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

Congratulations to the Philippine Journal of Pathology editorial staff on the publication of its 16th digital issue since being relaunched in 2016. It is a testament to the vigor of the editorial staff to see the PJP published regularly. Gone are the days when the publications could be counted on one hand.

Commendations must also be made to the pathology consultants, residents, and other researchers who took the time and effort to develop the research, write the manuscript, and publish it. However, one wishes to see residents and consultants from more training institutions represented in the publications. One does not need complicated equipment to produce a research paper worthy of publication. The world is interested in what Filipino pathologists observe, see, and experience locally. Who better to share this through research than us?

Here's to a more fruitful year (and thicker volume) of the PJP!

Maria Cecilia F. Lim, MD, FPSP
President, Philippine Society of Pathologists, Inc.

Quo Vadis: The Future of Pathology Practice in the Philippines

The field of pathology, often regarded as the bedrock of accurate medical diagnosis and treatment, stands at a pivotal juncture in the Philippines. With the rapid advancement of technology and evolving healthcare needs, we find ourselves asking: Quo Vadis? Where is the practice of pathology headed in our country, and what steps must we take to ensure its growth and relevance in the coming years?

Pathology in the Philippines has witnessed remarkable progress over the past decade. Advances in diagnostic techniques, improved laboratory infrastructure, and the dedication of our medical professionals have contributed to more accurate and timely diagnoses. However, significant challenges remain that must be addressed to ensure the continued development and effectiveness of pathology practice.

One of the most pressing issues is the unequal distribution of pathology services across the archipelago. While metropolitan areas boast well-equipped laboratories and a concentration of pathologists, many rural regions still lack access to basic diagnostic facilities. This disparity results in delayed diagnoses and suboptimal patient outcomes. Addressing this inequity requires strategic investments in telepathology and mobile diagnostic units to extend quality care to underserved areas.

Another challenge is the need for sustained funding and resources for pathology research and education. The rapid pace of technological advancements necessitates continuous learning and adaptation. However, financial constraints often hinder the ability of institutions to provide ongoing training and invest in cutting-edge diagnostic tools. Increased government support and private sector involvement are essential to bridge this gap and foster a culture of innovation within the pathology community.

Despite these challenges, the future of pathology in the Philippines is brimming with potential. Several transformative opportunities can propel the practice to new heights and ensure its alignment with global standards.

Digital pathology is one such opportunity that holds immense promise. By digitizing tissue slides and employing advanced imaging techniques, we can facilitate remote consultations and second opinions from experts around the world. This not only improves diagnostic accuracy but also reduces the turnaround time for critical diagnoses. Embracing digital pathology requires investment in the necessary infrastructure and training programs to equip pathologists with the skills needed to navigate this new paradigm.

Artificial intelligence (AI) is another frontier that can revolutionize pathology practice. AI-powered algorithms can assist in the interpretation of complex patterns in tissue samples, enhancing the precision and speed of diagnoses. Collaborative efforts between pathologists and data scientists can lead to the development of robust AI tools tailored to the unique needs of our healthcare system. These tools can augment the capabilities of pathologists, allowing them to focus on more complex and nuanced cases.

Furthermore, the integration of personalized medicine into pathology practice offers a transformative approach to patient care. By identifying specific genetic markers and tailoring treatment plans accordingly, pathologists can contribute to more effective and targeted therapies. This requires a concerted effort to incorporate genomic technologies into routine practice and to ensure that pathologists are proficient in interpreting genetic data.

To navigate the future of pathology practice in the Philippines effectively, several strategic initiatives must be undertaken. First and foremost, we need a comprehensive national strategy that prioritizes equitable access to diagnostic services. This includes expanding telepathology networks, establishing regional centers of excellence, and incentivizing pathologists to work in underserved areas.

Investment in education and training is equally crucial. Pathology residency programs should be enhanced to include training in digital pathology and AI applications. Continuing medical education (CME) programs should be made readily available to practicing pathologists to keep them abreast of the latest developments in the field.

Collaboration between government, academic institutions, and the private sector is essential to drive innovation and research. Public-private partnerships can facilitate the funding of research projects and the acquisition of advanced diagnostic tools. These collaborations can also help in developing standardized protocols and guidelines that ensure the consistent and accurate application of modern technologies.

The question of "Quo Vadis?"—where are we going? —invites us to envision a future where pathology practice in the Philippines is not only robust and innovative but also equitable and accessible to all. By addressing current challenges and embracing opportunities for transformation, we can chart a course toward a healthcare system where every Filipino has access to timely and accurate diagnoses. The journey ahead requires collective effort, strategic planning, and an unwavering commitment to excellence. Let us move forward with determination and optimism, knowing that the future of pathology in the Philippines holds great promise.

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

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Utilization of Artificial Intelligence in Breast Pathology: An Overview

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ABSTRACT

In the last decade, artificial intelligence (AI) has been increasingly used in various fields of medicine. Recently, the advent of whole slide images (WSI) or digitized slides has paved the way for AI-based anatomic pathology. This paper set out to review the potential integration of AI algorithms in the workflow, and the utilization of AI in the practice of breast pathology.

Key words: AI algorithm, anatomic pathology, artificial intelligence, breast cancer, digitized slides, whole slide images

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INTRODUCTION

The World Health Organization (WHO) has reported that there were 2.3 million women diagnosed with breast cancer, and 685,000 deaths globally in 2020.[1] Surprisingly, the Philippines has the highest incidence rate of breast cancer in Asia. Approximately 7% (1/13) of Filipino women will develop breast cancer in their lifetime and 25% (1/4) who were diagnosed with breast cancer die within the first 5 years.[2] Breast examination, mammogram, ultrasound, magnetic resonance imaging (MRI), and biopsy are some of the tests used to diagnose breast cancer.[3]

The recent and ongoing advances in artificial intelligence (AI), machine learning (ML), and deep learning (DL), have paved the way for the development of AI algorithms not just in pathology, but in the practice of medicine in general.[4] For a pathologist, these ML and DL approaches offer the potential to significantly improve workflow, reduce turnaround time, and increase diagnostic accuracy. In 2022, Sandbank and colleagues conducted a validation and real-world clinical application of an AI algorithm for breast cancer detection in biopsies. Their study focused on 4 main steps: (1) development of an AI algorithm for breast cancer detection using digitized slides; (2) algorithm internal testing; (3) blinded algorithm validation using an independent dataset and (4) algorithm deployment in routine clinical use. Galen™ Breast was used for the algorithm's internal testing and external validation. Based on the results of the study, it has been observed that the area under the curve (AUC) for invasive breast cancer detection was 0.990 (95% CI 0.984-0.997) with a sensitivity and specificity of 95.51% and 93.57%, respectively. On the other hand, the AUC for ductal carcinoma in situ (DCIS) detection was 0.980 (95% CI 0.967-0.993) with a sensitivity and specificity of 93.79% and 93.20%, respectively. The diagnostic performance for tumor-infiltrating lymphocytes (TILs) was remarkable with an AUC of 0.965.[5] Overall, based on this study, the deployment of an AI algorithm in the clinical setting was proven to be useful for breast cancer detection in biopsies.

KNOWING THE PAST TO UNDERSTAND THE PRESENT

In the 1950s, enzyme histochemistry was used to apply specific reactions on tissue sections with the aid of chemical dyes. In 1956, the term artificial intelligence was coined by John McCarthy. Three years later, machine learning was introduced by Arthur Samuel. Then in the 1960s, electron microscopy (EM) was first used to assist in the diagnosis of kidney diseases and neuropathies. In 1965, computerized image analysis of cells and chromosomes was introduced by Judith Prewitt and Mortimer Mendelsohn. Eventually in the 1980s, immunohistochemistry (IHC) became a major part of diagnosis for various malignancies. The term deep learning was coined by Rina Dechter in 1986 and two years later, the convolutional neural network (CNN) model was invented by Yann LeCun. In 1990, a whole slide scanner was introduced. In the 2000s, molecular diagnostics has been widely used for assessing diagnostic and therapeutic biomarkers. Examples of biomarkers used in clinical practice to guide diagnosis and therapeutic decisions for breast cancer include but are not limited to breast cancer gene (BRCA), estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PI3KCA), Oncotype DX, MammaPrint Test, and immunohistochemical 4 (IHC4). In 2013, a photoacoustic microscopy (PAM) imaging technique was developed. The following year, the generative adversarial network (GAN) was introduced by Ian Goodfellow. In 2016, microscopy with ultraviolet surface excitation (MUSE) microscopy was invented and the year after, Philips received approval for a digital pathology whole-slide scanning solution.[6-10]

COMPUTATIONAL PATHOLOGY

Machine learning is a subset of AI and is largely classified into 3 categories namely supervised learning, unsupervised learning, and semi-supervised learning. Examples of supervised learning include artificial neural networks (ANN), decision trees, k-nearest neighbors, and linear regression. Deep learning is a subset of ML and the commonly used model in anatomic pathology is supervised learning based on the convolutional neural network (CNN).[11] Training of neutral networks begins by dividing each whole slide image (WSI) into smaller patches. The reason for this is that the number of pixels for WSI is about 10^5 x 10^5 which is too big for processing. Each patch then goes through multiple convolutional kernels or filters, and the convolved patches are flattened and used as input. The weights and biases are adjusted, and the model is trained.[4] Examples of training goals and datasets using the CNN model are shown in Table 1.[12-21] Meanwhile, unsupervised learning is an ML method that takes unlabeled input data to form and generate patterns. Examples of unsupervised learning techniques include cluster analysis and principal component analysis.[11]

VALIDATING DIGITIZED SLIDES

Digital pathology has played an important role in the development and application of WSI. This technology utilized an automated scanner capable of digitizing the whole slide using state-of-the-art software. Validation of WSI is essential to ensure that diagnostic performance and quality assurance are in place. Hence, the College of American Pathologists (CAP) has set forth guidelines for validating WSI for diagnostic purposes.[22]

1. All pathology laboratories implementing WSI technology for clinical diagnostic purposes should carry out their own validation studies.
2. Validation should be appropriate for and applicable to the intended clinical use and clinical setting of the application in which WSI will be employed; Validation of WSI systems should involve specimen preparation types relevant to the intended use (e.g., formalin-fixed paraffin-embedded tissue, frozen tissue, immunohistochemical stains, cytology slides, hematology blood smears).
3. The validation study should closely emulate the real-world clinical environment in which the technology will be used.
4. The validation study should encompass the entire WSI system.
5. Revalidation is required whenever a significant change is made to any component of the WSI system.
6. A pathologist adequately trained to use the WSI system must be involved in the validation process.
7. The validation process should include a sample set of at least 60 cases for one application (e.g., hematoxylin-eosin-stained sections of fixed tissue, frozen sections, cytology, hematology) that reflects the spectrum and complexity of specimen types and diagnoses likely to be encountered during routine practice.
8. The validation study should establish diagnostic concordance between digital and glass slides for the same observer (i.e., intraobserver variability).
9. Digital and glass slides can be evaluated in random or nonrandom order (as to which is examined first and second) during the validation process.
10. A washout period of at least 2 weeks should occur between viewing digital and glass slides.

Table 1. Examples of training goals and datasets using CNN model

DL	Input	Training goal	Dataset	Authors
CNN	WSI	Diagnosis of breast cancer	Private	Mi et al., 2021
CNN	WSI	Genomic correlation of breast cancer	TCGA	Lu et al., 2021
CNN	WSI	Diagnosis of brain tumor	Private	Im et al., 2021
CNN	WSI	Diagnosis of gastric cancer	Private	Hu et al., 2021
CNN	WSI	Screening of cervical cancer	Private	Cheng et al., 2021
CNN	WSI	Diagnosis of ovarian cancer	TCGA	Shin et al., 2021
CNN	WSI	Classification of colon cancer	TCGA	Zhou et al., 2021
CNN	WSI	Segmentation of prostate gland	Private	Salvi et al., 2021
CNN	WSI	Classification of transplant kidney	Private	Kers et al., 2022
CNN	WSI	Prognosis of lung cancer	Private	Shim et al., 2021

CNN: convolutional neural network; DL: deep learning; TCGA: The Cancer Genome Atlas; WSI: whole slide image

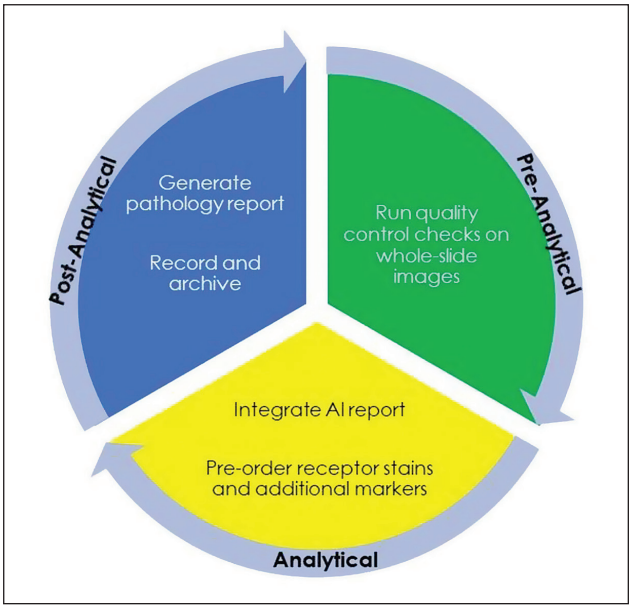

Figure 1. The three phases of laboratory testing.

11. The validation process should confirm that all the material present on a glass slide to be scanned is included in the digital image.
12. Documentation should be maintained recording the method, measurements, and final approval of validation for the WSI system to be used in the clinical laboratory.

EMBEDDING AI ALGORITHM IN THE WORKFLOW

The integration of AI into the workflow of anatomic pathology has many advantages and opens more opportunities in clinical practice. First, it can perform quality control in the 3 phases of the laboratory work processes namely, the pre-analytical phase, the analytical phase, and the post-analytical phase. Specifically, the AI algorithm can run quality control checks on the digitized slides using either frozen sections or formalin-fixed paraffin-embedded (FFPE) tissue blocks (Figure 1). After which, integrated diagnosis can be achieved through consolidation and correlation of clinical information, laboratory findings, and AI reports.[23] Furthermore, pertinent radiologic images and omics studies (e.g., genomics, transcriptomics, proteomics) may be integrated into the clinical data and would offer a wider perspective. Lastly, personalized patient-centric analysis and interpretation of the case, as well as the generation of a final pathology report and archiving can be incorporated into the workflow.

Whole slide images using hematoxylin and eosin (H&E)-stained slides can be easily scanned. The glass slides are usually scanned at 40x magnification with a resolution of 0.23 to 0.25 μm/pixel.[24-26] Some of the commonly used scanners that are now available in the market include IntelliSite (Philipps Digital Pathology Solutions, Netherlands); Aperio (Leica Biosystems, Germany); NanoZoomer (Hamamatsu, Japan); and Axioscan (Zeiss, Oberkochen, Germany). This has accelerated the deployment of WSI for clinical use mostly in developed countries. In particular, the IntelliSite has been approved for diagnostic purposes in the United States (US) and is licensed for in-vitro diagnostics (IVD) in the European Union (EU), Canada, Japan, Singapore, Korea, and the Middle East. These scanners can produce automated, high-speed, and high-resolution digitized slides.

The AI algorithm can help in the practice of breast pathology by:

1. Providing efficient workflow and improved turnaround time. This means that pathology cases can be prioritized in terms of diagnosis. Immunohistochemistry stains such as ER, PR, and HER2 can be pre-ordered. Additional levels such as calcifications can also be pre-ordered.
2. Detecting invasive cancers and DCIS with an AUC close to 1.0 as demonstrated by Sandbank and colleagues;
3. Distinguishing different subtypes of cancer. In particular, the AI algorithm can separate ductal and lobular cancers. This would then help the physician identify patients who are unlikely to respond to neoadjuvant therapies;
4. Detecting a special type of cancer of favorable prognosis. An example of this is the detection of tubular and mucinous carcinoma;.
5. Detecting metaplastic carcinomas;[5]
6. Detecting lymph nodes for metastasis;[27]
7. Quantitating various markers such as antigen Kiel 67 (Ki-67), programmed cell death ligand 1 (PD-L1), and TILs.[28]

HOW WILL AI ENHANCE THE PRACTICE OF BREAST PATHOLOGY?

AI has already been shown to be of value in prostate cancer and can be used as a critical adjunct to anatomic pathology.[29,30] The pathologist who used AI has these to offer:

1. Increase diagnostic accuracy through notification and alert system;
2. Improve interobserver concordance;
3. Provide an additional layer of quality control and improve patient safety.

CHALLENGES TO THE IMPLEMENTATION OF AI

The challenges for the implementation of AI in anatomic pathology include but are not limited to the following: algorithm validation and generalizability; digitalizing slides and storage of WSI requires huge and expensive data storage facilities; ethical issues; integration of other pertinent data such as radiology images and omics (e.g., genomics, transcriptomics, proteomics); regulatory considerations and technological infrastructure. Here, there is a need for laboratories to invest and develop the technological infrastructure to support whole-slide imaging using AI algorithms.

WILL AI REPLACE THE PATHOLOGISTS?

Charles Darwin, known for his theory of evolution by natural selection, published his landmark book on the Origin of Species in 1859. According to him, *it is not the strongest of the species that survives nor the most intelligent that*

survives. It is the one that is adaptable to change.[31] This means that AI will not replace the pathologists but instead, AI will enhance the practice of anatomic pathology. In other words, AI is the perfect companion for breast cancer detection in biopsies. This is in line with the perspective of a seasoned pathologist, Dr. Stuart J. Schnitt, Chief of Breast Oncologic Pathology, Dana-Farber Brigham Cancer Center, and Professor of Pathology, at Harvard Medical School. According to him, *instead of pathologists being replaced by AI, pathologists who use AI will replace those who don't use AI.*[32] Taken together, the integration of AI in the practice of breast pathology can significantly improve diagnostic accuracy and efficiency.

CONCLUSION

AI algorithms have been developed and validated for detecting breast cancer in biopsies. This offers the potential to increase accuracy, improve patient safety, and eventually help in providing better care and patient outcomes. Given the limited number of pathologists and the increasing caseloads, AI-based systems may ease the pressure on anatomic pathologists, reduce interobserver variability, and improve total turnaround time. Indeed, AI is increasingly a maturing technology, however, further evaluation in the clinical setting, as well as a demonstration of the clinical utility of AI in anatomic pathology is crucial to widespread adoption.

STATEMENT OF AUTHORSHIP

The author certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

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OPEN ACCESS – ORIGINAL ARTICLE

Coagulation and Platelet Profiles of COVID-19 Patients Admitted to a COVID Referral Center from March 2020 to December 2022

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ABSTRACT

Objective. This study aimed to determine the demographic profiles of admitted COVID-19 patients, the association of coagulation and platelet tests on COVID-19 severity and compare the coagulation and platelet profile across the spectrum of the disease in terms of severity among adult COVID-19 patients admitted to the Philippine General Hospital from March 2020 to December 2022.

Methodology. Medical records of a sample of adult COVID-19 patients admitted to the emergency room of the Philippine General Hospital from March 2020 to December 2022 were reviewed. The demographics, initial COVID-19 diagnosis and initial coagulation and platelet test results were gathered and tabulated. Comparison of the initial coagulation and initial platelet results were made per disease category.

Results. Three hundred eighty-five (385) patients were included; 194 were males, and 191 were females. The mean age of all patients was 56.18 years old. There was a total of 30 patients classified as mild and 105 patients are under moderate category. 141 patients were classified as severe, whereas 109 patients were classified as critical. Platelet count test and Activated Partial Thromboplastin Time (APTT) were mostly normal in all disease categories. Prothrombin time was normal in a majority of patients from the mild and severe categories. INR and D-dimer were all elevated mostly in all disease categories.

Conclusion. Platelet counts and APTT were mostly normal in all disease categories. Prothrombin time and D-dimer had a significant association with disease severity. Platelet count, APTT and INR did not show significant association with disease severity. Prothrombin time, APTT, INR and D-dimer means had significant differences versus disease categories.

Key words: coagulation, platelet test, activated partial thromboplastin time, APTT, COVID-19, disease severity

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INTRODUCTION

COVID-19 had been an emerging global pandemic since 2020. According to the World Health Organization (WHO), the pandemic caused over 768 million confirmed cases including more than 6.9 million deaths all over the world.[1] In the Philippines, there were more than 4 million total cases with more than 66 thousand recorded deaths since the pandemic started.[2]

Even if the WHO ended the COVID-19 global pandemic response,[3] it still constantly affected every nation because COVID-19 cases had been detected and some countries had increasing cases. Since COVID-19 cases have been continuously recorded and detected, the burden of the management and prevention of the spread of the disease is still a problem for those countries that are affected.

Coronavirus disease 2019 (COVID-19) was caused by SARS-CoV-2, similarly known as Acute Respiratory Syndrome Coronavirus 2, a novel beta coronavirus.[4] The disease could cause clinical features that vary from mild to fatal illness. The most common symptoms were nonspecific and include fever, cough, myalgia, core throat, headache, chills, nausea, vomiting, diarrhea, ageusia, and conjunctival congestion.[5] Protocols were made for risk stratification and management intervention as well as

isolation and quarantine rules to prevent overwhelming numbers of patients in health facilities and hospitals. Asymptomatic and mild symptoms required home isolation. For those with worse symptoms such as shortness of breath, tachypnea, and any signs of respiratory distress, hospitalization was advised.[6]

COVID-19 posed a global threat because it was not only a respiratory infection but a disease that affected multiple organ systems such as cardiovascular, gastrointestinal, neurological, immune, and hematopoietic systems. It also mostly affects hemostasis.[7] According to Abd El-Lateef AE et al., the most common hematological complication of COVID-19 was coagulopathy. Although the exact pathogenesis of coagulopathy of COVID-19 had not been properly explained, many factors were contributory such as cytokine storm, neutrophil activation, impaired endothelial function, platelet activation, tissue factor expression and coagulation induction.[8] According to Wool et al, the SARS-COV-2 virus did not have an intrinsic procoagulant effect. The coagulopathy could be a byproduct of profound COVID-19 inflammatory response and endothelial activation/change.[9]

The most important biomarkers indicating poor prognosis in COVID-19 patients were coagulopathy and abnormal coagulation parameters. The occurrence of coagulation dysfunction was seen in severe and critically ill patients. There was also an increased occurrence of intravascular disseminated coagulopathy in patients with COVID-19.[4] Arterial and venous thrombotic complications were seen in ICU patients. There was an increased level of D-dimer and fibrinogen degradation products in patients with severe COVID-19 infection than the milder forms. Those patients with severe COVID-19 have also lower platelet counts compared to those with milder disease.[10] Since coagulopathy in patients with critical and fatal disease was common, coagulation parameters should be thoroughly discussed for risk classification and predicting prognosis.[11] Moreover, blood coagulation and platelet profiles should be done for COVID-19 patients for improvement of management and health outcomes.[4] Finally, there have been no studies about coagulation and platelet profiles for COVID-19 patients here in the Philippines to date. This study aims to provide an initial report on the relationship between coagulation and platelet profiles of COVID-19 patients in a COVID-referral center.

