	1	0
Plexiform fibrohistiocytic tumor	0	0.0%	NA	NA	0	0	0	0	0	0	0
Giant cell tumour of soft parts NOS	1	0.1%	0.01%, 0.44%	NA	0	0	0	1	0	1	0
Malignant tenosynovial giant cell tumour	0	0.0%	NA	NA	0	0	0	0	0	0	0
Smooth muscle	68	10.0%	7.75, 12.25	53.5	0	2	17	39	10	14	54
Smooth muscle tumour of uncertain malignant potential	6	0.9%	0.18%, 1.59%	45.5	0	1	2	3	0	0	6
Leiomyosarcoma NOS	62	9.1%	6.95%, 11.28%	54	0	1	15	36	10	14	48
Skeletal muscle	31	4.6%	2.99, 6.13	10	22	5	1	3	0	15	16
Rhabdomyosarcoma[b]	12	1.8%	0.78%, 2.75%	12.5	7	3	0	2	0	5	7
Rhabdomyosarcoma, "Embryonal-Alveolar"[b]	1	0.1%	0.01%, 0.44%	NA	1	0	0	0	0	1	0
Embryonal rhabdomyosarcoma NOS	11	1.6%	0.67%, 2.57%	10	10	1	0	0	0	5	6
Embryonal rhabdomyosarcoma, pleomorphic	0	0.0%	NA	NA	0	0	0	0	0	0	0
Alveolar rhabdomyosarcoma	5	0.7%	0.09%, 1.38%	14	3	1	0	1	0	3	2
Pleomorphic rhabdomyosarcoma NOS	1	0.1%	0.01%, 0.44%	NA	0	0	1	0	0	1	0
Spindle cell rhabdomyosarcoma	1	0.1%	0.01%, 0.44%	NA	1	0	0	0	0	0	1
Congenital spindle cell rhabdomyosarcoma with VGLL2/NCOA2/CITED2 rearrangements	0	0.0%	NA	NA	0	0	0	0	0	0	0
MYOD1-mutant spindle cell/sclerosing rhabdomyosarcoma	0	0.0%	NA	NA	0	0	0	0	0	0	0
Intraosseous spindle cell rhabdomyosarcoma (with TFCP2/NCOA2 rearrangements)	0	0.0%	NA	NA	0	0	0	0	0	0	0
Ectomesenchymoma	0	0.0%	NA	NA	0	0	0	0	0	0	0

Table 3. Distribution of STTI and STS according to tumor classification, age and sex, Philippine General Hospital, 2014-2018 *(continued)*

Tumor Classification and Histologic Type	Number	Proportion[a]	95% CI[c]	Median age[c] (years)	Age Group, *n*					Sex, *n*	
					Pediatric <18 years	Young adult 18-24 years	Middle-aged Adult 25-44 years	Older Adult 45-64 years	Elderly ≥65 years	Male	Female
Vascular	18	2.6%	1.44, 3.85	30.5	1	2	9	4	2	11	7
Hemangioendothelioma[b]	3	0.4%	0.01%, 0.94%	49	0	0	1	1	1	1	2
Kaposiform hemangioendothelioma	1	0.1%	0.01%, 0.44%	NA	0	1	0	0	0	0	1
Retiform haemangioendothelioma	0	0.0%	NA	NA	0	0	0	0	0	0	0
Papillary intralymphatic angioendothelioma	0	0.0%	NA	NA	0	0	0	0	0	0	0
Composite haemangioendothelioma	0	0.0%	NA	NA	0	0	0	0	0	0	0
Neuroendocrine composite haemangioendothelioma	0	0.0%	NA	NA	0	0	0	0	0	0	0
Kaposi sarcoma	2	0.3%	0.01%, 0.70%	NA	0	0	2	0	0	2	0
Classic indolent Kaposi sarcoma	0	0.0%	NA	NA	0	0	0	0	0	0	0
Endemic African Kaposi sarcoma	0	0.0%	NA	NA	0	0	0	0	0	0	0
AIDS-associated Kaposi sarcoma	0	0.0%	NA	NA	0	0	0	0	0	0	0
Iatrogenic Kaposi sarcoma	0	0.0%	NA	NA	0	0	0	0	0	0	0
Pseudomyogenic (epithelioid sarcoma-like) haemangioendothelioma	0	0.0%	NA	NA	0	0	0	0	0	0	0
Epithelioid haemangioendothelioma NOS	3	0.4%	0.01%, 0.94%	64	0	0	1	1	1	3	0
Epithelioid haemangioendothelioma with WWTR1-CAMTA1 fusion	0	0.0%	NA	NA	0	0	0	0	0	0	0
Epithelioid haemangioendothelioma with YAP1-TFE3 fusion	0	0.0%	NA	NA	0	0	0	0	0	0	0
Angiosarcoma	9	1.3%	0.46%, 2.18%	30	1	1	5	2	0	5	4
Tumors of peripheral nerves	54	7.9%	5.91, 9.97	33	7	8	16	13	10	26	28
Malignant peripheral nerve sheath tumour NOS	54	7.9%	5.91%, 9.97%	33	7	8	16	13	10	26	28
Malignant peripheral nerve sheath tumour, epithelioid	0	0.0%	NA	NA	0	0	0	0	0	0	0
Melanotic malignant peripheral nerve sheath tumour	0	0.0%	NA	NA	0	0	0	0	0	0	0
Granular cell tumour, malignant	0	0.0%	NA	NA	0	0	0	0	0	0	0
Perineurioma, malignant	0	0.0%	NA	NA	0	0	0	0	0	0	0
Chondro-osseous	3	0.4%	0.01, 0.94	59	0	0	1	1	1	3	0
Osteosarcoma, extraskeletal	3	0.4%	0.01, 0.94	59	0	0	1	1	1	3	0
Gastrointestinal stromal tumors	91	13.4%	10.82, 15.94	62	0	0	9	44	38	41	50
Gastrointestinal stromal tumor	91	13.4%	10.82, 15.94	62	0	0	9	44	38	41	50
Tumors of uncertain differentiation	61	9.0%	6.82, 11.12	36	7	12	23	13	6	30	31
Haemosiderotic fibrolipomatous tumor	0	0.0%	NA	NA	0	0	0	0	0	0	0
Angiomyolipoma, epithelioid	0	0.0%	NA	NA	0	0	0	0	0	0	0
Atypical fibroxanthoma	0	0.0%	NA	NA	0	0	0	0	0	0	0
Angiomatoid fibrous histiocytoma	0	0.0%	NA	NA	0	0	0	0	0	0	0
Ossifying fibromyxoid tumour NOS	0	0.0%	NA	NA	0	0	0	0	0	0	0
Mixed tumour NOS	0	0.0%	NA	NA	0	0	0	0	0	0	0
Mixed tumour, malignant, NOS	0	0.0%	NA	NA	0	0	0	0	0	0	0
Myoepithelioma NOS	0	0.0%	NA	NA	0	0	0	0	0	0	0
Phosphaturic mesenchymal tumour, malignant	0	0.0%	NA	NA	0	0	0	0	0	0	0
NTRK-rearranged spindle cell neoplasm (emerging)	0	0.0%	NA	NA	0	0	0	0	0	0	0
Synovial sarcoma NOS	31	4.6%	2.99%, 6.13%	29	5	8	13	3	2	12	19
Synovial sarcoma, spindle cell	0	0.0%	NA	NA	0	0	0	0	0	0	0
Synovial sarcoma, biphasic	6	0.9%	0.18%, 1.59%	37	0	2	3	1	0	4	2
Synovial sarcoma, poorly differentiated	0	0.0%	NA	NA	0	0	0	0	0	0	0
Epithelioid sarcoma	1	0.1%	0.01%, 0.44%	NA	0	0	0	1	0	0	1
Proximal or large cell epithelioid sarcoma	0	0.0%	NA	NA	0	0	0	0	0	0	0
Classic epithelioid sarcoma	0	0.0%	NA	NA	0	0	0	0	0	0	0
Alveolar soft part sarcoma	3	0.4%	0.01%, 0.94%	28	1	0	2	0	0	2	1
Clear cell sarcoma NOS	1	0.1%	0.01%, 0.44%	NA	0	0	1	0	0	1	0
Extraskeletal myxoid chondrosarcoma	0	0.0%	NA	NA	0	0	0	0	0	0	0
Desmoplastic small round cell tumour	1	0.1%	0.01%, 0.44%	NA	1	0	0	0	0	1	0
Rhabdoid tumour NOS	1	0.1%	0.01%, 0.44%	NA	0	1	0	0	0	0	1
Perivascular epithelioid tumour, malignant	0	0.0%	NA	NA	0	0	0	0	0	0	0
Intimal sarcoma	0	0.0%	NA	NA	0	0	0	0	0	0	0
Ossifying fibromyxoid tumour, malignant	0	0.0%	NA	NA	0	0	0	0	0	0	0
Myoepithelial carcinoma	0	0.0%	NA	NA	0	0	0	0	0	0	0
Undifferentiated sarcoma	3	0.4%	0.01%, 0.94%	60	0	0	1	1	1	1	2
Spindle cell sarcoma, undifferentiated	0	0.0%	NA	NA	0	0	0	0	0	0	0
Pleomorphic sarcoma, undifferentiated	14	2.1%	0.99%, 3.13%	58	0	1	3	7	3	9	5
Round cell sarcoma, undifferentiated	0	0.0%	NA	NA	0	0	0	0	0	0	0
Undifferentiated small round cell sarcomas of bone and soft tissue	34	5.0%	3.36, 6.64	15.5	24	3	5	2	0	22	12
Ewing sarcoma	32	4.7%	3.11%, 6.30%	15.5	23	2	5	2	0	22	10
Round cell sarcoma with EWSR1-non-ETS fusions	0	0.0%	NA	NA	0	0	0	0	0	0	0
C/C-rearranged sarcoma	2	0.3%	0.01%, 0.70%	NA	1	1	0	0	0	0	2
Sarcoma with BCOR genetic alterations	0	0.0%	NA	NA	0	0	0	0	0	0	0
Sarcoma, NOS	149	21.9%	18.80. 25.02	50	9	17	38	59	26	73	76
Total	**680**	**100%**		**47[d]**	**90**	**61**	**175**	**240**	**114**	**309**	**371**

Notes:
a: Blue: proportion of each tumor classification to the total STTI and STS; White: proportion of tumor diagnosis to the total STTI and STS
b: STTI and STS diagnosis as reported in the surgical pathology report, not further subtyped.
c: Not applicable (NA) for entries that are too few for evaluation.
d: Median Age for all STTI and STS sarcomas in the study.

Table 4. Distribution of STTI and STS according to tumor site, Philippine General Hospital, 2014-2018

Tumor Classification and Histologic Type	Tumor Site, *n*									Total
	Head and neck	Extremities	Trunk	Gastro-intestinal	Genito-urinary	Visceral retroperitoneal	Gyneco-logic	Breast	Lung, pleura, mediastinum	
Adipocytic	**4**	**33**	**19**	**4**	**0**	**8**	**0**	**0**	**2**	**70**
Atypical lipomatous tumor	1	**10**	5	0	0	0	0	**0**	0	**16**
Liposarcoma, well-differentiated, NOS	1	7	2	2	0	4	0	0	0	**16**
Lipoma-like liposarcoma	0	0	0	0	0	0	0	0	0	**0**
Inflammatory liposarcoma	0	0	0	0	0	0	0	0	0	**0**
Sclerosing liposarcoma	0	0	0	0	0	0	0	0	0	**0**
Dedifferentiated liposarcoma	1	2	3	0	0	1	0	0	0	**7**
Myxoid liposarcoma	1	8	6	2	0	3	0	0	2	**22**
Pleomorphic liposarcoma	0	5	3	0	0	0	0	0	0	**8**
Epithelioid liposarcoma	0	1	0	0	0	0	0	0	0	**1**
Myxoid pleomorphic liposarcoma	0	0	0	0	0	0	0	0	0	**0**
Fibroblastic/Myofibroblastic	**35**	**26**	**31**	**3**	**0**	**2**	**0**	**1**	**2**	**100**
Fibromatosis	7	10	4	0	0	0	0	0	1	**22**
Solitary fibrous tumor, benign	0	0	0	0	0	0	0	0	0	**0**
Palmar/plantar-type fibromatosis	0	0	0	0	0	0	0	0	0	**0**
Desmoid-type fibromatosis	1	0	3	0	0	0	0	0	0	**4**
Extra-abdominal desmoid	0	1	0	0	0	0	0	0	0	**1**
Abdominal fibromatosis	0	0	0	1	0	0	0	0	0	**1**
Lipofibromatosis	0	0	0	0	0	0	0	0	0	**0**
Giant cell fibroblastoma	0	0	0	0	0	0	0	0	0	**0**
Dermatofibrosarcoma protuberans NOS	3	5	11	0	0	0	0	1	0	**20**
Pigmented dermatofibrosarcoma protuberans	0	0	0	0	0	0	0	0	0	**0**
Dermatofibrosarcoma protuberans, fibrosarcomatous	0	0	1	0	0	0	0	0	0	**1**
Myxoid dermatofibrosarcoma protuberans	1	0	1	0	0	0	0	0	0	**2**
Dermatofibrosarcoma protuberanswith myoid differentiation	0	0	0	0	0	0	0	0	0	**0**
Plaque-like dermatofibrosarcoma protuberans	0	0	0	0	0	0	0	0	0	**0**
Solitary fibrous tumor NOS	14	5	5	0	0	1	0	0	0	**25**
Fat-forming (lipomatous) solitary fibrous tumour	0	0	0	0	0	0	0	0	0	**0**
Giant cell-rich solitary fibrous tumour	0	0	0	0	0	0	0	0	0	**0**
Inflammatory myofibroblastic tumour	2	0	2	2	0	0	0	0	1	**7**
Epithelioid inflammatory myofibroblastic sarcoma	0	0	0	0	0	0	0	0	0	**0**
Myofibroblastic sarcoma	3	1	2	0	0	0	0	0	0	**6**
Superficial CD34-positive fibroblastic tumor	0	0	0	0	0	0	0	0	0	**0**
Myxoinflammatory fibroblastic sarcoma	0	0	0	0	0	0	0	0	0	**0**
Infantile fibrosarcoma	0	0	0	0	0	0	0	0	0	**0**
Solitary fibrous tumor, malignant	1	0	0	0	0	1	0	0	0	**2**
Fibrosarcoma NOS	2	1	1	0	0	0	0	0	0	**4**
Myxofibrosarcoma	0	2	1	0	0	0	0	0	0	**3**
Epithelioid myxofibrosarcoma	0	0	0	0	0	0	0	0	0	**0**
Low-grade fibromyxoid sarcoma	1	0	0	0	0	0	0	0	0	**1**
Sclerosing epithelioid fibrosarcoma	0	1	0	0	0	0	0	0	0	**1**
Fibrohistiocytic	0	0	1	0	0	0	0	0	0	**1**
Plexiform fibrohistiocytic tumor	0	0	0	0	0	0	0	0	0	**0**
Giant cell tumour of soft parts NOS	0	0	1	0	0	0	0	0	0	**1**
Malignant tenosynovial giant cell tumour	0	0	0	0	0	0	0	0	0	**0**
Smooth muscle	6	10	8	6	0	5	32	1	0	**68**
Smooth muscle tumour of uncertain malignant potential	0	0	0	0	0	0	6	0	0	**6**
Leiomyosarcoma NOS	6	10	8	6	0	5	26	1	0	**62**
Skeletal muscle	19	6	3	0	2	1	0	0	0	**31**
Rhabdomyosarcoma	5	3	2	0	1	1	0	0	0	**12**
Rhabdomyosarcoma, "Embryonal-Alveolar"	0	1	0	0	0	0	0	0	0	**1**
Embryonal rhabdomyosarcoma NOS	9	0	1	0	1	0	0	0	0	**11**
Embryonal rhabdomyosarcoma, pleomorphic	0	0	0	0	0	0	0	0	0	**0**
Alveolar rhabdomyosarcoma	4	1	0	0	0	0	0	0	0	**5**
Pleomorphic rhabdomyosarcoma NOS	0	1	0	0	0	0	0	0	0	**1**
Spindle cell rhabdomyosarcoma	1	0	0	0	0	0	0	0	0	**1**
Congenital spindle cell rhabdomyosarcoma with VGLL2/NCOA2/CITED2 rearrangements	0	0	0	0	0	0	0	0	0	**0**
MYOD1-mutant spindle cell/sclerosing rhabdomyosarcoma	0	0	0	0	0	0	0	0	0	**0**
Intraosseous spindle cell rhabdomyosarcoma (with TFCP2/NCOA2 rearrangements)	0	0	0	0	0	0	0	0	0	**0**
Ectomesenchymoma	0	0	0	0	0	0	0	0	0	**0**

Table 4. Distribution of STTI and STS according to tumor site, Philippine General Hospital, 2014-2018 *(continued)*

Tumor Classification and Histologic Type	Tumor Site, *n*									Total
	Head and neck	Extremities	Trunk	Gastro-intestinal	Genito-urinary	Visceral retroperitoneal	Gyneco-logic	Breast	Lung, pleura, mediastinum	
Vascular	6	8	2	2	0	0	0	0	0	**18**
Hemangioendothelioma	2	1	0	0	0	0	0	0	0	**3**
Kaposiform hemangioendothelioma	1	0	0	0	0	0	0	0	0	**1**
Retiform haemangioendothelioma	0	0	0	0	0	0	0	0	0	**0**
Papillary intralymphatic angioendothelioma	0	0	0	0	0	0	0	0	0	**0**
Composite haemangioendothelioma	0	0	0	0	0	0	0	0	0	**0**
Neuroendocrine composite haemangioendothelioma	0	0	0	0	0	0	0	0	0	**0**
Kaposi sarcoma	1	0	0	1	0	0	0	0	0	**2**
Classic indolent Kaposi sarcoma	0	0	0	0	0	0	0	0	0	**0**
Endemic African Kaposi sarcoma	0	0	0	0	0	0	0	0	0	**0**
AIDS-associated Kaposi sarcoma	0	0	0	0	0	0	0	0	0	**0**
Iatrogenic Kaposi sarcoma	0	0	0	0	0	0	0	0	0	**0**
Pseudomyogenic (epithelioid sarcoma-like) haemangioendothelioma	0	0	0	0	0	0	0	0	0	**0**
Epithelioid haemangioendothelioma NOS	0	2	0	1	0	0	0	0	0	**3**
Epithelioid haemangioendothelioma with WWTR1-CAMTA1 fusion	0	0	0	0	0	0	0	0	0	**0**
Epithelioid haemangioendothelioma with YAP1-TFE3 fusion	0	0	0	0	0	0	0	0	0	**0**
Angiosarcoma	2	5	2	0	0	0	0	0	0	**9**
Tumors of peripheral nerves	11	24	15	0	0	3	0	0	1	**54**
Malignant peripheral nerve sheath tumour NOS	11	24	15	0	0	3	0	0	1	**54**
Malignant peripheral nerve sheath tumour, epithelioid	0	0	0	0	0	0	0	0	0	**0**
Melanotic malignant peripheral nerve sheath tumour	0	0	0	0	0	0	0	0	0	**0**
Granular cell tumour, malignant	0	0	0	0	0	0	0	0	0	**0**
Perineurioma, malignant	0	0	0	0	0	0	0	0	0	**0**
Chondro-osseous	2	1	0	0	0	0	0	0	0	**3**
Osteosarcoma, extraskeletal	2	1	0	0	0	0	0	0	0	**3**
Gastrointestinal stromal tumors	0	1	10	76	0	2	2	0	0	**91**
Gastrointestinal stromal tumor	0	1	10	76	0	2	2	0	0	**91**
Tumors of uncertain differentiation	15	34	8	1	0	1	1	1	0	**61**
Haemosiderotic fibrolipomatous tumor	0	0	0	0	0	0	0	0	0	**0**
Angiomyolipoma, epithelioid	0	0	0	0	0	0	0	0	0	**0**
Atypical fibroxanthoma	0	0	0	0	0	0	0	0	0	**0**
Angiomatoid fibrous histiocytoma	0	0	0	0	0	0	0	0	0	**0**
Ossifying fibromyxoid tumour NOS	0	0	0	0	0	0	0	0	0	**0**
Mixed tumour NOS	0	0	0	0	0	0	0	0	0	**0**
Mixed tumour, malignant, NOS	0	0	0	0	0	0	0	0	0	**0**
Myoepithelioma NOS	0	0	0	0	0	0	0	0	0	**0**
Phosphaturic mesenchymal tumour, malignant	0	0	0	0	0	0	0	0	0	**0**
NTRK-rearranged spindle cell neoplasm (emerging)	0	0	0	0	0	0	0	0	0	**0**
Synovial sarcoma NOS	7	19	5	0	0	0	0	0	0	**31**
Synovial sarcoma, spindle cell	0	0	0	0	0	0	0	0	0	**0**
Synovial sarcoma, biphasic	3	2	1	0	0	0	0	0	0	**6**
Synovial sarcoma, poorly differentiated	0	0	0	0	0	0	0	0	0	**0**
Epithelioid sarcoma	0	1	0	0	0	0	0	0	0	**1**
Proximal or large cell epithelioid sarcoma	0	0	0	0	0	0	0	0	0	**0**
Classic epithelioid sarcoma	0	0	0	0	0	0	0	0	0	**0**
Alveolar soft part sarcoma	1	2	0	0	0	0	0	0	0	**3**
Clear cell sarcoma NOS	0	1	0	0	0	0	0	0	0	**1**
Extraskeletal myxoid chondrosarcoma	0	0	0	0	0	0	0	0	0	**0**
Desmoplastic small round cell tumour	0	0	0	1	0	0	0	0	0	**1**
Rhabdoid tumour NOS	0	1	0	0	0	0	0	0	0	**1**
Perivascular epithelioid tumour, malignant	0	0	0	0	0	0	0	0	0	**0**
Intimal sarcoma	0	0	0	0	0	0	0	0	0	**0**
Ossifying fibromyxoid tumour, malignant	0	0	0	0	0	0	0	0	0	**0**
Myoepithelial carcinoma	0	0	0	0	0	0	0	0	0	**0**
Undifferentiated sarcoma	0	1	0	0	0	0	1	1	0	**3**
Spindle cell sarcoma, undifferentiated	0	0	0	0	0	0	0	0	0	**0**
Pleomorphic sarcoma, undifferentiated	4	7	2	0	0	1	0	0	0	**14**
Round cell sarcoma, undifferentiated	0	0	0	0	0	0	0	0	0	**0**
Undifferentiated small round cell sarcomas of bone and soft tissue	9	12	10	1	1	0	0	0	1	**34**
Ewing sarcoma	9	11	10	0	1	0	0	0	1	**32**
Round cell sarcoma with EWSR1-non-ETS fusions	0	0	0	0	0	0	0	0	0	**0**
C/C-rearranged sarcoma	0	1	0	1	0	0	0	0	0	**2**
Sarcoma with BCOR genetic alterations	0	0	0	0	0	0	0	0	0	**0**
Sarcoma, NOS	19	89	21	3	4	6	3	2	2	**149**
Total	**126**	**244**	**128**	**96**	**7**	**28**	**38**	**5**	**8**	**680**

CONCLUSION

We extensively reviewed all diagnosed, as well probable, STTI and STS in PGH, from January 1, 2014 to December 31, 2018, and made apparent the significant number of tumors that need further histopathologic evaluation for a definitive assessment. Sarcoma, NOS was the most common diagnosis rendered, followed by gastrointestinal stromal tumor and leiomyosarcoma. The median age in the study was found to be 47 years, with a slight female predominance (0.83 male to female ratio), both of which were different from published WHO data. STTI and STS were found to be most commonly located in the extremities, which is consistent with available literature.