METHODOLOGY

The study employed a cross-sectional study design on the initial coagulation and platelet profiles of adult COVID-19 patients. Data sampling was done in this study based on the objectives. To determine the demographic profile of COVID-19 patients, a minimum sample size of 385 patients was computed with respect to sex with an expected proportion of 50% for the sex male, an error of 5% and a significance level of 0.05. A list of all COVID-19 patient case numbers admitted from March 2020 to December 2022 was gathered from the Health Information Management Division from the Medical Records. Random sampling was performed using a pseudo-random number-generating function in Microsoft Excel. Sampling was performed until the set sample size (N=385) was reached.

A list of all admitted COVID-19 patients from March 2020 to December 2022 was requested from the Health Information Management Division of the Medical Records. Random sampling using a random number-generating function from Microsoft Excel was made to select a minimum of 385 patients that were included in the study. Code numbers were assigned by the principal investigator to the records of patients who fulfilled the inclusion criteria. The clinical data was retrieved via the Radish platform while the coagulation and platelet results were reviewed in the OpenMRS platform.

Study population

All patients were 19 years old and above with or without co-morbidities; had been initially seen at the emergency room and subsequently admitted at the Philippine General Hospital; and diagnosed as COVID-19 by RT PCR positive with or without co-morbidities, were included.

Patients who were pregnant, who had missing data, or whose results were not continuous variables (e.g., INR <1, D-dimer >20) were not included in the study.

COVID-19 risk classification

The criteria for risk classification followed Department of Health (DOH) guidelines.[6]

- **Mild COVID-19** was classified as having an acute onset of fever and cough or any three (3) or more of the following: fever, cough, coryza, sore throat, diarrhea, anorexia/nausea/vomiting, loss of sense of smell or taste, general body weakness/malaise/fatigue, myalgia, headache with no pneumonia or desaturation.
- **Moderate COVID-19** is classified as pneumonia* but with no difficulty of breathing or shortness of breath, RR of less than 30 breaths/minute, $Sp0_2$ saturation of >94% at room air, or without pneumonia but with risk factors for progression: elderly (60 years old and above) and/or without co-morbidities.
- **Severe COVID-19** was classified as pneumonia* and any one of the following: signs of respiratory distress, $Sp0_2$ saturation of <94% at room air, respiratory rate of >30 breaths/minute, requiring oxygen supplementation.
- **Critical COVID-19** was as with pneumonia and any of the following: impending respiratory failure requiring high flow oxygen, non-invasive or invasive ventilation, acute respiratory distress syndrome, sepsis or shock, deteriorating sensorium, multi-organ failure, thrombosis.

Note: Pneumonia was diagnosed as evidence of lower respiratory disease during clinical assessment (e.g., cough, fever, plus crackles) and/or imaging such as chest x-ray, ultrasound or CT scan

Data analysis

Data gathered were encoded in Microsoft Excel 2019. Analysis was done using STATA 14.0. Descriptive statistics were employed in describing the socio-demographic and clinical characteristics of the patients. The mean was calculated for all continuous variables and proportions were calculated for all categorical variables. Pearson chi-square test was done to determine the association of the variables. Shapiro Wilk test was employed to test

Table 1. Demographic profile (age and sex) of all samples included in the study

Variable	Mean/Percentages n=385
Age (in years)	56.18
Sex	
Male	194 (50.39%)
Female	191 (49.61%)

Table 2. Demographic and clinical profiles of all samples across disease severity

Variable	Mild (n=30)	Moderate (n=105)	Severe (n=141)	Critical (n=109)
Age (in years)	46.73 ± 2.84	52.42 ± 1.66	57.10 ± 1.39	61.20 ± 1.43
Sex				
Male	(53.33%, 16)	(50.50%, 53)	(54.60%, 77)	(44.00%, 48)
Female	(46.67%, 14)	(49.50%, 52)	(45.40%, 64)	(56.00%, 61)
Co-morbidity				
Without co-morbidity (121, 31.43%)	14 (46.67%)	35 (33.33%)	41 (29.08%)	31 (28.44%)
With co-morbidity (264, 68.57%)	16 (53.33%)	70 (66.67%)	100 (70.92%)	78 (71.56%)
Any	6 (37.5%)	25 (35.71%)	38 (38.00%)	24 (30.77%)
Hypertension and Diabetes	2 (12.5%)	18 (25.71%)	28 (28.00%)	22 (28.21%)
Hypertension only	6 (37.5%)	20 (28.57%)	25 (25.00%)	19 (24.36%)
Diabetes only	1 (6.25%)	2 (2.86%)	7 (7.00%)	10 (12.82%)
Bronchial asthma only	1 (6.25%)	5 (7.14%)	2 (2.00%)	3 (3.85%)

if the data in each variable were normally distributed. If normally distributed, the means of the coagulation and platelet variables were compared across the categories of disease severity using One-way ANOVA. A post hoc test was performed. If the distribution was not normal, Kruskall-Wallis test was employed for comparison across disease severity.

Ethical considerations

This study was approved by the UPM Research Ethics Board committee (UPM REB Code 2023-0509-01). The study was done following the National Ethical Guidelines for Health-Related Research (NEGHHR). Patient information and all information regarding the identity of the patients were concealed ensuring patient privacy and confidentiality at all times.

Since this study involved a review of medical records, informed consent for the study was deemed unnecessary and impractical. A waiver of informed consent was requested and approved by the UPMREB panel since the study presented no more than minimal risk by the provisions (provision 11.2) stipulated in the 2017 National Ethical Guidelines for Health and Health-related Research.[12]

RESULTS

A total of 385 patients were included; 194 were males, and 191 were females. The mean age of all patients was 56.18 years old (Table 1). There was a total of 30 patients classified as mild. There were 105 patients under the moderate category. One hundred forty-one (141) patients were classified as severe, whereas 109 patients were classified as critical.

The mean age of patients in mild, moderate, severe, and critical COVID-19 patients was 46.73 years, 52.42 years, 57.10 years and 61.20 years, respectively (Table 2). The mean age was highest among patients with critical COVID-19 disease. The majority of the disease severity has the same sexual distribution as having more males than females except for those with critical COVID-19 (females>males). The proportion of patients with co-morbidities (68.57%) was higher than those without co-morbidities (31.34%). This was also observed across all disease categories. Those patients with critical COVID-19 had the highest percentage of patients with co-morbidities. The most common co-morbidities were hypertension and diabetes.

In the mild category, a majority of patients had normal platelet count (76.67%), normal prothrombin time (55.23%) and normal activated partial thromboplastin time (93.33%). INR (66.67%) and D-dimer (62.33%) were increased in a majority of mild COVID-19 patients. In the moderate category, most patients had normal platelet count (67.61%), and normal activated partial thromboplastin time (85.71%). There was an increased proportion of patients with prolonged prothrombin time (55.23%), increased INR (80%) and increased D-dimer (87.62%) in a majority of moderate COVID-19 patients. In the severe category, most patients had normal platelet count (75.23%), normal prothrombin time (50.46%), and normal activated partial thromboplastin time (88.07%). Moreover, there was a higher proportion of patients with increased INR and increased D-dimer in the critical category. Using the Pearson Chi-square test, there was a significant association between prothrombin time and d-dimer levels and the disease severity (p-values <0.05). There was no significant difference between platelet count, activated partial thromboplastin time, INR and disease severity (Table 3).

One of the objectives of the study was to compare the coagulation and platelet variables among the different COVID-19 disease categories. To compute this, the study used Kruskall-Wallis test to determine which among the mean of the variables had a significant difference with at least one pair of COVID-19 disease categories. Prothrombin time activated partial thromboplastin time, INR and D-dimer had a p <0.05 hence those variables had at least one pair of disease severity with a significant difference. To identify which pair of disease severity had significant differences, the study used the Dunn test. In the Dunn test, for the prothrombin time, there is a significant difference in the mean prothrombin time of mild vs moderate, mild versus critical and moderate versus severe category. In the activated partial thromboplastin time, no disease pairing had a significant difference. In the INR variable, there was a significance difference in the mild versus moderate, mild versus critical and moderate versus severe pairing categories. In the D-dimer variable, almost all the pairings have significant differences except moderate vs severe.

DISCUSSION

We described the demographic and clinical profiles as well as the coagulation and platelet profiles of patients with mild, moderate, severe and critical COVID-19 patients.

Table 3. Coagulation and platelet profiles of patients across disease severity

Variable	COVID-19 disease severity				
	Mild (n=30)	Moderate (n=105)	Severe (n=141)	Critical (n=109)	*p*
Platelet count					
Below normal (<150 x 10^9/L)	5 (16.67%)	19 (18.10%)	20 (14.18%)	14 (12.84%)	0.832
Normal (150-450 x 10^9/L)	23 (76.67%)	71 (67.61%)	104 (73.76%)	82 (75.23%)	
Above normal (>450 x 10^9/L)	2 (6.66%)	15 (14.29%)	17 (12.06%)	13 (11.93%)	
Prothrombin time					
Normal (≤11-4-13.9 sec)	24 (80.00%)	47 (44.76%)	85 (60.28%)	54 (49.54%)	**0.002**
Prolonged (>13.9 sec)	6 (20.00%)	58 (55.23%)	56 (39.72%)	55 (50.46%)	
APTT					
Normal (25-35 sec or less)	28 (93.33%)	90 (85.71%)	124 (87.94%)	96 (88.07%)	0.731
Prolonged (>35 sec)	2 (6.67%)	15 (14.29%)	17 (12.06%)	13 (11.93%)	
INR					
Normal (1 or less)	10 (33.33%)	21 (20.00%)	41 (29.08%)	26 (23.85%)	0.290
Increased (>1)	20 (66.67%)	84 (80.00%)	100 (70.92%)	83 (76.15%)	
D-dimer					
Normal (0-0.50 ug/mL)	11 (36.67%)	13 (12.38%)	6 (4.26%)	3 (2.75%)	**<0.001**
Increased (>0.50 ug/mL)	19 (63.33%)	92 (87.62%)	135 (95.74%)	106 (97.25%)	

Table 4. Kruskall-Wallis and Dunn Test on platelet and coagulation variables and disease severity

Variable	Kruskall-Wallis test (*p*)	Dunn test					
		Mild vs moderate	Mild vs severe	Mild vs critical	Moderate vs severe	Moderate vs critical	Severe vs critical
Platelet count	0.4382	-	-	-	-	-	-
Prothrombin time	0.0005	0.0012	0.2246	0.0085	0.0104	1.0000	0.1317
APTT	0.0306	0.1326	0.1545	1.0000	1.0000	0.0862	0.0950
INR	0.0009	0.0014	0.1959	0.0089	0.0189	1.0000	0.1723
D-dimer	0.0001	0.0003	0.0008	0.0000	1.0000	0.0073	0.0005

There was a total of 385 patients included in the study with a mean age of 56.18 years old. This was consistent with other studies, where the mean age is 53 years old or between (40-64 years old).[13] Most of the patients in this study were males, similar to other studies regarding COVID-19. A study by Ibrahim et al., attributed the X chromosome in women to play an essential role in innate and adaptive immunity, hence fewer women succumbed to COVID-19 than men. The study also reported that the majority of patients with COVID-19 had co-morbidities during the time of their admission.[14] Those in the critical category had the highest percentage of patients with co-morbidities. This was also a trend seen in other studies due to the increased susceptibility of patients with co-morbidities with COVID-19 severe infection. Among the co-morbidities, hypertension and diabetes were the most common co-morbidities which were seen also in other studies.[15]

Most of the patients exhibited normal platelet counts in all disease categories. This was also seen in the study by Abd El-Lateef et al., wherein the average platelet count was within normal limits. This could be due to the presence of lung inflammation that can increase the secretion of thrombopoietin which stimulates platelet production in COVID-19 patients.[8] There was no significant difference in the mean platelet count among the COVID-19 categories (mild, moderate, severe, critical). Other studies showed that lower platelet counts are seen in patients with more severe disease.[16]

The majority of patients with COVID-19 in this study had normal prothrombin and activated partial thromboplastin time. This was also seen in the study by Liao et al., where most of their patients had normal PT and APTT during the time of admission.[11] This may be attributed to hyper-coagulability occurring at the early stage of COVID-19. However, other studies showed elevated PT, APTT as well as INR at the time of admission.[4]

Most patients had increased INR across all categories which may be due to the activation of molecular pathways induced by SARS-CoV-2 infection which caused intrinsic and extrinsic coagulation cascades leading to increased APTT and INR.[8]

Another finding in the study was that D-dimer was elevated in all disease categories. This could be seen also in the study by Liao et al., showing non-survivors have increasing D-dimer and fibrin degradation products.[11] In the early stages of COVID-19 disease, damage to the endothelium caused by the SARS-CoV virus could lead to hypercoagulation with subsequent release of thrombin in the absence of fibrinolysis hence increasing the level of D-dimer.[8]

The investigators found that there was a significant association between prothrombin time, D-dimer and disease severity. Moreover, the means of prothrombin time, activated partial thromboplastin time, INR, and D-dimer had significant differences versus the disease category. And among the variables, D-dimer showed a significant difference in most of the categories compared. Other blood parameters in a similar study showed significant differences in the disease comparison were neutrophil count, lymphocyte count, neutrophil to lymphocyte ratio, fibrin degradation products, C-reactive protein, and lactate dehydrogenase and white blood cell count, IL-10, and serum ferritin.[11]

Our study had some limitations. Aside from the number of cases included, only the initial laboratory results for

the coagulation and platelet profiles of patients were analyzed. Blood parameters were not taken at different stages of the disease to see the dynamic changes in blood coagulation.

CONCLUSION AND RECOMMENDATIONS

In conclusion, the study population had a mean age of 56 years old. The majority were men and had co-morbidities during the time of COVID-19 admission. Platelet counts and APTT were within normal limits across all disease severity. The PT results were mostly normal in mild and severe cases, however, prolonged in those with moderate and critical categories. INR and D-dimer are increased in all disease categories. The prothrombin time and d-dimer had a significant association with disease severity. The means of prothrombin time, APTT, INR and d-dimer all have significant differences versus disease severity.

In future studies regarding coagulation, it is recommended to investigate the association of coagulation and platelet variables with survivorship and disease outcome. Additional coagulation parameters such as fibrinogen and other inflammatory biomarkers may complete the picture. Coagulation parameters measured at different disease stages or with treatment effect (before, during and after treatment) to see coagulation patterns would yield useful information.

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STATEMENT OF AUTHORSHIP

The authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

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OPEN ACCESS – ORIGINAL ARTICLE

Breast Panel Biomarker Changes After Neoadjuvant Chemotherapy in Breast Cancer: A Single-center Study

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ABSTRACT

Objectives. The aim of this study is to evaluate the breast panel biomarker changes and tumor intrinsic subtype after neoadjuvant chemotherapy among patients with residual invasive breast carcinoma whose breast specimens were processed at St. Luke's Medical Center - Quezon City (SLMC-QC) from 1 January 2017 to 30 June 2023.

Methodology. Cases of residual invasive breast carcinoma status post neoadjuvant systemic therapy were identified by retrospective review of cases. The baseline characteristics, type of biopsy and resection procedures, pre – and post–neoadjuvant ER, PR and HER2 status and pre – and post–neoadjuvant tumor intrinsic subtype were analyzed using frequency and percentage. The comparison of the changes in pre- and post-neoadjuvant breast panel biomarkers were analyzed by using McNemar test while the changes in the intrinsic tumor subtype was done using Wilcoxon signed-rank test.

Results. This study encompassed a total of 43 cases of residual invasive breast carcinoma following neoadjuvant systemic therapy. The data disclosed shifts in the breast molecular profile and intrinsic subtype post-administration of neoadjuvant systemic therapy. The alterations in hormone receptor status, ER and PR, were observed in 11.6% of cases, while HER-2 status exhibited changes in 2.3%. A 14% change in the tumor intrinsic subtype is observed. Among the initial 18 Luminal A cases, 1 transitioned to Luminal B, and among the 6 Luminal B cases, 2 become HER2 enriched subtypes. Furthermore, among the initial 12 HER2 enriched cases, three shifted to Luminal B, while all triple-negative cases remained unchanged after chemotherapy.

Conclusion. Based on our findings, alterations in the molecular profile of breast tumors, including shifts in intrinsic subtype after neoadjuvant chemotherapy (NAC), could impact patient prognosis. While the data generated from this study may not exhibit statistical significance, its clinical relevance is noteworthy. In summary, retesting of breast biomarkers in the resection specimen is recommended to accurately ascertain the appropriate use of targeted therapy.

Key words: residual invasive breast carcinoma, neoadjuvant systemic therapy, breast panel

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INTRODUCTION

Neoadjuvant chemotherapy (NACT) for invasive breast carcinoma is the standard of care for locally advanced and an alternative option for primary operable invasive breast carcinoma (IBCA). Changes in biomarker expression after neoadjuvant chemotherapy have been recorded, with an average prevalence rate reaching up to 18% for estrogen receptor, 32% for progesterone receptor, and 6% for HER2/Neu.[1,2] Currently, there is no universally agreed-upon guideline for the retesting of these biomarkers post-neoadjuvant chemotherapy, leading to considerable variability in practices.

A global working group was assembled to formulate practical guidelines for the pathological evaluation of residual disease in neoadjuvant clinical trials for breast cancer. The group suggests that the retesting of ER, PR, and HER2 should be based in the initial biopsy testing results, the pathological characteristics of the remaining tumor, and the potential influence on treatment decisions.[3] In 2020, a survey involving 26 pathologists across the United States revealed that 15 out of 25 respondents engage in routine retesting of NACT samples. Primarily,

the retesting of breast biomarkers was initiated either in response to clinical requests from oncologists or as a necessity dictated by clinical trial protocols.[4]

This study aimed to evaluate the breast panel biomarkers and tumor intrinsic subtype changes among patients with residual invasive breast carcinoma after neoadjuvant systemic therapy in a single center.

METHODOLOGY

This is an observational, descriptive, cohort, single-center study. This study was approved by the Institutional Ethics Review Committee (IERC) of St. Luke's Medical Center – Quezon City (SLMC-QC).

Patient selection

Eighty-six (86) cases of residual invasive breast carcinoma status post neoadjuvant systemic therapy were identified by retrospective review of cases from the records of surgical pathology reports in the laboratory information system from January 2017 to June 2023. The specimens include tissue breast biopsies (core needle, mammotome, incision), total mastectomies, modified radical mastectomies, needle localization excision surgeries, breast panel by immunohistochemical stains (IHC) and/or HER2 fluorescence in situ hybridization (FISH). To be included in this study, the following inclusion criteria were observed: (1) the breast specimens should have been processed or slides were reviewed at SLMC-QC; (2) patient's should have received neoadjuvant systemic therapy and must have residual invasive breast carcinoma on resection surgery; (3) all cases must have pre and post neoadjuvant breast biomarkers by IHC; and/or FISH, (4) the patient must have undergone partial mastectomy, total mastectomy, and or modified radical mastectomy. Patients of all groups were included. Cases with equivocal HER2/c-ERB result and with incomplete data in the laboratory information system were excluded.

Data analysis

Determination of the breast hormonal biomarker changes of post neoadjuvant chemotherapy were analyzed. Frequency and 95% confidence interval of the percentage were calculated. The significance of the change of hormonal biomarkers in pre-neoadjuvant chemotherapy and post- neoadjuvant chemotherapy was determined using McNemar test. The level of significance was set at $\alpha = 0.5$.

RESULTS

A total of 43 breast cancer patients with residual invasive tumor after receiving neoadjuvant systemic therapy were included in the study whose mean age is 53 years old (SD=11.5) while most common biopsy is via core needle (88.4%) and 9.3% are via incision. Around 93% of them underwent modified radical mastectomy while 4.7% had total mastectomy (Table 1).

Data on the estrogen receptor status (ER) reveals that prior to neoadjuvant chemotherapy, 48.8% were positive for ER, but it slightly increased to 55.8% after. Specifically, 11.6% of cases had changes in ER, but this difference is not significant (p =.375). Among the 21 positive ER in pre-NACT, only 1 turns out to be negative after treatment, while among the 22 negative ER in pre-NACT, 4 of them become positive (Table 2).

Data on the progesterone receptor status (PR) reveals that prior to neoadjuvant chemotherapy, 48.8% were positive for PR, but it slightly decreased to 46.5% after. Specifically, 11.6% of cases had changes in PR, but this difference is not significant (p = 1.000). Among the 21 positive PR in pre-NACT, 3 turn out to be negative, while among the 22 negative PR in pre-NACT, 2 become positive (Table 3).

The data on HER2 reveal that prior to neoadjuvant chemotherapy, 41.9% were positive for HER2 but it slightly increases 44.2% after. Specifically, 2.3% of cases had changes in HER2, but this difference is not significant (p=1.000).

Table 1. Profile of Breast Cancer Patient

	Values
Age (years), mean ± SD	53.0 ± 11.5
Sex, n, %	
Female	43 (100)
Biopsy, n, %	
Core needle biopsy	38 (88.4)
Mammotome biopsy	1 (2.3)
Incision biopsy	4 (9.3)
Surgery, n, %	
Total mastectomy	2 (4.7)
Modified Radical Mastectomy	40 (93.0)
Needle localization excision biopsy	1 (2.3)

Table 2. Changes in ER pre- and post-neoadjuvant chemotherapy

ER		N	%
Pre-NACT ER	Positive	21	48.8
	Negative	22	51.2
Post-NACT ER	Positive	24	55.8
	Negative	19	44.2
Change in ER	Yes	5	11.6
	No	38	88.4

Pre-NACT (Pre-neoadjuvant chemotherapy); Post-NACT (Post-neoadjuvant chemotherapy)

Pre-NACT ER	Post-NACT ER		*p* value
	Positive	Negative	
Positive	20	1	0.375
Negative	4	18	

Pre-NACT (Pre-neoadjuvant chemotherapy); Post-NACT (Post-neoadjuvant chemotherapy)

Table 3. Changes in PR pre- and post-neoadjuvant chemotherapy

PR		n	%
Pre-NACT PR	Positive	21	48.8
	Negative	22	51.2
Post-NACT PR	Positive	20	46.5
	Negative	23	53.5
Change in PR	Yes	5	11.6
	No	38	88.4

Pre-NACT (Pre-neoadjuvant chemotherapy); Post-NACT (Post-neoadjuvant chemotherapy)

Pre-NACT PR	Post-NACT PR		*p* value
	Positive	Negative	
Positive	18	3	1.000
Negative	2	20	

Pre-NACT (Pre-neoadjuvant chemotherapy); Post-NACT (Post-neoadjuvant chemotherapy)

Table 4. Changes in HER2 pre- and post-neoadjuvant chemotherapy

HER2		n	%
Pre-NACT HER2	Positive	18	41.9
	Negative	25	58.1
Post-NACT HER2	Positive	19	44.2
	Negative	24	55.8
Change in HER2	Yes	1	2.3
	No	42	97.7

Pre-NACT (Pre-neoadjuvant chemotherapy); Post-NACT (Post-neoadjuvant chemotherapy)

Pre-NACT HER2	Post-NACT HER2		*p* value
	Positive	Negative	
Positive	18	0	1.000
Negative	1	24	

Pre-NACT (Pre-neoadjuvant chemotherapy); Post-NACT (Post-neoadjuvant chemotherapy)

Table 5. Changes in Sub-Type pre- and post-neoadjuvant chemotherapy

Subtype		n	%
Pre-NACT Subtype	Luminal A	18	41.9
	Luminal B	6	14.0
	HER2 enriched	12	27.9
	Triple negative breast CA	7	16.3
Post-NACT Subtype	Luminal A	17	39.5
	Luminal B	8	18.6
	HER2 enriched	11	25.6
	Triple negative breast CA	7	16.3
Change in Subtype	Yes	6	14.0
	No	37	86.0

Pre-NACT (Pre-neoadjuvant chemotherapy); Post-NACT (Post-neoadjuvant chemotherapy)

Pre	Post				*p* value
	Luminal A	Luminal B	HER2 enriched	Triple negative	
Luminal A	17	1	0	0	1.000
Luminal B	0	4	2	0	
HER2 enriched	0	3	9	0	
Triple negative	0	0	0	7	

All 18 positive remains to be positive while the 24 negative prior, 1 becomes positive after chemotherapy (Table 4).