This study addresses the lack of locoregional data pertaining to soft tissue tumors in the Philippines and Asia. The advent of improved ancillary diagnostic methods will pave the way to an improved STTI and STS database that will better reflect its true epidemiology.

RECOMMENDATIONS

The authors recommend that slide review and further immunohistochemistry and/or cytogenetic studies be performed to the excluded cases. We also recommend that additional support should be given to make ancillary tests more accessible to our patient population. Furthermore, the data is available for perusal to determine what additional ancillary tests are needed to improve our diagnostic capability as a teaching and tertiary referral hospital. A formal research may also be performed to determine the various factors involved in the nonfulfillment of recommended immunohistochemistry studies.

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STATEMENT OF AUTHORSHIP

Both authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

Both authors declared no conflict of interest.

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None.

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APPENDICES

Appendix A. Data Collection Form

A Five-Year Review of Soft Tissue Sarcomas in a Tertiary Hospital: University of the Philippines – Philippine General Hospital
(Salise, JMM and Atun, JML)

ID Number: _____
Age: _____
Sex: _____

Tumor Site:

Final Histopathologic Diagnosis:

Appendix B. Master Spreadsheet

ID Number	Date (dd/mm/year)	Age	Sex	Tumor Site	Tumor Classification	Final Histopathologic Diagnosis

Appendix C. Diagrammatic Workflow

1. Permission obtained to conduct research from the Department of Laboratories, Section of Surgical Pathology.

↓

2. Laboratory technologist transfers anonymized data from surgical pathology reports into data collection forms.

↓

3. Research assistant encodes information from data collection forms into master spreadsheet.

↓

4. Data Analysis.

Appendix D. Coding Manual

Variable	Type	Code
ID Number	Field data	As is
Age	Field data	As is
Age group	Bracket code	0 = Pediatric (less than 18 years old) 1 = Young Adult (18-24 years old) 2 = Middle-aged Adult (25-44 years old) 3 = Older Adult (45-64 years old) 4 = Elderly (65 years old and above)
Sex	Listing code	0 = Male 1 = Female
Tumor site	Listing code	0 = Head and neck 1 = Extremities 2 = Trunk 3 = Gastrointestinal 4 = Genitourinary 5 = Visceral retroperitoneal 6 = Gynecologic 7 = Breast 8 = Lung, pleura, and mediastinum 9 = Other
Tumor classification	Listing code	0 = Adipocytic 1 = Fibroblastic/myofibroblastic 2 = Fibrohistiocytic 3 = Smooth muscle 4 = Pericytic (perivascular) 5 = Skeletal muscle 6 = Vascular 7 = Peripheral Nerve 8 = Chondro-osseous 9 = Gastrointestinal Stromal Tumors 10 = Tumors of uncertain differentiation **11 = Undifferentiated small round cell sarcomas of bone and soft tissue** 12 = Sarcoma, NOS 13 = Others
Tumor histologic type	Field data	As is

OPEN ACCESS – CASE REPORT

Mixed Small Cell and Large Cell Neuroendocrine Carcinoma involving the Endometrium: A Case Report and Literature Review*

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ABSTRACT

Neuroendocrine carcinoma (NEC) of the endometrium is a rare, aggressive subtype of endometrial cancer. We report a 61-year-old female with a history of breast cancer, s/p modified radical mastectomy, chemotherapy, radiotherapy and hormonal (tamoxifen) therapy, who presented with post-menopausal bleeding. Patient underwent TAH-BSO with lymph node dissection, and was diagnosed with a mixed small cell neuroendocrine carcinoma (SCNEC) and large cell neuroendocrine carcinoma (LCNEC), confirmed by positive immunohistochemical staining for neuroendocrine markers. No other lesions were identified on PET-CT, making a primary endometrial NEC the most likely diagnosis. We review the clinical and pathologic characteristics of endometrial neuroendocrine carcinomas.

Key words: endometrial neoplasms, neuroendocrine carcinoma, large cell carcinoma, small cell carcinoma

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Available from: https://www.enets.org/download/f.2030.pdf

INTRODUCTION

Studies by the International Agency for Research on Cancer have shown that endometrial cancer is the 6th most commonly diagnosed cancer, the 2nd most commonly diagnosed female genital cancer, and the 14th leading cause of cancer death in women worldwide. In the past 10 years, the rates of endometrial cancer have increased in several countries, particularly in Japan, the Philippines, Belarus, Singapore, Costa Rica, and New Zealand. The Philippine Cancer Society estimates that in 2015, there were 2451 new cases of cancers of the corpus uteri and 565 deaths due to said cancer, however, the specific proportion by tumor type is not reported.[1-3]

While the majority of primary uterine corpus malignancies are endometrial endometrioid adenocarcinomas (80-90%), other significant tumor types include serous carcinoma (10%), clear cell carcinoma (<10%), undifferentiated carcinoma (≈2%), and neuroendocrine carcinoma (<0.8%), as well as carcinosarcomas and mesenchymal malignancies of the uterus (≈5%). Neuroendocrine tumors (NETs) of the gynecologic tract are uncommon, comprising about only 2% of gynecologic malignancies. Among gynecologic organs, neuroendocrine carcinomas (NECs) are most likely to occur in the uterine cervix, while they rarely occur in the uterine corpus. Primary endometrial neuroendocrine carcinoma is extremely rare, estimated to comprise less than 0.8% of endometrial carcinomas, with approximately a hundred cases reported in the literature.[1-7]

A search for reports in local literature revealed only a single published case of "poorly differentiated adenocarcinoma with neuroendocrine differentiation." At the study center, an average of approximately 330 hysterectomy specimens are received per annum, out of which approximately 60 are diagnosed with primary malignancy

of the endometrium. In descending order of frequency, these cases were diagnosed as endometrial endometrioid adenocarcinoma (89%), serous carcinoma (5%), carcinosarcomas and malignant mesenchymal tumors (3%), and clear cell carcinomas (2%). In comparison, over a five year period, only 2 resection specimens (≈0.6%), including the present case, were diagnosed as neuroendocrine carcinomas of the endometrium.[3]

Clinically, endometrial neuroendocrine carcinomas affect mainly perimenopausal or postmenopausal females. These tumors run an aggressive course with a propensity to metastasize. Histologically, they may present with morphology similar to small cell and large cell neuroendocrine carcinomas of the lung, and may also present in a mixed pattern with other forms of endometrial malignancy. Pathologic examination and immunohistochemical staining with neuroendocrine markers are essential for the proper diagnosis of these lesions.[4-6]

Here, we report a case of a 61-year-old female diagnosed with mixed small and large cell neuroendocrine carcinoma (NEC) of the endometrium. The clinicopathologic characteristics, management and outcomes of neuroendocrine carcinomas of the endometrium are reviewed.

CASE

A 61-year-old, nulligravid, Filipino female presented with postmenopausal vaginal bleeding beginning four months prior to referral to our institution. She had a previous history of breast cancer stage IIA, right, for which she underwent modified radical mastectomy ten (10) years prior to consult, followed by adjuvant chemotherapy and radiotherapy followed by adjuvant endocrine treatment with Tamoxifen for five (5) years. No evidence of disease metastasis or recurrence was noted on regular surveillance with mammography, bone scintigraphy and serum CA 15-3 monitoring. The patient also had a history of uterine myoma, status post myomectomy eighteen (18) years prior to consult, endometrial polyp, status post transcervical polypectomy seven (7) years prior to consult, and type II diabetes mellitus and hypertension.

Three months prior to referral, the patient consulted with a gynecologist. A transvaginal ultrasound revealed a thickened endometrium, and the patient underwent an endometrial biopsy, with a histopathologic diagnosis of endometrial endometrioid adenocarcinoma. She was advised total abdominal hysterectomy with bilateral salpingo-oophorectomy (TAHBSO).

Physical examination on admission at our institution revealed no palpable abdominal mass or ascites. Internal examination showed a cervix that was firm, long and closed with no cervical motion tenderness and an anteverted uterus without palpable masses, enlargement or tenderness.

The patient underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy (TAHBSO) with bilateral lymph node dissection and peritoneal fluid sampling with intraoperative findings of a slightly enlarged uterus, as well as adhesions between the left anterolateral aspect of the uterus and the posterior bladder wall which were lysed by sharp and blunt dissection.

On gross examination of the TAHBSO specimen at the pathology laboratory, the uterine corpus (5.5 x 5 x 4 cm.) was observed to have a 1.6 cm. thick anterior myometrium and a 2 cm. thick posterior myometrium, with a 5 x 2 cm. endometrial canal lined by 0.3 cm. thick endometrium. Sectioning revealed a 3 x 2.2 x 1.8 cm. cream tan, necrotic, friable, fungating endometrial mass arising in the posterior fundic area, with extension into the right anterior endometrium, and grossly infiltrating into the posterior myometrium. The mass was located about 2 cm. from the internal cervical os, and did not grossly extend to the lower uterine segment or cervix. The remaining myometrium demonstrated pink tan, whorled to trabeculated, rubbery cut surfaces. There was note of dense adhesions between the distal portion of the left fallopian tube (2.5 x 0.5 x 0.5 cm.) and left ovary (2.8 x 1.7 x 1 cm.), such that the fimbriae were not grossly distinguishable. Sectioning of the left ovary revealed cream tan to brown, smooth cut surfaces. Additionally, the right ovary (2.7 x 2.5 x 0.8 cm.) had a smooth outer surface and a 0.6 cm. unilocular cyst filled with mucoid material on sectioning. The right fallopian tube (5.5 x 0.5 x 0.5 cm.) was fimbriated and grossly unremarkable.

Microscopic examination of the endometrial mass disclosed an invasive tumor composed of large areas of malignant cells with interspersed areas of intratumoral necrosis and scant residual normal endometrial glands and stroma. The tumor demonstrated a mixed population of cells with approximately 60% composed of small cells with small hyperchromatic, ovoid to pleomorphic nuclei, nuclear molding and scant, poorly defined cytoplasm, arranged in solid sheets and nests. The remaining 40% was composed of larger cells with large, hyperchromatic to vesicular, ovoid to elongated to pleomorphic nuclei with nuclear molding, with scant to moderate cytoplasm, arranged in nests, glandlike and cribriform patterns and rosettes. The small cells also demonstrated crushing artifact (Figure 1).

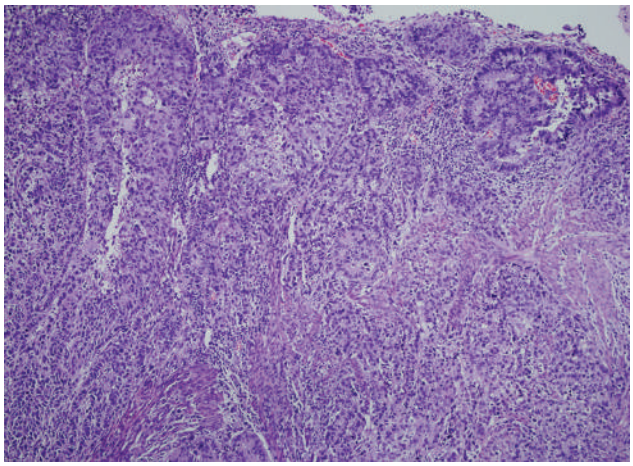

Figure 1. Low-power view of endometrial mass (H&E, 200x): Microsections reveal an infiltrative endometrial tumor composed of mixed small and large cells in nests, sheets, trabeculae, gland-like and rosette-like patterns.

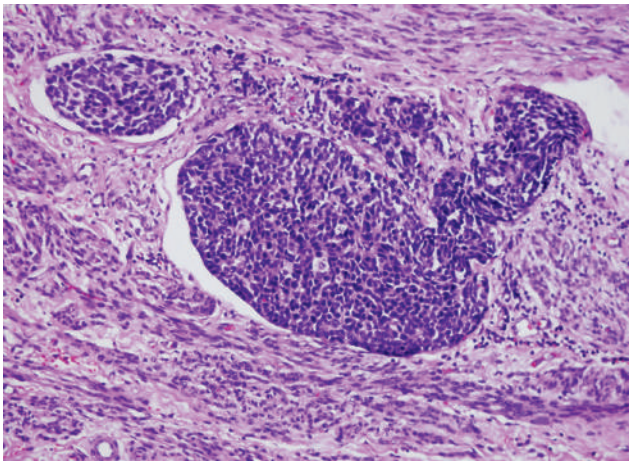

Figure 2. High-power view of infiltrating mass (H&E, 400x): Microsections of the myometrium demonstrate lymphovascular invasion by the small cell tumor component, with prominent nuclear crushing artifact.

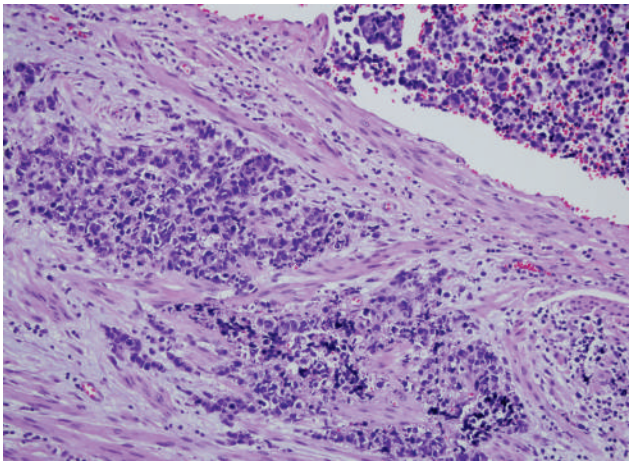

Figure 3. High-power view of infiltrating mass (H&E, 400x): Microsections of the myometrium demonstrate deep tumor infiltration (bottom) and extensive lymphovascular invasion (top) by the small cell tumor component, with prominent nuclear crushing artifact.

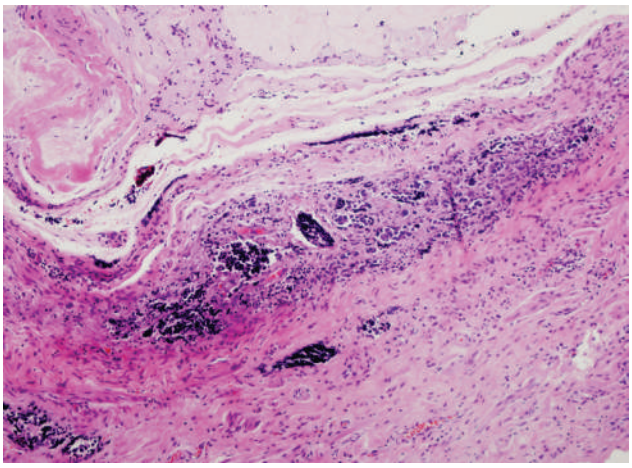

Figure 4. Low-power view of tumor in the adnexae (H&E, 100x): Microsections of the left ovary demonstrate nests of the small-cell tumor component present within the ovarian stroma and blood vessels (bottom), adjacent to a corpus albicans (top).

The tumor appeared to develop from within the endometrium, as the bulk of the tumor was located in the endometrial canal with invasion into the underlying myometrium. Microscopically, the invasion into the myometrium reached the subserosal area of the uterus, or more than 50% of the 2 cm thick myometrium. The tumor extended to the lower uterine segment but did not involve the cervix. The cervix showed benign endocervical and ectocervical epithelium with Nabothian cyst formation. There was also note of extensive lymphovascular invasion in the uterus, mainly of the small cell type (Figures 2 and 3). The left fallopian tube and ovary were also noted to have deposits of small cell type tumor cells, particularly within blood vessels (Figure 4). The right fallopian tube was unremarkable, and the right ovary was noted to have a mucinous cystadenoma. The parametria, right and left pelvic lymph nodes and peritoneal fluid were negative for tumor.

The initial impression was a poorly-differentiated endometrial carcinoma, to consider a high grade endometrioid carcinoma versus a dedifferentiated carcinoma versus a neuroendocrine carcinoma. Thus, immunohistochemistry was performed using the following stains to further characterize the tumor: CK (AE1/AE3), CD56 (CD564), Chromogranin (LK2H10), Synaptophysin (SP11), Pax8 (Biocare BC12) and WT-1 (6F-H2). The tumor cells showed diffuse positive staining for CK, CD56, Chromogranin and Synaptophysin, but were negative for Pax8 and WT-1 (Figure 5). Given the results, the tumor was diagnosed as a high grade neuroendocrine tumor with combined small cell and large cell histomorphology.

Given the presence of deep myometrial invasion and involvement of the left adnexa, the tumor was staged as pT3a by the AJCC 8th edition pathologic staging.

PET-CT scan performed three weeks after resection showed a prominent-sized hypermetabolic prebifurcation lymph node and normal-sized hypermetabolic mesenteric, para-aortic and left pelvic nodes which were suspicious for metastases. Thus, by FIGO 2015 staging the patient was considered as having a stage IIIC2 tumor. No other hypermetabolic areas were noted. Patient underwent adjuvant chemotherapy with six cycles of carboplatin and etoposide followed by radiotherapy to the pelvis (50 gy in 28 fractions) and paraaortic nodes (45 gy in 25 fractions). Treatment was generally well tolerated with few episodes of grade 1 thrombocytopenia. On reevaluation with PET-CT after eighteen months, no evidence of disease was noted and patient remained asymptomatic and stable.

DISCUSSION

Clinical Presentation

The 2020 WHO Classification of Tumors of Female Reproductive Organs divides primary neuroendocrine tumors of the female genital tract into low-grade neuroendocrine tumors (also known as carcinoid tumors) and high-grade neuroendocrine carcinomas (NEC), which includes small cell neuroendocrine carcinoma (SCNEC) and large cell neuroendocrine carcinomas (LCNEC) of the endometrium. SCNEC and LCNEC of the endometrium

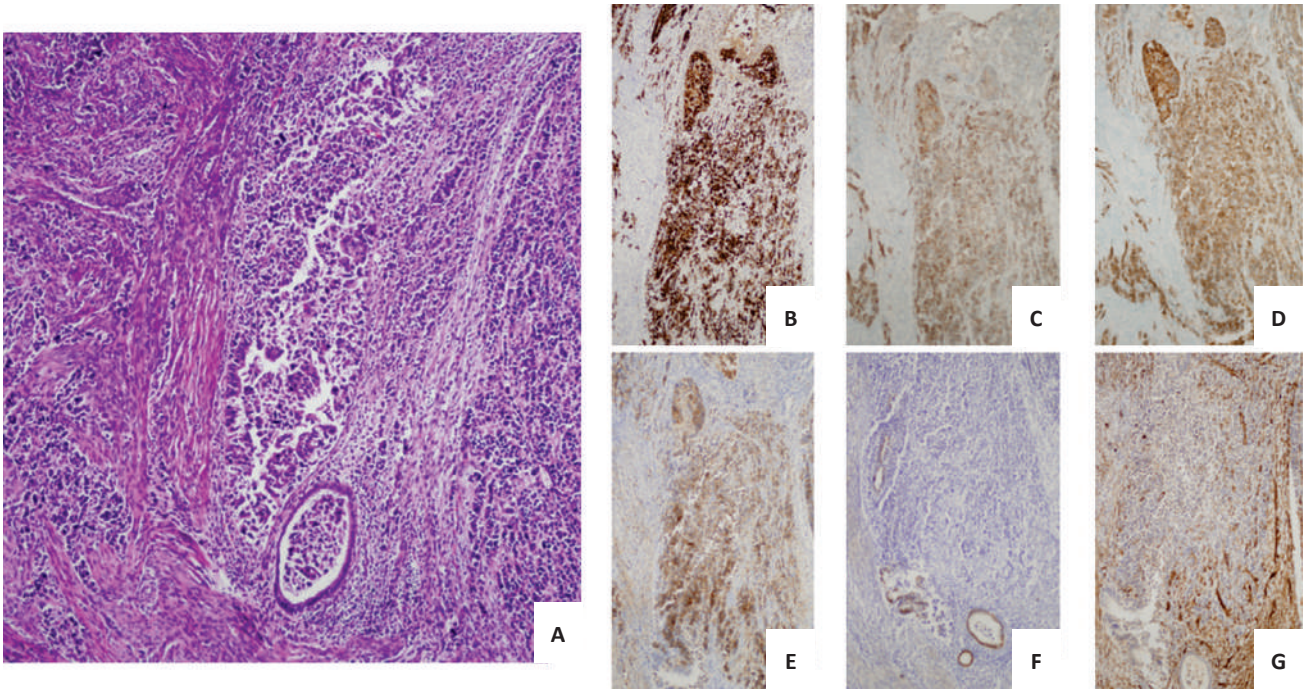

Figure 5. (A) Immunohistochemical Studies of Mass (H&E 100x) **(B)** Tumor cells are diffusely positive for staining with Cytokeratin; **(C)** Chromogranin; **(D)** Synaptophysin and **(E)** CD56; and **(F)** negative for staining with PAX8 and **(G)** WT-1.