Table 5 displays the molecular subtypes and their alterations. The predominant subtype before neoadjuvant chemotherapy is Luminal A (41.9%), followed by HER2-enriched (27.9%). Post-chemotherapy, Luminal A decreased to 39.5%, and HER2 decreased to 25.6%. Specifically, 6 out of 43 cases (14%) underwent changes, but this change did not reach statistical significance (p=1.000). Among the initial 18 Luminal A cases, one transitioned to Luminal B, and among the 6 Luminal B cases, two shifted to HER2-enriched. Furthermore, among the initial 12 HER2-enriched cases, three transitioned to Luminal B, while all triple-negative cases remained unchanged after chemotherapy.

DISCUSSION

Currently, there is no universally adopted reporting system for post-neoadjuvant chemotherapy breast carcinoma, and data from current practices show substantial diversity in the retesting of tumor biomarkers following neoadjuvant therapy.[5] According to the 2023 NCCN guidelines for invasive breast carcinoma, the status of ER/PR and HER2 may undergo changes during treatment and metastatic progression. In such cases, it is recommended to consider repeat testing on fresh samples, especially if it would lead to a modification of the treatment approach.[6]

Results from this study revealed that there are alterations in the molecular profile and subtypes of invasive breast carcinoma following neoadjuvant systemic therapy. The observed rates of change in hormone receptors, ER and PR, were both 11.6%, aligning with the deduced findings of Sahoo et al.,[5] of 3% to 8% across reported studies. Furthermore, HER2 status exhibited less frequent changes (1% to 7%) compared to hormone receptors in response to systemic treatment.[2,7-9] The discrepancy in biomarker status between pre-treatment and post-treatment tumors can be attributed to various factors. These include preanalytical variables such as cold ischemia, intratumoral heterogeneity, unsampled tumor regions, and the influence of targeted therapy. More precisely, this refers to situations in which HER2-positive tumors may change to HER2-negative state after receiving HER2-targeted treatments.[5]

Out of the total of 43 cases, only 6 cases (14%) had alterations in the molecular intrinsic subtype after NACT. However, these alterations did not achieve statistical significance, as indicated by a *p*-value of 1.000. Out of the original 18 cases classified as Luminal A, one case changed to Luminal B. Additionally, out of the 6 cases initially classified as Luminal B, two cases changed to HER2-enriched. In addition, out of the original 12 cases classified as HER2-enriched, three cases converted to Luminal B subtype, while all cases classified as triple-negative remained stable during chemotherapy.

Based on these findings, one case developed HER-2 expression. This case is of a 30-year-old female with a Luminal A type of tumor (ER+, PR+, HER2-) and was classified as Luminal B (ER+, PR+, HER2+) in the resection specimen. The patient received 4 cycles of paclitaxel prior to definitive surgery.

Another noteworthy case from this study involves a 72-year-old female who developed a second breast mass with exhibiting distinct histologic and molecular profile that is seen on the modified radical mastectomy specimen. The initial biopsy indicated a HER-2 enriched molecular subtype (ER-, PR-, HER2+), but upon excision, two masses were identified. One mass retained the HER-2 enriched type, while the other mass exhibited a Luminal A subtype (ER+, PR+, HER2-). The second mass may represent a synchronous malignancy that may or may not be present at the initial time of biopsy.

A clinically significant shift in ER, PR, or HER2 status from negative to positive post-neoadjuvant chemotherapy (NAC) might necessitate a modification in adjuvant treatment. For instance, if the initially reported hormone status changes from ER negative to positive, the patient could become eligible for endocrine therapy. Similarly, if the reported biomarker status shifts from HER2 negative to positive, the patient could become a candidate for trastuzumab. However, if there is no alteration in biomarker status or if the reported differences are not clinically relevant, then repeating the testing becomes an additional unnecessary healthcare cost.[8]

CONCLUSION

According to our results, changes in the molecular profile of breast tumors, including shifts in intrinsic subtypes after neoadjuvant chemotherapy (NAC), may influence patient prognosis. Although the data from this study may not demonstrate statistical significance, its clinical relevance is significant. In summary, retesting of breast biomarkers in the resection specimen is recommended to accurately ascertain the appropriate use of targeted therapy. Further research should be pursued to enhance our understanding of the biology and treatment strategies for breast cancer patients.

To optimize cost-effectiveness in resource-constrained settings, adherence to the recommendations of the I-SPY pathology working group is advised, wherein repeat testing is advocated for tumors with unknown or ambiguous biomarker status and if it is necessary for clinical trials. Repeat testing may be warranted for tumors demonstrating heterogeneity, those with multiple tumors with distinct histomorphology, and cases where tumors failed to respond to treatment.[5]

STATEMENT OF AUTHORSHIP

The authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declare no conflict of interest.

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OPEN ACCESS – ORIGINAL ARTICLE

Evaluation of Rapid Antigen Testing (Panbio™ COVID-19 Ag Rapid Test Device) for COVID-19 Diagnosis in a Tertiary Hospital

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ABSTRACT

Background. The Panbio™ COVID-19 Ag Rapid Test is a Food and Drug Administration (FDA)-approved point-of-care test (POCT) used for SARS-CoV-2 detection which has met minimum sensitivity and specificity requirements by the World Health Organization (WHO).

Objective. The study aimed to compare the clinical performance of a commercial lateral flow assay (LFA) to reverse transcriptase polymerase reaction (RT-PCR) in SARS-CoV-2 infection diagnosis.

Methodology. Clinical data and simultaneous LFA and RT-PCR samples collected from June 2021 to June 2022 were obtained to analyze the diagnostic accuracy of LFA compared to RT-PCR.

Results. A total of 265 samples was obtained. 34.45% of RT-PCR positive samples were reliably detected by LFA. COVID-19 was reliably ruled out by LFA in 99.32% RT-PCR negative samples. LFA sensitivity among symptomatic patients with ≤7 days of illness was 51.61%, slightly higher than those with >7 days of illness (18.92%), and significantly higher than asymptomatic patients (16.67%). Asymptomatic subjects have a varied range of Ct-values, indicating different stages of infection or viral loads. Individuals with symptoms for more than 7 days have higher Ct-values, suggesting they are in later stages of infection or have lower viral loads. The probability of a positive LFA result decreases significantly when the Ct-value is beyond 28-30.

Conclusion. The LFA evaluated in this study did not show significant sensitivity and specificity during the early disease course wherein viral loads are suggestively high. However, its utility to accurately rule out COVID-19 is quite reliable in subjects with symptoms that are >7 days since Ct-values are suggestively beyond 28-30 which implies a significantly decreased probability of a positive LFA result.

Key words: COVID-19 Antigen Test, Ct-value, LFA, RT-PCR, SARS-CoV-2

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INTRODUCTION

To date, SARS-CoV-2, the etiologic agent of COVID-19, has infected over 143 million and caused more than 3 million deaths worldwide. Accurate and prompt diagnosis and lately mass vaccination have become key measures in limiting the spread, preventing severe infection, and timely clinical management.

RT-PCR testing is the current diagnostic gold standard for the detection of SARS-CoV-2.[1] However, specialized instruments, dedicated laboratory supplies, and trained personnel are required to conduct the assays. Although the shortages of RT-PCR accredited laboratories and reagent supply have already been addressed, the current turnaround time is still longer than available POCTs such as antigen testing. This hinders early identification of infected individuals which is essential in the containment of transmission.

The Panbio™ COVID-19 Ag Rapid Test (Abbott, USA) is a lateral flow assay (LFA)-based POCT used for SARS-CoV-2 nucleoprotein detection in nasopharyngeal specimens for the diagnosis of COVID-19. It is simple, affordable, can generate results within 15-20 minutes, has been approved by the FDA, and has met the WHO minimum requirements

of sensitivity (≥80%) and specificity (≥97-100%) as specified by the Health Technology Assessment Council (HTAC).[2,3] And among all the published studies showing the comparison of its performance to RT-PCR, the results proved to be promising.

To the knowledge of the authors, no study comparing the performance of LFA and RT-PCR for SARS-CoV-2 detection, particularly Panbio™ COVID-19 Ag Rapid Test, has been done locally at the time of conception of this research. As such, the data on its specificity and sensitivity is limited to internationally published studies and all studies currently available have similar recommendations of utilizing this test only among symptomatic individuals.

METHODOLOGY

Research design

This study employed a retrospective cross-sectional analytical review of the results of simultaneous antigen and RT-PCR testing of patients at a tertiary hospital from June 2021 to June 2022.

Study population

This study involved all patients regardless of age, sex, or symptomatology who underwent simultaneous antigen and RT-PCR testing from June 2021 to June 2022. Symptomatic subjects were those who presented with clinical manifestations of COVID-19 such as fever, cough, dyspnea, etc. while asymptomatic subjects were those who did not present with clinical manifestations. All subjects during the study's duration were initially seen in the emergency room and subsequently discharged or admitted depending on their conditions at that time.

Sample size

All subjects who underwent simultaneous antigen and RT-PCR testing from June 2021 to June 2022 were identified using logbooks and laboratory information systems (LIS). The data collected included the age, symptomatology at the time of testing, duration of symptoms, and risk of exposure to SARS-CoV-2. A target of 100 positive LFA results comprised the minimum sample size required as recommended by the National Institute for Public Health and the Environment. Purposive sampling method was utilized. The collected data variables were encoded and tabulated accordingly. Descriptive data (frequency and percentages) and graphs or figures were constructed using Microsoft Excel Sheet Software ver. 16.66.1 (volume license 2019). Population characteristics were reported as mean. Difference testing for comparisons of groups was performed by Chi-square testing for categorical variables, independent samples Student's t-tests with Welch's correction for continuous normally distributed variables, and by using Mann-Whitney U tests for not non-normally distributed variables. Specificity and sensitivity with 95% confidence intervals and positive and negative predictive values of the LFA were calculated using the RT-PCR results as a reference test. Factors associated with LFA results were determined using logistic regression, using Nagelkerke's pseudo R2 as a measure of goodness-of-fit. Data were analyzed using a free, open-source software environment.

Ethical considerations

This study entailed a review of antigen and RT-PCR testing results through access to logbooks and LIS and a review of pertinent demographic and clinical data through access to case investigation forms. Only the investigators had access to the personal data of the participants. Data collection, gathering, and analysis commenced upon approval of the Research Ethics Committee and the study was conducted in accordance with Good Clinical Practice (GCP) principles and guidelines. Safeguarding patient information was ensured during data collection and encoding. Patient identifiers were excluded from the study. No patient interaction occurred throughout the study.

RESULTS

Table 1 presents the clinicodemographic profile of subjects who underwent simultaneous antigen and RT-PCR testing for SARS-CoV-2 at NKTI from June 2021 to June 2022.

Table 2 gives the overall diagnostic accuracy of LFA-based Panbio. The rapid antigen test was able to identify only slightly over a third of SARS-CoV-2 infected subjects (Sn: 34.45%); the rest were missed cases (65.55%). However, its ability to rule out COVID-19 correctly was near perfect (Sp: 99.32%). In terms of reliability of the Panbio results, nearly all that returned positive on this test were confirmed with COVID-19 (PPV: 97.62%), with only 1 (2.38%) instance of a false alarm. On the other hand, 34.98% of negative

Table 1. Clinicodemographic profile (n=265)

	All	PCR + (n=119)	PCR - (n=146)	*P*
	Median (Range); Frequency (%)			
Age, years	49.50 (12-82)	55 (16-82)	45 (12-81)	**<.001**§
Sex				
Female	132 (49.81)	58 (48.74)	74 (50.68)	.805†
Male	133 (50.19)	61 (51.26)	72 (49.32)	.805†
Declared as symptomatic	187 (70.57)	100 (84.03)	87 (59.59)	**<.001**†
Symptom duration, days				
≤7	74 (39.57)	31 (31.00)	43 (49.43)	**<.001**†
>7	49 (26.20)	36 (36.00)	13 (14.94)	**<.001**†
Unknown	64 (34.22)	33 (33.00)	31 (35.63)	**<.001**†
Ct-value				
ORF-1ab gene (n=103)	33.73 (17.80-40.81)	33.73 (17.80-40.81)	-	
N gene (n=117)	33.03 (14.19-40.00)	33.03 (14.19-40.00)	-	
LFA (Panbio™) result				
Positive	42 (5.85)	41 (34.45)	1 (0.68)	**<.001**§
Negative	223 (84.15)	78 (65.55)	145 (99.32)	**<.001**§

Statistical analysis used: §–Mann-Whitney; †–Fisher's exact test.

Table 2. Overall Diagnostic accuracy of LFA (Panbio™) test for SARS-CoV-2 (n=265)

LFA (Panbio™)	RT-PCR		Total
	Positive	Negative	
Positive	41	1	42
Negative	78	145	223
Total	119	146	265
Sensitivity, % (95% CI)	34.45 (25.98–43.72)	Positive LR (95% CI)	50.30 (7.02–360.32)
Specificity, % (95% CI)	99.32 (96.24–99.98)	Negative LR (95% CI)	0.66 (0.58–0.75)
PPV, % (95% CI)	97.62 (87.43–99.94)	Accuracy, % (95% CI)	70.19 (64.29–75.63)
NPV, % (95% CI)	65.02 (58.37–71.27)		

LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.
Likelihood ratios were estimated using the substitution formula where 0.5 was added to all cell frequencies before calculation.

Table 3. Diagnostic accuracy of LFA (Panbio™) test for SARS-CoV-2 (n=265) according to symptomatology and/or duration of symptoms

	Asymptomatic			≤7 days			>7 days		
	PCR								
Antigen	+	-	Total	+	-	Total	+	-	Total
+	3	1	4	16	0	16	7	0	7
-	15	57	72	15	44	59	30	13	33
Total	18	58	76	31	44	75	37	13	40
Sensitivity, % (95% CI)	16.67 (3.58-41.42)			51.61 (33.06-69.85)			18.92 (7.96-35.16)		
Specificity, % (95% CI)	98.28 (90.76-99.96)			100 (91.96-100.00)			100 (75.29-100.00)		
PPV, % (95% CI)	75.00 (24.94-96.44)			100 (79.41-100.00)			100 (59.04-100.00)		
NPV, % (95% CI)	79.17 (75.50-82.41)			74.58 (67.10-80.84)			30.23 (27.05-33.61)		
Positive LR (95% CI)	9.67 (1.07 to 87.29)			n/a			n/a		
Negative LR (95% CI)	0.85 (0.69 to 1.05)			0.48 (0.34-0.70)			0.81 (0.69-0.95)		
Accuracy, % (95% CI)	78.95 (68.08% to 87.46%)			80 (69.17-88.35)			40 (26.41-54.82)		

LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.
Likelihood ratios were estimated using the substitution formula where 0.5 was added to all cell frequencies before calculation.

Panbio tests were false results (NPV: 65.02%). Confirmed COVID-19 cases were about 50 times as likely to get positive Panbio results as non-COVID subjects did (LR+: 50.30). The former also tested negative in Panbio at a frequency of about two-thirds as much as non-COVID subjects did (LR-: 0.66).

Table 3 gives the diagnostic accuracy of LFA-based Panbio according to symptomatology and/or duration of symptoms. Discussion on this is in the next three paragraphs.

Among asymptomatic subjects, the results are as follows: less than one-fifth of SARS-CoV-2 infected subjects (Sn: 16.67%) were identified by rapid antigen testing while missed cases were more than four-fifths (83.33%). Its ability to rule out COVID-19 correctly was near perfect (Sp: 98.28%). In terms of reliability, only two-thirds read as positive on this test were confirmed with COVID-19 (PPV: 75.00%), with 15 (25.00%) instances of false positives. On the other hand, the probability that the SARS-CoV-2 was not present when the test was negative is higher (NPV: 79.17%). Confirmed COVID-19 cases were about 10 times as likely to get positive Panbio results as non-COVID subjects did (LR+: 9.67). The former also tested negative in Panbio at a frequency of more than four-fifths as much as non-COVID subjects did (LR-: 0.85).

Among subjects with symptoms for ≤7 days, the results are as follows: A little over half (Sn: 51.61%) were identified and less than half (48.39%) were not. COVID-19 was correctly ruled out in all SARS-Cov-2-negative cases (Sp: 100%). Positivity for SARS-CoV-2 was confirmed in all true SARS-CoV-2-positive cases (PPV: 100%), showing no false positives, while negativity was confirmed in three out of four SARS-Cov-2-negative cases (NPV: 74.58%). Confirmed COVID-19 cases tested negative in Panbio at a frequency of about half as much as non-COVID subjects did (LR-: 0.48).

Among subjects with symptoms for >7 days results are as follows: Around one-fifth (Sn: 18.92%) were detected and more than four-fifths (81.08%) were not. SARS-Cov-2-negative cases were ruled out completely in true negative cases (Sp: 100%). SARS-Cov-2 positive cases were confirmed within all cases (PPV: 100%), showing no false positive. SARS-Cov-2-negative cases were confirmed only in one out of three instances (NPV: 30.23%). Confirmed COVID-19 cases tested negative in Panbio™ at a frequency of more than four-fifths as much as non-COVID subjects did (LR-: 0.48).

Figure 1 shows that for both the E and N genes, cases that were negative on LFA but positive on RT-PCR denote potential false negatives of the LFA. With 62 and 76 cases for the E and N genes, respectively, this highlights instances where the LFA might have missed detecting the virus, even when the RT-PCR indicated a positive result. On the other hand, the positive LFA and positive RT-PCR cases, 41 for both genes, suggest instances where the LFA correctly identified the presence of the virus. The distribution of LFA-positive cases in both genes is along lower Ct-values in contrast to the distribution of LFA-negative cases along higher Ct-values.

In Figures 2 and 3, lower Ct-values, situated on the left end of the spectrum, predominantly correspond with positive LFA results (LFA=1), indicating a higher likelihood or

risk of the condition under study. Conversely, as Ct-value increases, there is a pronounced shift towards negative LFA outcomes (LFA=0), signaling a reduced risk or absence of the condition. The inflection point in the middle of the graph denotes a Ct-threshold (30 and 28 for E and N genes, respectively), beyond which the probability of a positive LFA result decreases significantly.

In Figures 4 and 5, the link between symptom duration and Ct-values is shown. The higher the Ct-value, the lower the viral load, which can suggest less severity or later stages of an infection. Asymptomatic subjects seem to have a varied range of Ct-values, indicating different stages of infection or viral loads. On the other hand, individuals who've shown symptoms for more than 7 days tend to have higher Ct-values, suggesting they might be in a later stage of infection or have a lower viral load.

DISCUSSION

Based on the latest interim guidelines released by the United States Centers for Disease Control and Prevention (US CDC), a positive antigen test can be reliably used for symptomatic patients due to the high specificity of the test.[4] However, relative precaution is warranted in the interpretation of the results of asymptomatic patients hence an algorithm was formulated for this population. He et al. inferred that COVID-19 infectiousness begins at 2-3 days prior to symptom onset, peaks around symptom onset, and takes 9-10 days in total.[5]

The results in this study agree with previous literature when plotting cycle threshold (Ct) values of nasopharyngeal swab specimens against the time after the symptom onset. Lowest Ct-values, which studies propose to correspond to

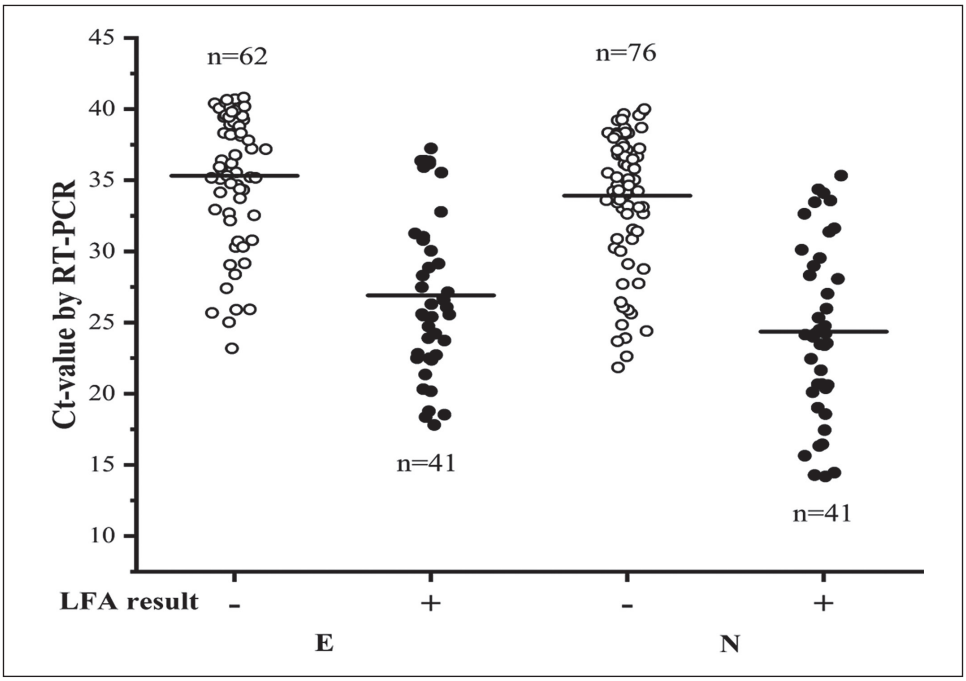

Figure 1. RT-PCR and LFA results of all participants.

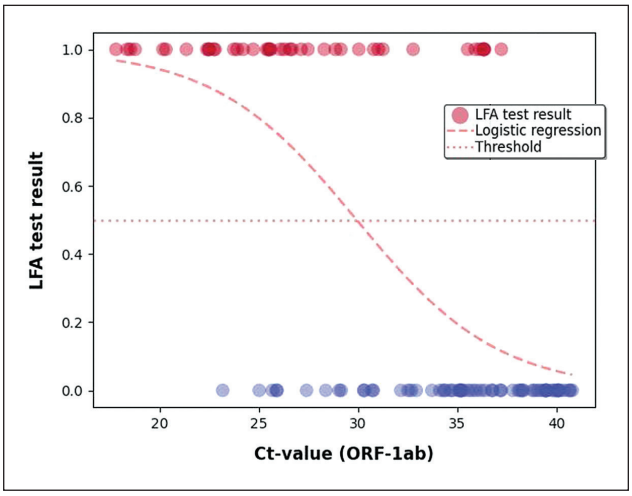

Figure 2. Association between LFA test results and E gene as the Ct-value.

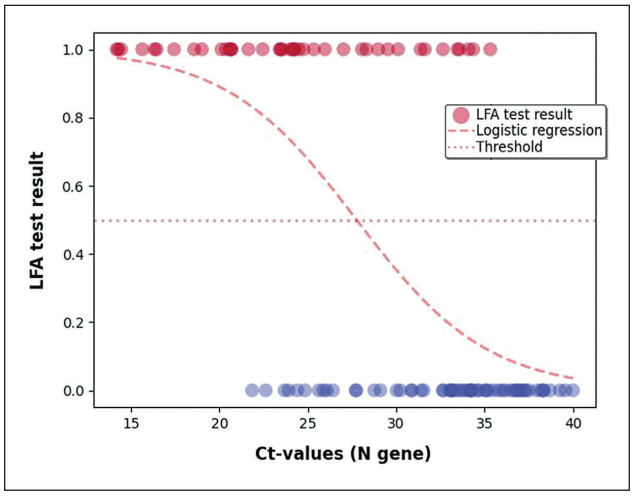

Figure 3. Association between LFA test results and N gene (N2 gene for GX) as the Ct-value.

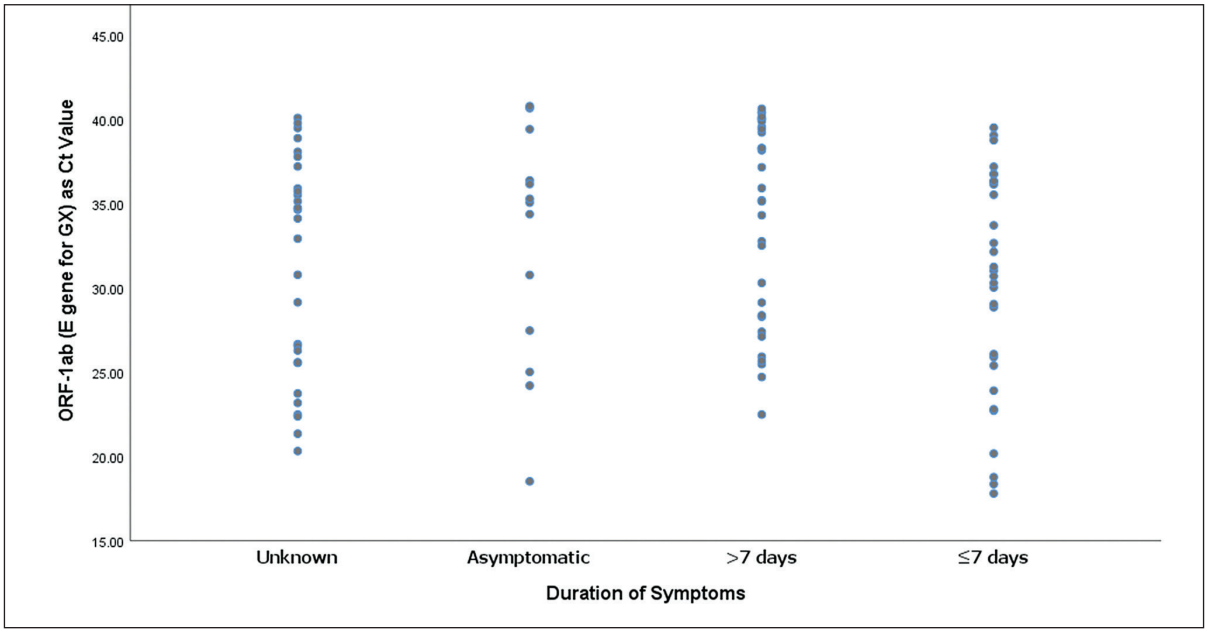

Figure 4. E gene as Ct-Value of RT-PCR positive subjects grouped by duration of symptoms.

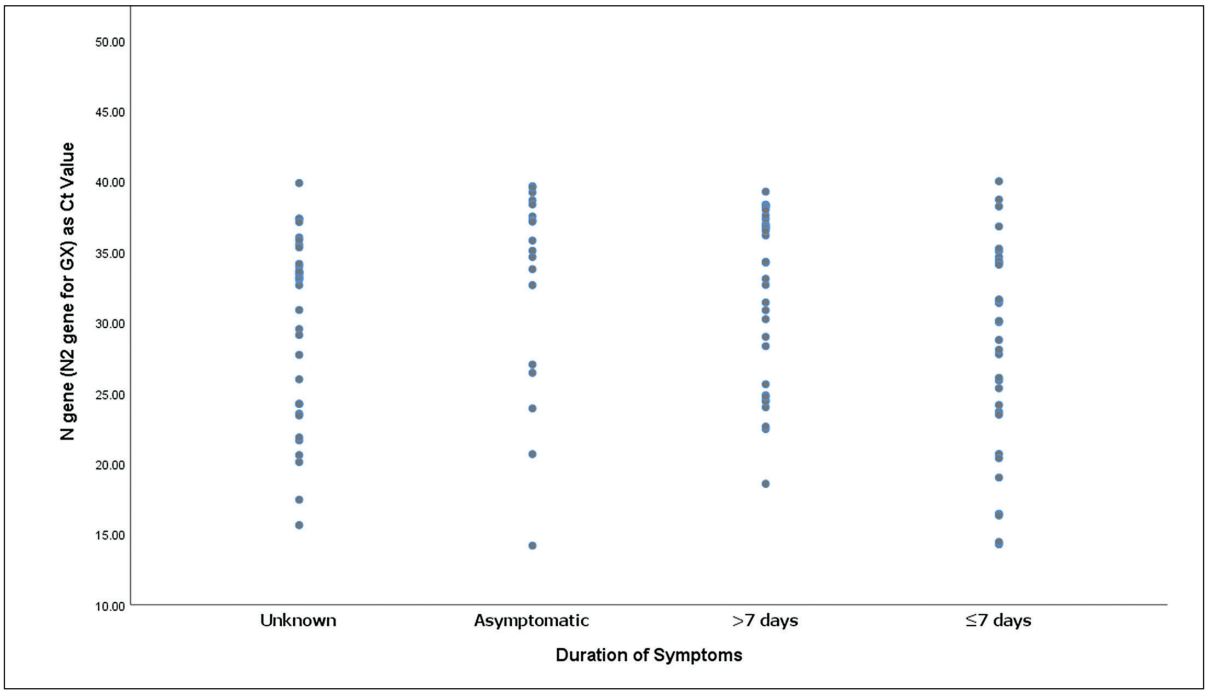

Figure 5. N gene as Ct-value of RT-PCR positive subjects grouped by duration of symptoms.

the highest virus loads, occurred early after the symptom onset, followed by a decline in virus load with increasing time after the symptom onset.[6-8] In addition, sensitivity is noted to decrease as duration of illness becomes more prolonged. This decreasing trend was not elucidated in previous studies although overall LFA sensitivity (34.45%) in this study is significantly lower than in previous studies which listed values over 70%.[8,9]

The reliability of LFA to detect SARS-CoV-2 positivity in truly infected patients (PPV: 97.62%) is comparable to previous literature showing that nearly all that tested positive on this test were truly positive for COVID-19.[8] On the other hand, the reliability of not detecting SARS-CoV-2 in truly uninfected patients is not as high (NPV: 65.02%).[8] These measures of reliability, as well as likelihood ratio, in relation to symptomatology, were not elucidated in previous studies.

As previously mentioned, studies propose that the lower the Ct-value, the higher the viral load, implying higher severity or earlier stages of an infection. However, this notion cannot be entirely supported since the association of the duration of symptoms and Ct-values was found to be weak.[9] In fact, this is supported by findings in the study showing symptomatic subjects of ≤7 days duration and

symptomatic subjects of >7 days duration both displaying a varied range of Ct-values. Although symptomatic subjects of >7 days duration tend to have higher Ct-values and during this period, Ct-values are suggestively beyond 28-30, which is near the range for clinically relevant levels of SARS-CoV-2 RNA,[9] thus probability of a positive LFA result decreases significantly. In addition, asymptomatic subjects also seem to have a varied range of Ct-values, indicating different stages of infection or viral loads, further weakening the association of duration of symptoms and Ct-values.

CONCLUSION

The LFA evaluated in this study did not show significant sensitivity and specificity in samples obtained during the early course of illness wherein viral loads are suggestively high. However, its utility to accurately rule out COVID-19 is quite reliable, particularly in subjects with symptoms that are >7 days since Ct-values are suggestively beyond 28-30 and the probability of a positive LFA result decreases significantly. Results show that LFA has a nearly perfect ability to rule out COVID-19 correctly in these situations.

STATEMENT OF AUTHORSHIP

The authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

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OPEN ACCESS – ORIGINAL ARTICLE

Proficiency Testing Program for Screening Drug Testing Laboratories in the Philippines, 2009-2019: Experience of the National Reference Laboratory of the East Avenue Medical Center

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ABSTRACT

Background. According to the guidelines of the Department of Health (DOH)'s Health Facilities and Services Regulatory Bureau (HFSRB), accreditation of drug testing laboratories (DTLs) requires annual participation in a proficiency testing (PT) program. Since 2009, the National Reference Laboratory for Environmental and Occupational Health, Toxicology and Micronutrient Assay of the East Avenue Medical Center (NRL-EAMC) has conducted the PT program for DTLs.

Objectives. This article aims to provide a general overview of the PT program conducted for screening drug testing laboratories (SDTLs) and to examine data on laboratories' participation and performance in the PT program.

Methodology. Laboratories registered for the PT program were given ten 3-mL synthetic urine specimens which may or may not contain drugs of abuse such as methamphetamine and tetrahydrocannabinol at or above the cut-off level. Laboratories analyzed the PT specimens using immunoassay test kits. The results of the analysis were reported back to NRL-EAMC. The performance of the laboratories in the PT depends on the number of incorrect responses.

Results. For ten years (2009-2019), 1102 ± 188 laboratories annually participated in the program. The mean passing rate was 96.6 ± 4.8%. The number of laboratories which initially failed the PT program significantly decreased from 2009 (15.1%) to 2012 (1.5%). From 2013 to 2019, only below 2.5% of the participating laboratories initially failed the PT. On average, 48.4 ± 18.4% of the laboratories achieved an excellent performance, 34.0 ± 13.6% had a highly satisfactory performance, and 14.3 ± 5.4% got an acceptable performance.

Conclusion. The continued decreasing number of laboratories which failed the PT signifies the improvement of laboratories in urine drug testing. In general, some laboratories participating in the PT for the first time are the ones which initially fail the PT which could be due to a lack of experience in handling PT test items. The PT program highlights the effectiveness of quality control procedures being implemented in a drug testing laboratory.

Key words: laboratories, quality control, accreditation, drug testing, methamphetamine, tetrahydrocannabinol, proficiency testing

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INTRODUCTION

Drug testing laboratories (DTLs) are important facilities in clinical practice to determine drug overdose, manage mental health and seizure cases, identify exposure risk to illicit drugs, and monitor medication in substance abuse treatment centers. They also play a vital role in forensic toxicology such as monitoring of drug abuse in workplaces.[1] Therefore, it is imperative that they provide high-quality, accurate, and precise test results. In the Philippines, only DOH-accredited DTLs are authorized to conduct drug testing. DTLs accredited by the DOH receive urine specimens and test this matrix to determine the presence and absence of illegal drugs. The laboratories are instruments in the diagnosis, treatment, and monitoring of substance use disorders.

Relative to the Republic Act (RA) 9165 or the "*Comprehensive Dangerous Drugs Act of 2002*", the Dangerous Drugs Board (DDB) issued Board Regulation No. 2 Series of 2003, "*Implementing Rules and Regulations Governing Accreditation of Drug Testing Laboratories in the Philippines*" which provides the technical and administrative requirements for the accreditation of DTLs.[2] Accreditation of DTLs shall be regulated by the DOH's Health Facilities and Services Regulatory Bureau (HFSRB). One of the requirements for DTL's renewal of accreditation is the annual participation and passing a proficiency testing (PT) program. The Board Regulation mandated the NRL-EAMC to conduct a continuing assessment of DTLs' proficiencies through the implementation of a PT program.

The DDB Board Regulation No. 3 Series of 2006, "*Guidelines for the Drug PT Program for DTLs*" defined Proficiency Testing as an "external assessment of a laboratory's performance using samples of known but undisclosed content, to assure competence and reliability of test results." Said unknown samples shall be provided by NRL-EAMC as part of its mandated function. Furthermore, the Board Regulation specified three objectives for the conduct of PT: (1) to assure competency of DTLs and their compliance with the standards of conduct of drug tests; (2) to provide assessment for the regulation of DTLs; and (3) to continually assure the public of Quality Drug Testing Services. It also reiterated that DTLs should participate and pass the annual PT as a requirement for the renewal of their accreditation.[3]

The NRL-EAMC has been providing proficiency testing samples to screening drug testing laboratories (SDTLs) since 2009. The objectives of this article are to provide a general overview of the PT program conducted for SDTLs and to present the results of PT program during the 2009-2019 period. The scope of this article is only limited to the presentation of several participating laboratories and their performance. Highlights on the 2019 PT program are also included.

METHODOLOGY

The NRL-EAMC's PT program for SDTLs follows a cycle from registration up to the reporting of results (Figure 1).

Registration

SDTLs are required to participate annually in the PT program. The registration for the succeeding year's PT starts in November of the current year. Initially, the registration deadline was in September of the succeeding year. Beginning 2018, the scheduled deadline is every May 31 of the current year PT according to the DOH Circular No. 2017-0173.[4] Registration form can be downloaded from NRL-EAMC's website or official Facebook Page. The properly filled out registration form together with the participation fee is sent personally to NRL-EAMC's office or via their preferred courier.

Specimen preparation

The PT specimens are prepared in such a way that they are like those normally tested and have similar levels of determinant (routine specimens). Since it is not feasible to collect large amounts of human urine (e.g., one cycle

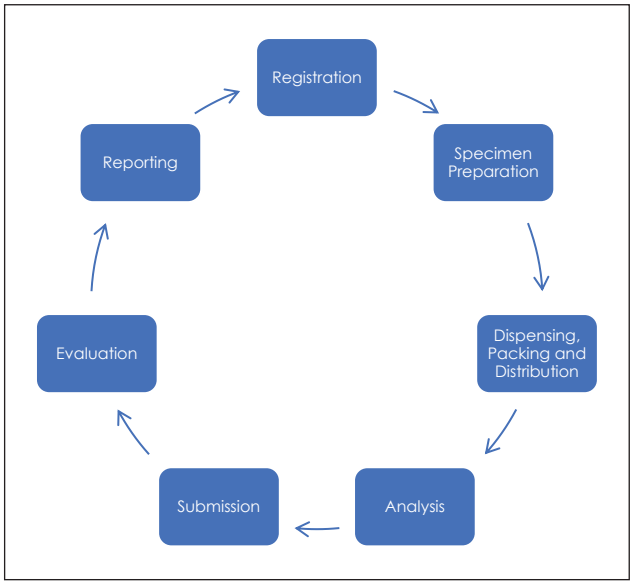

Figure 1. Process flow for the conduct of proficiency testing program for screening drug testing laboratories.

of PT for 500 laboratories requires at least 17 liters of urine), the NRL-EAMC prepares synthetic urine (SU). The SU resembles the common chemicals found in normal human urine. It has been widely used in laboratories for teaching urinalysis concepts[5], and analysis of creatinine and albumin.[6] For PT purposes, it is prepared by dissolving certain amounts of salts, urea, creatinine, acids, and bases (Merck, Darmstadt, Germany) in ultrapure water (18.2 MΩ·cm). Yellow color food dye (obtained from a local supermarket) is added to give its urine-like color. The pH (6 to 7.5) and specific gravity (1.005 to 1.030) are analyzed to be within the acceptable ranges.

The SU is spiked with standard solutions of common drugs of abuse: methamphetamine (Meth) and tetrahydrocannabinol (THC) obtained from Cerilliant®, Millipore-Sigma, Merck, KGaA, Darmstadt, Germany. The spiked concentration is ±50% of the common cut-off values in immunoassay test kits (1000 ng/mL and 500 ng/mL for Meth, and 50 ng/mL for THC). For Meth, synthetic urine is spiked with the standard solution to obtain the following final concentrations: 1500 ng/mL, 500 ng/mL, 750 ng/mL, and 250 ng/mL. For THC, 75 ng/mL and 25 ng/ml. Meth and THC remain to be the most common drugs to be abused.[7] The combinations of these values are used to obtain ten formulations. The formulations' concentrations are initially screened with immunoassay test kits and verified via analysis with gas chromatography-mass spectrometry (Agilent Technologies Inc., Santa Clara, CA, USA) and/or liquid chromatography-mass spectrometry (Thermo Scientific, Waltham, MA, USA).

The PT specimen must have sufficient bias, homogeneity, and stability. The PT bias is sufficient when the spiked concentrations of the analytes are within the acceptable recoveries. Homogeneity is tested by analyzing one PT package consisting of 10 proficiency test items for every 20 participating laboratories. Homogeneity is attained if at least 80% of the results are the same. In one PT cycle, around 30 PT packages are randomly selected

for homogeneity testing. To ensure the stability of the specimen, an aliquot of the formulations is stored in three different storage conditions: room temperature (≈ 25 ºC), cold temperature (≈ 8 ºC) and hot temperature (≈ 35 ºC). The formulations are tested after preparation up to 2 weeks or until all the laboratories have received the PT package. In general, the PT specimens are stable for up to three weeks.

Dispensing, packing and distribution

An aliquot of 3 mL from each formulation is dispensed to cryogenic polypropylene vials. Each PT specimen vial is randomly coded corresponding to each formulation. PT packages containing ten PT specimen vials are sent out to each participating laboratory. Each laboratory is assigned a unique laboratory code. The vial codes and laboratory codes are confidential. Also included in the PT package are the Instructions on handling the PT test items, as well as the Acknowledgement and Results Forms.

Although the PT specimens are synthetic urine, it is still considered to contain biological hazards. As per the International Air Transport Association (IATA), a triple packaging system is imposed. The triple packaging system is composed of plastic airtight cryovials (primary receptacle), zip plastic bag (secondary packaging) and aluminum insulator pouch and courier pouch (tertiary packaging). All packaging materials were obtained from a local supermarket hardware store. Depending on the number of successfully registered laboratories, the PT samples are distributed in 3 to 4 batches/cycles. The PT packages are expected to be delivered by courier within 1 to 2 weeks.

Analysis

The laboratories are expected to analyze the samples as soon as they receive the samples according to their laboratory procedures. Screening methods such as instrumented or immunoassay test kits can be used. Laboratories are advised to strictly follow instruments or test kits' instructions and to properly use quality control materials (negative and positive controls). For each PT specimen, the laboratory must identify the presence or absence of the analytes and report them as positive or negative. A total of twenty responses must be reported (two analytes for each PT specimen).

Submission

According to the DDB Board Regulation No. 3 Series of 2006, DTLs are instructed to submit results within 48 hours through the NRL-EAMC website or Google Forms. Hard copies shall also be submitted to NRL. Annex A. No. 4, of the Board Regulation also requires DTLs to submit test results in two modes: hard copy and online.[3] For the hard copy, the original signatures of the analyst and head of the laboratory are required. Furthermore, cut-off values for the method must be correctly indicated.

Evaluation

Participating laboratories' responses are evaluated according to the modified Metrology of Qualitative Chemical Analysis (MEQUALAN) method for binary responses[8] In this method, correct and incorrect responses are marked with "0" and "1", respectively. The marks are then added. Hence, "0" is the best score. Table 1 presents the ranges of the total score and their corresponding description. A total of 20 incorrect responses are possible (10 each for Meth and THC).

Table 1. Evaluation of PT performance according to total incorrect responses

Total Score (Incorrect Responses) out of 20	Performance Description	Remarks
0 to 2	Excellent	Passed
3 to 5	Highly Satisfactory	Passed
6 to 8	Acceptable	Passed
≥9	Questionable	Initially Failed
Passed Repeat PT	Acceptable	Passed
Failed Repeat PT	Failed	Failed

DDB Board Regulation No. 2 Series of 2003 states that failure in the PT shall result in the suspension of the laboratory's accreditation and must be given a repeat PT (to be included in the next PT cycle/batch). The laboratory's failure in the repeat PT shall result in the revocation of the DTL's accreditation.[2]

Reporting

Every fourth quarter of the year, NRL-EAMC submits the PT reports to HFSRB. These include the list of DTLS which passed the PT, failed the PT, and with pending status. Laboratories with pending status are those with deficiencies such as no or incorrect cut-off value indicated, no or not original signature (electronic or stamped), and no online submission. Laboratories are given two weeks to comply with their deficiencies. Meanwhile, the laboratories receive their certificates of proficiency, performance of laboratories, announcement of the next PT, and registration form.

Data analysis

MS Excel program was used to encode the data from laboratories, generate graphs and tables, and calculate the mean and standard deviation. Excluded in the analysis are the laboratories which 1) did not submit hard copy or online results, 2) did not provide the cut-off values of the method used, 3) indicated invalid cut-off values, 4) had no original signatures of analyst and/or head of the laboratory, and 5) did not follow instructions. The identities of the participating laboratories were kept confidential. Statistical analyses were performed on the aggregate data collected from 2009 to 2019.

RESULTS AND DISCUSSION

PT participation

The majority of SDTLs cater to drug testing services for driver's licenses, pre-employment, and random drug testing in workplaces. In the first year of PT program implementation in 2009, 1045 SDTLs successfully participated. Successful participation refers to the laboratories which registered in the PT program, received the PT package, analyzed the PT samples, and submitted their PT results. In 2010, it was not possible to provide PT samples due to logistical concerns. Thus, PT samples distributed in 2011 covered the 2010-2011 period. The average number of successfully participated laboratories nationwide from 2009 to 2019 is 1102 ± 188 (Figure 2).

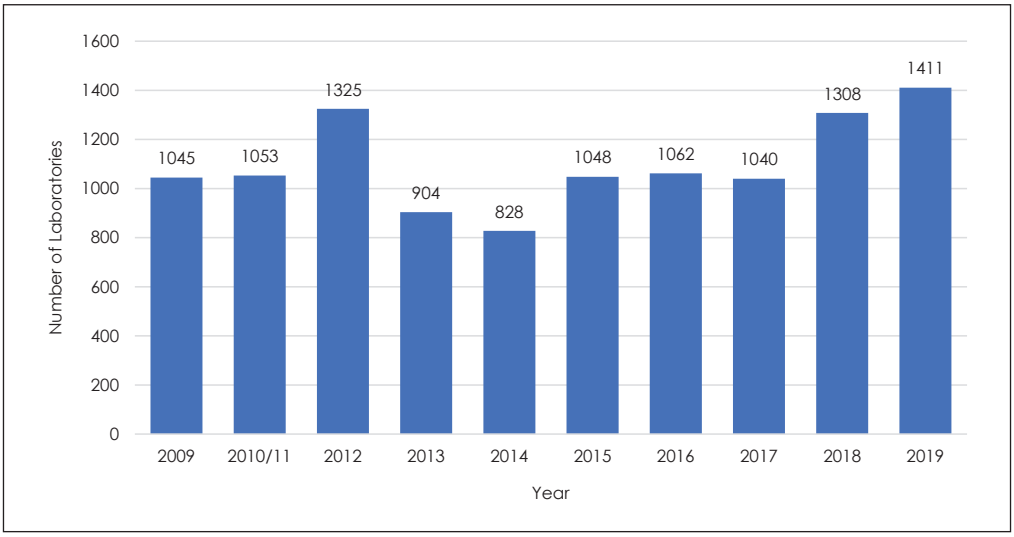

Figure 2. Annual number of participating laboratories during the 2009-2019 PT implementation period.