** (F) Pax8 stains the residual atrophic endometrial glands but not the tumor cells while (G) WT-1 stains the smooth muscle of the myometrium but not the tumor cells.*

are very uncommon, estimated to comprise only 0.8% of endometrial carcinomas. Low-grade neuroendocrine tumors of the endometrium are even less common, with only three reported cases.[4-7]

Endometrial NECs usually occur in postmenopausal or perimenopausal patients, with an average age at diagnosis of 60 for SCNEC and 55 for LCNEC. It may be presumed that factors associated with increased risk for endometrial endometrioid adenocarcinomas such as nulliparity, increased BMI, diabetes mellitus, and certain endocrine therapies such as tamoxifen may also increase the risk of endometrial NECs, although this is not yet well studied due to the rarity of endometrial NECs. In the present case, the patient's risk factors include age, nulliparity, diabetes mellitus and history of breast cancer and tamoxifen therapy.[1,8]

Cortesi et al., reported that women with a history of breast cancer have a standardized incidence ratio (SIR) of 2.15 for development of endometrial cancer compared to the general population, with a higher SIR in patients who were treated with tamoxifen (2.5 compared to 1.34). The effect is bidirectional, with women with a history of endometrial cancer also having a higher risk of developing breast cancer (1.62). The effect of chemoradiotherapy for breast cancer on risk for endometrial cancer is not yet known, however, it has been observed that radiotherapy is associated with increased risk for second primary breast cancer, thyroid cancer and hematopoietic malignancies, and chemotherapy is associated with increased risk for hematopoietic malignancies.[8]

The most common clinical presentations of SCNEC and LCNEC are abnormal uterine bleeding and abdominal pain. Other reported presentations include abdominal enlargement, fatigue, and abnormal Pap smear findings. Paraneoplastic syndromes such as Cushing syndrome presenting as fatigue or visual impairment have also been reported.[4-7,9-14]

Histopathologic Characteristics

Grossly, endometrial NECs are often noted to be bulky, fungating masses, and deep infiltration into the myometrium is often noted. Some are described as polypoid or arising in a polyp, and may possibly have a more favorable prognosis according to a case series by Albores-Saavedra et al. Extra-uterine spread has been noted in several cases, including ovarian or tubal involvement, vaginal involvement, lymph node metastasis, and involvement of other pelvic and abdominal organs.[9-10]

Microscopically, SCNEC is composed of ovoid, poorly cohesive cells which may be arranged in solid, nested, trabecular, pseudoglandular or rosette-like patterns. The tumor cells have condensed chromatin and scant cytoplasm, with frequent nuclear molding, numerous mitotic figures, necrosis and apoptotic bodies, resembling small cell carcinoma of the lung. Similarly, LCNEC is characterized as having tumor cells arranged in solid, trabecular, nested, pseudoglandular and rosette-like patterns, trabeculae or cords, with peripheral palisading, and large, polygonal tumor cells with vesicular or hyperchromatic nuclei and prominent nucleoli. High mitotic activity and extensive geographic necrosis are also appreciated.[4-7,9-14]

To establish the diagnosis of NEC, a neuroendocrine growth pattern should be present in at least part of the tumor, together with expression of one or more neuroendocrine markers (Chromogranin, Synaptophysin

or CD56) in >10% of the tumor cells. However, it is important to note that 25-50% of typical endometrial adenocarcinomas may have minor populations of endocrine cells that are also positive for neuroendocrine markers. Neuroendocrine markers may also be detected in 30-40% of undifferentiated carcinomas, however, they exhibit focal staining of less than 10% of the total tumor cells. Histologically, NECs of the endometrium may appear as pure small cell or large cell neuroendocrine carcinomas, mixed small and large cell neuroendocrine carcinomas, or mixed with other histologic subtypes of endometrial tumors. The most common other cellular component in mixed tumors is endometrioid adenocarcinoma. Rare mixed tumors with serous cell carcinoma and clear cell carcinoma have also been reported.[4-7,9-14]

The histomorphologic differential diagnoses for SCNEC and LCNEC include high grade endometrioid adenocarcinoma, undifferentiated carcinoma, primitive neuroectodermal tumor, and carcinosarcoma. Thus, immunohistochemical staining is essential for properly identifying the neuroendocrine nature of the lesions. The majority of endometrial SCNEC and LCNEC are positive for synaptophysin, chromogranin A, CD56, neuron specific enolase and CD57. For SCNEC, synaptophysin is the most consistently expressed marker, while chromogranin is the most specific. These tumors also demonstrate high proliferative activity, with >50% of cells staining positive for Ki-67. SCNEC and LCNEC are usually positive for broad-spectrum cytokeratins such as CK AE1/AE3 and CAM5.2, and negative for CK20. Not unlike undifferentiated endometrial carcinomas, other markers of gynecologic origin such as PAX8, estrogen and progesterone receptor may or may not be positive. Generally, SCNEC and LCNEC are negative for mesenchymal markers such as desmin and S100. Based on these patterns of staining, it is reasonable to recommend that an initial immunohistochemical panel include neuroendocrine markers and epithelial markers when a neuroendocrine tumor is suspected.[4-7,9-14]

In addition to the morphology on H and E staining and positivity for neuroendocrine markers, van Hoeven, et al. proposed an additional criteria for diagnosis of primary neuroendocrine tumors of the endometrium: unequivocal evidence of endometrial origin in order to exclude the possibility of invasion or transfer of neuroendocrine tumors from other parts of the body, such as the cervix or lungs.[7]

In this case, the cervix was thoroughly sampled and showed no tumor involvement, ruling it out as the primary tumor site. Immunohistochemical staining for p16 and HR-HPV in-situ hybridization (ISH) are generally positive in primary cervical NECs, and may be useful in cases which are not as clear cut.[4,5]

The patient had a history of invasive breast carcinoma, raising the possibility of a metastatic breast tumor. Unfortunately, the full histopathology report was not available to the authors. Metastatic carcinoma from extragenital tumors to the female genital tract is uncommon, and most commonly affects the ovaries and vagina. A case series by Kumar and Hart (1982) reported that the primary extragenital tumors that most commonly metastasize to the uterine corpus are breast (42.9%), colon (17.5%), stomach (11.1%), pancreas (11.1%), gallbladder (4.8%) and lung (4.8%). Invasive lobular carcinoma is the most frequent histologic type of breast cancer to metastasize to the uterus, possibly due to loss of expression of the E-cadherin adhesion molecule enhancing spread. Metastases are most commonly distributed in the myometrium without involving the endometrium (63.5%), and rarely affect the endometrium without myometrial involvement.[15,16]

Stewart et al., have devised guidelines to aid in the distinction of endometrial and metastatic carcinomas. Careful consideration of tumor histomorphology is important, as metastatic tumors generally maintain an appearance resembling the tumor at the primary organ. Clues to a metastatic lesion include multifocal involvement, poor differentiation, lack of an identifiable precursor lesion, and a pattern of infiltration surrounding normal glands. In the patient's case, the lesion demonstrates spread from the main tumor mass in the posterior fundic area, and a progression from glandlike and cribriform patterns to solid sheets and nests, all of which diffusely stained positive for neuroendocrine markers, favoring a primary endometrial tumor (Figure 1). If needed, immunohistochemical staining with GCDFP, GATA-3 and CK7 may help uncover a breast primary, however these were not done in the patient's case due to budget constraints. The patient's follow-up in showed no breast tumor recurrence or second primary breast tumor, and postoperative PET-CT showed only hyperactivity of mesenteric, para-aortic and left pelvic nodes suspicious for metastases, with no other hyperactivity in other organs to suggest another primary tumor. Therefore, while a metastatic lesion cannot be completely ruled out, it is less likely.[15-17]

Pathogenesis

The pathogenesis of neuroendocrine tumors of the endometrium is not yet fully understood, however, immunohistochemical stains and molecular diagnostics provide a glimpse into the nature of the tumor. Mulvany, et al., report that some cases of endometrial NECs have been noted to have positive staining for p16, however, these did not show HR-HPV reactivity. This may indicate that unlike neuroendocrine tumors of the cervix, endometrial neuroendocrine tumors are not associated with high-risk HPV infection. However, the INK4a pathway may be involved through other means, causing expression of p16.[10,18]

Ariura, et al., report a case of combined LCNEC and endometrioid carcinoma, in which both tumor components were analyzed for mutation status, and were found to have identical alterations in the PTEN, PIK3CA and FGFR3 genes, although the endometrioid component contained an additional missense mutation in FGFR3. This suggests that the two components arose from a common precursor lesion. Additionally, it raises the possibility that some neuroendocrine tumors of the endometrium may be of the "Type I" or "CN Low" TCGA category of endometrial carcinoma, which is characterized by mutations in the PTEN, ARID1A and PIK3CA genes and history of estrogen exposure. This may be the pathway in patients with a history of tamoxifen intake, and is the most likely

pathophysiology in our patient. It should be noted that in addition to a history of 5 years of tamoxifen therapy, the patient also had a history of an endometrial polyp seven years prior, suggesting endometrial proliferation stimulated by the "pseudo-estrogen" effect of the hormonal therapy on the endometrium. Based on the patient's endometrial biopsy results three months prior to consult, it is possible that she developed an endometrial endometrioid adenocarcinoma, which later transformed into or was overtaken by the growth of the SCNEC and LCNEC components from a common precursor.[14,18]

Pocrnich, et al., in their study of 25 cases, report NECs with positive staining for p53 and intact MMR status. This suggests that the a population of neuroendocrine tumors of the endometrium may be of the "Type II" or "CN High" TCGA category of endometrial carcinoma, which is characterized by mutations in the TP53, PICK3CA, FBXW7, CHD4 and PPP2R1A genes. This is supported by findings of association with serous carcinoma and high grade endometrioid carcinoma, and noted propensity to spread.[6,18]

Prognosis and Management

SCNEC and LCNEC are described as having an unfavorable prognosis due to the early development of vascular invasion, lymph node and distant organ metastasis resulting in poorer survival outcomes compared to other histopathological subtypes of endometrial carcinoma. The majority of these tumors are diagnosed at advanced stages, and the outcome of the disease is predictably correlated to the stage at time of diagnosis. Overall, in previously reported cases, the outcome for NECs is poorer than that of similarly staged endometrioid carcinoma.[4-7,9-14]

Due to their rarity, at present, there is no defined regimen for the management endometrial neuroendocrine carcinoma. Surgical staging with debulking, chemotherapy and radiotherapy are among the treatment strategies available. Given its aggressive nature, surgical resection and multi-modal systemic therapy is warranted, and a multidisciplinary team is important. The most common chemotherapy regimens involved cisplatin and etoposide, similar to the treatment of small cell carcinoma of the lung. Other agents used include octreotide, doxorubicin and irinotecan. However, despite treatment, the prognosis is often poor.[4-7,9-13]

CONCLUSION

Neuroendocrine carcinoma of the endometrium is a rare but aggressive disease. Extrauterine spread and early development of distant metastasis is common. It is important for clinicians and pathologists to consider this disease entity when a poorly differentiated endometrial tumor is suspected, and perform the appropriate diagnostic procedures to guarantee proper identification and management of this malignancy. An initial immunohistochemical panel of neuroendocrine markers and epithelial markers are suggested when a neuroendocrine tumor is suspected. It is also important to rule out a neuroendocrine tumor with an origin outside of the endometrium. A multidisciplinary team approach is also important in the management of these cases. Due to its rarity and limited data available, more studies are needed to establish the optimal treatment for this malignancy.

ETHICAL CONSIDERATION

The case has been received and registered by St. Luke's Institutional Ethics Review Committee.

STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

FUNDING SOURCE

None.

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OPEN ACCESS – CASE REPORT

HPV-Independent Gastric Type Adenocarcinoma of the Uterine Cervix presenting as Ovarian Masses: A Case Report and Review of Literature*

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ABSTRACT

Adenocarcinoma, HPV-independent, gastric type of the uterine cervix comprises only 10-15% of all cervical adenocarcinomas. A rare case of which, with metastasis to the uterine corpus and bilateral ovaries, is described. A 43-year-old female (G0P0) presented with menorrhagia and right flank pain radiating to the hypogastrium. Physical examination revealed an immovable, tender mass at the right lower quadrant with a nodular, firm cervix. Transabdominal ultrasound revealed multiseptated ovarian masses. The right and left ovaries were sent for frozen section and was diagnosed as Mucinous Cystadenoma and Mature Cystic Teratoma, respectively. Hysterectomy revealed a detached and fragmented cervix with irregular, abnormally shaped glands lined by a single layer of columnar cells with bland, basally located nuclei and clear cytoplasm associated with desmoplasia, findings which were also seen in the endomyometrium and ovaries. These tumor cells were CK7 positive and negative for ER, PR, CK20 and CDX2. The patient died six months after surgery. The presence of benign appearing glands is a diagnostic challenge. Despite the appearance, they may be malignant and should be investigated rigorously.

Key words: cervical adenocarcinoma, gastric-type cervical adenocarcinoma, hpv-independent cervical adenocarcinoma

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INTRODUCTION

Adenocarcinoma, HPV-independent, Gastric Type, initially called adenoma malignum, was first described by Gusserow in 1870.[1] It was named as such due to its resemblance to endocervical glands without malignant features.[2] In 1975, Silverberg proposed a designation of minimal deviation adenocarcinoma (MDA) to accurately represent its clinical behavior.[3] In 2014, the WHO classification grouped extremely well-differentiated mucinous adenocarcinoma with gastric type differentiation including Minimal Deviation Adenocarcinoma as Mucinous Carcinoma, Gastric Type.[4] The WHO Classification of Tumors for Female Genital Tumors updated the nomenclature to Adenocarcinoma, HPV-independent, gastric type in 2020, setting it apart from the other mucinous adenocarcinomas of the uterine cervix, which are HPV-dependent.[5] This variant of mucinous adenocarcinoma is considered to be a separate entity because it has distinct features and clinical course.[6] It is rare, accounting for only 10-15% of all cervical adenocarcinomas and is often misdiagnosed.[2,5,7-11] Although the pattern is benign, this type of tumor is highly malignant and has poor prognosis.[10] In this report, we describe a case of mucinous carcinoma with gastric differentiation metastasizing to the uterine corpus and bilateral ovaries and compare it with previous reports.

A 43-year-old female (G0P0) presented with a ten-month history of right flank pain radiating to the hypogastric area and a 6-month history of prolonged menstrual bleeding lasting ten to eleven days. On physical examination, the abdomen was flat and soft with a 5.0 x 5.0 cm fixed and tender palpable mass at the right lower quadrant.

The uterine cervix was closed, nodular and firm. Transabdominal ultrasound revealed an abdominopelvic septated, cystic mass, ovarian in origin measuring 11.9 x 9.1 x 7.5 cm. Total abdominal hysterectomy with bilateral salpingo-oophorectomy, appendectomy and frozen section were performed at our institution. The cervix was fragile and was damaged during surgery. On frozen section, a diagnosis of Mucinous Cystadenoma (right ovary) and Mature Cystic Teratoma (left ovary) was given. The fixed specimens were then processed for final diagnosis.

CASE

The uterus was previously opened, tan gray, rubbery measuring 9.0 x 7.0 x 6.0 cm. The uterine cavity was 4.0 cm in depth. Endometrial thickness measured 0.1 cm while the myometrium measured 4.0 cm with slit-like spaces (Figure 1A). The detached cervix was fragmented, tan gray and firm measuring 3.0 x 1.5 cm (Figure 1B). The right ovary was tan cream, smooth and rubbery measuring 17.0 x 13.5 x 9.0 cm. The cut surface was multiloculated with mucinous fluid. No solid areas were noted (Figure 1C). The fallopian tube was tan gray, smooth and rubbery measuring 6.0 x 0.9 cm. Cut section shows an empty lumen. The left ovary was tan cream to gray, smooth and soft in consistency measuring 12.0 x 8.5 x 7.0 cm. Cut section showed a biloculated mass, one measured 7.0 cm in diameter containing hair strands and sebum. The smaller cyst measuring 3.0 cm contained mucinous fluid (Figure 1D). Attached to the left ovary was a gray tan, fallopian tube measuring 4.0 x 0.9 cm, which was grossly unremarkable. The appendix appeared tan gray with smooth surface measuring 5.0 x 1.0 cm with brown materials seen on cut section.

Microscopically, the cervix was filled with widely spaced irregular and angulated glands infiltrating deep into the cervical stroma. They are lined by a single layer of columnar cells with bland, basally located nuclei and clear cytoplasm associated with desmoplasia. Some of them formed leaf-like patterns while others were cystically dilated (Figure 2A). Neoplastic glands were also seen in the endometrium and myometrium, majority of which were in the myometrium encroaching from the cervix (Figure 2B), as well as in both ovaries (Figures 2C and 2D). Sections taken from the right ovary showed glands in close proximity to thick-walled blood vessels (Figure 2C). The left ovary had areas showing hair follicles, skin, cartilage, smooth muscles, and sebaceous glands. Thick and thin-walled congested vessels interspersed with few mature adipose tissues were noted as well. The appendix showed proliferating lymphoid nodules with active germinal centers.

On immunohistochemical staining, the tumor cells in the cervix, endomyometrium and ovaries were positive for CK7 and negative for ER, PR, CK20 and CDX2.

Based on the above morphological and immunohistochemical findings, a final diagnosis of Mucinous Carcinoma, Gastric Type was made. The patient died six months after surgery.

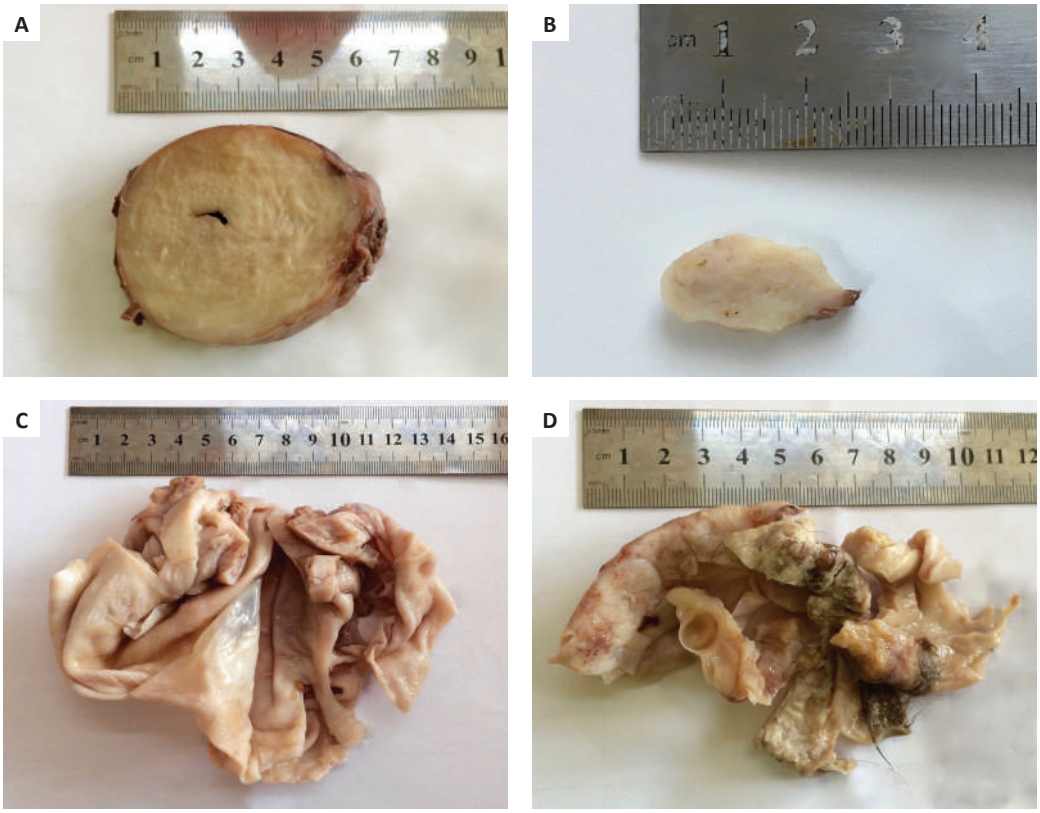

Figure 1. ***Gross examination:*** **(A)** Cross section of the uterus showing the endometrium and myometrium; **(B)** A fragment of the cervix, which was damaged during surgery; **(C)** Right ovary (opened) showing a multiloculated cyst; **(D)** Left ovary (opened) filled with hair strands and sebaceous material.

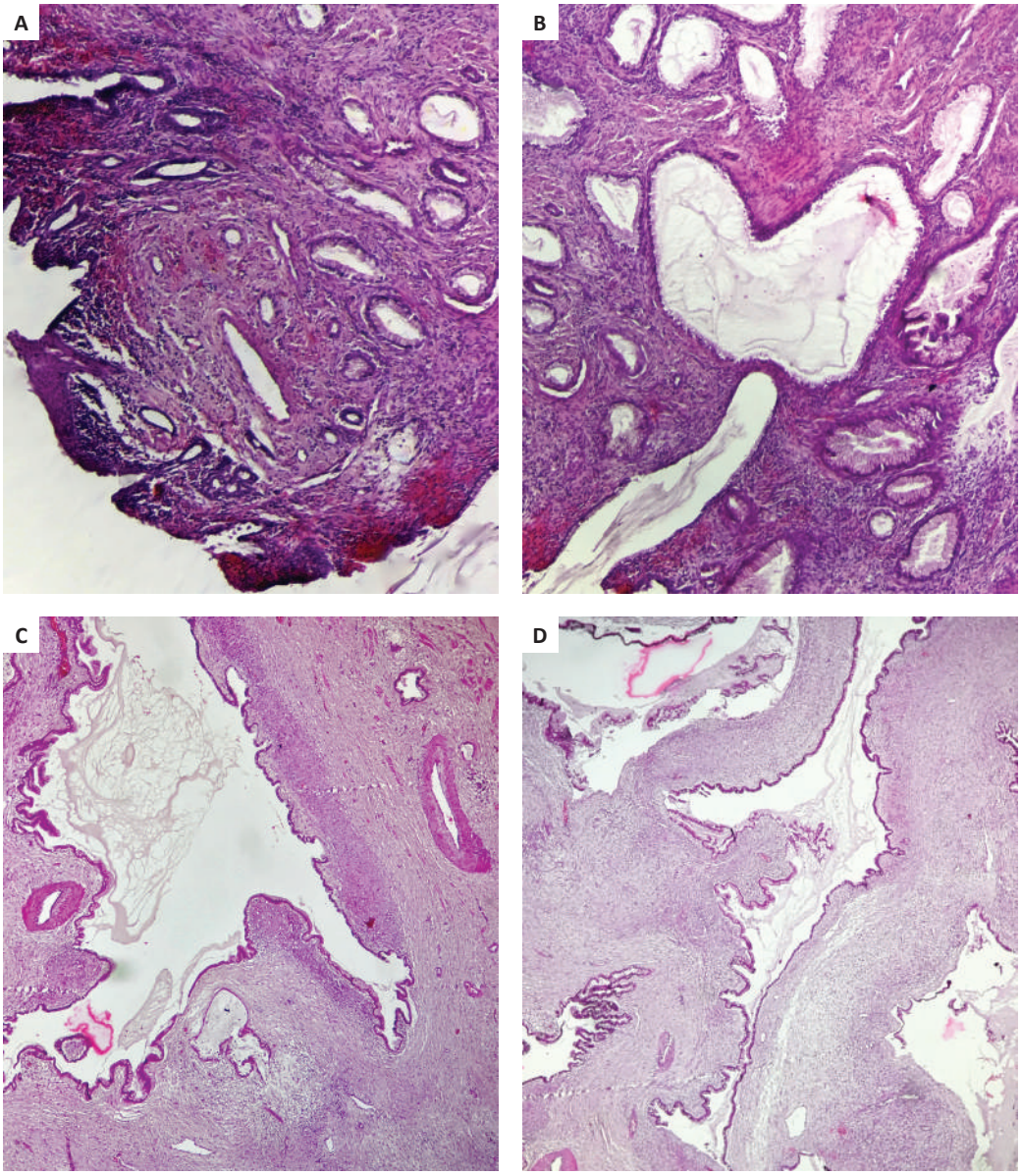

Figure 2. ***Histopathologic Features on H&E:*** **(A)** Cervix with widely spaced glands lined by a single layer of columnar cells. The cells have bland, basally located nuclei and clear cytoplasm surrounded by moderate desmoplasia, 4x; **(B)** Endomyometrium with similar neoplastic mucinous glands, 4x; **(C)** Benign appearing glands in the right ovary near thick-walled blood vessels, 4x; **(D)** Neoplastic glands in the left ovary with similar morphologic features as those seen in the cervix, 4x.

DISCUSSION

Adenocarcinoma, HPV-independent, gastric type of the uterine cervix accounts for only 10-15% of all cervical adenocarcinomas with the average age at diagnosis of 45 years.[2,5,7-11] Clinical symptoms may be abnormal vaginal discharge and uterine bleeding as well as an enlargement of the cervix associated with erosion and hardening.[2,12] Early physical examinations may not reveal any specific findings. At later stages, however, cervical hypertrophy with granular appearance of the cervical os may be present.[10] Due to the late diagnosis, metastasis to the ovaries may already be seen at presentation,[4] which is similar to our case. The patient presented with heavy menstrual bleeding lasting for ten to eleven days. The cervix was also nodular and firm on internal examination.