There was no significant difference in the number of laboratories between 2009 and 2010/2011. By 2012, a 25.8% increase in the number of participating SDTLs was noted. However, in 2013, it went down (-31.8%) to 904 laboratories. This could be due to the enactment of RA 10586 or the Anti-Drunk and Drugged Driving Act of 2013 which prohibits driving a motor vehicle under the influence of alcohol, dangerous drugs, and other similar substances.[9] Under this law, drug testing is only required for drivers when involved in a vehicular accident. Thus, drug testing for the renewal of a driver's license is no longer required. Many Land Transportation Office (LTO)-based SDTLs (laboratories near LTO branches) closed as a result. The decreased number of participating laboratories continued in 2014.

In 2015, there was a 26.6% increase (compared to 2014) in the number of participating laboratories. The number of laboratories remained to be more than 1000 in 2016 and 2017. The increased number of laboratories compared to the previous year (2014) was due to the country's situation and implementation of different drug policies. For instance, the Office of the President under the Aquino Administration (2015) released a memorandum regarding the implementation and institutionalization of the national anti-drug plan of action.[10] The memorandum reiterated the implementation of a drug-free workplace program in the government and private offices. Thus, more laboratories were established or re-established for random drug testing in workplaces. In 2016, then-President Rodrigo Duterte declared his war on drugs[11] which further increased the need for drug testing of individuals. In support of Duterte's drug policy, the Civil Service Commission (CSC) issued Memorandum Circular No. 13 series of 2017, providing guidelines for mandatory random drug testing for public officials and employees.[12] For the private sector, the Department of Labor and Employment (DOLE) reminded employers to comply with Department Order 53-03 regarding the implementation of a drug-free workplace.[13]

The implementation and reiteration of drug testing programs especially in workplaces whether public or private resulted in the need for more drug testing laboratories to conduct authorized random drug testing. In effect, the number of participating laboratories in the PT program continued to increase in 2018 and 2019.

SDTLs are categorized according to ownership, whether private or government. It is also classified according to the nature of the laboratories. Institution-based refers to laboratories which are under a main laboratory, or the laboratory simultaneously offers other clinical laboratory services (e.g., clinical chemistry, hematology). On the other hand, a free-standing laboratory solely offers drug testing services. SDTLs are further classified according to the status of their accreditation whether initial or renewal.

In 2019, 1411 successfully participated in the PT program. A majority (92.8%) of the participants were private laboratories while only 7.2% were government laboratories (Table 2). Of the 1309 private SDTLs, 72.4% were institution-based while 27.6% were free-standing laboratories. From the private institution-based laboratories, 10.4% and 89.6% were on initial and renewal accreditation status, respectively. On the other hand, from the private free-standing laboratories, 6.1% and 93.9% were on initial and renewal accreditation status, respectively. Of the 102 government SDTLs, 94.1% were institution based while 5.9% were free-standing laboratories. From the government institution-based SDTLs, 4.2% and 95.8% were on initial and renewal accreditation status, respectively. On the other hand, from the government free-standing SDTLs, 16.7% and 83.3% were on initial and renewal accreditation status, respectively. Throughout the implementation of the PT program during 2009-2019, the majority of the participating laboratories came from private institution-based SDTLs. Most of the free-standing laboratories were located near an LTO, when drug testing was a requirement for renewal of a driver's license.

According to the 2019 data, almost one-third or 32.6% (460/1411) of the SDTLs were from the National Capital Region (NCR). It was followed by Region IV-A and Region III, which registered 244 (17.3%) and 155 (11.0%)

Table 2. Number of participating laboratories in the 2019 PT program

Government	Institution- based	Initial	4
		Renewal	92
	Free- Standing	Initial	1
		Renewal	5
Private	Institution- based	Initial	99
		Renewal	849
	Free- Standing	Initial	22
		Renewal	339

SDTLs, respectively. The three regions with the highest participating laboratories corresponded with the three regions with the highest populations, according to the census by the Philippine Statistics Authority (PSA). Region VII and Region VI contributed more than 5% of the total SDTLs with 115 (8.2%) and 75 (5.3%) laboratories, respectively. They were followed by Regions XI, I, and X with 56 (4.0%), 45 (3.2%), and 41 (2.9%) participating laboratories, respectively. Regions V and XII both had 36 (2.6%) SDTLs which participated in the PT while Region II had 30 (2.1%). Regions with less than 30 participants were Region IX (27), Region VIII (24), Cordillera Administrative Region (CAR) (22), Region XIII- CARAGA (22), and Region IV-B (18). The region with the lowest number of participating laboratories was the Bangsamoro Autonomous Region of Muslim Mindanao (BARMM) with 5 or 0.4% only of the total SDTLs. The distribution of the participating laboratories in different regions was consistent with the other PT years.

Throughout the 2009-2019 PT implementation period, 100% of the participating laboratories used immunoassay test kits with dual (2-panel) test analytes: methamphetamine and tetrahydrocannabinol, with 1000 or 500 ng/mL and 50 ng/mL cut-off values, respectively. In 2019, around 90% used the drop test kits while the remaining 10% used the dip test kits.

Laboratory performance in PT

The performance of laboratories from 2009-2019 is shown in Figure 3. During the initial implementation of the PT program (2009), 16.6% (173/1045) of the participating laboratories achieved excellent performance. On the other hand, almost half of the participants, 43.9% (459/1045) had a highly satisfactory performance. The number of laboratories with excellent performance is lower than the number of laboratories with highly satisfactory performance since it was the first time that laboratories participated in a PT program. The number of excellent laboratories increased to 47.7% (502/1053) in 2010/2011, further increased to 56.3% (746/1325) in 2012, and peaked at 76.9% (695/904) in 2013. Beginning in 2014, a downward trend in the number of excellent laboratories was observed. In 2014, 65.8% (545/828) had excellent performance which slightly decreased to 63.6% (667/1048) in 2015; further decreased to 51.0% (542/1062) in 2016 until it reached 42.2% (439/1040) in 2017. The percentage of total laboratories with excellent performance in 2018 was 33.7% (441/1308) which decreased to 29.6% (418/1411) in 2019.

For the 2009-2019 period, the trend in the number of laboratories with highly satisfactory performance is opposite to the trend observed in the excellent performance. In 2009, 43.9% (459/1045) had highly satisfactory performance but decreased to 24.7% (260/1053) in 2010/2011. It further decreased to 24.1% (319/1325) in 2012 and 17.3% (156/904) in 2013, which was the lowest during the 2009-2019 period. In 2014, it increased to 20.4% (169/828) and further increased to 24.5% (257/1048) in 2015. The increasing trend in the number of laboratories with highly satisfactory performance continued in 2016 with

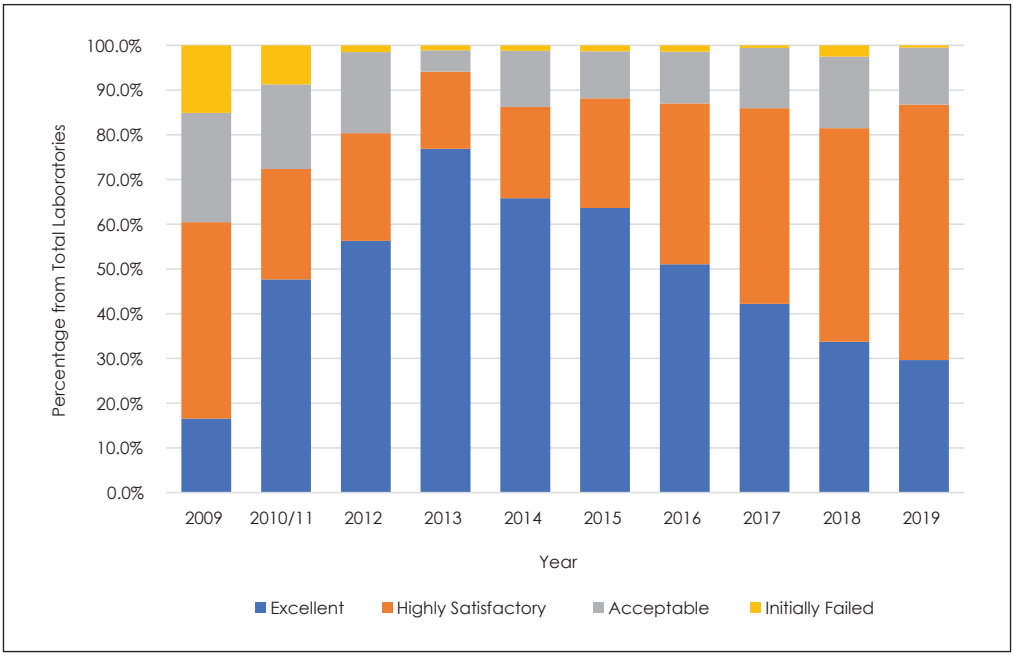

Figure 3. Annual percentage of laboratories achieving excellent, highly satisfactory, and acceptable performance in the PT program (2009-2019).

36.0% (382/1062); 43.8% (455/1040) in 2017; and 47.8% (625/1308) in 2018. The highest percentage was recorded in 2019 with 57.1% (806/1411).

A similar trend with highly satisfactory performance, the number of laboratories with acceptable performance decreased from 2009 to 2013. Laboratories with acceptable performance were lowest in 2013 (4.8%, 43/904) and highest in 2009 (24.4%, 255/1045). On the average, less than 20% of the participating laboratories had acceptable performance during the 2009-2019 period: 24.4% (255/1045) in 2009; 18.9% (199/1053) in 2010/2011; 18.1% (240/1325) in 2012; 4.8% (43/904) in 2013; 12.6% (104/828) in 2014; 10.5% (110/1048) in 2015; 11.6% (123/1062) in 2016; 13.5% (140/1040) in 2017; 16.0% (209/1308) in 2018; and 12.8% (180/1411) in 2019.

The upward and downward trend in the number of excellent laboratories could be correlated to the trend observed in the number of new participating laboratories. New participants tended to obtain highly satisfactory performance on their initial participation as they were not yet familiar with the PT procedures. Participants tend to commit more errors during their first participation in PT program.[14] Moreover, it could be due to the random formulations of the PT specimens every year, i.e., the number of positive and negative specimens would be different every year depending on the results of randomization, making it harder to achieve less than three incorrect responses. Nevertheless, the passing rate for the PT was consistently high: 84.9% (2009), 91.3% (2010/2011), 98.5% (2012), 98.9% (2013), 98.8% (2014), 98.7% (2015), 98.6% (2016), 99.4% (2017), 97.5% (2018), and 99.5% (2019). During the 2009-2019 period, the average passing rate was 96.6% ± 4.8%.

Focusing on the 2019 data (Figure 4), for every region except BARMM, the number of laboratories with highly satisfactory performance was greater than the number of laboratories with excellent performance. The percentages of laboratories with excellent performance in every region were as follows: I- 24.4%, II- 33.3%, III- 34.8%, IV-A- 32.8%, IV-B- 22.2%, V- 36.1%, VI- 36.0%, VII- 19.1%, VIII- 29.2%, IX- 33.3%, X- 14.6%, XI- 21.4%, XII- 33.3%, NCR- 29.8%, CAR- 31.8%, BARMM- 0%, and CARAGA- 31.8%. Region V had the highest percentage of excellent performance while the BARMM had the lowest.

For the highly satisfactory performance, the percentages of participating laboratories in the 2019 PT were: I- 60.0%, II- 56.7%, III- 52.9%, IV-A- 59.4%, IV-B- 72.2%, V- 55.6%, VI- 54.7%, VII- 58.3%, VIII- 45.8%, IX- 59.3%, X- 61.0%, XI- 66.1%, XII- 47.2%, NCR- 56.1%, CAR- 59.1%, BARMM- 100%, and CARAGA- 54.6%. The top three regions with the highest percentages of laboratories with highly satisfactory performance were BARMM, IV-B, and XI, while Region VIII had the lowest.

The percentages of laboratories with acceptable performance in every region were as follows: I- 15.6%, II- 10.0%, III- 11.6%, IV-A- 7.8%, IV-B- 5.6%, V- 8.3%, VI- 9.3%, VII- 21.7%, VIII- 25.0%, IX- 7.4%, X- 22.0%, XI- 12.5%, XII- 16.7%, NCR- 13.5%, CAR- 9.1%, BARMM- 0%, and CARAGA- 13.6%. Region VIII had the highest percentage of excellent performance while the BARMM had the lowest.

Failures in the PT program

According to the DDB Board Regulation, initial failure in PT will result in the suspension of the laboratory's accreditation. In the first year of implementation of PT (2009), 158 laboratories initially failed the PT (Figure 5), corresponding to 15.1% of the total participating laboratories (1045). Since it was the first time, it was expected to have many initially failed participants. Most participants were not yet familiar with the PT program, especially in the conduct of testing the proficiency test specimens and the quality assurance procedures were not yet fully established. In 2010/2011, the initial failed laboratories dropped to 92 or 8.7% of the total participating laboratories (1053). It further decreased to 20 (1.5% of 1325) laboratories in 2012 and remained to be less than 20 until 2017. On average, less than 1.5% of the total participating laboratories initially failed the

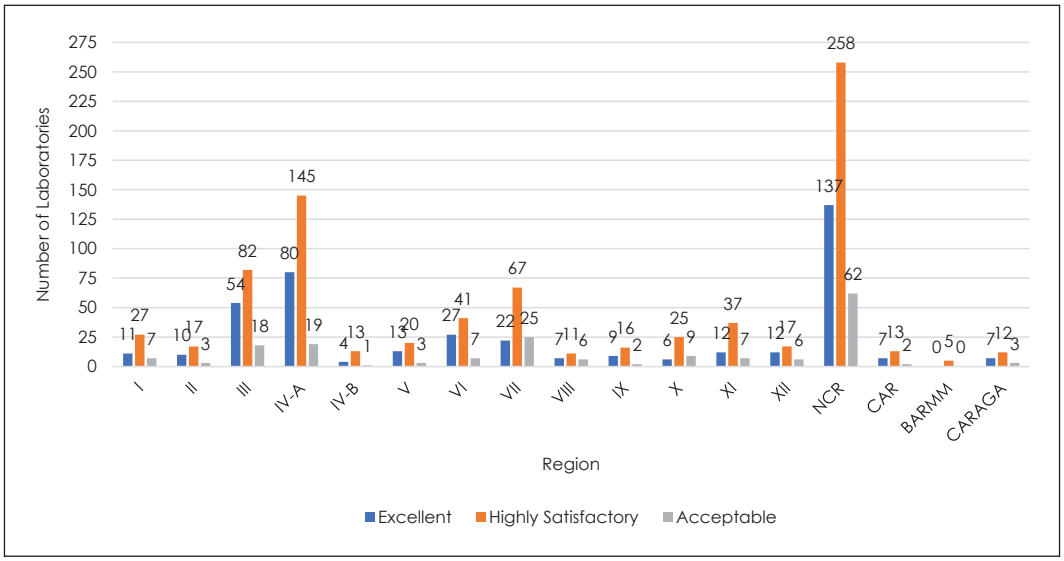

Figure 4. Number of laboratories and their performances in the 2019 PT program.

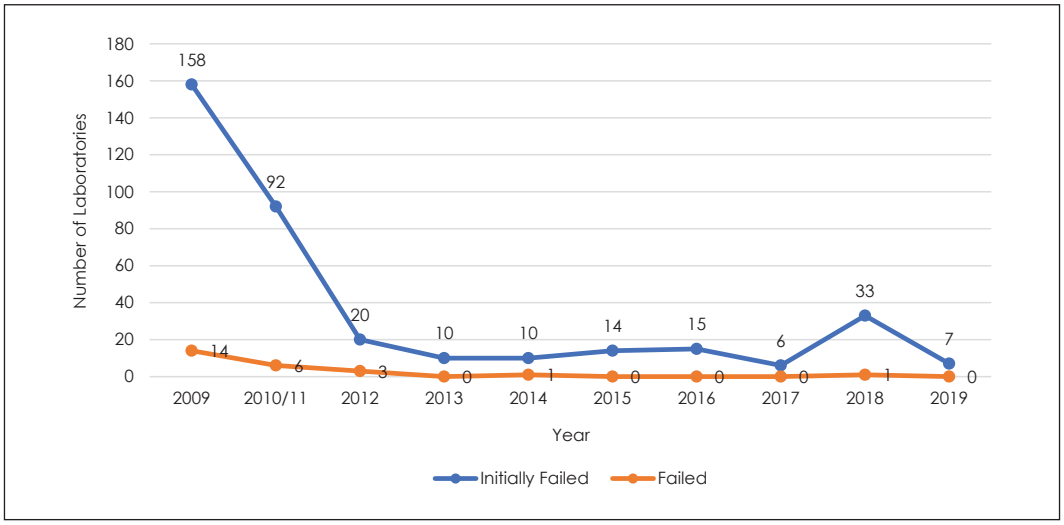

Figure 5. Annual number of initially failed and failed laboratories in the 2009-2019 PT implementation period.

PT during 2012-2019, except in 2018 which had 2.5%. Based on this trend, we can conclude that the proficiency testing program implemented for SDTLs has helped them improve the quality of their tests.

In 2019, seven laboratories initially failed the PT which were all private SDTLs (100%). Three of them came from NCR (two free-standing and one institution-based). On the other hand, one laboratory each from Regions III (institution-based), VII (institution-based), X (free-standing), and XII (institution-based) initially failed the PT. Since NCR had the largest number of participants, it was expected that they would have the highest rate of failure. The trend is similar to previous years.

A review of testing and quality control procedures of the initially failed laboratories revealed that they were not able to follow specific test kit instructions regarding the required temperature and reading time. The immunoassay test kits were also not stored properly which affected their performance. Mistakes in reading the test kits were also noted. Some analysts read the drug test kits as pregnancy test kits which have different interpretations (i.e., a line in the drug test kits means a negative result while it is a positive result in a pregnancy test). Some drug test kits consider a faint line as a negative, while others already consider it as positive. The expiry of drug test kits may also affect its performance, however, all the laboratories used test kits that were within their expiry dates.

Some initially failed laboratories did not handle and store the PT specimens properly. When the testing was not performed right away, the specimens were not put in the refrigerator. Furthermore, due to its high cost, quality control materials were not regularly run together with the PT specimens. Clerical errors in notetaking and record-keeping were also reasons for the laboratory's failure. Overall, the initially failed laboratories were not able to follow or maintain good laboratory practice.

Since the screening drug testing is qualitative, the experience, analytical and interpretive capabilities of the analyst are very important. Continued training and application of quality control procedures are needed by the drug test analysts.[15] NRL-EAMC is responsible for the training of drug testing analysts and re-training is available whenever needed.

To lift the suspension of accreditation of initially failed laboratories, they must immediately register for the next available PT cycle. The initially failed laboratories must improve their testing and quality assurance procedures prior to taking the repeat PT. On their second attempt, most of them passed the repeat PT. In 2009, 14 laboratories or 9.7% of the initially failed laboratories failed their second PT. This has resulted in the revocation of their accreditation as authorized drug testing laboratories. In 2010/2011, 7.0% of the initially failed laboratories failed their repeat PT while 17.7% in 2012. From 2013 to 2019, less than or equal to one laboratory failed their second attempt in PT. Furthermore, laboratories which initially failed their current PT tended to obtain acceptable/highly satisfactory or even excellent performance in their next year's participation. This has shown the improvements of the laboratories in their drug testing procedures and the effectiveness of the PT program implementation.

PT programs in other countries

In the United States, PT program for DTLs started in 1972 when 114 laboratories were invited to participate. Initially, morphine and methadone were spiked into water matrix and added with caramel and urea to achieve the specific gravity of urine. Around 50 – 70% of the participating laboratories were able to correctly identify the drugs.[16]

The American Association for Clinical Chemistry (AACC) Special Study on Drugs of Abuse in Urine initiated the Toxicology Surveys Plus in 1985 to check the capability of DTLs to assess the presence/absence of five drugs or drug classes of interest usually encountered in pre-employment drug testing. Forty-nine laboratories participated and were given eight 50 mL urine specimens spiked with tetrahydrocannabinol, benzoylecgonine, morphine/codeine, methamphetamine, and phencyclidine. They reported that 69 – 82% of the laboratories used enzyme immunoassay (EIA) test kits. The lowest accuracy achieved

by any participating laboratory was 75%. On the other hand, 100% accuracy was achieved for cannabinoids.[17]

Since 1987, the United Kingdom National External Quality Assessment Scheme (UK NEQAS) for Drugs of Abuse in Urine has been providing proficiency testing specimens for DTLs. Participants were supplied with three sets of freeze-dried aliquots of 25 mL of urine spiked with amphetamine, barbiturates, benzoylecgonine, benzodiazepines, methadone, and morphine. For the PT between March 1990 and August 1992, 131 laboratories participated wherein high-performance liquid chromatography (HPLC) was revealed to be the most sensitive technique.[15]

The first implementation of a proficiency testing program for DTLs in Spain was in 1987. Participating laboratories received six samples of urine specimens spiked with drugs of abuse (amphetamines, barbiturates, benzodiazepines, opiates, methadone, dextropropoxyphene, benzoylecgonine, and cannabinoids) four times a year. The mean percentage error of the 25 participating laboratories was 2.8%. For laboratories participating for the first time, the mean error was 3.6%. A majority (62%) of the laboratories used EIA test kits.[14]

In Italy, the Centre of Behavioral and Forensic Toxicology (CBFT) of the University of Padova initiated the PT program for DTLs in 1995. In batches, six urine specimens (spiked with amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, methadone, opiates, and interfering substances) were sent every three months to about 200 participating laboratories. Between 1995 – 1998, the average percentage of correct results was 96.8%.[18]

The United Nations Office on Drugs and Crime (UNODC) has organized the International Collaborative Exercises (ICE) program since 1995. Its primary objective is to aid DTLs worldwide in assessing their performance. The UNODC ships twice a year four unknown test specimens of drugs and their metabolites in urine to more than 300 laboratories worldwide.[19] The NRL-EAMC regularly participates in the said ICE program with satisfactory results.

For laboratories worldwide performing screening drug testing only, the College of American Pathologists (CAP) sends five 10mL liquid urine specimens thrice per year to registered laboratories. They provide specimens for testing of diverse analytes: acetaminophen, amphetamines, barbiturates, benzodiazepines, benzoylecgonine, buprenorphine, tetrahydrocannabinol, ethanol, fentanyl, lysergic acid diethylamide (LSD), methadone and metabolite, methamphetamines, methylenedioxymethamphetamine (MDMA), opiates, oxycodone, phencyclidine, propoxyphene, and tricyclic group. In their Urine Drug Testing, Screening (UDS) Proficiency Survey 2011-2017, more than 3000 laboratories participated in the screening of THC, while more than 1000 laboratories for METH/AMPH.[20] However, the survey did not provide details on the performance of the laboratories but rather focused on the cross-reactivities of immunoassays especially for synthetic opioids. The laboratories used different cut-offs for the immunoassays and the authors encouraged them to adjust their test services based on clinical needs.