Despite the deep infiltration of this type of carcinoma, a visible lesion is not seen in many cases,[7] which is also comparable to this case. Radiologic findings are not specific. Ultrasonography, CT and MRI may reveal intrauterine and/or vaginal fluid accumulation.[10] Moreover, multilocular lesions with solid components from the endocervical glands to the deep cervical stroma may be seen on MRI.[1,13] On contrast imaging using bolus intravenous gadolinium-diethylene triaminepentaacetic acid (Gd-DTPA), rough, irregular cyst walls with fine granular appearance may be detected.[10] A recent study by Mills using PET combined with CT showed that these lesions may be intensely hypermetabolic.[14]

Cytological evaluation and biopsies have low detection rates, which could delay accurate diagnosis, eventually

leading to poor prognosis.[1] As such, deep conization or hysterectomy may be necessary to make a proper diagnosis.[2] Kojima et al., established the criteria for histologic diagnosis wherein tumor cells must have clear or pale, voluminous, eosinophilic cytoplasm and distinct cell borders.[6] However, there is still a lack of criteria to differentiate benign hyperplastic lesions, MDA and common adenocarcinoma. There are also interobserver differences in the interpretations of cellular atypia and invasion.[15]

This neoplasm is a well-differentiated form in the spectrum of gastric type mucinous adenocarcinoma.[6] On low power, the glands have different sizes with irregular and distorted forms and angular projections. Complex outlines and desmoplasia are usually present. On higher magnification, the nuclei are basally located and bland without prominent nucleoli.[7] Some areas may show cribriform pattern, solid areas and infolded papillae.[6] Due to its benign histologic appearance, frequent diagnostic difficulties are encountered, especially in small biopsy specimens.[2] The most reliable criteria for assessing its malignant nature are the haphazard arrangement of the irregular and angulated glands extending beyond the level of the normal endocervical glands as well as the presence of occasional mitosis. The stromal reaction is a helpful indicator of its infiltrative nature.[7] Some of the major differential diagnoses are deeply positioned nabothian cysts, tunnel clusters, microglandular hyperplasia and mesonephric hyperplasia.[2]

In 2018, the International Endocervical Adenocarcinoma Criteria and Classification (IECC) published a new pathogenetic classification for invasive endocervical adenocarcinomas, wherein the tumors were classified as HPV-associated adenocarcinoma (HPVA) and no or limited HPVA features (NHPVA). Tumors with apical mitosis and/or apoptotic bodies were classified as HPVA while tumors without these features were classified as NHPVA. These groups were then subclassified using existing histomorphologic criteria and supported by p16 immunophenotype and HPV status. The study showed that mucinous carcinomas comprise a mixture of HPVA and NHPVA types and the gastric type was the major NHPVA type.[16] Compared to HPVA, NHPVAs were larger, occurred in older patients, presented at a higher stage and has different responses to standard therapy.[17]

In our case, immunohistochemical staining of the tumor cells in the cervix and endomyometrium were CK7 positive and negative for ER, PR, CK20 and CDX2. Both ovaries were also positive for CK7 and negative for ER, PR, CK20 and CDX2. Given their negative staining for ER and PR, a carcinoma of endometrial origin is not considered since tumor cells from the endometrium are expected to be positive. The negative staining for CK20 supports an endocervical primary. Furthermore, the possibility of an intestinal primary tumor is not considered because the tumor cells are negative for CK20 and CDX2. Gastric type adenocarcinoma does not express the characteristic Mullerian-type substances such as ER, PR and CA125 and a proportion of cells contain gastric epithelial substances such as gastric mucin and CEA.[7] Tumor cells also show neutral mucin production and positive staining for HIK1083 and MUC6 suggesting a gastric phenotype.[6] HIK1083 is an antibody directed against pyloric gland mucin and is negative in most cervical adenocarcinomas and endocervical glandular lesions.[18] P16 is usually negative or only focally positive, in contrast to HPV-associated adenocarcinomas where diffuse positive staining is seen.[19] Mutations in the STK11 are also observed in this type of adenocarcinoma making it commonly associated with Peutz-Jeghers syndrome.[20] This mutation has been noted in about half of this neoplasm and is more likely to lose expression of mullerian-type markers.[4,21] A clonal assay study by Li Gong et al., demonstrated that this tumor type was monoclonal, which makes it a true neoplastic lesion, whereas adenomatous hyperplasia and normal cervical tissues were polyclonal.[22] A clonality analysis also demonstrates an X chromosome inactivation in MDA.[23]

Surgical treatment is the most successful option. However, there is still no standard surgical treatment or adjuvant therapy established.[1] The use of neoadjuvant chemotherapy and radiotherapy has become an alternative approach and the response to this therapy before surgery can predict the prognosis.[6] Some authors have described purely surgical management while others have described the use of adjuvant therapy with chemotherapy or external beam radiation.[13]

Gastric type adenocarcinoma of the uterine cervix is reported to have an aggressive clinical course. The five-year survival rate of patients with gastric type adenocarcinoma (38%) is substantially lower than that of patients with the usual type of uterine cervical adenocarcinoma (74%). Moreover, a study by Kojima, et al. indicates that the 2-year survival rate of patients with any stage of minimal deviation adenocarcinoma is 20-30%, whereas that of patient with stage I cervical adenocarcinoma is around 50%.[6] Early peritoneal dissemination and distant metastasis is usually observed as this tumor has a propensity to spread to the ovaries, abdominal wall or peritoneum, omentum and urinary tract.[24,25] Similar findings were also seen by Nishio et al., in a multi-institutional study wherein this tumor is associated with histopathological predictors of poor outcomes, such as bulky mass, deep stromal invasion, lymphovascular space invasion, parametrial invasion, ovarian metastasis and positive ascitic fluid cytology.[26] In our case, the tumor has spread to the bilateral ovaries. Peritoneal invasion and distant metastasis were not noted. In contrast to the abovementioned statistics, some studies have reported conflicting results stating that survival rates and distribution of metastases are only similar to those of a classic well-differentiated adenocarcinoma.[7]

CONCLUSION

Adenocarcinoma, HPV-independent, gastric type, of the uterine cervix is a rare neoplasm which poses a diagnostic challenge because of its deceptively benign appearance. It is reported to be highly aggressive, prone to metastasize and has poor prognosis. Symptoms, physical examination, and radiologic evaluation are nonspecific. Also, cytological evaluation may not be useful and conization or hysterectomy is necessary to assess the invasion, which is one of the diagnostic criteria. The presence of bilateral ovarian masses associated with a cervical pathology

should be investigated rigorously because there is a high chance of malignancy and metastasis despite the benign appearance. Because it is often misdiagnosed, it is usually in its advanced stage at the time of diagnosis.

ETHICAL CONSIDERATION

An Ethics Approval was obtained from the institution. The hospital assumes full responsibility of the case in accordance with the Data Privacy Act of the Philippines under Republic Act Number 10173. The authors ensured that the case report has no identifying information and has been sufficiently anonymized.

STATEMENT OF AUTHORSHIP

Both authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

Both authors declared no conflict of interest.

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Disseminated Double-Hit Lymphoma in a Young Adult

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ABSTRACT

High-grade B-cell lymphoma with MYC and BCL2 and-or BCL6 rearrangements, also called double-hit lymphoma, is an aggressive mature B-cell lymphoma that carries both MYC and BCL2 and/or BCL6 translocations. This can present as a diagnostic challenge since clinical characteristics, morphology and immunophenotype are not accurate indicators of underlying genetic aberrations in this type of lymphoma. We report a case of a 25-year-old male who presented with a one-year history of cough, gradually increasing abdominal girth and jaundice. Definitive diagnosis was made post-mortem with additional ancillary studies using immunohistochemistry staining and fluorescent in-situ hybridization studies.

Key words: lymphoma, B-cell lymphoma, molecular diagnostic technique

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INTRODUCTION

In 2016, the World Health Organization (WHO) established a new criterion under the high-grade B-cell lymphoma which describes a tumor with a unique molecular pathogenesis. This lymphoma involves the presence of translocations in the MYC gene and BCL-2 or less frequently the BCL-6 genes, hence the name "double hit lymphoma" (DHL).[1] The diagnosis of DHL requires molecular techniques, most commonly by fluorescence is situ hybridization (FISH), without much regard for its clinical features and heterogenous morphologies. Double hit lymphomas confer poorer prognosis and lower response to usual chemotherapy regimens.[1] Here we present an autopsy case of a 25-year-old male who presented with one year history of cough, increasing abdominal girth, jaundice and abdominal pain.

CASE

The patient was a 25-year-old, male admitted for abdominal pain. Two years prior to admission, patient had repeated episodes of non-productive cough with accompanying undocumented, on and off fever. One year prior to admission, patient had more frequent febrile episodes still with non-productive cough. Over time, he began experiencing recurrent burning epigastric pain with increasing abdominal girth, early satiety, generalized weakness, progressive weight loss, bipedal edema and jaundice. Patient was eventually admitted at our institution with physical examination findings showing tachycardia, tachypnea, icteric sclerae, decreased breath sounds, a distended abdomen with right hemiabdominal tenderness, jaundice, and bipedal edema. Laboratory exams revealed anemia, thrombocytopenia, deranged coagulation parameters, hypercalcemia, hypoalbuminemia, elevated transaminases, elevated alkaline phosphatase, three-fold elevation in CA 19-9 and a non-reactive hepatitis profile. Radiographic studies revealed pulmonary changes and bilateral pleural effusion. Ultrasound findings of the liver revealed multiple hypoechoic nodules, largest at segment VIII measuring 3 x 3 x 6 cm with non-dilated intrahepatic ducts and common bile duct. Ascites was

minimal. Patient's condition deteriorated over the course of admission with persistent fever, progressing jaundice and anasarca. Biopsy of the liver nodules was not done due to the unstable condition of the patient. Despite medical management and intubation, patient succumbed on his ninth hospital day of admission.

AUTOPSY FINDINGS

The decedent was received for partial autopsy 7-hours postmortem. The head was normocephalic with note of facial edema, particularly evident at the periorbital area and lips. The sclerae were icteric. The neck was symmetrical with multiple palpable lymph nodes. The abdomen was soft and distended with a measured girth of 89 cm. There were multiple petechiae noted over the anterior abdomen.

Upon opening of the thoraco-abdominal area, the liver was studded with multiple, yellow, firm lesions. Straw-colored, ascitic fluid amounting to 1600 cc was drained. Bilateral serosanguinous pleural fluid amounting to 400 cc on the right and 360 cc on the left were also drained. The liver was enlarged, weighing 2,700 grams, well-above the normal reference range. Its surface was nodular and studded with multiple yellow, firm masses, the largest measuring 9 cm in greatest diameter. Cut sections revealed a red-brown parenchyma with yellow, firm nodules interspersed throughout the substance of the liver (Figure 1A). Microscopic sections of the liver revealed sheets of hyperchromatic cells with a diffuse growth pattern. On higher magnification, the cells were atypical and pleomorphic with abundant cytoplasm, large, hyperchromatic nuclei and prominent nucleoli (Figure 1B-C). Mitotic figures and apoptotic cells were also identified. The spleen was also enlarged, weighing 285 grams, above the normal reference range. The capsule was intact with multiple, yellow, firm nodules throughout. Cut sections showed multiple, yellow, firm nodules scattered throughout the entirety of the splenic parenchyma. Microscopic sections revealed similar tumor infiltrates as that of the liver (Figure 2A). The pancreas, gallbladder, bilateral kidneys, bilateral adrenal glands, bilateral lungs all showed variably-sized nodules of the same gross and tumor microscopic features as that of the liver and spleen. The bone marrow smears revealed atypical lymphoid population with large irregular nuclei, prominent nucleoli and moderate cytoplasm (Figure 2B). Enlarged pulmonary hilar lymph nodes with sizes ranging from 3 to 8 cm in greatest diameter were also noted on further dissection, cut sections of which showed tan-brown, fleshy to friable surfaces. Enlarged lymph nodes were also noted in the peripancreatic and inguinal areas. Microscopic examination revealed effacement of the usual lymph node architecture and replaced by sheets of atypical, pleomorphic, discohesive cells with large nucleus and prominent nucleolus, some cells exhibiting eosinophilic nucleoli (Figure 2C-D). Immunohistochemistry studies were done on the posterior mediastinal lymph node which revealed positivity for the following stains: Leukocyte common antigen (LCA), CD20 (diffuse, strong), PAX-5 (diffuse, strong), CD30 (patchy, weak) (Figure 3). Subsequent immunohistochemistry studies were done which revealed positivity for BCL-2 (>40%), C-MYC

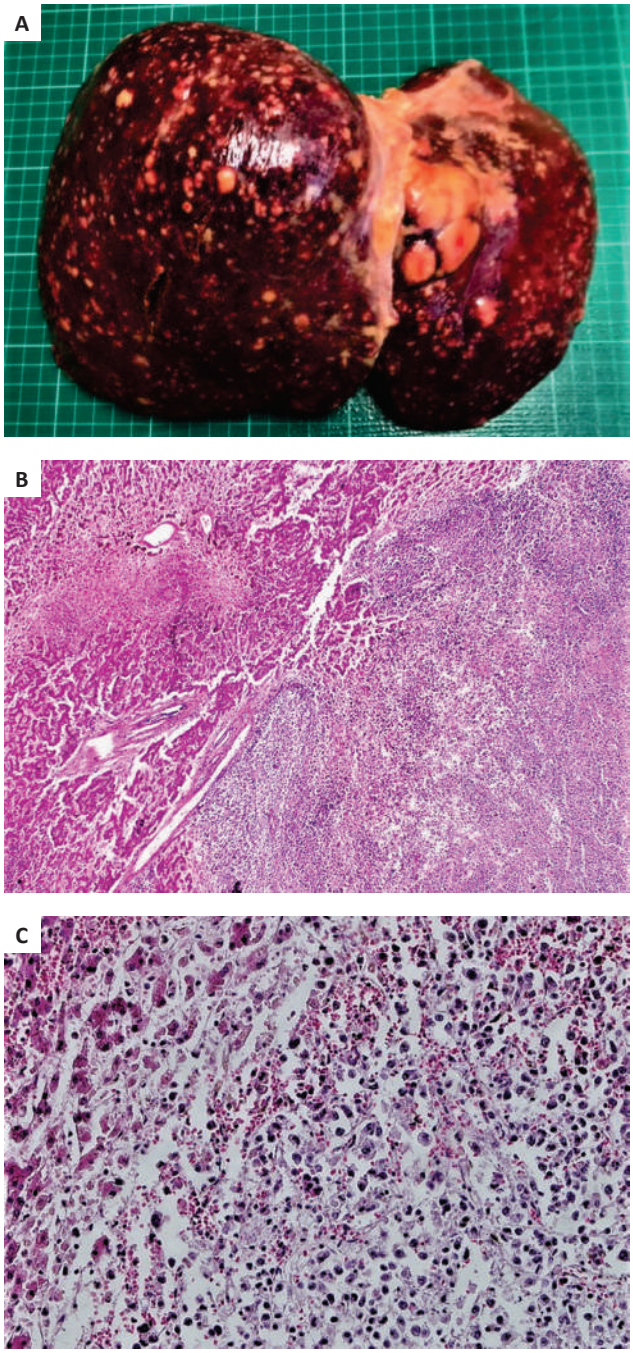

Figure 1. **(A)** Gross appearance of the liver showing variably-sized, firm, nodules throughout; **(B)** Photomicrographs of the liver showing sheets of tumor infiltrates within the parenchyma, H&E, 10x and **(C)** 40x.

(>50%) and a proliferative index of 50-60% using Ki-67. The tumor cells were negative for the following stains: CD5, TdT, Cyclin D1, CD10, BCL-6, MUM-1, pan-cytokeratin and HMB-45. Molecular analysis using FISH was done revealing translocation rearrangements in MYC (5.56%) and BCL-2 (8.25%), trisomy 8 (IgH/Myc fusion), and no BCL-6 rearrangement (Figure 4).

Given the said morphology, the immunophenotype and molecular characteristics, the final diagnosis given to this case was a high-grade B-cell lymphoma with MYC and BCL-2 rearrangements (double hit lymphoma).

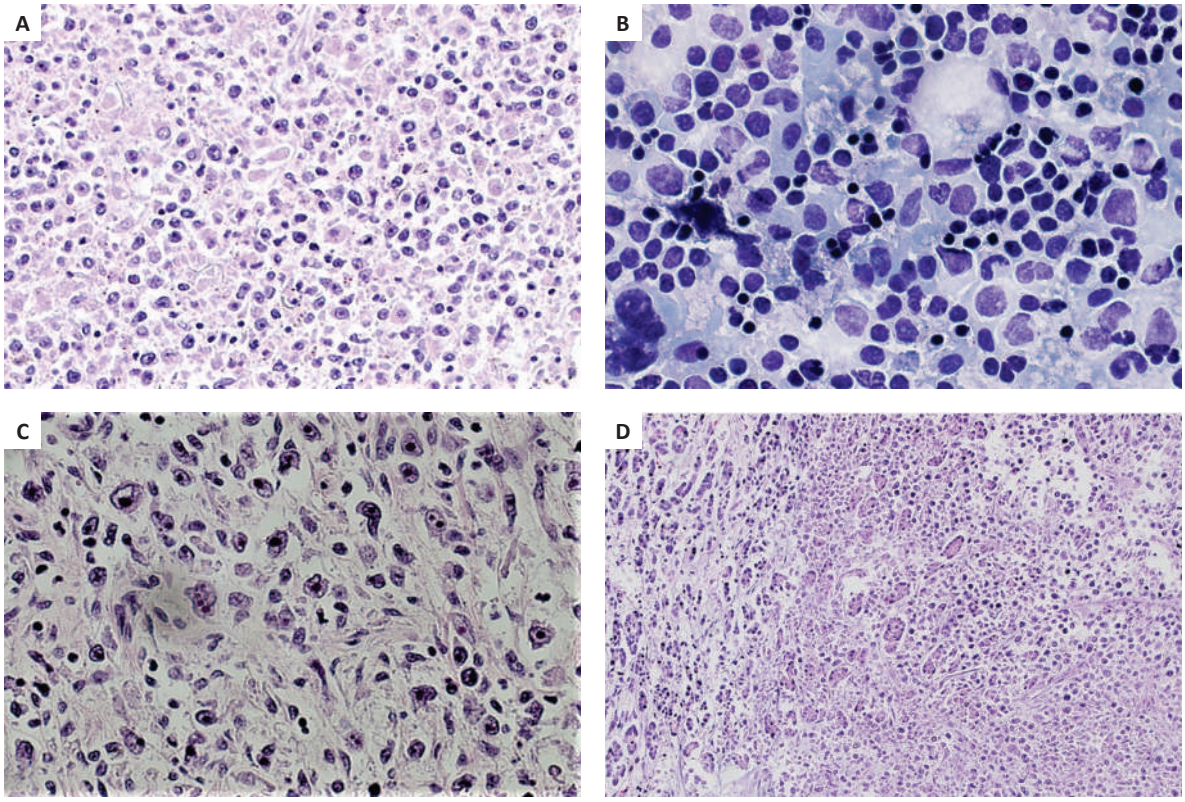

Figure 2. **(A)** Photomicrographs of the tumor infiltrates in the spleen, H&E, 40x; **(B)** bone marrow, Wright, 100x; **(C)** perihilar lymph node, H&E, 40x and **(D)** pancreas, H&E, 40x. The tumor cells show large, vesicular nuclei with prominent nucleoli and scant cytoplasm.

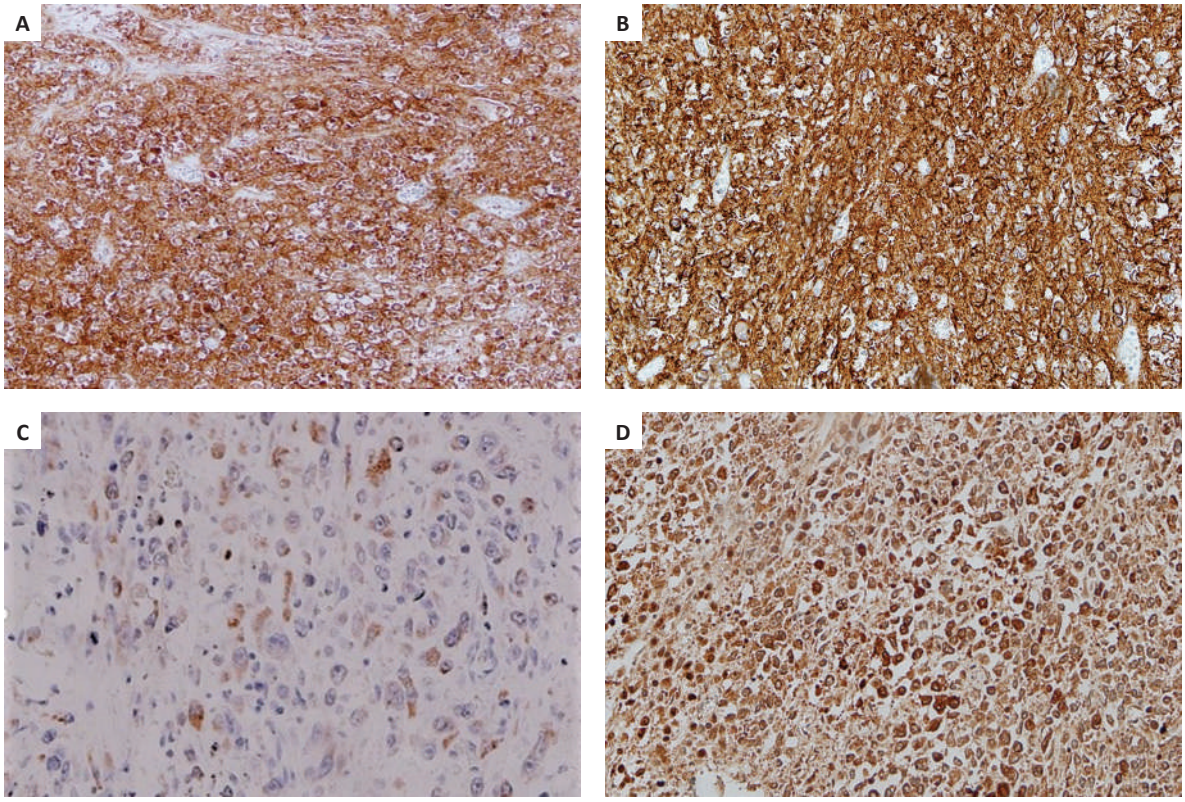

Figure 3. **(A)** Neoplastic cells showing positivity with the following immunohistochemistry studies: LCA, 40x; **(B)** CD20, 40x; **(C)** bcl-2, 40x; **(D)** and C-myc, 40X. LCA, CD 20 and C-myc show diffuse, strong positivity while bcl-2 showed variable staining intensity in >40% of the tumor cells.