Plans on improving the PT program

Most of the PT programs for DTLs in other countries use drug-free human urine as a matrix while the PT program in this article uses synthetic urine. As previously mentioned, the use of human urine for the PT specimen would be hard to achieve since a large volume is required and consent from healthy volunteers would be needed. The collected human urine must also be certified to be drug-free prior to use as PT specimen. Furthermore, the homogeneity and stability of collected human urine in large volumes would be harder to attain. Although there is commercially available drug-free human urine, it is very expensive.

Synthetic urine has also been used by the Thailand Association for Clinical Biochemists for their PT Program for urinalysis.[21] Advantages of the use of synthetic urine include easy preparation, longer stability, better homogeneity, assurance of being drug-free, and less hazardous. However, the formulation for synthetic urine could be further improved to better imitate human urine. Additional quality control measures such as measuring the infrared absorbance spectra of the synthetic urine and comparing it with human urine could be implemented.[22]

Although Meth and THC remain to be the top drugs to be abused in the Philippines, additional analytes such as methylenedioxymethamphetamine (MDMA, ecstasy), benzodiazepines, opiates and cocaine which are also requested for screening drug testing could be added to the proficiency test specimens in the future. Interfering substances or drugs with similar structures with the analytes of interest could also be added to the PT specimens to make the PT more challenging. Although currently there are only three confirmatory drug testing laboratories nationwide, an external quality assurance program for them could also be initiated by conducting interlaboratory comparisons or sending blind samples.

The evaluation of PT scores could also be improved. Although still fit-for-purpose, the scoring method currently used, which relies on the number of incorrect responses does not reflect the performance of a laboratory in comparison with other laboratories. Furthermore, the score is based on the total score and not per analyte. The PT program for SDTLs is qualitative and the calculation of z-scores is deemed to be impossible. A new method that mimics the calculation of z-scores based on the proportion of satisfactory results and consensus from laboratories could also be applied in the future PT program.[23]

Provided with enough funding, the PT program could also be improved by developing and maintaining a dedicated website for PT where laboratories can access from registration up to the releasing of results.

Ultimately, the NRL-EAMC aims to be an accredited proficiency testing provider that is compliant with the ISO 17043:2023 standard requirements.[24]

CONCLUSION

The proficiency testing program for SDTLs has been successfully implemented since 2009. Through its

implementation, the objectives defined by the DDB Board Regulation have been fulfilled. Firstly, the competency of the SDTLs was assured since passing the PT is a requirement to be recognized as an authorized SDTL. Secondly, the results of the PT have been the basis for the regulation of SDTLs (i.e., initial PT failure results in suspension of accreditation; second failure leads to revocation of accreditation). Finally, the public is assured that authorized SDTLs which passed the PT offer drug testing services of high quality.

The number of participating laboratories fluctuated during the 2009-2019 period. On average, around 1000 laboratories participate annually. The mean passing rate was high (>96%). Although there is a decreasing trend in the number of laboratories achieving excellent performance, the number of laboratories failing the PT has significantly decreased. This demonstrates the effectiveness of the PT program in improving the testing procedures of SDTLs. Initially failed laboratories improved by reviewing and implementing rigorously their quality control procedures and strict adherence to good laboratory practices.

While the laboratory is improving by participating in the PT program, there is still a need to improve the PT program itself to better assess the performance of drug testing laboratories.

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STATEMENT OF AUTHORSHIP

All authors certified fulfillment of ICMJE authorship criteria.

AUTHORS DISCLOSURE

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OPEN ACCESS – ORIGINAL ARTICLE

Agreement between Sonographic Features and Fine Needle Aspiration Cytology in the Diagnosis of Thyroid Nodules in a Tertiary Hospital

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ABSTRACT

Objective. Management of thyroid nodules relies on the Thyroid Imaging Recording and Data System (TIRADS) for sonographic findings and the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC). The proponents aimed to determine the concordance between sonographic TIRADS findings and cytological diagnosis by TBSRTC in the evaluation of malignancy of patients with thyroid nodules.

Methodology. Sonographic and cytology results collected from 2018 to 2022 were obtained to determine whether there is an agreement between TIRADS and TBSRTC findings.

Results. Two hundred sixty-two (262) samples were obtained. Overall accuracy of predicting TIRADS category was highest for echogenic foci. Thyroid nodule distribution was highest for TIRADS 3 and 4 sonographically and TBSRTC II cytologically. There is low agreement between TBSRTC and TIRADS in the categorization of nodules as benign, implying that nodules may show sonographic features suspicious of malignancy despite being categorized as TBSRTC I or II by cytology. However, nodules categorized as TBSRTC III to VI show sonographic features suspicious for malignancy at the very least.

Conclusion. The correctness of TIRADS prediction is highest for echogenic foci although not significantly higher than other parameters. The overall predicting power of TIRADS for the absence of malignancy is high for TIRADS 1 and 2, whereas TIRADS 5 predicts a 31.11% risk of malignancy making it a strong indication for FNAC. However, prediction of malignancy in TIRADS 3 and 4 nodules must be in association with other factors since a significant percentage may turn out to be TBSRTC II.

Key words: thyroid nodules, thyroid ultrasound, TIRADS, fine-needle aspiration cytology, TBSRTC

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INTRODUCTION

Thyroid nodules are focal well-defined lesions of altered echogenicity having estimated global prevalences of 4-8% and 19-67% by palpation and ultrasonography, respectively.[1,2] In the local setting, clinicians follow the 2015 criteria established by the American Thyroid Association (ATA) in managing thyroid nodules which recommends ultrasound-guided fine-needle aspiration as the mainstay for diagnosis.[3] The guideline stratifies thyroid nodules based on the thyroid imaging recording and data system (TIRADS) for sonographic findings and the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) for cytologic diagnosis which respectively categorize thyroid nodules into five and six categories.[1]

Although widely available, data on concordance of thyroid nodule ultrasound (US) and fine-needle aspiration cytology (FNAC) findings remains unsettled and scarcely available in the Philippines hence this study aims to provide local data on this matter by assessing these findings among patients with thyroid nodules in-a tertiary hospital setting.

METHODOLOGY

Research design

This study is a retrospective cross sectional analytical review of results of patients who underwent thyroid ultrasound and subsequent fine-needle aspiration cytology regardless of thyroid function test results in a five-year period from 2018 to 2022.

Sampling strategy

This study employed purposive sampling which is a non-probability approach that relied on the primary investigator's discretion in selecting patients who underwent thyroid ultrasound and subsequent fine-needle aspiration cytology regardless of thyroid function test results. Based on the institution's data from 2018 to 2022 which showed a total population size of 423, a sample size of 202 was calculated considering the following assumptions: a hypothesized frequency of 50%, a margin of error of 5% with a 95% confidence interval, and a design effect of 1. Two hundred sixty-two individuals (262) qualified for the study. Their demographic data and thyroid ultrasound and fine-needle aspiration cytology results were retrieved from hospital's radiology and laboratory information systems and recorded using Microsoft Excel Sheet Software ver. 16.66.1.

Analysis

Descriptive statistics were be used to assess the age, US findings and final diagnosis of the patients. Categorical variables were analyzed using frequency and percentage, while continuous variables were assessed using the mean and standard deviation.

The polychoric correlation coefficient was employed to assess the strength of the relationship between the ordinal variables under investigation (sonographic TIRADS findings and the cytological diagnosis determined by TBSRTC scoring). Subsequently, the dataset was divided into two subsets: training and testing data.

To establish a model for the training subset, Univariate Regression Analysis was conducted. The cutoff score will be derived from the area under the receiver operating characteristic of the training dataset. Following this, Sensitivity and Specificity, accompanied by 95% confidence intervals, along with positive (PPV) and negative (NPV) predictive values, were computed for each major ultrasound feature strongly indicative of malignancy, using cytology as the reference test.

All statistical tests were two-tailed tests. Null hypotheses were rejected at 0.05α-level of significance. RStudio version 4.2.0 software was used for data analysis.

RESULTS

Table 1 presents population characteristics of patients who underwent thyroid ultrasound and subsequent fine-needle aspiration cytology. The data is organized by age groups and gender, with a total of 262 patients. Noteworthy trends include a concentration of cases in the age groups of 45-54 and 55-64, which collectively represent a significant portion of the total cases.

Table 1. Population characteristics of patients who underwent thyroid US and subsequent FNAC

Age group (years)	Female (N = 217)	Male (N = 45)	Total (N = 262)
	Frequency (%)		
15-24	5 (1.9%)	1 (0.4%)	6 (2.3%)
25-34	18 (6.9%)	2 (0.8%)	20 (7.6%)
35-44	30 (11.5%)	1 (0.4%)	31 (11.8%)
45-54	51 (19.5%)	13 (5.0%)	64 (24.4%)
55-64	80 (30.5%)	18 (6.9%)	98 (37.4%)
Greater than or equal to 65	33 (12.6%)	10 (3.8%)	43 (16.4%)

Across all age groups, the number of female patients is notably higher than male patients. Specifically, in the age group of 55-64, there are 80 female patients (30.5%) compared to 18 male patients (6.9%).

Table 2 summarizes the findings from thyroid ultrasound, categorized by parameters such as TIRADS category, composition, echogenicity, echogenic foci, margin, and shape. Solid composition is prevalent in the majority of cases (73.7%), while complex (20.2%) and cystic (4.2%) compositions are also observed. Regarding echogenicity, a significant number of nodules are hypoechoic (60.3%), followed by isoechoic (20.6%) and hyperechoic (18.3%) types. Macro/microcalcifications are the most common echogenic foci findings accounting 45% of the population, while other foci such as peripheral calcifications (10%) and punctate echogenic foci (7%) are less frequent. Nodules with smooth margins are predominant (73.3%), and the majority exhibit a wider-than-tall shape (93.5%).

Table 3 presents the statistical characteristics of various radiologic parameters in predicting the TIRADS category of a patient. Each parameter (composition, echogenicity, echogenic foci, margin, and shape) has associated sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy. In terms of sensitivity, echogenic foci perform the best at 57.1%, indicating its ability to correctly identify patients with the TIRADS category. Specificity, measuring the ability to correctly identify patients without the TIRADS category, is highest for echogenic foci at 89.3%. PPV, representing the probability of a positive TIRADS prediction being accurate, is consistently matched with sensitivity for each parameter. NPV, indicating the probability of a negative TIRADS prediction being accurate, is also consistently high, ranging from 86.7% to 89.3%.

Table 4 provides a comprehensive overview of the ultrasound and cytology results, categorized by diagnostic classifications defined by both TIRADS and TBSRTC. The table presents the number and percentage of cases falling into specific intersections of TBSRTC and TIRADS categories.

The distribution across TIRADS classifications is as follows:

- 8 TBSRTC I nodules – TIRADS 1 to 4;
- 186 TBSRTC II nodules – TIRADS 1 to 5;
- 10 TBSRTC III nodules – TIRADS 1 and TIRADS 3;
- 14 TBSRTC IV nodules – TIRADS 1 and TIRADS 4 to 5;
- 17 TBSRTC V nodules – TIRADS 3 to 5; and
- 14 TBSRTC VI nodules – TIRADS 3 to 5.

Table 2. Thyroid ultrasound findings

Parameter	TIRADS category					Total
	1	2	3	4	5	
	Frequency (%)					
Composition						
Solid	-	-	47 (17.9%)	109 (41.6%)	37 (14.1%)	193 (73.7%)
Complex	1 (0.4%)	3 (1.1%)	20 (7.6%)	21 (8.0%)	8 (3.1%)	53 (20.2%)
Cystic	7 (2.7%)	1 (0.4%)	3 (1.1%)	-	-	11 (4.2%)
Spongiform	-	-	2 (0.8%)	2 (0.8%)	1 (0.4%)	5 (1.9%)
Echogenicity						
Hypoechoic	-	-	18 (6.9%)	96 (36.6%)	44 (16.8%)	158 (60.3%)
Isoechoic	4 (1.5%)	3 (1.1%)	27 (10.3%)	19 (7.3%)	1 (0.4%)	54 (20.6%)
Hyperechoic	2 (0.8%)	1 (0.4%)	27 (10.3%)	17 (6.5%)	1 (0.4%)	48 (18.3%)
Cystic	2 (0.8%)	-	-	-	-	2 (0.8%)
Echogenic foci						
Macro/Microcalcification	1 (0.4%)	-	16 (6%)	86 (33%)	14 (5%)	117 (45.0%)
Peripheral calcifications	-	-	1 (0.4%)	10 (4%)	15 (6%)	26 (10%)
Punctate echogenic foci	-	-	1 (0.4%)	3 (1%)	15 (6%)	19 (7%)
None/Comet Tail	7 (2.7%)	4 (1.5%)	54 (21.0%)	36 (14.0%)	4 (1.5%)	105 (40.0%)
Margin						
Smooth	8 (3.1%)	4 (1.5%)	63 (24.0%)	93 (35.5%)	24 (9.2%)	192 (73.3%)
Ill defined	-	-	7 (2.7%)	21 (8.0%)	7 (2.7%)	35 (13.4%)
Irregular	-	-	1 (0.4%)	18 (7.0%)	11 (4.2%)	30 (11.0%)
Extension	-	-	1 (0.4%)	0 (0.0%)	4 (1.5%)	5 (1.9%)
Shape						
Wider than tall	8 (3.1%)	4 (1.5%)	72 (27.5%)	126 (48.1%)	35 (13.4%)	245 (93.5%)
Taller than wide	-	-	-	6 (2.3%)	11 (4.2%)	17 (6.5%)

Table 3. Statistical characteristics of radiologic parameters in predicting TIRADS category of a patient

Parameter	Sensitivity	Specificity	PPV	NPV	Accuracy
Composition	46.7%	86.7%	46.7%	86.7%	78.7%
Echogenicity	52.4%	88.1%	52.4%	88.1%	81.0%
Margin	46.7%	86.7%	46.7%	86.7%	78.7%
Shape	48.6%	87.1%	48.6%	87.1%	79.4%
Echogenic foci	57.1%	89.3%	57.1%	89.3%	82.9%

The overall distribution across TIRADS categories indicates a substantial proportion of cases classified as TIRADS 3 (27.5%) and TIRADS 4 (49.6%).

The distribution across TBSRTC categories is as follows:
- TBSRTC I – 18 cyst fluid only (100%);
- TBSRTC II – 186 follicular nodular disease (71%);
- TBSRTC III – atypia of undetermined significance;
- TBSRTC IV – 3 follicular neoplasm (Hürthle cell type) (21.43%), 11 follicular neoplasms (78.57%);
- TBSRTC V – 15 suspicious for papillary carcinoma (88.24%), 1 suspicious for metastatic carcinoma (5.88%), 1 suspicious for lymphoma (5.88%);
- TBSRTC VI – 9 papillary thyroid carcinoma (64.29%), 1 high-grade follicular cell-derived non-anaplastic thyroid carcinoma (7.14%), 1 medullary thyroid carcinoma (7.14%), 1 undifferentiated (anaplastic) carcinoma (7.14%), 2 metastatic carcinoma (14.29%).

The overall distribution across TBSRTC categories indicates a substantial proportion of cases classified as TBSRTC II.

Table 5 provides an overview of the correlation between TIRADS classification and risk of malignancy which evidently shows a 4 to 5-fold and 15 to 16-fold estimated risk of malignancy for TIRADS 4 and 5 compared to category 3 with respective p-values of 0.05 and 0.0004, respectively. All TIRADS 1 and 2 and majority of TIRADS 3 cases turned out to be benign.

DISCUSSION

The institution utilizes the GE Logiq P9 ultrasound machine and employs TIRADS for classifying thyroid nodules based on composition, echogenicity, echogenic foci, margins, and shape, with each descriptor giving a point. Adding all points of all descriptors provides the TIRADS score which divides thyroid nodules into 5 categories namely TIRADS 1(benign), 2 (not suspicious for malignancy), 3 (mildly suspicious for malignancy), 4 (moderately suspicious for malignancy), and 5 (highly suspicious for malignancy) with respective malignancy risk of 0%, 1.7%, 3.3-72.4%, and 87.5% for categories 2-5.[4] Suspicious sonographic features include solid or mixed composition, hypoechogenicity, taller than wider in shape, irregular margins, and evidence of extrathyroid extension and risk of malignancy being 7-15%.[5-7]

The Bethesda System for Reporting Thyroid Cytopathology is utilized in classifying thyroid nodules into 6 categories namely I ("non-diagnostic" – cyst fluid only,

Table 4. Ultrasound findings (TIRADS) and FNAC correlation

Diagnostic Categories		TIRADS					Total
		1	2	3	4	5	
TBSRTC	I	3 (1.1%)	1 (0.4%)	10 (3.8%)	4 (1.5%)	-	18 (6.9%)
	II	3 (1.1%)	3 (1.1%)	59 (22.5%)	96 (36.6%)	25 (9.5%)	186 (71.0%)
	III	1 (0.4%)	-	1 (0.4%)	7 (2.7%)	1 (0.4%)	10 (3.8%)
	IV	1 (0.4%)	-	-	8 (3.1%)	5 (1.9%)	14 (5.3%)
	V	-	-	1 (0.4%)	10 (3.8%)	6 (2.3%)	17 (6.5%)
	VI	-	-	1 (0.4%)	5 (1.9%)	8 (3.1%)	14 (5.3%)
	Total	**8 (3.1%)**	**4 (1.5%)**	**72 (27.5%)**	**130 (49.6%)**	**45 (17.2%)**	**259 (98.9%)**
Polychoric coefficient					**0.4962 – Moderate**		

Table 5. Proportion of malignancy per TIRADS classification

TIRADS Classification	Benign, n (%)	Malignant, n (%)	Total, n	Risk of Malignancy	OR (95% CI)	*P*-value
1	8 (100%)	0 (0%)	8	**0.00%**	**1.66 (0.07-37.52)**	**0.75**
2	4 (100%)	0 (0%)	4	**0.00%**	**3.13 (0.13-75.53)**	**0.48**
3	70 (97.22%)	2 (2.78%)	72	**2.78%**	**Reference**	
4	115 (88.46%)	15 (11.54%)	130	**11.54%**	**4.57 (1.01-20.56)**	**0.05**
5	31 (68.89%)	14 (31.11%)	45	**31.11%**	**15.81 (3.39-73.79)**	**0.0004**
Total	228 (88.03%)	31 (11.97%)	259			

virtually acellular, other); II ("benign" – follicular nodular disease, chronic lymphocytic (Hashimoto) thyroiditis, granulomatous (subacute) thyroiditis, other); III ("atypia of undetermined" – nuclear type, other); IV ("follicular neoplasm" – oncocytic (Hürthle cell) type); V ("suspicious for malignancy" – papillary thyroid carcinoma, medullary thyroid carcinoma, metastatic carcinoma, lymphoma, other); and VI ("malignant" – papillary thyroid carcinoma, high-grade follicular cell-derived non-anaplastic thyroid carcinoma, medullary thyroid carcinoma, anaplastic carcinoma, squamous cell carcinoma, carcinoma with mixed features, metastatic malignancy, non-Hodgkin lymphoma, other), with respective risk of malignancy of 13%, 4%, 22%, 30%, 74%, and 97% based on follow-up of surgically resected nodules.[8]

The age groups 45-54 and 55-64 represent a significant portion of the total cases with the number of female patients being notably higher in this study which corroborated with previous literature.[9] Among all the ultrasound parameters, overall sensitivity, specificity, PPV, and NPV of predicting TIRADS category is highest for echogenic foci and lowest for composition in contrast to a previous study where values were highest for echogenic foci and lowest for composition.[10]

The 14 out of 18 (22.22%) TBSRTC I nodules exhibit TIRADS 3 to 5 categorization, suggesting that non-diagnostic nodules may also show sonographic features suspicious for malignancy. There is 3.23% agreement between TBSRTC and TIRADS in the categorization of TBSRTC II nodules as benign given that only 6 out of 186 TBSRTC category II nodules are under TIRADS 1 and 2 and the remaining 180 (96.77%) are under TIRADS 3 to 5, implying that despite being benign, majority of TBSRTC II nodules will show sonographic features suspicious for malignancy with considerable overlap between TIRADS 3 and 4. Among TBSRTC category III (9 out of 10 – 90%) and IV (13 out of 14 – 92.86%) nodules, the majority show TIRADS 3 to 5 categorizations, suggesting that nodules with atypia of undetermined significance and follicular nodules will primarily exhibit sonographic features suspicious for malignancy. All 17 (100%) TBSRTC category V and 14 (100%) TBSRTC category VI nodules are under TIRADS 3 to 5, implying that malignant nodules will probably show suspicious sonographic features at the very least.

The investigators found that results for nodules categorized as TBSRTC II, V, and VI are comparable to previous studies.[9-11] Findings for nodules categorized as TBSRTC I and III were not previously elucidated.

CONCLUSION

The correctness of TIRADS prediction is highest for echogenic foci although not significantly higher than other parameters. The overall predicting power of the TIRADS system for the absence and presence of malignancy is high in both ends of the spectrum and TIRADS 1 and 2 are reassuring whereas TIRADS 5 is a strong indication for FNAC. However, the decision to proceed with FNAC in TIRADS 3 and 4 nodules must only be indicated in association with other factors since a significant percentage may turn out to be TBSRTC II.

STATEMENT OF AUTHORSHIP

The authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

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OPEN ACCESS – CASE REPORT

Mucinous Tubular and Spindle Cell Carcinoma of the Kidney: A Case Report and Concise Review of Literature

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ABSTRACT

Mucinous tubular and spindle cell carcinoma (MTSCC) is a rare neoplasm of the kidney. Recognition of this rare entity is important with regards to a patient's prognosis and therapeutic management.

Key words: mucinous tubular and spindle cell carcinoma, kidney neoplasms, immunohistochemistry, surgical pathology

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INTRODUCTION

Mucinous tubular and spindle cell carcinoma (MTSCC) is a rare neoplasm of the kidney, accounting for less than 1% of renal cell carcinomas.[1] Females are more frequently affected than males, with a majority of cases diagnosed in adults. Most tumors are indolent and discovered incidentally.[1,2]

First described in 1996, MTSCCs were previously described as "low-grade collecting duct carcinoma," and formally introduced in the 2004 edition of the WHO Classification of Tumors.[1] Tumors are predominantly located in the renal cortex. Histologic features of MTSCCs include elongated tubules and spindle cells within a mucinous to myxoid stroma, with components occurring in varying proportions.[1,2] Tumors are generally low-grade, but high-grade features such as pleomorphism, nuclear atypia and extensive necrosis have been recognized.[3] Metastases are remarkably rare, especially in tumors with low-grade morphology. Despite its nonspecific clinical features, recognizing MTSCC's distinct histomorphology is important due to different therapeutic and prognostic implications.[1]

We present a case of a 50s female with an incidental finding of a renal mass on imaging, and describe its gross pathology, microscopic features and a concise review of literature.