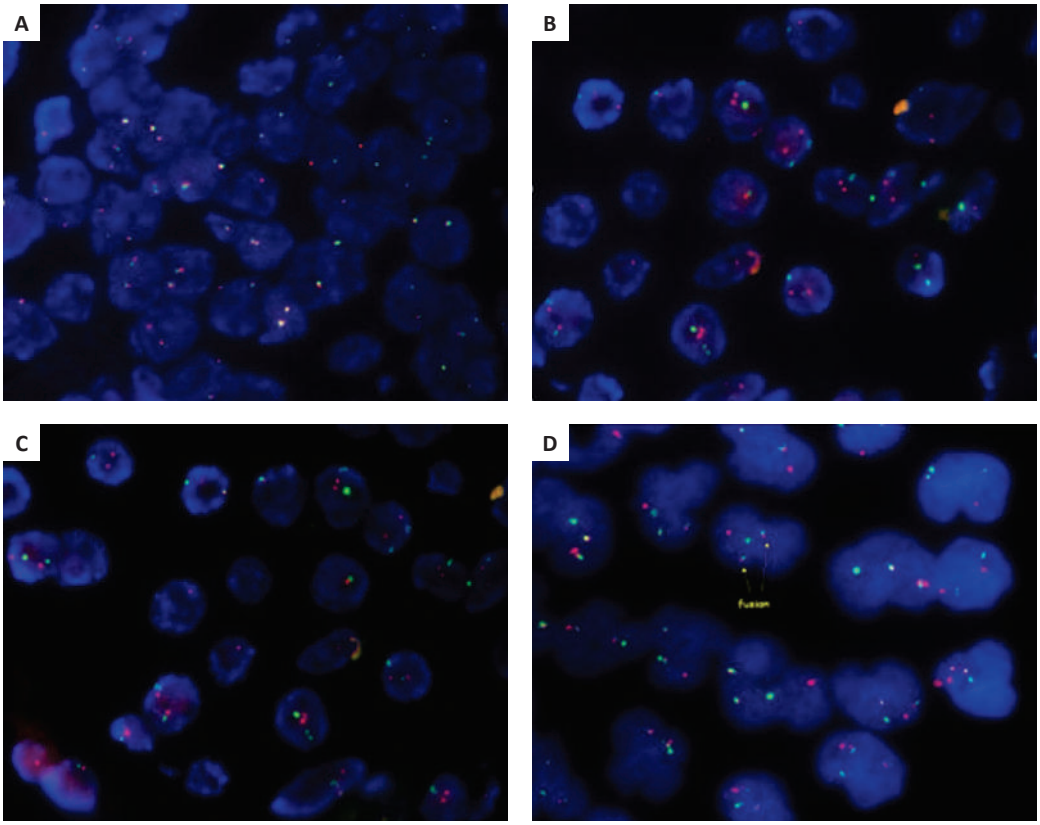

Figure 4. (A) Fluorescent in-situ hybridization study showing: Absence of BCL-6 rearrangement; **(B)** presence of MYC rearrangement (11.11%, cut-off value of >10%); **(C)** BCL-2 rearrangement (8.25%, cut-off value of >8%); **(D)** and, t(8:14) aberration on the MYC gene (8q24) (5.66%, cut-off value of 2%).

DISCUSSION

With the advent of more discoveries about the biology and genetics of hematolymphoid neoplasms, newer types of lymphomas have been identified and reclassified by the WHO.[1,2] Among these lymphomas are the so-called double-hit lymphomas. The term "double hit" as defined for this category refers only to the co-occurrence of MYC and BCL-2 and/or BCL-6 translocations. Exceptions for this category are lymphomas that do not harbor MYC translocations as well as MYC-associated translocations in other genes other than BCL-2 and/or BCL-6, transformed follicular lymphomas and B-lymphoblastic leukemia/lymphoma with MYC and BLC-2 translocations.[1] Much interest has been piqued by aggressive B-cell lymphomas, particularly DLBCLs, because a small subgroup of these patients were observed to be resistant to standard chemotherapy, had dismal overall survival despite treatment and preponderance of extranodal spread particularly in the central nervous system and bone marrow.[1-3] The advances in molecular testing has paved the way for the identification of certain genetic aberrations that have led to the establishment of this new category of aggressive B-cell lymphoma.[3] Diagnosis is made by detection of rearrangements of MYC, BCL-2, and BCL-6 by a cytogenetic or molecular method such as FISH.

A peculiarity in our case is the age at presentation of the patient. Majority of double-hit lymphoma cases present in elderly patients, with a median age at diagnosis ranging from 51-65 years, and a slight male predominance.[1] DHL are reported rarely in young adults, adolescents and children.[4,5] To date, there has been no reported case of DHL among the said population in the Philippines.

Most of the patients were diagnosed in the advanced stages and presented with widespread disease, with most cases involving the central nervous system (CNS) and bone marrow. A study by Niitsu, et al., reported several cases of double hit lymphoma with pleural effusion, similar to our case.[6] Niitsu also reported twelve cases which had three extranodal sites of involvement. A study by Guillermo et al., reported the distribution of disease according to nodal and extranodal sites and noted cases with liver involvement in only 2% of cases. He also reported only one case out of 382 with a double extranodal site of involvement, that being the bilateral breasts and ovary.[7] A literature search of multiorgan involvement by double hit lymphoma yielded no reports with more than three sites of involvement. To the best of our knowledge, no case has ever been reported with such extensive, widespread organ involvement such as ours. Presenting symptoms may be related to the rapidly enlarging mass depending upon the site of the mass; B symptoms such as fever, night sweats, weight loss may be seen in a number of patients. Our case was consistent with published reports, presenting with disseminated disease and accompanying signs and symptoms correlate to the sites of involvement. These include increasing abdominal girth, jaundice and anasarca due to hepatosplenomegaly and tachypnea due to pleural effusion and enlarging perihilar lymph nodes. Laboratory test results were also consistent with the autopsy findings,

with most tests having deranged results due to hepatic and pancreatic involvement by tumor. It is unfortunate that the autopsy was only a partial one which precluded us from further evaluation of the CNS.

Double hit lymphomas have a heterogenous morphologic appearance, with most authors agreeing to a higher-grade type of morphology- DLBCL-like, blastoid and Burkitt-like. Most of the reported cases appear to exhibit a DLBCL morphology with a diffuse growth pattern, variable nuclear size, prominence of nucleoli, variable mitotic figures and variable proliferation rate.[1,3,8] Our case also reports similar high-grade, diffuse large B-cell lymphoma-like morphologic features with that of previously reported cases.

Earlier studies have suggested the use of immunohistochemistry studies, particularly CD10, BCL-6, IRF4/MUM-1, BCL-2 and C-MYC as a surrogate test to identify patients who are likely candidates for further FISH testing. The first three mentioned stains are utilized to determine cell of origin (COO) wherein DLBCLs are classified according to the stage of differentiation where the malignant cells are derived.[3,9,10] The most commonly used method to determine cell of origin is the Hans algorithm, a binary classifier, assigning the DLBCLs into either germinal center or non-germinal center phenotype. Most studies report that DHLs are of germinal center origin, particularly those with the MYC/BCL-2 translocation. However, a small percentage of DHLs, specifically those with the MYC/BCL-6 translocation will have a non-germinal center differentiation. It is for this reason that using cell of origin as a surrogate test to determine cases which should undergo FISH may not be the most ideal because a small percentage of cases will be missed.[3,9] Another frequently suggested method to predict cases with DHL is thru the use of BCL-2 and MYC immunohistochemistry studies.[11-14] This comes from the premise that these two genes are overexpressed in DHL. However, more and more studies have shown that dual protein expression (DPE) among DLBCLs can occur without genetic aberrations.[1-3,11-15] The WHO 2016 reports that although this is not currently accepted as a new, separate entity, DPE in DLBCLs can confer a poorer prognosis with worse survival outcome compared to other DLBCL subtypes. Several studies also caution the use of DPE to identify which cases should undergo further FISH testing citing differences in cut-offs as a reason that cases can be missed.[3,10,11-15] Our case had an immunohistochemistry result which showed DPE with C-MYC and BCL-2. Given the staining result coupled with the aggressive clinical history, this prompted us to do further testing with FISH to confirm the presence of chromosomal aberrations. Most authors recommend FISH analysis for all cases of DLBCLs.[1,2] However, due to limited availability of this test as well as financial constraints from our patients, this will be very difficult to implement as a standard practice. Although selection by immunohistochemistry studies is not the most optimal way to determine which cases to FISH due to variable concordance, as reported by most authors, we opted to go this route given the above-mentioned limitations.

Double hit lymphomas have been given much attention of late due advancements in molecular pathology. Interest has been shown by both clinicians and pathologists alike because of its poor response to usual chemotherapeutic treatment protocols and overall poor survival. At present, no standard treatment protocol has been developed for this disease.[16] Clinical trials are still underway to determine which may confer better progression free survival and overall survival.

CONCLUSION

We present a case of an aggressive B-cell lymphoma that had an unusual clinical presentation. This case affirms the fact that lymphoma is a great mimic and that it should always be one of the considerations especially when faced with a clinical diagnostic dilemma. Definitive diagnosis was made post-mortem which highlights the value of autopsy and exhaustive but cost-effective work-up. Awareness that this type of lymphoma exists is a foreground which prompted us to report this case. Together with a careful evaluation of clinical, morphologic and immunophenotypic predictors, we recommend a sequential approach to the diagnosis of double hit lymphomas especially in a low-resource setting such as ours.

ACKNOWLEDGMENT

The authors would like to acknowledge the National Kidney and Transplant Institute for granting permission to use photos of the FISH analysis of the patient.

ETHICAL CONSIDERATION

Patient consent was obtained before submission of the manuscript.

STATEMENT OF AUTHORSHIP

Both authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

Both authors declared no conflict of interest.

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PSP

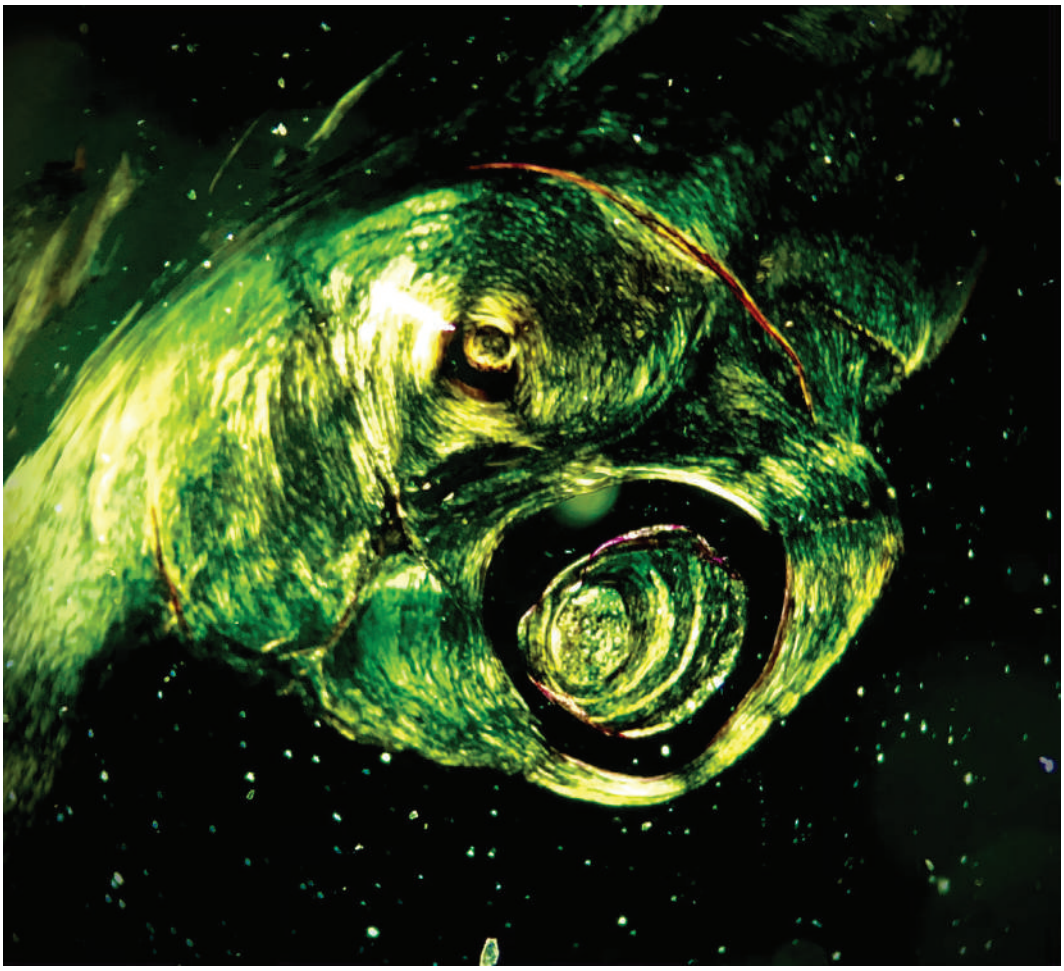
SNAPSHOTS

PHOTOMICROSCOPY CONTEST 2021

OMNIBUS

BIO-MEDICAL SYSTEMS, INC.

INSTITUTION CATEGORY



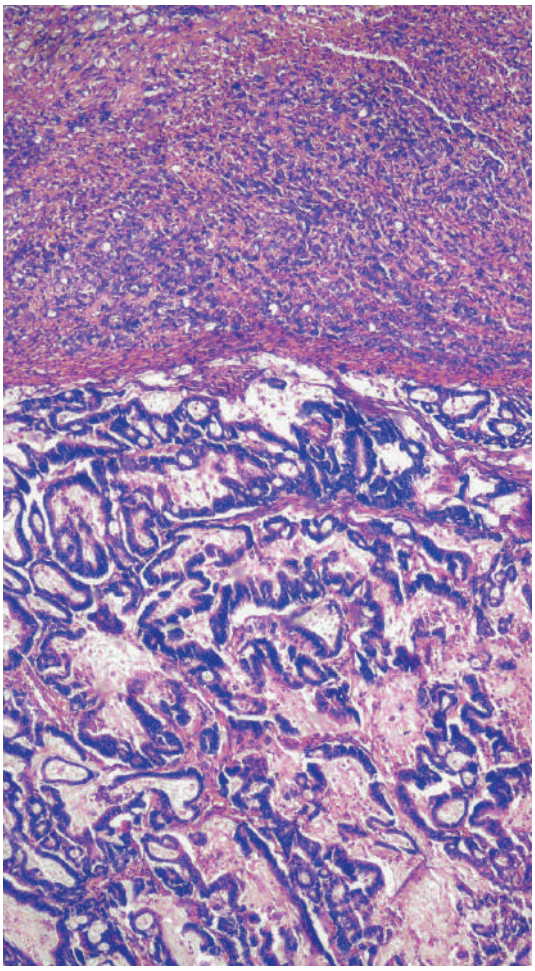
FIRST PLACE

PHILIPPINE HEART CENTER

"The Invasion Begins"

"A long time ago from a galaxy far, far away... a galactic strike ship from an unknown planet is ready to conquer and invade the universe."
This photomicrograph is a mitral valve from a patient with rheumatic heart disease with amyloid accumulation then stained with congo-red which shows the characteristic apple-green birefringence on polarizing microscope.
Captured using Olympus CX31-P at 100x magnification.

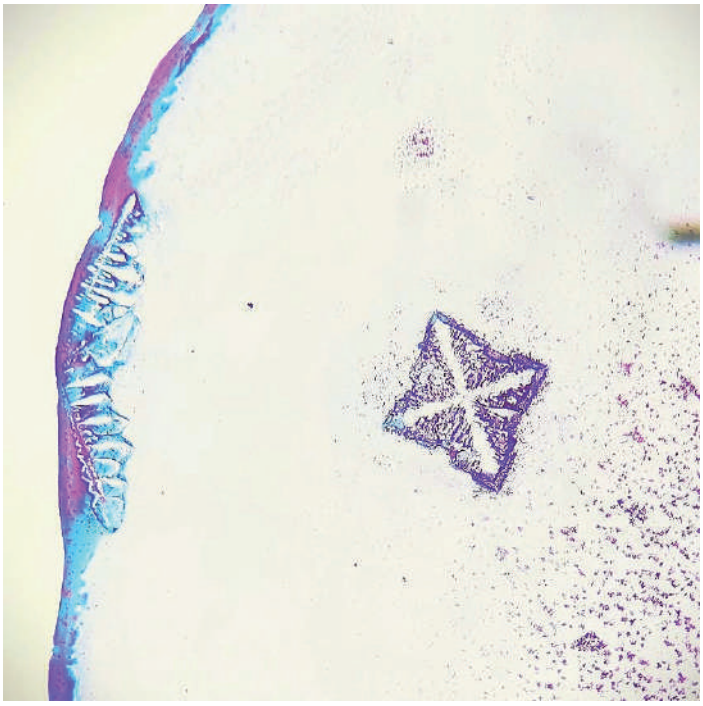
INSTITUTION CATEGORY



SECOND PLACE

WEST VISAYAS STATE UNIVERSITY MEDICAL CENTER

"Where the Sand Meets the Sea"
"Underneath the lens, one may find a place where serenity greets the uproar, where the ocean finds the shore, where the sand meets the sea, akin to the collision of these two distinct tumors."
Image total magnification: 40x.

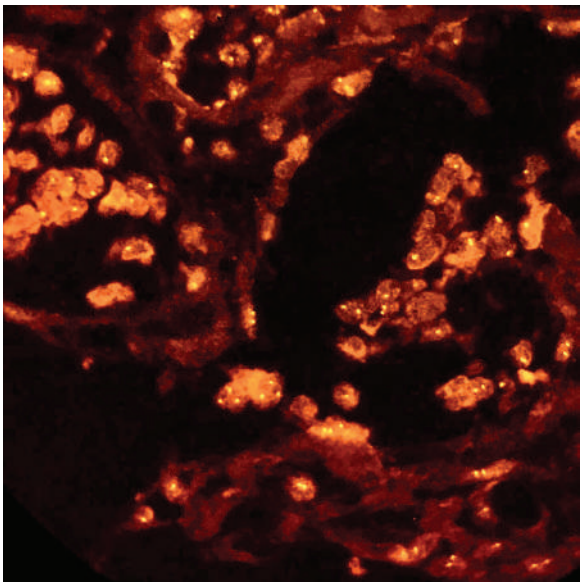


THIRD PLACE

JOSE B. LINGAD MEMORIAL GENERAL HOSPITAL

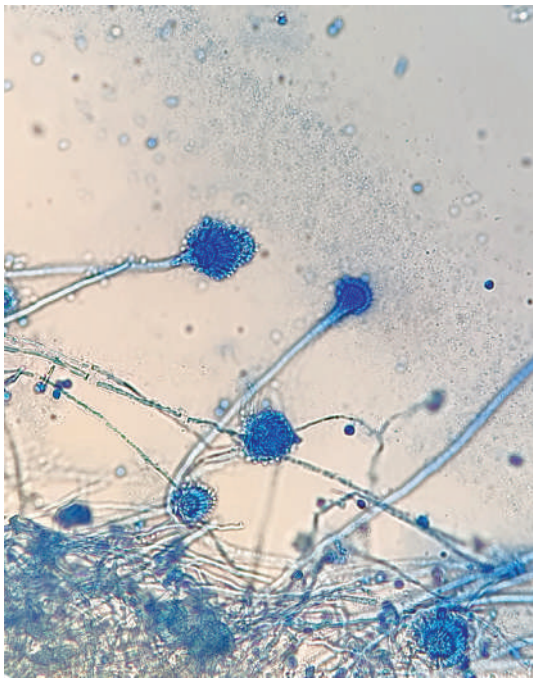
"At the Cross, I Bow My Knee"
"A glimmer of hope in this trying time, a reminder that amidst the fear, anxiety, and weariness in our daily battle against this pandemic, we have Him to hold on to."
Pleural fluid aspirate in 40x magnification.

INSTITUTION CATEGORY



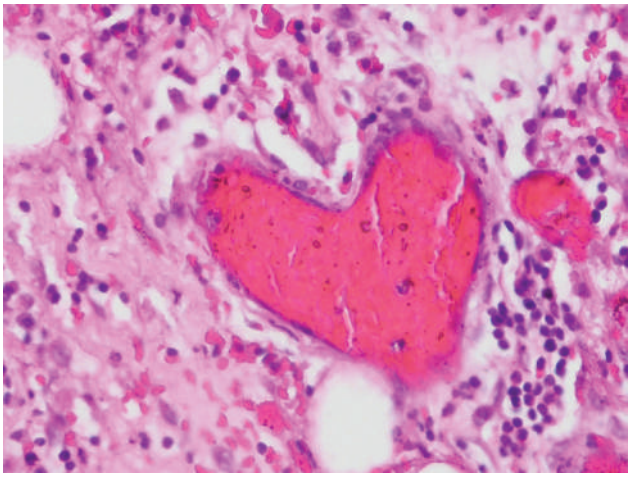
THE MEDICAL CITY

"Smoldering Fire"
A burst of 'flames' of invasive breast carcinoma cells in HER2/neu FISH testing.
Taken using BX41 Fluorescence Microscope - 40x objective.



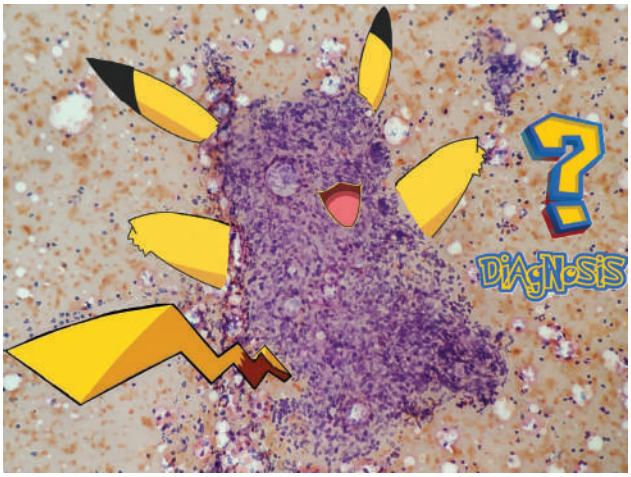
UERM

"Blue Flowers"
"There are always flowers for those who want to see them." - Henri Matisse



VALENZUELA MEDICAL CENTER

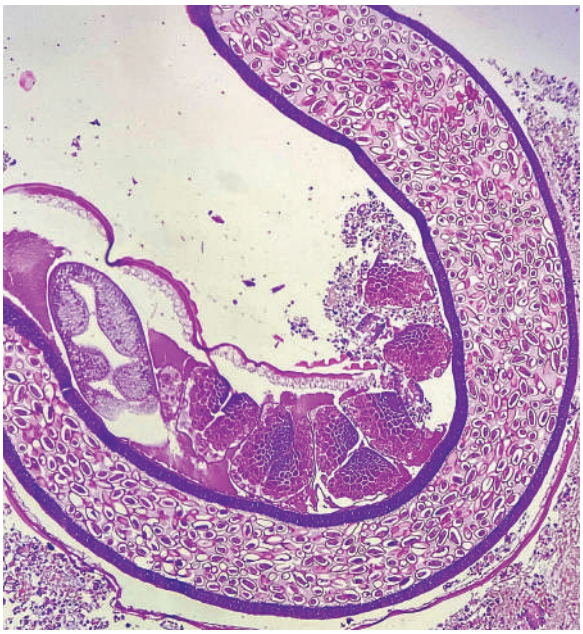
"My Heart Went OOPS"
"There are times when our heart stops when we arrive at a grave diagnosis. But we make extra time for additional sections, and pour our hearts into what we do - all for our patients."
Photo taken from an omentum, stained using Hematoxylin and Eosin.



ST. LUKE'S MEDICAL CENTER QUEZON CITY

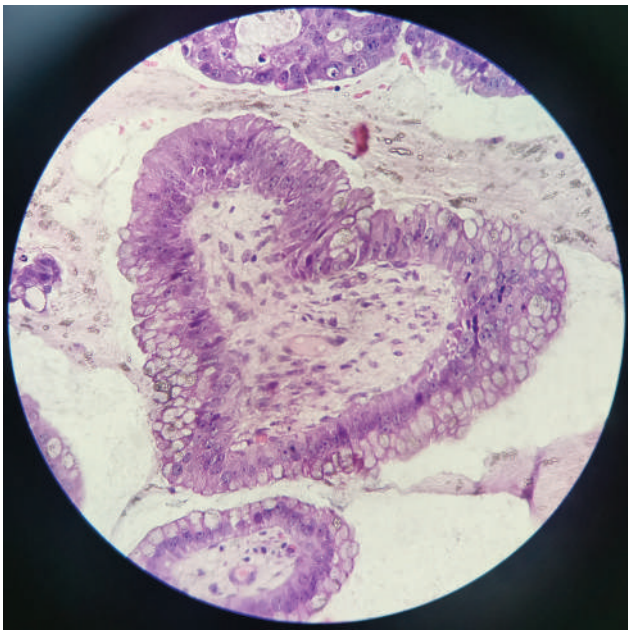
"TB or not TB, pick and choose"
"A lung nodule, it manifests, And causes so much fuss,
Epithelioid histiocytes, now can you guess?
To get it right is a must! The other cells just look so bland,
Necrosis cannot hide, Giant cells sometimes join the band,
With Nuclei on the side! Granuloma!
Gotta catch `em all!"

INSTITUTION CATEGORY



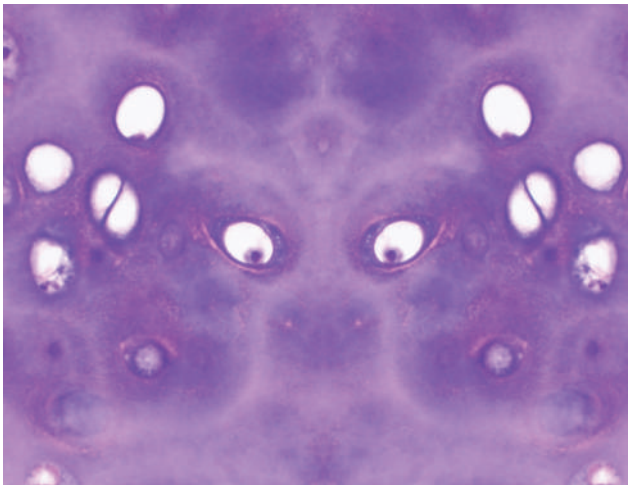
PHILIPPINE CHILDREN'S MEDICAL CENTER

"A Mother's Sacrifice"
"A gravid Enterobius in full circle - within her is the ability to create, cultivate and transmute. A fascinating life that was cut short."



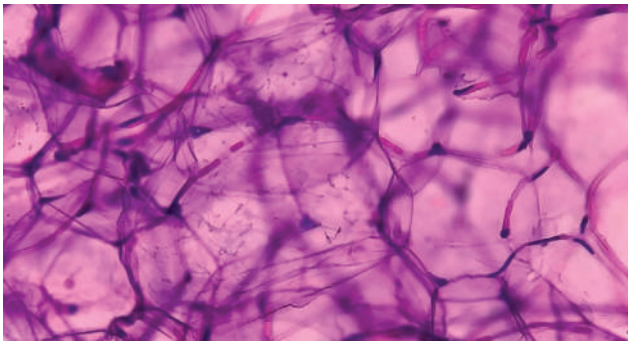
FEU-NRMF

"Put your heart in everything you do. Sometimes you might lose it, but you'll find it if you look close enough in high power view."
Captured using an Olympus CX-33 (40x).



VICENTE SOTTO MEMORIAL MEDICAL CENTER

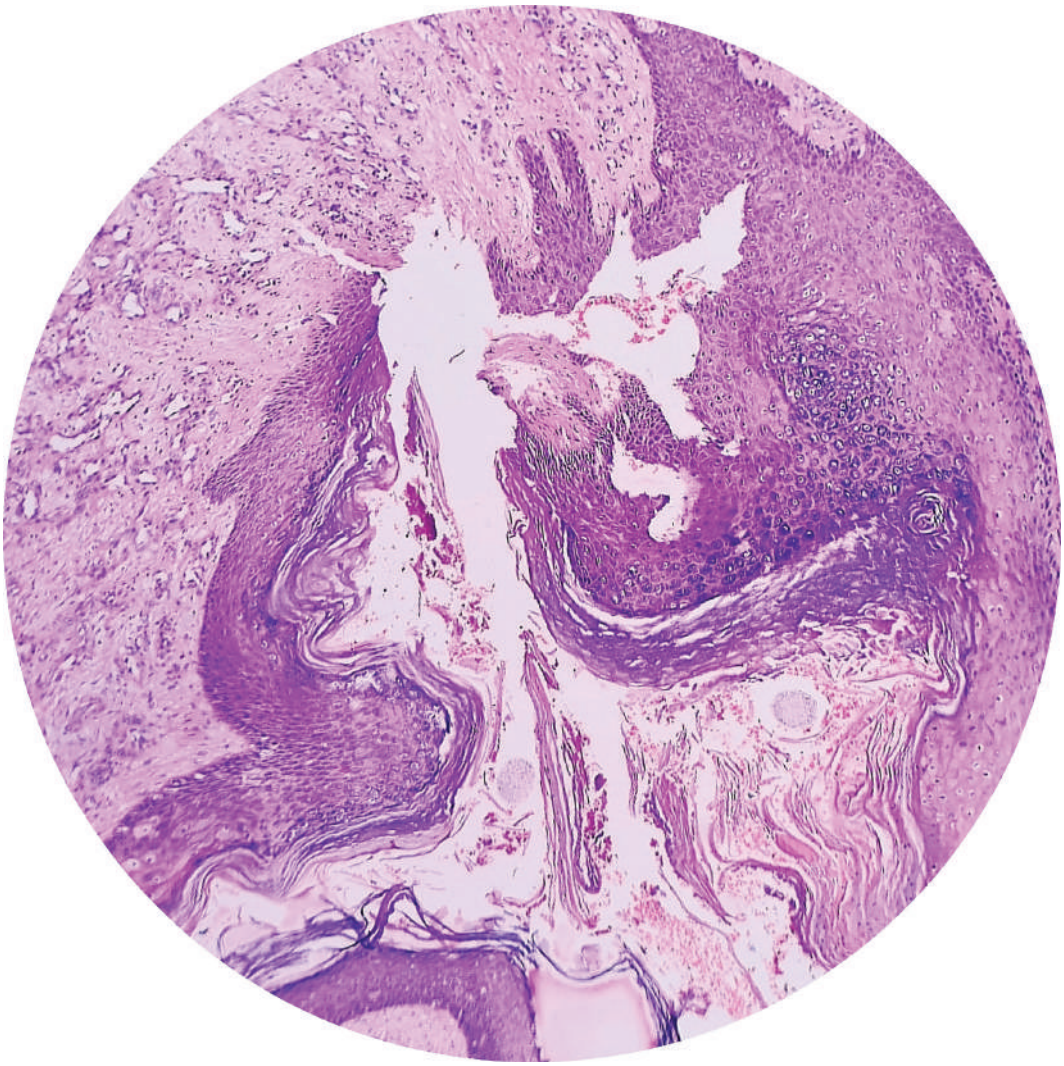
"Out there may be monsters, but in you there is a dragon"
Benign Cartilage at 40x magnification.
This image is captured by cellSens Imaging Software through an installed Olympus DP74 camera on a CX33 Olympus microscope.



PHILIPPINE GENERAL HOSPITAL

"Im not empty and plain. I just need to be on the right.... stain."
Adipose tissue unfortunately taken from a fine needle aspiration biopsy of the thyroid.
Captured using an IphoneXMax using a Olympus CX23

INDIVIDUAL CATEGORY

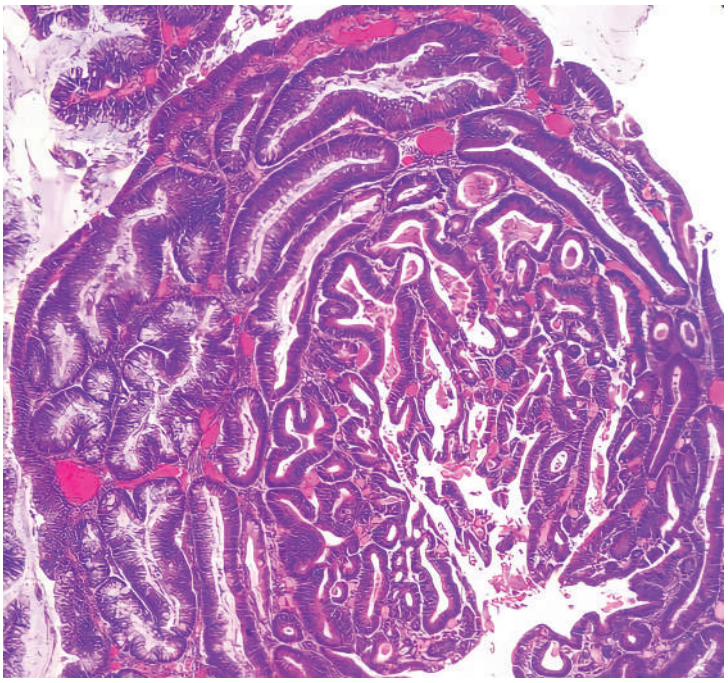


FIRST PLACE

SERREN LOR V. GALLINERO, MD
(WESTERN VISAYAS MEDICAL CENTER)

"The Rise of the Phoenix"
"Like a phoenix, prepare to be renewed after this period of crisis."

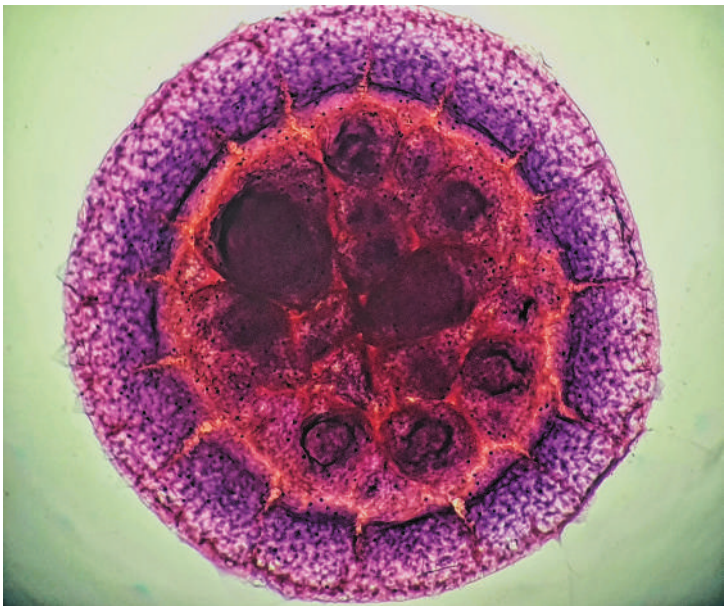
INDIVIDUAL CATEGORY



SECOND PLACE

ANNA KATRINA M. DONATO
(PHILIPPINE HEART CENTER)

A photomicrograph showing a Well-Differentiated Adenocarcinoma of the Colon, mimicking the numerous convolutions of the brain. A reminder of the infinite capability of the human mind to continuously adapt and evolve scientifically through the modern times.
Captured using Olympus BX53, H&E stain, 100X magnification.

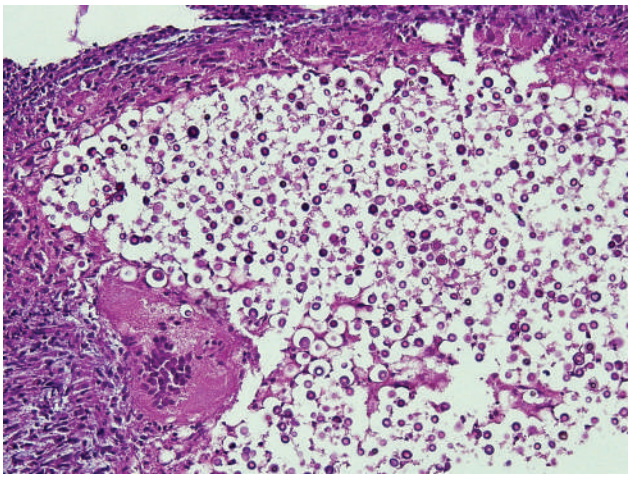


THIRD PLACE

JELA PATRICIA BANAYAT, MD
(OSPITAL NG MAYNILA)

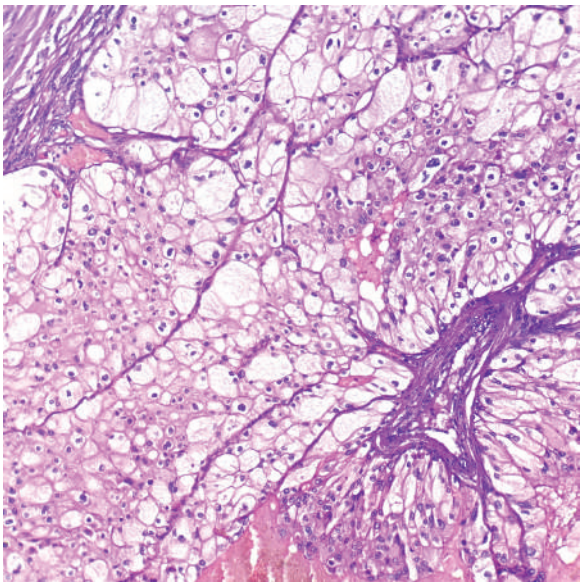
""And no matter how dark the night gets, the sun will rise again."
Thyroid cytology, 100x total magnification.

INDIVIDUAL CATEGORY



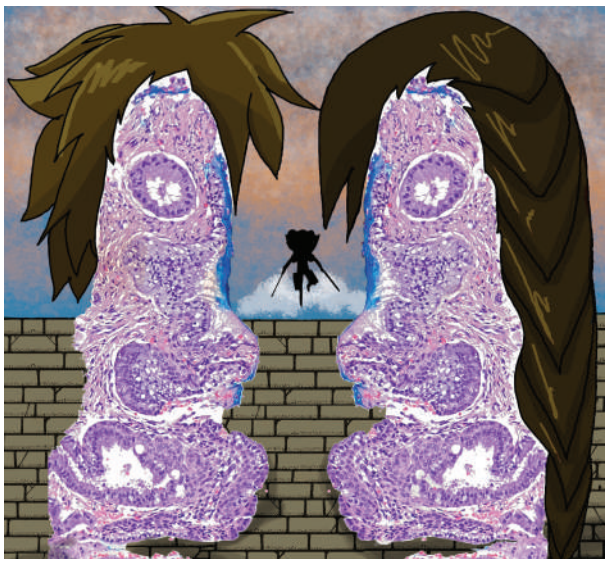
REBECCA R. NAGTALON
(ST. LUKE'S MEDICAL CENTER - GLOBAL CITY)

"The Last Stand"
"This brain's final response to these Cryptococcal invaders."
Captured using Olympus CX33 at 400x magnification



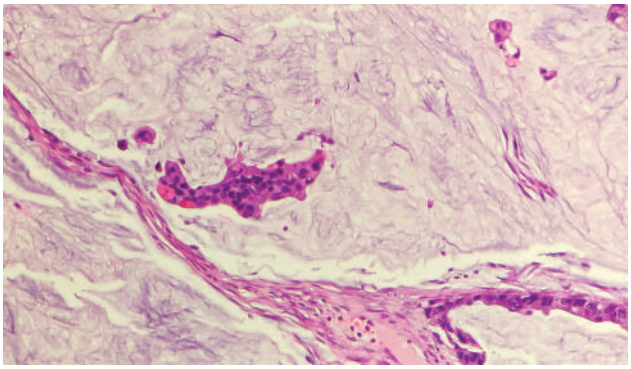
JANINE B. DALISAY, MD
(WESTERN VISAYAS MEDICAL CENTER)

"Botanical"
"Broad alveolar arrangement of the tumor with sharply defined borders and abundant cytoplasm with very prominent cell membranes reminiscent of our Botany days in this Classic ("Plant-like") Type of Chromophobe Renal Cell Carcinoma."
Captured using BX53 Olympus microscope at 400x magnification.



MA. PAULA ENGEDI M. DELMENDO
(ST. LUKE'S MEDICAL CENTER - QUEZON CITY)

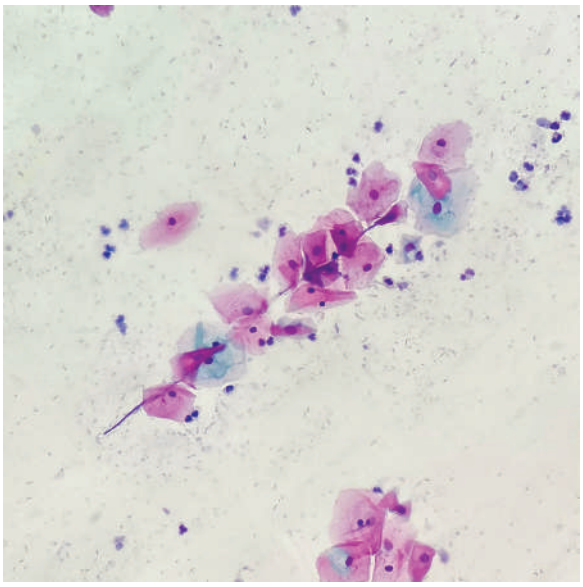
"Let's wipe that SMILE off your face (cervix)"
"Stratified mucin-producing intraepithelial lesion (SMILE) is an unusual premalignant lesion best classified as a variant of adenocarcinoma in-situ (AIS). They are akin to Titans trying to breach the Wall and wreak havoc in the city. However, if they are seen early, immediate intervention (by the Garrison/ your physician) can prevent this." Cervical tissue with H&E stain.
Visualized with Olympus BX53 (x200), photographed with iPhone 11 Pro and background edited and drawn with GNU Image Manipulation Program.



JOSHUA T. UYBOCO
(ST. LUKE'S MEDICAL CENTER - QUEZON CITY)

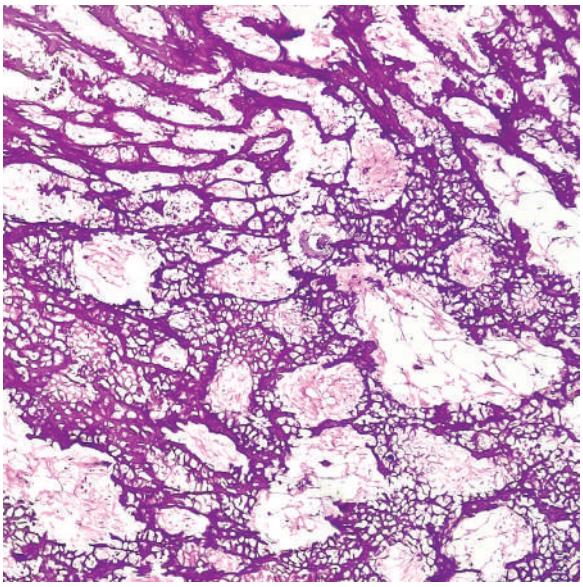
"Creature of the Night"
"Swooping in on clouds of mucin, a cluster of tumor cells forms a silhouette of a bat! Is this nocturnal animal a dark omen, or a sign of a better tomorrow? A keen pathologist must understand the significance of these findings to determine the patient's prognosis."
H&E stain, mucinous adenocarcinoma of the colon.
Captured on Olympus BX53 Microscope at 400x magnification.

INDIVIDUAL CATEGORY



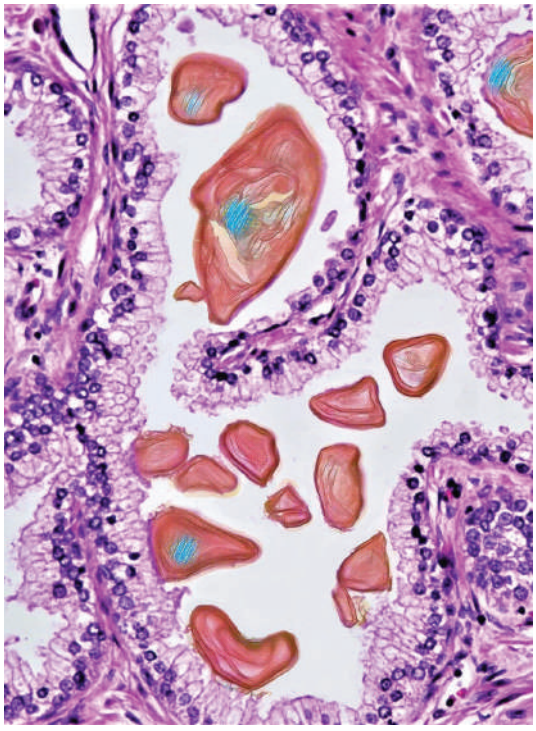
LEONNIE MAE G. GALLO
(WESTERN VISAYAS MEDICAL CENTER)

"Minuscule Bouquet"
"Beads of squamous cells pierced by a string of fungal pseudohyphae reminiscing a bouquet of carnations seen lying in a field of bacteria and inflammatory cells."



TERENCE MICHAEL M. NISMAL
(PHILIPPINE ORTHOPEDIC CENTER)

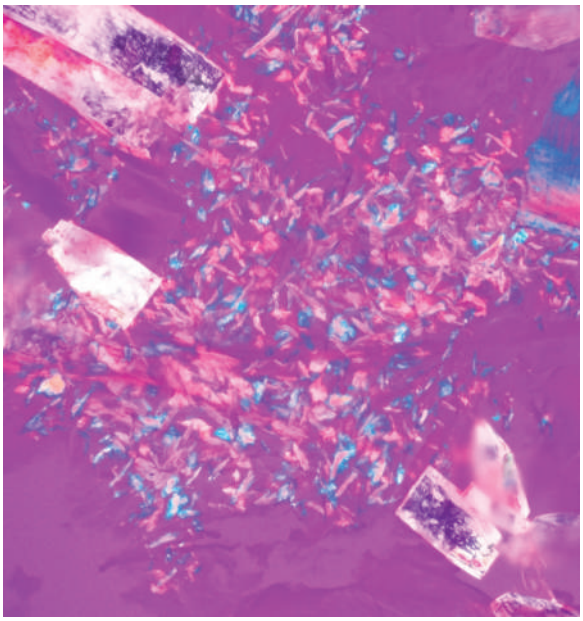
"Favorable Necrosis"
"Mineralized osteoid matrix which remained after neoadjuvant chemotherapy for a conventional osteosarcoma. This marks areas previously inhabited by malignant cells which is now replaced by an amorphous stromal material. Survival advantage is seen in patients with tumors that show >90% necrosis after neodjuvant chemotherapy. Always report tumor histologic response in these cases."



ARIAM O. GATPO
(ST. LUKE'S MEDICAL CENTER- QUEZON CITY)

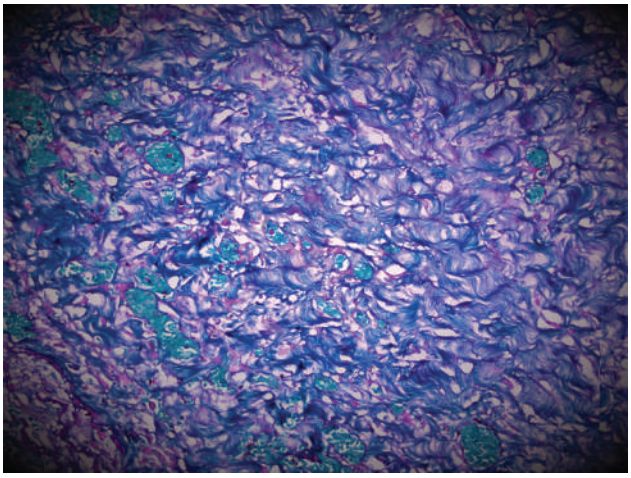
"Benign Hearty Prostate"
"This photomicrograph show benign prostate glands secretions composed of concentric lamellar rings called corpora amylacea. Improving cardiovascular risk lowers, one's odds of developing prostate problems. Regular exercise and weight loss reduces prostate cancer risk. Red-meat free diet significantly improves prostate health and cardiovascular health. Being "heart-healthy" promotes a healthy prostate."
Captured using OLYMPUS BX51, at 20X objective photographed with OLYMPUS DP70, corpora amylacea enhanced using LunaPic.