CASE REPORT

A female in her 50s with chronic kidney disease was found to have an incidental ultrasound finding of a left kidney mass. Further investigation with triple-phase computed tomography of the abdomen showed a predominantly endophytic mass with high complexity in the superior to middle pole of the left kidney (2+3+3+x+3 = 11+x by R.E.N.A.L. Nephrometry scoring). The patient was then admitted for surgery. A radical nephrectomy was performed, and the specimen received consisted of a left kidney with no ipsilateral adrenal gland attached. Serial sections of the kidney showed a well-circumscribed, cream-tan mass with a central hemorrhagic area, located in the superior to middle pole of the kidney and measuring 6.8

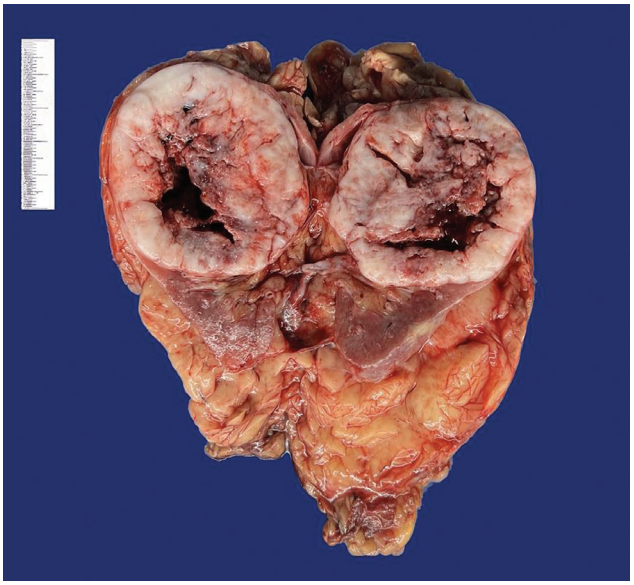

Figure 1. Gross appearance of the left kidney with a superior to mid-pole mass (bivalved).

x 5.2 x 5 cm (Figure 1). The renal pelvis, renal sinus soft tissues, perinephric fat, ureter and hilar blood vessels were grossly uninvolved by the mass.

Microscopic sections showed an unencapsulated, fairly defined cellular tumor (Figure 2) composed of elongated and anastomosing tubules seen within a basophilic myxoid to mucinous stroma (Figure 3). These structures were composed of cuboidal cells with low-grade nuclei, merging with bland spindle-shaped cells (Figure 4). No evidence of sarcomatoid or rhabdoid differentiation was seen.

Immunohistochemical studies using CK7, alpha-methylacyl-CoA racemase (AMACR), PAX8, CD10, and renal cell carcinoma antigen (RCCA) (Dako Agilent) were also performed. PAX8 showed moderate to strong, diffuse nuclear expression, while CK7, AMACR, and RCCA showed strong, diffuse, cytoplasmic expression in the tumor cells (Figure 5 A-D). No expression of CD10 was observed in the tumor (Figure 5 E).

DISCUSSION

The case described highlights the classic clinical and histologic features of mucinous tubular and spindle cell carcinoma. This tumor is mostly seen in adult patients, and predominantly affects females, with a reported female-to-male ratio of up to 4:1.[2] MTSCCs usually present asymptomatically as an incidental finding, which was the case for our patient. However, MTSCCs may also present as an abdominal mass, or with flank pain and hematuria.[1,2] It has also been proposed that there may be some association with nephrolithiasis and chronic kidney disease.[4]

Grossly, MTSCCs are well-circumscribed, sometimes partially encapsulated tumors located within the renal cortex.[2] Cut surfaces are solid, and homogenous tan to grey or pale yellow. Hemorrhage and necrosis are uncommonly present.[1] While MTSCC may be easy to recognize when it presents with classic histomorphology, a mucin-poor

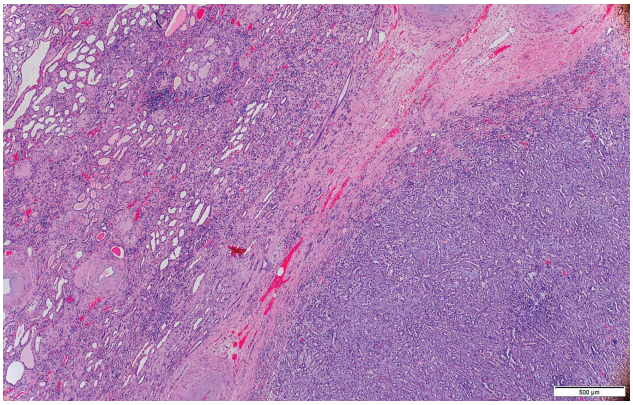

Figure 2. Well-delineated and unencapsulated cellular tumor with uninvolved renal parenchyma (H&E, 40x).

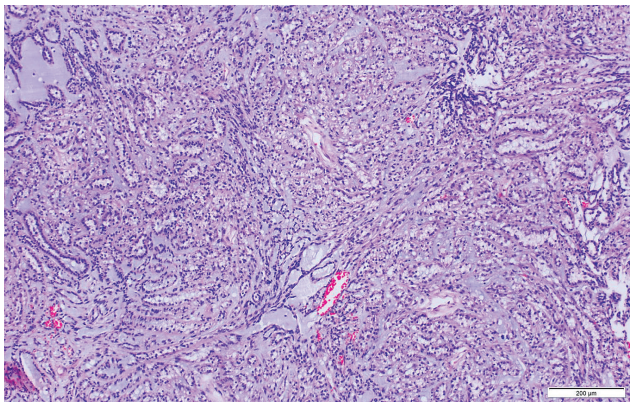

Figure 3. The tumor consists of tubules seen within a basophilic myxoid to mucinous stroma (H&E, 100x).

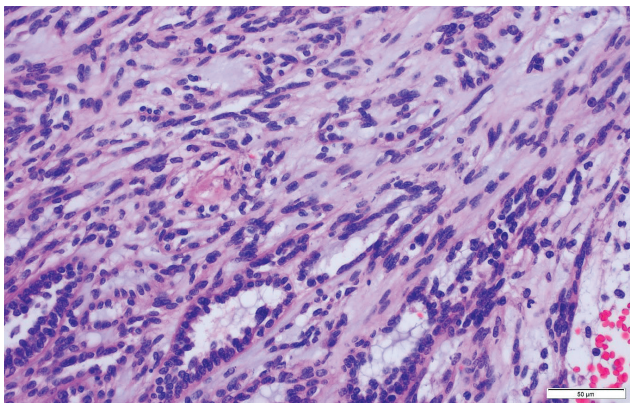

Figure 4. Tubules are composed of cuboidal cells with low-grade nuclei, merging with spindle-shaped cells (H&E, 400x).

variant has been described, which may be confused with a papillary renal cell carcinoma.[2,4] High-grade features such as sarcomatoid changes and necrosis have also been reported to occur in MTSCCs.[3,5] Other variations in the morphology of MTSCC may prompt consideration of other renal tumors. MTSCCs with predominantly spindle cells may be confused with sarcomatoid transformation of other renal cell carcinomas or mesenchymal neoplasms.[2] Clear cell changes have also been reported and may be confused with the more common clear cell renal cell

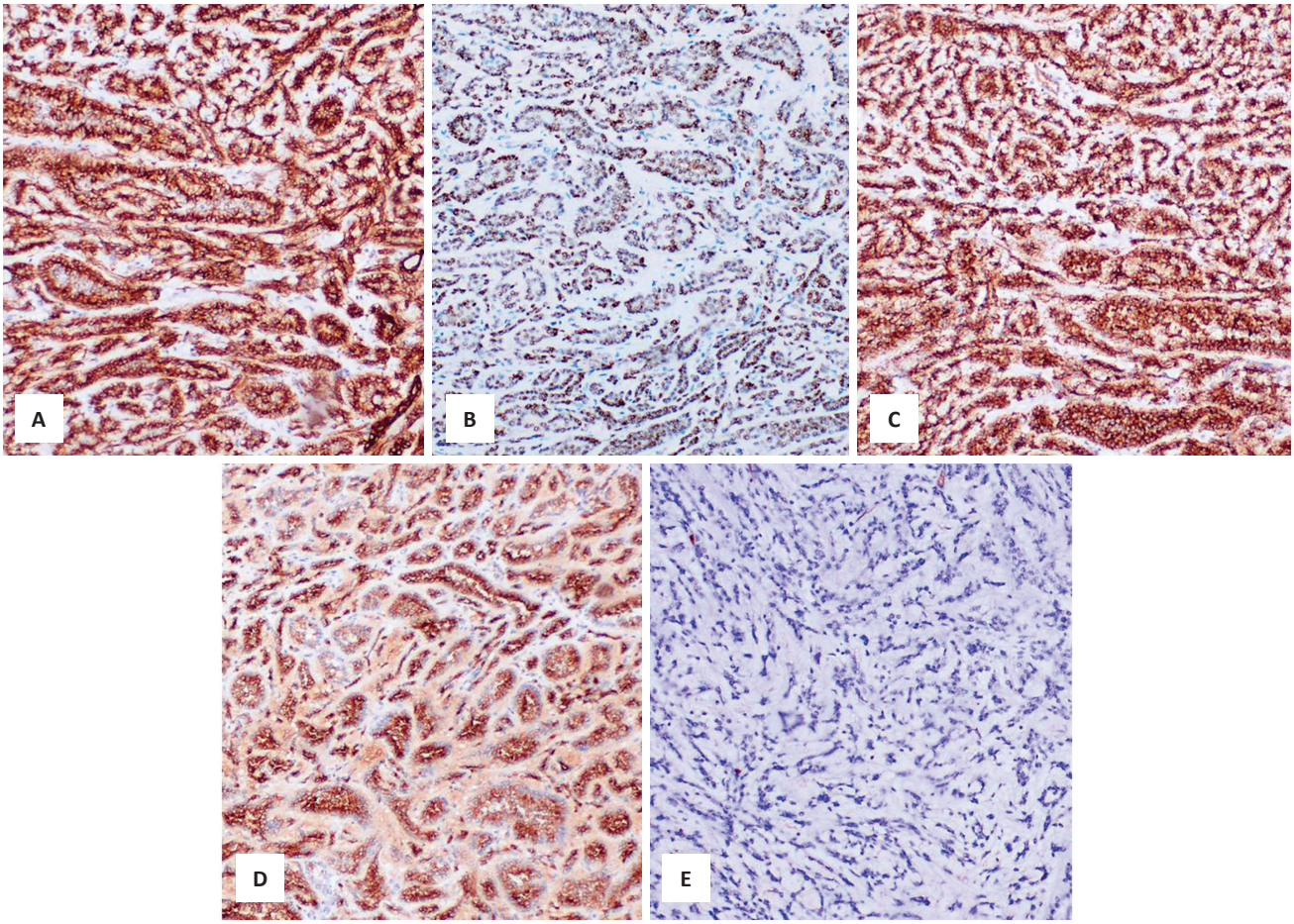

Figure 5. Immunohistochemistry expression of the renal tumor (400x). The tumor stained positively for CK7 **(A)**, PAX8 **(B)**, AMACR **(C)**, and RCCA **(D)**. No staining was seen using CD10 **(E)**.

carcinoma.[6] Psamomma bodies and aggregates of foamy macrophages may also be seen in MTSCCs, papillary renal cell carcinoma, and Xp11 translocation renal cell carcinoma.[7]

Immunohistochemistry studies may further complicate the distinction of MTSCCs from its closest differential. MTSCCs, like papillary renal cell carcinomas, typically demonstrate expression of CK7, AMACR, PAX8.[2,4,5,8] This immunohistochemical profile has led some authors to speculate the origin of MTSCCs from the proximal renal tubules,[1,2] but at present, the exact histogenesis of MTSCCs has remained unclear.[5,9] However, MTSCCs have also been reported to have lower expression of CD10 as compared to papillary renal cell carcinoma.[3,10]

When distinction cannot be made by examination of the histomorphology and immunohistochemical expression, MTSCCs may be distinguished from other renal neoplasms using molecular studies. Cytogenetic studies such as karyotyping, fluorescence in situ hybridization (FISH) and comparative genomic hybridization (CGH) studies frequently demonstrate chromosomal losses involving chromosomes 1, 4, 6, 8, 9, 13, 14, 15, and 22.[7,8,10-12] This is in contrast to papillary renal cell carcinomas, which are associated with trisomy 7, 17, or loss of Y chromosome.[7,8,10-12] Loss of 3p or mutations in the *VHL* gene, seen in clear cell renal cell carcinomas, are also not observed in MTSCCs.[7,11] Other investigations involving the use of novel markers, such as VSTM2A by RNA in situ hybridization, have also shown its possible use as a sensitive and specific marker for the diagnosis of MTSCC.[13]

The management of MTSCCs is surgery, with successful treatment via complete surgical excision or radical nephrectomy.[2,3,5,14] However, tumor recurrence, lymph node and/or distant metastasis have been occasionally reported, and these findings may be correlated with high-grade features.[1,2] Tumor progression, associated with loss of CDKN2A/B and additional complex genomic abnormalities, is seen in these more aggressive MTSCCs.[5,11] Nevertheless, the majority of MTSCCs are still considered to have favorable prognosis.[1-3]

CONCLUSION

Mucinous tubular and spindle cell carcinoma of the kidney is a rare, frequently indolent neoplasm of the kidney with recognizable histomorphologic features, as seen in the presented case. Immunohistochemical examinations done for this case also parallel previous studies. Molecular studies, such as karyotyping, FISH, or CGH analysis may be performed when distinction from more aggressive entities is difficult. Prompt recognition of this tumor is important, as it influences treatment and prognosis.

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OPEN ACCESS – CASE REPORT

Mucinous Cystadenocarcinoma of the Breast with Axillary Lymph Node Metastasis: An Entity with an Unusual Clinical Course

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ABSTRACT

This is a case of a 54-year-old, perimenopausal, Asian, woman, who presented with an enlarging left breast mass associated with whitish to bloody nipple discharge. A core needle biopsy, done in another institution, showed histologic findings of a mucinous carcinoma with triple negative "basal-like" biomarker status (ER, PR, HER2/neu). Six cycles of neoadjuvant chemotherapy were given after which the subsequent modified radical mastectomy revealed a centrally located, 10.0 cm, well-circumscribed, nodular, ovoid mass on gross examination. Microscopic findings showed tall columnar cells in stratification, tufts and papillary formations, with surrounding abundant extracellular mucin. The individual tumor cells exhibit enlarged, hyperchromatic, basally located nuclei with prominent nucleoli, abundant amphophilic and occasionally oncocytic cytoplasm which contains intracytoplasmic mucin. Based on the histologic features, "basal-like" biomarker expression, and additional immunohistochemical studies (positive CK7, negative CK20 and CDX2), this case demonstrates a pure mucinous cystadenocarcinoma of the breast. In addition to the rare histologic type, this case is exceptional since, despite multiple cycles of neoadjuvant chemotherapy, presence of extensive lymphovascular invasion and axillary lymph node involvement with extranodal extension remain evident.

Key words: mucinous cystadenocarcinoma, breast cancer, axillary lymph node metastasis

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INTRODUCTION

Mucinous cystadenocarcinoma is a primary mucin-producing carcinoma. Histopathologically, it is described as cystic structures lined by columnar cells with abundant intracellular and extracellular mucin and is most commonly seen in the ovary, pancreas and appendix.[1] Its occurrence on the breast has been included as a new entity in the recent 5th edition of the World Health Organization (WHO) classification of tumors of the breast.[1] More than 30 cases of primary MCA have been reported in the literature. Additionally, several cases of mixed MCA with Invasive ductal carcinoma, not otherwise specified (IDC NOS) have been documented. Furthermore, with similar histomorphological features to its ovarian, pancreatic and gastrointestinal counterparts, demonstration of this tumor on the breast warrants primarily excluding a metastatic lesion as well as the more common primary mucinous carcinoma. In line with this, immunohistochemical studies have been proven to play a crucial role in ruling out the possibility of metastasis. Despite limited information on clinical course and follow-up, case reports on mucinous cystadenocarcinoma of the breast mostly show good prognosis. Most publications report long survivability with no or little occurrence of relapse and lymph node metastasis despite having a triple negative (ER, PR, HER2/neu) biomarker status.[2]

In the Philippines, no known local studies have been reported with the same features as similar to this case. Since there are only about five (5) cases that have been reported with lymph node metastases, most cases have benign lymph nodes. The purpose of this manuscript is to report a rare case of pure mucinous cystadenocarcinoma of the breast presenting with lymph node metastasis

and lymphovascular invasion. Additional workups such as immunohistochemical and molecular studies are imperative tools to confirm the final diagnosis.

CASE

This is a case of a 54-year-old female who presented with an enlarging left breast mass. Two years prior to admission, she noticed a "ping-pong ball"-sized mass on her left breast with associated whitish to bloody nipple discharge. There was no pain, tenderness or skin dimpling noted hence she did not seek consultation. Six months prior to admission, the patient noticed an increase in the size of the said mass, prompting consultation with her primary attending physician whose assessment was invasive breast cancer, hence initial work-up (ultrasound, mammogram and core needle biopsy) was advised. Performed in another institution, the core needle biopsy result revealed mucinous breast carcinoma and further immunohistochemistry studies for biomarkers (ER, PR, and HER2neu) demonstrated triple negative or "basal-like" expression. The patient had a metastatic work-up (whole abdominal ultrasound, chest X-ray, bone scan and bone densitometry) which revealed unremarkable results. Due to the diagnosis of triple-negative breast cancer, she underwent six (6) cycles of neoadjuvant chemotherapy which included Docetaxel and Carboplatin, followed by modified radical mastectomy of the left breast as the definitive surgical procedure.

On gross examination, a modified radical mastectomy of the left breast was received with the following measurements: left breast - 24.5 x 19.5 x 5.5 cm; axillary tail - 24.5 x 19.0 x 5.5 cm. The overlying skin ellipse is smooth and without any signs of skin dimpling, ulceration, or discoloration. The nipple-areola complex is stretched out to resemble a dark-gray plaque which measures 4.5 x 4.0 cm in greatest dimension. There is no skin dimpling, thickening, or other skin lesions grossly defined. Serial sections show a well-circumscribed ovoid mass located along the central retroareolar aspect (Figure 1) which measures 10.0 x 6.5 cm in greatest dimension with a cream-pink, firm, solid cut surface with scattered cystic spaces (1.0 to 6.0 cm in diameter) filled with yellow to brown thick mucoid material. The mass is 0.1 cm away from the nearest basal resection margin, and 3.5 to 11.5 cm away from the other peripheral resection margins. The unaffected surrounding tissues show a yellow-tan and glistening, with interspersed tan-white fibrous areas. No other masses or nodules were identified grossly. Multiple pink-tan lymph nodes were isolated from the axillary fat which measure from 0.2 to 0.8 cm in widest diameter. Depicts the representative gross appearance of the left breast mass. A well-circumscribed solid-cystic mass directly underneath the skin and exhibits cream to pink firm cut surfaces and scattered cystic spaces filled with yellow to brown thick mucoid material (Figure 1).

Microscopic examination of the left breast mass showed a cystic tumor described to have intracystic papillary structures, some forming tufts and stratifications. The individual tumor cells are tall columnar cells with increased nucleus to cytoplasm ratio, enlarged round to oval nuclei with prominent nucleoli, and abundant eosinophilic cytoplasm (Figures 2A to 2F). Some representative sections

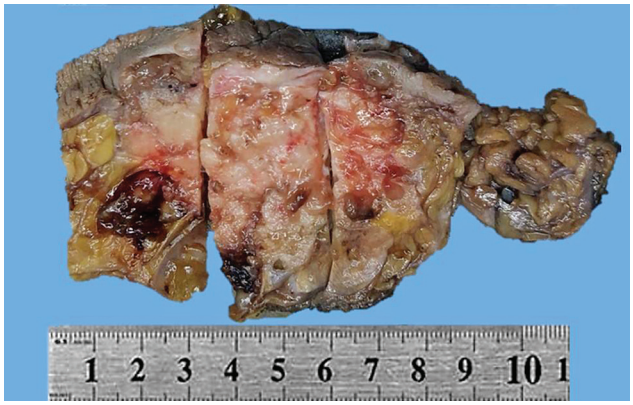

Figure 1. ***Representative gross appearance of the left breast mass.*** A well-circumscribed solid-cystic mass directly underneath the skin and exhibits cream to pink firm cut surfaces and scattered cystic spaces filled with yellow to brown thick mucoid material.

of axillary lymph nodes show macrometastases with positive extracapsular invasion (Figures 3A, 3B, and 3C). Dermal lymphatic and lymphovascular invasion were also noted (Figures 3E and 3F).

Immunohistochemistry studies were done revealing positive membranous and cytoplasmic expression for keratin 7 (CK7), but negative for keratin 20 (CK20), and CDX2 (Figures 4D, 4E, and 4F). Companion diagnostic testing for Estrogen receptor (ER), Progesterone receptor (PR), and HER2/neu (clone 4B5) protein were also done which revealed triple negative or "basal-like" status (Figures 4A, 4B, and 4C). The ki-67 index was not measured for this case.

Based on histomorphologic and immunohistochemical profiles, this case was signed-out as a Mucinous cystadenocarcinoma of the breast, Nottingham histologic grade II, central (left) breast with accompanying positive for lymphovascular and dermal lymphatic invasion, as well as axillary nodal macrometastases. No evidence of ductal carcinoma in situ (DCIS) was found on the representative sampled sections. Following the College of American Pathologist Protocol, Nottingham over-all histologic grade II was established, assigning Glandular/Tubular differentiation with a score of 2, Nuclear pleomorphism with a score of 2, and Mitotic rate with a score of 2.

In accordance with the American Joint Committee on Cancer (AJCC) 8th edition staging, the pathologic stage is as follows: ypT3 (post treatment with tumor of 10 cm in greatest dimension), ypN2a (macrometastases in 7 harvested axillary lymph nodes), ypM not applicable (no specimen submitted). Assuming that there is no distant metastasis, the Anatomic Stage Group is IIIA while the Prognostic Stage Group is IIIC.

The patient received adjuvant treatment (18 sessions of radiotherapy) which she completed three months after surgery. Four months after her surgery, she had a follow-up consultation and underwent additional metastatic work-up which included whole abdominal ultrasound, chest x-ray, bone scan as well as bone densitometry (non-institutional).

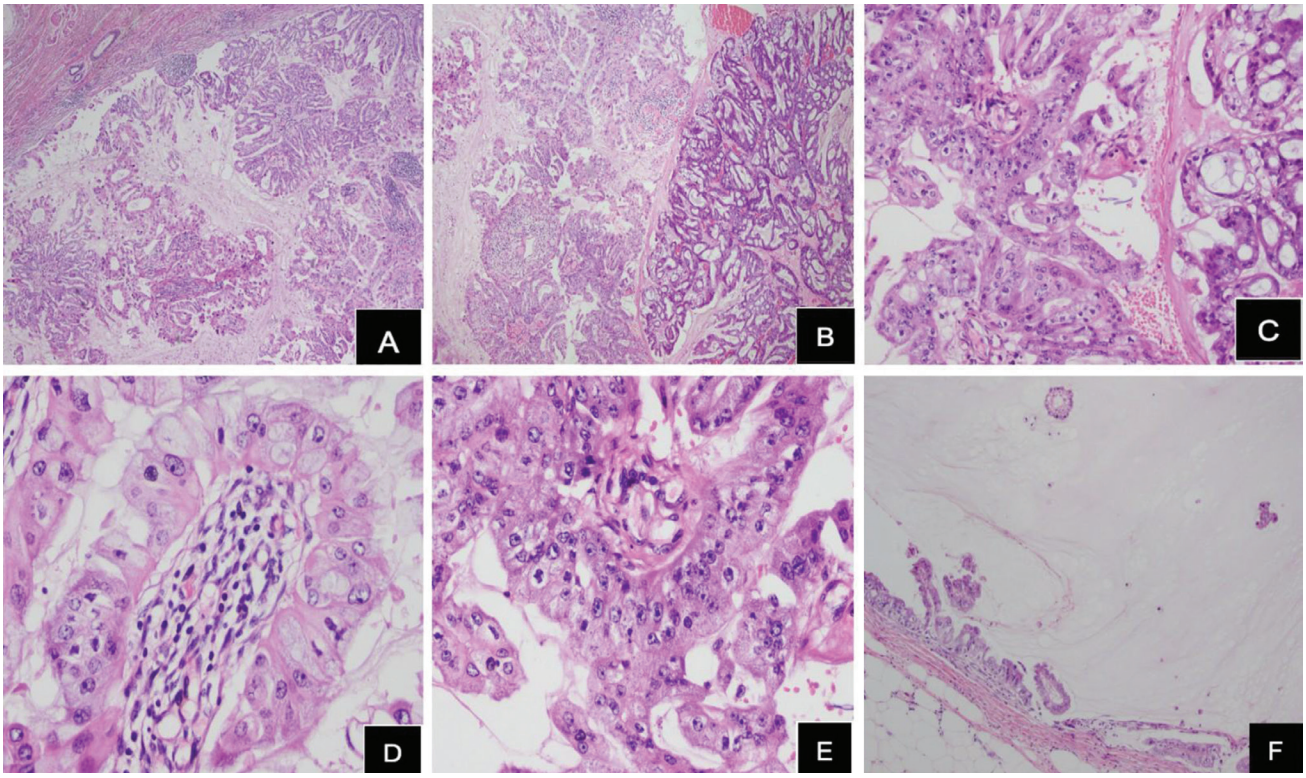

Figure 2. ***Representative microscopic sections of the mass [Hematoxylin-eosin stain].*** **(A)** Intracystic papillary formation [50x], **(B and C)** Tufting and stratification [100x], **(D)** Tall columnar cells with oncocytic cytoplasm and abundant intracytoplasmic mucin [400x], **(E)** Pseudostratified columnar cells with conspicuous mitotic figures, **(F)** Hypocellular cystic areas filled with abundant mucin mimicking mucinous carcinoma [100x].

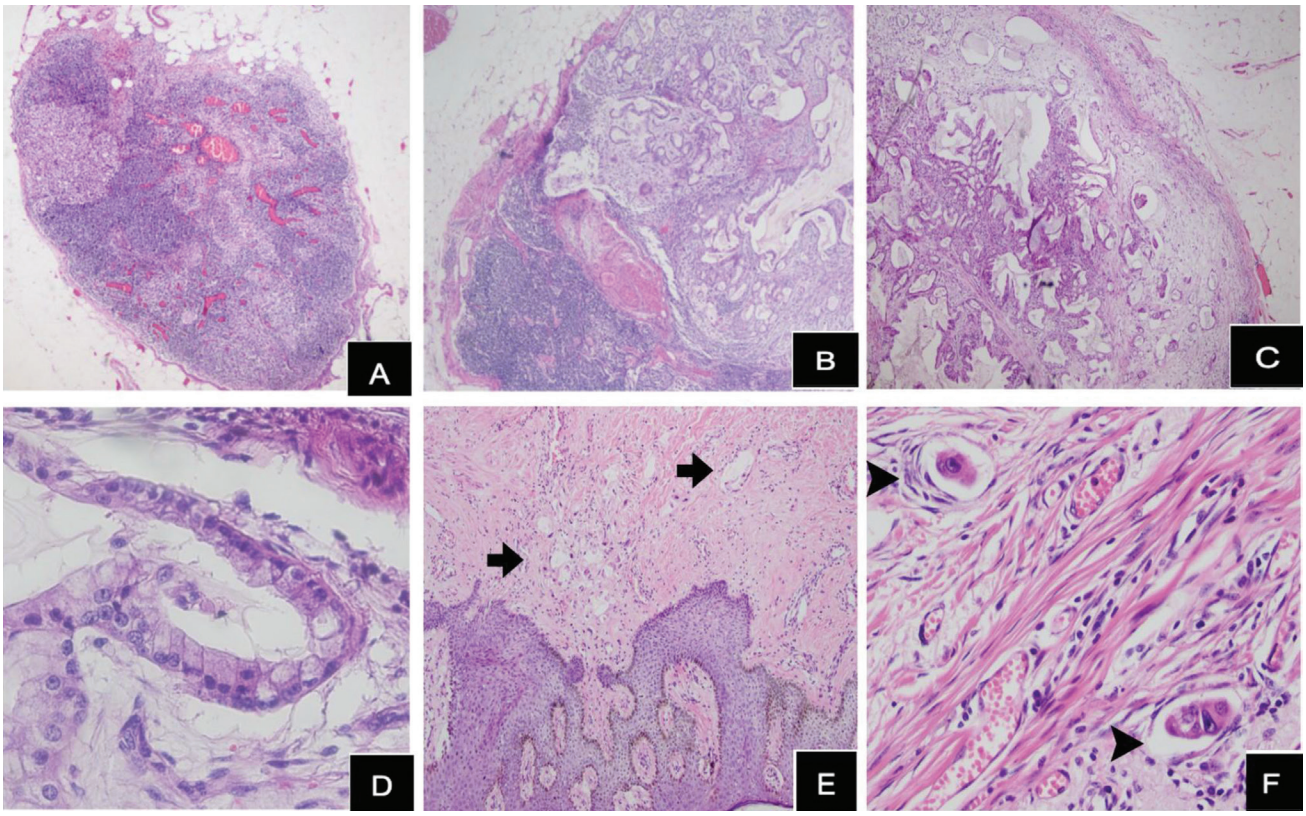

Figure 3. ***Representative prognostically significant findings [Hematoxylin and eosin stain].*** **(A and B)** Sections of axillary lymph nodes with macrometastasis [40x], **(C)** Positive lymph node with extracapsular invasion [100x], **(D)** Higher magnification of metastatic focus show histomorphologic features with the breast mass [400x], **(E and F)** Dermal lymphatic invasion (arrow) [100x] and lymphovascular space invasion (arrowhead) [400x].

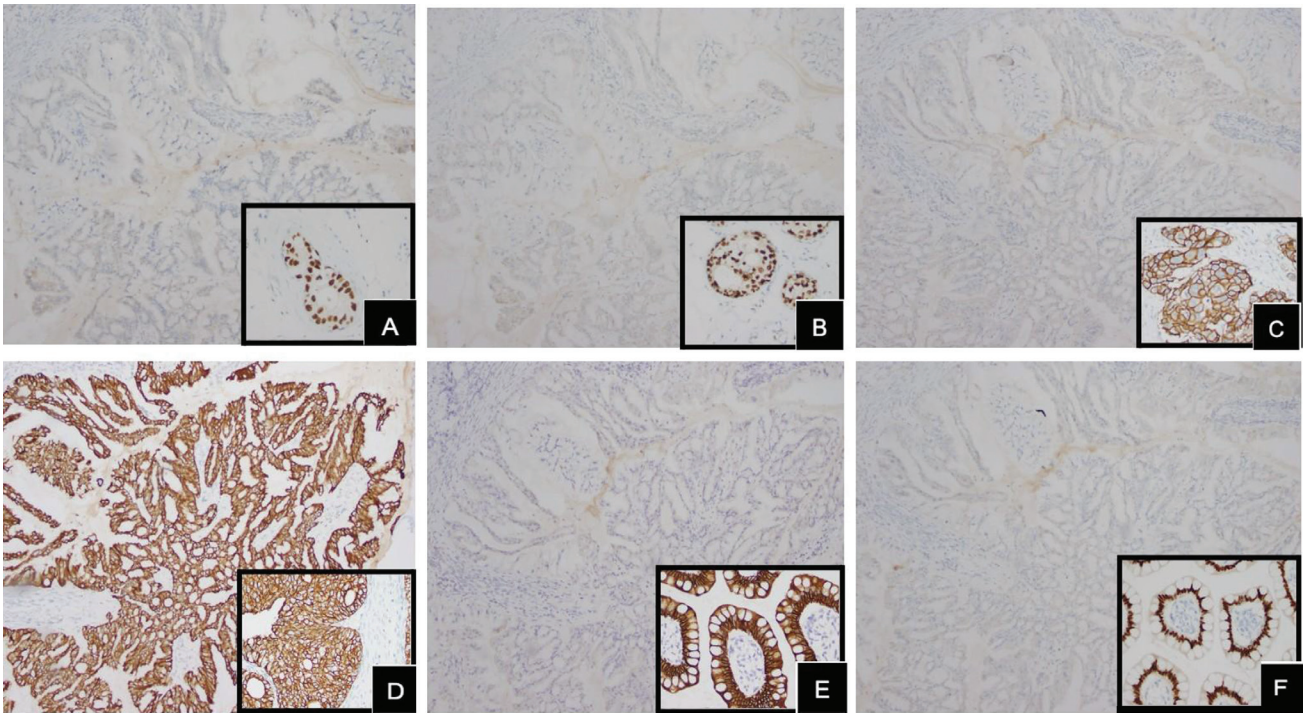

Figure 4. ***Immunohistochemistry stains [Horseradish peroxidase method, 100x] with respective positive controls (inset):*** **(A)** ER, **(B)** PR, **(C)** HER2, **(D)** CK7, **(E)** CK20, **(F)** CDX2.

There were no reports of recurrence or any metastasis. At present, the patient is doing well and remains disease-free.

DISCUSSION

Mucinous cystadenocarcinoma (MCA) is a rare primary malignancy of the breast. It was first described in 1998 by Koenig and Tavassoli,[3] reported to be a variant of invasive ductal carcinoma of low-grade usually seen in post-menopausal women. With the recent 5th edition publication of the WHO Classification Breast Tumors, it now belongs as a separate entity under the epithelial tumors of the breast.

Based on literature, most cases of MCA may belong in the peri-and postmenopausal women. According to the World Health Organization, it has an average age of 61 years old which may range from 41 to 96 years.[4] The described patient is in the perimenopausal age of 54 years old.

MCA, like what was described in this case, is grossly a well-circumscribed solid and cystic mass with gelatinous material found along the cystic spaces. The size of the breast masses reported ranges from 3 to 10 cm in length[1,5,6] with a well-circumscribed soft cut surface. Consistently, this case shows a similar gross description with the greatest dimension of 10.0 cm.

Histologically, MCA is a carcinoma composed of generally tall, columnar cells with architectural features showing stratification, tufting and papillae. It is accompanied by nuclei that are located basally with the accumulation of intracytoplasmic mucin and extracellular mucin found in the surrounding cystic spaces.[4] With consideration of being a low-grade variant, it was reported to have a favorable prognosis. Microscopically can be described as tall columnar mucinous cells with either papillary, cribriform and fused glandular features. Nuclei atypia may be evident, together with the presence of mitosis with a Nottingham score of 1, accompanied by mucinous lakes in the surrounding stroma.[2]

The differential diagnoses for MCA of the breast would usually include the following: mucinous carcinoma of the breast and encapsulated papillary carcinoma of the breast. The similarity between mucinous cystadenoma carcinoma and mucinous carcinoma of the breast is evidence of large pools of mucin production. However, the main difference between the two entities is that the latter entity does not show any evidence of intracytoplasmic mucin accumulation, rather, it shows clusters of epithelial tumor cells floating in pools of extracellular mucin.[4] Furthermore, biomarker status plays a major role in differentiating MCA from mucinous carcinoma. The latter can be described as ER and/or PR positive under the luminal group, while the biomarker status of MCA is triple-negative (ER, PR, and HER2/neu negative) or "basal-like" as similarly described in most literature.[2,5,7-12] Although ER, PR and/or HER2/neu positivity in MCA has been reported, it is considered exceptionally rare.[1,6,7,13] On the other hand, similarities between MCA and encapsulated papillary carcinoma of the breast can be seen based on its architecture, exhibiting papillary-like fronds within cystic spaces as well as columnar epithelial cell lining that are arranged in single or multiple cell layers, able to form micropapillary or cribriform structures that fill in the gaps and separating adjacent papillae.[14] But unlike mucinous cystadenocarcinoma, encapsulated papillary carcinoma lacks intracytoplasmic mucin, and is described to mostly show ER and/or PR positivity.

Aside from primary carcinomas of the breast, another factor that must be considered is an MCA originating from either the ovary, pancreas or gastrointestinal tract that eventually metastasized to the breast. In this scenario, immunohistochemical markers play a major role in ruling out this possibility. The typical immunohistochemical studies profile of primary breast MCA is the following: positive CK7 expression, but negative for CK20 and CDX2 expression, as reported in most literature,[2,5,8,9,13] and cited in the 5th edition of WHO[3] which is like our case. As opposed to immunohistochemistry profile of MCA originating from the ovary which would show positive expression of CK7 and variable expression for both CK20 and CDX2[15,16] and MCA originating from the pancreas and gastrointestinal tract which may show positive expression for CK7, but more consistently show positive expression for both CK20 and CDX2.[15,17-19]

For this type of carcinoma, the presence of metastasis to the axillary lymph node, with only four (4) reported cases at present[4] while lymphovascular invasion also had minimal reporting in the literature. Despite few reported cases globally, in the Philippines, MCA with axillary lymph nodes and lymphovascular invasion with extranodal extension is the first known case up to this date. The rarity of the said case can explore the possibility of such metastasis despite it being considered a low-grade variant. In our case, seven out of twenty harvested lymph nodes showed evidence of metastasis and presence of lymphovascular space invasion.

Due to the rarity of the case, standard treatments have yet to be established. According to the National Comprehensive Cancer Network (NCCN) guidelines for invasive breast carcinoma, the recommended treatment for breast cancer, in general, is either breast-conserving therapy (lumpectomy) or mastectomy.[20] The documented surgical plan these patients usually received was either lumpectomy or mastectomy with axillary lymph node dissections.[2,6,8,11,12] In our case since the tumor was measured to be 10 cm in widest diameter, a lumpectomy procedure was contraindicated, hence the surgical procedure of choice was mastectomy. Also stated in the NCCN guidelines, adjuvant radiation therapy is highly advised especially for those individuals who had findings of positive results for axillary lymph node metastasis after mastectomy. Meta-analysis has also proven the reduction of both recurrence and breast cancer mortality in women even if systemic therapy was administered, hence, adjuvant radiation therapy was offered to the patient.[20] Studies regarding the benefits of chemotherapy in this case are still quite unclear and limited. Our patient received six cycles of neoadjuvant chemotherapy followed by a modified radical mastectomy and despite this, the tumor on resection was still quite large and histopathologic findings still showed extensive lymphovascular invasion and axillary lymph node involvement with extranodal extension. Despite the extensive findings mentioned up to this day, the patient is well and still for metastatic work-up. The possibility of local recurrence is unusual for MCA, although it has been mentioned in a few publications.[4]

The majority of published reports showed negative HER2/neu receptor status, which is like this case. To date, there were only two reported cases that have shown strong membranous HER2/neu immunohistochemistry expression.[21] The use of Her2/neu gene amplification using fluorescence in situ hybridization studies has been reported and achieved concordant results with its HER2/neu staining.[21] Genomic alterations testing and other gene amplification studies (such as using c-MYC and ZNF217 genes) for breast cancer progression for MCA of the breast are quite limited in the literature review, thus, no known pathognomonic molecular alterations are currently noted.[22]

CONCLUSION

Diagnosing MCA of the breast involves a combination of clinicopathologic and immunohistochemical modalities. Awareness of its existence and ruling out the possibility of metastasis are necessary for proper documentation, prognostication, and management. Due to the rarity of the case, standard treatments have yet to be established but it is reported to have a favorable overall prognosis with rare reports of local recurrence and distant metastasis. In addition, months after her surgery, the patient continued her usual follow-ups and underwent metastatic work-up (bone scan and bone densitometry) with her attending physician. There were no reports of recurrence or any metastasis. At present, the patient is doing well and remains disease-free. This case is exceptional since, despite the MCA histologic type and multiple cycles of neoadjuvant chemotherapy, the presence of extensive lymphovascular invasion and axillary lymph node involvement with extranodal extension remains evident, resulting in an advanced stage at presentation despite favoring good prognostic outcomes.

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ETHICAL CONSIDERATIONS

Patient consent was obtained prior to submission.

STATEMENT OF AUTHORSHIP

The authors certified fulfillment of ICMJE authorship criteria.

AUTHOR DISCLOSURE

The authors declared no conflict of interest.

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None.

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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

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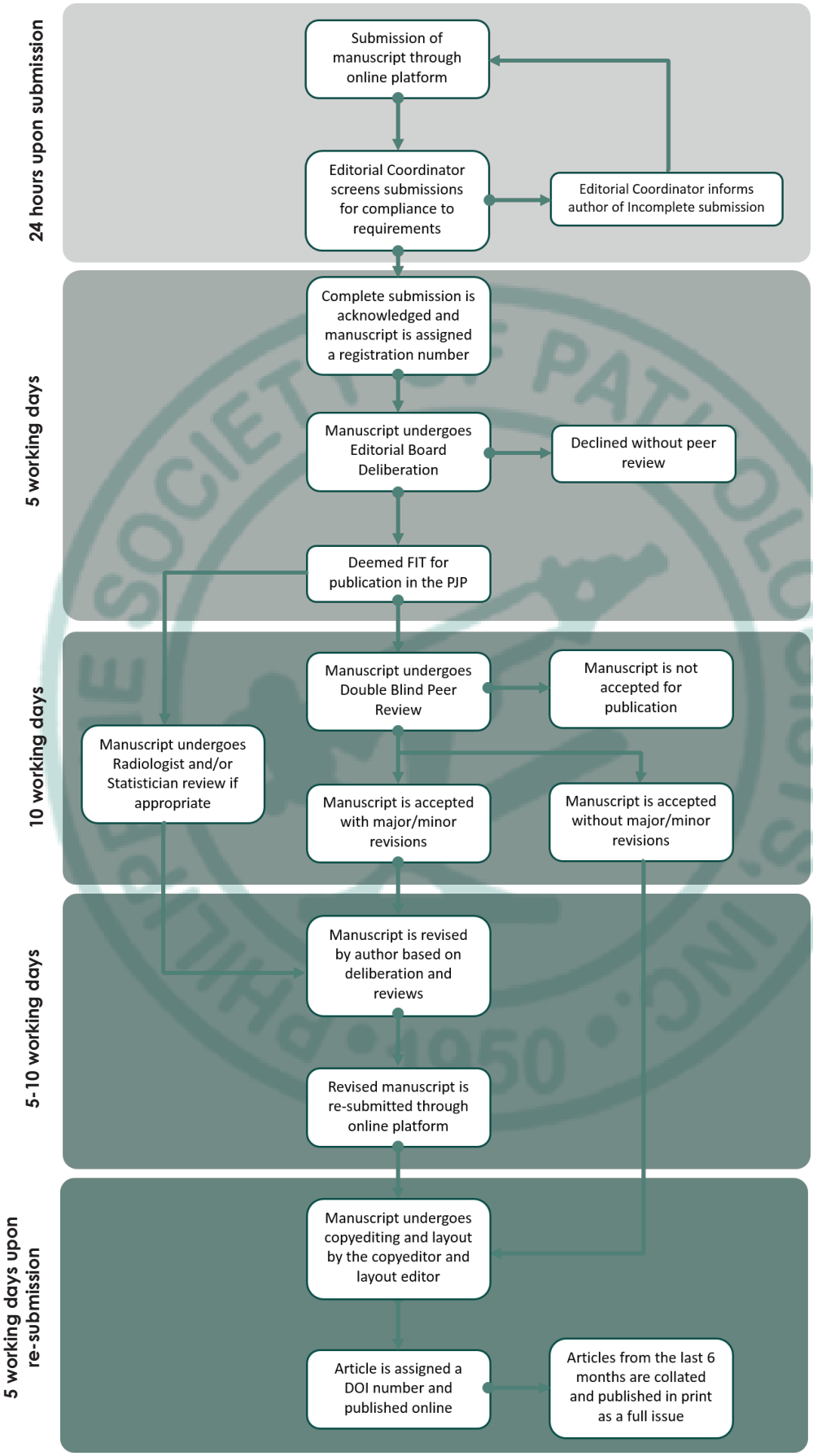

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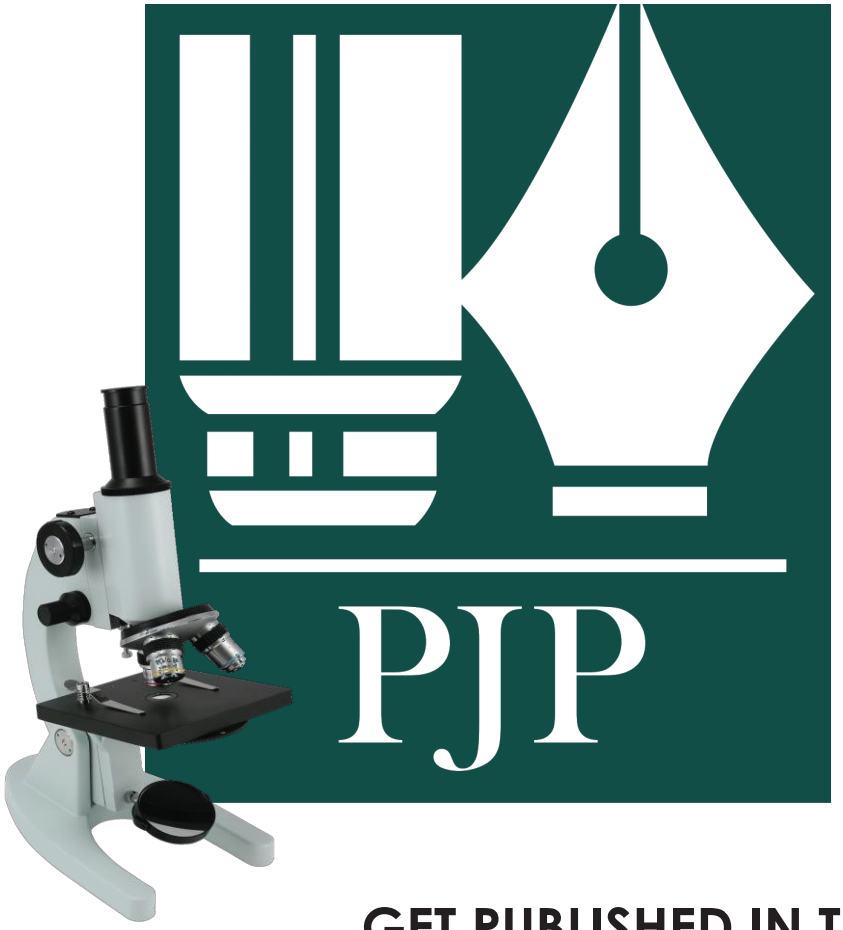

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