INDIVIDUAL CATEGORY



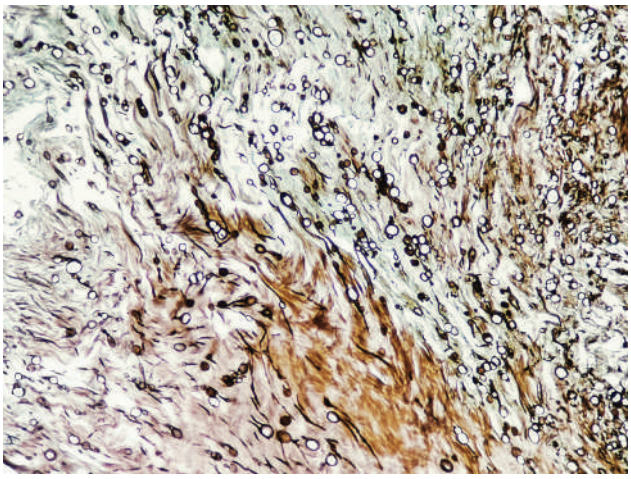
JOSA PINOL
(ST. LUKE´S MEDICAL CENTER, QUEZON CITY)

"Take a Trip"
"Psychedelic crystals blast off on a spectrum of vivid brights right into the 80´s inspired galaxy of tophaceous gout."
Captured with Olympus BX53 light microscope at 100x (low power objective) magnification.



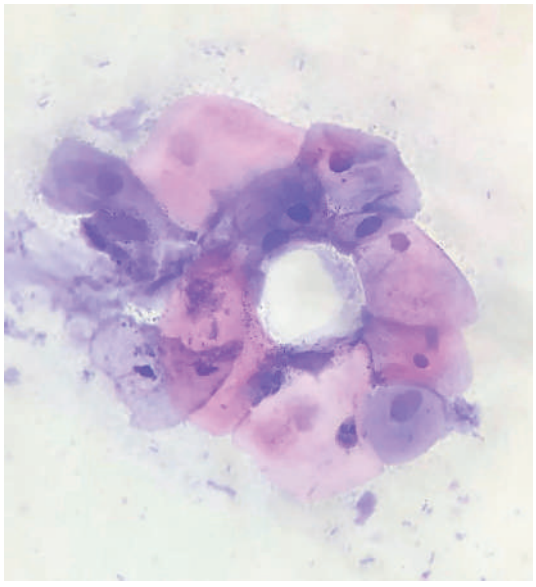
GLENN NATHANIEL S.D. VALLOSO, MD
(PHILIPPINE HEART CENTER)

"Lucid dreams need only three colors."
Captured using Olympus DP22.



KATHRINA ASEANNE C. ACAPULCO
(PERPETUAL SUCCOUR HOSPITAL)

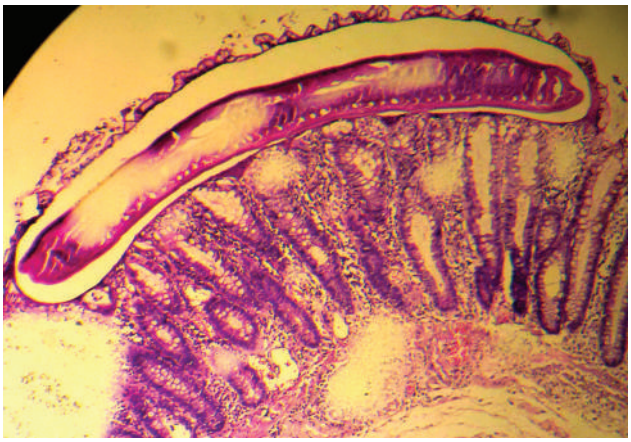
"AspARTgilloma"
"Accidentally discovered abstract art in disease upon staining with Grocott-Gomori Methenamine Stain. Sections disclosed hyphae, sporangia, and sporangiospores in a granulomatous lesion derived from a "lung mass" specimen."



RAY V. OLAC, JR., MD
(WEST VISAYAS STATE UNIVERSITY MEDICAL CENTER)

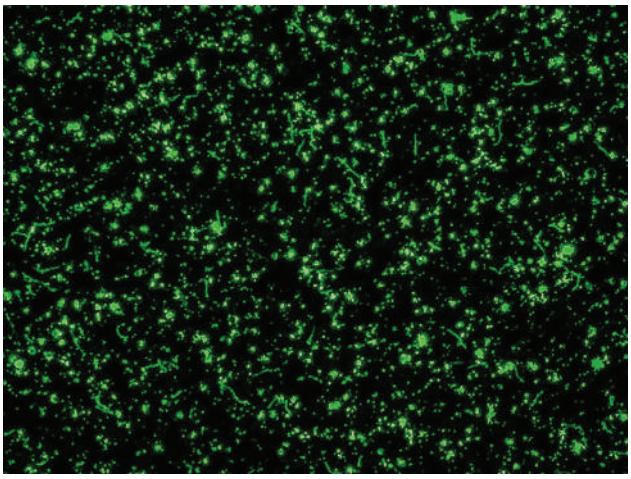
"Cervical Rosette"
"Mirroring the shadows of adornments past,
Whose sires and maidens deem to last.
Finite to thought and deeply beset,
The once glory of eminent rosette."
A rosette of cervical epithelium in a routine Pap smear is seen. A true "lumen" is appreciated where the cells themselves surround a central hollow point.

INDIVIDUAL CATEGORY



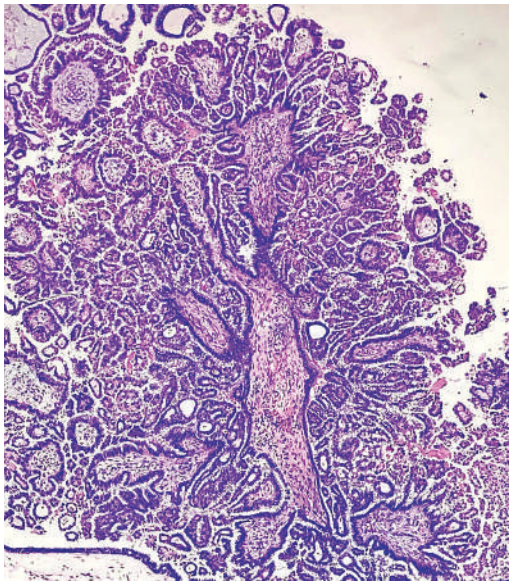
REDEN A. PATALINJUG, MD
(VICENTE SOTTO MEMORIAL MEDICAL CENTER)

"I came discreetly, I crept stealthily. I whipped gently, and you prolapsed excruciatingly." -Trichuris trichiura.
Captured using an Olympus cx23, LPO.



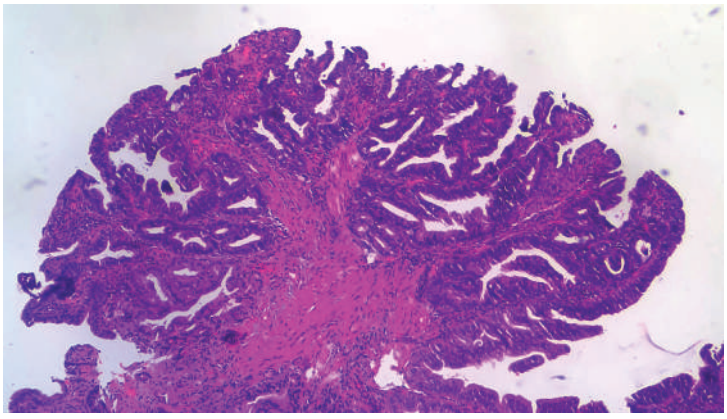
DAVID JEROME ONG, MD, MBA
(THE MEDICAL CITY)

"A Spiral Night"
"A bright sky filled with green fluorescent spirochetes and other debris." This specimen is positive for spirochetes (T. pallidum) in FTA-ABS.
Captured using Olympus BX41 - 40x objective.



KATRINA INOFERIO
(THE MEDICAL CITY)

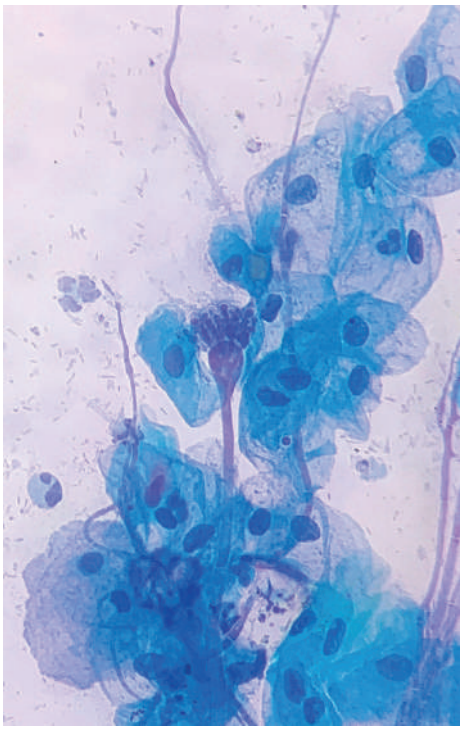
"Tree of Life"
"Rise up and stand firm against adversity. Remain steadfast and you shall live."
Borderline serous tumor (H&E).
Captured using Olympus BX43 - 10x objective.



VICTORIA E. CRUZ, MD
(UERM)

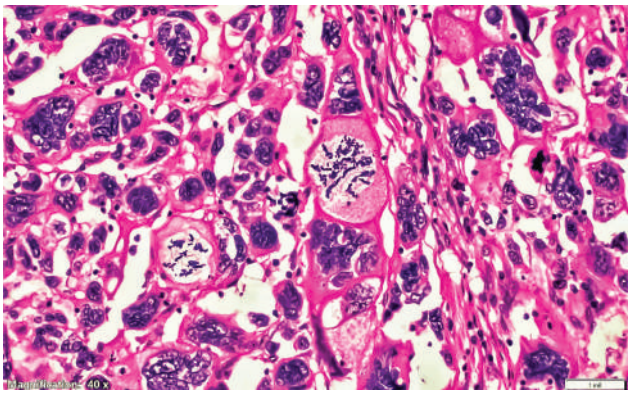
"The Tree of Love"
"For love is like a tree; it grows of itself; it sends its roots deep into our being, and often continues to grow green over a heart in ruins." - Victor Hugo

INDIVIDUAL CATEGORY



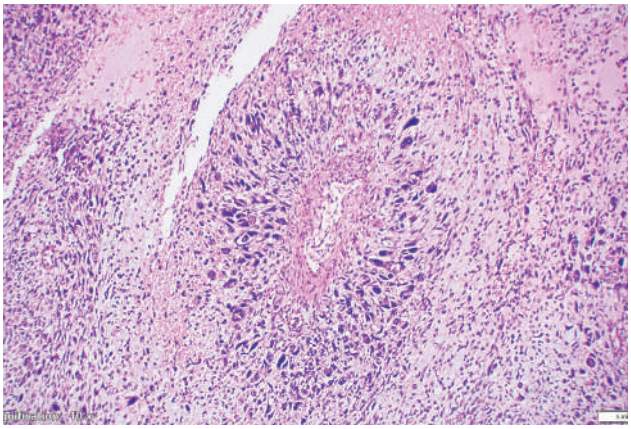
BLESSING DILLA
(WEST VISAYAS STATE UNIVERSITY MEDICAL CENTER)

"A queen with a crown of pearls or a beautiful blue flower in spring. Preserved forever as she sits idly with time's passing."



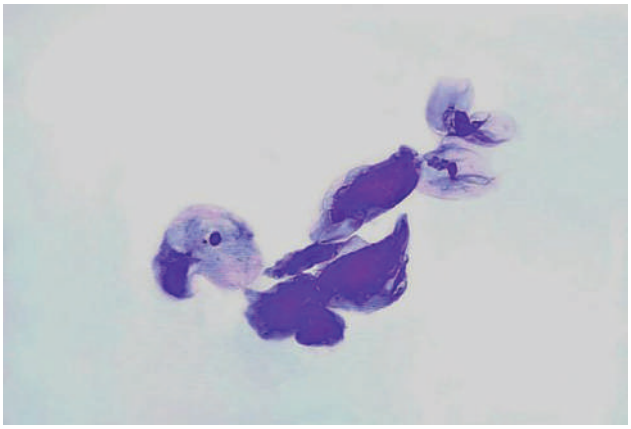
LOVELY EDIZA W. DOLLOSA
(VICENTE SOTTO MEMORIAL MEDICAL CENTER)

"Mother and Daughter Sticks Together"
Captured using Olympus CX33 with attached DP74 Olympus Camera.



ALEXIS J. ARCENAS-AMOROSO
(VICENTE SOTTO MEMORIAL MEDICAL CENTER)

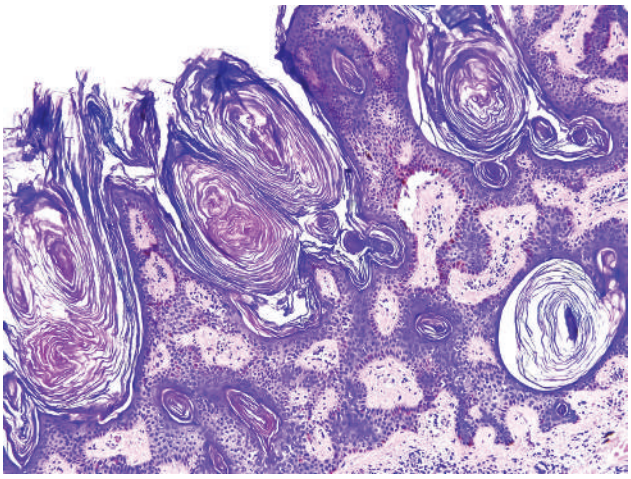
"My Crown of Tumor Cells"
40x magnification.



JULIAN MARIE AGAPE M. GRAGEDA, MD
(VALENZUELA MEDICAL CENTER)

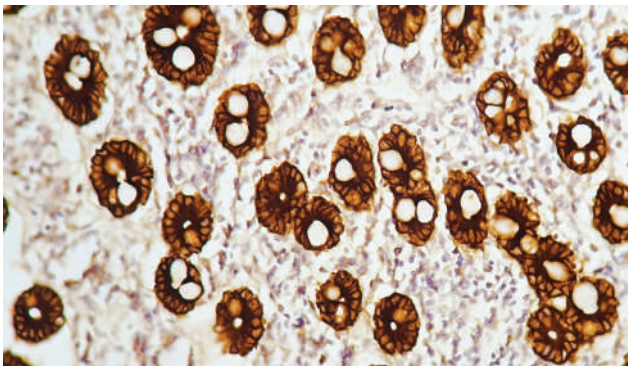
"Dreams of Flight"
"It was in pre-residency when this happy coincidence came by in a routine pap smear (December 2019). I hope we'd all be as free as this bird as soon as this pandemic ends."

INDIVIDUAL CATEGORY



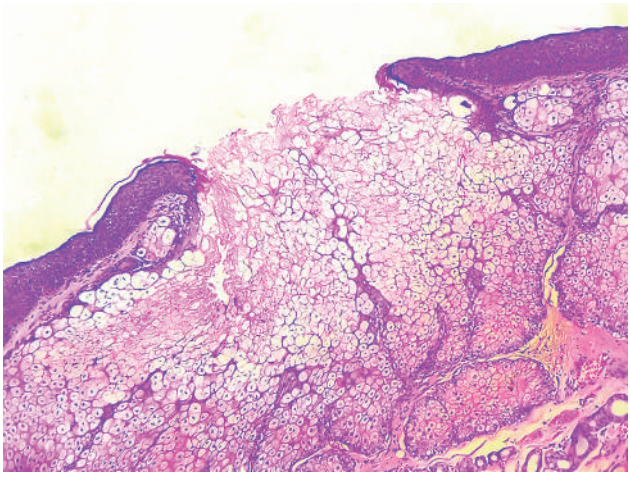
ANNA THERESE R. DATUIN
(BAGUIO GENERAL HOSPITAL AND MEDICAL CENTER)

"Quartz"
"Like geodes with layers and layers of crystals, horn pseudocysts found in Seborrheic Keratosis are made of loose swirls of delicate acellular keratin."



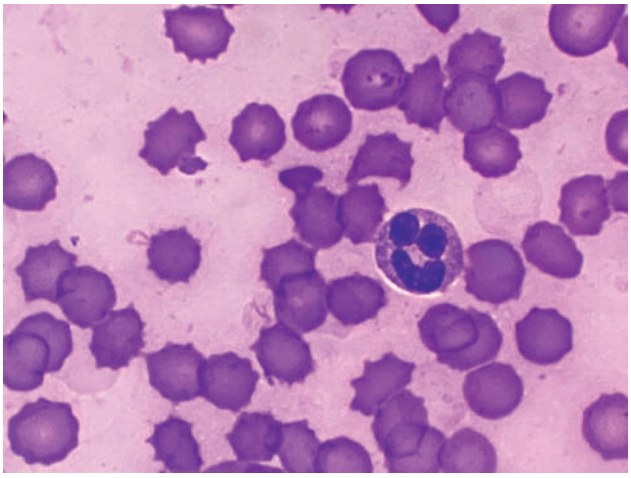
KAMILLE DELA CRUZ
(QUIRINO MEMORIAL MEDICAL CENTER)

"With Great Power, Comes Great Responsibility"
Captured using Olympus CX21LED, x400.



MA. REENA H. RANARIO, MD
(EASTERN VISAYAS REGIONAL MEDICAL CENTER)

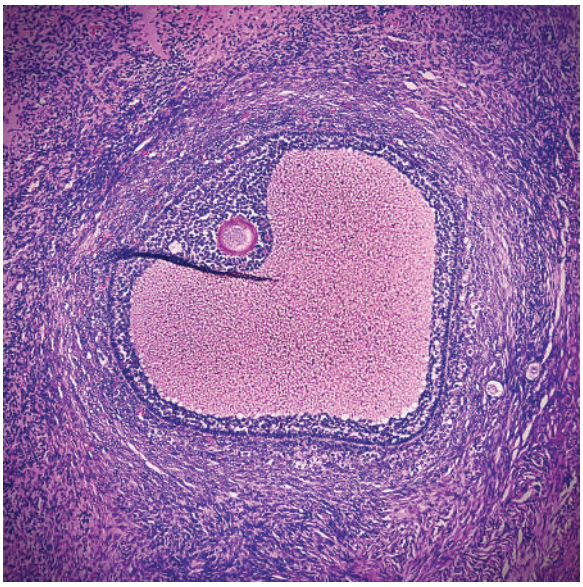
"A Volcano within a Tumor"
Astonishing eruption of the sebaceous glands to the epidermis from a Mature Teratoma of the Ovary.



CHINO PAOLO M. SAMSON
(REGION 1 MEDICAL CENTER)

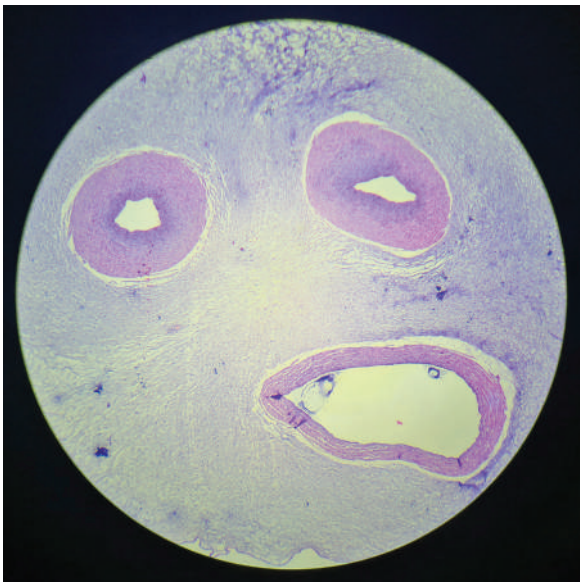
"There are many reasons to be wearisome during this pandemic; but this photo of a blood smear from a patient with COVID-19 reminds us that even the tiniest cells in our body are not giving up – and neither should we. Dear Frontliners (Macro and microscopic), continue fighting with a smile!"

INDIVIDUAL CATEGORY



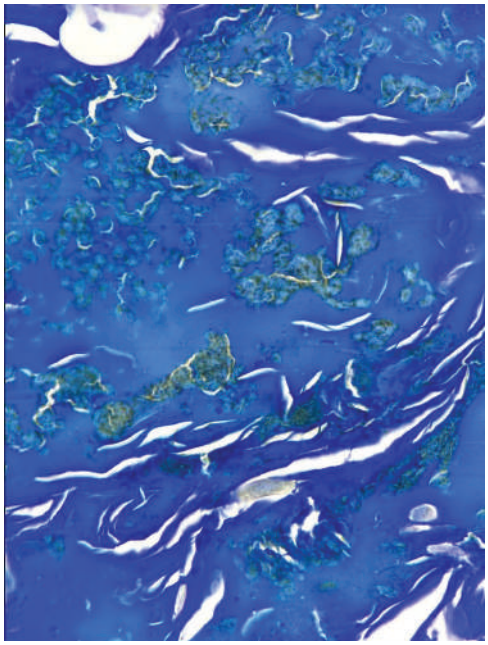
JOEANNE MARIE M. SALISE
(UP-PHILIPPINE GENERAL HOSPITAL)

"Beauty found in a seemingly unremarkable ovary, a pleasant sight to behold during a time of uncertainty."
Image captured using Olympus CX23 and Iphone 12 Pro Max.



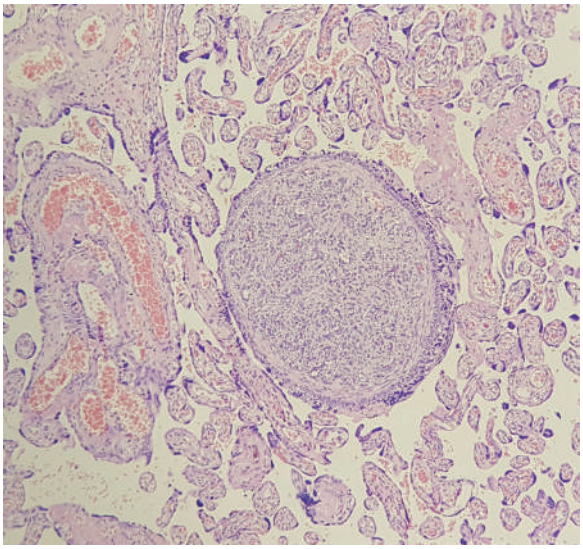
ZOE PHYLLIS C. CARPISO, MD
(FEU-NRMF)

"Frustrations. Me, after one year of being in a pandemic. Two arteries and one vein of an umbilical cord cross section."



JOSEPH MICHAEL R. ESPIRITU
(BULACAN MEDICAL CENTER)

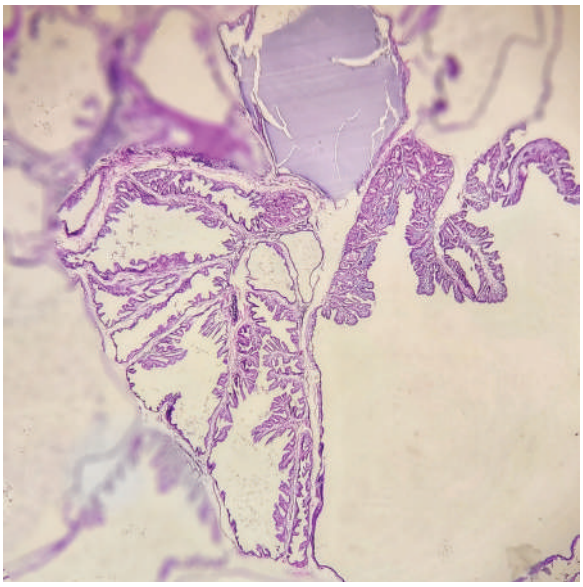
This is a rare case of cutaneous histoplasmosis from an HIV-AIDS patient referred to our institution. Just imagine yourself looking outside the window of an airplane. We all miss this. Visualize the Histoplasma as islands surrounded by the deep blue sea, as depicted by the Giemsa stain.



JAN ROMAN M. AYCO
(PHILIPPINE CHILDREN'S MEDICAL CENTER)

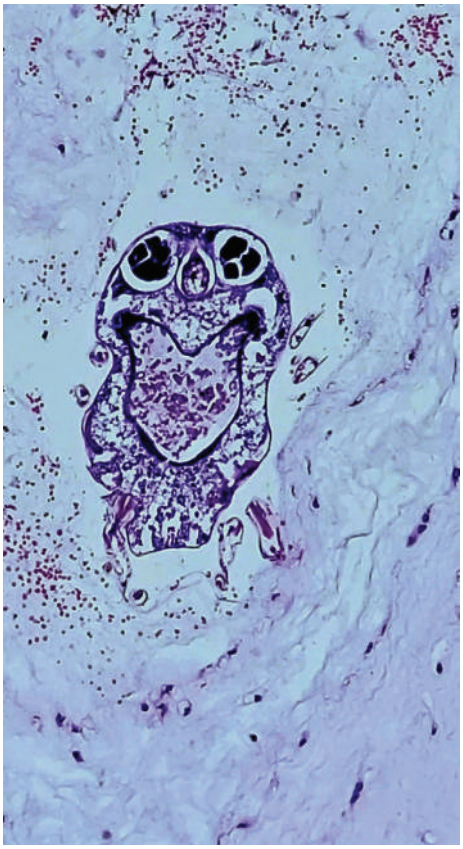
"The Seed of Destiny"
"Deep in the placenta I await,
brought forth by maladies of old mother.
The fate of the pregnancy at stake,
to grow forth or regress this I ponder."
A photomicrograph of a chorangioma occurring in a placenta of an elderly primigravid with history of diabetes mellitus and hypertension.

INDIVIDUAL CATEGORY



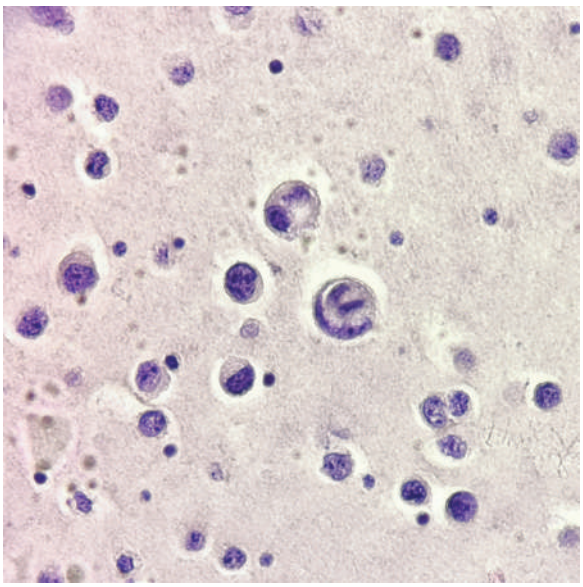
JILL J. JAIME
(PHILIPPINE CHILDREN'S MEDICAL CENTER)

"The Mundane and the Divine"
We embrace the mundane, Lest there's nothing beyond.
In hidden patterns we turn, To catch a glimpse of the divine.
Scanning view; iris blur via Adobe Photoshop CS6.



RAQUEL P. NGOHO
(EASTERN VISAYAS REGIONAL MEDICAL CENTER)

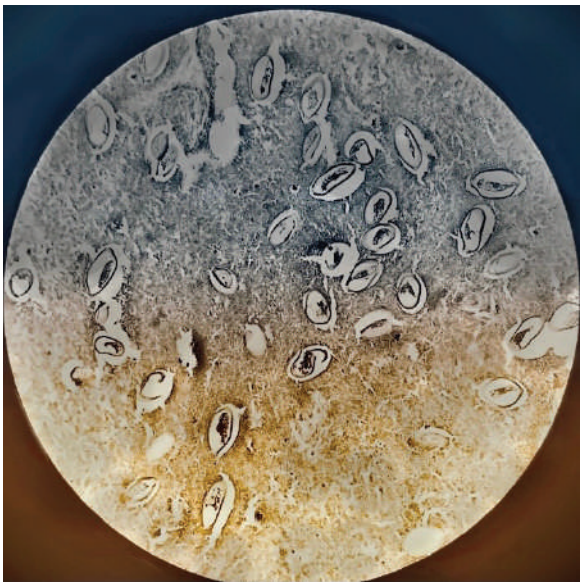
"Hey, if you don't have a smile,
I'll give you one of mine!"
An image captured using a light microscope from an extraplacental membrane, magnified 100x.



JOAN MARIE INFANTE
(FEU-NRMF)

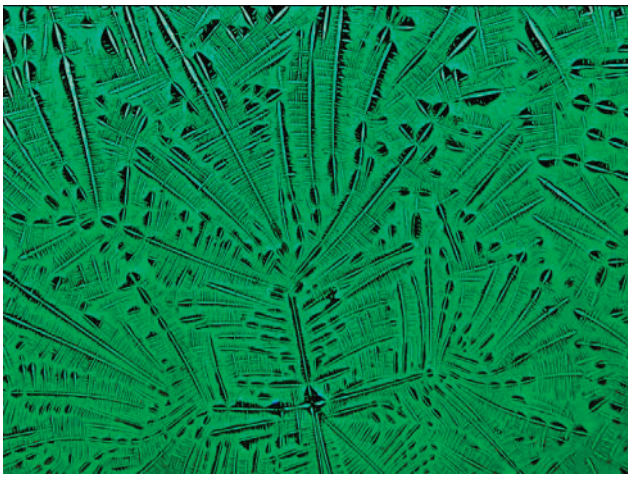
""The Nightmare Before Christmas."
An atypical cell seen in a pleural fluid cytology smear.

INDIVIDUAL CATEGORY



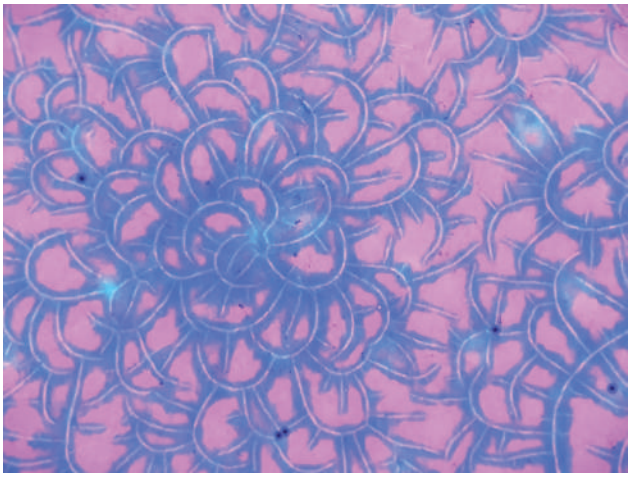
JOAN MARIZ T. PUNSALAN
(JOSE B. LINGAD MEMORIAL GENERAL HOSPITAL)

"Midnight Sun"
"Be the light in someone's hopeless night
For all great beginnings start in the dark."
(Fecal Material with Schistosoma ovas in an Appendectomy Specimen at 40x magnification).



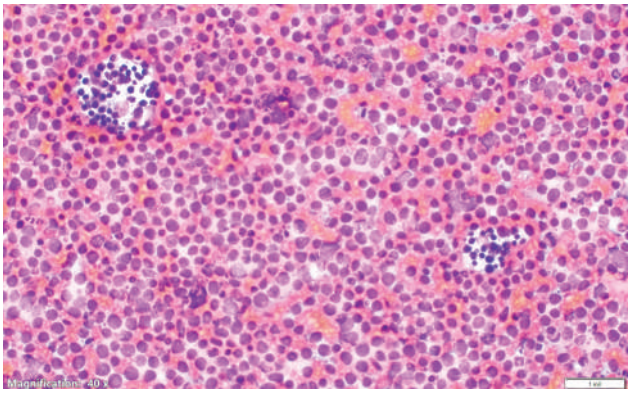
KATRINA JEZZELA M. DELA PENA
(JOSE B. LINGAD MEMORIAL GENERAL HOSPITAL)

"Breathe Easy"
Salt in the air, Breeze in the sea
How wonderful would it be, If trees can grow in the city.
(Pleural fluid cytology at 40x magnification).



LIAA MARIE G. DIMACALI
(JOSE B. LINGAD MEMORIAL GENERAL HOSPITAL)

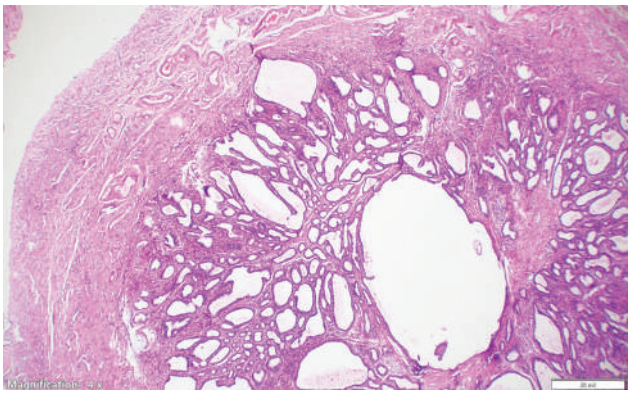
"Today too, I Bloom"
Today too, I face my fears. Today too, I learned mistakes.
But today too, I move forward at my own pace.
And today too, I grow and bloom with grace.
(Pleural fluid aspirate at 40x magnification.)



DAPHNE MARIE BORROMEO
(VICENTE SOTTO MEMORIAL MEDICAL CENTER)

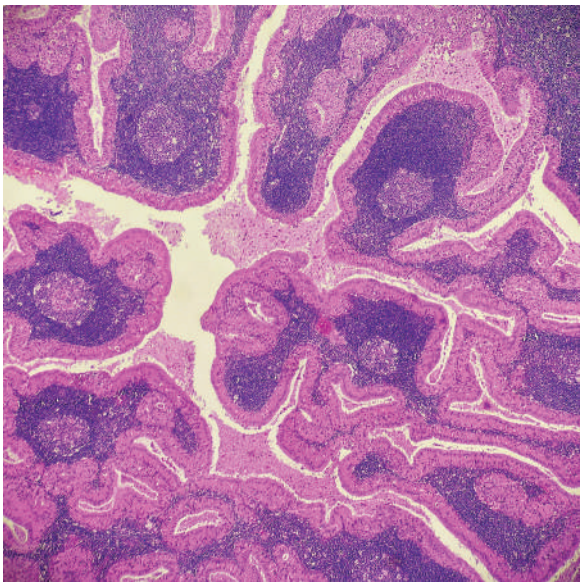
"Two Galaxies amidst the Sea of Stars"
This is a taken from a Fine Needle Aspiration Cytology of a Submandibular Mass. 40x Magnification.
The image is captured by cellSens Imaging Software through an installed Olympus DP74 camera on a CX33 Olympus microscope.

INDIVIDUAL CATEGORY



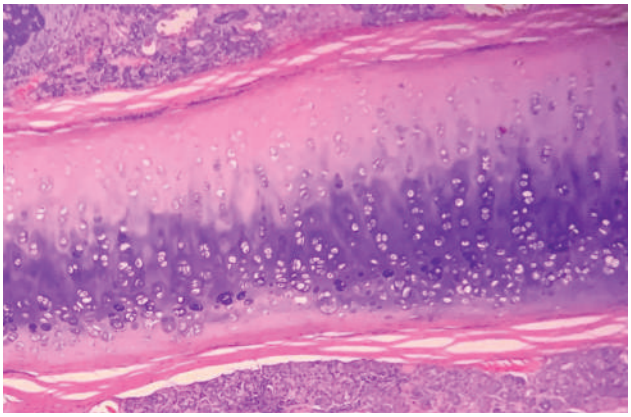
JOSEPH GARY C. SANCHEZ JR.

Don't underestimate the fallopian tube:
a case of salphingitis isthmica nodosa.



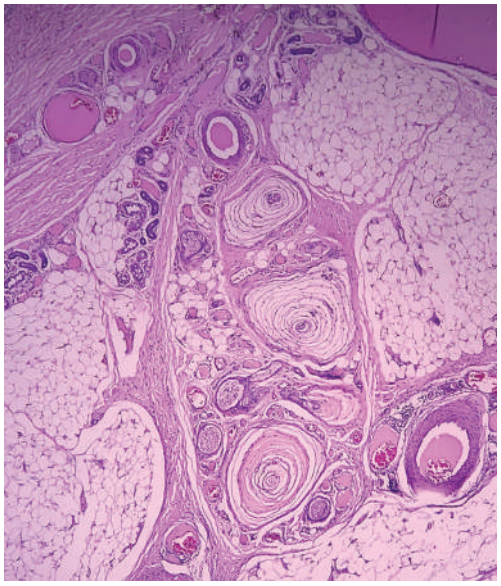
EUNICE DANICA FE
(JOSE B. LINGAD MEMORIAL GENERAL HOSPITAL)

"Who Am I?"
"Let smoke kiss your lips, I'll grow beneath your cheeks. Bigger than pears, I come from the tissue of defenders."
(Warthin's tumor at 10x magnification).



SANDY MAGANITO
(PHILIPPINE GENERAL HOSPITAL)

"The Ripening"
"Isnt it odd? We tend to lose our potential as we mature." Cartillage maturation of the nasal septum.
Captured using an IphoneXMax using a Olympus CX23.



CECILE DUNGOG
(PHILIPPINE GENERAL HOSPITAL)

"Come here, let me feel you no more"
Bundles of Pacinian corpuscle from an amputated hand.

PHILIPPINE JOURNAL OF PATHOLOGY

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ARTICLE SECTIONS

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

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

Original articles

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

Reviews

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

Case Reports

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

Feature articles

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

Autopsy Vault

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

Images in Pathology

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

Brief Communications

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

Editorials

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

Letters to the Editor

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

Special Announcements

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

COVER LETTER

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

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- Authors must use the standard PJP templates for each type of manuscript. These templates are aligned with the most current versions of the EQuaToR Network guidelines and checklists (http://equatornetwork.org).
- The manuscript should be encoded on the template using Microsoft Word (2007 version or later version), single-spaced, 2.54 cm margins throughout, on A4 size paper. Preferred fonts may include Century Gothic (template default), Times New Roman, or Arial.
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- References should pertain directly to the work being reported. Within the text, references should be indicated using Hindu-Arabic numerals in superscripts.

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- The title should be as concise as possible.
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  - **Final approval of the version to be published; AND**
  - **Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.**
- The highest educational attainment or title of the authors should be included as an attachment whenever appropriate (MD, PhD, et cetera).
- Name and location of no more than one (1) institutional affiliation per author may be included.
- If the paper has been presented in a scientific forum or convention, a note should be provided indicating the name of the forum or convention, location (country), and date of its presentation.

Abstract

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

Keywords

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

Text

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

References

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

One to Six Authors

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

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

More than Six Authors

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

Authors Representing a Group

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

Book

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

World Wide Web

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

Tables

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

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- Provide a title and brief caption for each figure or graph. Caption should not be longer than 15-20 words.
- All identifying data of the subject/s or patient/s under study such as name or case numbers, should be removed.
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- Where appropriate, all illustrations/photographic images should be at least 800 x 600 dpi and submitted as image files (preferably as .png, .jpeg, .tif, .psd or .pdf files).
- For photomicrographs, the stain used (e.g. H & E) and magnification (e.g. 400X) should be included in the description.
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EDITORIAL PROCESS (Figure 1)

- The Editorial Coordinator shall review each submission to check if it has met aforementioned criteria and provide feedback to the author within 24 hours.
- Once complete submission is acknowledged, the manuscript undergoes Editorial Board Deliberation to decide whether it shall be considered or not for publication in the journal. Within five (5) working days, authors shall be notified through e-mail that their manuscript either (a) has been sent to referees for peer-review or (b) has been declined without review.
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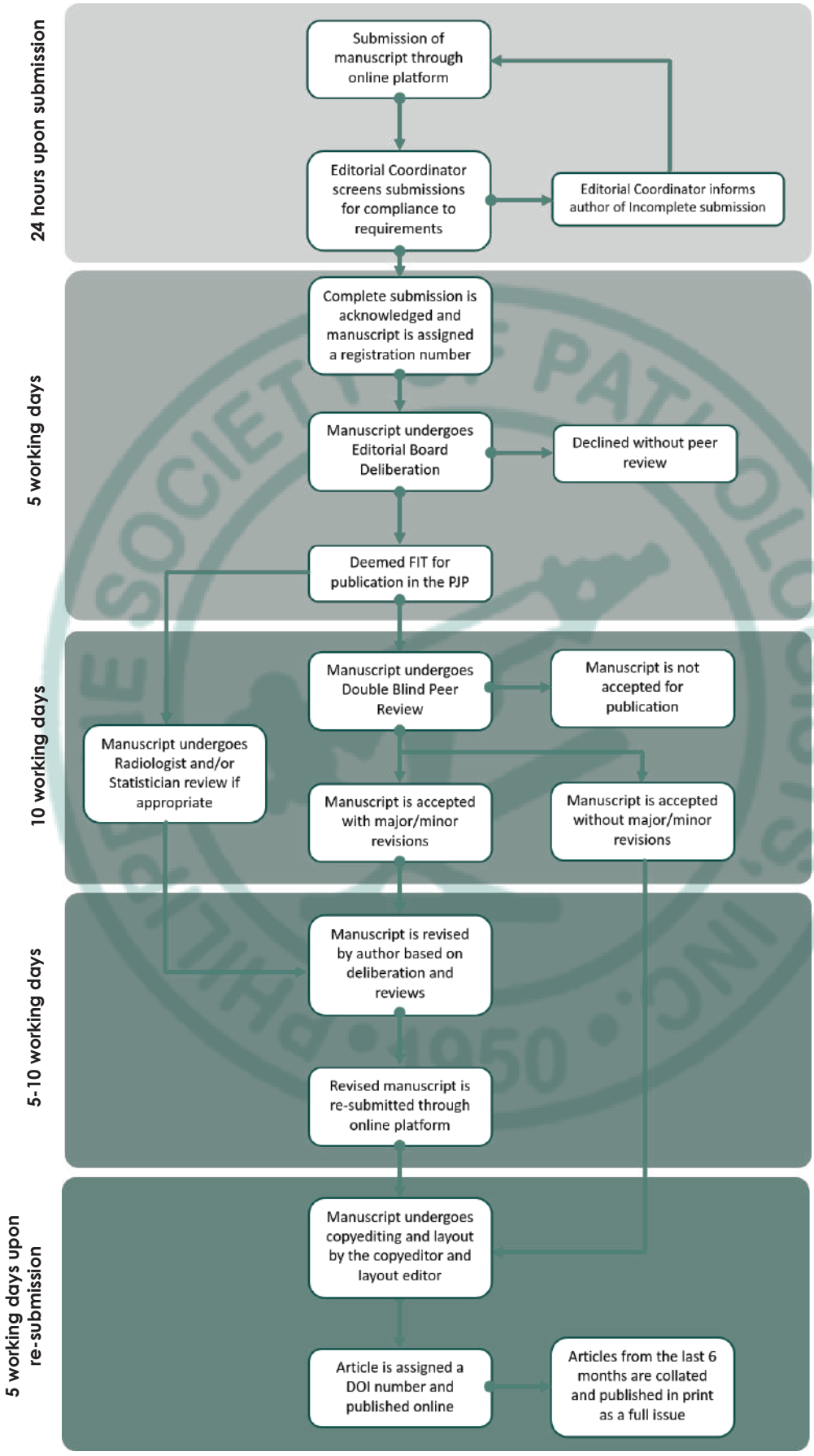

Figure 1. Editorial Process Flow.

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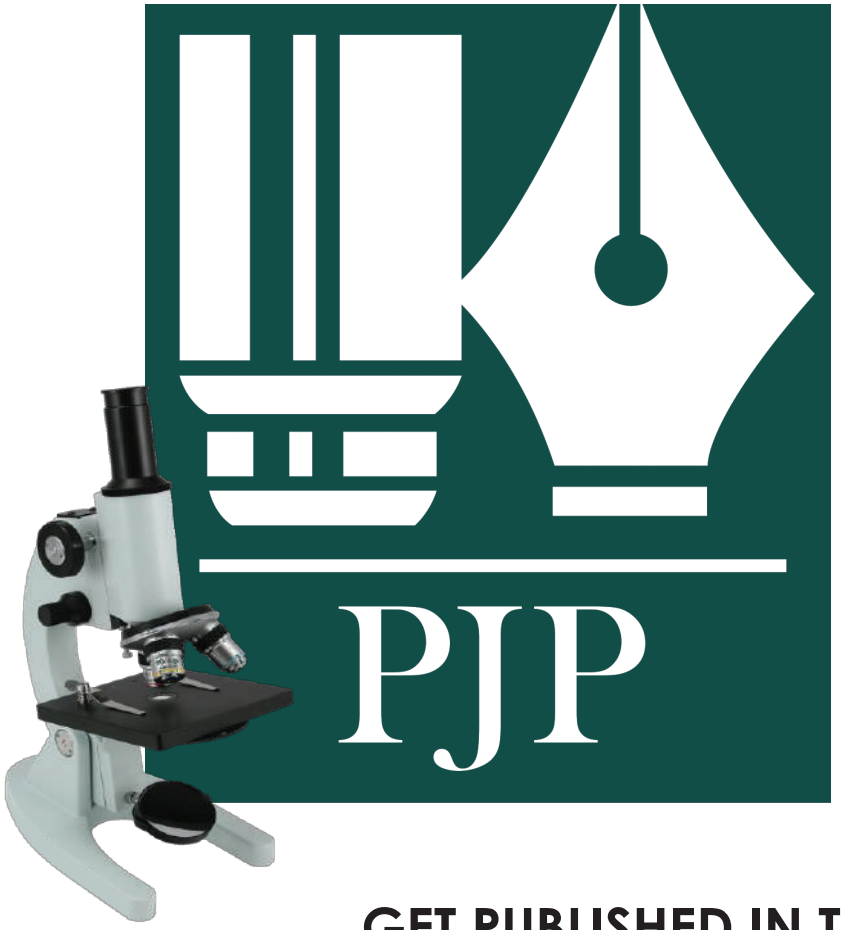

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